Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 2727–2731, April 1992
Biophysics

Symmetry conditions for binding processes

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Contributed by Jeffries Wyman, December 27, 1991

ABSTRACT **Symmetry conditions are derived for global and local binding processes in biological macromolecules. It is shown that the conditions applying in the case of the macromolecule as a whole are decoupled from those referring to individual sites. In the case of two sites, the global binding curve is always symmetric, and the individual-site binding curves are always asymmetric, unless the two sites are identical or independent. In the case of three sites or more, individual-site binding curves can show symmetric or asymmetric behavior. The conditions derived for symmetry in the local description of binding processes also apply to the case of linkage among different ligands and to steady-state kinetics. Application to the analysis of oxygen binding to human hemoglobin under physiological conditions provides a model-independent interpretation of the asymmetric nature of the binding curve. Asymmetry of the global binding curve can coexist with symmetric or asymmetric binding to the individual α and β chains. If the binding curves of the two chains are symmetric, then subunit heterogeneity and asymmetric interactions must exist in the hemoglobin tetramer. On the other hand, if the binding curves of the two chains are asymmetric, then subunit heterogeneity and asymmetric interactions are not necessary for global asymmetric binding.**

Symmetry permeates many different aspects of biological structure and function, ranging from the spatial arrangement of protein subunits to the thermodynamic conditions involving response functions of the system or else the shape of a binding isotherm. In some of these aspects symmetry is an inherent component and arises as a consequence of thermodynamic principles. The mutual interference of different ligands or binding sites is governed by linkage relationships (1, 2) that reflect the intrinsic symmetry of the abstract metric space associated with a system at equilibrium (3, 4). In other cases symmetry may or may not be present as a phenomenological aspect of the system. Group symmetry is observed in the three-dimensional structure of a number of multimeric proteins (5), and in many cases functional aspects of biological function and regulation reflect the structural symmetry of the system. The idea of relating structural and functional symmetry in biological macromolecules is a very old one (6, 7) and played a major role in the development of allosteric models (8, 9). Interest in functional symmetry, especially when dealing with binding and linkage processes, stems from the considerable simplification of the mathematical expressions involved. Symmetry introduces constraints among the parameters defining the partition function of the system and provides a convenient test for a number of mechanistic models. Our purpose in this report is to draw attention to some general aspects of functional symmetry in binding and linkage processes that can be used in practical applications.

General Considerations on Symmetry

From a mathematical point of view, a function $F(z)$, with $-\infty \leq z \leq \infty$, can have a number of symmetry properties. If $F(z)$ is an even function, then $F(z) = F(-z)$ and any point in the positive half-plane at z has an image at $-z$. Symmetry in this case is specular in the sense that $F(z)$ in one half-plane is the specular image of $F(z)$ in the other half. On the other hand, if $F(z)$ is an odd function, then $F(z) = -F(-z)$ and symmetry is rotational, for $F(z)$ is reproduced exactly when rotated 180° around $z = 0$. These two types of symmetry are related by differentiation. If $F(z)$ has rotational symmetry, then the derivative $dF(z)/dz$ has specular symmetry since it has the same value at z and $-z$. In view of this fact, it is sufficient to focus our attention on one type of symmetry only. Rotational symmetry is the one of major practical interest when dealing with binding and linkage processes.

Consider a function $F(x)$, where $0 \leq x \leq \infty$ and x_m is the value of x at the center of symmetry. Then, $F(x)$ plotted versus ln x has rotational symmetry if, and only if,

$$F(x_m\lambda) + F(x_m\lambda^{-1}) = F(0) + F(\infty), \quad [1]$$

for any $\lambda \geq 0$. At the center of symmetry the value of $F(x)$ is by definition halfway between the limiting values $F(0)$ and $F(\infty)$; i.e.,

$$F(x_m) = [F(0) + F(\infty)]/2 \quad [2]$$

so that

$$F(x_m\lambda) + F(x_m\lambda^{-1}) = 2F(x_m), \quad [3]$$

which gives the general definition of symmetry to be used in what follows. It is rather instructive to notice that shifting the origin of axes to the point $[x_m, F(x_m)]$ yields an odd function if $F(x)$ is symmetric. In other words, the function $F(x) - F(x_m)$ plotted versus $\ln(x/x_m)$ is odd around $x = x_m$, as can be seen from Eq. 3 by letting $\lambda = x/x_m$.

