Biophysical Chemistry, 30 (1988) 159–172
Elsevier

BPC 01255

Linked-function origins of cooperativity in a symmetrical dimer

Gregory D. Reinhart

Department of Chemistry, University of Oklahoma, Norman, OK 73019, U.S.A.

Received 23 December 1987
Accepted 2 February 1988

Cooperativity; Hill coefficient; Allosterism; Thermodynamic linkage; Protein-ligand interaction

The thermodynamic origins of substrate binding cooperativity in a dimeric enzyme that can bind one substrate (A) and one allosteric ligand (X) to each of two identical subunits are discussed. It is assumed that maximal activity is not subject to allosteric modification and that the substrates and allosteric ligands achieve binding equilibrium in the steady state. Each uniquely ligated form is assumed to be capable of exhibiting unique binding properties, and only the principles of thermodynamic linkage are used to constrain the system further. The explicit relationship between the Hill coefficient, the concentration of X, and the magnitudes of the relevant coupling free energies and dissociation constants is derived. In the absence of X only the homotropic coupling between substrate sites contributes to a nonhyperbolic substrate saturation profile. An allosteric ligand, X, can alter the cooperativity in two distinct ways, one mechanism being manifested when X is saturating and the other only when X is present at subsaturating concentrations. By evaluating the concentration of substrate required to produce half-maximal velocity as a function of [X], as well as the Hill coefficients when X is absent and fully saturating, the dissociation and coupling constants most important for understanding the mechanisms of allosteric action in an enzyme of this type can be determined.

1. Introduction

Allosteric modification of enzyme activity often involves the generation of cooperativity in the enzyme's response to substrate concentration. In this communication cooperativity is defined to be a nonhyperbolic substrate or ligand saturation profile. While a cooperative response function is not a defining characteristic of allosterism, it often is taken to be such because of the frequency with which it is a feature of allosteric behavior.

Several different mechanisms can lead to apparent cooperativity in a substrate saturation profile. Mechanistically trivial reasons include concentration-dependent changes in the activity coefficient of the substrate, substrate-induced changes in the association state (that leads to a change in activity) of the enzyme, and a heterogeneous population of isozymes with differing affinities for the substrate. We are primarily interested in cooperativity that results from ligand-mediated alterations of the enzyme structure.

Allosteric ligands can influence either the maximal turnover rate (V-type effectors) or the affinity of the enzyme for substrate (K-type effectors) through conformational effects, and both of these modes of action can lead to cooperativity. Since K-type effectors are commonly encountered, we feel it useful to examine more closely the nature of the cooperative effect that can result from ligand-ligand interactions on an enzyme that induce alterations in ligand-protein affinity.

Largely because of the extensive literature on the allosteric properties of hemoglobin, which is strictly a binding protein, most modeling of enzymatic K-type allosteric behavior adopts a thermodynamic premise. In other words, the rapid-equilibrium assumption is usually invoked, either

Correspondence address: G.D. Reinhart, Department of Chemistry, University of Oklahoma, Norman, OK 73019, U.S.A.

0301-4622/88/$03.50 © 1988 Elsevier Science Publishers B.V. (Biomedical Division)

explicitly or implicitly. Such an approach is reasonable; the rapid-equilibrium assumption provides a simplification that usually describes observable data satisfactorily and greatly simplifies otherwise exceedingly complex steady-state rate equations that would otherwise be virtually useless. Nevertheless, one must be cognisant of the assumption and the possible deviations in behavior that might be introduced by a steady-state mechanism in which thermodynamic relationships do not hold. By thoroughly knowing the behavior characteristics predicted by a thermodynamic-based mechanism one is in a better position to recognize these potential deviations.

The purpose of this communication is to consider in particular the nature of the interactions that exist in a symmetrical dimeric enzyme with a single binding site for substrate, A, and allosteric ligand, X, on each of the two identical subunits, and how the interactions between these ligands contribute to cooperativity. Our analysis will not invoke the possible existence of tautomeric forms with identical states of ligation existing in true equilibrium because they do not impact the form of the rate equation and cannot be revealed from kinetics or binding measurements [1]. Rather, we will only utilize the principles of thermodynamic linkage [2–6] to simplify the resulting equations.

Most saturation profiles that exhibit cooperative behavior can be presented in the form of a 'Hill plot' of $\log(v/(V_{max}-v))$ vs. log[substrate]. This plot is convenient because it provides a linear presentation of rate data obtained over most of the saturation curve. The 'degree' of cooperativity is usually described with reference to the 'Hill coefficient' that is equal to the slope of a Hill plot at half-saturation. At very high and very low substrate concentrations a Hill plot will asymptotically approach a slope of unity regardless of the nature and degree of cooperativity. Interpreting a Hill plot by analyzing the distance between asymptotes has been discussed previously [4]. However, in practice this approach is rarely utilized because of the large error in the Hill plot associated with data that approach asymptotic behavior. We will evaluate how the more easily obtainable Hill coefficient relates to the magnitude of ligand-ligand interactions on a protein dimer.

2. Thermodynamic linkage

Thermodynamic linkage refers to the fact that if two ligands, X and Y, bind to discrete sites on a protein, and if the affinity of the protein for X is altered when Y is bound, then the affinity of the protein for Y must be altered by the same amount when X is bound. Consequently, the binding interactions of these two ligands with the protein are 'linked' by the magnitude of this mutual, protein-mediated interaction. This concept was first articulated by Wyman [2–4], but its ramifications have been more recently discussed by Weber [5,6], who also made the important contribution of restating the argument in terms of free energy.

In the discussion that follows, we first extend the principles of thermodynamic linkage to the analysis of a protein that can bind either two, three or four different ligands to corresponding individual binding sites. We then utilize the resulting relationships to consider specifically a symmetrical, nondissociating dimeric enzyme binding one substrate and one allosteric ligand per subunit, paying particular attention to the origins of nonhyperbolic substrate saturation behavior. In so doing, several behavioral characteristics are illuminated that are not anticipated by analyses in which the ramifications of thermodynamic linkage are obscured.

2.1. Two-ligand couplings

The binding of two ligands, X and Y, to different sites on a protein, P, can be represented by the reaction:

$$P + X + Y \rightleftharpoons X - P - Y \qquad (1)$$

In reality, reaction 1 never occurs via a concerted mechanism, but rather it is accomplished in steps with either ligand X binding first, followed by Y, or Y binding first followed by X. The total standard free energy change, ΔG_t^0, that results from reaction 1 is equal, therefore, to the sum of the standard free energy changes for each step in either mechanism:

$$\Delta G_t^0 = \Delta G_x^0 + \Delta G_{y/x}^0 = \Delta G_y^0 + \Delta G_{x/y}^0 \qquad (2)$$

where ΔG_x^0 and ΔG_y^0 equal the standard binding free energies of X and Y with free (unligated) protein, respectively, and $\Delta G_{x/y}^0$ and $\Delta G_{y/x}^0$ equal the standard binding free energies of X and Y, respectively, with protein to which the other ligand, Y and X, respectively, has already bound.

