Chapter 12 Enzyme Kinetics, Inhibition, and Regulation

This chapter introduces chemical kinetics—the study of reaction rates—followed by the kinetics of enzymatic reactions. An enzyme-catalyzed reaction can be described by the Michaelis–Menten equation, which expresses the reaction velocity in terms of its Michaelis constant, K_M, and its maximum velocity, V_{max}. Detailed knowledge of the kinetics of a reaction can contribute to the understanding of its step-by-step reaction mechanism. The effects of different substrates, inhibitors, and other factors may also reveal an enzyme's physiological function. This knowledge can be exploited to develop drugs that are enzyme inhibitors. In this chapter, the three types of reversible enzyme inhibition and the equations that describe them are presented. Finally, the chapter describes the allosteric regulation of enzymes, using aspartate transcarbamoylase as an example.

Essential Concepts

Reaction Kinetics

1. A chemical reaction may proceed through several simple steps, called elementary reactions. The overall reaction pathway may therefore involve several short-lived intermediates.

2. The rate, or velocity (v), at which a reactant is consumed or a reaction product appears can be mathematically described. Thus, for the conversion of reactant A to product P,

$$v = \frac{d[\mathrm{P}]}{dt} = -\frac{d[\mathrm{A}]}{dt} = k[\mathrm{A}]$$

3. The rate of an elementary reaction varies with the concentration(s) of the reacting molecule(s). For example, for a single-reactant reaction (a unimolecular or first-order reaction), the rate is directly proportional to the concentration of the reactant. For a two-reactant reaction (a bimolecular or second-order reaction), the rate is directly proportional to the product of the concentrations of the reactants or to the square of the concentration of a reactant that reacts with itself.

4. The proportionality constant in the equation above, which is known as the rate constant, k, can be determined graphically. The rate equation for a first-order reaction is

$$\ln[\mathrm{A}] = \ln[\mathrm{A}]_0 - kt$$

and that for a second-order reaction is

$$\frac{1}{[\mathrm{A}]} = \frac{1}{[\mathrm{A}]_0} + kt$$

where $[A]_o$ is the initial concentration of the reactant and t is time. Consequently, if a plot of ln [A] versus t yields a straight line, the reaction is first-order, and if a plot of 1/[A] versus t yields a straight line, the reaction is second-order. The slope of the line reveals the value of the corresponding rate constant, k.

5. The kinetics of enzyme-catalyzed reactions are more complicated because the enzyme and substrate (reactant) combine to form a complex that then decomposes to product and free enzyme. For reactions involving a single substrate, S, the reaction velocity is typically measured under conditions where [S] » [E]. At very high substrate concentrations, the velocity is independent of [S] and the enzyme is said to be saturated with substrate.

6. The Michaelis–Menten equation, which describes an enzymatic reaction, is based on the assumption that the enzyme–substrate complex maintains a steady state; that is, its concentration does not change. This assumption is valid over most of the course of a typical enzymatic reaction.

7. The Michaelis–Menten equation is

$$v_o = \frac{V_{max}[S]}{K_M + [S]}$$

where v_o is the initial velocity of the reaction (before more than ~10% of the substrate has been consumed), V_{max} is the maximum rate of the reaction, and K_M is the Michaelis constant. This equation describes a rectangular hyperbola (the shape of the curve generated by a plot of v_o versus [S]) whose asymptote is V_{max}.

8. The Michaelis constant, K_M, is unique to each enzyme–sustrate pair. Its value is the substrate concentration at which the reaction velocity is half-maximal. It is therefore a measure of the affinity of the enzyme for its substrate.

9. The catalytic constant (k_{cat}), or turnover number, of an enzyme can be derived from V_{max}:

$$k_{cat} = \frac{V_{max}}{[E]_T}$$

where $[E]_T$ is the total enzyme concentration. The overall catalytic efficiency of an enzyme can be expressed as k_{cat}/K_M, which is an apparent second-order rate constant for the enzymatic reaction.

10. The kinetic parameters for an enzymatic reaction can be determined by taking the reciprocal of the Michaelis–Menten equation:

$$\frac{1}{v_o} = \left(\frac{K_M}{V_{max}}\right)\frac{1}{[S]} + \frac{1}{V_{max}}$$

A plot of $1/v_o$ versus 1/[S], a so-called Lineweaver–Burk or double-reciprocal plot, yields a straight line whose slope and intercepts yield the values of K_M and V_{max}.

11. Steady state kinetics cannot unambiguously establish a reaction mechanism because there are an infinite number of mechanisms that are consistent with a given set of kinetic data. However, mechanisms that are not consistent with the kinetic data can be ruled out.

12. Many enzymes have multiple (usually two) substrates and products. For example, transferase reactions are bisubstrate reactions. In a Sequential reaction, all the substrates bind to the enzyme before products are formed. In Sequential reactions, a particular order of substrate addition may be obligatory (an Ordered mechanism) or not (a Random mechanism).

13. In a Ping Pong reaction, one or more products of a transferase reaction are released before all substrates bind.

Enzyme Inhibition

14. Many drugs and pharmaceutical compounds alter the activities of specific enzymes. Detailed information about the mechanism of an enzyme can aid in the design of drugs with the desired properties. Studies of the kinetics of enzymes in the presence of specific inhibitors help reveal how the inhibitor acts and provide a way to quantitatively compare the effects of different inhibitors. An inhibitor may reversibly interact with an enzyme to interfere with its substrate binding, its catalytic activity, or both.

15. Three modes of reversible enzyme inhibition can be distinguished by their effects on the kinetic behavior of enzymes: competitive, uncompetitive, and mixed (noncompetitive) inhibition. Double-reciprocal plots of data collected in the presence of different concentrations of an inhibitor reveal the value of K_I, the dissociation constant of the inhibitor from the enzyme.

16. A competitive inhibitor competes with a normal substrate for binding to the enzyme. It therefore reduces the apparent affinity of the enzyme for its substrate (increases K_M). A large excess of substrate can overcome the effect of the inhibitor, so V_{max} is not affected.

17. An uncompetitive inhibitor binds only to the enzyme–substrate complex and apparently distorts the active site. It decreases the apparent K_M and V_{max}.

18. A mixed inhibitor binds to both free and substrate-bound enzyme and may interfere with both substrate binding and catalysis. As a result, the apparent V_{max} decreases, and the apparent K_M may increase or decrease. When only V_{max} is affected, the inhibition is said to be noncompetitive.

Regulation of Enzyme Activity

19. Enzyme activity can be controlled either by altering the amount of enzyme available for reaction or by modifying its catalytic activity through allosteric effects (or by covalent modification). For example, in feedback regulation, the end product of a metabolic pathway inhibits the first committed step in the pathway. This ensures that the pathway is active when product concentrations are low but is inactive when product concentrations exceed the levels needed by the cell.

20. Aspartate transcarbamoylase (ATCase) provides an example of allosteric regulation that includes feedback inhibition by CTP (the ultimate product of the pyrimidine synthesis pathway, which begins with ATCase) and activation by ATP (which ensures that the concentrations of pyrimidine nucleotides keep pace with those of purines). The binding of either effector molecule (ATP or CTP) to the regulatory subunits of ATCase induces changes in the enzyme's quaternary structure that alter the activity of the catalytic subunits. ATP stabilizes the R (high activity) state, whereas CTP stabilizes the T (low activity) state of this allosteric enzyme.

Key Equations

$$\ln [A] = \ln [A]_o - kt$$

$$\frac{1}{[A]} = \frac{1}{[A]_o} + kt$$

$$v_o = \frac{V_{max}[S]}{K_M + [S]}$$

$$k_{cat} = \frac{V_{max}}{[E]_T}$$

$$\frac{1}{v_o} = \left(\frac{K_M}{V_{max}}\right)\frac{1}{[S]} + \frac{1}{V_{max}}$$

Guide to Study Exercises (text p. 348)

1. First-order reaction: $\ln[A] = \ln[A]_o - kt$ or $[A] = [A]_o e^{-kt}$
   Second-order reaction: $1/[A] = 1/[A]_o + kt$
   (Section 12-1A)

2. Instantaneous velocity is the rate of a reaction at a particular point in time. It varies over the course of the reaction. The initial velocity of a reaction, v_o, is defined as the rate when $t = 0$, but really refers to the rate at a point after the enzymatic reaction has achieved a steady state (usually less than 1 sec). In practice, v_o is taken to be the reaction velocity before more than ~10% of the substrate has been converted to product. The maximal velocity is a property of enzyme-catalyzed reactions and refers to the velocity when [S] » K_M, that is, at substrate concentrations at which the enzyme is saturated with substrate. (Sections 12-1A and B)

3. See pp. 327–328.

12. At pH 0,

$$\tfrac{1}{2}\,O_2 + 2\,H^+ + 2\,e^- \rightleftharpoons H_2O \qquad \mathscr{E}^\circ = 1.23\ V$$

Is oxygen reduction more favored at pH 0 or at pH 7? Explain in electrochemical terms as well as in terms of chemical equilibria.

13. Consider the reaction in which acetoacetate is reduced by NADH to β-hydroxybutyrate:

$$\text{Acetoacetate} + \text{NADH} + H^+ \rightleftharpoons \beta\text{-hydroxybutyrate} + NAD^+$$

Calculate ΔG for this reaction at 25°C when [acetoacetate] and [NADH] are 0.01 M, and [β-hydroxybutyrate] and [NAD^+] are 0.001M.

Experimental Approaches to the Study of Metabolism

14. Radioactive isotope tracers and metabolic inhibitors have been essential to the elucidation of metabolic pathways. Can both kinds of agents be used to determine the order of metabolites in a metabolic pathway?

15. Many biosynthetic pathways have been elucidated by the analysis of genetic mutations in organisms such as *Neurospora crassa* and *Escherichia coli*. How would you elucidate the steps of a hypothetical biosynthetic pathway in *N. crassa* in which compound A leads to compound Z?

16. You have isolated four mutants in amino acid metabolism of the mold *N. crassa*. Mutant 1 requires two compounds for growth, X and Z. Mutant 2 only requires X. Mutants 3 and 4 require only Z. Mutant 3 accumulates a compound, W, that supports the growth of Mutant 4 but not that of Mutants 1 or 2. Mutant 4 accumulates a compound, Y, that alone supports the growth of Mutant 1.
    (a) Diagram the biosynthetic pathway connecting compounds W, X, Y, and Z, indicating the step at which each mutant is blocked.
    (b) According to the diagram in (a), what is the first committed step in the synthesis of Z?

Answers to Questions

1. The fundamental processes by which living organisms acquire and use free energy depend on oxidation–reduction reactions. For example, the oxidation of a metabolic fuel generates a reduced cofactor, such as NADH, whose subsequent reoxidation generates ATP. Rapid (i.e., enzyme-catalyzed) oxidation–reduction reactions therefore would be consistent with (but would not prove) the presence of life on Mars.

4. K_M, the substrate concentration at which the rate of an enzyme-catalyzed reaction is half-maximal, is a measure of an enzyme's affinity for a substrate. The smaller the K_M, the higher the affinity.

   The value of k_{cat}/K_M is indicative of an enzyme's catalytic efficiency. It is an apparent second-order rate constant for the reaction E + S → P. Hence, it takes into account the enzyme's affinity for the substrate (K_M) and well as the rate that the enzyme converts ES to P (k_{cat}). (Section 12-1B)

5. The Lineweaver–Burk plot is $1/v_o = (K_M/V_{max})(1/[S]) + 1/V_{max}$. Therefore, if $1/v_o$ is plotted as a function of 1/[S], the slope of the line is K_M/V_{max}, the extrapolated intercept on the 1/[S] axis is $-1/K_M$, and the intercept on the $1/v_o$ axis is $1/V_{max}$. (Section 12-1C)

6. See p. 334.

7. A competitive inhibitor increases K_M but does not affect V_{max}. An uncompetitive inhibitor decreases both K_M and V_{max}. A mixed inhibitor decreases V_{max} and may increase or decrease K_M. (Section 12-2)

8. An inhibitor binds reversibly to an enzyme, whereas an inactivator reacts irreversibly with an enzyme to inactivate it. (Section 12-2)

9. Enzyme activity can be regulated by (a) altering the amount of enzyme present through changes in its rate of synthesis or degradation, and (b) influencing its substrate-binding or catalytic properties through allosteric effectors or by covalent modification. (Section 12-3)

10. In ATCase, three dimers of regulatory subunits link the subunits of two catalytic trimers. Allosteric effects are made possible by intersubunit contacts that communicate changes in one subunit to the other 11 subunits. The activator ATP preferentially binds to and stabilizes the R (high activity) conformation of ATCase, whereas the inhibitor CTP preferentially binds to and stabilizes the T (low activity) conformation. Hence, ATP or CTP binding to a regulatory subunit alters the substrate affinity of all the catalytic subunits. The quaternary structural change of the T → R transition is primarily a counter-rotation of the regulatory dimers of ~15° that is accompanied by the separation of the catalytic trimers by ~11 Å. In the R state, the two domains of each catalytic subunit have swung together to assume a catalytically active conformation. (Section 12-3)

Questions

Reaction Kinetics

1. For each of the following reactions, write a rate equation and determine the reaction order.
   (a) A → P
   (b) A + B → P + Q
   (c) 2A → P

2. List two different ways to measure the progress of a chemical reaction.

3. A first-order reaction has a $t_{1/2}$ of 20 minutes.
   (a) What is the rate constant k?
   (b) What time is required to form 20% of the product?
   (c) What time is required to form 80% of the product?
   (d) How much starting material remains after 15 min?
   (e) Compare the rate constant for this reaction to that of the decay of ^{32}P, which has a half-life of 14 days.

4. The energy of binding a transition state complex ($X^{\ddagger}$) can be determined by writing an equilibrium expression for the formation of the complex.
   (a) For the reaction $A \rightleftharpoons X^{\ddagger}$, what is the equilibrium expression?
   (b) What is the expression for the free energy of binding to the transition state ($\Delta G^{\ddagger}$)?
   (c) What factors are needed to equate the rate constant k to the free energy of activation?

5. For the following reaction:

$$E + S \underset{k-1}{\overset{k1}{\rightleftharpoons}} ES \xrightarrow{k2} P + E$$

   (a) What is meant by the term "enzyme–substrate complex"?
   (b) Write a rate equation for the production of ES.
   (c) What is the rate of product formation from ES?
   (d) If all the enzyme is bound to substrate, what is the effect of adding more substrate on the forward rate of the reaction?

6. What is meant by (a) the steady state assumption, (b) K_M, (c) k_{cat}, (d) turnover number, (e) catalytic efficiency, and (f) diffusion-controlled limit?

7. The following data were obtained for the reaction $A \rightleftharpoons B$, catalyzed by the enzyme Aase. The reaction volume was 1 mL and the stock concentration of A was 5.0 mM. Seven separate reactions were examined, each containing a different amount of A. The reactions were initiated by adding 2.0 μL of a 10 μM solution of Aase. After 5 minutes, the amount of B was measured.

Reaction	*Volume of A added (μL)*	*Amount of B present at 5 minutes (nmoles)*
1	8	26
2	10	29
3	15	39
4	20	43
5	40	56
6	60	62
7	100	71

(a) Calculate the initial velocity of each reaction (in units of $\mu M \cdot min^{-1}$)
(b) Determine the K_M and V_{max} of Aase from a Lineweaver–Burk plot.
(c) Calculate k_{cat}.

8. Can you use kinetic data to prove that a particular model for an enzymatic reaction mechanism is correct? Explain.

9. Why is it possible for Sequential bisubstrate reactions to be Ordered or Random, whereas a Ping Pong reaction always has an invariant order of substrate addition and product release?

Enzyme Inhibition

10. There are three general mechanisms for the reversible inhibition of enzymes that follow the Michaelis–Menten model. How does the mode of inhibitor–enzyme binding differ among the three mechanisms?

11. The catalytic behavior of an enzyme may depend on ionizable amino acids. Therefore, a change in pH may influence an enzyme's catalytic behavior. How can you tell whether pH affects substrate binding or catalytic activity?

12. The movement of glucose across the erythrocyte membrane is "catalyzed" by a transport protein (Section 10-4B and Box 10-2).
(a) What is the kinetic behavior of this process?
(b) Can glucose transport be subject to competitive, uncompetitive, or mixed inhibition? Explain.

13. In hen egg white lysozyme (Section 11-4), the substitution of Ala for Asn at position 37 or for Trp at position 62 may alter the enzyme's kinetics. What changes would you predict and why?

Regulation of Enzyme Activity

14. Draw velocity versus [Asp] curves for the reaction catalyzed by the ATCase catalytic trimer and by the intact enzyme. Explain why the curves differ.

15. How do carbamoyl phosphate, aspartate, ATP, and CTP affect the T ⇌ R equilibrium of ATCase?

Answers to Questions

1. (a) $v = -d[A]/dt = k[A]$ This is a first-order reaction.
(b) $v = -d[A]/dt = -d[B]/dt = k[A][B]$ This is a second-order reaction (A and B must collide to form product).
(c) $v = -d[A]/dt = k[A]^2$ This is also a second-order reaction (A must collide with another molecule of A for the reaction to proceed).

2. The progress of a reaction can be followed by measuring the rates of the appearance of the product(s) or the disappearance of the reactant(s). In practice, any physical property, such as light absorbance, pH, or an NMR signal, can be followed, provided that it changes in proportion to the concentration(s) of the reactant(s) or product(s).

3. (a) For a first-order reaction, $t_{1/2} = 0.693/k$ (Equation 12-9). Therefore, $k = 0.693/20$ min $= 0.035$ min^{-1}.
   (b) Since $\ln([A]/[A]_o) = -kt$ (from Equation 12-6), $t = \ln([A]/[A]_o)/-k$. When 20% of A has been converted to product, $[A]/[A]_o = 0.8$ and $t = (\ln 0.8)/(-0.035\ \text{min}^{-1}) = (-0.22)/(-0.035\ \text{min}^{-1}) = 6.4$ min.
   (c) When 80% of A has been converted to product, $[A]/[A]_o = 0.2$ and $t = (\ln 0.2)/(-0.035\ \text{min}^{-1}) = (-1.61)/(-0.035\ \text{min}^{-1}) = 46$ min.
   (d) When $t = 15$ min, $\ln([A]/[A]_o) = (-0.035\ \text{min}^{-1})(15\ \text{min}) = -0.525$. Since $e^{-0.525} = 0.59$, 59% of A remains at 15 minutes.
   (e) For the decomposition of ^{32}P, $k = (0.693/14\ \text{days})\ (1\ \text{day}/1440\ \text{min}) = 3.4 \times 10^{-5}\ \text{min}^{-1}$. This is ~1000 times slower than the reaction described above.

4. (a) $K^{\ddagger} = [X^{\ddagger}]/[A]$.
   (b) $\Delta G^{\ddagger} = -RT \ln K^{\ddagger}$.
   (c) As described in Box 12-2, $d[P]/dt = k[A] = k'[X^{\ddagger}]$ where k' is the rate constant for the decomposition of $X^{\ddagger}$ to from products. k' can be expressed in terms of the Boltzman constant (k_B) and Planck's constant (h): $k' = k_B T/h$. Thus, since $[X^{\ddagger}] = K^{\ddagger}[A]$ and $K^{\ddagger} = e^{-\Delta G^{\ddagger}/RT}$,

$$\frac{d[P]}{dt} = \frac{k_B T}{h} e^{-\Delta G^{\ddagger}/RT} [A]$$

and

$$k = \left(\frac{k_B T}{h}\right) e^{-\Delta G^{\ddagger}/RT}$$

5. (a) The enzyme–substrate (ES) complex is the species formed by the interaction between an enzyme and its substrate.
   (b) The rate equation for the net formation of ES is the rate of formation of ES minus the rate of ES degradation: $d[ES]/dt = k_1[E][S] - k_{-1}[ES] - k_2[ES]$.
   (c) The rate of product formation, $d[P]/dt = v = k_2[ES]$.
   (d) Increasing substrate concentration when all the enzyme is in the ES complex does not increase the rate of the reaction since then $v = V_{max} = k_2[E]_T$ where $[E]_T$ is the total enzyme concentration; that is, the enzyme is working at its maximal rate; it can work no faster.

6. (a) The steady state assumption assumes that during the course of an enzyme-catalyzed reaction, the concentration of the ES complex does not change.

(b) K_M is the Michaelis constant: $K_M = (k_{-1} + k_2)/k_1$. K_M is the substrate concentration at which the reaction velocity is half-maximal.

(c) The k_{cat} is the maximum velocity divided by the total enzyme concentration: $k_{cat} = V_{max}/[E]_T$. It is the number of reaction processes (turnovers) that each active site catalyzes per unit time. For the simple kinetic scheme used to derive the Michaelis–Menten equation, $k_{cat} = k_2$.

(d) The turnover number is the same as k_{cat}.

(e) Catalytic efficiency, calculated as k_{cat}/K_M, is the apparent second-order rate constant for the reaction of E + S and indicates how often the enzyme catalyzes a reaction upon encountering its substrate.

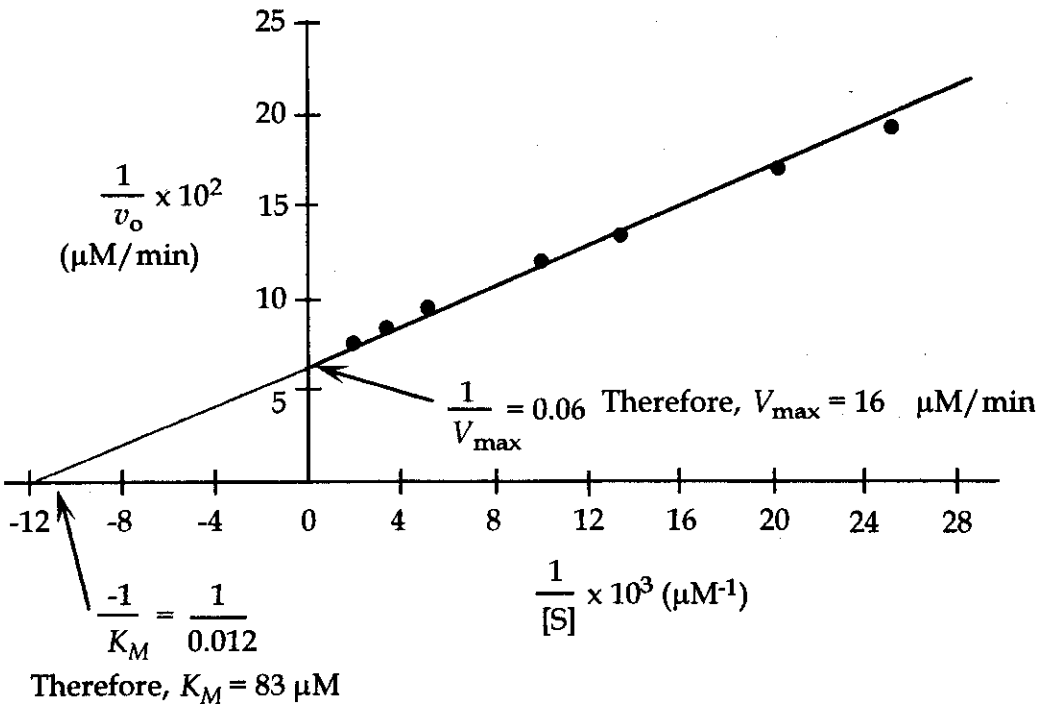
(f) If an enzyme catalyzes a reaction every time it collides with its substrate, it has reached catalytic perfection and the rate is controlled by how often the molecules collide, that is, by their rate of diffusion. At this point, the rate is said to have reached its diffusion-controlled limit.

7. (a) To calculate v_o for Reaction 1, for example,
v_o = (26 nmol/5 min)/(1.0 mL) × (10^3 mL/1 L) × (0.001 μmol/nmol) = 5.2 $\mu M \cdot min^{-1}$

Reaction	v_o ($\mu M \cdot min^{-1}$)
1	5.2
2	5.8
3	7.8
4	8.6
5	11.2
6	12.4
7	14.2

(b) First, calculate [S] for each reaction. In Reaction 1, for example, [A] = (0.008 mL)(5 mM)/(1 mL) × (1000 μM/1 mM) = 40 μM. Next, convert the data to values of 1/[S] and 1/v.

Reaction	*[S] (μM)*	*1/[S] (μM^{-1})*	*v ($\mu M \cdot min^{-1}$)*	*1/v ($min \cdot \mu M^{-1}$)*
1	40	0.025	5.2	0.192
2	50	0.02	5.8	0.172
3	75	0.0133	7.8	0.128
4	100	0.010	8.6	0.0116
5	200	0.005	11.2	0.089
6	300	0.0033	12.4	0.081
7	500	0.002	14.2	0.070



(c) First calculate $[E]_T$ = (0.002 mL)(10 μM Aase)/(1 mL) = 0.02 μM.
Using the value of V_{max} determined above and Equation 12-27, $k_{cat} = V_{max}/[E]_T$ = (16 μM·min^{-1})/(0.02 μM) = 800 min^{-1}.

8. Kinetics can support but cannot prove a particular reaction mechanism. A single kinetic model may explain several mechanisms, so additional experiment must be performed to establish a particular mechanism. However, kinetic data can rule out mechanisms that are inconsistent with the observed behavior.

9. In a Sequential reaction, both substrates must bind before any product is released. Hence it is possible for the two substrates to bind in an Ordered or Random fashion. In a Ping Pong reaction, a group is transferred from the first substrate to the enzyme to form the first product; then a second substrate binds and is converted to a second product. Consequently, the second product cannot bind first or yield the second product until the first substrate has bound and been converted to the first product.

10. The three general mechanisms of inhibition are competitive, uncompetitive, and mixed inhibition. In competitive inhibition, the inhibitor competes with the substrate for binding to the active site, increasing the apparent K_M. The inhibitor binds only to the free enzyme and not to the ES complex. In uncompetitive inhibition, the inhibitor binds only to the ES complex to inhibit the enzyme. Although the inhibitor does not directly affect substrate binding, it decreases the apparent K_M. In mixed inhibition, the inhibitor can bind to both the free enzyme and the ES complex. Binding of a mixed inhibitor may alter the binding of the substrate and therefore may alter the apparent K_M. If the inhibitor does not alter the apparent K_M, inhibition is said to be noncompetitive.

11. Aside from extremely low or high pH's, which can denature an enzyme, changes in pH may affect the protonation/deprotonation of residues involved in substrate binding and/or catalysis. Constructing a Lineweaver–Burk plot at several different pH values should yield a set of lines that indicate whether K_M (substrate binding) and/or V_{max} (catalytic activity) is affected (just as Lineweaver–Burk plots can reveal the different types of enzyme inhibition).

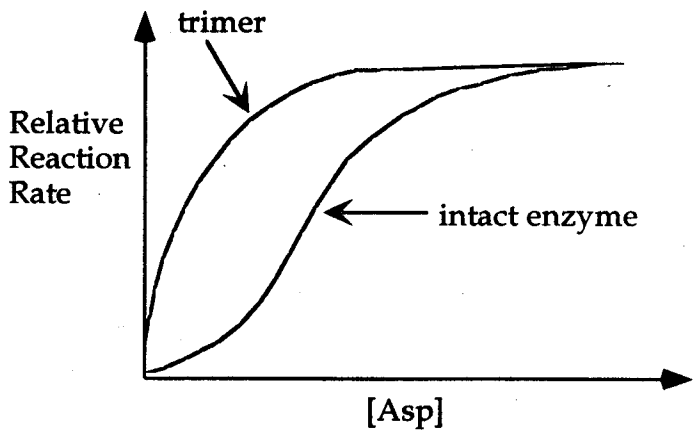
12. (a) The process obeys Michaelis–Menten (saturation) kinetics:

$$\text{Glucose}_{out} + \text{T}_{out} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \text{glucose}_{out}\cdot\text{T}_{out} \xrightarrow{k_2} \text{glucose}_{in}\cdot\text{T}_{in} \longrightarrow \text{glucose}_{in} + \text{T}_{in}$$

(b) Yes. All three inhibitory modes are possible. An inhibitor could compete with glucose for binding to the transport protein (competitive inhibition); it could bind to the transporter–glucose complex and interfere with the conformational change that exposes glucose to the other side of the membrane (uncompetitive inhibition); or it could interfere with the transporter's function with glucose bound or not bound (mixed inhibition).

13. These substitutions would weaken substrate binding to lysozyme by removing hydrogen bonds that hold the substrate in place. The K_M would increase, but V_{max} would remain nearly the same, since the catalytic residues Asp 52 and Glu 35 are not changed.

