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1 Jardetzky, O., Thielmann, H., Arata, Y., Markley, J. L., and Williams, M. N., *Cold Spring Harb. Symp. quant. Biol.*, **36**, 257-261 (1971).
2 Anfinsen C. B., Haber, E., Sela, M., and White, jun., F. H., *Proc. natn. Acad. Sci., U.S.A.*, **47**, 1309-1314 (1961).
3 Anfinsen, C. B., *Biochem. J.*, **128**, 737-749 (1972).
4 Ikai, A., Fish, W. W., and Tanford, C., *J. molec. Biol.*, **73**, 165-184 (1973).
5 Guzzo, A. V., *Biophys. J.*, **5**, 809-822 (1965).
6 Prothero, J. W., *Biophys. J.*, **6**, 367-370 (1966).
7 Schiffer, M., and Edmundson, A. B., *Biophys. J.*, **8**, 29-39 (1968).
8 Kotelchuck, D., and Scheraga, H. A., *Proc. natn. Acad. Sci., U.S.A.*, **62**, 14-21 (1969).
9 Lewis, P. N., Go, N., Go, M., Kotelchuck, D., and Scheraga, H. A., *Proc. natn. Acad. Sci., U.S.A.*, **65**, 810-815 (1970).
10 Ptitsyn, O. B., and Finkelstein, A. V., *Dokl. Akad. Nauk SSSR*, **195**, 221-224 (1970).
11 Ptitsyn, O. B., and Finkelstein, A. V., *Biofizika* (*USSR*), **15**, 757-768 (1970).
12 Leberman, R., *J. molec. Biol.*, **55**, 23-30 (1971).
13 Lewis, P. N., Momany, F. A., and Scheraga, H. A., *Proc. natn. Acad. Sci., U.S.A.*, **68**, 2293-2297 (1971).
14 Robson, B., and Pain, R. H., *J. molec. Biol.*, **58**, 237-259 (1971).
15 Wu, T. T., and Kabat, E. A., *Proc, natn. Acad. Sci., U.S.A.*, **68**, 1501-1506 (1971).
16 Finkelstein, A. V., and Ptitsyn, O. B., *J. molec. Biol.*, **62**, 613-624 (1971).
17 Nagano, K., *J. molec. Biol.*, **75**, 401-421 (1973).
18 Kabat, E. A., and Wu, T. T., *Proc. natn. Acad. Sci., U.S.A.*, **70**, 1473-1477 (1973).
19 Burgess, A. W., Ponnuswamy, P. K., and Scheraga, H. A., *Israel J. Chem.* (in the press).
20 Nagano, K., *J. molec. Biol.* (in the press).
21 Chou, P. Y., and Fasman, G. D., *Biochemistry*, **13**, 211-222 (1974).
22 Chou, P. Y., and Fasman, G. D., *Biochemistry*, **13**, 222-245 (1974).
23 Lim, V. I., *J. molec. Biol.*, (in the press).
24 Noda, L., *The Enzymes* (edit. by Boyer, P. D.), **8**, 279-305 (Academic Press, New York, 1973).
25 Heil, A., Müller, G., Noda, L. H., Pinder, T., Schirmer, I., Schirmer, R. H., and v. Zabern, I., *Eur. J. Biochem*, **43**, 131 (1974).
26 Schulz, G. E., Elzinga, M., Marx, F., and Schirmer, R. H., *Nature*, **250**, 120-123 (1974).
27 Schulz, G. E., and Schirmer, R. H., *Nature*, **250**, 142-144, (1974).
28 Lewis, P. N., and Scheraga, H. A, *Archs. Biochem. Biophys.*, **144**, 576-583 (1971).
29 Ptitsyn, O. B., Lim, V. I., and Finkelstein, A. V., *Proc. 8th FEBS Meeting*, Vol. 25 (edit. by Hess, B.), 421-429, (North-Holland, Amsterdam, 1972).
30 Wetlaufer, D. B., *Proc. natn. Acad. Sci., U.S.A.*, **70**, 697-701 (1973).
31 Ptitsyn, O. B., *Biophys. Chem.* (in the press).
32 Krigbaum, W. R., and Knutton, S. P., *Proc. natn. Acad. Sci., U.S.A.*, **70**, 2809-2813 (1973).