The coupling free energy, ΔG_{xy}, between two ligands, X and Y, that bind to different sites on a single protein molecule has been defined by Weber [5] to be equal to the difference between the actual sum of the two individual binding free energies (i.e., the free energy change for reaction 1) and the sum of each individual binding free energy to free protein:

$$\Delta G_{xy} = \left(\Delta G_x^0 + \Delta G_{y/x}^0\right) - \left(\Delta G_x^0 + \Delta G_y^0\right)$$
$$= \left(\Delta G_y^0 + \Delta G_{x/y}^0\right) - \left(\Delta G_x^0 + \Delta G_y^0\right) \quad (3)$$

$$\text{or } \Delta G_{xy} = \Delta G_{y/x}^0 - \Delta G_y^0 = \Delta G_{x/y}^0 - \Delta G_x^0 \quad (4)$$

Thus, it is apparent that the coupling free energy quantitatively describes the degree to which the binding of one ligand influences the standard free energy of binding of the other ligand and vice versa. Consequently, the order of ligand binding that is explicitly considered, and hence the order in which the subscripts are written for the coupling free energy expression, is arbitrary. By convention, and according to the above definition, if the two ligands antagonize one another's binding, then the coupling free energy will be positive, and if they enhance one another's binding affinity then ΔG_{xy} will be negative. (Note that the superscript denoting standard state is dropped from the coupling free energy notation by convention [6].)

2.2. Multiple ligand couplings

The general situation when three or more different ligands bind is analogous. A coupling term exists that represents the difference between the actual free energy change upon the binding of all ligands and that calculated from each ligand's interaction with free (unligated) protein.

For three ligands, X, Y and Z, this coupling term, denoted ΔG_{xyz}, would be given by:

$$\Delta G_{xyz} = \left(\Delta G_x^0 + \Delta G_{y/x}^0 + \Delta G_{z/xy}^0\right) - \left(\Delta G_x^0 + \Delta G_y^0 + \Delta G_z^0\right) \quad (5)$$

where $\Delta G_{z/xy}^0$ equals the binding free energy of Z after X and Y have already bound. Once again, any order of ligand binding may be considered with equivalent results.

For a protein binding three ligands, the magnitude of the coupling between each two ligands may in principle be dependent upon whether the third ligand is bound. That is to say, ΔG_{yz}, for example, will not in general be equal to $\Delta G_{yz/x}$ where $\Delta G_{yz/x}$ is equal to the coupling between Y and Z when X is present on the protein. Accordingly, $\Delta G_{yz/x}$ is defined by the following:

$$\Delta G_{yz/x} = \Delta G_{y/xz}^0 - \Delta G_{y/x}^0 = \Delta G_{z/xy}^0 - \Delta G_{z/x}^0 \quad (6)$$

By combining eqs. 4–6 one can easily derive the following identity that describes the relationship between ΔG_{xyz} and the two ligand couplings that exist when a total of three ligands bind (as described previously [7]):

$$\Delta G_{xyz} = \Delta G_{xy} + \Delta G_{xz} + \Delta G_{yz/x} \quad (7)$$

If four ligands, W, X, Y and Z, bind to a protein molecule, the four ligand coupling free energy, ΔG_{wxyz}, is equal to the actual binding free energy of all ligands minus the sum of the free energies for each ligand binding to free protein.

$$\Delta G_{wxyz} = \left(\Delta G_w^0 + \Delta G_{x/w}^0 + \Delta G_{y/wx}^0 + \Delta G_{z/wxy}^0\right) - \left(\Delta G_w^0 + \Delta G_x^0 + \Delta G_y^0 + \Delta G_z^0\right) \quad (8)$$

Reducing ΔG_{wxyz} to two ligand coupling terms using the same procedures as used for deriving eq. 6 results in:

$$\Delta G_{wxyz} = \Delta G_{wx} + \Delta G_{wy} + \Delta G_{wz} + \Delta G_{xy/w} + \Delta G_{xz/w} + \Delta G_{yz/wx} \quad (9)$$

where $\Delta G_{yz/wx}$ represents the coupling free energy between Y and Z when W and X are already bound. By combining eqs. 7 and 9, the following, somewhat more general, relationship can also be derived:

$$\Delta G_{wxyz} = \Delta G_{wxy} + \Delta G_{wxz} + \Delta G_{yz/wx} - \Delta G_{wx} \quad (10)$$

Finally, rate equations, being akin to saturation expressions, do not contain free energy terms but rather the corresponding equilibrium constants.

The comparable equilibrium form of a coupling free energy is defined to be Q such that:

$$\Delta G_{xy} = -RT \ln(Q_{xy}) \tag{11}$$

where R is the gas constant and T the absolute temperature. It should be noted that Q terms are multiplied to arrive at an expression equivalent to one in which ΔG terms are added. For example, eq. 9 is equivalent to:

$$Q_{wxyz} = Q_{wx}Q_{wy}Q_{wz}Q_{xy/w}Q_{xz/w}Q_{yz/wx} \tag{12}$$

Similarly, the definition of a two ligand coupling free energy given in eq. 4 is equivalent to:

$$Q_{xy} = \frac{K_x}{K_{x/y}} = \frac{K_y}{K_{y/x}} \tag{13}$$

where K_x and K_y are dissociation constants of X and Y, respectively. $K_{x/y}$ and $K_{y/x}$ are dissociation constants of X and Y, respectively, from protein to which the other ligand remains bound.

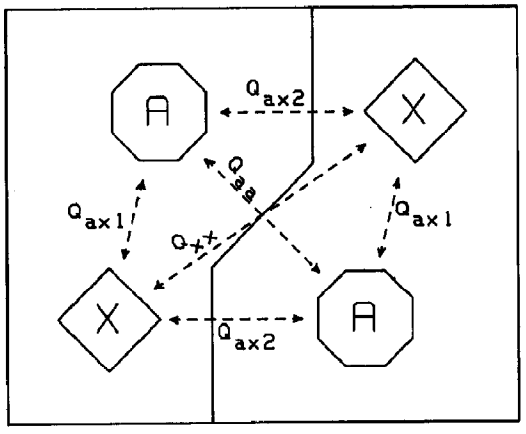

Fig. 1. Schematic diagram depicting the possible two ligand interactions (dashed double-headed arrows) on a symmetrical dimer with one binding site for substrate A and one binding site for allosteric ligand X on each subunit. The coupling constants (Q terms) are related to the corresponding coupling free energies between the indicated ligands by eq. 11 as described in the text. Note that two different types of A-X interactions exist: intrasubunit, denoted Q_{ax1}, and intersubunit, denoted Q_{ax2}. All couplings, except the intrasubunit A-X couplings (Q_{ax1}), must cross the subunit-subunit interface. The magnitude of each of the interactions depicted is potentially altered by the occupancy of one or both of the other two binding sites not involved directly in the interaction.

2.3. Couplings in a symmetrical dimeric enzyme

The two ligand couplings that exist in a symmetrical dimer of identical subunits containing a single substrate (A) site and a single allosteric (X) site per subunit are depicted in fig. 1. There are four different basic types of two ligand couplings: A-A, X-X, intrasubunit A-X, and intersubunit A-X and the corresponding coupling constants are denoted as Q_{aa}, Q_{xx}, Q_{ax1}, and Q_{ax2}, respectively. The magnitude of each of these two ligand couplings may be altered when ligands are bound to the other sites. Consequently, in the general case the following couplings must also be considered: $Q_{aa/x}$, $Q_{aa/xx}$, $Q_{xx/a}$, $Q_{xx/aa}$, $Q_{ax1/a}$, $Q_{ax1/x}$, $Q_{ax1/ax}$, $Q_{ax2/a}$, $Q_{ax2/x}$ and $Q_{ax2/ax}$. Fortunately, these constants are not all independent, but are related by the multiple ligand coupling constants, according to the general relationships just described, as follows.

