

On the Nature of Allosteric Transitions: A Plausible Model

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“It is certain that all bodies whatsoever, though they have no sense, yet they have perception; for when one body is applied to another, there is a kind of election to embrace that which is agreeable, and to exclude or expel that which is ingrate; and whether the body be alterant or altered, evermore a perception precedeth operation; for else all bodies would be like one to another.”

*Francis Bacon
(about 1620)*

1. Introduction

Ever since the haem-haem interactions of haemoglobin were first observed (Bohr, 1903), this remarkable phenomenon has excited much interest, both because of its physiological significance and because of the challenge which its physical interpretation offered (cf. Wyman, 1948, 1963). The elucidation of the structure of haemoglobin (Perutz *et al.*, 1960) has, if anything, made this problem more challenging, since it has revealed that the haems lie far apart from one another in the molecule.

Until fairly recently, haemoglobin appeared as an almost unique example of a protein endowed with the property of mediating such indirect interactions between distinct, specific, binding-sites. Following the pioneer work of Cori and his school on muscle phosphorylase (see Helmreich & Cori, 1964), it has become clear, especially during the past few years, that, in bacteria as well as in higher organisms, many enzymes are electively endowed with specific functions of metabolic regulation. A systematic, comparative, analysis of the properties of these proteins has led to the conclusion that in most, if not all, of them, *indirect* interactions between *distinct* specific binding-sites (allosteric effects) are responsible for the performance of their regulatory function (Monod, Changeux & Jacob, 1963).

By their very nature, allosteric effects cannot be interpreted in terms of the classical theories of enzyme action. It must be assumed that these interactions are mediated by some kind of molecular transition (allosteric transition) which is induced or stabilized in the protein when it binds an “allosteric ligand”. In the present paper, we wish to submit and discuss a general interpretation of allosteric effects in terms of certain features of protein structure. Such an attempt is justified, we believe, by the fact that, even though they perform widely different functions, the dozen or so allosteric systems which have been studied in some detail do appear to possess in common certain remarkable properties.

Before summarizing these properties, it will be useful to define two classes of allosteric effects (cf. Wyman, 1963):

- (a) “*homotropic*” effects, i.e. interactions between *identical* ligands;
- (b) “*heterotropic*” effects, i.e. interactions between *different* ligands.

The general properties of allosteric systems may then be stated as follows:

- (1) Most allosteric proteins are polymers, or rather oligomers, involving several identical units.
- (2) Allosteric interactions frequently appear to be correlated with alterations of the *quaternary* structure of the proteins (i.e. alterations of the bonding between subunits).
- (3) While heterotropic effects may be either positive or negative (i.e. co-operative or antagonistic), homotropic effects appear to be always co-operative.
- (4) Few, if any, allosteric systems exhibiting *only* heterotropic effects are known. In other words, co-operative homotropic effects are almost invariably observed with at least one of the two (or more) ligands of the system.
- (5) Conditions, or treatments, or mutations, which alter the heterotropic interactions also simultaneously alter the homotropic interactions.

By far the most striking and, physically if not physiologically, the most interesting property of allosteric proteins is their capacity to mediate homotropic co-operative interactions between stereospecific ligands. Although there may be some exceptions to this rule, we shall consider that this property characterizes allosteric proteins. Furthermore, given the close correlations between homotropic and heterotropic effects, we shall assume that the same, or closely similar, molecular transitions are involved in both classes of interactions. The model which we will discuss is based upon considerations of molecular symmetry and offers primarily an interpretation of co-operative homotropic effects. To the extent that the assumptions made above are adequate, the model should also account for heterotropic interactions and for the observed correlations between the two classes of effects.

We shall first describe the model and derive its properties, which will then be compared with the properties of real systems. In conclusion, we shall discuss at some length the plausibility and implications of the model with respect to the quaternary structures of proteins.

2. The Model

Before describing the model, since we shall have to discuss the relationships between subunits in polymeric proteins, we first define the terminology to be used as follows:

- (a) A polymeric protein containing a *finite*, relatively small, number of *identical* subunits, is said to be an *oligomer*.
- (b) The *identical* subunits associated within an oligomeric protein are designated as *protomers*.
- (c) The term *monomer* describes the fully dissociated protomer, or of course any protein which is not made up of *identical* subunits.
- (d) The term “subunit” is purposely undefined, and may be used to refer to any chemically or physically identifiable sub-molecular entity within a protein, whether identical to, or different from, other components.

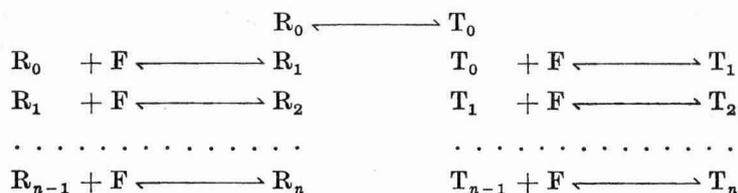
Attention must be directed to the fact that these definitions are based exclusively upon considerations of identity of subunits and do not refer to the number of different peptide chains which may be present in the protein. For example, a protein made up of two different peptide chains, each represented only once in the molecule, is a monomer according to the definition. If such a protein were to associate into a molecule which would then contain two chains of each type, the resulting protein would be a dimer (i.e., the lowest class of oligomer) containing two protomers, each protomer in turn being composed of two different peptide chains. Only in the case where an oligomeric protein contains a single type of peptide chain would the definition of a protomer coincide with the chemically definable subunit. An oligomer the protomers of which all occupy exactly equivalent positions in the molecule may be considered as a "closed crystal" involving a fixed number of asymmetric units each containing one protomer.

The model is described by the following statements:

- (1) Allosteric proteins are oligomers the protomers of which are associated in such a way that they all occupy equivalent positions. This implies that the molecule possesses at least one axis of symmetry.
- (2) To each ligand able to form a *stereospecific* complex with the protein there corresponds one, and only one, site on each protomer. In other words, the symmetry of each set of stereospecific receptors is the same as the symmetry of the molecule.
- (3) The conformation of each protomer is constrained by its association with the other protomers.
- (4) Two (at least two) states are reversibly accessible to allosteric oligomers. These states differ by the distribution and/or energy of inter-protomer bonds, and therefore also by the conformational constraints imposed upon the protomers.
- (5) As a result, the affinity of one (or several) of the stereospecific sites towards the corresponding ligand is altered when a transition occurs from one to the other state.
- (6) When the protein goes from one state to another state, its molecular symmetry (including the symmetry of the conformational constraints imposed upon each protomer) is conserved.

Let us first analyse the interactions of such a model protein with a single ligand (F) endowed with differential affinity towards the two accessible states. In the absence of ligand, the two states, symbolized as R_0 and T_0 , are assumed to be in equilibrium. Let L be the equilibrium constant for the $R_0 \leftrightarrow T_0$ transition. In order to distinguish this constant from the dissociation constants of the ligand, we shall call it the "allosteric constant". Let K_R and K_T be the microscopic dissociation constants of a ligand F bound to a stereospecific site, in the R and T states, respectively. *Note that by reason of symmetry and because the binding of any one ligand molecule is assumed to be intrinsically independent of the binding of any other, these microscopic dissociation constants are the same for all homologous sites in each of the two states.* Assuming n protomers (and therefore n homologous sites) and using the notation $R_0, R_1, R_2, \dots, R_n; T_0, T_1, T_2,$

... T_n , to designate the complexes involving 0, 1, 2, ... n molecules of ligand, we may write the successive equilibria as follows:



Taking into account the probability factors for the dissociations of the R_1, R_2, \dots, R_n and T_1, T_2, \dots, T_n complexes, we may write the following equilibrium equations:

$$T_0 = LR_0$$

$$\begin{array}{ll}
 R_1 = R_0 n \frac{F}{K_R} & T_1 = T_0 n \frac{F}{K_T} \\
 R_2 = R_1 \frac{n-1}{2} \frac{F}{K_R} & T_2 = T_1 \frac{n-1}{2} \frac{F}{K_T} \\
 \dots & \dots \\
 R_n = R_{n-1} \frac{1}{n} \frac{F}{K_R} & T_n = T_{n-1} \frac{1}{n} \frac{F}{K_T}
 \end{array}$$

Let us now define two functions corresponding respectively to:

(a) the fraction of protein in the R state:

$$\bar{R} = \frac{R_0 + R_1 + R_2 + \dots + R_n}{(R_0 + R_1 + R_2 + \dots + R_n) + (T_0 + T_1 + T_2 + \dots + T_n)}$$

(b) the fraction of sites actually bound by the ligand:

$$\bar{Y}_F = \frac{(R_1 + 2R_2 + \dots + nR_n) + (T_1 + 2T_2 + \dots + nT_n)}{n[(R_0 + R_1 + R_2 + \dots + R_n) + (T_0 + T_1 + T_2 + \dots + T_n)]}$$

Using the equilibrium equations, and setting

$$\frac{F}{K_R} = \alpha \quad \text{and} \quad \frac{K_R}{K_T} = c$$

we have, for the "function of state" \bar{R} :

$$\bar{R} = \frac{(1 + \alpha)^n}{L(1 + c\alpha)^n + (1 + \alpha)^n} \quad (1)$$

and for the "saturation function" \bar{Y}_F :

$$\bar{Y}_F = \frac{Lc\alpha(1 + c\alpha)^{n-1} + \alpha(1 + \alpha)^{n-1}}{L(1 + c\alpha)^n + (1 + \alpha)^n} \quad (2)$$

In Fig. 1(a) and (b), theoretical curves of the \bar{Y}_F function have been drawn, corresponding to various values of the constants L and c . In such graphs the co-operative homotropic effect of the ligand, predicted by the symmetry properties of the model, is expressed by the curvature of the lower part of the curves. The graphs illustrate the fact that the "co-operativity" of the ligand depends upon the values of L and c . The co-operativity is more marked when the allosteric constant L is large (i.e. when the