14. The catalytic trimer does not display cooperativity in the absence of the regulatory subunits. Hence the rate profile is hyperbolic, much like the binding of O_2 to myoglobin. Intact ATCase exhibits a sigmoidal curve characteristic of cooperative substrate binding, which is possible when the regulatory subunits mediate conformational changes between catalytic subunits.



15. ATP has higher affinity for and stabilizes the R (more active) state, and CTP has higher affinity for and stabilizes the T (less active) state.

Chapter 13 Introduction to Metabolism

This chapter provides a brief overview of the biological strategies and thermodynamics of metabolism. Metabolism is the overall biochemical processes that living systems use to acquire and use free energy. Organisms break down macromolecules to a common set of smaller molecules, or metabolites, which then serve as precursors for new biosynthesis. This chapter introduces some basic thermodynamic features of metabolic pathways and the mechanisms that have evolved to allow an organism to control the flow of a few common metabolites through different metabolic pathways.

Organisms harness the free energy from the degradation of macromolecules by trapping it in certain nucleotides (ATP, NAD^+, and FAD) and certain thioesters, which then make free energy available to energy-requiring pathways. The chapter describes these energy transmitters in terms of their thermodynamic features and their chemical properties. Some phosphorylated compounds have significant negative free energies of hydrolysis, which are described as their phosphoryl group-transfer potentials, their tendency to transfer their phosphoryl group to another compound. ATP, with its intermediate phosphoryl group-transfer potential, is the principal energy currency of life. Thioesters are also "high-energy" compounds. One of these, coenzyme A, shuttles acyl groups in metabolic processes.

Oxidation–reduction reactions are the most important process through which living organisms acquire and use free energy. The chapter reviews the principles of redox reactions, including the Nernst equation, and describes the mathematical relationship between free energy and reduction potentials. The chapter concludes with a brief look at the methods used to map the labyrinth of biochemical pathways in living cells and to understand the regulation of biochemical pathways.

Essential Concepts

1. Metabolism, the network of all biochemical reactions in cells, can be divided into two parts:
   (a) Catabolism (degradation), in which free energy is released as organic molecules are broken down into smaller constituents.
   (b) Anabolism (biosynthesis), in which biological molecules are synthesized from smaller, simpler molecules.

2. In general, catabolic reactions release free energy, which can then be used to drive endergonic synthetic reactions. This coupling requires free energy transmitters including nucleotides (ATP, NADH, NADPH, and $FADH_2$) and thioesters (e.g., coenzyme A).

Overview of Metabolism

3. Organisms use different strategies for capturing free energy from their environment:
   (a) Autotrophs synthesize all their macromolecules from simple molecules obtained from their environment. The chemolithotrophs oxidize inorganic compounds, and the photoautotrophs use light to drive synthetic reactions.

(b) Heterotrophs obtain free energy from the oxidation of organic compounds (usually produced by autotrophs).

4. Organisms can be further classified by their requirements for oxygen. Obligate aerobes require oxygen for the oxidation of nutrients. Obligate anaerobes are poisoned by oxygen and must use another electron acceptor for the oxidation of nutrients. Facultative anaerobes can oxidize nutrients both in the absence and presence of oxygen.

5. Metabolic pathways are compartmentalized in the cytosol of prokaryotic and eukaryotic cells. Eukaryotic cells further compartmentalize metabolic pathways in membrane-bound organelles. In multicellular organisms, many metabolic pathways are compartmentalized in different tissues.

6. In any given metabolic pathway, most reactions are near equilibrium, such that the law of mass action largely dictates the flow rate (flux) of metabolites.

7. In each metabolic pathway, there is at least one reaction that is far from equilibrium, in which the reactants accumulate above their equilibrium values and $\Delta G \ll 0$. Such reactions are referred to as rate-determining steps since they control flux in the pathway. The flux changes only with a change in the enzyme's ability to increase the reaction rate.

8. Metabolic pathways have three key characteristics:
   (a) They are irreversible.
   (b) They have an exergonic step that serves as the first committed step and ensures irreversibility.
   (c) Catabolic and anabolic pathways involving the interconversion of two metabolites differ in key exergonic reactions.

9. Control of flux in the rate-determining step requires control of the enzyme catalyzing it by one or more of the following mechanisms:
   (a) Allosteric control by feedback regulation from an end product of the pathway.
   (b) Covalent modification of the enzyme, which may increase or decrease its ability to accelerate a reaction.
   (c) Substrate cycles in which interconversion of two substrates utilizes different rate-determining enzymes.
   (d) Genetic control, which regulates the steady-state levels of the enzyme.

   Mechanisms (a)–(c) respond quickly to changes in physiological states (seconds to minutes), whereas mechanism (d) involves slower, long-term adaptive changes to new physiological states (minutes to days).

"High-Energy" Compounds

10. The free energy derived from the degradation of organic compounds is transiently captured in "high-energy" compounds whose subsequent breakdown provides the free energy to drive otherwise endergonic reactions.

11. ATP is the primary energy currency of cells. Its energy resides in the thermodynamic instability of its two phosphoanhydride bonds. The free energy of hydrolysis of ATP, in which the phosphate group is transferred to water, is called its phosphoryl group-transfer potential. ATP has an intermediate phosphoryl group-transfer potential, making it a conduit for the transfer of free energy from higher-energy compounds to lower-energy compounds.

12. The high-energy character of the phosphoanhydride bonds results from
    (a) Increased resonance stabilization of the hydrolysis products.
    (b) The destabilizing effect of electrostatic repulsions between the charged phosphates at neutral pH.
    (c) Increased solvation energy of the hydrolysis products.

13. Many endergonic reactions in cells are coupled to the hydrolysis of ATP or pyrophosphate (PP_i) so that the net reaction is exergonic. In some biosynthetic reactions, the transfer of a nucleotidyl group "activates" the substrate for further reaction (e.g., the polymerization reactions of polysaccharides and the formation of aminoacyl–tRNA for protein synthesis).

14. ATP can be replenished by transfer of a phosphoryl group to ADP from a compound with a higher phosphoryl group-transfer potential. Such a transfer is called substrate-level phosphorylation. The concentrations of ATP and other nucleotides are maintained in part by the activity of kinases.

15. The transfer of acyl groups requires their "activation" by formation of a thioester bond to a sulfur-containing compound such as coenzyme A. The hydrolysis of thioesters is about as exergonic as the hydrolysis of ATP. Hence, thioester cleavage drives the otherwise endergonic transfer of the acyl group.

Oxidation–Reduction Reactions

16. Oxidation–reduction reactions are the principal source of free energy for life. The oxidation of organic compounds is coupled to the reduction of the nucleotide cofactors NAD^+ (and $NADP^+$) and FAD.

17. A measure of the potential electrical energy (electromotive force or reduction potential) in an electrochemical cell is described by the Nernst equation:

$$\Delta\mathscr{E} = \Delta\mathscr{E}^{\circ\prime} - \frac{RT}{n\mathscr{F}} \ln\left(\frac{[A_{red}][B_{ox}^{n+}]}{[A_{ox}^{n+}][B_{red}]}\right)$$

Here, $\Delta\mathscr{E}$ is the reduction potential, $\Delta\mathscr{E}^{\circ\prime}$ is the reduction potential when all the components are in their biochemical standard states, $\mathscr{F}$ is the faraday (96,485 $J \cdot V^{-1} \cdot mol^{-1}$), n is the number of moles of electrons transferred per mole of reactants reduced, and R is the gas constant.

18. $\Delta\mathscr{E}$ is related to the free energy change in a redox reaction by the following relationship:

$$\Delta G = -n\mathscr{F}\Delta\mathscr{E}$$

Electrons flow spontaneously from a compound with the lower reduction potential to a compound with the higher reduction potential.

Experimental Approaches to the Study of Metabolism

19. Metabolites can be traced by labeling them with isotopes of certain atoms (e.g., C, S, P, and H) that can be detected by their radioactivity or through nuclear magnetic resonance spectroscopy. Radioactive tracers are especially useful for establishing precursor–product relationships and for examining the rates of biochemical transformation in living cells and tissues.

20. Other methods for analyzing a metabolic pathway include the use of metabolic inhibitors, which inhibit specific enzymes, and genetic mutations in enzymes involved in the pathway. In recent years, genetic engineering has become a powerful new tool for studying metabolism, as the gene for a specific enzyme can be added, deleted, or specifically altered.

Key Equations

$$\Delta G = -n\mathscr{F}\Delta\mathscr{E}$$

$$\Delta\mathscr{E} = \Delta\mathscr{E}^\circ - \frac{RT}{n\mathscr{F}}\ln\left(\frac{[A_{red}][B^{n+}{}_{ox}]}{[A^{n+}{}_{ox}][B_{red}]}\right)$$

$$\Delta\mathscr{E}^\circ = \mathscr{E}^\circ_{(e^-\text{ acceptor})} - \mathscr{E}^\circ_{(e^-\text{ donor})}$$

Guide to Study Exercises (text p. 380)

1. Autotrophs can synthesize all the molecules they require from simple molecules that are available from their environment. They obtain the free energy to do so by oxidizing inorganic compounds or by absorbing light energy.

   Heterotrophs cannot synthesize all their molecules from environmentally available precursors, nor can they harness the energy of inorganic compounds or the sun. Instead, they rely on autotrophs to supply them with organic compounds that they break down to obtain free energy and precursors for synthesizing other compounds. (Section 13-1A)

2. Reactions that operate near equilibrium are freely reversible. Since their ΔG values are close to zero, flux in either direction is possible, with the direction of flux determined by the

relative concentrations of reactants and products (at equilibrium there is no net flow). Such a reaction does not offer an opportunity for metabolic control, but because it operates in either direction, it can participate in two opposing pathways.

Reactions that function far from equilibrium have $\Delta G \ll 0$ and are therefore irreversible. These steps provide control points for metabolic pathways. Because the enzyme that catalyzes such a reaction is insensitive to changes in substrate concentrations, it can control the flux of substrate through the pathway in response to other factors, such as changes in the amount of enzyme present, the presence of allosteric effectors, and covalent modification. (Sections 13-1C and D)

3. ATP is a "high-energy" compound because its breakdown is highly exergonic. The free energy of hydrolysis of one of its phosphoanhydride bonds (which approximates the free energy of cleaving one of these bonds as part of another reaction) is ~30 $kJ \cdot mol^{-1}$ under standard biochemical conditions. Cleavage of these bonds is thermodynamically favored because the reaction products are resonance stabilized, experience less electrostatic repulsion, and are better solvated (Section 13-2A)

4. An exergonic process can drive an endergonic process only if the two processes are linked by a common intermediate. This can happen when a product of an exergonic reaction is a reactant for an endergonic reaction, or when a product of an endergonic reaction is a reactant for an exergonic reaction. Thus, the exergonic reaction can either "push" or "pull" the endergonic reaction. The coupled reactions will proceed if the net change in free energy is negative; that is, if the magnitude of ΔG for the exergonic reaction is greater than that of the endergonic reaction. (Section 13-2B)

5. The coenzymes NAD^+ and FAD are reduced with electrons obtained from the exergonic oxidation of metabolic fuels, so that their reduced forms (NADH and $FADH_2$) are a form of stored free energy. When the reduced coenzymes give up their electrons in order to return to their oxidized forms, that free energy is harvested for the endergonic synthesis of ATP from ADP + P_i. This is accomplished through the exergonic movement of the electrons through a series of electron carriers that establish a transmembrane proton concentration gradient whose dissipation drives ADP phosphorylation (oxidative phosphorylation). (Section 13-3A)

6. The Nernst equation (Equation 13-7) expresses the electromotive force (or reduction potential; $\Delta\mathscr{E}$, in units of volts) of an oxidation–reduction reaction as the difference between the standard reduction potential ($\Delta\mathscr{E}^\circ$, or $\Delta\mathscr{E}^{\circ\prime}$ when all components are in their biochemical standard state) and a variable term that takes into account the concentrations of the reactants (the logarithmic term), the temperature (T, in Kelvin), and the number of electrons transferred (n). These quantities are scaled by the gas constant (R = 8.3145 $J \cdot K^{-1} \cdot mol^{-1}$) and the faraday ($\mathscr{F}$ = 96,485 $J \cdot V^{-1} \cdot mol^{-1}$). (Section 13-3B)

7. $\Delta\mathscr{E}$ is related to ΔG as $\Delta G = -n\mathscr{F}\Delta\mathscr{E}$ so that the free energy change of an oxidation–reduction reaction depends on the number of electrons transferred (n, multiplied by a constant, the faraday, $\mathscr{F}$) and the reduction potential difference between the electron

acceptor and the electron donor ($\Delta\mathscr{E}$). ΔG is negative, and the reaction is spontaneous, only when $\Delta\mathscr{E}$ is positive, that is, when electrons move from a substance with a low reduction potential to a substance with a high reduction potential. (Sections 13-3B and C)

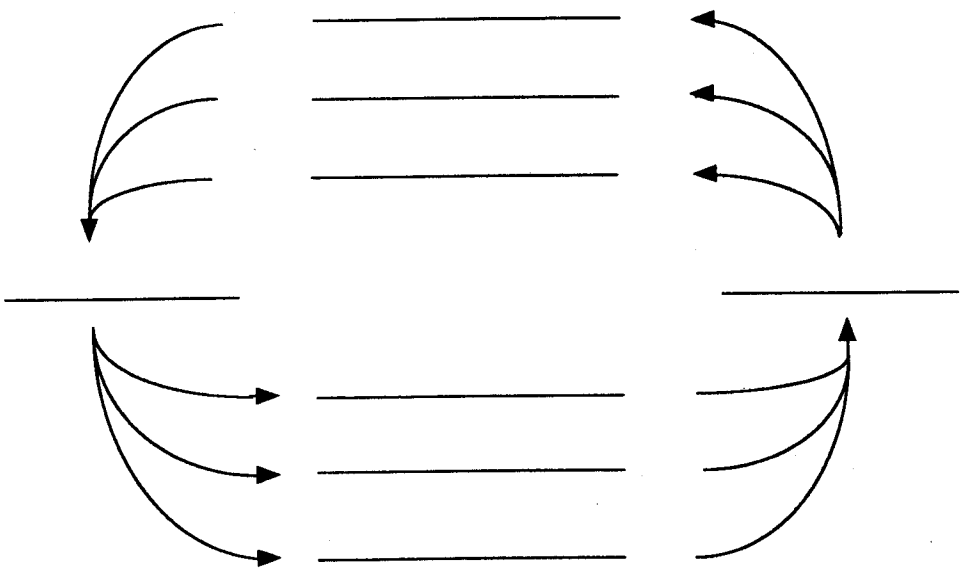
8. The transformation of a precursor compound to a product can be followed if an atom in the precursor that also appears in the product is replaced by an uncommon isotope. This allows the precursor, intermediates, and product to be detected through NMR or radioactivity. A small amount of labeled compound is added to the experimental system (cell extract, tissue culture, or whole organism) and the compounds containing the label are followed over a period of time. In this way, it is possible to trace the decrease in precursor concentration and the increase in concentration of the product. (Section 13-4A)

Questions

Overview of Metabolism

1. In searching for life on Mars in the 1970s, the Viking spacecraft tested the Martian soil for rapid oxidation–reduction reactions. Explain why such reactions might indicate the presence of life.

2. Use the following terms to fill in the diagram below: ADP, P_i, $NADP^+$, ATP, NADPH, carbohydrates, proteins, lipids, acetyl-CoA, catabolism, anabolism.



3. Phosphofructokinase (PFK) catalyzes the reaction

$$\text{ATP} + \text{fructose-6-phosphate (F6P)} \rightleftharpoons \text{fructose-1,6-bisphosphate (FBP)} + \text{ADP}$$

Explain why the reaction rate is relatively insensitive to changes in the concentrations of F6P or FBP. What does this tell you about PFK?

"High-Energy" Compounds

4. In the cell, divalent cations such as Mg^{2+} bind to the anionic phosphate groups of ATP. How would the removal of the divalent cations affect the ΔG for the hydrolysis of ATP?

5. What processes maintain the cellular concentration of ATP?

6. Why don't "high-energy" compounds such as phosphoenolpyruvate and phosphocreatine (Figure 13-7) break down quickly under physiological conditions?

7. Explain how phosphocreatine acts as an ATP "buffer."

8. Many metabolic reactions are actually coupled reactions. A common coupled reaction is substrate phosphorylation by ATP. For example, the oxidation of glucose begins with its phosphorylation to glucose-6-phosphate (G6P).
   (a) For the reaction

$$\text{Glucose} + P_i \rightleftharpoons \text{glucose-6-phosphate} + H_2O \qquad \Delta G = 14\ \text{kJ·mol}^{-1}$$

Calculate the ratio of [G6P]/[glucose][P_i] at equilibrium at 25°C.

   (b) In muscle cells at 37°C, the steady-state ratio of [ATP]/[ADP] is 12. Assuming that glucose and G6P achieve equilibrium values in muscle, what is the ratio of [G6P] to [glucose]?

9. Aldolase catalyzes the reaction

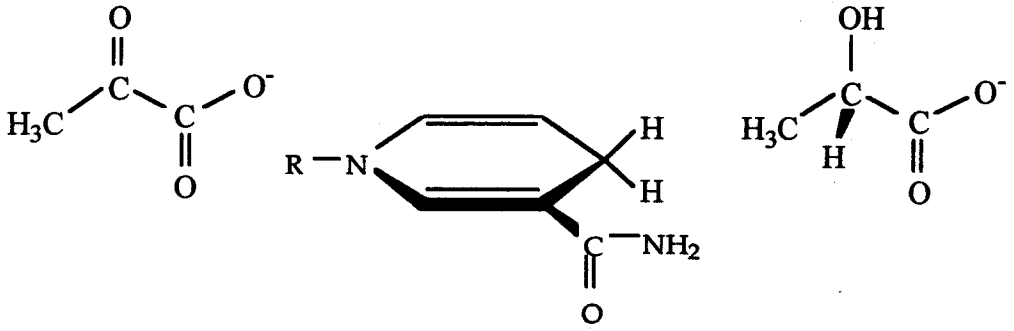
$$\text{Fructose-1,6-bisphosphate (FBP)} \rightleftharpoons \text{glyceraldehyde-3-phosphate (GAP)} + \text{dihydroxyacetone phosphate (DHAP)}$$

$\Delta G°'$ for this reaction is 22.8 kJ·mol^{-1}. In the cell at 37°C, the ΔG for this reaction is –5.9 kJ·mol^{-1}. What is the ratio [GAP][DHAP]/[FBP]?

Oxidation–Reduction Reactions

10. Using the curved-arrow convention, show the transfer of electrons in the reduction of pyruvate to lactate in the presence of NADH + H^+.

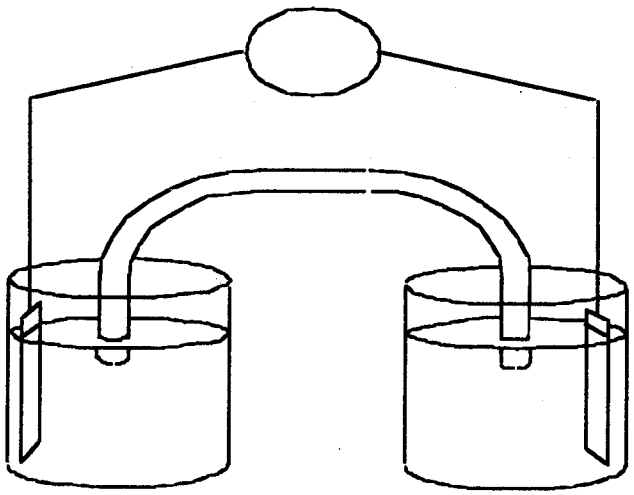
pyruvate NADH lactate



11. In the diagram below, indicate the direction of flow of electrons and the voltage (on the meter) for the following half-reactions under standard conditions:

$Zn^{2+} + 2e^- \rightarrow Zn$ $\mathscr{E}^\circ = -0.763$ V

$Ni^{2+} + 2e^- \rightarrow Ni$ $\mathscr{E}^\circ = -0.250$ V



Ni half-reaction Zn half-reaction

12. At pH 0,

$$\tfrac{1}{2}\,O_2 + 2\,H^+ + 2\,e^- \rightleftharpoons H_2O \qquad \mathscr{E}^\circ = 1.23\ V$$

Is oxygen reduction more favored at pH 0 or at pH 7? Explain in electrochemical terms as well as in terms of chemical equilibria.

13. Consider the reaction in which acetoacetate is reduced by NADH to β-hydroxybutyrate:

$$\text{Acetoacetate} + \text{NADH} + H^+ \rightleftharpoons \beta\text{-hydroxybutyrate} + NAD^+$$

Calculate ΔG for this reaction at 25°C when [acetoacetate] and [NADH] are 0.01 M, and [β-hydroxybutyrate] and [NAD^+] are 0.001M.

Experimental Approaches to the Study of Metabolism

14. Radioactive isotope tracers and metabolic inhibitors have been essential to the elucidation of metabolic pathways. Can both kinds of agents be used to determine the order of metabolites in a metabolic pathway?

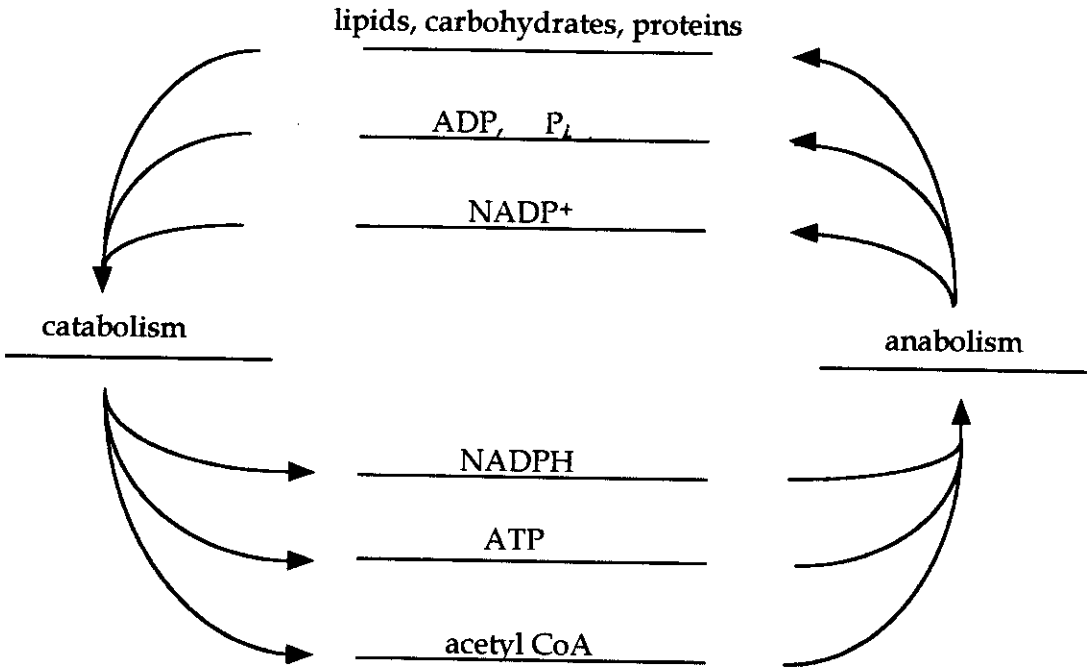
15. Many biosynthetic pathways have been elucidated by the analysis of genetic mutations in organisms such as *Neurospora crassa* and *Escherichia coli*. How would you elucidate the steps of a hypothetical biosynthetic pathway in *N. crassa* in which compound A leads to compound Z?

16. You have isolated four mutants in amino acid metabolism of the mold *N. crassa*. Mutant 1 requires two compounds for growth, X and Z. Mutant 2 only requires X. Mutants 3 and 4 require only Z. Mutant 3 accumulates a compound, W, that supports the growth of Mutant 4 but not that of Mutants 1 or 2. Mutant 4 accumulates a compound, Y, that alone supports the growth of Mutant 1.
    (a) Diagram the biosynthetic pathway connecting compounds W, X, Y, and Z, indicating the step at which each mutant is blocked.
    (b) According to the diagram in (a), what is the first committed step in the synthesis of Z?

Answers to Questions

1. The fundamental processes by which living organisms acquire and use free energy depend on oxidation–reduction reactions. For example, the oxidation of a metabolic fuel generates a reduced cofactor, such as NADH, whose subsequent reoxidation generates ATP. Rapid (i.e., enzyme-catalyzed) oxidation–reduction reactions therefore would be consistent with (but would not prove) the presence of life on Mars.

2.



3. PFK operates far from equilibrium, so it is not sensitive to changes in substrate concentrations. It most likely catalyzes a rate-determining step of a metabolic pathway. (In fact, it is part of the glycolytic pathway, and its activity is under allosteric control.)

4. ΔG would become more negative because, without the shielding effect of the divalent cations, the phosphate groups in ATP would experience more repulsion.

5. Processes that maintain the cellular concentration of ATP are oxidative phosphorylation (the synthesis of ATP from ADP + P_i as driven by the free energy of dissipation of a transmembrane proton concentration gradient); substrate-level phosphorylation (direct transfer of a phosphoryl group from a "high-energy" compound such as phosphoenolpyruvate to ADP); and reactions catalyzed by kinases such as nucleoside diphosphate kinase and adenylate kinase.

6. These "high-energy" compounds have large negative values for ΔG of hydrolysis. Therefore, their breakdown is thermodynamically spontaneous (exergonic). However, the kinetics of their breakdown depend on the availability and activity of enzymes to catalyze such reactions. In the absence of the appropriate enzymatic activity, the compounds are quite kinetically stable.

7. The reaction catalyzed by creatine kinase

$$\text{ATP} + \text{creatine} \rightleftharpoons \text{phosphocreatine} + \text{ADP}$$

is at equilibrium and is freely reversible in the cell. Hence, the concentration of phosphocreatine is directly sensitive to changes in [ATP]. When ATP is plentiful, phosphocreatine is formed by the forward reaction. When [ATP] drops, the reaction proceeds in reverse, so that phosphocreatine can transfer its phosphoryl group to ADP to produce more ATP. The extent of this reaction depends on the decrease in [ATP].