A symmetrical dimer generates two types of three ligand couplings, Q_{aax} and Q_{axx}, which can be reduced to two ligand couplings by referring to eq. 7. Therefore, we can write the following identities:

$$\begin{aligned} Q_{aax} &= Q_{aa}Q_{ax1}Q_{ax2/a} \\ &= Q_{aa}Q_{ax2}Q_{ax1/a} \\ &= Q_{ax1}Q_{ax2}Q_{aa/x} \end{aligned} \tag{14}$$

$$\begin{aligned} Q_{axx} &= Q_{xx}Q_{ax1}Q_{ax2/x} \\ &= Q_{xx}Q_{ax2}Q_{ax1/x} \\ &= Q_{ax1}Q_{ax2}Q_{xx/a} \end{aligned} \tag{15}$$

The multiple identities arise because the order of the subscripts is arbitrary due to the equivalence of the various sequences of ligand binding steps which can result in three bound ligands.

Similarly, the unique four ligand coupling parameter, Q_{aaxx}, can generate several identities, of which the following will prove to be the most useful. From eq. 10 we can write:

$$\begin{aligned} Q_{aaxx} &= \frac{Q_{axx}Q_{axx}Q_{aa/xx}}{Q_{xx}} \\ &= \frac{Q_{aax}Q_{aax}Q_{xx/aa}}{Q_{aa}} \end{aligned} \tag{16}$$

Substituting the final identity depicted in eq. 14 into eq. 16 yields:

$$Q_{aaxx} = \frac{Q_{ax1}^2 Q_{ax2}^2 Q_{aa/x}^2 Q_{xx/aa}}{Q_{aa}} \tag{17}$$

Finally, by combining the various identities in eqs. 14–16 one can derive the following relationship:

$$\frac{Q_{aa} Q_{aa/xx}}{(Q_{aa/x})^2} = \frac{Q_{xx} Q_{xx/aa}}{(Q_{xx/a})^2} \tag{18}$$

This last identity now enables us to rewrite eq. 17 as follows:

$$Q_{aaxx} = Q_{ax1}^2 Q_{ax2}^2 Q_{xx/a}^2 \left[\frac{Q_{aa/xx}}{Q_{xx}} \right] \tag{19}$$

3. Rate equations

The rate equation for a rapid-equilibrium dimeric enzyme with two substrate (A) binding sites and two allosteric ligand (X) binding sites can be derived using conventional approaches [8]. Briefly, the numerator consists of a sum that will contain a term for each ligation state of the enzyme that can potentially lead to turnover, i.e., all enzyme forms containing at least one bound A. Each of these terms is multiplied by a parameter corresponding to the potentially unique maximal velocity or turnover number associated with that enzyme form (denoted by V_a, V_{ax1}, V_{ax2}, V_{axx}, V_{aa}, V_{aax1}, V_{aax2}, and V_{aaxx}). In the case of the enzyme form containing two bound As and one bound X, two maximal velocity terms exist, V_{aax1} and V_{aax2}, corresponding to turnover of the A occurring on the same or opposite subunit, respectively, as that to which the X is bound. The denominator is also a sum of terms representing all possible ligation forms of the enzyme including those that do not contain A. Each term in the numerator and denominator will consists of the product of the free concentration of each bound ligand divided by its actual dissociation constant and multiplied by the integral number of ways that enzyme form can be achieved. For example, one X can bind to either of two sites on the enzyme and so the terms containing a single [X] factor will also be multiplied by 2. The product of actual dissociation constants for ligands in a multiligated enzyme form is obtained by dividing the product of the dissociation constants for each ligand when bound singly to unligated enzyme by the corresponding coupling constant.

For example, let K_a^0 equal the reciprocal of the binding constant of a single A binding to free enzyme in the absence of X, the affinity of either site being initially identical. Let K_{ix}^0 be equal to the comparable dissociation constant of a single X from either binding site when [A] = 0. The product of the actual dissociation constants of A and X when they are bound to the same subunit is then given by $K_a^0 K_{ix}^0 / Q_{ax1}$ according to eqs. 3 and 11. Similarly, the product of the actual dissociation constants pertaining to the enzyme form containing two As and one X bound is $(K_a^0)^2 K_{ix}^0 / Q_{aax}$ according to eqs. 5 and 11.

Following this general outline, the following rate equation will be obtained:

$$\begin{aligned} v = {} & \{ (V_a + [V_{ax1} Q_{ax1} + V_{ax2} Q_{ax2}] X \\ & + V_{axx} Q_{axx} X^2) A + (V_{aa} Q_{aa} \\ & + [V_{aax1} + V_{aax2}] Q_{aax} X + V_{aaxx} Q_{aaxx} X^2) A^2 \} \\ & \times \{ (1 + 2X + Q_{xx} X^2) \\ & + 2(1 + [Q_{ax1} + Q_{ax2}] X + Q_{axx} X^2) A \\ & + (Q_{aa} + 2Q_{aax} X + Q_{aaxx} X^2) A^2 \}^{-1} \end{aligned} \tag{20}$$

where

$$A = [A]/K_a^0$$

$$X = [X] K_{ix}^0$$

If all of the intersubunit couplings depicted in fig. 1 were nonexistent, only ΔG_{ax1} would retain a finite value and the coupling constants appearing in eq. 20 would assume the following values: $Q_{aa} = Q_{xx} = Q_{ax2} = 1$, $Q_{aax} = Q_{axx} = Q_{ax1}$ and $Q_{aaxx} = Q_{ax1}^2$. If, in addition, turnover was similarly unaffected by the presence of bound ligands on the opposite subunit, then $V_{aax1} = V_{axx} = V_{aaxx} = V_{ax1}$ and $V_{aa} = V_{ax2} = V_{aax2} = V_a$. Substituting

these identities into eq. 20 reduces it to the following:

$$v = \frac{V_a A + V_{ax1} Q_{ax1} XA}{1 + X + A + Q_{ax1} XA} \tag{21}$$

Eq. 21 is equal to the rapid-equilibrium rate equation describing the case of a single substrate and a single allosteric ligand binding to a monomeric enzyme [7,9,10]. No cooperativity is predicted by this equation for either K-type ($Q_{ax1} \neq 1$) or V-type ($V_{ax1} \neq V_a$) allosteric effects [5–7,9,10]. Consequently, we see immediately that cooperativity, unlike the allosterically induced V-type or K-type activation or inhibition per se, is the specific result of intersubunit interactions in a dimeric enzyme.