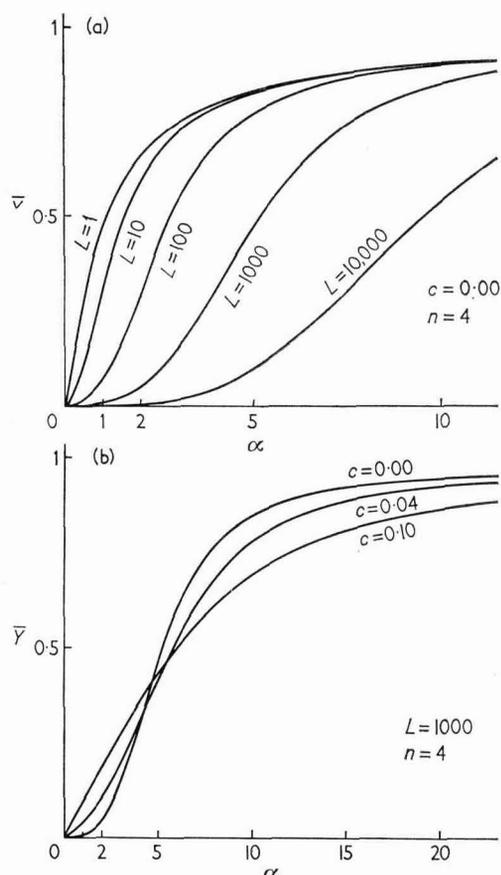


FIG. 1(a) and (b). Theoretical curves of the saturation function \bar{Y} (equation (2)) drawn to various values of the constants L and c , with $n = 4$ (i.e. for a tetramer).

$R_0 \leftrightarrow T_0$ equilibrium is strongly in favour of T_0) and when the ratio of the microscopic dissociation constants ($c = K_R/K_T$) is small.†

It should be noted that for $c = 1$ (i.e. when the affinity of both states towards the ligand is the same) and also when L is negligibly small, the \bar{Y}_F function simplifies to:

$$\bar{Y}_F = \frac{\alpha}{1 + \alpha} = \frac{F}{K_R + F}$$

that is, to the Michaelis-Henri equation.

The model therefore accounts for the homotropic co-operative effects which, as we pointed out, are almost invariably found with allosteric proteins. Let us now analyse the properties of the model with respect to heterotropic interactions between different allosteric ligands. For this purpose, consider a system involving three stereospecific ligands, each binding at a different site. Assume that one of these ligands is the substrate (S) and, for simplicity, that it has significant affinity only for the sites in one of the two states (for example R). Assume similarly that, of the two other ligands,

† When c is very small, equation (2) simplifies to:

$$\bar{Y}_F = \frac{\alpha(1 + \alpha)^{n-1}}{L + (1 + \alpha)^n}$$

one (the inhibitor I) has affinity exclusively for the T state, and the other (the activator A) for the R state. Let \bar{Y}_S be the fractional saturation of the enzyme with S.

According to the model, heterotropic effects would be due exclusively to displacements of the spontaneous equilibrium between the R and T states of the protein. The saturation function for substrate in the presence of activator and inhibitor may then be written as:

$$\bar{Y}_S = \frac{\alpha(1 + \alpha)^{n-1}}{L' + (1 + \alpha)^n} \quad (3)$$

where α is defined as above and L' is an "apparent allosteric constant", defined as:

$$L' = \frac{\sum_0^n T_I}{\sum_0^n R_A}$$

where $\sum_0^n T_I$ and $\sum_0^n R_A$ stand respectively for the sum of the different complexes of the T state with I and of the R state with A. Following the same derivation as above, it will be seen that:

$$L' = L \frac{(1 + \beta)^n}{(1 + \gamma)^n}$$

with $\beta = \frac{I}{K_I}$ and $\gamma = \frac{A}{K_A}$, where K_I and K_A stand for the microscopic dissociation

constants of activator and inhibitor with the R and T states respectively. Substituting this value of L' in equation (3) we have:

$$\bar{Y}_S = \frac{\alpha(1 + \alpha)^{n-1}}{L \frac{(1 + \beta)^n}{(1 + \gamma)^n} + (1 + \alpha)^n}. \quad (4)$$

This equation[†] expresses the second fundamental property of the model, namely, that the (heterotropic) effect of an allosteric ligand upon the saturation function for another allosteric ligand should be to modify the homotropic interactions of the latter. When the substrate itself is an allosteric ligand (as assumed in the derivation of equation (4)), the presence of the effectors should therefore result in a change of the *shape* of the substrate saturation curve. As is illustrated in Fig. 2, the inhibitor increases the co-operativity of the substrate saturation curve (and also, of course, displaces the half-saturation point), while the activator tends to abolish the co-operativity of substrate (also displacing the half-saturation point). Both the activator and the inhibitor, as well as the substrate, exhibit co-operative homotropic effects.

The model therefore accounts for both homotropic and heterotropic interactions and for their interdependence. Its main interest is to predict these interactions solely on the basis of symmetry considerations. No particular assumption has been, or need be, made about the structure of the specific sites or about the structure of the protein, except that it is a symmetrically bonded oligomer, the symmetry of which is *conserved* when it undergoes a transition from one to another state. It is therefore a fairly stringent, even if abstract model, since co-operative interactions are not only allowed but even required for any ligand endowed with differential affinity towards the two states of the protein, and heterotropic interactions are predicted to occur between any ligands showing homotropic interactions.

[†] A much more complicated, albeit more realistic, equation would apply if the ligands were assumed to have significant affinity for *both* of the two states.

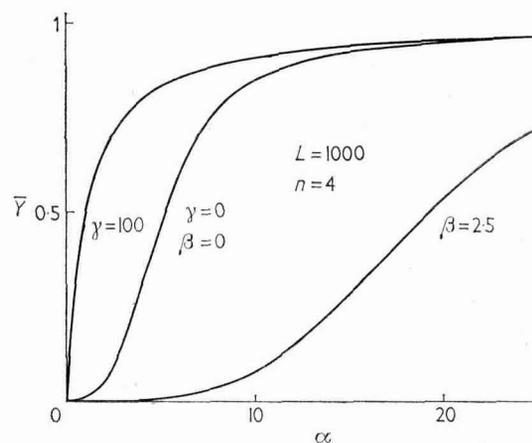


FIG. 2. Theoretical curves showing the heterotropic effects of an allosteric activator (γ) or inhibitor (β) upon the shape of the saturation function for substrate (α) according to equation (3).

3. Application to the Description of Real Systems

(a) *The kinetics of allosteric systems*

In Fig. 3, results for the fractional saturation of haemoglobin by oxygen at different partial pressures (Lyster, unpublished work) have been fitted to equation (2). While the fit is satisfactory, we feel that strict quantitative agreement is neither sufficient

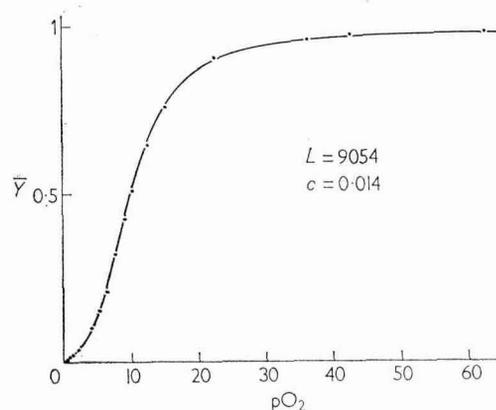


FIG. 3. Saturation of haemoglobin with oxygen. Results (points) obtained by R. W. J. Lyster (unpublished work) with horse haemoglobin (4.6%) in 0.6 M-phosphate buffer (pH 7) at 19°C. Solid line drawn to equation (2) using the values of the constants L and c given on the graph.

nor necessary as a test of the basic assumptions of the model. It must be borne in mind that in almost all enzyme systems, the saturation functions with respect to substrate or effectors cannot be determined directly, and are only inferred from kinetic measurements. (This of course does not apply to the case of haemoglobin just cited.) Very often it is difficult to judge to what extent the inference is correct, and the interpretation of kinetic results in terms of saturation functions sometimes depends upon assumptions about the mechanism of the reaction itself. It is to be expected, then, that most real systems will exhibit appreciable deviations from the theoretical functions, as indeed is very often the case for the much simpler Michaelis-Henri saturation law.

We shall therefore discuss only the most characteristic qualitative predictions of the model in its application to real systems.

In any enzyme system, activating or inhibitory effects are measured in terms of variations of the two classical kinetic constants (K_M and V_M), as a function of the concentrations of substrate (S) and effector(s) (F). Two classes of effects may then be expected in allosteric systems.

(a) "K systems." Both F and S have differential affinities towards the T and R states (i.e. both F and S are *allosteric* ligands). Then evidently the presence of F will modify the apparent affinity of the protein for S, and conversely.

(b) "V systems." S has the same affinity for the two states. Then there is no effect of F on the binding of S, nor of S on the binding of F. F can exert an effect on the reaction only if the two states of the protein differ in their *catalytic* activity. Depending on whether F has maximum affinity for the active or for the inactive state, it will behave as an activator (positive V system) or as an inhibitor (negative V system).

It should be noted that this classification of allosteric systems is compatible with other mechanisms and does not depend upon the specific properties of the model.

The following predictions, however, are based on the distinctive properties of the model.

- (a) In an allosteric enzyme system, an *allosteric* effector (i.e. a specific ligand endowed with different affinities towards the two states) should exhibit co-operative homotropic interactions.
- (b) In those systems in which an allosteric effector modifies the apparent affinity of the substrate, the substrate also should exhibit co-operative homotropic interactions.
- (c) In those systems in which the effector does not modify the affinity of the substrate, the latter should not exhibit homotropic co-operative interactions.

