J. Mol. Biol. (1965) **12**, 88–118

On the Nature of Allosteric Transitions: A Plausible Model

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(*Received 30 December 1964*)

"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 $R_0 \rightleftharpoons T_0$ 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 $R_0, R_1, R_2, \ldots R_n$; $T_0, T_1, T_2,$

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

$$\begin{array}{lll} & R_0 \longleftrightarrow T_0 & \\ R_0 + F \longleftrightarrow R_1 & & T_0 + F \longleftrightarrow T_1 \\ R_1 + F \longleftrightarrow R_2 & & T_1 + F \longleftrightarrow T_2 \\ \cdots\cdots\cdots\cdots & & \cdots\cdots\cdots\cdots \\ R_{n-1} + F \longleftrightarrow R_n & & T_{n-1} + F \longleftrightarrow T_n \end{array}$$

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

$$T_0 = LR_0$$

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

Let us now define two functions corresponding respectively to:

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

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

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

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

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" $\bar{R}$:

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

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

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

In Fig. 1(a) and (b), theoretical curves of the 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