For the following discussion we assume that the maximal velocity is the same for each of these enzyme forms and equal to V_m. In addition, let us define the parameter Q_{ax} to be equal to the geometric mean of the inter- and intrasubunit couplings between A and X.

$$Q_{ax} = (Q_{ax1} Q_{ax2})^{1/2} \tag{22}$$

The following expression results from substituting the identities, eqs. 14, 15, and 19 and 22 into eq. 20.

$$\begin{aligned}\frac{v}{V_m} = {} & \left\{\left(1 + [Q_{ax1} + Q_{ax2}]X + Q_{ax}^2 Q_{xx/a} X^2\right)A \right. \\ & + \left(Q_{aa} + 2Q_{ax}^2 Q_{aa/x} X \right. \\ & \left. \left. + Q_{ax}^4 Q_{xx/a}^2 \left[Q_{aa/xx}/Q_{xx}\right] X^2\right) A^2 \right\} \\ & \times \left\{\left(1 + 2X + Q_{xx} X^2\right) \right. \\ & + 2\left(1 + [Q_{ax1} + Q_{ax2}]X + Q_{ax}^2 Q_{xx/a} X^2\right)A \\ & + \left(Q_{aa} + 2Q_{ax}^2 Q_{aa/x} X \right. \\ & \left. \left. + Q_{ax}^4 Q_{xx/a}^2 \left[Q_{aa/xx}/Q_{xx}\right] X^2\right) A^2 \right\}^{-1}\end{aligned} \tag{23}$$

4. Cooperativity

4.1. Hill coefficient

An expression relating the Hill coefficient to the parameters appearing in eq. 23 can be derived as follows. Eq. 23 can be rewritten in the form:

$$\frac{v}{V_m} = \frac{\alpha A + \alpha\beta A^2}{1 + 2\alpha A + \alpha\beta A^2} \tag{24}$$

where

$$\alpha = \frac{\left(1 + [Q_{ax1} + Q_{ax2}]X + Q_{ax}^2 Q_{xx/a} X^2\right)}{\left(1 + 2X + Q_{xx} X^2\right)} \tag{25}$$

$$\beta = \frac{\left(Q_{aa} + 2Q_{ax}^2 Q_{aa/x} X + Q_{ax}^4 Q_{xx/a}^2 \left[\frac{Q_{aa/xx}}{Q_{xx}}\right] X^2\right)}{\left(1 + [Q_{ax1} + Q_{ax2}]X + Q_{ax}^2 Q_{xx/a} X^2\right)} \tag{26}$$

Let R be the quantity defined by eq. 27:

$$R = \frac{v/V_m}{1 - v/V_m} = \frac{\alpha A + \alpha\beta A^2}{1 + \alpha A} \tag{27}$$

A Hill plot is constructed by plotting log R vs. log[A]. The slope of the Hill plot is then given by:

$$\begin{aligned}\frac{\partial(\log R)}{\partial(\log[A])} &= \left[\frac{\partial([A])}{\partial(\log[A])}\right]\left[\frac{\partial(\log R)}{\partial(R)}\right]\left[\frac{\partial(R)}{\partial([A])}\right] \\ &= \frac{1 + 2\beta A + \alpha\beta A^2}{1 + (\alpha + \beta)A + \alpha\beta A^2}\end{aligned} \tag{28}$$

Note that eq. 28 predicts that the slope of a Hill plot will approach a value of unity as [A] becomes either very small or very large.

The Hill coefficient is defined to be equal to the slope of the Hill plot when $v = V_m/2$, in which case $R = 1$. Solving eq. 27 for A when $R = 1$ yields:

$$A = \frac{1}{(\alpha\beta)^{1/2}} \tag{29}$$

Therefore, by substituting eq. 29 into eq. 28 one obtains the general analytical expression for calculating the Hill coefficient:

$$\text{Hill coefficient} = n_H = \frac{2(\beta/\alpha)^{1/2}}{1 + (\beta/\alpha)^{1/2}} \tag{30}$$

4.2. Homotropic cooperativity

Homotropic cooperativity occurs when the binding of one A to one of the two sites with

initially identical affinity alters the affinity of the remaining site for the second A to bind. Positive cooperativity results if the affinity is increased. Negative cooperativity arises if the subsequent affinity decreases. The magnitude by which the binding of the first A alters the affinity of the second A is equal to the coupling constant Q_{aa} by definition (see eq. 13). Since homotropic cooperativity arises from a direct coupling between the binding of the two substrate molecules on the dimer; i.e., when $\Delta G_{aa} \neq 0$ ($Q_{aa} \neq 1$); this coupling will produce cooperativity at all concentrations of X. However, only homotropic coupling remains in the general rate expression when $X = 0$:

$$\lim_{X \to 0}\left[\frac{v}{V_m}\right] = \frac{A + Q_{aa}A^2}{1 + 2A + Q_{aa}A^2} \tag{31}$$

Therefore, according to eq. 30 and recognizing that $\alpha = 1$ and $\beta = Q_{aa}$ in eq. 31, the Hill coefficient in the absence of X is given by:

$$n_H = \frac{2(Q_{aa})^{1/2}}{1 + (Q_{aa})^{1/2}} \tag{32}$$

Eq. 32 is consistent with the conventional interpretation of Hill coefficients greater than 1 in which the Hill coefficient is interpreted as being equal to or less than the number of interacting sites on the enzyme. In fact, only an infinite, negative coupling free energy would result in a Hill coefficient of 2 for a protein dimer. It should also be noted that eqs. 31 and 32 also hold for antagonistic couplings ($Q_{aa} < 1$) which give rise to negative cooperativity and Hill coefficients of less than 1 and for which conventional quantitative interpretations are not as straightforward. A plot of the magnitude of the Hill coefficient vs. the value of the coupling constant, Q_{aa}, or the coupling free energy, ΔG_{aa}, that is predicted by eq. 32 is shown in fig. 2.

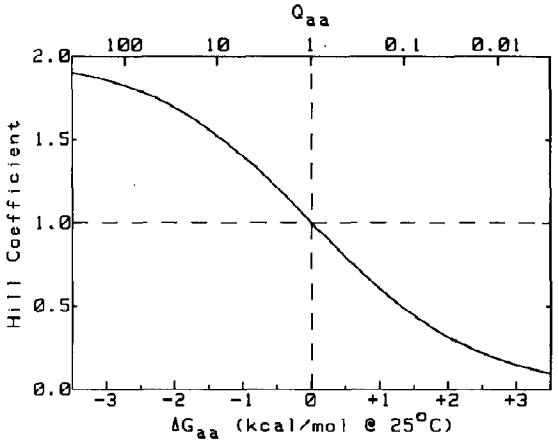

Fig. 2. Hill coefficient induced by homotropic coupling between two substrate binding sites in the absence of X vs. the magnitude of the coupling expressed in terms of either the coupling free energy at 25°C (bottom axis) or the coupling constant Q_{aa} (top axis). Note that an antagonistic coupling ($\Delta G_{aa} > 0$, $Q_{aa} < 1$) produces a Hill coefficient less than 1 whereas a coupling that facilitates binding ($\Delta G_{aa} < 0$, $Q_{aa} > 1$) produces a Hill coefficient that is greater than 1. The curve was calculated according to eq. 32 described in the text.

4.3. Heterotropic cooperativity

Heterotropic cooperativity is by definition a cooperative response that is generated by the binding of an allosteric ligand. There are two distinct classes of heterotropic cooperativity that need to be considered: subsaturating heterotropic cooperativity and heterotropically induced homotropic cooperativity.