As may be seen from inspection of Table 1, where the properties of a number of systems have been summarized, all four classes of effects (positive and negative K and V systems) have been found among the dozen or so allosteric enzymes adequately studied. In inspecting Table 1, it should be borne in mind that the published data concerning allosteric enzymes are very heterogeneous and often do not provide the kind of information which we are now seeking. Reasonably adequate kinetic data are available, however, for the systems numbered 1 to 8, 10, 13, 14, 16, 18, 19 and 20. In all but two of these 15 systems, homotropic co-operative interactions of at least one of the ligands have been observed. Three of these systems (18, 19 and 20) show no K effect of the inhibitor and no co-operative interactions of substrate, while the K for systems 2 to 8 and 16 show evidence of homotropic interactions for *both* substrate and effector(s), as predicted by the model.†

It is somewhat difficult to judge whether systems 13 and 14 represent true exceptions or not. One of these (glycogen synthetase, no. 14) is a "positive K system", where the occurrence of homotropic interactions might easily be missed. The other (glutamine—F6P transaminase, no. 13) is a negative K system which has not yet been studied extensively. The possible significance of these exceptions will be considered in the general discussion.

† Attention must be directed to the fact that the homotropic effect of a ligand may not be expressed in the absence of an antagonistic ligand. For example, the co-operative interactions of G-1-P, in the case of phosphorylase *b*, are visible only in the presence of ATP (Madsen, 1964).

TABLE I

Summary of properties of various allosteric systems†

Enzyme	Substrate	Inhibitor	Activator	V System	K System	Subunits	References
1. Haemoglobin (vertebrates) (invertebrates)	Oxygen +					+	Bohr, 1903; Wyman, 1963; Manwell, 1964
2. Biosynthetic L-threonine deaminase (<i>E. coli</i> K12) and (yeast)	L-Threonine +	L-Isoleucine +	L-Valine +		+	(+)	Umberger & Brown, 1958a; Changeux, 1961,1962,1963,1964a,b; Freundlich & Umberger, 1963; Cennamo <i>et al.</i> , 1964
3. Aspartate transcarbamylase (<i>E. coli</i>)	Aspartate + Carbamyl phosphate	CTP +	ATP		+	+	Gerhart & Pardee, 1962,1963,1964
4. Deoxycytidylate aminohydrolase (ass spleen)	dCMP +	dTTP +	dCTP +		+		Scarano <i>et al.</i> , 1963,1964; Scarano, 1964; Maley & Maley, 1963,1964
5. Phosphofructokinase (guinea pig heart)	Fructose-6-phosphate ATP	ATP (+)	3'-5' AMP		+		Passoneau & Lowry, 1962; Mansour, 1963; Vinuela <i>et al.</i> , 1963
6. Deoxythymidine kinase (<i>E. coli</i>)	Deoxythymidine ATP + or GTP -	(dTTP)	dCDP	(+)	+		Okazaki & Kornberg, 1964
7. DPN-isocitric dehydrogenase (<i>N. crassa</i>)	D-Isocitrate + DPN	(α -Ketoglutarate)	Citrate		+		Sanwal <i>et al.</i> , 1963,1964
8. DPN-isocitric dehydrogenase (yeast)	D-Isocitrate + DPN		5' AMP		+		Hataway & Atkinson, 1963

9. Homoserine dehydrogenase (<i>E. rubrum</i>)	Homoserine (-) Aspartate semialdehyde TPN-TPNH	L-Threonine	L-Isoleucine (+) L-Methionine	+	+	Sturani <i>et al.</i> , 1963; Datta <i>et al.</i> , 1964
10. L-Threonine deaminase (<i>C. tetanomorphum</i>)	L-Threonine		ADP +	+	+	Hayaishi <i>et al.</i> , 1963
11. Acetolactate synthetase (<i>E. coli</i>)	Pyruvate (-)	L-Valine		+	+	Umbarger & Brown, 1958b
12. "Threonine" aspartokinase (<i>E. coli</i>)	Aspartate (-)	L-Threonine		+	+	Stadtman <i>et al.</i> , 1961
13. L-Glutamine-D-fructose-6-P transaminase (rat liver)	L-Glutamine - D-Fructose-6-P	UDP-N acetyl-glucosamine -		+	+	Kornfield <i>et al.</i> , 1964
14. Glycogen synthetase (yeast) (lamb muscle)	UDP-glucose -		Glucose-6-P -	+	+	Algranati & Cabib, 1962; Traut & Lipmann, 1963
15. Glutamate dehydrogenase (beef liver)	Glutamate	ATP GTP DPNH Oestrogens + Thyroxine	ADP Leucine + Methionine	+	(+)	(Ref. in Tomkins <i>et al.</i> , 1963)
16. Phosphorylase b (rabbit muscle)	Glucose-1-P + Glycogen P _i (+)	ATP	5' AMP +	+	+	Helmreich & Cori, 1964; Madsen, 1964; Schwartz (personal communication); Ullmann <i>et al.</i> , 1964
17. UDP-N acetyl-glucosamine-2-epimerase (rat liver)	UDP-N acetyl-glucosamine	CMP-N acetyl-neuraminic acid +		+	+	Kornfield <i>et al.</i> , 1964
18. Homoserine dehydrogenase (<i>E. coli</i>)	Homoserine - Aspartate semialdehyde TPN-TPNH	L-Threonine +		+	+	Patte <i>et al.</i> , 1963; Cohen <i>et al.</i> , 1963; Patte & Cohen, 1964

Enzyme	Substrate	Inhibitor	Activator	V System	K System	Subunits	References
19. "Lysine"-aspartokinase (<i>E. coli</i>)	Aspartate — ATP	L-Lysine +		+			Stadtman <i>et al.</i> , 1961; Patte & Cohen, 1964
20. Fructose-1-6-diphosphatase (frog muscle) (rat liver)	Fructose-1-6-diphosphate (—)	5' AMP +		+			Krebs, 1964; Salas <i>et al.</i> , 1964; Taketa & Pogell, 1965
21. ATP-PRPP-pyrophosphorylase (<i>S. typhimurium</i>)	ATP — PRPP	Histidine		+		+	Martin, 1962
22. "Tyrosine" 3-deoxy-D-arabinoheptulosonic-acid-7-phosphate synthetase (<i>E. coli</i>)	Phosphoenol-pyruvate — D-Erythrose-4-P —	L-Tyrosine		+			Smith <i>et al.</i> , 1962
23. "Phenylalanine" 3-deoxy-D-arabinoheptulosonic-acid-7-phosphate synthetase (<i>E. coli</i>)	Phosphoenol-pyruvate — D-Erythrose-4-P —	L-Phenylalanine		+			Smith <i>et al.</i> , 1962
24. Acetyl-CoA carboxylase (rat adipose tissue)	Acetyl CoA ATP, CO ₂		Citrate +			+	Martin & Vagelos, 1962

† The + and — signs against the name of the substrate(s) and effector(s) of each system indicate whether or not co-operative homotropic effects occur with the corresponding compound. A blank implies no relevant data, while (+) or (—) implies uncertainty. The + signs in the "K" and "V" columns indicate whether K or V effects have been observed. In the "subunit" column we have noted with a + those systems for which some evidence (direct or indirect) of the existence of subunits (not necessarily proved to be identical) has been obtained.

Note that (a) this summary is not claimed to be complete; (b) many of the systems listed have been described only recently and as yet incompletely; (c) the properties assigned to many systems represent our (rather than the original authors') interpretation of the data. We therefore assume responsibility for interpretative mistakes.

Let us now examine some of the more specific predictions of the model. According to the theory developed above, the V systems are described by the "function of state" (\bar{R} or $1 - \bar{R}$), assuming that the two states differ in their catalytic activity towards the substrate. We shall mostly discuss the properties of the K systems, of which there are more examples and for which the predictions of the model are particularly interesting and characteristic.

According to the model, the complex kinetics of such systems simply result from displacements of the $R \leftrightarrow T$ equilibrium, and their properties are described by equation (3). We shall examine only a few typical experimental situations and compare them with predictions based on the model.

Consider first a K system involving a substrate and an allosteric inhibitor. Assume that the R state binds the substrate, and the T state binds the inhibitor. We may expect that in any such system, the allosteric constant will be very different from 1. In other words, one of the two states (R or T) will be greatly favoured. Threonine deaminase of *E. coli* is a K system, threonine being the substrate, and isoleucine the inhibitor (Umbarger, 1956). In the presence of inhibitor and substrate, the rate-concentration curve for *both* is S-shaped. In the *absence* of inhibitor, the substrate saturation curve is *still* S-shaped. According to the model, this indicates that the favoured state (in the absence of both ligands) is the one that has minimum affinity for threonine and maximum affinity for isoleucine. It is therefore expected that the saturation curve for inhibitor *in the absence of substrate* should be Michaelian, exhibiting no co-operative effect. This prediction has been verified experimentally (Changeux, 1964*a*). Moreover, as shown in Fig. 4, the co-operativity of the inhibitor increases with the concentration of substrate.

More generally, in any K system, we expect heterotropic effects to be expressed essentially as alterations of the homotropic "co-operativity" of any one allosteric ligand when in the presence of another. As a measure of homotropic effects, it is convenient to use the Hill approximation:

$$\bar{Y} = \frac{\alpha^n}{Q + \alpha^n}$$

where Q is a constant and n (the Hill coefficient) is *not* the number of interacting sites (which we write n), but an interaction coefficient. It has been shown by one of us (Wyman, 1963) that under certain conditions the Hill coefficient can be interpreted as measuring the free energy of interaction between sites. As it may be seen from Table 2, the Hill coefficients for the substrate of the allosteric system deoxycytidine deaminase are modified, in the expected direction, when the concentration of the other ligands (activator or inhibitor) varies. Another specific prediction of the model has been verified in the case of threonine deaminase, namely, the fact that a *true* competitive inhibitor (allothreonine), i.e. a substrate analogue (able to inhibit the enzyme by binding at the same site as the substrate), should exert the same effect as the substrate itself (L-threonine) as an antagonist of the allosteric inhibitor (isoleucine) (Changeux, 1964*a*). Another prediction, concerning the effect of analogues, is that at very low concentrations of substrate low concentrations of analogue should activate, rather than inhibit, the enzyme. This is observed with aspartic transcarbamylase (Gerhart & Pardee, 1963) (Fig. 5) and also with threonine deaminase (Changeux, 1964*a*).