8. (a) At equilibrium, $\Delta G^{\circ\prime} = -RT \ln K_{eq}$,

$$\text{Hence } K_{eq} = \frac{[\text{G6P}]}{[\text{glucose}][P_i]} = e^{-\Delta G^{\circ\prime}/RT}$$
$$= e^{-(14{,}000\ \text{J·mol}^{-1})/(8.3145\ \text{J·K}^{-1}\text{·mol}^{-1})(298\text{K})}$$
$$= e^{-5.65}$$
$$= 0.0035$$

(b) The phosphorylation of glucose coupled to ATP hydrolysis is the sum of the following reactions:

$\text{ATP} + \text{H}_2\text{O} \rightleftharpoons \text{ADP} + P_i$	$\Delta G^{\circ\prime} = -30.5\ \text{kJ·mol}^{-1}$ (Table 13-2)
$\text{glucose} + P_i \rightleftharpoons \text{glucose-6-phosphate} + \text{H}_2\text{O}$	$\Delta G^{\circ\prime} = 14\ \text{kJ·mol}^{-1}$
$\text{ATP} + \text{glucose} \rightleftharpoons \text{glucose-6-phosphate} + \text{ADP}$	$\Delta G^{\circ\prime} = -16.5\ \text{kJ·mol}^{-1}$

At equilibrium, $\Delta G = 0$ and $\Delta G^{\circ\prime} = -RT \ln K_{eq}$. Thus,

$$K_{eq} = e^{-\Delta G^{\circ\prime}/RT} = \frac{[\text{ADP}][\text{G6P}]}{[\text{ATP}][\text{glucose}]}$$

Since [ATP]/[ADP] = 12,

$$\frac{[\text{G6P}]}{[\text{glucose}]} = 12\, e^{-\Delta G^{\circ\prime}/RT}$$

$$= 12\, e^{-(-16{,}500\ \text{J·mol}^{-1})/(8.3145\ \text{J·K}^{-1}\text{·mol}^{-1})(310\text{K})}$$

$$= 12\, e^{6.40}$$

$$= 7233$$

9. Use Equation 13-1:

$$\Delta G = \Delta G^{\circ\prime} + RT \ln \left(\frac{[\text{GAP}][\text{DHAP}]}{[\text{FBP}]} \right)$$

$$\frac{\Delta G - \Delta G^{\circ\prime}}{RT} = \ln \left(\frac{[\text{GAP}][\text{DHAP}]}{[\text{FBP}]} \right)$$

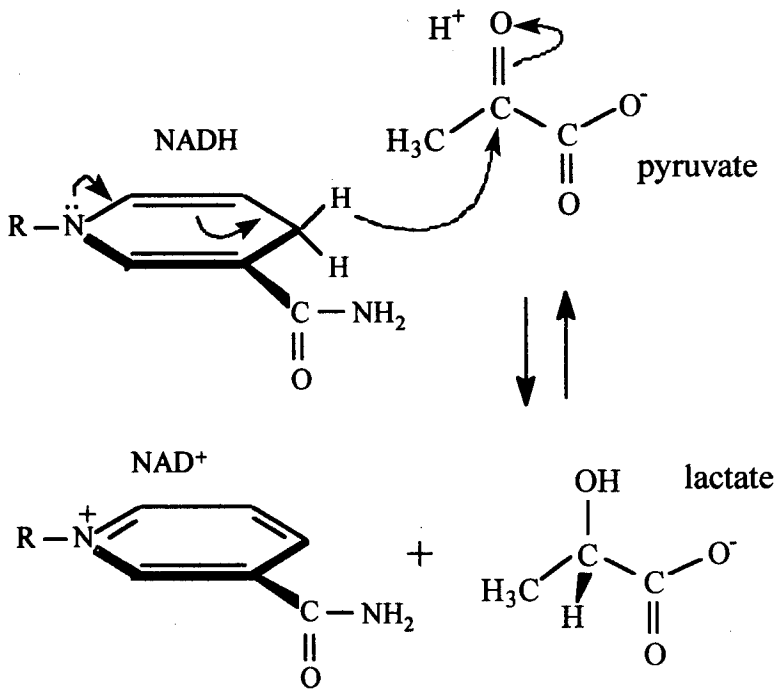
$$\frac{(-5900 \text{ kJ·mol}^{-1}) - (22{,}800 \text{ kJ·mol}^{-1})}{(8.3145 \text{ J·K}^{-1}\text{·mol}^{-1})(310\text{K})} = \ln \left(\frac{[\text{GAP}][\text{DHAP}]}{[\text{FBP}]} \right)$$

$$-11.13 = \ln \left(\frac{[\text{GAP}][\text{DHAP}]}{[\text{FBP}]} \right)$$

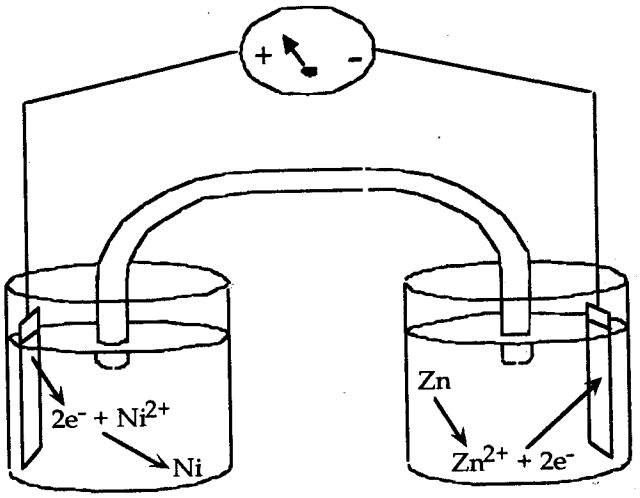
$$e^{-11.13} = \frac{[\text{GAP}][\text{DHAP}]}{[\text{FBP}]}$$

$$1.46 \times 10^{-5} = \frac{[\text{GAP}][\text{DHAP}]}{[\text{FBP}]}$$

10.



11. Electrons flow from substances of lower reduction potential to substances of higher reduction potential. $\Delta\mathscr{E}^{\circ} = -0.250 \text{ V} - (-0.763 \text{ V}) = 0.513 \text{ V}$.



12. At pH 7.0, $\mathscr{E}^{\circ\prime} = 0.815$ V (Table 13-3). Therefore, reduction is more favorable at pH 0 (where $\mathscr{E}^{\circ} = 1.23$ V) since the more positive the reduction potential, the more negative the ΔG (Equation 13-6). The law of mass action dictates that an increase in the concentration of one of the reactants (H^+) will shift the equilibrium toward product. Thus, decreasing the pH, which increases $[H^+]$, will favor the reduction of oxygen.

13. The reduction of acetoacetate by NADH is a coupled redox reaction, where the half-reactions (shown in Table 13-3) are

$$\text{Acetoacetate} + 2\,H^+ + 2\,e^- \rightleftharpoons \beta\text{-hydroxybutyrate} \qquad \mathscr{E}^{\circ\prime} = -0.346 \text{ V}$$

$$NAD^+ + H^+ + 2\,e^- \rightleftharpoons NADH \qquad \mathscr{E}^{\circ\prime} = -0.315 \text{ V}$$

For the overall reaction direction specified, the NAD^+/NADH half-reaction is the electron donor, and the acetoacetate/β-hydroxybutyrate half-reaction is the electron acceptor. Use the Nernst equation to calculate $\Delta\mathscr{E}$:

$$\Delta\mathscr{E} = \Delta\mathscr{E}^{\circ\prime} - \frac{RT}{n\mathscr{F}} \ln \left(\frac{[\beta\text{-hydroxybutyrate}][NAD^+]}{[\text{acetoacetate}][\text{NADH}]} \right)$$

$$= (\mathscr{E}^{\circ\prime}_{e^-\text{ acceptor}} - \mathscr{E}^{\circ\prime}_{e^-\text{ donor}}) - \frac{(8.3145 \text{ J}\cdot\text{K}^{-1}\cdot\text{mol}^{-1})(298\text{K})}{(2)(96{,}485 \text{ J}\cdot\text{V}^{-1}\cdot\text{mol}^{-1})} \ln \left[\frac{(0.001)(0.001)}{(0.01)(0.01)} \right]$$

$$= (-0.346 \text{ V} + 0.315 \text{ V}) - (0.01284 \text{ V}) \ln (0.01)$$
$$= -0.031 \text{ V} - (0.01284 \text{ V})(-4.605)$$
$$= 0.028 \text{ V}$$

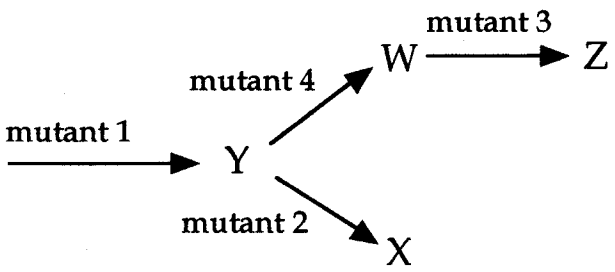
Next, use Equation 13-6 to calculate ΔG:

$$\begin{aligned}\Delta G &= -n\mathcal{F}\Delta\mathcal{E} \\ &= -(2)(96{,}485\ \text{J}\cdot\text{V}^{-1}\cdot\text{mol}^{-1})(0.028\ \text{V}) \\ &= -5403\ \text{J}\cdot\text{mol}^{-1} \\ &= -5.4\ \text{kJ}\cdot\text{mol}^{-1}\end{aligned}$$

14. Radioactive tracers can be used to determine the order of metabolic transformations in a pathway. An inhibitor that blocks a step of the pathway causes earlier intermediates to accumulate but does not necessarily reveal their order, and cannot reveal information about the order of metabolites following the blocked step.

15. To study the metabolic steps between compounds A and Z, first generate mutants by irradiating the cells or treating them with a chemical mutagen. Next, screen cells for their inability to grow in the absence of compound Z, the end product of the pathway. This would yield cells with defects in the A→Z pathway. To identify mutations in enzymes that catalyze individual steps of the pathway, screen the mutant cells for their inability to grow in the absence of each of the compounds suspected to be intermediates in the transformation of A to Z.

16. (a) Since Mutant 1 requires two compounds for growth, the pathway for their synthesis is probably branched, such that both are derived from a common precursor lacking in Mutant 1. Mutant 2 is blocked in the pathway leading to X, while Mutants 3 and 4 are blocked in the pathway leading to Z. The step blocked in Mutant 4 precedes that blocked in Mutant 3, since Mutant 3 accumulates compound W, which supports the growth of Mutant 4. Since compound Y, which accumulates in Mutant 4, supports the growth of Mutant 1, it must be the common precursor of X and Z. Therefore, the most likely pathway is



(b) The first committed step in the synthesis of Z is the step that converts Y to W, the step missing in Mutant 4.

Chapter 14 Glucose Catabolism

Glycolysis, or the biochemical conversion of glucose to pyruvate, is one of the best understood metabolic pathways. The mechanisms and structures of the ten glycolytic enzymes are known in some detail, and they serve as models for the study of other enzymes with similar reaction mechanisms. The regulation of metabolic pathways, a topic introduced in Chapter 13, is illustrated here. This chapter also describes the fate of pyruvate (the end product of glycolysis), the entry of other sugars into the glycolytic pathway, and the pentose phosphate pathway that also catabolizes glucose.

Essential Concepts

Overview of Glycolysis

1 Glucose is a major source of metabolic energy in many cells. The energy released during its conversion (oxidation) to pyruvate is conserved in the form of ATP and the reduced coenzyme NADH. Ten enzymes catalyze the glycolytic pathway, which occurs in both prokaryotes and eukaryotes and is almost universal.

2. Stage I of glycolysis is a preparatory state in which glucose is "activated" by phosphorylation by ATP and broken down into two C_3 sugars. Stage II produces "high-energy" intermediates that phosphorylate ADP to form ATP, for a net gain of 2 ATP. One glucose molecule yields two pyruvate molecules and requires the oxidizing power of two NAD^+. The overall equation for glycolysis is

$$\text{Glucose} + 2\,NAD^+ + 2\,ADP + 2\,P_i \rightarrow 2\,\text{pyruvate} + 2\,NADH + 2\,ATP + 2\,H_2O + 4\,H^+$$

The Reactions of Glycolysis

3. Hexokinase, the first enzyme in the pathway, transfers a phosphoryl group from ATP to the C6-OH group of glucose to produce glucose-6-phosphate (G6P). Hexokinase undergoes a large conformational change on binding glucose, which excludes water from the active site and promotes the specific transfer of the phosphoryl group from ATP to glucose.

4. Phosphoglucose isomerase catalyzes the conversion of glucose-6-phosphate to fructose-6-phosphate (F6P). This reaction proceeds via an enediolate intermediate.

5. Phosphofructokinase (PFK) converts fructose-6-phosphate to fructose-1-6-bisphosphate (FBP) by another phosphoryl-group transfer from ATP. This reaction, which is irreversible, is the first committed step of the pathway. The phosphofructokinase reaction is the rate-determining step of glycolysis and the principal regulatory point.

6. Aldolase cleaves fructose-1,6-bisphosphate to two C_3 compounds: glyceraldehyde-3-phosphate (GAP) and dihydroxyacetone phosphate (DHAP). Class I aldolases (in animals and plants) operate via Schiff base and enamine intermediates. Class II aldolases use Zn^{2+} or Fe^{2+} to stabilize the enolate intermediate

7. Triose phosphate isomerase catalyzes the interconversion of glyceraldehyde-3-phosphate and dihydroxyacetone phosphate through an enediolate intermediate. This α/β barrel enzyme has achieved catalytic perfection. Its activity allows the products of the first stage of glycolysis to proceed through the second stage as glyceraldehyde-3-phosphate.

8. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) catalyzes the formation of the first glycolytic intermediate that has sufficient free energy to synthesize ATP from ADP. Glyceraldehyde-3-phosphate is converted to 1,3-bisphosphoglycerate (1,3-BPG) by the reduction of NAD^+ and the addition of inorganic phosphate. The sulfhydryl group of an enzyme Cys residue attacks GAP, which is then reduced by NAD^+ to form an acyl thioester intermediate that is attacked by P_i.

9. 1,3-Bisphosphoglycerate, a "high-energy" mixed anhydride of a phosphate and a carboxylic acid, is the substrate for phosphoglycerate kinase, which transfers the phosphoryl group at C1 to ADP to generate 3-phosphoglycerate (3PG) and the first ATP product of glycolysis.

10. 3-Phosphoglycerate is converted to 2-phosphoglycerate (2PG) by phosphoglycerate mutase. The active form of this enzyme contains a phosphohistidine residue whose phosphoryl group is transferred to 3PG to produce 2,3-bisphosphoglycerate (2,3-BPG), which then transfers the phosphoryl group at the 3 position back to the histidine, yielding 2PG.

11. Enolase dehydrates (removes water from) 2-phosphoglycerate to form phosphoenolpyruvate (PEP). This reaction produces the second "high-energy" intermediate of glycolysis.

12. The free energy of phosphoenolpyruvate is released in the reaction catalyzed by pyruvate kinase. The transfer of the phosphoryl group to ADP produces the second ATP product of glycolysis and an enol product whose tautomerization to the keto form yields pyruvate. Most of the free energy of the pyruvate kinase reaction is supplied by this tautomerization step. Keep in mind that because the initial substrate of glycolysis is a C_6 compound that is converted to two C_3 compounds, the first stage of glycolysis consumes 2 ATP but the second stage generates 4 ATP (2 for each GAP), for a net yield of 2 ATP.

Fermentation: The Anaerobic Fate of Pyruvate

13. The NADH produced by glycolysis must be converted back to NAD^+ in order for the glyceraldehyde-3-phosphate dehydrogenase reaction to proceed. Consequently, pyruvate is not the end product of glucose metabolism but can undergo one of three processes to regenerate NAD^+: homolactic fermentation, alcoholic fermentation, or oxidative metabolism. In oxidative metabolism, pyruvate is oxidized to CO_2 via the citric acid cycle.

14. In muscle cells, when O_2 is in short supply, lactate dehydrogenase reduces pyruvate to lactate, with the concomitant oxidation of NADH to NAD^+. The lactate that builds up in muscles upon strenuous exertion can either be reconverted to pyruvate later or carried by the blood to the liver, where it can be converted back to glucose by a process called gluconeogenesis.

15. Under anaerobic conditions, yeast carry out alcoholic fermentation. In the first reaction of this process, pyruvate is decarboxylated to acetaldehyde and carbon dioxide. Pyruvate decarboxylase catalyzes this reaction with the aid of the coenzyme thiamine pyrophosphate, which stabilizes the reaction's carbanion intermediate. In the second reaction, acetaldehyde is reduced with NADH to form ethanol and NAD^+, as catalyzed by alcohol dehydrogenase. These two reactions have been known for thousands of years: The released CO_2 raises bread, and the ethanol is used to make alcoholic beverages.

16. The anaerobic catabolism of glucose can be 100 times faster than the catabolism of glucose in the presence of oxygen. However, fermentation produces 2 ATP per glucose, whereas oxidative metabolism (via the citric acid cycle and oxidative phosphorylation) generates 38 ATP for each glucose molecules that is converted to CO_2 and H_2O.

Control of Glycolysis

17. The reaction catalyzed by phosphofructokinase, which has a large negative ΔG, is the first committed step in glycolysis and the primary control point for the pathway. PFK is allosterically inhibited by ATP, which binds to an inhibitory site and stabilizes PFK's T (less active) state. This is an example of feedback inhibition, since ATP is a product of the pathway. AMP, ADP, and fructose-2,6-bisphosphate (F2,6P) relieve the inhibition of PFK by ATP by preferentially binding to the R (more active) state. PFK thereby senses the energy state of the cell and adjusts the flux through glycolysis accordingly.

18. Additional control of glycolytic flux is provided by a substrate cycle. PFK catalyzes the reaction F6P + ATP $\rightarrow$ FBP + ADP, whereas fructose-1,6-bisphosphatase (FBPase) catalyzes the opposing reaction FBP + $H_2O \rightarrow$ F6P + P_i. The sum of these reactions is the hydrolysis of ATP. Both enzymes exist in the same cell and their relative activity is under hormonal and neuronal control. Substrate cycling, which produces heat, can provide a form of nonshivering thermogenesis.

Metabolism of Hexoses Other than Glucose

19. Three other sugars—fructose, galactose, and mannose—are major sources of cellular energy. In muscle, fructose can be directly phosphorylated by hexokinase to F6P. However, liver glucokinase cannot directly phosphorylate fructose. In the liver, fructokinase phosphorylates C1 to generate fructose-1-phosphate. Fructose-1-phosphate aldolase generates dihydroxyacetone phosphate and glyceraldehyde by an aldol cleavage. Glyceraldehyde kinase phosphorylates C3 of glyceraldehyde to produce the glycolytic intermediate GAP. Three other enzymes (alcohol dehydrogenase, glycerol kinase, and

glycerol phosphate dehydrogenase) convert glyceraldehyde to dihydroxyacetone phosphate, which is then converted to GAP by triose phosphate isomerase. Individuals who have defective fructose-1-phosphate aldolase have fructose intolerance and quickly develop a strong distaste for anything sweet.

20. Galactose, which differs from glucose in the configuration of the OH group at C4, requires four reactions to enter glycolysis. First, galactose is converted to galactose-1-phosphate by galactokinase. Next, galactose-1-phosphate uridylyl transferase transfers the UMP group of UDP–glucose to produce UDP–galactose and glucose-1-phosphate. An epimerase converts UDP–galactose to UDP–glucose. Finally, phosphoglucomutase converts glucose-1-phosphate to glucose-6-phosphate.

21. Mannose, the C2 epimer of glucose, is recognized by hexokinase. The resulting mannose-6-phosphate is then converted to the glycolytic intermediate fructose-6-phosphate by mannose isomerase.

The Pentose Phosphate Pathway

22. Besides ATP, cells require the reducing power of NADPH for the biosynthesis of macromolecules (anabolism). NADPH is used in biosynthesis, whereas NADH is used in oxidative metabolism (catabolism). Cells keep the $[NAD^+]/[NADH]$ ration near 1000 (which favors metabolite oxidation) and the $[NADP^+]/[NADPH]$ ratio near 0.01 (which favors reductive biosynthesis). The oxidation of glucose by the pentose phosphate pathway generates NADPH.

23. The first stage of the pentose phosphate pathway consists of three steps, its oxidative reactions:
    (a) Glucose-6-phosphate is oxidized to 6-phosphoglucono-δ-lactone by glucose-6-phosphate dehydrogenase, producing the first NADPH.
    (b) 6-Phosphogluconolactonase hydrolyzes the lactone (cyclic ester) to yield 6-phosphogluconate.
    (c) 6-Phosphogluconate dehydrogenase then catalyzes the oxidative decarboxylation of 6-phosphogluconate by $NADP^+$ to yield ribulose-5-phosphate, CO_2, and the second NADPH.

24. Stage two of the pentose phosphate pathway is catalyzed by two enzymes that act on ribulose-5-phosphate (Ru5P): Ribulose-5-phosphate epimerase converts Ru5P to xylulose-5-phosphate (Xu5P), and ribulose-5-phosphate isomerase converts Ru5P to ribose-5-phosphate (R5P). The R5P can be used to produce nucleosides for RNA and DNA synthesis.

25. In the third stage of the pentose phosphate pathway, 3 five-carbon sugars are converted to 2 fructose-6-phosphate and 1 glyceraldehyde-3-phosphate. First, transketolase transfers a two-carbon unit from Xu5P to R5P, yielding the seven-carbon sugar sedoheptulose-7-phosphate (S7P) and glyceraldehyde-3-phosphate (GAP). Next, transaldolase transfers a

three-carbon unit from S7P to GAP to form fructose-6-phosphate (F6P) and the four-carbon sugar erythrose-4-phosphate (E4P). Finally, another transketolase reaction converts Xu5P and E4P to F6P and GAP.

26. The reversible nature of the second and third stages of the pentose phosphate pathway permits the cell to meet its need for R5P (a nucleic acid precursor) and NADPH. For example, if the need for NADPH is greater than that for R5P, the excess R5P is converted to F6P and GAP for consumption via glycolysis. Conversely, if the demand for R5P outstrips the need for NADPH, the glycolytic intermediates F6P and GAP can be diverted to the pentose phosphate pathway to synthesize R5P.

27. The flux of glucose-6-phosphate through the pathway is controlled by the rate of the glucose-6-phosphate dehydrogenase (G6PD) reaction. This enzyme is regulated by the availability of its substrate $NADP^+$ so that the pathway flux increases in response to increasing levels of $NADP^+$ (which indicates increased cellular demand for NADPH).

28. A deficiency of G6PD is the most common human enzyme deficiency. The resulting shortage of NADPH increases the sensitivity of red blood cells to oxidative stress, since NADPH is required to maintain the supply of reduced glutathione, which removes organic hydroperoxides that occasionally form. Certain compounds such as the antimalarial drug primaquine stimulate peroxide formation, which induces hemolytic anemia in G6PD-deficient individuals. However, mutations in G6PD confer resistance to malaria in heterozygous females.

Guide to Study Exercises (text p. 424)

1. See Figure 14-1.

2. The fate of pyruvate depends on the energy needs of the cell and the availability of oxygen. Under aerobic conditions, pyruvate can be completely oxidized to CO_2 by the citric acid cycle, which ultimately generates large amounts of ATP. Under anaerobic conditions, pyruvate is metabolized to a lesser extent and no additional free energy is recovered in the form of ATP. In yeast, alcoholic fermentation converts pyruvate to CO_2 and ethanol by the actions of pyruvate decarboxylase and alcohol dehydrogenase. In muscle, pyruvate may be reversibly converted to lactate by the action of lactate dehydrogenase. (Section 14-3)

3. Phosphofructokinase (PFK) is an allosteric enzyme. The inhibitor ATP (which is also a substrate) binds to an inhibitory site, thereby stabilizing the T state of PFK, which has a lower affinity for its other substrate, fructose-6-phosphate. ADP, AMP, and fructose-2,6-bisphosphate reverse the inhibitory effects of ATP. The R (more active) state of PFK is stabilized by F6P, whose phosphoryl group forms an ion pair with an Arg side chain in another subunit. The R→T conformational change, which replaces this ion pair with unfavorable electrostatic interactions between F6P and the enzyme, is prevented when the activator AMP or ADP is bound to the R state enzyme. (Section 14-4A)

4. A substrate cycle permits greater variations in flux than regulation of a single enzyme. The coordinated regulation of two opposing reactions in a substrate cycle creates a situation that resembles a near-equilibrium reaction, whose flux can vary widely. The simultaneous operation of the two reactions results in the apparently wasteful consumption of ATP. This loss of free energy is offset by the ability to dramatically alter the flux through the pathway on short notice. An additional advantage of a substrate cycle is that two—rather than one—enzymes are involved, which allows a greater variety of hormonal or neuronal control mechanisms to fine-tune the activity of the pathway. (Section 14-4B)

5. Fructose enters the glycolytic pathway in a straightforward manner in muscle, by being converted to fructose-6-phosphate by the action of hexokinase. In liver, which has glucokinase rather than hexokinase, fructose is first converted to fructose-1-phosphate, which then undergoes aldol cleavage to dihydroxyacetone phosphate (which is converted to the glycolytic intermediate glyceraldehyde-3-phosphate) and glyceraldehyde. The glyceraldehyde may be phosphorylated to GAP for entry into glycolysis or it may be transformed into dihydroxyacetone phosphate by a three-reaction pathway that converts it first to glycerol and then glycerol-3-phosphate. This latter compound is required for lipid synthesis.

   Galactose requires four enzymatic reactions to enter glycolysis. First, galactose is phosphorylated at C1. The resulting galactose-1-phosphate is transferred to the UMP group of UDP–glucose, thereby releasing glucose-1-phosphate. The UDP–galactose undergoes epimerization to regenerate UDP–glucose. The glucose-1-phosphate released from UMP is converted by hexokinase to the glycolytic intermediate glucose-6-phosphate.

   Mannose is recognized by hexokinase, which produces mannose-6-phosphate. An isomerization reaction yields fructose-6-phosphate, which can continue through glycolysis. (Section 14-5)

6. The pentose phosphate pathway consists of three sets of reactions (see Figure 14-29). In the first stage, glucose-6-phosphate is oxidized and decarboxylated in three reactions to produce 2 NADPH, CO_2, and ribulose-5-phosphate. In the second stage, ribulose-5-phosphate is converted to ribose-5-phosphate and xylulose-5-phosphate. If ribose-5-phosphate is not siphoned off for nucleotide biosynthesis, these five-carbon sugars are produced in a ratio of two xylulose-5-phosphate to one ribose-5-phosphate so that they can be quantitatively converted to glycolytic intermediates. In the third stage, the five-carbon sugars undergo rearrangements catalyzed by transaldolase and transketolase so that 3 five-carbon sugars are converted to 2 fructose-6-phosphate and 1 glyceraldehyde-3-phosphate. (Section 14-6)

7. When ribose-5-phosphate is not needed, the pentose phosphate pathway yields 2 NADPH for each glucose-6-phosphate, and the carbon skeleton of the G6P substrate is entirely recycled back to glycolytic intermediates by the activities of ribulose-5-phosphate isomerase, ribulose-5-phosphate epimerase, transaldolase, and transketolase.

   When R5P is needed for nucleotide biosynthesis, the ribulose-5-phosphate produced by the oxidative reactions is converted to R5P rather than xylulose-5-phosphate. In addition, the glycolytic intermediates fructose-6-phosphate and glyceraldehyde-3-phosphate can be

converted to R5P by the reversible reactions catalyzed by transaldolase and transketolase. (Section 14-6D)

Questions

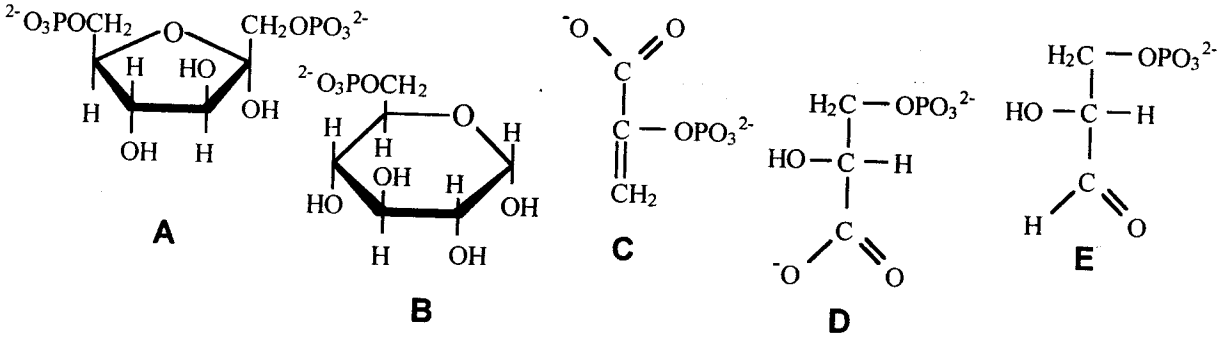
Overview of Glycolysis

1. Describe the two-stage "chemical strategy" of glycolysis and write a balanced equation for each phase.

2. The two "high-energy" compounds produced in glycolysis are _____ and _____.

The Reactions of Glycolysis

3. Examine the following five glycolytic intermediates:



(a) Name each intermediate
(b) Write the order in which they appear in glycolysis.
(c) Which intermediate is a reactant in substrate-level phosphorylation?
(d) List the glycolytic enzyme for which each intermediate is a substrate.
(e) Which phosphorylated intermediates of glycolysis are not shown above?

4. List the four kinases of glycolysis.

5. There are _____ isomerization reactions in glycolysis. The enzymes that catalyze them are _____.

6. How do Class II aldolases differ from Class I aldolases?

7. For the reaction catalyzed by triose phosphate isomerase, what is the equilibrium ratio of reactants and products under standard biochemical conditions? How does this ratio differ from the ratio observed in the cell at 37°C?

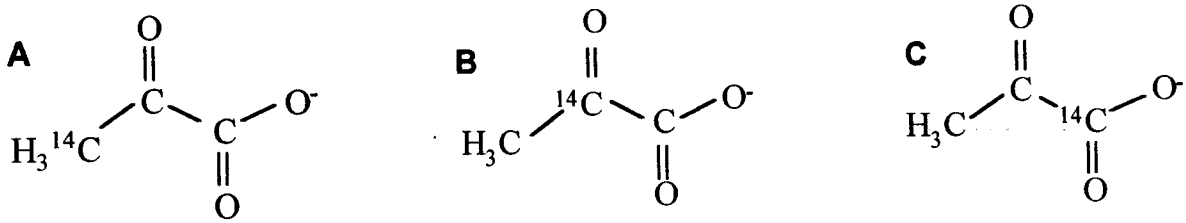
8. How does the GAPDH-catalyzed exchange of ^{32}P between P_i and 1,3-bisphosphoglycerate corroborate the existence of an acyl–enzyme intermediate in the GAPDH reaction?