The general condition 3 has two important consequences.

COROLLARY 1. *If* F(x) *is symmetric, then the integral*

$$\int_{x_m\lambda^{-1}}^{x_m\lambda} F(x)d(\ln x) = 2F(x_m)\ln \lambda \quad [4]$$

holds for any $\lambda \geq 0$.

COROLLARY 2. *If* F(x) *is symmetric with a center of symmetry* x_m, *then the function*

$$G(x) = \ln\left\{\frac{F(x) - F(0)}{[F(\infty) - F(x)]x}\right\} \quad [5]$$

is symmetric and has the same center of symmetry.

The validity of *Corollary 1* hinges on the properties of the function $F(x) - F(x_m)$ that is odd around $x = x_m$. This implies that the integral of $F(x) - F(x_m)$ from $x_m\lambda^{-1}$ to x_m must equal (in absolute value) the integral of $F(x) - F(x_m)$ from x_m to $x_m\lambda$, since from Eq. 3 it follows that $F(x_m\lambda) - F(x_m)$ equals $F(x_m) - F(x_m\lambda^{-1})$ for any $\lambda \geq 0$. Consequently, the integral

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$$\int_{x_m\lambda^{-1}}^{x_m\lambda} [F(x) - F(x_m)]d(\ln x) = 0 \quad [6]$$

holds for any $\lambda \geq 0$ and hence *Corollary 1*. The validity of *Corollary 2* implies that

$$G(x_m\lambda) + G(x_m\lambda^{-1}) = 2G(x_m) = -2 \ln x_m \quad [7]$$

or else that

$$\left[\frac{F(x_m\lambda) - F(0)}{F(\infty) - F(x_m\lambda)}\right]\left[\frac{F(x_m\lambda^{-1}) - F(0)}{F(\infty) - F(x_m\lambda^{-1})}\right] = 1, \quad [8]$$

which is always true if Eq. 3 holds.

Global Binding Processes

The expressions derived above can be used to find the conditions for symmetry in ligand binding processes. We shall consider symmetry of the binding isotherm reflecting global processes and symmetry of the binding curve reflecting local or site-specific binding processes. The general aspects of the global and local descriptions are dealt with in detail elsewhere (2) and will not be repeated here. The partition function $Z(x)$ for the global description is

$$Z(x) = \sum_{j=0}^{t} A_j x^j, \quad [9]$$

where A_j is the overall equilibrium constant for binding j ligand molecules to the free macromolecule ($A_0 = 1$) and the summation runs from zero to the total number of sites t. The form of $Z(x)$ in Eq. 9 implies that the macromolecule does not change aggregation state upon ligand binding. The binding isotherm in the global description is a measure of the average number of ligand sites as a function of ln x; i.e.,

$$\bar{X}(x) = \frac{d[\ln Z(x)]}{d(\ln x)} = \frac{\sum_{j=0}^{t} jA_j x^j}{\sum_{j=0}^{t} A_j x^j}. \quad [10]$$

Symmetry of $\bar{X}(x)$ necessarily demands $\bar{X}(x_m) = t/2$ and application of Eq. 4 yields

$$\sum_{j=0}^{t} A_j x_m^j \lambda^j = \sum_{j=0}^{t} A_j x_m^j \lambda^{t-j}. \quad [11]$$