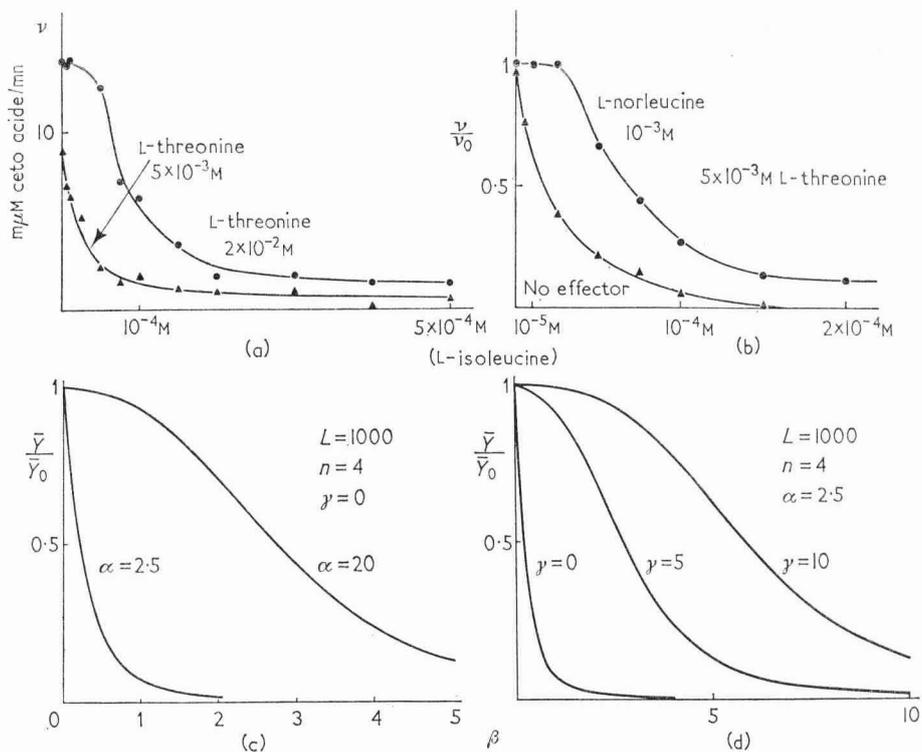


FIG. 4. Effects of the allosteric inhibitor L-isoleucine upon the activity of L-threonine deaminase. (a) In the presence of two different concentrations of the substrate (L-threonine). (b) At low concentration of substrate in the presence or absence of the allosteric activator L-norleucine.

Compare with theoretical curves (c and d) describing similar situations according to equation (3). Note that at low concentrations of substrate the co-operative effect of the inhibitor is scarcely detectable either in the theoretical or in the experimental curves. An increase of the concentration of substrate, or the addition of an activator, both reveal the co-operative effects of the inhibitor.

TABLE 2

Hill coefficients of homotropic interactions with respect to substrate (\underline{n}), inhibitor (\underline{n}') and activator (\underline{n}'') observed with dCMP deaminase

(From Scarano et al., 1963; Scarano, 1964)

		\underline{n}
Substrate (dCMP)	No effector	2.0
	+ dTTP 1.25 μ M	3.0
	„ 2.25 μ M	4.1
	„ 10.00 μ M	3.9
	+ dCTP 100.00 μ M	1.0
		\underline{n}'
Inhibitor (dTTP)	Substrate concentration 4 mM	3.4
		\underline{n}''
Activator (dCTP)	Substrate concentration 67 μ M	2.0

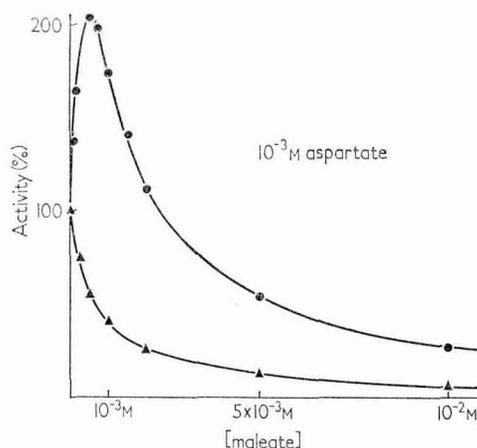


FIG. 5. Effect of a substrate analogue (maleate) upon the activity of aspartic transcarbamylase at relatively low concentration of substrate (aspartate).

Upper curve: native enzyme. Lower curve: desensitized enzyme.

Note the large increase of activity at low maleate concentration which occurs with the native enzyme, but not with the desensitized enzyme (data from Gerhart & Pardee, 1963).

The effect of an allosteric *activator* in a *K* system should be, according to the model, to decrease or abolish the substrate-substrate interactions. This has been observed in several different systems. As illustrated in Fig. 6, the effect is particularly striking because, as expected, at saturating concentration of activator it results in converting the S-shaped rate-concentration curve for substrate into a Michaelian hyperbola. Moreover, of course, the presence of an activator should increase the co-operativity of an inhibitor, and conversely. Both effects are observed (see Figs 4 and 7).

It is clear from the model and the equations that the homotropic interactions of an allosteric ligand are independent of the absolute values of the microscopic dissociation constants. One may therefore expect that two sterically closely analogous ligands could bind to the same sites with the same interaction coefficient, even though their affinities might be widely different. For example, with haemoglobin, the functionally significant steric features of the prosthetic groups must be virtually the same, whether the haems are bound to oxygen or to carbon monoxide. Therefore, although the affinity of carbon monoxide for the haem is known to be nearly 250 times that of oxygen, we should expect the interaction coefficients to be the same for both, as indeed they are (Wyman, 1948). When, however, the binding of two analogous ligands depends very much on steric factors, it may be expected that the *ratios* of the affinities of each ligand towards the two states of the protein (i.e. the constant *c* in equation (1)) will be different. If so, the two ligands might bind to the same sites with widely different interaction coefficients. This appears to be the case, according to the observations of Okazaki & Kornberg (1964) for various triphosphonucleosides which act as phosphoryl donors in the deoxythymidine-kinase reaction. With ATP, for example, the rate-concentration curve is strongly co-operative, whereas with dATP the curves exhibit scarcely any evidence of homotropic effects. Furthermore, this enzyme, as shown by the same authors, is allosterically activated by CDP (Fig. 6(c)). It is easily seen that if this effect conforms to the model, activation should be observed only with those substrates that show evidence of homotropic effects (ATP), and not with those that do not (dATP). This, actually, is the observed result.

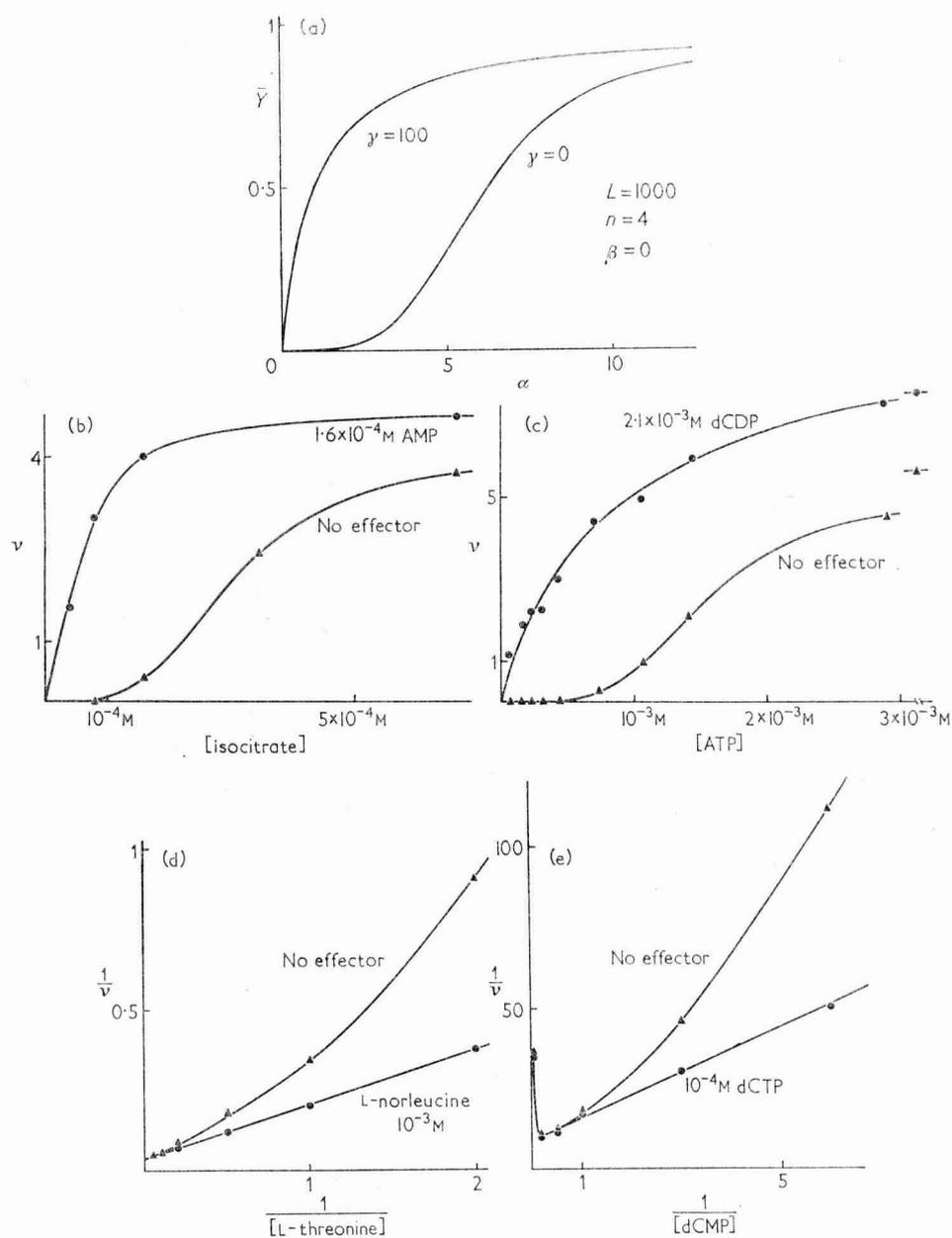


FIG. 6. Activity of various allosteric enzymes as a function of substrate concentration in the presence or absence of their respective activators.