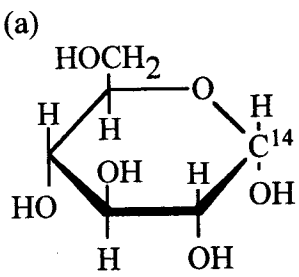
9. 2,3-BPG is an intermediate in the reaction catalyzed by phosphoglycerate mutase. Why does the cell require trace amounts of 2,3-BPG?

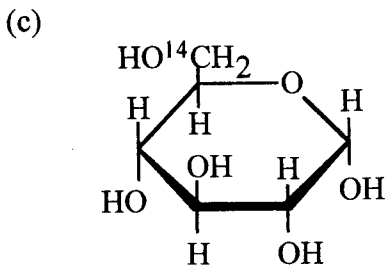
10. If the cytosolic $[NAD^+]/[NADH]$ ratio is 100 and the $[ATP]/[ADP][P_i]$ ratio is 10, what is the actual (not equilibrium) ratio of [GAP]/[3PG] at 37°C in the cell? Assume that $[H^+] = 1$, its value in the biochemical standard state.

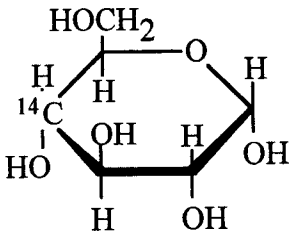
11. Fluoride ions specifically inhibit enolase in the presence of P_i in cell extracts.
    (a) Explain why both 2PG and 3PG accumulate in the presence of F^- and P_i.
    (b) Explain why 1,3-BPG does not accumulate.

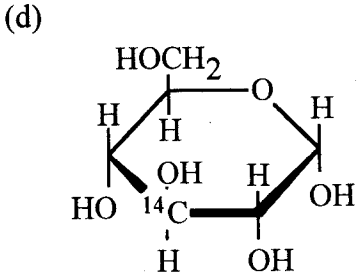
12. Match each ^{14}C-labeled glucose with the ^{14}C-labeled pyruvate produced by glycolysis.











Fermentation: The Anaerobic Fate of Pyruvate

13. How does a muscle cell maintain the [NAD^+]/[NADH] ratio during the catabolic breakdown of glucose?

14. Which of the carbon(s) of glucose must be labeled with ^{14}C for the end products of alcoholic fermentation to be unlabeled ("cold") ethanol and $^{14}CO_2$?

15. Compare the rates of ATP production in fermentation versus oxidative phosphorylation. Which process is utilized in rapid bursts of muscular activity?

Control of Glycolysis

16. In many metabolic pathways the first reaction is the rate-determining step of the pathway.
    (a) What is the rate-determining step of glycolysis?
    (b) What rationale can you offer for this "unusual" regulation?

17. What is meant by a futile cycle?

Metabolism of Hexoses Other than Glucose

18. The catabolism in the liver of which sugar—mannose, fructose, or galactose—bears the closest similarity to the first two reactions of glycolysis?

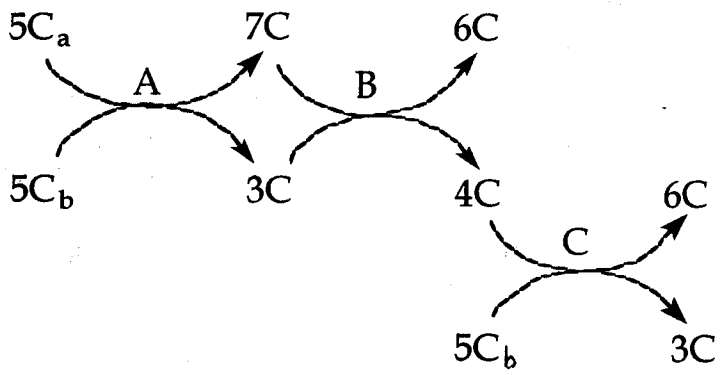
19. Defend or refute the following statement: The association of NAD^+ with UDP–galactose-4-epimerase suggests the generation of an additional NADH in the oxidation of galactose to pyruvate.

20. Why is galactosemia especially dangerous to nursing infants?

21. Some organisms use glycerol as a carbon energy source, and it is also an intermediate in fructose metabolism.
    (a) Write equations for the reactions required to oxidize glycerol to pyruvate.
    (b) Compare the ATP yield of two molecules of glycerol versus one molecule of glucose in glycolysis.
    (c) Does the anaerobic fermentation of glycerol maintain the redox balance of the cell? Explain.

The Pentose Phosphate Pathway

22. The diagram on the next page shows the interconversions of the nonoxidative reactions of the pentose phosphate pathway.



(a) Which sugar phosphates correspond to the 5C compounds?
(b) Which sugar phosphate corresponds to the 6C compound?
(c) Which sugar phosphate corresponds to the 3C compound?
(d) Which reaction(s) involves transketolase? Transaldolase?

23. The nonoxidative reactions of the pentose phosphate pathway convert pentose phosphates into hexose phosphates.
    (a) For every 3 glucose phosphates that enter the pentose phosphate pathway, how many fructose-6-phosphates are recovered?
    (b) For every 3 glucose-6-phosphates that enter the pentose phosphate pathway, how many glyceraldehyde-3-phosphates are recovered?

24. Ribulose-5-phosphate is converted to xylulose-5-phosphate and ribose-5-phosphate by an epimerase and an isomerase, respectively. What distinguishes these isomerizations?

25. You obtain a mutant transketolase from yeast that binds R5P and E4P poorly. What unique side product of the reaction catalyzed by this enzyme might you find in these cells?

26. Circle the appropriate choice in parentheses: Transaldolase transfers a (1, 2, 3) - carbon unit from a(n) (ketose / aldose) to a(n) (ketose / aldose) to form a(n) (ketose / aldose).

27. Circle the appropriate choice in parentheses: Transketolase transfers a (1, 2, 3) - carbon unit from a(n) (ketose / aldose) to a(n) (ketose / aldose) to form a(n) (ketose / aldose).

28. Which step commits glucose to oxidation via the pentose phosphate pathway? How is this enzyme regulated?

29. The conversion of G6P to R5P via the reactions of glycolysis and the pentose phosphate pathway, *without* the production of NADPH, can be summarized as

$$5\ \text{G6P} + \text{ATP} \rightleftharpoons 6\ \text{R5P} + \text{ADP} + \text{H}^+$$

What reaction requires ATP? How do you account for the stoichiometry?

Answers to Questions

1. In the energy investment phase, glucose is phosphorylated and cleaved to yield two molecules of glyceraldehyde-3-phosphate. Two ATPs are consumed in this stage so that the second stage can produce "high-energy" compounds whose breakdown drives ATP synthesis. The initial hexose is split, in the first stage, to two trioses. These undergo the reactions of the second stage, thereby generating four ATPs for a net "profit" of two ATPs per glucose. The equation for Stage I is

$$\text{Glucose} + 2\,\text{ATP} \rightarrow 2\,\text{GAP} + 2\,\text{ADP} + 2\,H^+$$

The equation for Stage II is

$$2\,\text{GAP} + 2\,NAD^+ + 4\,\text{ADP} + 2\,HPO_4^{2-} \rightarrow 2\,\text{pyruvate} + 2\,\text{NADH} + 4\,\text{ATP} + 2\,H_2O + 2\,H^+$$

2. ATP and NADH

3. (a) **A** Fructose-1,6-bisphosphate
   B Glucose-6-phosphate
   C Phosphoenolpyruvate
   D 3-Phosphoglycerate
   E Glyceraldehyde-3-phosphate
   (b) **B, A, E, D, C**
   (c) **C**
   (d) **A** Aldolase
   B Phosphoglucose isomerase
   C Pyruvate kinase
   D Phosphoglycerate mutase
   E Glyceraldehyde-3-phosphate dehydrogenase
   (e) Fructose-6-phosphate, dihydroxyacetone phosphate, 1,3-bisphosphoglycerate, and 2-phosphoglycerate.

4. Hexokinase, phosphofructokinase, phosphoglycerate kinase, and pyruvate kinase.

5. There are three isomerization reactions in glycolysis. The enzymes that catalyze them are phosphoglucose isomerase, triose phosphate isomerase, and phosphoglycerate mutase.

6. Class II aldolases stabilize the enolate intermediate by using a metal ion to polarize the carbonyl oxygen, rather than forming a covalently bound Schiff base intermediate, as in Class I aldolases.

7. Triose phosphate isomerase catalyzes the interconversion of GAP and DHAP. Its $\Delta G°' = 7.9\ kJ \cdot mol^{-1}$ and $\Delta G = 4.4\ kJ \cdot mol^{-1}$ (Table 14-1). Since $\Delta G = -RT \ln K$, $\ln K = -\Delta G/RT$ and $K = e^{-\Delta G/RT}$. Under standard conditions,

$$K = \frac{[GAP]}{[DHAP]} = e^{-(7900\ J \cdot mol-1)/(8.3145\ J \cdot K-1 \cdot mol-1)(310K)}$$

$$= 0.047$$

In the cell, where the reaction is not at equilibrium,

$$\Delta G = \Delta G°' + RT \ln \frac{[GAP]}{[DHAP]}$$

$$\frac{[GAP]}{[DHAP]} = e^{(\Delta G - \Delta G°')/RT)}$$

$$\frac{[GAP]}{[DHAP]} = e^{(4400 - 7900\ J \cdot mol-1)/(8.3145\ J \cdot K-1 \cdot mol-1)(310K)}$$

$$= 0.26$$

Therefore, the cellular ratio [GAP]/[DHAP] is nearly 6 times larger than under standard conditions, so that in the cell, formation of GAP is favored.

8. This isotope exchange reaction is consistent with the existence of an acyl–enzyme intermediate through the following series of events: For exchange to occur, 1,3-bisphosphoglycerate (1,3-BPG) must react with GAPDH to form an acyl–enzyme intermediate and eliminate P_i. (Fig. 14-9, Reaction 5 in reverse). In this reaction, the ^{32}P-labeled P_i reacts with the acyl-enzyme intermediate to yield ^{32}P-labeled 1,3-BPG.

9. 2,3-BPG is occasionally released by bisphosphoglycerate mutase. The resulting enzyme is inactive because it requires a phospho-His residue to phosphorylate 2PG. The presence of trace amounts of 2,3-BPG permits this substance to rebind to the enzyme and thereby activate it by forming the phospho-His residue.

10. The combined glyceraldehyde-3-phosphate dehydrogenase/phosphoglycerate kinase reaction is

$$GAP + NAD^+ + ADP + P_i \rightarrow 3PG + NADH + ATP + H^+$$

$\Delta G°' = -16.7\ kJ \cdot mol^{-1}$ and $\Delta G = -1.1\ kJ \cdot mol^{-1}$ (Table 14-1).

Since $\Delta G = \Delta G^{\circ\prime} + RT \ln Q$, where

$$Q = \frac{[3PG][NADH][ATP][H^+]}{[GAP][NAD^+][ADP][P_i]}$$

$$Q = e^{(\Delta G - \Delta G^{\circ\prime})/RT}$$
$$= e^{(-1100 + 16{,}700\ J \cdot mol^{-1})/(8.3145\ J \cdot K^{-1} \cdot mol^{-1})(310K)}$$
$$= 425$$

Since $$Q = \frac{[3PG][NADH][ATP][H^+]}{[GAP][NAD^+][ADP][P_i]} = \frac{[3PG]}{[GAP]} \times \frac{1}{100} \times 10 \times 1 = 425$$

$$\frac{[GAP]}{[3PG]} = \frac{1}{4250} = 2.35 \times 10^{-4}$$

11. (a) Inhibition of enolase causes its substrate, 2PG, to accumulate. Because the preceding reaction (catalyzed by phosphoglycerate mutase) is near equilibrium, 2PG equilibrates with 3PG so that [3PG] increases as [2PG] increases.
    (b) The formation of 3PG from 1,3-BPG is endergonic, so this reaction (catalyzed by phosphoglycerate kinase) does not proceed in reverse. Therefore, an increase in [3PG] does not cause [1,3-BPG] to increase.

12. (a) A
    (b) C
    (c) A
    (d) C

13. During the catabolic breakdown of glucose, NAD^+ is reduced to NADH, thereby lowering the $[NAD^+]/[NADH]$ ratio. Under anaerobic conditions, pyruvate can be converted to lactate with the concomitant oxidation of NADH to NAD^+. Under aerobic conditions, oxidative phosphorylation regenerates the NAD^+. Both of these processes will increase the $[NAD^+]/[NADH]$ ratio.

14. C3 or C4 of glucose must be labeled with ^{14}C.

15. The rate of ATP production during anaerobic fermentation can be as much as 100 times greater than during oxidative phosphorylation. Therefore, anaerobic fermentation provides the bulk of ATP during rapid bursts of muscle activity, even though the yield of ATP per glucose is much lower than in oxidative phosphorylation.

16. (a) The phosphofructokinase reaction is the rate-determining step.
    (b) The first reaction of glycolysis, catalyzed by hexokinase, is not a suitable control point for the overall pathway because significant amounts of sugar enter glycolysis as glucose-6-phosphate, the product of this reaction. For example, galactose is converted

to glucose-6-phosphate to enter glycolysis. Not all this glucose-6-phosphate proceeds through glycolysis: Some is shunted to the pentose phosphate pathway. Furthermore, mannose and, in muscle, fructose enter glycolysis as fructose-6-phosphate, which freely interconverts with glucose-6-phosphate. Therefore, the production of fructose-1,6-bisphosphate by PFK is the first committed step of glycolysis and the most effective point for regulation.

17. Substrate cycles were originally referred to as futile cycles because of their apparent futile waste of free energy.

18. Mannose.

19. No additional NADH is produced. The NAD^+ is probably reduced and then reoxidized in the sequential oxidation and reduction of C4 during epimerization of the hexose.

20. The primary sugar in human milk is lactose, a disaccharide of glucose and galactose.

21. (a) Glycerol can be converted to pyruvate through reactions catalyzed by glycerol kinase, glycerol phosphate dehydrogenase, triose phosphate isomerase, and the enzymes of Stage II of glycolysis (see Fig. 14-26):

$$\text{Glycerol} + \text{ATP} \rightarrow \text{glycerol-3-phosphate} + \text{ADP}$$
$$\text{Glycerol-3-phosphate} + \text{NAD}^+ \rightarrow \text{DHAP} + \text{NADH} + \text{H}^+$$
$$\text{DHAP} \rightarrow \text{GAP}$$
$$\text{GAP} + \text{NAD}^+ + 2\,\text{ADP} + P_i \rightarrow \text{pyruvate} + \text{NADH} + 2\,\text{ATP} + H_2O + 2\,H^+$$

$$\text{Glycerol} + 2\,\text{NAD}^+ + \text{ADP} + P_i \rightarrow \text{pyruvate} + 2\,\text{NADH} + \text{ATP} + H_2O + 3\,H^+$$

(b) The net yield of ATP is the same for the oxidation of 1 glucose or 2 glycerol to pyruvate.

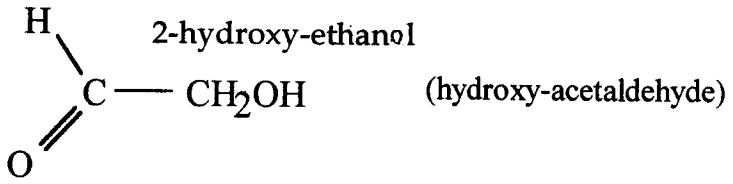
(c) For every pyruvate formed from glycerol, 2 NAD^+ are reduced to NADH. Alcoholic fermentation regenerates only 1 NAD^+ per pyruvate, so this pathway does not maintain the redox balance of the cell.

22. (a) $5C_a$ is ribose-5-phosphate, and $5C_b$ is xylulose-5-phosphate.
(b) 6C is fructose-6-phosphate.
(c) 3C is glyceraldehyde-3-phosphate.
(d) A and C are transketolase reactions, and B is a transaldolase reaction.

23. (a) Two
(b) One directly, although four more can be produced via glycolysis from the two F6P molecules also produced.

24. The conversion of Ru5P to Xu5P is a racemization that inverts the configuration at the C3 chiral center. The isomerization of Ru5P to R5P converts a ketose to an aldose by shifting the position of a double bond.

25.



As in the pyruvate decarboxylase reaction, an aldehyde might be expected to be released from the TPP cofactor of transketolase in the absence of the second substrate.

26. (3); (ketose); (aldose); (ketose and an aldose)

27. (2); (ketose); (aldose); (ketose and an aldose)

28. The committed step is the exergonic reaction mediated by G6PD, which is regulated by the availability of $NADP^+$.

29. (1) The 5 G6P are converted to 5 F6P by phosphoglucose isomerase.
(2) One F6P is converted to FBP, which requires ATP for the reaction catalyzed by phosphofructokinase.
(3) The FBP is converted to 2 GAP by aldolase and triose phosphate isomerase.
(4) The transaldolase and transketolase reactions of the pentose phosphate pathway operate in reverse to convert 2 F6P + 1 GAP to 2 Xu5P and 1 R5P. Since the starting materials are 4 F6P and 2 GAP, the result is 4 Xu5P and 2 R5P.
(5) The 4 Xu5P can be isomerized to 4 R5P, for a total yield of 6 R5P from the original 5 G6P.

Chapter 15 Glycogen Metabolism and Gluconeogenesis

This chapter discusses glycogen breakdown and synthesis, gluconeogenesis, and oligosaccharide synthesis, with an emphasis on the mechanisms that regulate these metabolic pathways. Six major enzymes are involved in the breakdown and synthesis of glycogen: glycogen phosphorylase, glycogen debranching enzyme, and phosphoglucomutase for glycogen breakdown, and UDP–glucose pyrophosphorylase, glycogen synthase, and glycogen branching enzyme for glycogen synthesis. The chapter explains how the synthesis of glycogen from glucose-1-phosphate requires the free energy of nucleotide hydrolysis in a reaction that is the opposite of the exergonic breakdown of glycogen. The mechanisms of glycogen phosphorylase and glycogen synthase are examined, including the role of the oxonium ion transition state in each case. Box 15-1 explores the strategies for optimizing the branched structure of glycogen. The chapter then discusses the regulation of glycogen phosphorylase and glycogen synthase by allosteric effectors and covalent modification. Here you are introduced to the notion of an enzyme cascade where extracellular signals (hormones) initiate the activation of successive kinases, resulting in the reciprocal activation of glycogen phosphorylase and inactivation of glycogen synthase. In this context, you are introduced to the second messengers cyclic AMP and Ca^{2+}. The role of phosphoprotein phosphatase-1 in modulating the ratio of phosphorylated to dephosphorylated enzymes is also explored as an additional layer of complexity in the regulation of the enzymes involved in glycogen breakdown and synthesis. Box 15-2 shows how inherited metabolic diseases contribute to our understanding of glycogen metabolism.

The chapter then moves on to gluconeogenesis, the process by which pyruvate and related metabolites can be converted to glucose. Gluconeogenesis uses many of the same enzymes as glycolysis but requires other enzymes to bypass the exergonic steps of glycolysis. The chapter discusses the interesting role of the allosteric effector fructose-2,6-bisphosphate in the regulation of gluconeogenesis and glycolysis in liver and heart muscle. The last section of this chapter discusses the roles of nucleotide sugars and dolichol pyrophosphate in oligosaccharide synthesis.

Essential Concepts

1. The mobilization of glucose from glycogen stores in the liver provides a constant supply of glucose to the central nervous system and red blood cells, which use glucose as their sole energy source. Under fasting conditions, amino acids (mainly from muscle protein degradation) serve as precursors for new glucose synthesis (gluconeogenesis).

2. Glucose-6-phosphate (G6P) is a key branch point in glucose metabolism in the liver, as it can be polymerized to glycogen, degraded to pyruvate, converted to ribose-5-phosphate, or hydrolyzed to glucose.

Glycogen Breakdown

3. Glycogen breakdown (glycogenolysis) utilizes three enzymes:

(a) Glycogen phosphorylase, which catalyzes the phosphorolysis at the nonreducing ends of glycogen to yield glucose-1-phosphate (G1P).
(b) Glycogen debranching enzyme, which transfers a trisaccharide and hydrolyzes the $\alpha(1 \rightarrow 6)$ linkage at branch points.
(c) Phosphoglucomutase, which converts G1P to G6P.

4. Phosphorylase utilizes the cofactor pyridoxal-5′-phosphate (PLP) in the general acid–base catalytic mechanism in glycogen phosphorolysis. Inorganic phosphate attacks the terminal glucose residue, which passes through an oxonium ion intermediate before being released as G1P. The activity of the dimeric enzyme is regulated by allosteric effectors and by phosphorylation/dephosphorylation of the protein at Ser 14.

5. Glycogen debranching enzyme acts as an $\alpha(1 \rightarrow 4)$ transglycosylase by transferring a trisaccharide from a limit branch (a four- or five-glucose-residue segment that phosphorylase cannot further degrade) to the nonreducing end of another branch. The remaining glucosyl residue, which is attached to glycogen by an $\alpha(1 \rightarrow 6)$ linkage, is hydrolyzed at a separate active site on the enzyme to release free glucose.

6. Phosphoglucomutase converts G1P to G6P by way of a G1,6P intermediate. A phosphate group from a Ser residue is transferred to C6 of G1P, followed by transfer of the C1 phosphate back to the Ser residue, in a manner similar to the phosphoglycerate mutase reaction.

7. The liver expresses glucose-6-phosphatase, an enzyme that hydrolyzes G6P. The resulting free glucose equilibrates with glucose in the blood so that the breakdown of liver glycogen leads to an elevation of blood glucose levels.

Glycogen Synthesis

8. Glycogen synthesis requires three enzymes to covert G1P to glycogen. First, UDP–glucose pyrophosphorylase catalyzes the transfer of UMP from UTP to the phosphate group of G1P to form UDP–glucose and PP_i. PP_i is eventually hydrolyzed to P_i by inorganic pyrophosphatase, which provides the exergonic push for this reaction.

9. Glycogen synthase catalyzes a transfer reaction in which the glucosyl residue of UDP–glucose is added to the nonreducing end of glycogen through an $\alpha(1 \rightarrow 4)$ bond. Glycogen synthase can only extend a pre-existing $\alpha(1 \rightarrow 4)$-linked chain. The glycogen molecule originates through the action of the protein glycogenin, which assembles a seven-residue glycogen "primer" for glycogen synthase to act on.

10. Glycogen branching enzyme transfers a seven-residue segment from the end of an $\alpha(1 \rightarrow 4)$-linked glucan chain to the C6-hydroxyl group of a glucosyl residue on the same chain or another chain, thereby forming an $\alpha(1 \rightarrow 6)$-linked branch.

Control of Glycogen Metabolism

11. The opposing processes of glycogen breakdown and synthesis are coordinately controlled by allosteric regulation and covalent modification of key enzymes. The major allosteric regulators are ATP, AMP, and G6P. Covalent modification occurs with the transfer of P_i from ATP to certain enzymes by the action of specific kinases.

12. Glycogen phosphorylase exists in two forms: phosphorylase *a* (the phosphorylated, more active enzyme) and phosphorylase *b* (the dephosphorylated, less active enzyme). Each form also has two conformations: the T (relatively inactive) state and the R (relatively active) state. AMP promotes the T $\rightarrow$ R conformational change and thereby activates phosphorylase *b*, whereas ATP and G6P inhibit this conformational change. Phosphorylase *a* is less sensitive to allosteric effectors and is mainly in the R state. However, high concentrations of glucose promote the R $\rightarrow$ T transition.

13. Covalent modification regulates glycogen phosphorylase and glycogen synthase in a reciprocal fashion. Glycogen phosphorylase tends to be more active when it is phosphorylated, so the ratio of phosphorylase *a* to phosphorylase *b* largely determines the rate of glycogen phosphorolysis. This ratio is set by the activity of phosphorylase kinase, which is regulated by cAMP-dependent protein kinase (cAPK), and by protein phosphatase-1 (PP-1). In contrast, glycogen synthase is activated by dephosphorylation so that cAPK promotes glycogen breakdown while inhibiting glycogen synthesis, and PP-1 inhibits glycogen breakdown while promoting glycogen synthesis.

14. The hormones glucagon and epinephrine activate adenylate cyclase, a transmembrane protein that converts ATP to cyclic AMP (cAMP). Elevated levels of cAMP bind to the regulatory subunits of cAPK, which causes its two catalytic subunits to dissociate from its regulatory dimer. This dissociation activates the catalytic subunits, which phosphorylate phosphorylase kinase, thereby activating it.

15. Phosphorylase kinase is also activated by Ca^{2+}, which binds to calmodulin, a ubiquitous Ca^{2+}-binding protein that interacts with numerous proteins and is a subunit of phosphorylase kinase. Elevated calcium levels can arise hormonally via epinephrine or from the neuronal impulses that trigger muscle contraction.

16. Protein phosphatase-1 (PP-1) can dephosphorylate glycogen phosphorylase, phosphorylase kinase, and glycogen synthase. In muscle, insulin-stimulated protein kinase activates PP-1 by phosphorylating its G subunit, which binds to glycogen in muscle . The cAPK-mediated phosphorylation of another site on the G subunit causes PP-1 to be released in the cytoplasm, where it cannot dephosphorylate glycogen-bound enzymes. In addition, phosphoprotein phosphatase inhibitor 1 (inhibitor-1) inhibits PP-1. Inhibitor-1 activity is stimulated by phosphorylation by cAPK, which helps preserve the phosphorylated (active) forms of phosphorylase kinase and phosphorylase *a*.

17. Glycogen synthase is inactivated by phosphorylation by the same enzyme system that phosphorylates glycogen phosphorylase. Hence, activation of phosphorylase kinase, which activates phosphorylase *a*, inactivates glycogen synthase. This regulatory mechanism provides a rapid and large-scale control of flux in the substrate cycle that links glycogen and G1P. Glycogen synthase activity is also controlled by other kinases.

18. Hormones, including glucagon, insulin, epinephrine, and norepinephrine, ultimately control glycogen metabolism. These hormones bind to transmembrane protein receptors and initiate a series of reactions that lead to the production of molecules called second messengers (e.g., cAMP and Ca^{2+}), which modulate the activities of numerous intracellular proteins. Glucagon binding to its receptor in the liver results in an elevation of cAMP, which favors glycogen breakdown. Epinephrine and norepinephrine bind to α- and β-adrenergic receptors in the liver and to β-adrenergic receptors in muscle. The binding of these hormones to β-adrenergic receptors increases [cAMP], whereas their binding to α-adrenergic receptors increases cytosolic [Ca^{2+}]. Insulin binding to its receptor in tissues other than the liver decreases [cAMP] and promotes glycogen synthesis.

Gluconeogenesis

19. The principal noncarbohydrate precursors of glucose are lactate, pyruvate, and amino acids. In animals, these compounds (except for leucine and lysine) are converted, at least in part, into oxaloacetate, which is required for gluconeogenesis.

20. The conversion of pyruvate (or lactate) to glucose follows a pathway that is the reverse of glycolysis except where it bypasses the exergonic steps catalyzed by pyruvate kinase, phosphofructokinase, and hexokinase.

21. To bypass the pyruvate kinase reaction, pyruvate is first carboxylated by pyruvate carboxylase in a reaction that is driven by ATP hydrolysis. The enzyme's biotin prosthetic group is converted to a carboxybiotinyl group in order to transfer CO_2 to pyruvate. The product of this reaction is oxaloacetate, which is subsequently decarboxylated to phospho-enolpyruvate (PEP). The HCO_3^- added to pyruvate therefore leaves as CO_2. This second reaction, catalyzed by PEP carboxykinase (PEPCK) is driven by the hydrolysis of GTP.

22. Oxaloacetate is produced in the mitochondria, while the reactions that convert PEP to glucose occur in the cytosol. In species with mitochondrial PEPCK, the PEP formed in the mitochondria is exported to the cytosol via a specific transporter. In species with cytosolic PEPCK, oxaloacetate is first converted to malate or aspartate, each of which has a transporter that allows mitochondrial-cytosolic exchange, and, in the cytosol, is reconverted to oxaloacetate.

