Name_____

CHEM 3511

April 10, 2009

Exam 3

1. (12 points) Give the name of your favorite Tech professor and in one sentence describe why you like him/her.

2. (10 points) An enzyme cleaves a chemical bond of a substrate with a rate of 50 s^{-1}. The nonenzymatic rate for this reaction is 1.0×10^{-7} s^{-1}. By approximately how much does this enzyme decrease the activation free energy (i.e., what is $\Delta\Delta G^{\ddagger}$, the difference in activation energy, in kcal mol^{-1}) of this bond cleavage at 25°C? (R = 2 x 10^{-3} kcal K^{-1} mol^{-1})?

$\Delta\Delta G^{\ddagger} = -RT\ln(k_e/k_{ne})$ $= -(2 \times 10^{-3}\ \text{kcal K}^{-1}\ \text{mol}^{-1})(298\ \text{K})\ln(50/10^{-7})$
$= -(2 \times 10^{-3}\ \text{kcal K}^{-1}\ \text{mol}^{-1})(298\ \text{K})(20)$
$= 11.92\ \text{kcal mol}^{-1}$

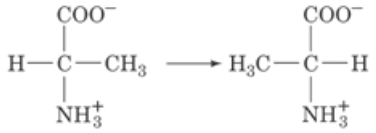
3. (10 points) Draw a section of an RNA backbone (the RNA bases can be represented as "Base"). In this drawing, also draw the key catalytic residues of RNase A in the initial state of the enzyme mechanism. Also indicate the electron flow, with arrows, to illustrate the start of the backbone cleavage mechanism.

4. (9 points) In the blank next to each enzyme listed below, write the number(s) for the mechanism(s) listed that are employed by the particular enzyme.
1. Acid–base catalysis
2. Covalent catalysis
3. Metal ion catalysis
4. Proximity and orientation effects
5. Preferential binding of the transition state complex

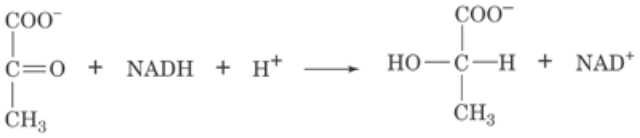
RNase A_1_(2 OK)__4_(5 OK)_ Lysozyme_1__2__(4 OK)_5_ Trypsin _1__2__(4 OK)_5_

All three enzymes should have "1" listed. With regards to "2", lysozme and trypsin for sure, but maybe not RNase A, as I am not sure that all covalent intermediates constitute covalent catalysis, but perhaps they do. For "3", none use a metal catalytically. For "4", all use proximal and orientational effects, as they place catalytic functional groups in the correct positions. For "5", it is very clear, as spelled out in the text, that lysozyme and trypsin bind preferentially to transition states (the carboxylic acid that stabilizes the oxonium ion in lysozyme and the oxyanion hole in trypsin), maybe not Rnase A. I am sure that it does, but will not not count off it a student lists it. This is a complicated question to grade, but it gets students thinking about all the ways that enzymes catalyze reactions.

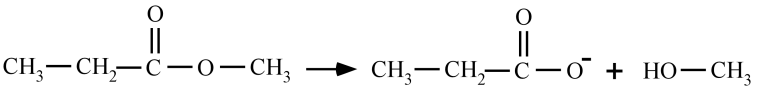
5. (6 points) Fill in the blanks with the type of enzyme that you would most expect to catalyze the reaction shown. Choose from the following: Oxidoreductase, Transferase, Hydrolase, Protease, Lyase, Isomerase, Ligase.



Isomerase



Oxidoreductase



Hydrolase or Protease

6. (10 points) For an enzyme obeying Michaelis-Menten kinetics, at what concentration of S (substrate, expressed as a multiples of K_M) will the initial velocity be equal to 75% of V_{max}? You must write the equation and show the basis of your calculation for credit.

$1/V_o = (K_m/V_{max}[S]) + 1/V_{max}$

$1/(0.75V_{max}) = (K_m/V_{max}[S]) + 1/V_{max}$

$1/0.75 = (Km/[S]) + 1$

$[S] = 3K_m$

7. (10 points) For an enzyme-catalyzed reaction obeying Michaelis-Menten kinetics, V_{max} = 5.0 x 10^{-9} M/s and K_m = 1.0 x 10^{-3} M. Calculate the rate of this enzymatic reaction when [S] = 2.0 x 10^{-3} M. You must show your work for credit.