Topological comparison of adenyl kinase with other proteins

A COMBINATION of amino acid sequence analysis[1] and X-ray analysis has yielded the atomic structure of the enzyme adenyl kinase[2] (adenylate kinase, EC 2.7.4.3). No protein of which the structure is known is so closely related to adenyl kinase that an amino acid sequence comparison[3] or an exact geometrical comparison of the structures[4-6] can be expected to indicate a relationship. Similarities with other proteins become apparent, however, if one restricts the comparison to topologies, that is, to chain folds without reference to the exact geometry. Since chain folds are particularly well conserved during evolution[7] such a procedure might reveal distant relationships.

The most important structural feature of adenyl kinase is its central five-stranded parallel pleated sheet[2]; therefore, we shall only discuss proteins containing a similar motif. Central parallel sheets with more than three strands are present in subtilisin[8-10], flavodoxin[11,12], and various dehydrogenases[13-16]. The sheets of these proteins are sketched in Fig. 1 according to a proposal of Rossmann (personal communication). The drawings contain no geometrical detail. Only the sheet strands and the approximate path of the connections between these strands (through the upper and lower side of the sheet), that is, only the sheet topology, is given. In such a presentation the topology is fully described by the strand sequence in the sheet and the above-below pattern of the connections. Since there are finite, calculable numbers of possible strand sequences and possible connection patterns, the number of possible topologies (M) is also finite and calculable.

This number can be used to define a quantitative measure of relationship between two structures. Assuming, for instance, each of two proteins contains an n-stranded parallel sheet with identical topology; since such a sheet can exist in M different topologies, the probability that by chance both sheet topologies are identical is $1/M$. Or, in other words, the significance of their relationship, which one might call 'figure of topological relatedness', is M:1. We shall now derive such figures within the group of protein structures shown in Fig. 1.

First we have to evaluate the number of possible strand sequences in an n-stranded parallel sheet. This is equal to $n!/2$, the number of permutations of n items reduced by a factor of 2. The halving is necessary because symmetrically related strand sequences as $ABCD$ and $DCBA$ can be superimposed by rotation. Second, we take into account that the $n-1$ connections between the strands run either through the upper or the lower side of the sheet, giving rise to 2^{n-1} possible above-below connection patterns. Combining strand sequences and connection patterns we find that there are $M=2^{n-1}\times n!/2=2^{n-2}\times n!$ possible topologies. For simplicity this counting scheme disregards further topological differences which arise whenever connections cross each other as, for example, connections A-B and B-C in subtilisin.

One can use this formula for a comparison within the dehydrogenase family, for example, for a comparison between

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Table 1 'Figures of topological relatedness' between protein structures

Compared sheet topologies		No. of strands in common for both sheets	No. of connections in common for both sheets	Uncorrected figure of topological relatedness	Reduction factor	Corrected figure of topological relatedness	Figure of topological relatedness including binding region
Adenyl kinase	Subtilisin	5	3*	480 : 1	1×2	240 : 1	2400 : 1
Adenyl kinase	Flavodoxin	4	3	96 : 1	2×2	24 : 1	240 : 1
Adenyl kinase	Any dehydrogenase	4	3	96 : 1	2×3	16 : 1	160 : 1
Subtilisin	Flavodoxin	5	4	960 : 1	2×1	480 : 1	4800 : 1
Subtilisin	Any dehydrogenase	5	4	960 : 1	2×2	240 : 1	2400 : 1
Flavodoxin	Any dehydrogenase	5	4	960 : 1	1×2	480 : 1	4800 : 1
A dehydrogenase	Another dehydrogenase	6	5	11520 : 1	1×1	11520 : 1	115200 : 1

* Adenyl kinase and subtilisin have five strands but only three (and not four) connections in common because connections *B–C* are different. No reduction factor for this disagreement has been applied, however, since connection *B–C* has only slipped around a sheet edge.

lactate dehydrogenase[13] and liver alcohol dehydrogenase[15]. Both structures contain six-stranded parallel sheets with identical topology (Fig. 1), although there exist $M=2^{6-2}\times 6!=$ 11,520 possible topologies for such a sheet. Therefore, their "figure of topological relatedness" is 11,520 : 1.

The reasoning can be extended to topologies that are identical in the central strands of the sheet but that do show differences at the sheet edges. The procedure is best described by comparing adenyl kinase with flavodoxin. Both structures have a four-stranded sheet (*ACDE*) and three connections (*A–B*=*A–C*, *C–D*, *D–E*) in common. This yields an "uncorrected figure of topological relatedness" of $2^{4-2}\times 4!:1$ or 96:1. For the comparison, however, one strand in each protein has been discarded. When selecting these strands an arbitrary choice of one out of two possibilities in each protein (left or right edge) has been made. Since such a choice reduces the significance, a reduction factor of $2\times 2=4$ was applied to the "uncorrected figure of topological relatedness", yielding a "corrected figure of topological relatedness" of 24:1 (see Table 1). An analogous procedure has been followed when comparing other pairs of structures (Table 1).