4.3.1. Heterotropically induced homotropic cooperativity

Heterotropically induced homotropic cooperativity arises when the binding of X alters the magnitude of the direct coupling between the two A sites. While Q_{aa} represents this coupling in the absence of X, $Q_{aa/x}$ and $Q_{aa/xx}$ are equal to the homotropic coupling when either one or two allosteric ligands are bound, respectively. These three homotropic coupling parameters need not be equal. If $Q_{aa} = 1$ and $Q_{aa/xx} \neq 1$, then fully saturating with X will induce homotropic cooperativity. If $Q_{aa} \neq 1$ but $Q_{aa/xx} = 1$ then X abolishes homotropic cooperativity. If both $Q_{aa} \neq 1 \neq Q_{aa/xx}$ then saturating X simply alters the cooperativity by this mechanism.

Heterotropically induced homotropic cooperativity is the only linked-function contributor to

cooperativity when X is saturating, as can be seen by considering eq. 23 as X approaches infinity:

$$\lim_{X\to\infty}\left[\frac{v}{V_m}\right] = \left\{Q_{ax}^2 Q_{xx/a} A + Q_{ax}^4 Q_{xx/a}^2 \left[Q_{aa/xx}/Q_{xx}\right] A^2\right\} \times \left\{Q_{xx} + 2Q_{ax}^2 Q_{xx/a} A + Q_{ax}^4 Q_{xx/a}^2 \left[Q_{aa/xx}/Q_{xx}\right] A^2\right\}^{-1} \quad (33)$$

Eq. 33 can be written in the form of eq. 24 where

$$\alpha = Q_{ax}^2\left[\frac{Q_{xx/a}}{Q_{xx}}\right]$$

$$\beta = Q_{aa/xx}Q_{ax}^2\left[\frac{Q_{xx/a}}{Q_{xx}}\right]$$

The Hill coefficient is therefore given by the following according to eq. 30:

$$n_H = \frac{2\left(Q_{aa/xx}\right)^{1/2}}{1+\left(Q_{aa/xx}\right)^{1/2}} \quad (34)$$

Since $Q_{aa/xx}$ can be either greater or less than 1, X can induce either positive or negative cooperativity. Also, since $Q_{aa/xx}$ may be either greater or less than Q_{aa}, the cooperativity at saturating concentrations of X can be either increased or decreased relative to the cooperativity exhibited when [X] = 0. Note that since eq. 34 is analogous to eq. 32, the curve in fig. 2 also describes the relationship between the Hill coefficient and $Q_{aa/xx}$ or $\Delta G_{aa/xx}$ when [X] is saturating.

4.3.2. Subsaturating heterotropic cooperativity

Subsaturating heterotropic cooperativity arises as a direct consequence of a nonzero intrasubunit and intersubunit heterotropic coupling free energy between A and X; i.e., Q_{ax1} and $Q_{ax2} \neq 1$ implying that $Q_{ax} \neq 1$. The effects of this type of cooperativity are most easily revealed if we assume for the moment that no homotropic couplings exist, i.e., that $Q_{aa} = Q_{aa/x} = Q_{aa/xx} = Q_{xx} = Q_{xx/a} = 1$. Under such a circumstance no cooperativity will be apparent in the substrate binding profile when [X] = 0 or [X] is saturating as was shown above. However, the condition that Q_{ax1} and $Q_{ax2} \neq 1$ is sufficient to ensure that the Hill coefficient will differ from unity at subsaturating concentrations of X. Moreover, this cooperativity will always be positive regardless of the nature of the interaction between A and X, as Weber first pointed out [5,6], as long as both intra- and intersubunit coupling free energies have the same sign (see below). Both an antagonistic relationship ($Q_{ax} < 1$) and a facilitating relationship ($Q_{ax} > 1$) will produce $n_H > 1$.

This latter prediction can be intuitively rationalized in the following manner. If the binding of X lowers the dissociation constant for A, then the converse must also be true and the binding of A facilitates the binding of X. In the presence of a subsaturating concentration of X, as the first equivalent of A binds, the binding of X is encouraged and hence a greater degree of X saturation will exist after the first equivalent of A has bound. Consequently, the dissociation constant for the second equivalent of A will be smaller, resulting in apparent positive cooperativity. When A and X antagonize each others binding, the binding of the first equivalent of A will decrease the degree of X saturation. Since the influence of an antagonizer of binding has been diminished, the binding of the second equivalent of A will be facilitated in comparison to the binding of the first equivalent, and once again apparent positive cooperativity will result.

This result can be derived formally by substituting our boundary conditions into eqs. 25, 26 and 30:

$$\alpha = \frac{1+\left[Q_{ax1}+Q_{ax2}\right]X+Q_{ax}^2X^2}{1+2X+X^2} = \frac{(1+Q_{ax1}X)(1+Q_{ax2}X)}{(1+X)^2} \quad (35)$$

$$\beta = \frac{1+2Q_{ax}^2+Q_{ax}^4X^2}{1+\left[Q_{ax1}+Q_{ax2}\right]X+Q_{ax}^2X^2} = \frac{\left(1+Q_{ax}^2X\right)^2}{(1+Q_{ax1}X)(1+Q_{ax2}X)} \quad (36)$$

$$n_H = \frac{2+2\left(Q_{ax}^2+1\right)X+2Q_{ax}^2X^2}{2+\left(Q_{ax}^2+Q_{ax1}+Q_{ax2}+1\right)X+2Q_{ax}^2X^2} \quad (37)$$

Eq. 37 predicts that cooperativity resulting from a finite A-X coupling will disappear when [X] = 0 or [X] is saturating because the Hill coefficient will approach a value of unity as these two limiting conditions are approached. It can be easily shown that $2(Q_{ax}^2 + 1) > (Q_{ax}^2 + Q_{ax1} + Q_{ax2} + 1)$ for all values of Q_{ax1} and Q_{ax2} provided that the following relationship holds true:

$$(Q_{ax1} - 1)(Q_{ax2} - 1) > 0 \tag{38}$$

Therefore, the Hill coefficient at subsaturating concentrations of X is always greater than 1 whether A and X antagonize each other's binding (Q_{ax1}, $Q_{ax2} < 1$) or A and X facilitate one another's binding (Q_{ax1}, $Q_{ax2} > 1$) as long as the natures of the intra- and inter-subunit couplings are the same (see fig. 3). In addition, if there is no A-X coupling either within each subunit or between subunits (i.e., if either $Q_{ax1} = 1$ or $Q_{ax2} = 1$) then no subsaturating heterotropic cooperativity will occur.

If one assumes that $Q_{ax1} = Q_{ax2} = Q_{ax}$, then the concentration of X that produces the maximum Hill coefficient can be determined by taking the partial derivative of eq. 37 with respect to X,

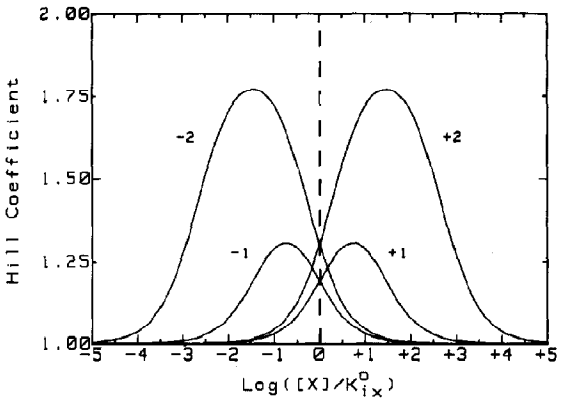

Fig. 3. Hill coefficient arising strictly from coupling between A and X ligands vs. log([X]/K_{ix}^{0}). Curves were calculated according to eq. 37 for an average A-X coupling free energy (ΔG_{ax}) equal to −2, −1, 1 and 2 kcal/mol at 25 °C as indicated on the figure. Note how the cooperativity vanishes at very low and very high concentrations of X in all cases.