23. PEP is converted to fructose-1,6-bisphosphate (FBP) by the enzymes of glycolysis operating in reverse. FBP is then hydrolyzed to fructose-6-phosphate and P_i by the action of fructose bisphosphatase (FBPase-1). Similarly, G6P is hydrolyzed by glucose-6-phosphatase, yielding

glucose and P_i. These reactions therefore bypass the exergonic hexokinase and phosphofructokinase reactions.

24. Gluconeogenesis and glycolysis are reciprocally regulated in the liver. The principal allosteric regulator is the metabolite fructose-2,6-bisphosphate (F2,6P), which is a potent activator of PFK-1 and inhibitor of FBPase-1. F2,6P is formed and degraded by a bifunctional protein referred to as PFK-2/FBPase-2. cAPK phosphorylates this protein, thereby activating FBPase-2 and inactivating PFK-2, which results in a net decrease in F2,6P (and favors gluconeogenesis). In heart muscle, the situation is reversed, so that phosphorylation activates PFK-2, which facilitates the muscle's ability to extract energy from glucose via glycolysis.

25. Glucose metabolism is also regulated by other mechanisms:
    (a) Acetyl-CoA activates pyruvate carboxylase.
    (b) Alanine inhibits pyruvate kinase. The amino group of alanine is transferred to an α-keto acid by transamination to yield pyruvate and a new amino acid. The resulting pyruvate then serves as a substrate for gluconeogenesis.
    (c) Long-term regulation of gluconeogenesis occurs via changes in gene expression. Prolonged low concentrations of insulin or high concentrations of cAMP stimulate the transcription of the genes for PEPCK, FBPase, and glucose-6-phosphatase, and repress the transcription of the genes for glucokinase, PFK, and the PFK-2/FBPase-2 bifunctional enzyme.

Other Carbohydrate Biosynthetic Pathways

26. The formation of glycosidic bonds in oligo- and polysaccharides is facilitated by nucleotide sugars, as in the polymerization of glucose by glycogen synthase. The principal nucleotides used are ADP and CDP. Nucleotide sugars are glycosyl donors in the synthesis of *O*-linked oligosaccharides and in the processing of *N*-linked oligosaccharides.

27. *N*-linked oligosaccharides are initially built on dolichol, an isoprenoid lipid carrier in the endoplasmic reticulum (ER). This process begins in the cytosol but finishes in the lumen of the ER, as the dolichol-oligosaccharide flips back and forth across the ER membrane. After the oligosaccharide reaches 14 residues, it is transferred to a protein, leaving dolichol pyrophosphate.

28. Lactose synthesis in mammals involves the mammary gland protein α-lactalbumin, which changes the substrate specificity of a galactosyltransferase so that it synthesizes lactate rather than *N*-acetyllactosamine.

Guide to Study Exercises (text p. 464)

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1. Glucose-6-phosphate is obtained by the phosphorylation of glucose by hexokinase, by the phosphorolysis of glycogen (catalyzed by glycogen phosphorylase) followed by

isomerization (catalyzed by phosphoglucomutase), and by gluconeogenesis. G6P can be converted to glucose in the liver by the action of glucose-6-phosphatase; it can enter the catabolic pathways of glycolysis and the pentose phosphate pathway; and it can be used to synthesize glycogen after being converted to G1P by phosphoglucomutase.

2. The structure of glycogen, a branched polymer whose chains each have two branches of 8–14 residues, satisfies several (often conflicting) criteria. First, glycogen must be a polymer in order to accommodate a large number of glucose residues in a small volume. The large number of branches in the outermost layer provides a large number of points of attack for glycogen phosphorylase to mobilize glucose. However, the branching cannot be so dense that the enzyme cannot access the glycosidic bonds. Furthermore, the branches must be long enough to support the rapid release of many glucose residues before debranching is required. (Box 15-2)

3. Glycogen degradation is catalyzed by three enzymes. Glycogen phosphorylase catalyzes the phosphorolysis of the $\alpha(1\rightarrow4)$ glycosidic bonds, thereby removing glucose residues (as glucose-1-phosphate) to within four or five residues of a branch point. Glycogen debranching enzyme (acting as a transglycosylase) transfers a trisaccharide from a shortened branch to the end of another branch. The same enzyme, now acting as an $\alpha(1\rightarrow6)$ glucosidase, hydrolyzes the residue remaining at the branch point and releases it as glucose. Phosphoglucomutase reversibly converts G1P to glucose-6-phosphate, which can enter glycolysis or the pentose phosphate pathway.

   Glycogen synthesis requires three other enzymes. G1P is "activated" by the attachment of a UTP-derived UMP group to produce UDP–glucose, in a reaction catalyzed by UDP–glucose pyrophosphorylase. The reaction is driven by the subsequent hydrolysis of the PP_i released from UTP. Next, glycogen synthase catalyzes formation of $\alpha(1\rightarrow4)$ glycosidic bonds to extend a chain in a pre-existing glycogen molecule. Branches are created when branching enzyme (a transglycosylase) removes a seven-residue segment from the end of a chain and reattaches it in an $\alpha(1\rightarrow6)$ linkage at a point farther up the same chain or another chain. (Sections 15-1 and 2)

4. Opposing metabolic pathways must differ in at least one step because each process must be exergonic. This means that an exergonic step of the degradative pathway cannot simply operate in reverse for the biosynthetic pathway (for which this would be an endergonic step) but must be bypassed by another, exergonic reaction. For example, in glycogen degradation, the exergonic step is catalyzed by glycogen phosphorylase. Therefore, in glycogen synthesis, this step is bypassed by linking the glucose to a nucleotide (UDP–glucose), which can then serve as a substrate for glycogen synthase. These two steps are exergonic. (Section 15-2).

5. In a simple allosteric system, the activity of each enzyme in a metabolic pathway varies in response to the presence of allosteric activators and inhibitors. These compounds bind to the enzyme in a stoichiometric fashion, so their concentrations must remain constant for their effects on the enzyme to persist. A phosphorylation/dephosphorylation system allows more sensitive regulation of a pathway for two reasons. First, it can operate in addition to allosteric effects, which permits a greater range of regulatory responses. Second,

phosphorylation and dephosphorylation, which result from a hormonal or neuronal signal, can be amplified or dampened in a catalytic fashion. Thus, the state of phosphorylation of an enzyme does not require a constant stoichiometric concentration of another substance. (Section 15-3)

6. In muscle, phosphoprotein phosphatase-1 (whose action deactivates glycogen phosphorylase and activates glycogen synthase) is active only when it associates with glycogen through its G subunit. Insulin (which signals glucose availability) prevents this association and therefore promotes glycogen synthesis. Epinephrine (which opposes the action of insulin) leads to the dissociation of PP-1 from glycogen and therefore promotes glycogen breakdown. PP-1 is also inhibited by phosphoprotein phosphatase inhibitor 1, which is activated by cAMP (which also activates glycogen phosphorylase). Thus, the increased inhibition of phosphoprotein phosphatase-1 leads to increased glycogen breakdown.

   In liver, PP-1 is inhibited by binding to glycogen phosphorylase *a*. When phosphorylase *a* is in its active R form, its Ser 14 phosphoryl group is inaccessible to PP-1. When phosphorylase *a* converts to the less active T form, the Ser 14 phosphoryl group becomes accessible to PP-1, which then catalyzes its dephosphorylation, thereby converting phosphorylase *a* to phosphorylase *b*. Because liver cells contain ten times more glycogen phosphorylase than PP-1, ~90% of the glycogen phosphorylase must be converted to the *b* form before PP-1 is released and can then dephosphorylate and activate glycogen synthase. (Section 15-3B)

7. Gluconeogenesis from pyruvate uses seven of the ten glycolytic enzymes, operating in reverse (see Figure 15-22). Four other enzymes are required to bypass the three exergonic reactions of glycolysis. These are pyruvate carboxylase and PEPCK, which together convert pyruvate to PEP at the expense of 2 ATP equivalents; fructose bisphosphatase, which converts fructose-1,6-bisphosphate to fructose-6-phosphate; and glucose-6-phosphatase, which converts glucose-6-phosphate to glucose. (Section 15-4)

8. Oxaloacetate, the product of the pyruvate carboxylase reaction (it is also an intermediate of the citric acid cycle), is produced in the mitochondrion. Depending on the species, oxaloacetate may be converted to PEP in the mitochondrion or in the cytosol, and the remaining gluconeogenic reactions occur in the cytosol. In species with cytosolic PEPCK, mitochondrial oxaloacetate must be converted to malate or aspartate, both of which have specific transporters. In the cytosol, the malate or aspartate is converted back to oxaloacetate When the malate shuttle is used, NADH is oxidized in the mitochondrion (to reduce oxaloacetate to malate), and NAD^+ is reduced in the cytosol. The net result is the transfer of a reducing equivalent from the mitochondrion to the cytosol. Thus, the malate shuttle supplies the cytosolic NADH required for gluconeogenesis (in the glyceraldehyde-3-phosphate dehydrogenase reaction). If gluconeogenesis begins with lactate rather than pyruvate, its oxidation to pyruvate generates the required NADH, so that the aspartate shuttle (which does not involve any redox reactions) can by used instead of the malate shuttle to transport oxaloacetate from the mitochondrion to the cytosol. (Section 15-4A)

9. Fructose-2,6-bisphosphate (F2,6P) allosterically activates phosphofructokinase (PFK, a glycolytic enzyme) and inhibits FBPase (a gluconeogenic enzyme). Therefore, the balance between glycolysis and gluconeogenesis depends on the concentration of F2,6P, which is synthesized and degraded by a bifunctional enzyme containing PFK-2 and FBPase-2 activities. The balance between F2,6P synthesis and degradation in turn depends on the phosphorylation state of this enzyme, which is ultimately under hormonal control. For example, glucagon binding to liver cell receptors leads to an increase in cAMP, which activates cAPK to phosphorylate the bifunctional enzyme. This activates FBPase-2 and inactivates PFK-2 so that [F2,6P] decreases. As a result, PFK activity decreases and FBPase activity increases, which favors gluconeogenesis rather than glycolysis. (Section 15-4C)

Questions

Glycogen Breakdown

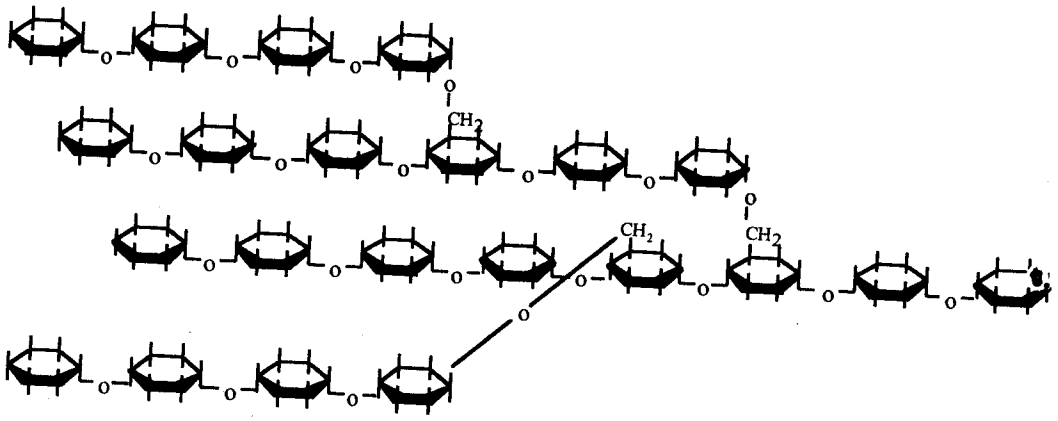
1. List the three enzymes required for the breakdown of glycogen.

2. What feature of the structure of glycogen phosphorylase is consistent with the observation that the enzyme cannot cleave a glycosidic bond within five residues of a branch point?

3. What role does pyridoxal phosphate (PLP) play in the mechanism of glycogen phosphorylase?

4. What would be the product of the nonenzymatic phosphorolysis of glycogen?

5. In the diagram of glycogen shown below, circle the substrates for glycogen debranching enzyme.



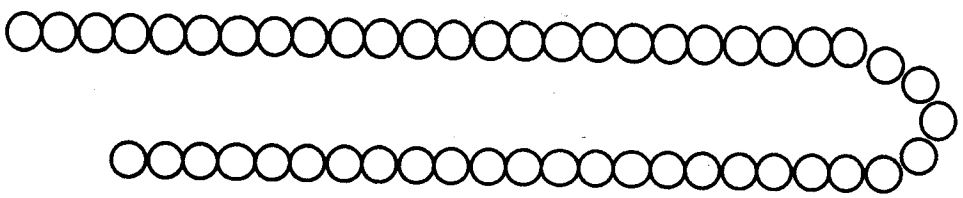
6. The rate of debranching is much slower than that of phosphorolysis. Explain how highly branched glycogen molecules release glucose-1-phosphate at a greater rate than relatively unbranched ones.

7. Write an equation for the equilibrium constant for the phosphorylase reaction. $\Delta G^{\circ\prime}$ for this reaction is +3.1 kJ·mol^{-1}, yet glycogen breakdown in the liver and muscle is thermodynamically favored at 37°C. What is the minimum ratio of $[P_i]/[G1P]$ required to make the phosphorylase reaction exergonic? Assume that the concentration of glycogen does not change significantly.

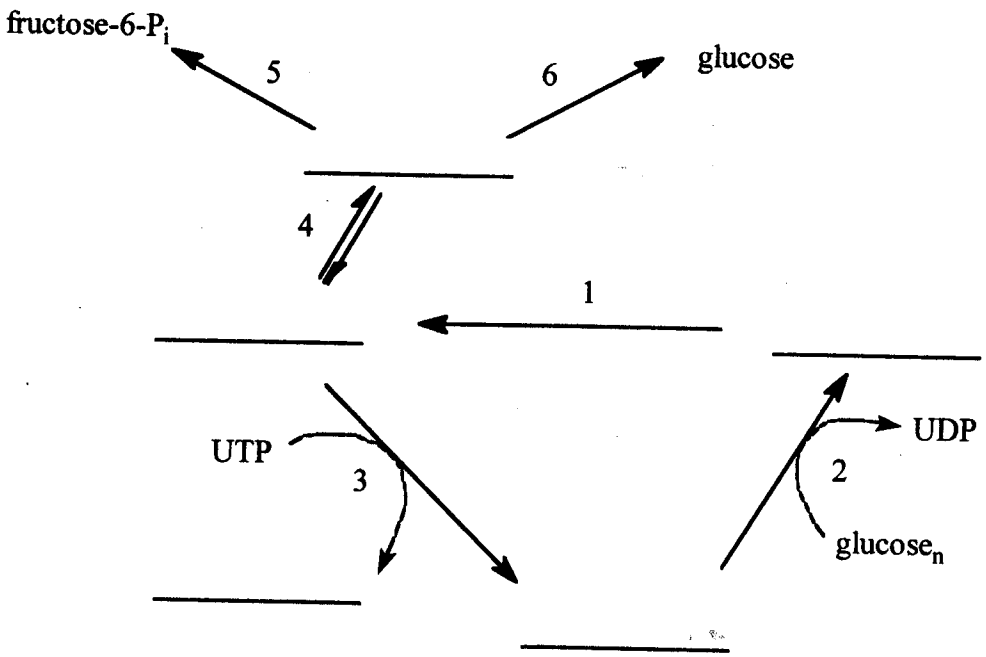
Glycogen Synthesis

8. How is the thermodynamic barrier to glycogen synthesis overcome by cells?

9. In the diagram below, each circle represents a glycosyl unit. By the action of branching enzyme, show the most branched structure this molecule can assume. Indicate the reducing and nonreducing ends of the molecule and the position where a glycogenin molecule would be found. There are 50 glycosyl residues.



10. Below is a diagram showing the synthesis and breakdown of glycogen $(glucose)_n$. Fill in the blanks and identify the enzyme that catalyzes each numbered reaction.



Control of Glycogen Metabolism

11. What structural features distinguish the T conformation of glycogen phosphorylase from the R conformation of the enzyme?

12. The addition of a mild detergent to a liver extract elevates cAPK activity and renders it insensitive to cAMP. Explain.

13. Glycogen phosphorylase is activated in vigorously active muscle without significant changes in intracellular [cAMP]. Explain.

14. Compare the structures of calmodulin (Figure 15-16) and troponin C (Figure 7-30). (a)How do their structures differ? (b) How are their functions similar?

15. The presence of epinephrine results in the stimulation of PFK-2 in heart muscle. How does epinephrine affect glycolysis in this organ?

16. Which target enzyme in glycogen metabolism requires both α- and β-adrenergic receptors to be activated for full enzyme activity?

17. Which of the hereditary glycogen storage diseases results in a pronounced decrease of stored glycogen?

Gluconeogenesis

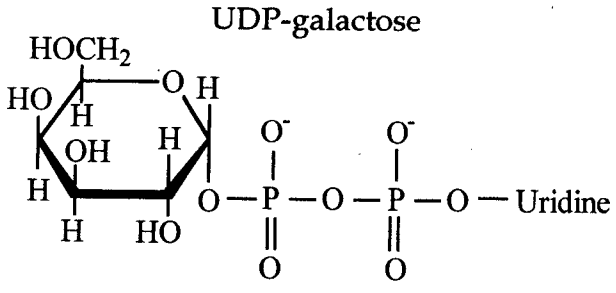
18. What is the fate of $H^{14}CO_3^-$ added to a liver homogenate that is active in gluconeogenesis?

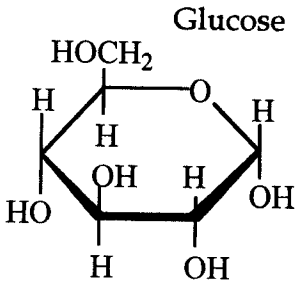
19. Write a balanced equation for the formation of PEP from pyruvate and compare it with the reverse reaction of glycolysis.

20. While acetyl-CoA, the end product of fatty acid oxidation, cannot be converted into glucose, another product of fat degradation, glycerol, can be converted to glucose. Where does glycerol enter the gluconeogenic pathway?

Other Carbohydrate Biosynthetic Pathways

21. UDP–galactose can donate is sugar residue to glucose to form the disaccharide lactose. For the structures of UDP–galactose and glucose below, indicate the reactive electrophilic and nucleophilic centers and the products of the reaction.





Answers

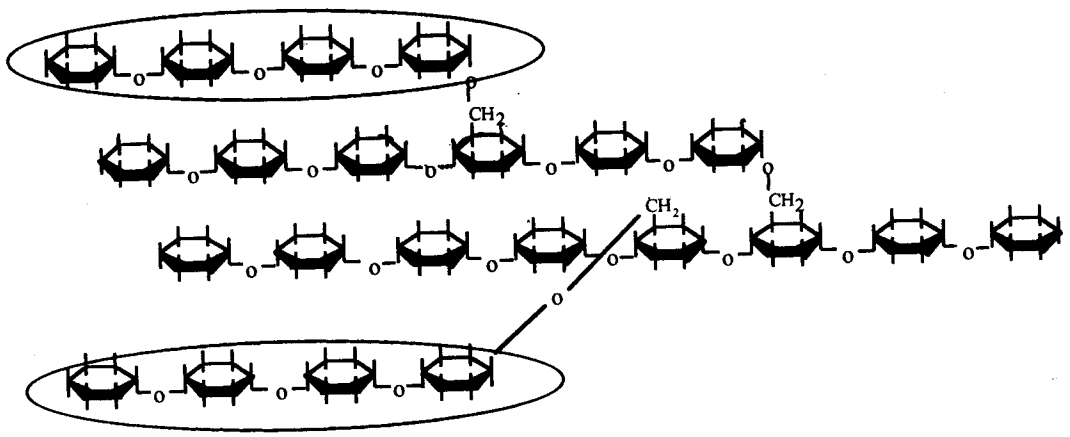
1. Glycogen phosphorylase, glycogen debranching enzyme, and phosphoglucomutase.

2. A narrow crevice on the surface of glycogen phosphorylase, which connects the glycogen storage site and the active site, can accommodate up to five residues of an unbranched glycogen chain but cannot accommodate a branched chain.

3. The phosphoryl group of PLP participates in general acid–base catalysis by donating a proton to the anionic P_i that reacts with glycogen to release G1P.

4. The reaction product would be a mixture of α and β anomers of G1P because the reaction intermediate is an oxonium ion whose C1 can react with phosphate approaching either face of the sugar residue.

5.



Glycogen debranching enzyme has two catalytic functions: (1) It transfers three α(1→4)-linked glucose residues from a "limit branch" of glycogen to the nonreducing end of another branch, and (2) it hydrolyzes the α(1→6) bond of the remaining residue to form free glucose. In the diagram above, the top and bottom branches serve as substrates in which the trisaccharide is transferred and the remaining glucose residue is released. Once these branches have been eliminated, continued phosphorolysis would produce additional "limit branch" substrates for debranching enzyme.

6. Highly branched glycogen molecules have more nonreducing ends available for phosphorylase to produce G1P, so the rate of G1P release remains high until a limit branch is encountered.

7. $$K_{eq} = \frac{[\text{glycogen}_{n-1}][\text{G1P}]}{[\text{glycogen}_n][P_i]} \approx \frac{[\text{G1P}]_{eq}}{[P_i]_{eq}}$$

$$\Delta G = \Delta G^{\circ\prime} + RT \ln \frac{[\text{G1P}]}{[P_i]}$$

In order for ΔG to be less than zero, $-RT \ln ([\text{G1P}]/[P_i])$ must be at least as great as $\Delta G^{\circ\prime}$.

$-RT \ln ([\text{G1P}]/[P_i]) \geq \Delta G^{\circ\prime}$

$-\ln ([\text{G1P}]/[P_i]) \geq \Delta G^{\circ\prime}/RT$

$\ln ([\text{G1P}]/[P_i]) \leq -\Delta G^{\circ\prime}/RT$

$[\text{G1P}]/[P_i] \leq e^{-\Delta G^{\circ\prime}/RT}$

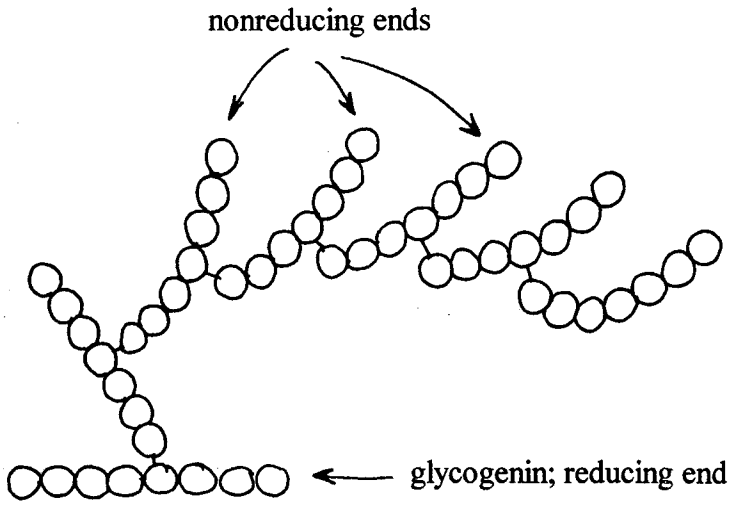
$[\text{G1P}]/[P_i] \leq e^{-(3100\ \text{J·mol}-1)/(8.3145\ \text{J·K}-1\text{·mol}-1)(310\text{K})}$

$[\text{G1P}]/[P_i] \leq 0.30$

Therefore, $[\text{G1P}]/[P_i]$ can be no greater than 0.30, so $[P_i]/[\text{G1P}]$ must be at least 1/0.30 = 3.33.

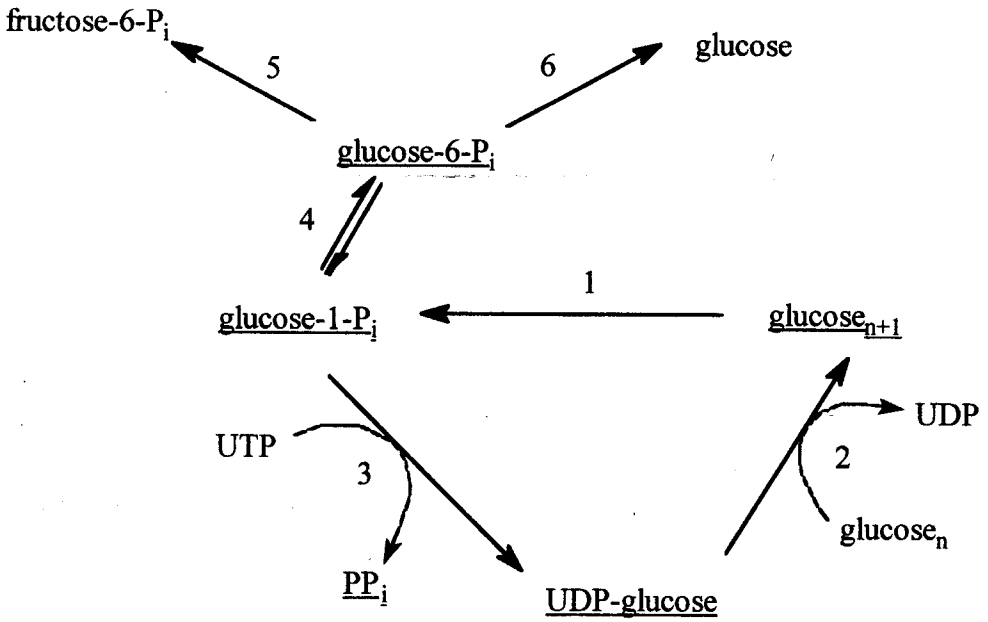
8. A "high-energy" compound is required to bypass the exergonic glycogen phosphorylase reaction. The formation of UDP–glucose from G1P and UTP yields PP_i, whose hydrolysis provides the thermodynamic "pull" to add another glucose residue to glycogen.

9.



Branching enzyme has three constraints: (1) Branches must be separated by four glucosyl residues; (2) seven residues are transferred at a time to make a branch; and (3) the donating chain must be at least 11 residues long. The first branch is made by moving a seven-residue segment from the reducing end. The most highly branched structure results when branches are added to branches for a final product with six $\alpha(1\rightarrow6)$ branch points, as shown below.

10.



1 Glycogen phosphorylase
2 Glycogen synthase
3 UDP–glucose pyrophosphorylase
4 Phosphoglucomutase
5 Phosphoglucose isomerase
6 Glucose-6-phosphatase

11. In the T form, the active site is less accessible to its substrates compared to the R form due to the presence of a loop that covers the T state active site so as to prevent access of substrate to it. In the R state, the tower helices have tilted and pulled apart relative to their positions in the T state, thereby inducing an ~10° counter-rotation of the two subunits. This also displaces and disorders the loop covering the active site, thus making the active site accessible to substrate. In addition, the side chain of Arg 569, which is located in the active site, rotates in such a way that it increases the R-state enzyme's affinity for its P_i substrate.

12. The mild detergent probably causes the dissociation of the subunits of cAPK (a process which normally requires cAMP) such that the freed catalytic subunits are catalytically active.

13. Ca^{2+} released during muscle contraction activates phosphorylase kinase via binding of Ca^{2+} to calmodulin (the δ subunit of phosphorylase kinase). Phosphorylase kinase is therefore active even without cAPK-catalyzed phosphorylation of its α and β subunits.

14. (a) In troponin C, two globular domains are connected by a nine-turn helix. The two Ca^{2+}-binding domains differ such that one domain binds two Ca^{2+} at low [Ca^{2+}] and the other domain binds two Ca^{2+} only at higher [Ca^{2+}]. In calmodulin, a seven-turn helix connects two highly similar Ca^{2+}-binding domains.

(b) In both proteins, Ca^{2+} binding induces conformational changes that are necessary to alter the activities of other proteins with which troponin C and calmodulin interact.

15. Epinephrine-dependent stimulation of PFK-2 leads to an increase in [F2,6P], which activates PFK-1. The result is increased flux through glycolysis.

16. The full activity of phosphorylase kinase requires both the presence of Ca^{2+} (whose concentration increases in response to hormone binding to α-adrenergic receptors) and phosphorylation by cAPK (which is activated by the binding of the cAMP second messenger whose synthesis is stimulated by hormone binding to β-adrenergic receptors).

17. Only Type 0 (glycogen synthase deficiency) causes a decrease in stored glycogen.

18. The $H^{14}CO_3^-$ is added to pyruvate to yield oxaloacetate via the pyruvate carboxylase reaction. The ^{14}C is then released as CO_2 by the PEP carboxykinase reaction, which converts oxaloacetate to PEP + CO_2.