Since Eq. 11 must hold for any λ, one necessarily has $x_m = A_t^{-1/t}$. This implies that the center of symmetry is the same as the mean ligand activity of the system (1, 2), where the unligated and fully ligated forms of the macromolecule have the same concentration. Elimination of x_m from Eq. 11 yields the condition of symmetry for the overall equilibrium constants as follows:

$$A_j A_t^{-j/t} = A_{t-j} A_t^{j/t-1}, \quad [12]$$

which must hold for any j. The above condition is a well known result (6) and implies that the partition function $Z(x)$ is a symmetric polynomial when x is expressed in x_m units. For $t = 1$ or $t = 2$, the condition is a mere tautology. The condition for $t = 3$ is $A_3 = (A_2/A_1)^3$, and the condition for $t = 4$ is $A_4 = (A_3/A_1)^2$ and does not depend on A_2. In general, the condition of symmetry involves all As in the partition function for t odd and all As but $A_{t/2}$ for t even. Therefore, the doubly ligated species makes no contribution to the symmetry or asymmetry of the binding curve of a protein such as hemoglobin, and the same applies to the half-saturated species of a protein containing an even number of binding sites.

Local Binding Processes

For local binding processes, we are interested in the behavior of individual sites of the macromolecule considered as subsystems open to interactions with other sites. The connection between global and local processes is provided by the conservation relationship

$$\bar{X}(x) = X_1(x) + X_2(x) + \cdots + X_t(x), \quad [13]$$

where $X_s(x)$ is the binding isotherm of site s and is bounded from zero to 1. The relationship above allows one to draw some qualitative conclusions about the symmetry properties of individual sites. When all sites are independent from one another, then each $X_s(x)$ behaves just as $\bar{X}(x)$ for $t = 1$ and is always symmetric. When all sites are identical and interact equally, then $\bar{X}(x)$ is merely $tX(x)$, where $X(x)$ is any $X_s(x)$ in Eq. 13, and the symmetry properties of an individual site coincide with those of the macromolecule as a whole. In general the symmetry properties of $\bar{X}(x)$ cannot be defined uniquely from those of individual sites and vice versa. The sum of symmetric functions is not necessarily symmetric and conversely the sum of asymmetric functions is not necessarily asymmetric. Even in the simplest case of interest arising for $t = 2$, nothing can be said from Eq. 13 on the symmetry properties of $X_1(x)$ and $X_2(x)$ separately, if $X_1(x) \neq X_2(x)$. All we know is that the sum $X_1(x) + X_2(x)$ will always be symmetric.

To investigate the symmetry properties of local binding processes, each $X_s(x)$ should conveniently be defined in terms of contracted partition functions (2) that are obtained from $Z(x)$ when site s is kept in a particular ligation state. The partition function $Z(x)$ in the local description can be written (2) as

$$Z(x) = {}^0Z_s(x) + K_s x {}^1Z_s(x), \quad [14]$$

where ${}^0Z_s(x)$ and ${}^1Z_s(x)$ contain all configurations with site s unligated and ligated, respectively, and K_s is the association constant for ligand binding to site s when all other sites are unligated. The local binding isotherm of site s is given by

$$X_s(x) = K_s x \frac{{}^1Z_s(x)}{Z(x)} = 1 - \left[\frac{{}^0Z_s(x)}{Z(x)}\right] = \frac{K_s(x)x}{1 + K_s(x)x} \quad [15]$$

and unlike $\bar{X}(x)$ cannot be obtained from $Z(x)$ by differentiation with respect to ln x [except in the trivial case where all $X_s(x)$ in Eq. 13 are identical]. The function $K_s(x)$ reflects the change in the association constant for binding to site s as a function of x and is given by

$$K_s(x) = K_s \frac{{}^1Z_s(x)}{{}^0Z_s(x)}. \quad [16]$$

The mathematical form of $X_s(x)$ makes *Corollary 1* of very little practical use. On the other hand, *Corollary 2* proves most useful since it states that if $X_s(x)$ is symmetric, then the function

$$G_s(x) = \ln\left\{\frac{X_s(x)}{[1 - X_s(x)]x}\right\}$$

$$= \ln K_s + \ln\left[\frac{{}^1Z_s(x)}{{}^0Z_s(x)}\right] = \ln K_s(x) \quad [17]$$