(a) Theoretical curve according to equation (3).

(b) DPN-isocitrate dehydrogenase from *Neurospora crassa* (results from Hataway & Atkinson, 1963).

(c) Deoxythymidine kinase from *Escherichia coli* (results from Okazaki & Kornberg, 1964).

(d) Biosynthetic L-threonine deaminase from *E. coli* (Lineweaver-Burk plot) (results from Changeux, 1962, 1963).

(e) dCMP deaminase from ass spleen (Lineweaver-Burk plot) (results from Scarano *et al.*, 1963).

Note that in all these instances, the presence of the allosteric activator abolishes the co-operative interactions of the substrate.

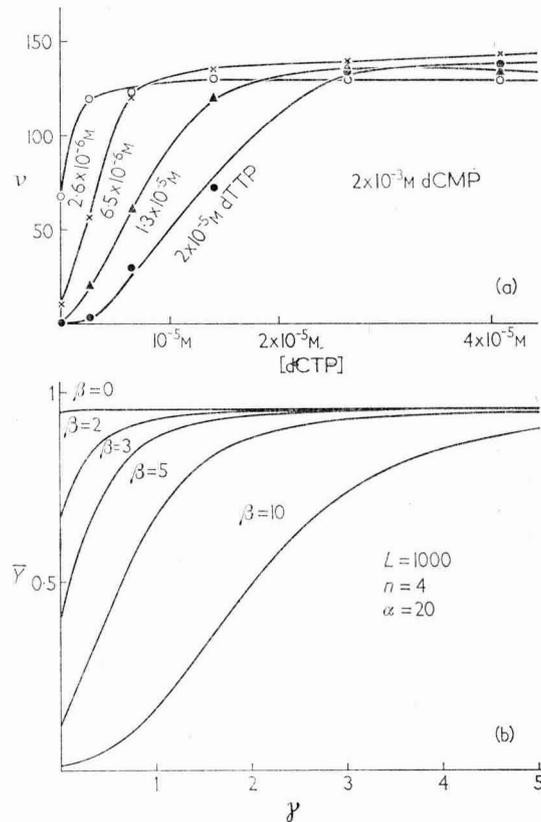


FIG. 7. (a) Activity of dCMP deaminase as a function of the concentration of its allosteric activator dCTP in the presence of substrate (dCMP) at near saturating concentration and at various concentrations of the allosteric inhibitor dTTP (results from Scarano, personal communication).

(b) Theoretical curves of equation (3) corresponding to a similar situation.

Note that the co-operative effects of the activator are revealed only at relatively high concentration of the inhibitor.

Since, again, the homotropic interactions are independent of absolute affinities, certain conditions or agents may modify the affinity of an allosteric ligand without altering its interaction coefficient. This is apparently the case for the Bohr effect shown by haemoglobin: as is well known, the oxygen saturation curves obtained at different values of pH can all be superimposed by a simple, adequately chosen, change of the abscissa scale. In terms of the model, this would mean that the binding of the "Bohr protons" does not alter the equilibrium between the two hypothetical states of the protein. Hence also the Bohr protons themselves would *not* be allosteric ligands, and their own binding is not expected to be co-operative. This, again, appears to be the case, at least for human and horse haemoglobin (Wyman, *loc. cit.*).

In the preceding paragraph, we have discussed only the more straightforward predictions of the model. It should be pointed out that the model could also account for more complicated situations, and for certain effects which were not considered here. For example, it seems possible that, in some instances, the phenomenon of inhibition by excess substrate might be due to an allosteric mechanism (rather than to the classically invoked direct interaction between two substrate molecules at the active site).

This effect could be described on the basis of our model by assuming two states with different affinities for the substrate, the one with higher affinity being catalytically inactive. The equation for such a situation would be of the form:

$$\frac{V}{V_m} = \frac{L S/K_a (1 + S/K_a)^{n-1}}{L(1 + S/K_a)^n + (1 + S/K_I)^n}$$

with K_I (dissociation constant of S with the inactive state) smaller than K_a (dissociation constant with the active state).

(b) *Desensitization and dissociation*

One of the most striking facts about allosteric enzymes is that their regulatory properties may be lost as a result of various treatments, without loss (indeed often with increase) of activity (Changeux, 1961; Gerhart & Pardee, 1961). That it should be so is understandable on the basis of the model, since conservation of the interactions should depend upon the integrity of the whole native structure, including in particular the inter-protomer binding, whereas conservation of activity should depend only on the integrity of the active site. Also, according to the model, the homotropic and heterotropic interactions should in general be simultaneously affected, if at all, by alterations of protein structure. This was first observed with threonine deaminase (Changeux, 1961) and ATCase (Gerhart & Pardee, 1962), and similar observations have since then been made with several other systems. These observations constituted the main initial basis for the assumption that regulatory interactions in general may be indirect (Changeux, 1961; Monod & Jacob, 1961; Monod *et al.*, 1963).

According to the model, loss of the interactions would follow from any structural alteration that would make one of the two states (R or T) virtually inaccessible. Now, one of the events most likely to result from various treatments of the protein is that quaternary (inter-protomer) bonds may be broken, completely or partially. One may therefore expect that:

- (a) Under any condition, or following any alteration, such that the protein is (and remains) dissociated, *both* types of interactions should disappear.
- (b) Conversely treatments, or mutations, which abolish the interactions should frequently be found to result in stabilization of a monomeric state.

These expectations are verified by observations made with at least two different systems (Gerhart & Pardee, 1963, 1964; Patte, Le Bras, Loviny & Cohen, 1963; Cohen & Patte, 1963).

Furthermore, since it is assumed from the model that in one of the two alternative states (R) the protomers are less constrained and therefore closer to the conformation of the monomer than in the other state (T), we expect that, under conditions where the protein is monomeric, it may exhibit high affinity for the ligand which stabilizes the R state, and little or no affinity for the ligand which stabilizes the T state. Hence, if the experiment can be performed, one may deduce *which* of the two states (R or T) is stabilized by a given ligand.

If conditions can be set up such that reversible dissociation of the protein actually occurs, one may expect that an allosteric ligand (i.e. any ligand exhibiting homotropic interactions) should now prove to act as a specific associative or dissociative agent. Actually, there is now clear evidence that under conditions where human haemo-

globin shows a detectable amount of dissociation (low pH, high ionic strength), dissociation is favoured by oxygenation (Antonini, Wyman, Belleli & Caputo, unpublished experiments, 1961; Benesch, Benesch & Williamson, 1962; Gilbert & Chionione, recent unpublished experiments). Lamprey haemoglobin, in the oxygenated form, exists primarily as a monomer under all conditions, but when deoxygenated shows a strong tendency to polymerize (see Table 3) (Briehl, 1963; Rumen, 1963). Myoglobin, which may be thought of as an isolated (and therefore relaxed) protomer of haemoglobin, has a much higher oxygen affinity, as would be expected on the basis of these two facts regarding human and lamprey haemoglobin.

TABLE 3
Sedimentation coefficients of oxygenated and reduced lamprey haemoglobin
(from Briehl, 1963)

$t^{\circ}\text{C}$	pH	Haemoglobin concentration (E. 275)	Sedimentation coefficient ($S_{20,w}$)	
			Oxygenated	Reduced
5.5	6.8	15.7	2.02	3.68
5.0	7.3	21.0	1.90	2.98

Similarly, Changeux (1963) has found that in the presence of urea (1.5 M) threonine deaminase is reversibly dissociable. As expected, under these conditions, all three types of allosteric ligands active in the system, namely the substrate (threonine or analogue of threonine), the activator (valine) and the inhibitor (isoleucine) powerfully affect the dissociation, the inhibitor favouring the associated state, whereas both the substrate and the activator appear to stabilize the dissociated state. Hence, under normal conditions, the substrate and the activator presumably stabilize an R state, while the inhibitor favours a T state.

The observations of Datta, Gest & Segal (1964) on homoserine dehydrogenase from *Rhodospirillum rubrum* provide a further striking example of the effects of allosteric ligands upon dissociation of the protein. This enzyme is activated by both methionine and isoleucine, and inhibited by threonine. Both activators, as well as the substrate, promote dissociation of the protein, whereas the inhibitor favours an aggregated state.

We may conclude from the preceding discussions that the characteristic, unusual, apparently complex functional properties of allosteric systems can be adequately systematized and predicted on the basis of simple assumptions regarding the molecular symmetry of oligomeric proteins. In the next section, we shall examine the structural implications and the plausibility of these assumptions from a more general point of view.

4. Quaternary Structure and Molecular Symmetry of Oligomeric Proteins

(a) *Geometry of inter-protomer bonding*

The first major assumption of the model is that the association between protomers in an oligomer may be such as to confer an element of symmetry on the molecule. The plausibility of this assumption has already been pointed out by Caspar (1963) and by Crick & Orgel (1964, and unpublished manuscript). We will analyse the

implications of this assumption in terms of the possible or probable modes of bonding between protomers. Although next to nothing is known, from direct evidence, regarding this problem, the following statements would seem to be generally valid.

- (a) A large number, probably a majority, of enzyme proteins are oligomers involving several *identical* subunits, i.e. protomers (see: Schachman, 1963; Reithel, 1963; Brookhaven Symposium, 1964, *Subunit Structure of Proteins*, no. 17).
- (b) In most cases the association between protomers in such proteins does not appear to involve covalent bonds.
- (c) Yet most oligomeric proteins are stable as such (i.e. do not dissociate into true monomers, or associate into superaggregates), over a wide range of concentrations and conditions.
- (d) The specificity of association is extreme: monomers of a normally oligomeric protein will recognize their identical partners and re-associate, even at high dilution, in the presence of other proteins (e.g. in crude cell extracts).