19. ***gluconeogenesis:***

$$\text{Pyruvate} + HCO_3^- + \text{ATP} \rightleftharpoons \text{oxaloacetate} + \text{ADP} + P_i$$

$$\text{Oxaloacetate} + \text{GTP} \rightleftharpoons \text{PEP} + \text{GDP} + CO_2$$

$$\text{Pyruvate} + HCO_3^- + \text{ATP} + \text{GTP} \rightleftharpoons \text{PEP} + \text{ADP} + \text{GDP} + P_i + CO_2$$

glycolysis:

$$H^+ + \text{PEP} + \text{ADP} \rightleftharpoons \text{ATP} + \text{pyruvate}$$

The formation of PEP from pyruvate requires the investment of two ATP equivalents ("high-energy" phosphoanhydride bonds), whereas PEP's reaction to form pyruvate yields only one ATP.

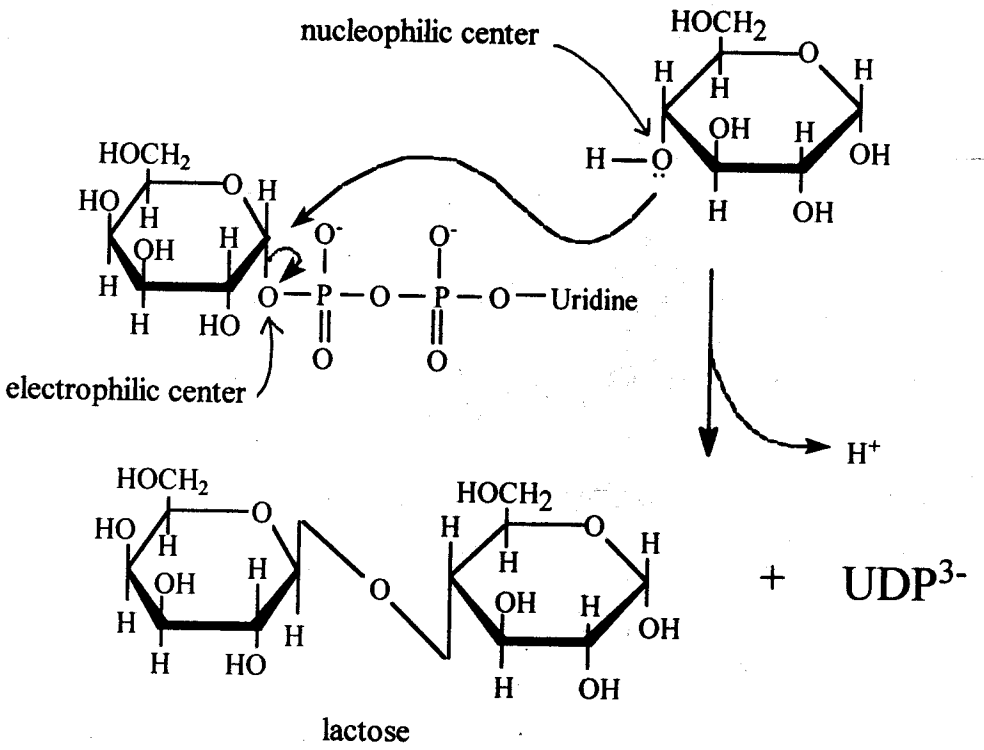
20. Glycerol enters the gluconeogenic pathway as dihydroxyacetone phosphate (DHAP; see Figure 14-26 on p. 413). Glycerol is a substrate for glycerol kinase, which catalyzes the reaction

$$\text{Glycerol} + \text{ATP} \rightleftharpoons \text{glycerol-3-phosphate} + \text{ADP}$$

Glycerol phosphate dehydrogenase then catalyzes the reaction

$$\text{Glycerol-3-phosphate} + NAD^+ \rightleftharpoons \text{DHAP} + \text{NADH} + H^+$$

21.


nucleophilic center
HOCH2
O
H
OH
Uridine
electrophilic center
H+
lactose
+ UDP3-

Chapter 16 Citric Acid Cycle

This chapter discusses the citric acid cycle as both a catabolic and an anabolic process. The citric acid cycle was elucidated by several investigators, but the key insights were provided by Hans Krebs, so the cycle is often referred to as the Krebs cycle. It is also called the tricarboxylic acid (TCA) cycle, which refers to its first intermediate, citrate. The chapter first provides an overview of the key features of the citric acid cycle. It then takes a closer look at each step of the cycle, beginning with a discussion of the pyruvate dehydrogenase complex that converts pyruvate to acetyl-CoA, the fuel molecule that enters the cycle. In this chapter, you will encounter several coenzymes that are critical to the functioning of the citric acid cycle, all derived from water-soluble vitamins. A discussion of the regulation of the citric acid cycle then ensues, in which you will encounter familiar regulatory mechanisms as well as new ones. The chapter then turns to the anabolic features of the citric acid cycle. The citric acid cycle is one of the key metabolic hubs in the cell: Its intermediates are generated by a variety of degradative reactions, and they provide precursors for several biosynthetic pathways. As citric acid cycle intermediates are diverted to anabolic pathways, they are replaced through anaplerotic reactions. The final section of the chapter introduces the glyoxylate cycle found in plants. This pathway is the only known pathway for the net conversion of acetyl-CoA to glucose. The chapter also discusses arsenic poisoning in the citric acid cycle (Box 16-1) and the stereospecificity of the cycle's enzymes (Box 16-2). Box 16-3 introduces the metabolon hypothesis, which centers around the idea of substrate channeling and the organization of large multienzyme complexes that carry out several different catalytic activities.

Essential Concepts

Overview of the Citric Acid Cycle

1. The citric acid cycle is a central pathway for recovering energy from the three major metabolic fuels: carbohydrates, fatty acids, and amino acids. These fuels are broken down to yield acetyl-CoA, which enters the citric acid cycle by condensing with the C_4 compound oxaloacetate. The citric acid cycle is a series of reactions in which 2 CO_2 are released for every acetyl-CoA that enters the cycle, so that oxaloacetate is always reformed. Hence, the cyclical series of reactions acts catalytically to process acetyl-CoA continuously.

2. The oxidation of the acetyl carbon skeleton in the citric acid cycle is coupled to the reduction of NAD^+ and FAD. Oxidation of the resulting NADH and $FADH_2$ by the electron-transport chain supplies free energy for ATP synthesis and regenerates NAD^+ and FAD for the oxidation of additional acetyl-CoA. In aerobic respiration, O_2 serves as the terminal acceptor of the acetyl group's electrons. In anaerobic respiration, this function is carried out by molecules such as NO_3^-, SO_4^{2-}, and Fe^{3+}.

3. One complete round of the cycle yields 2 CO_2, 3 NADH, 1 $FADH_2$, and 1 GTP (which is the equivalent of 1 ATP). Hence, the net reaction of the citric acid cycle is

$$3\,NAD^+ + FAD + GDP + P_i + \text{acetyl-CoA} + 2\,H_2O \rightarrow 3\,NADH + FADH_2 + GTP + CoA + 2\,CO_2 + 3\,H^+$$

The carbon atoms of the acetyl group entering the cycle do not exit the cycle as CO_2 in the first round but do so in subsequent rounds.

Synthesis of Acetyl-Coenzyme A

4. In eukaryotes, all the enzymes of the citric acid cycle (and the pyruvate dehydrogenase complex) occur in the inner compartment or matrix of the mitochondrion. The pyruvate produced by glycolysis in the cytosol is transported into the mitochondrion via a pyruvate–H^+ symport protein.

5. The pyruvate dehydrogenase complex is a large multienzyme complex containing multiple copies of three enzymes (E_1, E_2, and E_3) organized in a polyhedral array. For example, the *E. coli* pyruvate dehydrogenase complex consists of 24 copies of E_1 and 24 copies of E_2 arranged about the corners of concentric cubes, and 12 copies of E_3.

6. The pyruvate dehydrogenase complex catalyzes five reactions in the oxidative decarboxylation of pyruvate. Five different coenzymes are involved.
   (a) Pyruvate dehydrogenase (E_1) decarboxylates pyruvate in a reaction identical to that catalyzed by pyruvate decarboxylase in alcoholic fermentation (Figure 14-10). However, in the pyruvate dehydrogenase reaction, the hydroxyethyl group remains linked to the thiamine pyrophosphate (TPP) prosthetic group rather than being released as acetaldehyde.
   (b) Dihydrolipoyl transacetylase (E_2) transfers the hydroxyethyl group from TPP to a lipoic acid residue that is tethered to an enzyme Lys side chain via an amide linkage (lipoamide) to form acetyl-dihydrolipoamide.
   (c) E_2 then transfers the acetyl group to CoA to form acetyl-CoA and dihydrolipoamide.
   (d) Dihydrolipoyl dehydrogenase (E_3) oxidizes the dihydrolipoamide group of E_2 via the reduction of E_3's reactive Cys—Cys disulfide bond.
   (e) E_3 is then reoxidized by NAD^+, in a reaction involving the transient reduction and reoxidation of the enzyme's FAD group, to regenerate the reactive disulfide bond, thereby preparing the pyruvate dehydrogenase complex for another round of hydroxyethyl transfer and oxidation.

Enzymes of the Citric Acid Cycle

7. Citrate synthase catalyzes the condensation of acetyl-CoA and oxaloacetate to form citrate in a highly exergonic reaction. The enzyme undergoes a large conformational change as part of an Ordered Sequential reaction mechanism.

8. Aconitase catalyzes the isomerization of citrate to isocitrate via stereospecific dehydration and rehydration.

9. Isocitrate dehydrogenase catalyzes the oxidative decarboxylation of isocitrate to produce α-ketoglutarate and the first CO_2 and NADH of the citric acid cycle.

10. α-Ketoglutarate dehydrogenase (a multienzyme complex similar to the pyruvate dehydrogenase complex) catalyzes the oxidative decarboxylation of α-ketoglutarate by a mechanism identical to that of the pyruvate dehydrogenase complex. The reaction products are succinyl-CoA and the second CO_2 and second NADH of the cycle. Note that the two carbons released as CO_2 in this round of the citric acid cycle are not the carbons that entered the cycle as acetyl-CoA.

11. Succinyl-CoA synthetase catalyzes a coupled reaction in which thioester hydrolysis is coupled to the phosphorylation of GDP via succinyl-phosphate and phospho-His intermediates. This enzyme therefore catalyzes substrate-level phosphorylation. The GTP produced in this step is easily interconverted with ATP by nucleoside diphosphate kinase.

12. The remaining reactions of the citric acid cycle regenerate oxaloacetate.
    (a) Succinate dehydrogenase catalyzes the stereospecific dehydrogenation of succinate to fumarate. The enzyme's FAD prosthetic group is reduced in this redox reaction and is reoxidized when it gives up its electrons to the respiratory electron-transport chain.
    (b) Fumarase catalyzes the hydration of fumarate to malate.
    (c) Malate dehydrogenase catalyzes the oxidation of malate, which regenerates oxaloacetate and produces the third NADH of the cycle. This highly endergonic reaction is driven by the exergonic reaction that follows, namely the condensation of oxaloacetate with acetyl-CoA, which begins the next round of the citric acid cycle.

Regulation of the Citric Acid Cycle

13. The citric acid cycle generates ATP through substrate-level phosphorylation (GTP production) and by the subsequent reoxidation of its 3 NADH and $FADH_2$ products by the electron-transport chain. Each NADH generates ~3 ATP and $FADH_2$ generates ~2 ATP, so each round of the citric acid cycle yields ~12 ATP.

14. The pyruvate dehydrogenase complex is regulated by product inhibition by NADH and acetyl-CoA, and, in eukaryotes, by covalent modification via the phosphorylation/dephosphorylation of E_1. Pyruvate dehydrogenase kinase represses E_1 activity by phosphorylating it at a specific Ser residue, and pyruvate dehydrogenase phosphatase stimulates E_1 activity by dephosphorylating it.

15. The citric acid cycle is regulated principally at the steps catalyzed by citrate synthase, isocitrate dehydrogenase, and α-ketoglutarate dehydrogenase. Regulatory mechanisms include:
    (a) Substrate availability. The most critical regulators are acetyl-CoA, oxaloacetate, and the ratio of [NADH] to [NAD^+].
    (b) Product inhibition. Citrate competes with oxaloacetate in the citrate synthase reaction, and succinyl-CoA and NADH inhibit α-ketoglutarate dehydrogenase.
    (c) Competitive feedback inhibition. Succinyl-CoA competes with acetyl-CoA in the citrate synthase reaction.
    (d) Allosteric regulation. ADP activates isocitrate dehydrogenase, while ATP allosterically inhibits it. Ca^{2+} activates pyruvate dehydrogenase, isocitrate dehydrogenase, and α-ketoglutarate dehydrogenase.

Reactions Related to the Citric Acid Cycle

16. The citric acid cycle is amphibolic (both anabolic and catabolic). As an anabolic cycle, the citric acid cycle provides intermediates for gluconeogenesis (oxaloacetate, which must be first converted to malate for export from the mitochondrion), amino acid biosynthesis (oxaloacetate, α-ketoglutarate), porphyrin synthesis (succinyl-CoA), and lipid biosynthesis (citrate). The citric acid cycle also functions catabolically to complete the degradation of carbohydrates and fatty acids (which yield acetyl-CoA) and amino acids that are converted to fumarate, succinyl-CoA, α-ketoglutarate, and oxaloacetate.

17. Anaplerotic reactions replenish citric acid cycle intermediates that have been siphoned off into anabolic reactions. The most important of these reactions is catalyzed by pyruvate carboxylase:

$$\text{Pyruvate} + CO_2 + \text{ATP} + H_2O \rightarrow \text{oxaloacetate} + \text{ADP} + P_i$$

18. Acetyl-CoA cannot serve as a precursor for gluconeogenesis in animals, but it can do so in plants via the glyoxylate pathway. This pathway is a variation of the citric acid cycle that takes place in two organelles, the mitochondrion and the glyoxysome (a specialized peroxisome found in plants). The glyoxysome contains isocitrate lyase (which cleaves isocitrate to succinate and glyoxylate) and malate synthase (which condenses glyoxylate with acetyl-CoA to form malate). The net reaction for the glyoxylate pathway is

$$2\ \text{Acetyl-CoA} + 2\ NAD^+ + FAD + 3\ H_2O \rightarrow \text{oxaloacetate} + 2\ \text{CoA} + 2\ \text{NADH} + FADH_2 + 4\ H^+$$

Note that in the glyoxylate pathway, no carbons are lost as CO_2.

Guide to Study Exercises (text p. 491)

1. The five reactions of the pyruvate dehydrogenase complex are:
   (1) Pyruvate dehydrogenase (E_1) decarboxylates pyruvate, releasing CO_2 and leaving a hydroxyethyl group bound to its TPP prosthetic group.
   (2) Dihydrolipoyl transacetylase (E_2) transfers the hydroxyethyl group from TPP to its lipoamide prosthetic group, producing acetyl-dihydrolipoamide.
   (3) E_2 then transfers the acetyl group to CoA, yielding acetyl-CoA and a dihydrolipoamide group.
   (4) Dihydrolipoyl dehydrogenase (E_3) oxidizes the dihydrolipoamide group of E_2 by a disulfide interchange with two E_3 Cys residues.
   (5) The two reduced Cys residues are oxidized by NAD^+ through the intermediacy of FAD, producing NADH and regenerating E_3. (Section 16-2B)

2. See Figure 16-2.

3. $$\text{Pyruvate} + 4\,NAD^+ + FAD + GDP + P_i + 2\,H_2O \rightarrow 4\,NADH + FADH_2 + GTP + 3\,CO_2 + 3\,H^+$$

$$\text{Acetyl-CoA} + 3\,NAD^+ + FAD + GDP + P_i + 2\,H_2O \rightarrow 3\,NADH + FADH_2 + GTP + CoA + 2\,CO_2 + 3\,H^+$$

$$\text{Glucose} + 10\,NAD^+ + 2\,FAD + 2\,ADP + 2\,GDP + 4\,P_i + 2\,H_2O \rightarrow 10\,NADH + 2\,FADH_2 + 2\,ATP + 2\,GTP + 10\,H^+ + 6\,CO_2$$

(Section 16-1)

4. Flux through the citric acid cycle is regulated at four points. Entry of acetyl units into the cycle is controlled by product inhibition and covalent modification of the pyruvate dehydrogenase complex. The flux of metabolites through the cycle itself is regulated at the steps catalyzed by citrate synthase, isocitrate dehydrogenase, and α-ketoglutarate dehydrogenase. The activity of these enzymes is controlled by substrate availability and feedback inhibition by products of that reaction or a reaction farther along the cycle. (Section 16-4)

5. Ca^{2+} activates the citric acid cycle by activating isocitrate dehydrogenase and α-ketoglutarate dehydrogenase. It also activates the pyruvate dehydrogenase complex by inhibiting pyruvate dehydrogenase kinase and activating pyruvate dehydrogenase phosphatase. NADH inhibits citrate synthase and inhibits isocitrate dehydrogenase and α-ketoglutarate dehydrogenase by product inhibition. It competes with NAD^+ for binding to pyruvate dehydrogenase, thereby slowing this reaction. In combination with acetyl-CoA, NADH drives the E_2 and E_3 reactions of the pyruvate dehydrogenase complex backward, preventing the entry of additional acetyl-CoA into the citric acid cycle. NADH and acetyl-CoA also inactivate pyruvate dehydrogenase by activating the kinase that phosphorylates it. Acetyl-CoA itself regulates the citrate synthase reaction according to its availability and competes with CoA for binding to the pyruvate dehydrogenase complex. (Section 16-4)

6. The intermediates of a cyclic pathway that are removed to serve as precursors for other pathways must be replenished by anaplerotic reactions that synthesize these intermediates from other compounds. (Sections 16-5A and B)

7. See Figure 16-16. The glyoxylate pathway, which operates in plants, requires the enzymes of the citric acid cycle as well as the glyoxysome enzymes isocitrate lyase and malate synthase. The conversion of isocitrate to succinate and the C_2 compound glyoxylate, which is catalyzed by isocitrate lyase, bypasses the two CO_2-generating steps of the citric acid cycle. Malate synthase then combines glyoxylate with acetyl-CoA to form malate. This allows the net synthesis of oxaloacetate, a gluconeogenic precursor, from acetyl-CoA. (Section 16-5C)

Questions

Overview of the Citric Acid Cycle

1. The citric acid cycle can be divided into two phases with respect to the oxidation of acetyl-CoA. Describe each phase and write its balanced equation.

2. Early experiments showed that malonate, which inhibits succinate dehydrogenase, blocks cellular respiration. This led to the idea that succinate participates in oxidative metabolism as an intermediate and not as just another metabolic fuel. Using isotopically-labeled reagents, what observation(s) would demonstrate the validity of this interpretation?

Synthesis of Acetyl-Coenzyme A

3. Name the three enzymes that form the pyruvate dehydrogenase complex.

4. For each reaction listed below, indicate the appropriate enzyme(s) in the pyruvate dehydrogenase complex and the relevant cofactor(s), if applicable.

	Reaction	***Enzyme***	***Cofactor***
(a)	Oxidative formation of an enzymatic disulfide bond	_____	_____
(b)	Transfer of hydroxyethyl group bound to TPP	_____	_____
(c)	Liberation of CO_2	_____	_____
(d)	Oxidation of dihydrolipoamide	_____	_____
(e)	Formation of acetyl-CoA	_____	_____

5. What are the roles of FAD and NAD^+ in the pyruvate dehydrogenase catalytic mechanism?

6. You are given two preparations of purified pyruvate dehydrogenase complex enzymes with all the required cofactors You add pyruvate to each preparation and then measure the rate of production of acetyl-CoA and acetaldehyde under aerobic conditions.

		Acetyl-CoA (molecules/sec.)	***Free acetaldehyde (molecules/sec.)***
Preparation	**A**	10^5	10^{-6}
Preparation	**B**	10^{-2}	10^3

How might the pyruvate dehydrogenase complex enzymes differ in each preparation?

7. Which of the following labeled glucose molecules would yield $^{14}CO_2$ following glycolysis and the pyruvate dehydrogenase reaction?
   (a) 1-[^{14}C]-glucose
   (b) 3-[^{14}C]-glucose
   (c) 4-[^{14}C]-glucose
   (d) 6-[^{14}C]-glucose

Enzymes of the Citric Acid Cycle

8. What is the energetic function of the thioester bond of CoA in the citrate synthase reaction?

9. For the half-reaction

$$FAD + 2\,e^- + 2\,H^+ \rightleftharpoons FADH_2$$

$\mathscr{E}^{\circ\prime} \approx 0.00$ V for FAD bound to succinate dehydrogenase.

(a) Calculate $\Delta G^{\circ\prime}$ for the oxidation of succinate to fumarate by enzyme-bound FAD.
(b) How does this compare to the $\Delta G^{\circ\prime}$ for the oxidation of succinate to fumarate by free NAD^+?
(c) Based on these results, explain why nature chose FAD rather than NAD^+ as the oxidizing agent in the succinate dehydrogenase reaction.

10. What is the fate of C4 (the carboxyl that is β to the carbonyl) of oxaloacetate?

Regulation of the Citric Acid Cycle

11. How would the rapid accumulation of succinyl-CoA affect the rate of glucose oxidation?

Reactions Related to the Citric Acid Cycle

12. List four anabolic pathways that utilize citric acid cycle intermediates as starting material.

13. How does acetyl-CoA affect the activity of pyruvate carboxylase? Why is this advantageous for the cell?

14. Write a balanced equation for the synthesis of glucose from acetyl-CoA via the glyoxylate cycle.

15. Which reactions of the glyoxylate cycle deviate from the citric acid cycle?

16. Which pathway intermediates pass between the plant mitochondrion and the glyoxysome during the functioning of the glyoxylate cycle?

Answers to Questions

1. The first phase involves the net oxidation of acetyl-CoA to two molecules of CO_2 and the regeneration of CoA (Reactions 1–5 of the cycle).

$$\text{Acetyl-CoA} + \text{oxaloacetate} + GDP + 2\,NAD^+ + H_2O + P_i \rightarrow$$
$$\text{succinate} + GTP + CoASH + 2\,NADH + 2\,CO_2 + 2\,H^+$$

The second phase involves the regeneration of oxaloacetate from succinate (Reactions 6–8).

$$\text{Succinate} + \text{FAD} + \text{NAD}^+ + \text{H}_2\text{O} \rightarrow \text{oxaloacetate} + \text{NADH} + \text{FADH}_2 + \text{H}^+$$

2. To demonstrate that succinate is an intermediate in glucose oxidation, show that, in the presence of malonate, cell extracts containing newly added ^{14}C-labeled acetyl-CoA (labeled at either of the acetyl C atoms) and any of the other citric acid cycle intermediates yield ^{14}C-labeled succinate.

3. Pyruvate dehydrogenase (E_1), dihydrolipoyl transacetylase (E_2), and dihydrolipoyl dehydrogenase (E_3).

4.

	Reaction	***Enzyme***	***Cofactor***
(a)	Oxidative formation of an enzymatic disulfide bond	E_3	FAD, NAD^+
(b)	Transfer of hydroxyethyl group bound to TPP	E_2	lipoic acid
(c)	Liberation of CO_2	E_1	TPP
(d)	Oxidation of dihydrolipoamide	E_2, E_3	
(e)	Formation of acetyl-CoA	E_2	CoA, lipoic acid

5. FAD functions to oxidize the E_3 sulfhydryl groups that were reduced as they oxidized the sulfhydryl groups of dihydrolipoamide. NAD^+ then oxidizes the $FADH_2$. Both oxidants serve to regenerate the reactive functional groups of E_3.

6. The data suggest that in preparation B, E_1 (pyruvate dehydrogenase) is somehow unable to transfer its bound hydroxyethyl carbanion to the lipoamide cofactor of E_2 (dihydrolipoyl transacetylase). Instead, free acetaldehyde is released from the TPP group of E_1 (as occurs in the pyruvate decarboxylase reaction; Section 14-3B).

7. (b) and (c) would yield $^{14}CO_2$ after the pyruvate dehydrogenase reaction since the C3 and C4 atoms of glucose both become C1 of glyceraldehyde-3-phosphate and then pyruvate. Pyruvate dehydrogenase liberates C1 of pyruvate as CO_2.

8. The large, negative free energy of hydrolysis of the thioester bond provides the thermodynamic force for the otherwise endergonic condensation of the acetyl group and oxaloacetate under conditions of low oxaloacetate concentration. The oxaloacetate concentration is low because of the endergonic nature of the preceding malate dehydrogenase reaction.

9. (a) For the reaction succinate + FAD → fumarate + $FADH_2$, succinate is the electron donor and FAD is the electron acceptor. $\mathscr{E}^{\circ\prime}$ for the succinate → fumarate half-reaction is 0.031 V (Table 13-3). Therefore,

$$\Delta\mathscr{E}^{\circ\prime} = \mathscr{E}^{\circ\prime}_{\text{FAD}} - \mathscr{E}^{\circ\prime}_{\text{succinate}} = 0.00\text{ V} - 0.031\text{ V} = -0.031\text{ V}$$

$$\Delta G^{\circ\prime} = -n\mathcal{F}\Delta\mathscr{E}^{\circ\prime}$$
$$= -(2)(96{,}485\ \text{J}\cdot\text{V}^{-1}\cdot\text{mol}^{-1})(-0.031\ \text{V})$$
$$= 5982\ \text{J}\cdot\text{mol}^{-1} = 6.0\ \text{kJ}\cdot\text{mol}^{-1}$$

(b) When NAD^+ is the electron acceptor ($\mathscr{E}^{\circ\prime} = -0.315$ V),
$\mathscr{E}^{\circ\prime} = -0.315\ \text{V} - 0.031\ \text{V} = -0.346\ \text{V}$
$\Delta G^{\circ\prime} = -(2)(96{,}485\ \text{J}\cdot\text{V}^{-1}\cdot\text{mol}^{-1})(-0.346\ \text{V})$
$= 66768\ \text{J}\cdot\text{mol}^{-1} = 67\ \text{kJ}\cdot\text{mol}^{-1}$

Therefore, under standard biochemical conditions, the oxidation of succinate by FAD is slightly disfavored ($\Delta G^{\circ\prime} > 0$). However, the oxidation of succinate by NAD^+ is strongly disfavored ($\Delta G^{\circ\prime} \gg 0$).

(c) Succinate lacks the reducing power (has a reduction potential that is too high) to reduce NAD^+ to NADH but has sufficient reducing power to reduce FAD to $FADH_2$.

10. It is lost as CO_2 in the isocitrate dehydrogenase reaction in the first turn of the cycle (see Figure 16-2).

11. The rate of glucose oxidation would decrease because succinyl-CoA inhibits its own synthesis by the α-ketoglutarate dehydrogenase reaction, and it inhibits the citrate synthase reaction via feedback inhibition. The rapid accumulation of succinyl-CoA would also deplete the mitochondrial pool of CoA, thereby slowing the production of acetyl-CoA from glucose-derived pyruvate.

12. Glucose synthesis (gluconeogenesis) utilizes oxaloacetate; lipid biosynthesis utilizes acetyl-CoA derived from citrate; amino acid biosynthesis utilizes α-ketoglutarate and oxaloacetate, and porphyrin biosynthesis utilizes succinyl-CoA (Figure 16-15).

13. Acetyl-CoA activates pyruvate carboxylase. Increases in [acetyl-CoA] are indicative of an inability of the citric acid cycle to oxidize acetyl-CoA as fast as it is being produced (from glycolysis and fatty acid oxidation). Hence, activating pyruvate carboxylase, which adds more oxaloacetate to the pool of citric acid cycle intermediates will catalytically accelerate acetyl-CoA oxidation.