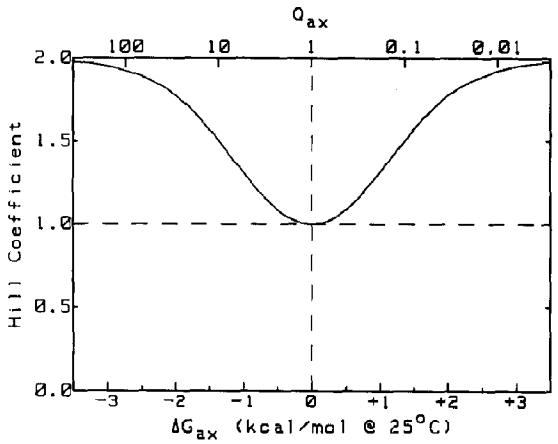

Fig. 4. Maximum Hill coefficient resulting from subsaturating heterotropic cooperativity vs. the average magnitude of the coupling between A and X. The curve was calculated according to eq. 40 when [X] = K_{ix}^{0}/Q_{ax} as described in the text. Note that both an antagonistic coupling ($\Delta G_{ax} > 0$, $Q_{ax} < 1$) and a facilitating coupling ($\Delta G_{ax} < 0$, $Q_{ax} > 1$) induce a Hill coefficient greater than 1.

setting the resulting expression equal to zero, and solving for X. The result is:

$$X = \frac{1}{Q_{ax}} \tag{39}$$

Substituting eq. 39 back into eq. 37 yields an expression describing the relationship between the maximum Hill coefficient and Q_{ax}:

$$n_H = \frac{2(1 + Q_{ax})(1 + 1/Q_{ax})}{4 + (1 + Q_{ax})(1 + 1/Q_{ax})} \tag{40}$$

It is immediately apparent from eq. 40 that identical values for the maximum Hill coefficient are obtained for any value of Q_{ax} and its reciprocal as realized by Weber [6] and illustrated graphically in fig. 4.

In general, subsaturating heterotropic cooperativity causes homotropic cooperativity to increase, but this does not necessarily imply that the Hill coefficient will become greater than 1. In the case of strongly negative homotropic cooperativity ($Q_{aa} \ll 1$), the subsaturating effect will only increase the Hill coefficient to a larger value that may still be less than unity. This can be quantita-

tively seen by solving for n_H when $Q_{aa} = Q_{aa/xx} \neq 1$ and $X = 1/Q_{ax}$ which yields:

$$n_H = \frac{2(1+Q_{ax})(1+1/Q_{ax})(Q_{aa})^{1/2}}{4+(1+Q_{ax})(1+1/Q_{ax})(Q_{aa})^{1/2}} \quad (41)$$

5. Evaluation of coupling and dissociation constants

It is apparent that the couplings depicted in eq. 23 give rise to all of the allosteric alterations in enzymatic behavior, both cooperativity and the underlying inhibition or activation. In other words, if all of the indicated coupling free energies were zero, then the enzyme would exhibit hyperbolic saturation behavior and would not be influenced by X. In addition, these couplings have very real biophysical origins that are responsible for the interactions that are experienced. It is important, therefore, to determine the magnitude of these couplings and their associated dissociation constants as a necessary first step in studying the biophysical cause of these interactions as well as to provide a basis for quantitatively describing enzymatic function. Fortunately, the most important of these parameters can be determined from the systematic evaluation of kinetic (or saturation) behavior as shown below.

5.1. Magnitude of Q_{aa} and $Q_{aa/xx}$

The magnitude of the intrinsic homotropic coupling constant between the two bound A ligands can be determined by evaluating the value of the Hill coefficient when [X] = 0 according to eq. 32 that has been solved for Q_{aa}:

$$Q_{aa} = \left[\frac{n_H}{2-n_H}\right]^2 \quad (42)$$

As is evident from eq. 34, the above relationship will also yield a value for $Q_{aa/xx}$ if the Hill coefficient is determined when [X] is saturating.

5.2. Magnitude of Q_{xx} and $Q_{xx/aa}$

Information pertaining to the magnitude of the average homotropic couplings between bound allosteric ligands in the absence and presence of A, Q_{xx} and $Q_{xx/aa}$, respectively, can be derived from experiments in which the concentration of A that produces half-maximal velocity, $K_{1/2}$, is evaluated as a function of the concentration of X. For a rate equation in the form of eq. 24, $K_{1/2}$ is given by:

$$K_{1/2} = \frac{K_a^0}{(\alpha\beta)^{1/2}} \quad (43)$$

From the definitions in eqs. 25 and 26 we can determine the following limits:

$$\lim_{X\to 0} K_{1/2} = \frac{K_a^0}{(Q_{aa})^{1/2}} \quad (44)$$

$$\lim_{X\to \infty} K_{1/2} = \left[\frac{K_a^0}{(Q_{aa/xx})^{1/2}}\right]\left[\frac{Q_{xx}}{Q_{ax}^2 Q_{xx/a}}\right] \quad (45)$$

Let us define the parameter Q to be equal to the ratio of these two limits:

$$Q = Q_{ax}^2\left[\frac{Q_{xx/a}}{Q_{xx}}\right]\left[\frac{Q_{aa/xx}}{Q_{aa}}\right]^{1/2} \quad (46)$$

Note that Q represents an experimentally determinable quantity. Substituting eqs. 25, 26 and 46 into eq. 43 yields the following:

$$K_{1/2} = K_a^{0\prime}\left[\frac{1+2X+Q_{xx}X^2}{1+2Q\left[\frac{Q_{xx}}{Q_{xx/aa}}\right]^{1/2}X+Q^2Q_{xx}X^2}\right]^{1/2} \quad (47)$$

where $K_a^{0\prime} = K_a^0/(Q_{aa})^{1/2}$. By determining $K_{1/2}$ as a function of [X], and fitting these data to eq. 47 via nonlinear regression analysis, the parameters Q_{xx} and $Q_{xx/aa}$; as well as $K_a^{0\prime}$, K_{ix}^0, and Q; can be unambiguously determined.

If no homotropic coupling or heterotropically induced homotropic coupling exists between the

two allosteric ligands, i.e., if $Q_{xx} = Q_{xx/aa} = 1$, then eq. 47 reduces to:

$$K_{1/2} = K_a^{0\prime}\left[\frac{1+X}{1+QX}\right] \tag{48}$$

Eq. 48 has exactly the same form as that predicted by eq. 21 which describes an enzyme binding only a single substrate and a single allosteric ligand [7,11]. Thus, only a homotropic coupling between the allosteric ligands can cause the dependence of $K_{1/2}$ on [X] for a dimeric enzyme to deviate from the form predicted by the monomeric case. This result probably explains in large measure the general utility of eq. 48 in describing allosteric behavior even in highly cooperative multimeric allosteric enzymes such as rat liver phosphofructokinase [11,12].