is always symmetric. If x_s is the center of symmetry of $X_s(x)$, then by definition $X_s(x_s) = 1/2$ and $K_s(x_s)x_s = 1$. Furthermore,

x_s coincides with the mean ligand activity of site s, as seen in the case of the global description, and is also the center of symmetry of $G_s(x)$. The definition in Eq. **3** applied to $G_s(x)$ yields

$$\frac{{}^1Z_s(x_s\lambda){}^1Z_s(x_s\lambda^{-1})}{{}^0Z_s(x_s\lambda){}^0Z_s(x_s\lambda^{-1})} = K_s^{-2}x_s^{-2} = \omega^2, \quad [18]$$

which is the condition for symmetry of $X_s(x)$. The solution for an arbitrary number of sites, t, is obtained by writing down the polynomial expansions for each contracted partition function as

$${}^0Z_s(x) = \sum_{j=0}^{t-1} \alpha_j x^j \quad [19]$$

$${}^1Z_s(x) = \sum_{j=0}^{t-1} \beta_j x^j, \quad [20]$$

where summations run from zero to $t-1$ and $\alpha_0 = \beta_0 = 1$. The coefficients of the contracted partition functions are related to the overall equilibrium constants as in Eq. **9** by the simple relationship $A_i = \alpha_i + K_s\beta_{i-1}$ for $0 \le i \le t-1$ and $A_t = K_s\beta_{t-1}$. Substitution of Eqs. **19** and **20** into Eq. **18** yields

$$\sum_{i=0}^{t-1}\sum_{j=0}^{t-1} (\beta_i\beta_j - \omega^2\alpha_i\alpha_j)x_m^{i+j}\lambda^{i-j} = 0. \quad [21]$$

Since Eq. **21** must hold for any λ, one necessarily has

$$\sum_{i=0}^{t-1}\sum_{j=0}^{t-1} (\beta_i\beta_j - \omega^2\alpha_i\alpha_j)x_m^{i+j} = 0, \quad [22]$$

for any i and j, such that $i-j$ is constant. The relationships above are invariant upon the substitution of i for j, so that only half of them are truly independent. If $i-j = n$ in Eq. **22**, then

$$\sum_{j=0}^{t-1-n} (\beta_{j+n}\beta_j - \omega^2\alpha_{j+n}\alpha_j)x_m^{2j+n} = 0. \quad [23]$$

There are t such conditions, corresponding to the different values of $n = 0, 1, 2, \ldots, t-1$. Each condition taken separately for a given value of n is necessary but not sufficient. It is also sufficient to the extent of which the other conditions hold. On the other hand, if $X_s(x)$ is symmetric, then any of the conditions **23** necessarily applies. One of them is particularly important and is obtained for $n = t-1$, i.e., $\beta_{t-1} - \omega^2\alpha_{t-1} = 0$ or else

$$x_s = K_s^{-1}\sqrt{\alpha_{t-1}/\beta_{t-1}}, \quad [24]$$

which provides a simple and analytical expression for the center of symmetry and, hence, the mean ligand activity of site s when its binding curve is symmetric. No such simple relationship exists for the mean ligand activity of an individual site in the general case, which is given by the integral equation (2)

$$\ln\langle x_s\rangle = \int_0^1 \ln x\,dX_x(x) = -\int_0^1 \ln x\,d[{}^0Z_s(x)/Z(x)]. \quad [25]$$

Solution of the integral above for any value of t is straightforward, although somewhat tedious, and involves logarithmic and/or inverse trigonometric functions (10). Symmetry greatly simplifies the solution since it makes $\langle x_s\rangle$ equal to the center of symmetry x_s in Eq. **24** and yields an expression for $\langle x_s\rangle$ that is of considerable practical use, unlike Eq. **25**.