14. Two rounds of the glyoxylate cycle are necessary to yield two oxaloacetate, which give rise to one glucose by gluconeogenesis.

glyoxylate cycle:

$$4\ \text{Acetyl-CoA} + 4\ NAD^+ + 2\ \text{FAD} + 6\ H_2O \rightarrow$$
$$2\ \text{oxaloacetate} + 4\ \text{CoA} + 4\ \text{NADH} + 2\ FADH_2 + 8\ H^+$$

gluconeogenesis:

$$2\ \text{Oxaloacetate} + 2\ \text{GTP} + 2\ \text{ATP} + 2\ \text{NADH} + 4\ H_2O + 6\ H^+ \rightarrow$$
$$\text{glucose} + 2\ \text{GDP} + 2\ \text{ADP} + 2\ NAD^+ + 4\ P_i + 2\ CO_2$$

net:

$$4\ \text{Acetyl-CoA} + 2\ \text{GTP} + 2\ \text{ATP} + 2\ \text{NAD}^+ + 2\ \text{FAD} + 10\ \text{H}_2\text{O} \rightarrow$$
$$\text{glucose} + 4\ \text{CoA} + 2\ \text{CO}_2 + 2\ \text{GDP} + 2\ \text{ADP} + 2\ \text{NADH} + 2\ \text{FADH}_2 + 2\ \text{H}^+ + 4\ \text{P}_i$$

15. *Isocitrate lyase*: isocitrate → succinate + glyoxylate

    Malate synthase: acetyl-CoA + glyoxylate → malate + CoA

16. Aspartate and α-ketoglutarate move from the mitochondrion to the glyoxysome, and succinate and glutamate move from the glyoxysome to the mitochondrion.

Chapter 17 Electron Transport and Oxidative Phosphorylation

This chapter introduces you to the remarkable process by which cells harness the free energy of oxidation and use it to synthesize ATP. (You have already seen how ATP can be synthesized by the phosphorylation of ADP by a metabolite with a higher phosphoryl group-transfer potential.) The reduced cofactors generated in metabolic reactions, NADH and $FADH_2$, are reoxidized in the mitochondrion by a set of reactions in which electrons flow through a series of redox carriers, finally reducing oxygen to water. During electron transport, an electrochemical potential is developed across the inner mitochondrial membrane by the vectorial transfer of protons. This proton gradient is stable because the inner mitochondrial membrane is impermeable to ions. The free energy of the electrochemical proton gradient is utilized by an ATP synthase to catalyze the endergonic reaction ADP + P_i $\rightarrow$ ATP. The coupling of the electrochemical gradient to ATP synthesis is described by the chemiosmotic hypothesis, which is supported by considerable evidence.

Essential Concepts

1. The complete oxidation of glucose carbons by glycolysis and the citric acid cycle can be written as

$$C_6H_{12}O_6 + 6\,H_2O \rightarrow 6\,CO_2 + 24\,H^+ + 24\,e^-$$

The reducing equivalents (electrons) are captured in the form of reduced coenzymes (NADH and $FADH_2$), which eventually transfer the electrons to molecular oxygen:

$$6\,O_2 + 24\,H^+ + 24\,e^- \rightarrow 12\,H_2O$$

This process regenerates NAD^+ and FAD and generates a proton concentration gradient across the inner mitochondrial membrane, whose dissipation provides the free energy for ATP synthesis. This process is known as oxidative phosphorylation.

The Mitochondrion

2. The mitochondrion is surrounded by a relatively porous outer membrane. An inner membrane is folded to form cristae and encloses the gel-like matrix, which contains the enzymes of the citric acid cycle and fatty acid oxidation. The matrix also contains genetic machinery (DNA, RNA, and ribosomes), reflecting the bacterial origin of this organelle. The proteins involved in electron transport and oxidative phosphorylation are located in the inner mitochondrial membrane. The inner and outer membranes also contain proteins that mediate the transport of ions and metabolites.

3. NADH produced in the cytosol as a result of glycolysis must enter the mitochondrion to be aerobically oxidized. There are two shuttles for NADH.
   (a) The malate–aspartate shuttle allows NADH to be indirectly transported into the mitochondrion by reducing oxaloacetate to malate in the cytosol and transporting it into the mitochondrion, where it is reoxidized to produce oxaloacetate and NADH. The oxaloacetate is converted by transamination to aspartate and transported out again.
   (b) The glycerophosphate shuttle first reduces cytosolic dihydroxyacetone phosphate to 3-phosphoglycerate and NAD^+. The 3-phosphoglycerate is oxidized by an inner mitochondrial membrane enzyme, flavoprotein dehydrogenase, which introduces electrons directly into the electron-transport pathway.

4. Most of the ATP generated in the mitochondria is used in the cytosol. The ADP–ATP translocator exports ATP out of the matrix while importing ADP. ATP has one more negative charge than ADP, so transport is electrogenic. Transport is driven by the electrochemical potential of the proton concentration gradient (positive outside). The proton gradient also favors the transport of P_i into the matrix by a P_i–H^+ symport system.

5. The mitochondrial $[Ca^{2+}]$ is controlled by two transporters. The influx transporter responds to cytosolic $[Ca^{2+}]$, and transport is driven by the membrane potential (negative inside). Ca^{2+} exits the mitochondrion in exchange for Na^+ by an antiport mechanism that operates at its maximum velocity. Thus, the mitochondrion acts as a Ca^{2+} buffer. Large influxes of Ca^{2+} activate citric acid cycle enzymes.

Electron Transport

6. In an electron transfer reaction, electrons flow from a substance with a lower reduction potential to a substance with a higher reduction potential. The standard reduction potential, $\mathscr{E}^{\circ\prime}$, is a measure of a substance's affinity for electrons. For a redox reaction, $\Delta\mathscr{E}^{\circ\prime} = \mathscr{E}^{\circ\prime}_{(e^-\text{ acceptor})} - \mathscr{E}^{\circ\prime}_{(e^-\text{ donor})}$. When $\Delta\mathscr{E}^{\circ\prime}$ is positive, the reaction is spontaneous, since $\Delta G^{\circ\prime} = -n\mathscr{F}\Delta\mathscr{E}^{\circ\prime}$, where n is the number of electrons transported and $\mathscr{F}$ is the faraday (96,485 $J \cdot V^{-1} \cdot mol^{-1}$). The transfer of electrons from NADH to O_2 ($\Delta\mathscr{E}^{\circ\prime} = 1.13$ V and $\Delta G^{\circ\prime} = -218$ $kJ \cdot mol^{-1}$) provides enough free energy to synthesize three ATP molecules.

7. Four large protein complexes in the inner mitochondrial membrane are involved in transferring electrons from reduced coenzymes to O_2. Complexes I and II transfer electrons to the lipid-soluble electron carrier ubiquinone (coenzyme Q or CoQ), which transfers electrons to Complex III. From there, electrons pass to cytochrome *c*, a peripheral membrane protein with a heme prosthetic group, which transfers electrons to Complex IV. The reactions of Complexes I–IV are as follows:

   (I) $NADH + CoQ\,(ox) \rightarrow NAD^+ + CoQ\,(red)$
   $\Delta\mathscr{E}^{\circ\prime} = 0.360$ V and $\Delta G^{\circ\prime} = -69.5$ $kJ \cdot mol^{-1}$

(II) $FADH_2 + CoQ\ (ox) \rightarrow FAD + CoQ\ (red)$
$\Delta\mathscr{E}^{\circ\prime} = 0.085$ V and $\Delta G^{\circ\prime} = -16.4$ kJ·mol^{-1}

(III) $CoQ\ (red) +$ cytochrome $c\ (ox) \rightarrow CoQ\ (ox) +$ cytochrome $c\ (red)$
$\Delta\mathscr{E}^{\circ\prime} = 0.190$ V and $\Delta G^{\circ\prime} = -36.7$ kJ·mol^{-1}

(IV) Cytochrome $c\ (red) + \frac{1}{2}\ O_2 \rightarrow$ cytochrome $c\ (ox) + H_2O$
$\Delta\mathscr{E}^{\circ\prime} = 0.580$ V and $\Delta G^{\circ\prime} = -112$ kJ·mol^{-1}

8. Complex I is an enormous protein complex containing flavin mononucleotide (FMN, which is FAD minus its AMP group) and multiple iron–sulfur clusters (which are one-electron carriers). The two electrons donated by NADH are transferred through these redox-active prosthetic groups and then to CoQ. As electrons are transferred, four protons are pumped from the matrix to the intermembrane space, most likely by protein conformational changes similar to those in bacteriorhodopsin.

9. Complex II, which contains the citric acid cycle enzyme succinate dehydrogenase, transfers electrons from succinate to FAD and then to CoQ. No protons are translocated by Complex II, which serves mainly to feed electrons into the electron transport chain.

10. Complex III (cytochrome bc_1 or cytochrome c reductase) contains two b-type cytochromes, cytochrome c_1, and an iron–sulfur protein, which contains a [2Fe–2S] cluster. Electron flow from CoQ through Complex III follows a bifurcated cyclic pathway known as the Q cycle. In the first round of the Q cycle, fully reduced ubiquinone (ubiquinol; QH_2) donates one electron to the iron–sulfur protein, which then transfers it to cytochrome c_1 and then to cytochrome c. This one-electron donation yields the ubisemiquinone anion ($Q\cdot^-$), which donates its remaining electron to the low potential cytochrome b (b_L), and then to the high potential cytochrome b (b_H). The resulting ubiquinone diffuses to the other side of the membrane, where it accepts the electron from b_L to reform $Q\cdot^-$. A second round of electron transfers completes the cycle: Another QH_2 donates its electrons, one to the iron–sulfur protein and one to cytochrome b_L. The net result is that two electrons are transferred, one at a time, to two cytochrome c molecules, and four protons are transferred from the matrix to the intermembrane space, two from each QH_2 that participate in the Q cycle.

11. Cytochrome c shuttles electrons between Complexes III and IV. Cytochrome c is a small water-soluble protein whose heme group is largely buried in a crevice surrounded by a ring of Lys residues. Both cytochrome c_1 and cytochrome c oxidase (Complex IV) have a corresponding patch of negatively charged amino acid residues to facilitate cytochrome c binding and electron transfer.

12. Complex IV (cytochrome c oxidase) has four redox centers [cytochrome a, cytochrome a_3, Cu_A (which contains two Cu ions), and Cu_B], and it carries out the following reaction:

$$4\ \text{Cytochrome}\ c\ (Fe^{2+}) + 4\ H^+ + O_2 \rightarrow 4\ \text{cytochrome}\ c\ (Fe^{3+}) + 2\ H_2O$$

O_2 reduction takes place at the cytochrome a_3–Cu_B binuclear complex, which mediates four one-electron transfer reactions. Four protons are consumed in the production of H_2O, and four additional proteins are pumped from the matrix to the intermembrane space (two for each pair of electrons that enter the electron-transport chain).

Oxidative Phosphorylation

13. ATP synthase (Complex V) phosphorylates ADP by a mechanism driven by the free energy of electron transport, which is conserved in the formation of an electrochemical proton gradient across the inner mitochondrial membrane. The two processes are coupled as described by the chemiosmotic hypothesis. Four observations support this hypothesis:
    (a) Mitochondrial ATP formation requires an intact inner membrane.
    (b) The inner membrane is impermeable to ions, so an electrochemical gradient across the membrane can be sustained.
    (c) Electron transport pumps protons out of the mitochondrion to create a measurable electrochemical gradient.
    (d) Agents that increase the permeability of the inner mitochondrial membrane to protons inhibit ATP synthesis but not electron transport.

14. The protonmotive force results from the difference in concentration of protons (pH) in the matrix and the intermembrane space and from the difference in charge (membrane potential, $\Delta\Psi$) across the membrane. Thus, $\Delta G = 2.3\ RT\ [\text{pH}(in) - \text{pH}(out)] + Z\mathcal{F}\Delta\Psi$, where Z is the charge of the proton. $\Delta\Psi$ is positive when a proton is transported from negative to positive, or against its potential. Thus, pumping protons out of the matrix (against the gradient) is an endergonic process, whereas transporting them back in (with the gradient) is an exergonic process. About three protons are needed to supply sufficient energy to synthesize one ATP from ADP + P_i.

15. ATP synthase, also called F_1F_0-ATPase, has two functional units. The F_0 component comprises the transmembrane proton channel. Dicyclohexylcarbodiimide and oligomycin binding to F_0 inhibits proton translocation and thereby inhibits ATP synthesis. The F_1 component is a water-soluble protein of subunit composition $\alpha_3\beta_3\gamma\delta\varepsilon$ that associates with the membrane via F_0 to form a lollipoplike structure.

16. The 3 α, 3 β, and γ subunits of F_1 form a pseudo-symmetrical structure of alternating α and β subunits in a ring with the elongated γ subunit in its central hole. The three pairs of $\alpha\beta$ subunits exhibit pseudo-threefold rotational symmetry.

17. The binding change mechanism describes ATP synthesis in terms of three processes:
    (a) Translocation of protons carried out by F_0.
    (b) Formation of the phosphoanhydride bond of ATP, catalyzed by F_1.
    (c) Interaction of F_0 and F_1 to couple the dissipation of the proton gradient to formation of the phosphoanhydride bond.

18. According to the binding change mechanism, ATP is synthesized as each αβ protomer shifts though three conformations in sequence. The three possible conformations are called open (O), loose (L), and tight (T). ADP and P_i bind to a protomer with the L conformation and are converted to ATP when the conformation shifts to the T state. The free energy of the proton concentration gradient converts the T state to the O state (this is the rate-limiting step), thereby releasing ATP. This three-step mechanism is consistent with the pseudo-threefold axis of rotation. The γ subunit rotates with respect to the $\alpha_3\beta_3$ assembly, and the geometric relationship of individual αβ protomers to the γ subunit dictates their conformational state. Three protons, each of which promotes one conformational shift, are required to synthesize one ATP.

19. The ratio of the amount of ATP produced to the amount of substrate oxidized (measured as oxygen consumed) is called the P/O ratio. [The P/O ratio refers to atomic oxygen, O, rather than molecular oxygen, O_2, because each substrate (NADH or $FADH_2$) transfers two electrons, not four.] Depending on where a substrate's electrons enter the electron-transport chain, the P/O ratio is ~3, ~2, or ~1. For example, the two electrons transferred from NADH through Complexes I, III, and IV pump 10 protons, which yields ~ 3 ATP, whereas the two electrons transferred from $FADH_2$ through Complexes II, III, and IV pump 6 protons, which yields ~2 ATP. The complete oxidation of glucose therefore yields 38 ATP. The P/O ratio is not necessarily a whole number, because protons are contributed to the gradient by more than one process and some protons leak back into the matrix.

20. Electron transport and oxidative phosphorylation are normally strongly coupled processes; that is, neither process occurs in the absence of the other. This is because, if the rate of electron transport were to outpace the rate of ATP synthesis, the proton gradient would build up to the level that it would resist additional proton pumping by Complexes I, III, and IV and hence the rate of electron transport would be slowed. However, when uncoupling agents, which dissipate the proton gradient, are added to respiring mitochondria, electron transport proceeds unchecked while ATP synthesis stops. The free energy of electron transport is then redirected from ATP synthesis to generate heat. 2,4-Dinitrophenol is an uncoupling agent because it carries protons through the membrane from the intermembrane space to the matrix, thereby providing a route for dissipation of the gradient that bypasses F_0.

Control of ATP Production

21. Electron transfer from NADH to cytochrome *c* is nearly at equilibrium. In contrast, the cytochrome *c* oxidase reaction is irreversible and hence its rate depends on the concentration of its substrate, reduced cytochrome *c*. Increased NADH concentrations and decreased ATP concentrations lead to the production of more reduced cytochrome *c* and hence to increased electron transfer rates. Thus, the overall rate of oxidative phosphorylation depends on the ratios $[NADH]/[NAD^+]$ and $[ATP]/[ADP][P_i]$, which in turn may depend on the activities of the respective mitochondrial transporters.

22. The coordinated control of oxidative metabolism centers on several key enzymes: hexokinase (HK), phosphofructokinase (PFK), pyruvate kinase (PK), pyruvate dehydrogenase (PDH), citrate synthase (CS), isocitrate dehydrogenase IDH) and α-ketoglutarate dehydrogenase (KDH). High levels of ATP inhibit PFK and PK while high [NADH]/[NAD^+] ratios inhibit PDH, IDH, and KDH. Citrate inhibits both PFK and CS. The need for ATP, represented by high concentrations of either AMP or ADP, activates PFK, PK, PDH, and IDH, while Ca^{2+} stimulates PDH, IDH, and KDH.

Physiological Implications of Aerobic Metabolism

23. Anaerobic glycolysis produces 2 ATP per glucose consumed, whereas oxidative metabolism generates 38 ATP per glucose, a 19-fold increase. However, there are several drawbacks of oxygen-based metabolism. Many organisms depend on oxidative metabolism and would perish without a steady supply of oxygen. Reactive oxygen species generated by incomplete oxygen reduction are potentially dangerous.

24. Oxygen is used by the enzyme cytochrome P450 to detoxify many harmful compounds such as polycyclic aromatic hydrocarbons, polychlorinated biphenyls, phenobarbital, and steroids. Cytochrome P450 catalyzes the addition of one atom of diatomic oxygen into the substrate as a hydroxyl group, while the other atom is converted to water. Hydroxylation generally renders the substrate more water-soluble and hence more easily excreted.

25. Reactive oxygen species include the superoxide radical $O_2\cdot^-$ (produced by the reaction $O_2 + e^- \rightarrow O_2\cdot^-$), which is a precursor of even more powerful oxidizing species such as $HO_2\cdot$ and ·OH. These free radicals readily extract electrons from other substances, creating a chain reaction. Neurodegenerative conditions such as Parkinson's, Alzheimer's, and Huntington's diseases are associated with mitochondrial oxidative damage. Free radical reactions arising from normal oxidative metabolism appear to be partially responsible for the aging process.

26. Antioxidants limit oxidative damage by destroying free radicals. Superoxide dismutase (SOD) catalyzes the production of water and hydrogen peroxide from superoxide:

$$2\ O_2\cdot^- + 2\ H^+ \rightarrow H_2O_2 + O_2$$

This enzyme electrostatically guides its substrate to the active site to catalyze a reaction near the diffusion-controlled limit. Mutations in Cu,Zn SOD are associated with amyotrophic lateral sclerosis (Lou Gehrig's disease).

27. Catalase and glutathione peroxidase degrade hydroperoxides. Some types of glutathione peroxidase require selenium, so Se also appears to be an antioxidant.

Key equation

$$\Delta G = 2.3\ RT\ [\mathrm{pH}(in) - \mathrm{pH}\ (out)] + Z\mathcal{F}\Delta\Psi$$

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Guide to Study Exercises (text p.527)

1. In the oxidation of glucose to 2 pyruvate by glycolysis, four electrons are transferred to 2 NAD^+ to produce 2 NADH at the step catalyzed by glyceraldehyde-3-phosphate dehydrogenase. The conversion of 2 pyruvate to 2 acetyl-CoA by pyruvate dehydrogenase transfers four more electrons to 2 NAD^+. During two rounds of the citric acid cycle (which completes the oxidation of the carbon atoms originally from glucose), 12 electrons are transferred to 6 NAD^+ (in the reactions catalyzed by isocitrate dehydrogenase, α-ketoglutarate dehydrogenase, and malate dehydrogenase), and four electrons are transferred to 2 FAD in the reactions catalyzed by succinate dehydrogenase. Thus, the 24 electrons from glucose yield 10 NADH and 2 $FADH_2$. These reduced cofactors are reoxidized by the electron-transport chain. NADH gives up its electrons to Complex I, and the $FADH_2$ is part of Complex II, whose electrons are transferred to the electron carrier CoQ (which also receives electrons from Complex I). Electrons then travel from CoQ to Complex III, to cytochrome *c*, and then to Complex IV, which caries out the four-electron reduction of O_2 to H_2O. (Chapter introduction and Section 17-2B)

2. The two electrons carried by NADH are transferred together to FMN, which is a two-electron acceptor group in Complex I. $FMNH_2$ then transfers one electron at a time to the first of a series of iron–sulfur clusters. Each electron is then passed through the iron–sulfur clusters to the mobile electron carrier coenzyme Q, which is a two-electron carrier. Reduced CoQ then transfers its two electrons to Complex III. The flow of electrons follows a bifurcated cyclic pathway (the Q cycle) in which the first electron reduces the Rieske iron–sulfur protein and then cytochrome c_1, which in turn reduces cytochrome *c*. Simultaneously, the second electron passes from $CoQ\cdot^-$ to cytochrome b_L and then to cytochrome b_H and back to $CoQ\cdot^-$. A second round of the cycle involving a second reduced CoQ results in the reduction of a second cytochrome c_1 and the reduction of the $CoQ\cdot^-$ back to reduced CoQ. In this way, the electrons of the two-electron carrier NAD^+ are transferred to two molecules of the one-electron carrier cytochrome *c*. (Sections 17-2C and E)

3. The translocation of protons from the matrix to the intermembrane space by Complexes I and IV depends on protein conformational changes that occur in conjunction with the reduction and reoxidation of its redox centers. In contrast, the protons translocated by Complex III are ferried from the matrix to the intermembrane space via the Q cycle by binding to a redox cofactor—CoQ—rather than the protein. (Sections 17-2C, E, and F)

4. According to the chemiosmotic theory, the free energy of electron transport is conserved in the formation of a transmembrane proton concentration gradient that is established when protons are pumped from the mitochondrial matrix to the intermembrane space by the action of the electron-transport complexes. The free energy of the gradient is harnessed to drive the phosphorylation of ADP to produce ATP. (Section 17-3A)

5. In the binding change mechanism, each of the three αβ protomers of F_1F_0-ATPase assumes one of three conformations. The free energy of proton translocation through F_0 causes each protomer to shift its conformation so that its transit through the three conformations in

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5. In the binding change mechanism, each of the three αβ protomers of F_1F_0-ATPase assumes one of three conformations. The free energy of proton translocation through F_0 causes each protomer to shift its conformation so that its transit through the three conformations in

succession accomplishes ATP synthesis. Thus, a protomer in the L conformation binds the substrates ADP and P_i loosely. It then changes to the T conformation, which binds the substrates tightly and is catalytically active. The resulting ATP is released when the protomer shifts to the O conformation. (Section 17-3B)

6. ATP is synthesized when reduced molecules donate their electrons to the electron-transport chain, which ultimately reduces O_2 to H_2O. However, the amount of ADP phosphorylated is indirectly related to the amount of O_2 reduced because phosphorylation is driven by the free energy of a transmembrane proton concentration gradient that is established by the action of more than one electron-transport protein and may be dissipated by more than one mechanism. (Section 17-3C)

7. Oxidative phosphorylation is linked to electron transport by the arrangement of protein complexes in the inner mitochondrial membrane such that electron transport through Complexes I, III, and IV generates a transmembrane proton concentration gradient whose dissipation through the F_0 channel drives ADP phosphorylation by F_1F_0-ATPase. This coupling depends on the impermeability of the membrane, which allows the electron-transport complexes to increase the concentration of protons on the cytoplasmic side of the membrane, and which prevents the protons from re-entering the matrix except through F_1F_0-ATPase. Electron transport and oxidative phosphorylation can be uncoupled by an agent that dissipates the proton concentration gradient. The result is that electron transport proceeds without the buildup of the proton gradient, and hence no ATP is synthesized. (Section 17-3D)

8. The primary advantage of O_2-based metabolism is that under aerobic conditions, the complete catabolism of 1 glucose yields 38 ATP, whereas the oxidation of glucose to lactate under anaerobic conditions yields only 2 ATP. One disadvantage of aerobic metabolism is that O_2 must be constantly available to serve as the terminal acceptor of electrons from substrate oxidation. Another disadvantage is that reactive oxygen species produced by the incomplete reduction of O_2 can damage cellular lipids, proteins, and DNA. (Section 17-5)

Questions

The Mitochondrion

1. Draw a cross-section of a mitochondrion and label the following structural features:

Outer membrane (OM)	Inner membrane (IM)
Matrix (M)	Intermembrane space (IMSP)
ATP synthase complex (ASC)	Direction of proton flux
ATP and P_i transporters (T)	Cristae (CR)

2. Match the following enzyme or other molecule with its location:

____ Pyruvate dehydrogenase	A. Cytosol
____ 3-Phosphoglycerate dehydrogenase	
____ Flavoprotein dehydrogenase	B. Mitochondrial outer membrane
____ Malate dehydrogenase	
____ Cytochrome *c*	C. Mitochondrial inner membrane
____ Cytochrome c_1	
____ Fatty acid oxidation enzymes	D. Mitochondrial intermembrane space
____ Mitochondrial DNA	
____ ADP–ATP translocator	E. Mitochondrial matrix
____ Mitochondrial porin	

3. About half the volume of the mitochondrial matrix is water, and the rest is protein. If a single protein of molecular mass 40,000 were as concentrated, what would be its molar concentration? Assume the protein's density is 1.37 $g \cdot mL^{-1}$.

4. Oxidative phosphorylation requires the transfer of electrons donated by NADH. (a) Is NADH imported directly into the mitochondria? Explain. (b) Describe two import mechanisms that transfer cytosolic electrons from NADH into the mitochondrion. (c) Why is it important to maintain a relatively constant level of cytosolic NAD^+?

5. What controls the rate of Ca^{2+} influx into the mitochondrial matrix? How does muscle activity change the respiration rate?

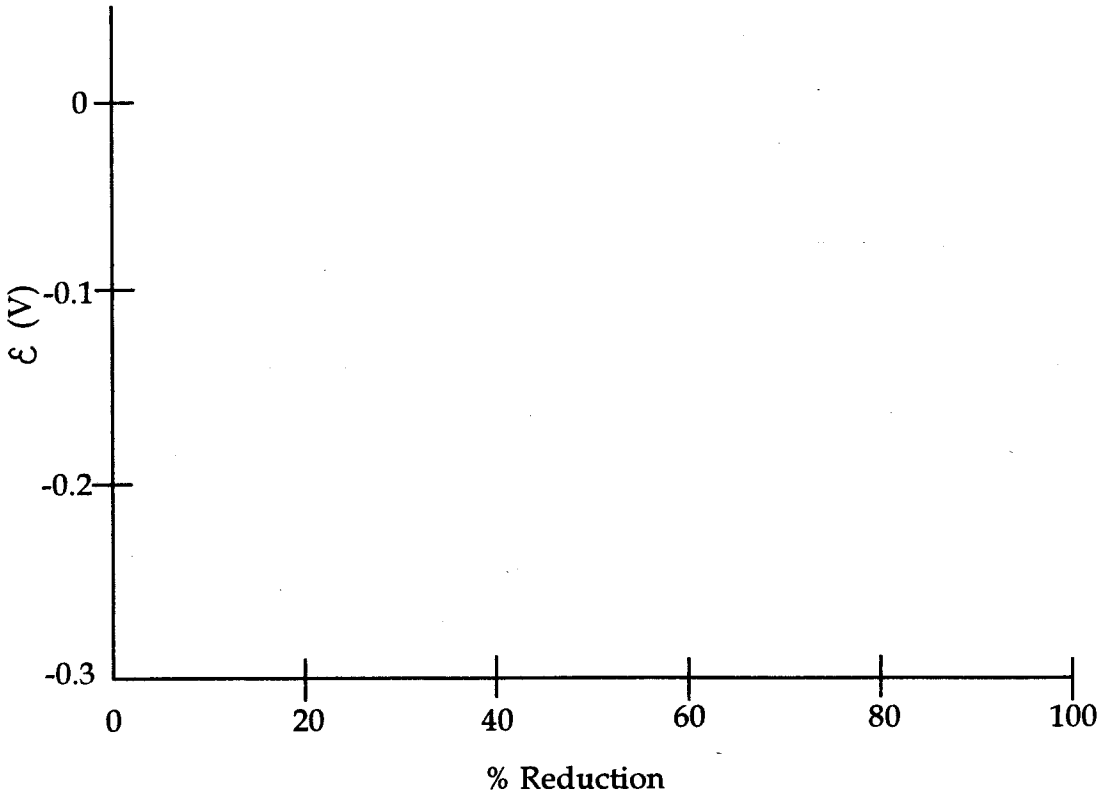
Electron Transport

6. Which reactions of the citric acid cycle donate electron pairs to the mitochondrial electron-transport chain?

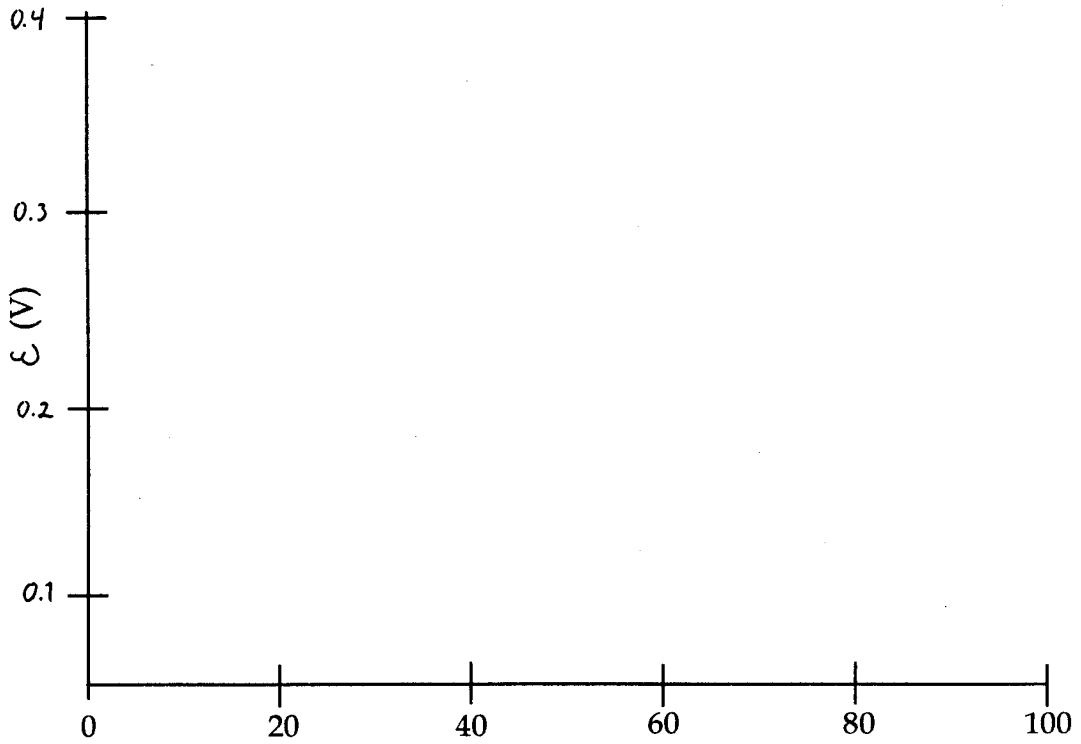
7. The half-cell reduction potential is provided by the Nernst equation (Equation 13-8):

$$\mathscr{E}_A = \mathscr{E}^\circ_A - \frac{RT}{n\mathscr{F}} \ln\left(\frac{[A_{red}]}{[A_{ox}^{n+}]}\right)$$

(a) On the graph on the following page, plot the reduction potentials for the $FADH_2$/FAD half-cell ($\mathscr{E}^{\circ\prime} = -0.219$ V) when the [$FADH_2$]/[FAD] ratios are 100, 10, 5, 2, 1, 0.5, 0.2, 0.1, and 0.01 at 25°C versus the percent reduction.