5.3. Magnitude of K_a^0 and K_{ix}^0

K_{ix}^0 can be determined by fitting data to either eq. 47 or 48 as just described. Note that eq. 48 can be transformed to a linear form for estimating the value of K_{ix}^0 (and Q) without resorting to nonlinear regression [7].

$K_a^{0\prime}$ is the geometric mean or average dissociation constant of A when [X] = 0 and hence is equal to the concentration of A required to produce half-maximal velocity (half-saturation) in the absence of X. However, the value of K_a^0, the reciprocal of the binding constant between a single equivalent of A and free enzyme, can be determined from the value of Q_{aa} obtained from evaluating the degree of cooperativity when [X] = 0 as discussed above. Thus, by referring to eq. 42, K_a^0 is given by:

$$K_a^0 = K_a^{0\prime}\left[\frac{n_H}{2-n_H}\right] \tag{49}$$

Of course the binding constant of the second equivalent of A to bind is given by Q_{aa}/K_a^0 by definition (see eq. 13).

5.4. Magnitude of Q_{ax1} and Q_{ax2}

In general it is not possible to distinguish between Q_{ax1} and Q_{ax2}. However, the average A-X coupling, Q_{ax}, can be determined when the direct coupling between the A binding sites is not changed with the binding of X and the direct coupling between the X binding sites is not changed by the binding of A; in which case $Q_{aa} = Q_{aa/xx}$, $Q_{xx} = Q_{xx/a}$, and

$$Q_{ax} = Q^{1/2} \tag{50}$$

according to eq. 46 as recognized by Weber [6]. In the more general case, when $Q_{aa} \neq Q_{aa/xx}$, and $Q_{xx} \neq Q_{xx/aa}$, the value of Q_{ax} can at least be bounded to within a finite range of values.

It is evident from eq. 46 that eq. 50 will not hold if the binding of X alters the homotropic cooperativity between the A sites or vice versa. The extent to which saturating the enzyme with X alters the interaction between A sites is given by the ratio of $Q_{aa/xx}$ to Q_{aa} and these parameters can be obtained by evaluating the Hill coefficient when [X] is saturating and [X] = 0, respectively, as discussed above. What cannot be determined explicitly is the influence on the homotropic interaction between X sites induced by the binding of a single A ligand, i.e., the ratio of $Q_{xx/a}$ to Q_{xx}, as required by eq. 46. We can, however, place limits on the likely value of this ratio by considering the following argument.

Although it is mathematically possible, it is physically unreasonable for the binding of one A ligand to have an effect on Q_{xx} that is opposite in nature to the effect of binding both A ligands. If $Q_{xx/aa} > Q_{xx}$ then neither A ligand individually is likely to diminish the value of Q_{xx}. In other words, we assume that:

$$Q_{xx} \leq Q_{xx/a} \leq Q_{xx/aa}$$

or

$$Q_{xx} \geq Q_{xx/a} \geq Q_{xx/aa} \tag{51}$$

Within the framework of this assumption, one extreme possibility is obtained if the heterotropically induced homotropic change is completely accomplished with the binding of the first A (similar to the concerted transition proposed by Monod, et al. [13]) in which case $Q_{xx/a} = Q_{xx/aa}$ and eq. 46 becomes:

$$Q = Q_{ax}^2\left[\frac{Q_{xx/aa}}{Q_{xx}}\right]\left[\frac{Q_{aa/xx}}{Q_{aa}}\right]^{1/2} \tag{52}$$

The other extreme is obtained if one assumes that the binding of one molecule of A is totally ineffectual and only the second A causes the change in Q_{aa}. In this case eq. 46 becomes:

$$Q = Q_{ax}^2 \left[\frac{Q_{aa/xx}}{Q_{aa}} \right]^{1/2} \tag{53}$$

Of course between these two extremes lies the 'average' position that the binding of each ligand contributes equivalently to the overall effect, in which case:

$$\left[\frac{Q_{xx/a}}{Q_{xx}} \right]^2 = \left[\frac{Q_{xx/aa}}{Q_{xx}} \right]$$

and eq. 46 becomes:

$$Q = Q_{ax}^2 \left[\frac{Q_{xx/aa}}{Q_{xx}} \right]^{1/2} \left[\frac{Q_{aa/xx}}{Q_{aa}} \right]^{1/2} \tag{54}$$

If the ratio $Q_{xx/aa}/Q_{xx}$ is significantly different from unity, then the fit to eq. 47 will yield this value. One can therefore estimate the value of Q_{ax} from the experimentally determined values of Q, Q_{aa}, $Q_{aa/xx}$ and the ratio $Q_{xx/aa}/Q_{xx}$ in the general case by utilizing eq. 54, recognizing that the actual value of Q_{ax} will lie within the range

$$Q \left[\frac{Q_{aa}}{Q_{aa/xx}} \right]^{1/2} \leq Q_{ax}^2 \leq Q \left[\frac{Q_{aa}}{Q_{aa/xx}} \right]^{1/2} \left[\frac{Q_{xx}}{Q_{xx/aa}} \right]$$

or

$$Q \left[\frac{Q_{aa}}{Q_{aa/xx}} \right]^{1/2} \geq Q_{ax}^2 \geq Q \left[\frac{Q_{aa}}{Q_{aa/xx}} \right]^{1/2} \left[\frac{Q_{xx}}{Q_{xx/aa}} \right] \tag{55}$$

as indicated by eqs. 52 and 53.

6. Discussion

Thermodynamic explanations of allosteric regulation of oligomeric proteins often involve the a priori assumption that the enzyme can exist in a finite number of conformational states to which particular attributes, such as binding properties, are ascribed. The two most popular formalisms were originally proposed by Monod et al. [13] (MWC model) and by Koshland et al. [14] (KNF model). The MWC model assumes that the conformations of all subunits in an oligomer are the same and subject to a concerted conformational transition equilibrium that can be displaced by the binding of ligands to either of the two or three possible forms that can exist. In the KNF model, ligand binding induces the conformational transitions and they can occur one subunit at a time as ligands sequentially bind. In either case the resulting equations consist of conformationally specific ligand binding or dissociation constants plus the equilibrium constants governing the conformational transition per se [15]. The number of protein forms considered in these equations is restricted to only those representing different functional conformational states, and the functional properties of these forms are independent of whether ligands are bound, rather than considering all different ligand-bound forms as having potentially different properties.

These models are usually invoked to simplify the interpretation of the very complex binding interactions that occur in large multimeric proteins and enzymes. However, the validity of these simplifications requires that the underlying assumption be correct, namely, that the functional properties of the protein can be attributed to only two or three functional states. Since such models usually gain credence because they are easy to conceptualize, the inherently arbitrary nature of the original construct often tends to become obscured. Even in the well studied case of hemoglobin, for example, where the original two-state model was prompted by the determination by Perutz [16] of two distinct X-ray crystal structures for oxygenated and unoxygenated forms, careful analysis of binding data in recent years has necessitated the reformulation of its mechanism in terms of at least several 'forms' in order to describe adequately hemoglobin's solution properties [17–21]. Thus, we are left with the realization that even the most carefully acquired data, if interpreted on the basis of arbitrary a priori assumptions, will give rise to highly precise values for parameters that may have no objective meaning.

The alternative approach, at least in the case of a dimeric enzyme of the type discussed herein, is

to utilize an analysis incorporating the principles of thermodynamic linkage. This approach has two distinct advantages. First, the parameters involved must have an absolute meaning. That is to say, the analysis is not based upon mechanistic assumptions but rather upon thermodynamic principles that proteins, like all molecules in solution, must obey when subject to detailed balance.