We can now explicitly solve the problem of symmetry for local binding processes using two simple examples. For $t = 2$, the global description yields a binding curve that is always symmetric. Does this apply to $X_1(x)$ and $X_2(x)$ as well? Consider the case of $X_1(x)$. The relevant partition functions are (2)

$$Z(x) = 1 + (K_1 + K_2)x + c_{12}K_1K_2x^2, \quad [26]$$

$${}^0Z_1(x) = 1 + K_2x, \quad [27]$$

and

$${}^1Z_1(x) = 1 + c_{12}K_2x, \quad [28]$$

where c_{12} is the interaction constant between the sites. Hence, $\alpha_1 = K_2$ and $\beta_1 = c_{12}K_2$ and the conditions **23** are given by

$$\beta_1 - \omega^2\alpha_1 = 0 \qquad (n = 1) \quad [29]$$

and

$$1 - \omega^2 + (\beta_1^2 - \omega^2\alpha_1^2)x_1^2 = 0 \qquad (n = 0) \quad [30]$$

and are both satisfied only if $\alpha_1 = \beta_1$ or $\alpha_1\beta_1x_1^2 = 1$, i.e., if $K_1 = K_2$ or $c_{12} = 1$. This means that $X_1(x)$ is symmetric only if the two sites have the same association constant or if they do not interact. The former case corresponds to $X_1(x) = X_2(x) = \bar{X}(x)/2$, and the latter corresponds to independent sites. Thus, for $t = 2$, a site-specific binding curve is never symmetric, unless the two sites are identical or independent.

For $t = 3$, the global description yields a binding curve that is symmetric if $A_3 = (A_2/A_1)^3$. The relevant partition functions for $X_1(x)$ are (2)

$$Z(x) = 1 + (K_1 + K_2 + K_3)x + (c_{12}K_1K_2 + c_{13}K_1K_3 + c_{23}K_2K_3)x^2 + c_{123}K_1K_2K_3x^3, \quad [31]$$

$${}^0Z_1(x) = 1 + (K_2 + K_3)x + c_{23}K_2K_3x^2, \quad [32]$$

and

$${}^1Z_1(x) = 1 + (c_{12}K_2 + c_{13}K_3)x + c_{123}K_2K_3x^2, \quad [33]$$

where the constants c are appropriate interaction constants. Hence, $\alpha_1 = K_2 + K_3$, $\alpha_2 = c_{23}K_2K_3$, $\beta_1 = c_{12}K_2 + c_{13}K_3$, and $\beta_2 = c_{123}K_2K_3$, and the conditions **23** are given by

$$\beta_2 - \omega^2\alpha_2 = 0 \qquad (n = 2), \quad [34]$$

$$\beta_1 - \omega^2\alpha_1 + (\beta_1\beta_2 - \omega^2\alpha_1\alpha_2)x_1^2 = 0 \qquad (n = 1), \quad [35]$$

and

$$1 - \omega^2 + (\beta_1^2 - \omega^2\alpha_1^2)x_1^2 + (\beta_2^2 - \omega^2\alpha_2^2)x_1^4 = 0 \qquad (n = 0), \quad [36]$$

which demand $\beta_2\alpha_1^2 = \alpha_2\beta_1^2$ and $K_1^2\beta_1 = \alpha_1\alpha_2$, or else.

$$c_{123}(K_2 + K_3)^2 = c_{23}(c_{12}K_2 + c_{13}K_3)^2 \quad [37]$$

and

$$K_1^2(c_{12}K_2 + c_{13}K_3) = c_{23}K_2K_3(K_2 + K_3). \quad [38]$$

Therefore, for $t = 3$, it is possible for a site-specific binding curve to be symmetric. The trivial case, where all constants c are equal to 1, which corresponds to independent sites, is embodied by the conditions above as a special case. The other special case where all sites and interaction constants are identical leads to $c_{123} = c^3$, where $c = c_{12} = c_{13} = c_{23}$.

Other special cases can be tested using Eqs. **37** and **38**. In general, for t sites, there will be t conditions to be satisfied among the site-specific parameters.