These properties indicate that within oligomeric proteins the protomers are in general linked by a *multiplicity* of non-covalent bonds, conferring both specificity and stability on the association. Clearly also the steric features of the bonded areas must play a major part.

Let us now distinguish between two *a priori* possible modes of association between two protomers. For this purpose we define as a "binding set" the spatially organized collection of all the groups or residues of *one* protomer which are involved in its binding to *one* other protomer. Considered together, the two linked binding sets through which two protomers are associated will be called the *domain of bonding* of the pair.

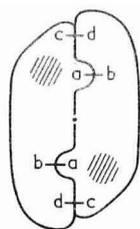
The two modes of association which we wish to distinguish may then be defined as follows.

- (a) *Heterologous associations*: the domain of bonding is made up of two *different* binding sets.
- (b) *Isologous associations*: the domain of bonding involves two *identical* binding sets.

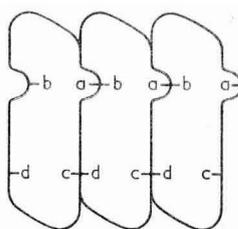
These definitions imply the following consequences.†

(1) In an isologous association (Figs 8 and 9), the domain of bonding has a two-fold axis of rotational symmetry. Along this axis, homologous residues (i.e. identical residues occupying the same position in the primary structure) face each other (and may form unpaired "axial" bonds). Anywhere else, within the domain of bonding, any bonded group-pair is represented *twice*, and the two pairs are symmetrical with respect to each other. Put more generally: in an isologous association, any group which contributes to the binding in one protomer furnishes precisely the same contribution in the other protomer. Isologous associations will therefore tend to give rise to "closed" i.e. *finite* polymers since, for example, an isologous dimer can further polymerize only by using "new" binding sets (i.e. areas and groups not already satisfied in the dimer). Note that this mode of association can give rise only to *even numbered* oligomers.

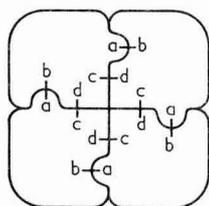
† The validity of the statements that follow can be visualized and demonstrated best with the use of models. The interested reader may find it helpful to use a set of dice for this purpose.



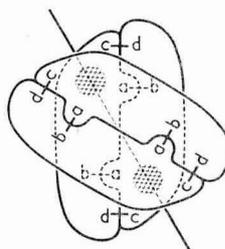
I-Isologous association



II-Heterologous association



III-Heterologous tetramer



IV-Isologous tetramer
(pseudotetrahedral)

FIG. 8. Isologous and heterologous associations between protomers.

Upper left: an isologous dimer. The axis of symmetry is perpendicular to the plane of the Figure.

Upper right: "infinite" heterologous association.

Lower left: "finite" heterologous association, leading to a tetramer with an axis of symmetry perpendicular to the plane of the Figure.

Lower right: a tetramer constructed by using isologous associations only. Note that two different domains of bonding are involved.

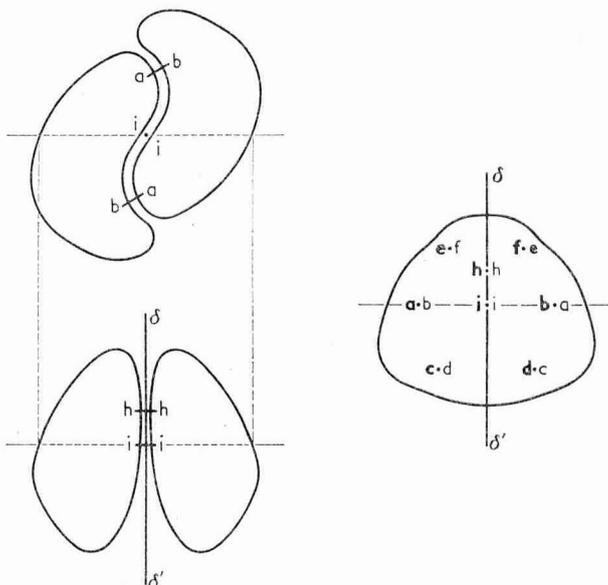


FIG. 9. Topography of the domain of bonding in an isologous association.

Upper left: represented in a plane perpendicular to the axis of symmetry.

Lower left: the same viewed in a plane of the axis of symmetry.

Right: projection of the domain of bonding in a plane of the axis of symmetry.

hh and ii, axial bonds; ab, ba, cd, dc, ef, fe, antiparallel bonds. It should be understood that in this Figure the bonding residues a, b, c, etc. are supposed to project from under and from above the plane of the Figure.

(2) In a heterologous association (Fig. 8), the domain of bonding has no element of symmetry; each bonded group-pair is unique. Heterologous associations would, in general, be expected to give rise to polydisperse, eventually large, helical polymers except, however, in two cases.

- (a) If polymerization is stopped at some point by steric hindrance, giving rise to a "hinged helix". Such aesthetically unpleasant structures should have less stability than "closed" structures.
- (b) If a "closed" structure can be achieved such that any binding set which is used by one protomer is also satisfied in all the others. This is impossible of course in a dimer, but it is possible for trimers, tetramers, pentamers, etc., provided that the angles defined by the domains of bonding are right or nearly so. Such an oligomer would necessarily possess an axis of symmetry.

On the basis of these considerations, it is reasonable to assume that, if an oligomeric protein possesses a wide range of stability, it consists of a closed structure where all the protomers use the same binding sets; which implies, as we have just seen, that the molecule should possess at least one axis of symmetry.

Direct experimental evidence on this important problem is available for haemoglobin. As is well known, although made up of four subunits, haemoglobin is not, strictly speaking, a tetramer, since the α and β chains are not identical. For our present purposes, however, we may consider the four subunits as equivalent protomers. The work of Perutz *et al.* (1960) has shown that these are associated into a pseudotetrahedral structure which possesses a twofold axis of symmetry.

Three further examples of oligomers possessing an element of molecular symmetry have recently been provided. Green & Aschaffenburg (1959) find that β -lactoglobulin (a dimer) has a dyad axis. Lactic dehydrogenase M4 (Pickles, Jeffery & Rossmann, 1964) and glyceraldehyde-phosphate-dehydrogenase (Watson & Banaszak, 1964), both tetrameric, appear to possess one (at least) axis of symmetry.

From the preceding discussion, and on the strength of these examples, it appears that oligomeric proteins are not only capable of assuming molecular symmetry, but also that this may be a fairly general rule.

Assuming this conclusion to be correct, it is of interest to enquire which mode of association (isologous or heterologous) may be most frequently used in Nature. For the reasons pointed out above, stable dimers, of which many examples are known, must represent isologous associations. Moreover, it may be pointed out that a symmetrical (isologous) dimer can further polymerize into a *closed* structure in two ways only.

(a) By again using isologous associations, thereby forming an isologous tetramer. Isologous polymerization, however, must stop at this point, since no further closed structure could be built by polymerization of such a tetramer.

(b) By using heterologous associations when the next closed structure would necessarily consist of three isologous dimers, and hence be an hexamer.

It follows from these remarks that (1) the exclusive use of isologous associations can lead to dimers and tetramers only; (2) the use of *both* isologous and heterologous domains of bonding should lead to even-numbered oligomers containing a *minimum* of six protomers; (3) the exclusive use of heterologous domains of bonding could lead to oligomers containing any number of protomers (except two). On this basis, the

apparently rather wide prevalence of dimers and tetramers among oligomeric enzymes suggests rather strongly that the quaternary structures of these proteins are mostly built up by isologous polymerization.

(b) *Protomer conformation: "quaternary constraints"*

The formation of stable, specific associations involving multiple bonds and strict complementarity between protein protomers is likely to imply in most cases a certain amount of re-arrangement of the tertiary structures of the monomers. Certain observations seem to confirm this assumption.

(1) The artificially prepared monomers of enzymes that are normally oligomeric generally exhibit functional alterations, suggesting that the structure of the active site in each protomer depends upon a conformation which exists only in the native oligomeric associated state (see Brookhaven Symposium, 1964, *Subunit Structure of Proteins*, 17).

(2) The rate of reactivation of oligomeric enzymes inactivated by dissociation into monomers is markedly dependent on temperature (alkaline phosphatase, Levinthal, Signer & Fetherolf, 1962; β -galactosidase, Perrin, manuscript in preparation; phosphorylase b, Ullmann, unpublished work). Since the association reaction does not involve the formation of covalent bonds, the temperature dependence of the rate is to be attributed, presumably, to a "conformational" transition state.

(3) The phenomenon of intra-cistronic complementation between different mutants of the same protein appears, as pointed out by Crick & Orgel (1964), to be due to a repair of altered structures which results from association between differently altered monomers of a (normally oligomeric) protein. Note that this interpretation necessarily implies, as pointed out by the authors, that the domain of bonding has an axis of symmetry.

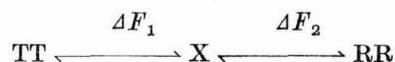
It is reasonable therefore to consider that the conformation of each protomer in an oligomer is somewhat "constrained" by, and dependent upon, its association with other protomers. (An excellent discussion of this concept, as applied to haem proteins, is given by Lumry (1965).) In a symmetrical oligomer, all the protomers are engaged by the same binding sets and submitted to the same "quaternary constraints"; they should therefore adopt the same conformations. By contrast, in any non-symmetrical association, identical monomers would, as protomers, assume somewhat *different* conformations and cease to be truly equivalent. Thus, symmetry of bonding is to be regarded as a condition, as well as a result, of the structural equivalence of subunits in an oligomer.

These remarks justify the assumption that the specific biological properties of an oligomer depend in part upon its quaternary structure, and that the protomers will be functionally as well as structurally equivalent if, and only if, they are symmetrically associated within the molecule.