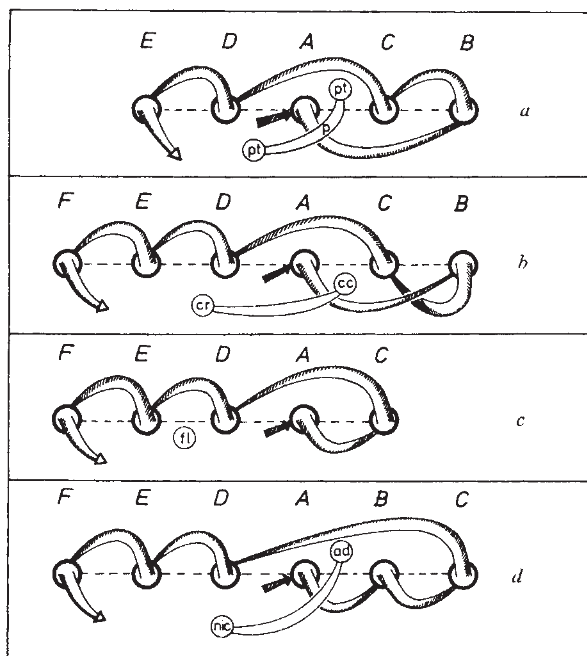

Fig. 1 Topologies of proteins containing a parallel sheet. *a*, Adenyl kinase[2]; *b*, subtilisin[8–10]; *c*, flavodoxin[11,12]; *d*, NAD-binding globules (domains) of lactate dehydrogenase[13], s-malate dehydrogenase[14], liver alcohol dehydrogenase[15], and D-glyceraldehyde-3-phosphate dehydrogenase[16]; pt, hydrophobic pockets, that is, presumed binding sites for adenine[2]; p, phosphate position in the catalytic centre of adenylate kinase; cr, hydrophobic crevice, that is, presumed binding site for aromatic or apolar side chain[10]; cc, catalytic centre; fl, FMN; nic-ad, nicotinamide and adenine moieties of the dinucleotide NAD. In the case of adenyl kinase the five heavy circles are the five strands in the sheet. (The drawing corresponds to the linear diagram given in Fig. 3 of the adenyl kinase structure description[2], if one views this figure from the top along the strands in the sheet, that is, along the plane of the paper, to the bottom.) The carbonyl ends of these strands point toward the viewer. The arrows indicate the direction of the polypeptide chain[3], that is strands *ABCDE* in the sheet are ordered alphabetically along the course of the chain. The sheet topologies of the other proteins have been drawn correspondingly. The topologies of the NAD-binding globules of the four dehydrogenases are identical[16]. The same topology might be present in horse muscle phosphoglycerate kinase[17]. The location of substrates (pt, cr), cosubstrates (fl, nic-ad), and catalytic centres (cc, p) are all in front of the carbonyl ends of the central strands in the sheet, that is above the plane of the paper.

The structural relationship within the dehydrogenase family is not only indicated by correspondence of sheet topologies but also by correspondence of nucleotide binding positions[16]. As shown in Fig. 1, the other proteins contain similar sites at similar positions relative to the sheet. Therefore, we shall include this kind of information about relationship into the proposed quantitative measure.

For sake of simplicity we do not distinguish between different kinds of sites but merely refer to them as being a "binding region". In the view given in Fig. 1, for all proteins this binding region is located in front of the carbonyl ends of the central sheet strands, that is, above the plane of the paper. In order to use the same reasoning as before, we estimate how many positions of such a binding region we would have accepted as being different. For instance, we would have been able to distinguish between left side, centre, and right side of the sheet as well as between above, in front, in the rear, and below the sheet, that is, we would have differentiated between about 10 positions.

Since all proteins show the same binding region position out of these roughly estimated 10 possibilities, there is an additional significance of their relationship of 10:1. Thus, the 'corrected figure of topological relatedness' (Table 1) may be multiplied by a factor of 10, yielding the overall 'figure of topological relatedness including binding region' between the structures (Table 1). If a closer correspondence of the binding regions could be established, there would be more than 10 distinguishable positions and therefore a larger additional factor.