Symmetry in the global description does not necessarily imply symmetry in the local description. For a macromolecule containing two binding sites, the global binding isotherm is always symmetric, whereas the site-specific isotherms are always asymmetric, except in a few special cases. For a macromolecule containing three sites, the conditions for symmetry in the global description are completely decoupled from those applying in the local description. When all interaction constants vanish, the global isotherm is the sum of three symmetric isotherms but is itself symmetric only if one of the site-specific association constants is the geometric mean of the other two (e.g., if $K_1^2 = K_2K_3$). When all site-specific equilibrium constants are identical, the global isotherm is symmetric when $27c_{123} = (c_{12} + c_{13} + c_{23})^3$, and $X_1(x)$ is symmetric when $c_{123} = c_{23}^3$, provided $2c_{23} = c_{12} + c_{13}$. For larger values of t, it becomes increasingly evident the lack of a clear connection between global and local patterns of symmetry and the considerable complexity of the local description of binding processes is readily appreciated.

The Case of Hemoglobin

Is there a unique code that translates structural organization into functional behavior as expressed by binding properties? The local description of binding processes provides a rigorous framework to address the fundamental question of how functional symmetry is related to structural features. Human hemoglobin is a case in point. The oxygen binding curve of human hemoglobin under conditions of physiological interest is strongly asymmetric and displays higher cooperativity at high saturation (11, 12). From a model-dependent analysis, Weber (13) and Peller (14) have concluded that the asymmetry is a consequence of the existence of two types of chains in the hemoglobin tetramer and is due to either asymmetric pairwise interactions between $\alpha\alpha$ and $\beta\beta$ pairs or binding heterogeneity of the two subunits. The local description of oxygen binding to hemoglobin provides a model-independent framework and is based on the partition function

$$Z(x) = 1 + 2(K_\alpha + K_\beta)x + [c_{\alpha\alpha}K_\alpha^2 + c_{\beta\beta}K_\beta^2 + 2(c_{\alpha\beta} + c'_{\alpha\beta})K_\alpha K_\beta]x^2 + 2(c_{\alpha\alpha\beta}K_\alpha + c_{\alpha\beta\beta}K_\beta)K_\alpha K_\beta x^3 + c_{\alpha\alpha\beta\beta}K_\alpha^2K_\beta^2x^4, \quad [39]$$

where K_α and K_β are the binding affinities of the two chains and the constants c are appropriate interaction constants. A distinction between $c_{\alpha\beta}$ and $c'_{\alpha\beta}$ must be made to take into account differential pairwise interactions between $\alpha_1\beta_2$ and $\alpha_1\beta_1$ pairs. The existence of two pairs of identical subunits in the hemoglobin tetramer reduces the number of independent parameters of the local description from 15 to 9 (2). Symmetry of the global binding curve requires $A_4 = (A_3/A_1)^2$. This condition *per se* does not involve the coefficient of the second power of x in Eq. **39** and, therefore, symmetry (or lack of it) cannot depend on any of the second-order or pairwise interaction constants $c_{\alpha\alpha}$, $c_{\beta\beta}$, $c_{\alpha\beta}$, or $c'_{\alpha\beta}$. Symmetry requires

$$c_{\alpha\alpha\beta\beta}(K_\alpha + K_\beta)^2 = (c_{\alpha\alpha\beta}K_\alpha + c_{\alpha\beta\beta}K_\beta)^2 \quad [40]$$