The last assumption of the model, namely, that in an allosteric transition the symmetry of quaternary bonding, and therefore the equivalence of the protomers, should tend to be conserved, may now be considered. Let us analyse the meaning and evaluate the possible range of validity of this postulate.

Consider a symmetrical oligomer (for simplicity, a dimer) wherein the conformation of each protomer is constrained and stabilized by the quaternary bonds (T). If these constraints were relaxed (i.e. the bonds broken) each protomer would tend towards

an alternative conformation (R), involving certain tertiary bonds which were absent in the other configuration. The transition may be written:



where TT and RR stand for two symmetrical configurations and X for one (or several) non-symmetrical intermediate states. To say that symmetry should "tend to be conserved" is to imply that the occurrence of the R \leftrightarrow T transition in one of the protomers should facilitate the occurrence of the same transition in the other. This would be the case of course if the intermediate state(s) X were less stable than either one of the symmetrical states; but it would also be the case, even if the X state were more stable than one of the symmetrical states, provided only that the ΔF of the first transition (from one of the symmetrical states to the intermediate state) were more positive than the second.

It is easy to see that the dissociation of a symmetrical oligomer should in general satisfy this condition. This may conveniently be symbolized as in Fig. 10, where each subunit is represented as an arrow and only a minimum number of bonds is shown—

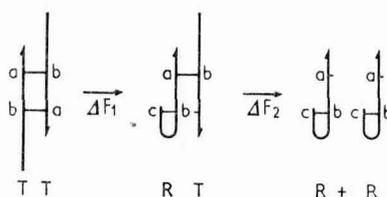


Fig. 10

actually two symmetrical (antiparallel) inter-protomer bonds (ab and ba) and one intra-chain bond (cb) the presence or absence of which is taken to characterize two distinct conformations (R and T) available to each subunit.

Although the symmetry of the protomers would not be conserved after dissociation into monomers, their equivalence would be, and the transition itself is symmetrical since it involves the breaking (or formation) of symmetrical bonds and symmetrical suppression (or creation) of identical quaternary constraints. The free energy of each of the two transitions may then be considered to involve two contributions: one (ΔF_b), assignable to the breaking and formation of individual bonds, the other (ΔF_x) associated with the freedom gained or lost by the protomers in respect to one another. By reason of symmetry, ΔF_b would be the same for both transitions, while ΔF_x would not. Since, in the example chosen, the second transition involves dissociation, the entropy gained in this step would be larger than in the first, and the sum of the two contributions would give $\Delta F_1 > \Delta F_2$, satisfying the condition of co-operativity. A ligand able to stabilize either the R or the T state would in turn exert homotropic co-operative effects upon the equilibrium.

There are examples in the literature of co-operative effects of this kind. The best illustration may be the muscle phosphorylase conversions which involve, as is well known, the formation of a tetrameric molecule (phosphorylase *a*) from the dimeric phosphorylase *b*. The conversion occurs when phosphorylase *b* is phosphorylated (in the presence of ATP and phosphorylase kinase). As expected since it is a tetramer, phosphorylase *a* contains four phosphoryl groups. Krebs & Fischer (personal communication) have observed that, when the amount of ATP used in the reaction is

sufficient to phosphorylate only a fraction of the (serine) acceptor residues, a *stoichiometric amount* of fully phosphorylated tetrameric phosphorylase *a* is formed, while the excess protein remains dimeric and unphosphorylated. Another striking illustration of co-operative effects upon dissociation is provided by the work of Madsen & Cori (1956), who observed that phosphorylase *a* would dissociate into monomers in the presence of parachloromercuribenzoate, and showed that when the amounts of mercurial used were insufficient to dissociate all the protein, the remaining non-dissociated fraction did not contain any mercuribenzoate.

Reversible allosteric transitions however do not, in the majority of known cases, involve actual dissociation of the protomers. A transition between two undissociated symmetrical states of an oligomer would, nevertheless, be co-operative if it were adequately symbolizable, for example as in Fig. 11, which expresses the assumption

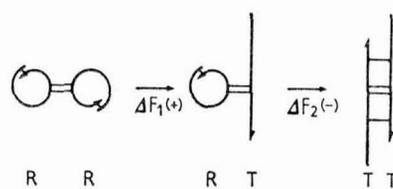


FIG. 11

that one of the alternative conformations (T in this case) is stable only when held by quaternary bonds which could be formed only at the price of breaking *symmetrically* certain tertiary bonds present in the other configuration (R).

In such a system, the ΔF of the first transition would be positive, the second negative, and the intermediate state (RT) therefore less stable than either one of the symmetrical states. Such a system could be very highly co-operative, and the strong homotropic interactions observed with many real systems† suggests that they may conform to such a pattern.

However, Fig. 12 symbolizes a much more general pattern of symmetrical transitions which is interesting to consider.

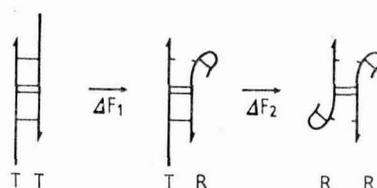


FIG. 12

Here again the free energy assignable to the formation and breaking of individual bonds is the same in both transitions. Whether the $RR \leftrightarrow TT$ transition will be co-operative, non-co-operative, or anti-co-operative, should then depend entirely on the entropy term associated with the degrees of mutual freedom gained or lost by the protomers in each transition. If these entropy terms were equal in absolute value and of the same sign for both transitions (or if they were negligible) the system would be non-co-operative. In general, however, one would expect these two terms to be

† That is, when the Hill coefficient (n) approaches the value corresponding to the actual number of protomers.

unequal and of significant magnitude in at least one of the transitions. The system would then be co-operative whenever the second entropy term was more positive than the first, and anti-co-operative in the reverse case.

The first possibility appears more likely on general grounds, since it seems reasonable to believe that certain degrees of mutual freedom, in a symmetrical dimer, may be held by either one of two (or more) symmetrical quaternary bonds, and liberated only when *both* are broken. Such a system would be closely comparable to a dissociating system, and it is interesting to note in this respect that in certain allosteric systems actual dissociation is observed under certain "extreme" conditions, whereas it is not seen under more normal conditions (see p. 104).

The possibility should also be considered that certain allosteric transitions might not involve a non-symmetrical intermediate. Such transitions would have to involve the initial breaking of axial bonds, eventually perhaps leading to, or allowing, the symmetrical breaking of symmetrical bonds as pictured in Fig. 13. Such a mechanism would necessarily be co-operative.

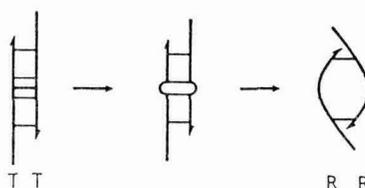


FIG. 13

It is impossible to say, at the present time, whether the co-operative homotropic effects observed with real systems are better described by one or other pattern of symmetrical transitions. One might hope, however, to identify or eliminate some of these mechanisms by adequate thermodynamic and kinetic studies (using fast-mixing techniques) of the transition itself. It is clear in any event that none of these descriptions could apply to a non-symmetrically bonded oligomer, the protomers of which would have to assume different conformations and could not therefore undergo co-operatively the same transitions. On this basis, the fact that allosteric ligands appear invariably to exert co-operative homotropic effects may be taken as experimental evidence that the transitions which they stabilize occur in a symmetrical structure; indeed it was pointed out several years ago by one of us (Allen, Guthe & Wyman, 1950) that the symmetry properties of the oxygen saturation function for haemoglobin appeared to reveal the existence of elements of structural symmetry in the molecule itself. This inference was proved correct when the structure of haemoglobin was elucidated. Moreover, the recent work of Muirhead & Perutz (1963) and Perutz, Bolton, Diamond, Muirhead & Watson (1964) has shown that while the *quaternary* structure of haemoglobin is very significantly different in the oxygenated *versus* the reduced state, the molecular symmetry of the tetramer is conserved in both states. These observations would give a virtually complete illustration of the model if the X-ray pictures also showed some evidence of concomitant alterations of the tertiary structure of the protomers. This has not been observed; but it is reasonable to assume that a functionally significant allosteric transition need not involve more than a very small structural alteration of the protomers. In other words, given the very close and

numerous intra-chain interactions, it would not be surprising that the quaternary constraints, even if strong, should not be expressed at the present level of resolution (5.5 Å) of the X-ray pictures. It is also possible that the quaternary constraints might not force any significant *sensu stricto* "conformational" alteration of the protomers, but only, for example, a (symmetrical) redistribution of charge within the molecule. We wish to point out that the assumptions of the model would remain valid also in such a case, and that the adjective "conformational" which we have used extensively (for lack of a better one) to qualify allosteric transitions, should be understood in its widest connotation.

5. General Discussion

In the preceding discussion we have tried to show, first that the functional properties of regulatory enzymes could be accounted for on the assumption that the quaternary structures of oligomeric proteins involve an element of symmetry in many, if not most, proteins made up of identical subunits (that is, presumably, in the majority of enzymes). We may now consider the problem in reverse and ask why molecular evolution should have so frequently favoured the appearance and maintenance of oligomeric globular proteins.

That it should be so must mean that there are functional advantages of some kind, inherent in the oligomeric state, and absent or difficult to achieve in the monomeric state. If most or all oligomeric proteins were endowed with the property of mediating allosteric interactions, especially homotropic interactions, we might believe that we had an answer to the question. Actually most of the enzymes known to be oligomeric are not, or at least are not known to be, allosteric. One should note, however, that the capacity to mediate physiologically significant interactions might be more frequent and widespread among proteins than has been realized so far. As we have seen, these properties are frequently very labile and may easily be lost during extraction and purification of an enzyme. Furthermore, it is conceivable that the effector for certain proteins may be an unknown or simply an improbable metabolite, if not, in some cases, another cellular protein (cf. Lehninger, 1964).