The results in Table 1 show that adenyl kinase is rather closely related to subtilisin and less closely to flavodoxin and to the dehydrogenase family. This corresponds to the strand sequence reversal *BC* compared with *CB* between adenyl kinase and subtilisin on the one hand and the dehydrogenases on the other. A relationship between the dehydrogenases, flavodoxin and subtilisin is clearly indicated.

These relationships point to either divergent or convergent evolution. Convergent evolution would be expected if the resulting topology is energetically favoured or if the topology is favoured in the dynamical folding process[18] of the polypeptide chain. But this does not seem very likely. In the case of divergent evolution, however, we have to postulate a common ancestor for enzymes as distant from each other as the

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bacterial extracellular protease subtilisin and the mammalian intracellular adenyl kinase.

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1 Heil, A., Müller, G., Noda, L. H., Pinder, T., Schirmer, I., Schirmer, R. H., and v. Zabern, I., *Eur. J. Biochem.*, **43**, 131-144 (1974).
2 Schulz, G. E., Elzinga, M., Marx, F., and Schirmer, R. H., *Nature*, **250**, 120-123 (1974).
3 Dayhoff, M. O., *Atlas of Protein Sequence and Structure*, **5**, (National Biochemical Research Foundation, Washington DC, 1972).
4 McLachlan, A. D., *Nature New Biol.*, **240**, 83-85 (1972).
5 Rao, S. T., and Rossmann, M. G., *J. molec. Biol.*, **76**, 241-256 (1973).
6 Rossmann, M. G., and Liljas, A., *J. molec. Biol.* **85**, 177-181 (1974).
7 Timkovich, R., and Dickerson, R. E., *J. molec. Biol.*, **79**, 39-56 (1973).
8 Schubert Wright, C., Alden, R. A., Kraut, J., *Nature*, **221**, 235-242 (1969).
9 Drenth, J., Hol, W. G. J., Jansonius, J. N., and Koekoek, R., *Cold Spring Harbor Symp. quant. Biol.*, **36**, 107-116 (1971).
10 Kraut, J., Robertus, J. D., Birktoft, J. J., Alden, R. A., Wilcox, P. E., and Powers, J. C., *Cold Spring Harbor Symp. quant. Biol.*, **36**, 117-123 (1971).
11 Watenpaugh, K. D., Sieker, L. C., Jensen, L. H., Legall, J., and Dubourdieu, M., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 3185-3188 (1972).
12 Andersen, R. D., Apgar, P. A., Burnett, R. M., Darling, G. D., Lequesne, M. E., Mayhew, S. G., and Ludwig, M. L., *Proc. natn. Acad. Sci. U.S.A.*, **69**, 3189-3191 (1972).
13 Rossmann, M. G., Adams, M. J., Buehner, M., Ford, G. C., Hackert, M. L., Lentz, jun., P. J., McPherson, jun., A., Schevitz, R. W., and Smiley, I. E., *Cold Spring Harbor Symp. quant. Biol.*, **36**, 179-191 (1971).
14 Hill, E., Tsernoglou, D., Webb, L., and Banaszak, L. J., *J. molec. Biol.*, **72**, 577-591 (1972).
15 Brändén, C. I., Eklund, H., Nordström, B., Boiwe, T., Söderlund, G., Zeppezauer, E., Ohlsson, I., and Åkeson, Å., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 2439-2442 (1973).
16 Buehner, M., Ford, G. C., Moras, D., Olsen, K. W., and Rossmann, M. G., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 3052-3054 (1973).
17 Blake, C. C. F., and Evans, P. R., *J. molec. Biol.* **84**, 585-601 (1974).
18 Wetlaufer, D. B., *Proc. natn. Acad. Sci. U.S.A.*, **70**, 697-701 (1973).

Stimulatory capacity of human T and B lymphocytes in the mixed leukocyte culture

LYMPHOCYTES can be separated into thymus-dependent (T) and thymus-independent (B) cells on the basis of ontogeny, surface membrane differentiation markers, and function. Thus, some functions can be attributed solely to T or B cells, whereas others require T/B cell cooperation[1,2]. When two populations of allogeneic lymphoid cells are cultured together in the mixed leukocyte culture assay (MLC), cellular interaction leads to blastic transformation and subsequent proliferation of a portion of the cultured cells. This proliferation response originates in T lymphocytes[3-5,30,31]. But secondary T cell-mediated B cell proliferation may in some species contribute to the overall proliferative response of T and B cells[6-9]. The question then arises: which cell type acts as stimulator in MLC? Is the stimulus to proliferate equally transferred by T and B cells, or are the antigenic determinants responsible for MLC activation represented exclusively or preferentially on the surface of only T or B lymphocytes? Studies in mice[9-11] indicated that T and B cells possess equal stimulatory capacities. However, in other reports the stimulatory capacity in mouse MLC was almost exclusively attributed to B lymphocytes (refs 3 and 12, and personal communication from D. H. Sachs).