(b) Using the same [Reduced]/[Oxidized] ratios, plot the reduction potentials of cytochrome *c* ($\mathscr{E}^{\circ\prime}$ = 0.235 V) on the graph below.



(c) What is $\Delta\mathscr{E}$ for the oxidation of $FADH_2$ by cytochrome *c* when the $[FADH_2]/[FAD]$ ratio is 10 and the [cytochrome *c* (Fe^{2+})]/[cytochrome *c* (Fe^{3+})] ratio is 0.1?

8. Inhibitors of electron transport have been used to determine the order of electron carriers. What would be the expected redox states of cytochromes *a*, b_L, and *c* when (a) myxothiazol, (b) antimycin A, or (c) rotenone is added to succinate-driven respiring mitochondria?

9. What is the most abundant type of redox carrier in Complex I?

10. Which mitochondrial electron carriers are potentially proton carriers? Which are more abundant—proton carriers or electron carriers? What does this suggest about the mechanism of transmembrane proton transport?

11. What amino acid residues would you expect to find at the cytochrome *c*–binding sites of Complex III and Complex IV?

12. Which redox group(s) in Complex IV accepts electrons from cytochrome *c*? Which redox group(s) binds oxygen during its four-electron reduction?

Oxidative Phosphorylation

13. What key observations support the chemiosmotic hypothesis?

14. Can a pH gradient exist without $\Delta\Psi$? Can $\Delta\Psi$ exist without a ΔpH?

15. (a) What is a P/O ratio? (b) What happens to oxygen consumption when electron donors and inorganic phosphate are present in a suspension of mitochondria in the absence of ADP? (c) What happens when ADP is added?

16. (a) Valinomycin, an antibiotic ionophore, allows the free passage of only K^+ ions across a membrane. If K^+ and valinomycin are added to respiring, fully coupled ATP-synthesizing mitochondria, what happens to the pH gradient and the $\Delta\Psi$? (b) Nigericin, another ionophore, exchanges one K^+ for one H^+. How does this affect ATP synthesis and electron transport in mitochondria? (c) Gramicidin allows the free passage of many small molecules and ions across the membrane. What happens to ATP production and electron transport in the presence of gramicidin?

17. The free energy-requiring step in the synthesis of ATP is not the formation of ATP from ADP and P_i ($\Delta G \approx 0$), but the release of tightly bound ATP. Explain why this is not inconsistent with the +30.5 $kJ \cdot mol^{-1}$ free energy of formation of ATP in solution.

18. What happens to the electron transport rate when DCCD is added to actively respiring mitochondria?

19. What does an H^+/P ratio measure? Why would it be impractical to determine an H^+/P ratio?

Control of ATP Production

20. The conversion of glucose to 2 lactate has $\Delta G^{\circ\prime} = -196$ kJ·mol^{-1}. The complete oxidation of glucose to 6 CO_2 has $\Delta G^{\circ\prime} = -2823$ kJ·mol^{-1}. Compare the efficiencies of ATP synthesis by each of these processes under standard conditions.

21. On an average day, an adult dissipates about 7000 kJ of free energy. Assuming that this occurs under standard conditions, (a) how many moles of ATP must be hydrolyzed to provide this quantity of free energy? (b) What is the mass of this quantity of ATP? (c) If the amount of ATP in an adult is about 0.1 mole, how many times per day, on average, is a molecule of ADP recycled? The molecular mass of ATP is 507.

22. What is the irreversible step in electron transport and how is its rate controlled?

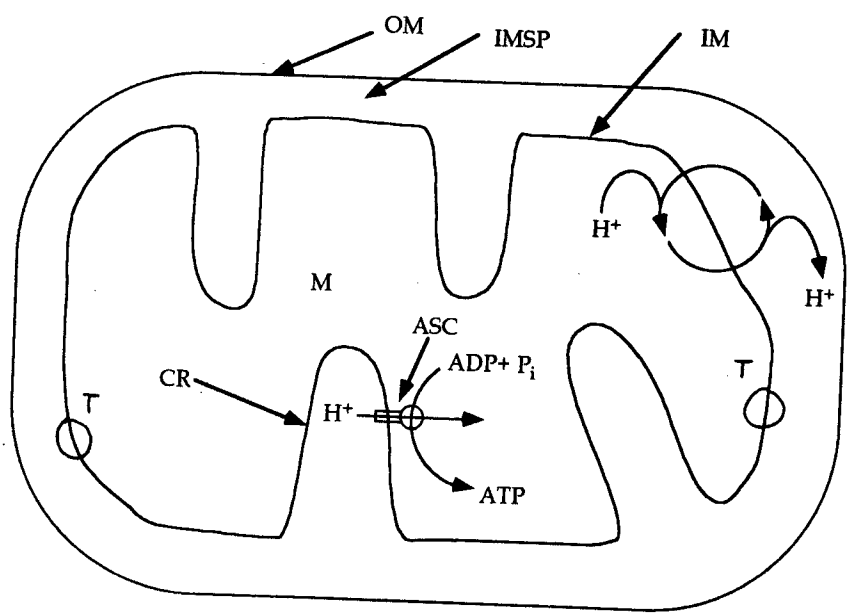
Physiological Implications of Aerobic Metabolism

23. Cytochrome P450 catalyzes a reaction in which two electrons supplied by NADPH reduce the heme Fe atom so that it can then reduce O_2 preparatory to the hydroxylation of a substrate molecule. Substrate binding to the enzyme displaces a water molecule that forms a ligand to the heme iron atom. This changes the reduction potential of the Fe from –0.300 V to –0.170 V. Why is this change necessary for efficient catalysis?

24. Rats that are fed a "cafeteria" diet (in which food is always available) tend to die sooner than rats whose dietary intake is limited. Propose an explanation for this observation.

Answers to Questions

1.



2. E Pyruvate dehydrogenase
A 3-Phosphoglycerate dehydrogenase
C Flavoprotein dehydrogenase
E, A Malate dehydrogenase
D Cytochrome *c*
C Cytochrome c_1
E Fatty acid oxidation enzymes
E Mitochondrial DNA
C ADP–ATP translocator
B Mitochondrial porin

3. The protein's molar concentration would be

$$\frac{1}{40\,000\ \text{g}\cdot\text{mol}^{-1}} \times \frac{1.37\ \text{g}}{\text{mL}} \times \frac{1000\ \text{mL}}{\text{L}} \times 50\%$$

$$= 0.0171\ \text{mol}\cdot\text{L}^{-1}$$
$$= 17.1\ \text{mM}$$

4. (a) The negatively charged phosphate groups of NADH (Fig. 3-4) prevent its diffusion across the inner mitochondrial membrane, and there are no NADH transport proteins to facilitate its transport.

(b) The malate–aspartate shuttle allows the indirect import of NADH reducing equivalents (Fig. 15-27). The reduction of oxaloacetate to malate by NADH followed by the facilitated transport of malate across the inner mitochondrial membrane yields NADH in the mitochondrial matrix when the malate is reoxidized to oxaloacetate. Oxaloacetate then returns to the cytosol by being converted to aspartate, for which there is a transporter. The glycerophosphate shuttle utilizes NADH to convert dihydroxyacetone phosphate to 3-phosphoglycerate by a flavoprotein dehydrogenase that donates electrons to the electron-transport chain in a manner similar to succinate dehydrogenase (Fig. 17-4).

(c) Cytosolic NAD^+ is required for the glyceraldehyde-3-phosphate dehydrogenase reaction of glycolysis. Limited [NAD^+] would shut down glycolysis.

5. The Ca^{2+} influx into the mitochondrial matrix is regulated by cytosolic [Ca^{2+}], which is less than the K_M for Ca^{2+} transport, so that Ca^{2+} influx is approximately first-order. During muscle activity, cytoplasmic [Ca^{2+}] increases, which then increases the mitochondrial [Ca^{2+}]. Ca^{2+} stimulates the enzymes of the citric acid cycle, leading to an increase in production of reduced coenzymes, whose reoxidation demands an increase in the respiration rate.

6. Isocitrate dehydrogenase, α-ketoglutarate dehydrogenase, and malate dehydrogenase produce NADH, which transfers its electrons to Complex I. Succinate dehydrogenase, whose FAD group is reduced in the oxidation of succinate to fumarate, is a component of Complex II.

7. (a) The percent reduction is

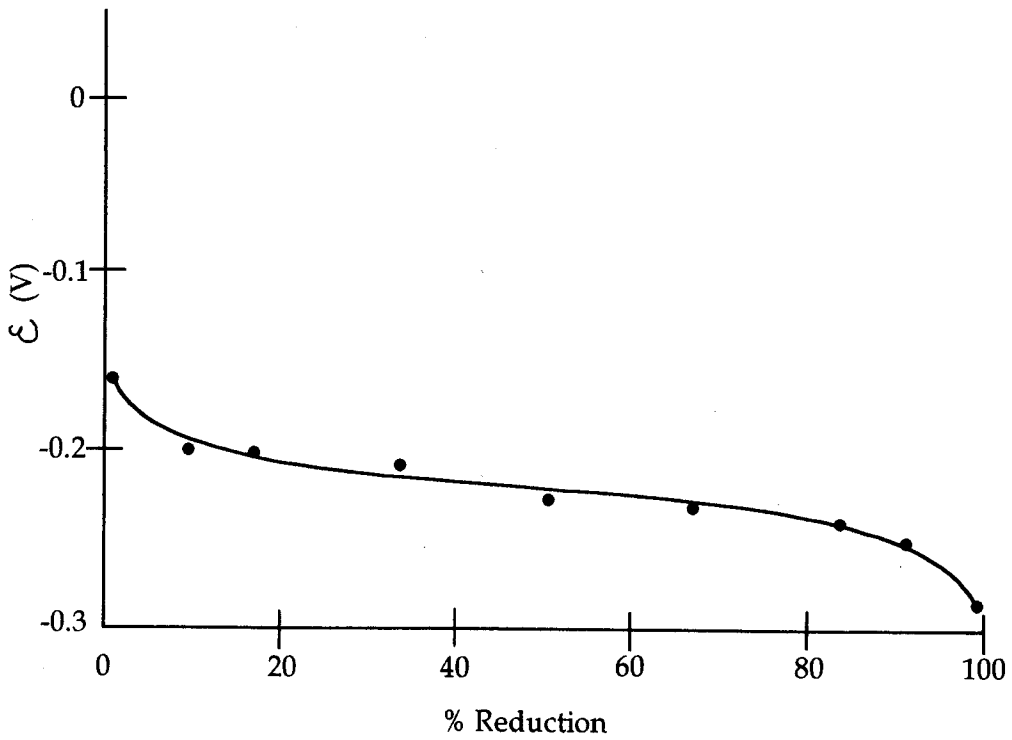
$$\frac{[\text{Reduced}]}{[\text{Reduced}] + [\text{Oxidized}]} \times 100 \quad \text{or} \quad \frac{[FADH_2]}{[FADH_2] + [FAD]} \times 100$$

For the $FADH_2/FAD$ half-cell, the Nernst equation is

$$\mathscr{E} = (-0.219\ \text{V}) - \frac{(8.3145\ \text{J}\cdot\text{K}^{-1}\cdot\text{mol}^{-1})(298\ \text{K})}{(2)(96{,}485\ \text{J}\cdot\text{V}^{-1}\cdot\text{mol}^{-1})} \ln\left(\frac{[FADH_2]}{[FAD]}\right)$$

$$\mathscr{E} = -0.219\ \text{V} - (0.0128\ \text{V}) \ln\left(\frac{[FADH_2]}{[FAD]}\right)$$

[$FADH_2$]/[FAD]	*% Reduction*	*$\mathscr{E}$ (V)*
100	99.0	−0.278
10	90.9	−0.248
5	83.3	−0.240
2	66.7	−0.228
1	50.0	−0.219
0.5	33.3	−0.210
0.2	16.7	−0.198
0.1	9.1	−0.190
0.01	0.99	−0.160

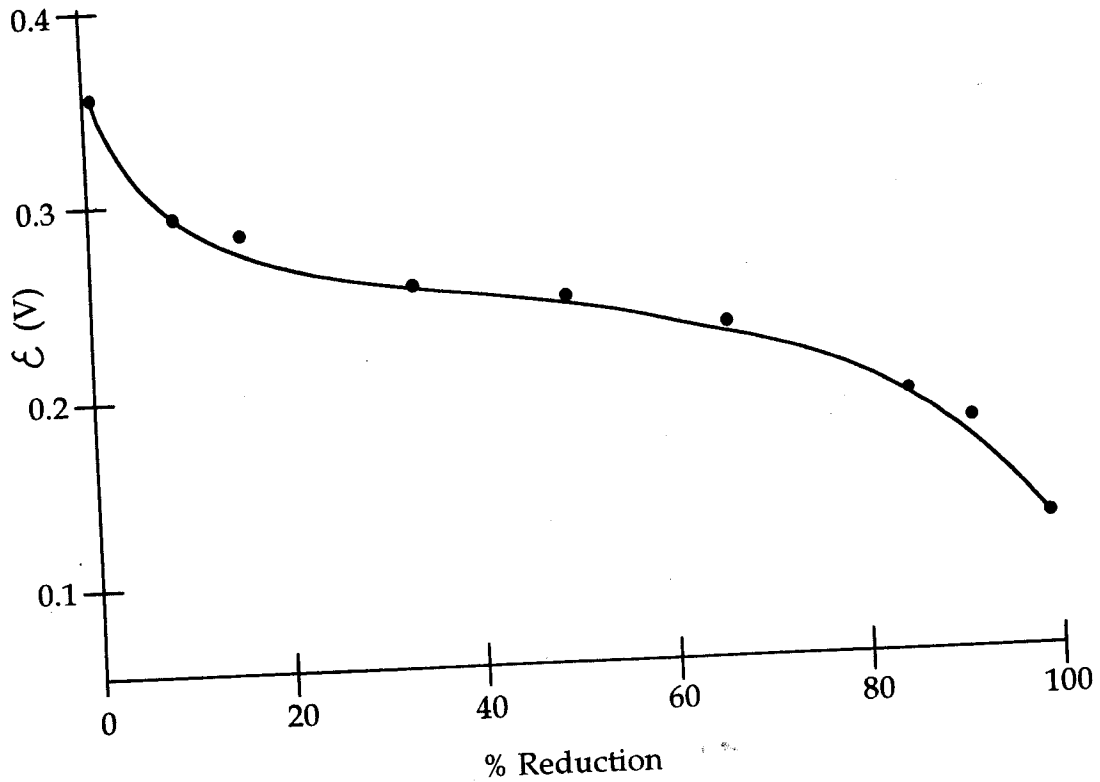


(b) For cytochrome *c*,

$$\mathscr{E} = (0.235\ \text{V}) - \frac{(8.3145\ \text{J}\cdot\text{K}^{-1}\cdot\text{mol}^{-1})(298\ \text{K})}{(1)(96{,}485\ \text{J}\cdot\text{V}^{-1}\cdot\text{mol}^{-1})} \ln\left(\frac{[\text{cyto } c\ (\text{Fe}^{2+})]}{[\text{cyto } c\ (\text{Fe}^{3+})]}\right)$$

$$\mathscr{E} = 0.235\ \text{V} - (0.0257\ \text{V}) \ln\left(\frac{[\text{cyto } c\ (\text{Fe}^{2+})]}{[\text{cyto } c\ (\text{Fe}^{3+})]}\right)$$

[cyto c (Fe^{2+})]/[cyto c (Fe^{3+})]	*% Reduction*	*$\mathscr{E}$ (V)*
100	99.0	0.117
10	90.9	0.176
5	83.3	0.194
2	66.7	0.217
1	50.0	0.235
0.5	33.3	0.253
0.2	16.7	0.276
0.1	9.1	0.294
0.01	0.99	0.353



(c) When $[FADH_2]/[FAD] = 10$, $\mathscr{E}_{FAD} = -0.248$ V
When [cyto *c* (Fe^{2+})]/[cyto *c* (Fe^{3+})] = 0.1, $\mathscr{E}_{cyto\ c} = 0.294$ V
According to Equation 13-10,

$$\begin{aligned}\Delta\mathscr{E} &= \mathscr{E}_{(e^-\text{ acceptor})} - \mathscr{E}_{(e^-\text{ donor})} \\ &= \mathscr{E}_{cyto\ c} - \mathscr{E}_{FAD} \\ &= 0.294\text{ V} - (-0.248\text{ V}) \\ &= 0.542\text{ V}\end{aligned}$$

8. (a) Myxothiazol inhibits the electron flow from $CoQH_2$ to Complex III. Hence cytochrome *a* (a component of Complex IV) cannot obtain electrons and would be largely in its oxidized state. Similarly, cytochrome b_L (part of Complex II) and cytochrome *c* (which links Complexes III and IV) would be largely in their oxidized states.
(b) Antimycin A blocks electron transport in Complex III from heme b_H to CoQ or $CoQ\cdot^-$. Thus, cytochrome b_L would be reduced since it could not pass its electrons on to cytochrome b_H. However, cytochromes *a* and *c* would be oxidized since they are both downstream of the block.
(c) Rotenone blocks electron transport in Complex I. However, since electrons are being introduced into the electron-transport chain at Complex II, electron transport can proceed all the way to O_2. Therefore, all three cytochromes would be predominantly in their reduced forms.

9. The most abundant type of electron carrier in Complex I is iron–sulfur clusters.

10. Only two electron carriers in the mitochondrial electron-transport chain can also carry protons, FMN and ubiquinone (CoQ). The paucity of proton carriers compared to electron carriers suggests that transmembrane proton transport involves some sort of protein-mediated proton pumping rather than only direct transport by the proton carriers.

11. Since cytochrome *c* contains positively charged Lys residues around its heme crevice, its redox partners must have negatively charged residues such as Glu and Asp at their cytochrome *c*–binding sites.

12. The Cu_A center accepts the first electron from cytochrome *c*. Oxygen binds to the partially reduced Fe(II)–Cu(I) form of the cytochrome a_3–Cu_B binuclear complex.

13. Key observations that support the chemiosmotic hypothesis are: (a) Oxidative phosphorylation requires an intact inner membrane; (b) the inner mitochondrial membrane is impermeable to ions such as H^+, OH^-, K^+, and Cl^-; (c) electron transport results in the transport of H^+ out of the mitochondrial matrix; and (d) compounds that increase proton permeability across the membrane uncouple phosphorylation from electron transport and inhibit ATP synthesis.

14. A pH gradient can exist without $\Delta\Psi$. If a counterion such as Cl^- moved in the same direction as the H^+, or if K^+ moved in the opposite direction, a $[H^+]$ gradient could form

(c) When $[FADH_2]/[FAD] = 10$, $\mathscr{E}_{FAD} = -0.248$ V
When [cyto c (Fe^{2+})]/[cyto c (Fe^{3+})] = 0.1, $\mathscr{E}_{\text{cyto } c} = 0.294$ V
According to Equation 13-10,

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14. A pH gradient can exist without $\Delta\Psi$. If a counterion such as Cl^- moved in the same direction as the H^+, or if K^+ moved in the opposite direction, a $[H^+]$ gradient could form

without altering the net charge on either side of the membrane. Similarly, the transmembrane movement of ions other than H^+ could generate $\Delta\Psi$ without generating a proton concentration gradient.

15. (a) The P/O ratio is a measure of how many ATPs are synthesized for every O atom reduced.
    (b) Oxygen consumption stops when the production of ATP from ADP cannot be carried out. This is because electron transport and proton translocation cannot take place independently. Hence, when the proton gradient has built up to the point that electron transport has insufficient free energy to translocate additional protons across the inner mitochondrial membrane, electron transport must stop.
    (c) When ADP is added, ATP can be synthesized. O_2 consumption then resumes because the dissipation of the proton concentration gradient by ATP synthase reduces the free energy of proton translocation across the inner mitochondrial membrane to the point that electron transport can continue.

16. (a) The pH gradient would remain unchanged. However, the $\Delta\Psi$ would collapse because K^+ would equilibrate across the membrane in response to the $\Delta\Psi$.
    (b) Nigericin would dissipate the proton gradient by exchanging protons for K^+ and hence arrest ATP synthesis. The rate of electron transport would increase because there would be no buildup of a proton gradient to hold electron transport in check. However, $\Delta\Psi$ would remain intact because there would be no net change in charge across the membrane.
    (c) Gramicidin would cause the collapse of both the $\Delta\Psi$ and the pH gradient, so ATP synthesis would stop but electron transport would accelerate.

17. In solution, the ATP-forming reaction is $ADP + P_i \rightarrow ATP + H_2O$. Recall that an enzymatic reaction may progress through several steps, but it cannot alter the ΔG for a reaction. For the ATP synthase–catalyzed reaction, the binding of substrates and release of products are just two of the steps in the overall process. Letting E represent the enzyme, the reaction can be written as

$$ADP + P_i + E \rightarrow ADP{\cdot}P_i{\cdot}E \rightarrow ATP{\cdot}H_2O{\cdot}E \rightarrow ATP + H_2O + E$$

where each step has a different ΔG value. However, the overall reaction remains

$$ADP + P_i + E \rightarrow ATP + H_2O + E$$

and hence the overall free energy change is still 30.5 $kJ{\cdot}mol^{-1}$.

18. DCCD reacts with a Glu residue in a subunit of F_0 that forms the proton channel through the membrane. DCCD binding inhibits ATP formation. The proton gradient therefore builds up tot he point that it arrests further electron transport.

19. The H^+/P ratio is a measure of the number of protons transported across the inner membrane for each ATP molecule synthesized. This could be difficult to determine since the measurement of pH would not take into account $\Delta\Psi$, which also contributes to the protonmotive force. In addition, the measurement would be highly sensitive to cytosolic pH changes that were not involved in mitochondrial electron transport.

20. $\Delta G^{\circ\prime} = +30.5$ kJ·mol^{-1} for the synthesis of ATP from ADP + P_i. The conversion of glucose to lactate by glycolysis is accompanied by the synthesis of 2 ATP. Thus, the efficiency of ATP production under standard conditions is [(2 × 30.5 kJ·mol^{-1})/(196 kJ·mol^{-1})] × 100 = 31%. When glucose is completely oxidized, the yield is 38 ATP. The efficiency of this process is [(38 × 30.5 kJ·mol^{-1})/(2823 kJ·mol^{-1})] × 100 = 41%. Therefore, not only does oxidative phosphorylation yield more ATP than glycolysis, but at least under standard conditions, it does so with greater efficiency.

21. (a) The energy is supplied by 7000 kJ/30.5 kJ·mol^{-1} = 230 moles of ATP.
    (b) 230 moles × 507 g·mol^{-1} = 11700 g = 117 kg
    (c) Since 230 moles are needed each day, 0.1 mol of ADP must recycled 230/0.1 = 2300 times.

22. The irreversible step in electron transport is the formation of water from oxygen. The rate of cytochrome *c* oxidase is controlled by the ratio of reduced to oxidized cytochrome *c*, which is in turn controlled by the [NADH]/[NAD^+] and [ATP]/[ADP][P_i] ratios.

23. The change in redox potential of the Fe atom promotes its ability to accept the electrons from NADPH ($\mathscr{E}^{\circ\prime}$ = –0.320). Recall that electrons flow spontaneously from a substance with a more negative redox potential to a substance with a more positive redox potential.

24. Rats that consume large quantities of metabolic fuels have a higher rate of oxidative metabolism and hence generate more oxygen radicals than rats that consume less food and have lower rates of oxidative metabolism. The cumulative oxidative damage would be greater in the cafeteria-fed rats, which therefore die sooner.

Chapter 18 Photosynthesis

In the preceding chapters, you learned that free energy is derived from reduced foodstuffs such as glucose and that the energy produced from catabolic pathways is used to generate both ATP and NAD(P)H. Photosynthesis, an ancient and important process, allows energy to be harvested directly form the most abundant and renewable source, the sun. Photosynthesis is a light-driven process in which carbon dioxide is "fixed" to produce carbohydrates. This occurs in two phases: (1) The light reactions (requiring light) produce ATP and NADPH, and (2) the dark reactions (not requiring light) use ATP and NADPH to synthesize carbohydrates. This chapter describes how different pigments (e.g., chlorophylls in plants and bacteria) efficiently capture light energy and redistribute it to specific reaction centers. Purple photosynthetic bacteria contain one photosystem that recycles its electrons, whereas higher plants have two photosystems that use water as a source of electrons to reduce NADPH. The oxidation of water in higher plants generates O_2 as a by-product of photosynthesis. As in mitochondria, the topology of chloroplasts is central to the biochemistry of photosynthesis, starting with the light-driven reactions in the thylakoid membrane and finishing with the dark reactions in the stroma. The dark reactions occur via the Calvin cycle, a set of reactions that synthesize glyceraldehyde-3-phosphate from 3 CO_2. The chapter also discusses the control of the Calvin cycle along with a variant called the C_4 pathway.

Essential Concepts

1. Photosynthesis is divided into two processes:
   (a) In the light reactions, organisms capture light energy to synthesize ATP and generate reducing equivalents in the form of NADPH.
   (b) In the dark reactions, carbon dioxide is converted to carbohydrates using the ATP and NADPH generated in the light reactions. Although the dark reactions are not light-driven, they only occur when it is light and hence are better described as light-independent.

Chloroplasts

2. Plants differ from bacteria by providing a separate organelle for the photosynthetic machinery, the chloroplast. A chloroplast is enveloped by a highly permeable outer membrane and a nearly impermeable inner membrane. The inner membrane encloses the stroma, which contains the soluble enzymes of carbohydrate synthesis, and the thylakoid membrane, which is organized in stacks of pancake-like disks (grana) that enclose the thylakoid compartment and that are linked by unstacked stromal lamellae. The proteins that capture light energy and mediate electron-transport processes are embedded in the thylakoid membrane.

3. Various pigment molecules absorb light of different wavelengths. The principal photosynthetic pigment is chlorophyll, a cyclic tetrapyrrole that ligands a central Mg^{2+} ion. Photosynthetic organisms also contain other pigments, such as carotenoids, phycoerythrin,