The second major advantage of this approach is that it emphasizes the importance of coupling parameters. It is precisely the various protein-mediated interactions between ligands that give rise to the defining characteristics of allosteric behavior. By recasting the multiple ligand coupling constants in terms of the equivalent two ligand coupling constant expressions, the mechanistic origins of allosteric behavior become more intuitively meaningful. The two ligand coupling parameters specifically quantify the biophysical perturbations that cause various allosteric attributes and therefore are the proper focus of attention in the design and interpretation of experiments aimed at illuminating the biophysical origins of these allosteric phenomena.

One characteristic of most allosterically regulated dimeric enzymes, cooperativity in substrate binding, requires some type of coupling across the subunit interface linking the two substrate sites. The most obvious mechanism is a direct, or homotropic, coupling, represented by Q_{aa}, in which the first A allosterically affects the second A binding. We have also seen that a nonsubstrate ligand, X, can induce or alter the effective A-A coupling in two distinct ways. After X binds, the enzyme is essentially a different species that may provide a different degree of interaction between the A sites as A binds. Hence, the binding of X can modify the direct A-A interaction. We have termed such an effect 'heterotropically induced (or altered) homotropic cooperativity'.

Another effect is predicted to occur only at subsaturating concentrations of X as a consequence of the influence that X has on the affinity of A per se. Interestingly, as recognized by Weber [5,6], this effect must lead to positive cooperativity whether X antagonizes or facilitates the binding of A provided that the effect of X on the binding of both As is consistent. (In the improbable event that X might inhibit the binding of the intrasubunit A while facilitating the binding of the intersubunit A or vice versa, the subsaturating effect will lead to a transient decrease rather than increase in the Hill coefficient as a function of X). However, for this type of cooperativity to be expressed, both A sites must still be coupled, in this case indirectly via an intervening X. As is apparent from eq. 38, both inter- and intrasubunit A-X couplings are required. Thus, the absence of a transient increase in the Hill coefficient that is only apparent at subsaturating concentrations of X, relative to that predicted by the homotropic and heterotropically induced homotropic limits, implies the absence of either intra- or intersubunit A-X couplings.

Aside from the recognition that a rate equation containing concentration terms with nonunitary powers can result in nonhyperbolic binding curves, little insight is gained from inspection of eq. 20 about the 'degree' of cooperativity predicted (as commonly quantified by the Hill coefficient). However, the extent of allosteric influence on cooperativity may be estimated by considering the probable magnitudes of these two-ligand couplings constants. It is unlikely that a coupling free energy between two ligands would assume a value greater than a moderate fraction of the binding free energy for either ligand [6]. Indeed, in those cases where coupling free energies have been measured for ligands with binding free energies in the range 4–8 kcal/mol (dissociation constants in the micro- to millimolar range), the absolute values of the coupling free energies are usually less than 2 kcal/mol, in keeping with this expectation [6,11,12]. According to eq. 11, therefore, we can anticipate that each of the 12 two ligand coupling constants will likely fall within the range 0.03–30, with values less than 1 reflecting antagonistic couplings ($\Delta G_{ax} > 0$) and values greater than 1 indicating facilitating interactions ($\Delta G_{ax} < 0$).

Upon examination of eq. 30, it becomes apparent that the Hill coefficient resulting from ligand-ligand couplings on a dimer has an absolute upper limit of 2 and a lower limit of 0, but these limits are only achieved as the coupling free energies approach minus or plus infinity, respectively (or the corresponding Q values approach

infinity or 0 respectively). If one considers ± 2 kcal/mol to be the more probable upper limit on ligand-ligand coupling free energies, then eqs. 32 and 34 would predict homotropic Hill coefficients (when X is absent or saturating) to fall within the range 1.7–0.3 (see fig. 2). Similarly, a ± 2 kcal/mol average coupling between A and X would lead to a maximum Hill coefficient of 1.8, achieved when $[X] = K^0_{ix}/Q_{ax}$ for either positive or negative couplings, from subsaturating heterotropic cooperativity alone (see fig. 3 and 4). Of course, cooperativity mechanisms can combine, at subsaturating but nonzero concentrations of X, to yield more extreme Hill coefficients that might more closely approach the limits of 2 and 0 (e.g., see eq. 41).

Although several linkage mechanisms can contribute to cooperativity, the preceding analysis suggests a systematic way to differentiate and quantitate the relative contributions of these mechanisms. Cooperativity at $[X] = 0$ and $[X] = \infty$ and the behavior of the $K_{1/2}$ as a function of [X] should yield estimates of the values for Q_{aa}, $Q_{aa/xx}$, Q_{xx}, $Q_{xx/aa}$, Q_{ax}, K^0_a and K^0_{ix}, which in turn correspond to the fundamental ligand-ligand and ligand-protein interactions that are responsible for the K-type allosteric regulation of a dimeric enzyme.

Acknowledgements

I thank Dr. Gregorio Weber and Dr. David Jameson for critically reading a preliminary version of this manuscript. This work was supported by grant GM 33216 from the National Institutes of Health and a grant from the Juvenile Diabetes Foundation International.

References

1 C. Tanford, Proc. Natl. Acad. Sci. U.S.A. 78 (1981) 270.
2 J. Wyman, Adv. Protein Chem. 4 (1948) 407.
3 J. Wyman, Adv. Protein Chem. 19 (1964) 223.
4 J. Wyman, J. Am. Chem. Soc. 89 (1967) 2202.
5 G. Weber, Biochemistry 11 (1972) 864.
6 G. Weber, Adv. Protein Chem. 29 (1975) 1.
7 G.D. Reinhart, Arch. Biochem. Biophys. 224 (1983) 389.
8 S. Cha, J. Biol. Chem. 243 (1968) 820.
9 J. Botts and M. Morales, Trans. Faraday Soc. 49 (1953) 696.
10 C. Frieden, J. Biol. Chem. 239 (1964) 3522.
11 G.D. Reinhart, Biochemistry 24 (1985) 7166.
12 G.D. Reinhart and S.B. Hartleip, Biochemistry 25 (1986) 7308.
13 J. Monod, J. Wyman and J.P. Changeux, J. Mol. Biol. 12 (1965) 88.
14 D.E. Koshland, Jr, G. Nemethy and D. Filmer, Biochemistry 5 (1966) 365.
15 D.E. Koshland, Jr, in: The enzymes, 3rd edn., vol. 1, ed. P.D. Boyer (Academic Press, New York, 1970) p. 342.
16 M.F. Perutz, Nature 228 (1970) 726.
17 G. Weber, Proc. Natl Acad. Sci. U.S.A. 81 (1984) 7098.
18 S.J. Edelstein and J.T. Edsall, Proc. Natl. Acad. Sci. U.S.A. 83 (1986) 3796.
19 S.J. Gill, E. Di Cera, M.L. Doyle, G.A. Bishop and C.H. Robert, Biochemistry 26 (1987) 3995.
20 E. Di Cera, C.H. Robert and S.J. Gill, Biochemistry 26 (1987) 4003.
21 G.K. Ackers and F.R. Smith, Annu. Rev. Biophys. Biophys. Chem. 16 (1987) 583.