It probably remains true, however, that most oligomeric proteins are not endowed with specific regulatory functions. One must therefore presume that there are some other, more general, advantageous properties associated with the oligomeric state.

This problem may be related to the even more general question: Why should enzymes be so large, as compared with the size of their stereospecific sites? It seems reasonable to believe that two factors in particular contribute to determining a minimum size for enzymes. One is the requirement of fixing a very precise position in space for the several residues which together constitute the stereospecific site. Not only does this involve the necessity of a peptide chain with enough degrees of freedom (i.e. long enough) to allow the precise relative arrangement of these residues, but also the use of a further length of peptide to freeze these degrees of freedom, thereby conferring enough rigidity (i.e. specificity) upon the site. Another factor probably is the requirement that a given protein should *not* tend to associate more or less indiscriminately with other cellular proteins. As Pauling has pointed out, proteins are inherently "sticky", and the structure of enzymes must have been selected against the tendency to form random aggregates. Such a "purpose" may be, in part, fulfilled by decreasing the surface-volume ratio, and also by putting the polar groups on the surface, thereby increasing the solubility.

Now, association between monomers may evidently also contribute both to the fixation of an adequate structure and to a decrease in the surface-volume ratio, as well as to the covering-up of the hydrophobic areas of the monomers. Moreover, it is evidently more economical to achieve such results, whenever possible, by associating monomers rather than by increasing the unit molecular weight (i.e. the molecular weight per active centre).

These selective factors should therefore have favoured in general the appearance of closed (i.e. symmetrical) oligomers, since "open" structures (potentially infinite and polydisperse) would be disadvantageous in the case of most enzymes. Isologous (rather than heterologous) polymerization may have been frequently preferred for the same reasons, since this type of association leads to closed structures exclusively and, in the process of evolution from a monomeric to a polymeric state, it is evidently easier to start at the dimer stage (at which a heterologous association is still necessarily open), rather than right away at a higher stage.

However, the most decisive factor in the emergence and selective maintenance of symmetrical oligomeric proteins may have been the inherent co-operativity of their structure. To illustrate this point, consider schematically the events which may lead to the formation of a primitive dimer from a monomer.

On the surface of a protein monomer, any particular area contains a variety of randomly distributed groups, many of which may possess inherent chemical affinity for another one in the area. Since the *distance* between any two such groups is necessarily the same in two individual monomers, antiparallel association of the two pairs whenever possible would satisfy simultaneously two such valencies, creating a dimer involving two bonds and possessing a dyad axis. Furthermore, since this applies to *any* pair of groups capable of forming a bond, the monomers have a choice of *any one* of the mutually attractive pairs to achieve such a structure. Even so, the primitive dimer may not be formed, or might remain very unstable, because of the presence, within the area of contact of the protomers, of mutually repulsive groups. These pairs of groups would be distributed symmetrically about the dyad axis defined by the first two, mutually attractive, pairs. Therefore any mutation of *one* residue, conferring upon it the capacity to form a bond with its partner, would result in *two* new bonds being achieved in the dimer. Because of the interactions through "quaternary constraints" between the conformation of each protomer and the structure of their common domain of bonding, any such mutational event would affect symmetrically and co-operatively the functional properties of each of the two protomers. It is clear that, because of these reciprocal interactions, the same general reasoning applies to any mutation which might, even very discretely, affect the conformation of the protomers, including in particular the steric features of the domain of bonding which must of course play an important part in the stability of the association. Thus the structural and functional effects of single mutations occurring in a symmetrical oligomer, or allowing its formation, should be greatly amplified as compared with the effects of similar mutations in a monomer or in a non-symmetrical oligomer. In other words, because of the inherent co-operativity of their structure, symmetrical oligomers should constitute particularly sensitive targets for molecular evolution, allowing much stronger selective pressures to operate in the random pursuit of functionally adequate structures.

We feel that these considerations may account, in part at least, for the fact that most enzyme proteins actually are oligomeric; and if this conclusion is correct, the

homotropic co-operative effects which seem at first to "characterize" allosteric systems should perhaps be considered only as one particular expression of the advantageous amplifying properties associated with molecular symmetry.

The same general argument may account for the fact that (apart from one or two possible exceptions) allosteric proteins have invariably been found to mediate *both* heterotropic and homotropic interactions, which implies of course that they are oligomeric. It should be clear from the discussion of the model that heterotropic interactions could *a priori* be mediated by a monomeric protein possessing two (necessarily different) binding sites, associated with two different "tautomeric" states of the molecule. If, for example, one of the states were stabilized by the substrate and the other by some other specific ligand, the latter would act as a competitive inhibitor. The saturation function (\bar{Y}_s) would then simplify into:

$$\bar{Y}_s = \frac{\alpha}{L(1 + \beta) + 1 + \alpha}$$

which we write only to indicate that, for $n = 1$ (i.e. for a monomer) the model *formally* allows heterotropic effects to occur, but not of course, homotropic effects.

Just as the effect of a single amino-acid substitution will be greater in a symmetrical oligomer than in a monomer, the stabilization by a specific ligand of an alternative conformation, implying a significant increase of potential, may be possible in an oligomer when it would not be, for lack of co-operativity, in a monomeric protein. The fact that *both* heterotropic and homotropic interactions disappear when an allosteric protein is "desensitized" as a result of various treatments may be considered to illustrate this point, and actually constitutes one of the main experimental justifications of the model. It might be said in other words, that the molecular symmetry of allosteric proteins is used to amplify and effectively translate a very low-energy signal. †

In addition, it is clear that the sigmoidal shape of the saturation curve characteristic of homotropic interactions may in itself offer a significant physiological advantage, since it provides the possibility of threshold effects in regulation. This property is of course essential in the case of haemoglobin, and it seems very likely that it has an important role in most, if not all, regulatory enzymes. Selection, in fact, must have operated on these molecules, not only to favour the structures which allow homotropic interactions, but actually to determine very precisely the energy of these interactions according to metabolic requirements.

The selective "choice" of oligomers as mediators of chemical signals therefore seems to be justified (*a posteriori*) by the fact that certain desirable physical and physiological properties are associated with symmetry, and therefore inaccessible to a monomeric protein.

We should perhaps point out here again that in the present discussion, as in the model, we accept the postulate that a monomeric protein or a protomer does not possess more than *one stereospecific site* able to bind a given ligand. That this postulate

† Consider for example an allosteric system with an intrinsic equilibrium constant ($L = T_0/R_0$) of 1000. Assume, that the R state has affinity $1/K_R$ for a ligand F, and set $F/K_R = \alpha$. In the presence of the ligand, the ratio of the two states will be: $\frac{\sum T}{\sum R} = \frac{1000}{(1 + \alpha)^n}$. Taking $\alpha = 9$, for

example, we would have, for a tetramer, $\frac{\sum T}{\sum R} = 0.1$. In order to reach the same value for the T/R ratio with a monomeric system, the concentration of F would have to be more than one thousand times larger.

does apply to stereospecific sites is amply documented (cf. Schachman, 1963) and need not be discussed at length here. It is obvious of course that, lacking symmetry, a monomer or an individual protomer cannot present two or more *identical* elements of tertiary structure of any kind.

The postulate, however, does not apply to *group-specific*, as opposed to *stereospecific*, ligands. Homotropic interactions of various kinds (not necessarily co-operative) may therefore occur in the binding of group-specific ligands (such as SH reagents, detergents, ions, etc.) whether the protein is monomeric or not. As is well known, the vast literature on the denaturation of proteins is replete with descriptions of multimolecular effects exerted by various group-specific reagents. It may be worth noting in this respect that in the last analysis, the co-operative effects of such reagents are accounted for by the simultaneous attack of numerous bonds occupying functionally similar (although not geometrically symmetrical) positions in the molecule.

The significance of this generalization may be made clear by considering the melting of double-stranded DNA. This is a typically co-operative phenomenon the co-operativity of which is evidently dependent upon and expresses the (helical) symmetry of the "domain of bonding" between the two strands in the Watson-Crick model. In the last analysis therefore, the axial symmetry requirement for homotropic co-operative effects to occur with a globular protein, when *stereospecific* ligands are concerned, reflects essentially the fact that, in general, only one stereospecific site able to bind such a ligand exists on a protein monomer or protomer.

Gerhart (1964) and Schachman (1964) have recently reported the successful separation, from crystalline aspartic transcarbamylase, of two different subunits, one of which bears the specific receptor for aspartate, and the other the receptor for CTP. It is very tempting to speculate on the possibility that this remarkable and so far unique observation may in fact correspond to a general rule, namely, that a protein should contain as many different subunits (peptide chains) as it bears stereospecifically different receptor sites. The emergence and evolution of such structures, by association of primitively distinct entities, would be much easier to understand than the acquisition of a new stereospecific site by an already existing and functional enzyme made up of a single type of subunit.

We have so far not discussed one of the major assumptions of the model, namely, that allosteric effects are due to the displacement of an equilibrium between discrete states assumed to exist, at least potentially, apart from the binding of a ligand. The main value of this treatment is to allow one to define, in terms of the allosteric constant, the contribution of the protein itself to the interaction, as distinct from the dissociation constants of the ligands. This distinction is a useful and meaningful one, as we have seen, and its validity is directly justified by the fact that the affinity of a ligand may vary widely without any alteration of its homotropic interaction coefficient (cf. page 103). But it should be understood that the "state" of the protein may not in fact be exactly the same whether it is actually bound, or unbound, to the ligand which stabilizes it. In this sense particularly, the model offers only an over-simplified first approximation of real systems, and it may prove possible in some cases to introduce corrections and refinements by taking into consideration more than two accessible states.

We feel, however, that the main interest of the model which we have discussed here does not reside so much in the possibility of describing quantitatively and in detail the complex kinetics of allosteric systems. It rests rather on the concept, which we have

tried to develop and justify, that a general and initially simple relationship between symmetry and function may explain the emergence, evolution and properties of oligomeric proteins as "molecular amplifiers", of both random structural accidents and of highly specific, organized, metabolic interactions.

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