$1/V_o = (K_m/V_{max}[S]) + 1/V_{max}$
$1/V_o = [(10^{-3})/(5.0 \times 10^{-9})(2.0 \times 10^{-3})] + (1/5.0 \times 10^{-9})$
$V_o = 3.3 \times 10^{-9}$ M/s

8. (8 points) In the Briggs-Haldane (steady state) derivation of the Michaelis-Menten equation that we discussed in class, write the mathematical expression that defines K_m.

$K_m = (k_{-1}+k_2)/k_1$

$K_m = K_s + k_2/k_1$

9. (5 points) K_M can be considered to be the same as the dissociation constant K_S for an enzymatic reaction if:

1) this statement cannot be completed because K_M can never approximate K_S.
2) ES → E + P is fast compared to ES → E + S.
3) the turnover number is very large.
4) $k_2 << k_{-1}$.
5) k_{cat}/K_M is near the diffusion-controlled limit.

10. (10 points) A competitive inhibitor for an enzyme is studied by the Lineweaver-Burke method. The slope of a plot of 1/v vs 1/[S] for the enzyme catalyzed reaction without inhibitor is 0.51. In the presence of 4.1 mM inhibitor, the slope is 0.67. Calculate the K_I value of the inhibitor.

$1/V_o = (K_m/V_{max}[S]) + 1/V_{max}$ $\quad K_m/V_{max} = 0.51$

$1/V_o = (\alpha K_m/V_{max}[S]) + 1/V_{max}$ $\quad \alpha K_m/V_{max} = 0.67$ $\quad \alpha = 0.67/0.51 = 1.31$

$\alpha = 1 + [I]/K_I$ $\quad 0.31 = 4.1 \times 10^{-3}/K_I$ $\quad K_I = 1.3 \times 10^{-2}$

11. (10 points) Benzamidine is a competitive inhibitor of trypsin and has a K_I value of 235 µM. Calculate the activity of trypsin (%) or fraction of active trypsin in a 35 nM solution of trypsin containing 71 µM benzamidine.

Method 1

From General Chemistry, make an ICE Chart

	E	I	EI
I	35×10^{-9}	71×10^{-6}	0
C	-x	-x	x
E	$35 \times 10^{-9} - x$	$71 \times 10^{-6} - x$	x

$K_I = [E][I]/[EI]$

$235 \times 10^{-6} = (35 \times 10^{-9} - x)(71 \times 10^{-6} - x)/x$

assume $x << 10^{-6}$

$235 \times 10^{-6} = (35 \times 10^{-9} - x)(71 \times 10^{-6})/x$

$x = (35 \times 10^{-9} - x)(71 \times 10^{-6})/235 \times 10^{-6}$

$x = 1.1 \times 10^{-8} - 0.30x$

$1.3x = 1.1 \times 10^{-8}$

$x = 8.5 \times 10^{-9}$

$[EI] / [E] + [EI] = 8.5 \times 10^{-9} / 35 \times 10^{-9}$

24% inactive

76% active

Method 2

$K_I = [E][I]/[EI]$

Since $[E] << [I]$ then [I] doesn't change

$235 = [E]71/[EI]$

$3.3 = [E]/[EI]$

$[E] + [EI] = 1$ in fractions or $[E] + [EI] = 100\%$ in percent

combining

$3.3 [EI] + [EI] = 1$

$4.3 [EI] = 1$

$[EI] = 0.23$

$[E] = 0.77$

Check: substituting values in $K_I = [E][I]/[EI]$

$K_I = .77 \times 71/.23$

$K_I = 238$ millimolar.

12. (10 points) Draw the complete structure for reasonable competitive inhibitors of each of the following two enzymes.

a) succinate dehydrogenase
(must have > 4 carbons)
Any reasonable molecule
with a $-COO^-$ group

b) a new serine protease
which hydrolyzes after
Glu or Asp residues in
Proteins
Any reasonable molecule with a $-COO^-$ group