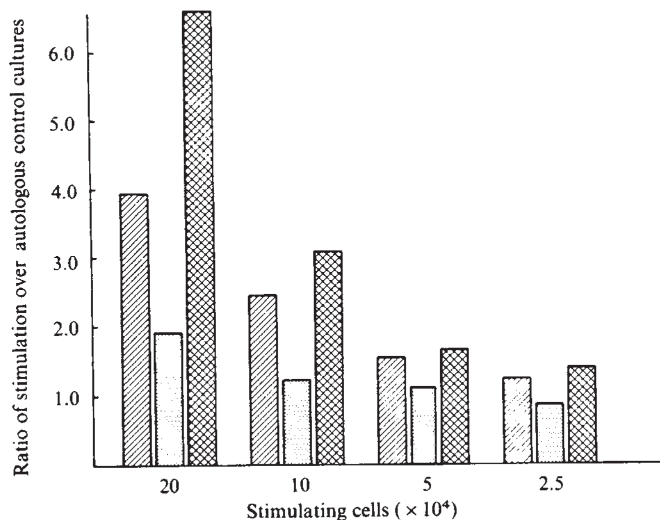

Fig. 1 Stimulatory capacity of unseparated T and B peripheral blood lymphocytes in human MLC. Lymphocytes (2×10^5) were stimulated with decreasing numbers of irradiated allogeneic cells. Ordinate: ratio of stimulation (^{3}H-TdR uptake in allogeneic cultures over ^{3}H-TdR uptake in corresponding autologous cultures), as calculated from the means of triplicate cultures. Abscissa; number of stimulating cells. Left bar, Unseparated lymphocytes; Centre bar, T lymphocytes; Right bar, B lymphocytes.

We have attempted to answer this same question for human peripheral blood lymphocytes and report here that the proliferative response in human MLC is largely the result of stimulation by B cell-bound alloantigens.

Cells responding in MLC were prepared from heparinised venous blood by centrifugation over a Ficoll-Hypaque gradient[13]. This preparation regularly contained more than 90% lymphocytes, the rest being monocytes and an occasional granulocyte.

Rosette formation of human T lymphocytes with unsensitised sheep erythrocytes (SRBC)[14] was utilised to separate peripheral blood lymphocytes, used as stimulating cells in MLC, into rosetting (T) and nonrosetting (B) cells. To remove monocytes, which stimulate, although weakly, in MLC[15], heparinised whole blood was incubated with sterile carbonyl iron powder for 60–90 min at 37° C with continuous agitation. Subsequent centrifugation over a Ficoll-Hypaque gradient yielded lymphocyte preparations containing less than 0.1% monocytes. Using a modification of the method of Wybran *et al.*[16], these lymphocytes were rosetted with neuraminidase-pretreated SRBC (N-SRBC) which bind more firmly to human T lymphocytes than do untreated SRBC[17,18]. Samples of 0.25 ml lymphocytes in Hanks balanced salt solution (HBSS) (10^7 ml^{-1}), 0.25 ml of foetal calf serum (absorbed with SRBC; Microbiological Associates) and 0.5 ml of N-SRBC (10^8 ml^{-1}) were added to plastic test tubes, centrifuged for 8 min at 200g, and further incubated at room temperature for 60 min. The gently resuspended pellets of all tubes (containing rosetted and nonrosetted lymphocytes) were pooled, and centrifuged over a Ficoll-Hypaque gradient (30 min at 400g); rosetting cells formed the pellet and nonrosetting cells remained at the interface between the layers. The pellet lymphocytes (termed T cells here) were washed twice in HBSS, and resuspended in culture medium (see below) to the concentration needed. No efforts were made to lyse or remove the N-SRBC so as to manipulate the lymphocytes as little as possible: orienting experiments showed that the capacity of lymphocytes to stimulate or respond in MLC was unaltered by addition of similar numbers of N-SRBC. The interface cells were washed, rerosetted and again centrifuged over a Ficoll-Hypaque layer, to remove residual rosetting cells. The inter-

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