and depends on the association constants of the two chains and third- and fourth-order interaction constants. Even if $K_\alpha = K_\beta$, asymmetry is observed whenever $4c_{\alpha\alpha\beta\beta} \neq (c_{\alpha\alpha\beta} + c_{\alpha\beta\beta})^2$, which can be satisfied even if $c_{\alpha\alpha\beta} = c_{\alpha\beta\beta}$. Therefore, symmetry or asymmetry demands neither subunit heterogeneity nor asymmetric interactions. On the other hand, if $c_{\alpha\alpha\beta\beta}$, $c_{\alpha\alpha\beta}$, and $c_{\alpha\beta\beta}$ are modeled in terms of pairwise interactions so that $c_{\alpha\alpha\beta\beta} = c_{\alpha\alpha}c_{\beta\beta}c_{\alpha\beta}^2c'^2_{\alpha\beta}$, $c_{\alpha\alpha\beta} = c_{\alpha\alpha}c_{\alpha\beta}c'_{\alpha\beta}$, and $c_{\alpha\beta\beta} = c_{\beta\beta}c_{\alpha\beta}c'_{\alpha\beta}$, then asymmetry necessarily demands $c_{\alpha\alpha} \neq c_{\beta\beta}$, regardless of subunit heterogeneity, which is the important result of Weber (13). Does Eq. **40** introduce any model-independent limitation on the binding properties of the chains? If the oxygen binding curves $X_\alpha(x)$ and $X_\beta(x)$ of the two chains are symmetric, then one necessarily has from Eq. **24** that the two centers of symmetry are $x_\alpha = K_\alpha^{-1}(c_{\alpha\beta\beta}/c_{\alpha\alpha\beta\beta})^{1/2}$ and $x_\beta = K_\beta^{-1}(c_{\alpha\alpha\beta}/c_{\alpha\alpha\beta\beta})^{1/2}$. These centers are identical to the mean ligand activities of the chains and, therefore, the square root of their product gives $(c_{\alpha\alpha\beta\beta}K_\alpha^2K_\beta^2)^{-1/4}$ [i.e., the mean ligand activity of the system as a whole (2)]. Hence, $c_{\alpha\alpha\beta\beta} = c_{\alpha\alpha\beta}c_{\alpha\beta\beta}$ and substitution into Eq. **40** shows that asymmetry necessarily demands $c_{\alpha\alpha\beta} \neq c_{\alpha\beta\beta}$, again regardless of subunit heterogeneity. However, in the specific case of interest for hemoglobin, the left-hand side of Eq. **40** far exceeds the right-hand side (11, 12), which necessarily implies that either $(K_\alpha/K_\beta)^2 > c_{\alpha\beta\beta}/c_{\alpha\alpha\beta} > 1$ or $(K_\alpha/K_\beta)^2 < c_{\alpha\beta\beta}/c_{\alpha\alpha\beta} < 1$. Since the ratio K_α/K_β is about 4 under physiological conditions, the first inequality applies and the ratio $c_{\alpha\beta\beta}/c_{\alpha\alpha\beta}$ cannot exceed 16. The first inequality also implies that $x_\alpha > x_\beta$, so that the binding curve $X_\beta(x)$ is shifted to the right with respect to $X_\alpha(x)$. If $X_\alpha(x)$ and $X_\beta(x)$ are not symmetric, there is no need to invoke asymmetric interactions to obtain a resulting global binding curve that is itself asymmetric, regardless of subunit heterogeneity. Knowledge of the shape of chain-specific oxygen binding curves under physiological conditions is, therefore, necessary to establish whether the asymmetric oxygen binding curve of human hemoglobin is due to asymmetric interactions.

Symmetry and Linkage

The symmetry conditions derived in the case of local binding processes also apply in the analysis of linkage effects. The function $K_s(x)$ in Eq. **16** is a change in association constant for ligand binding to site s due to a change in the ligand activity. In the local picture the remaining $t - 1$ sites of the macromolecule act as "allosteric effectors" of site s. Accordingly, the derivative $d[\ln K_s(x)]/d(\ln x)$ gives the change in ligands bound to the remaining $t - 1$ sites when site s is ligated and is directly related to the Hill coefficient of site s (2); i.e.,

$$d[\ln K_s(x)]/d(\ln x) = {}^1\bar{X}(x) - {}^0\bar{X}(x) = n_s(x) - 1. \quad [41]$$

The difference in saturation between the remaining $t - 1$ sites other than s provides the driving force for cooperative binding to site s. Linkage relationship **41** is mathematically equivalent to the change in the equilibrium constant K for binding ligand Y to the macromolecule due to a change in the activity of a second ligand X (1), with ${}^1\bar{X}(x)$ and ${}^0\bar{X}(x)$ being the amount of ligand X bound to the unligated and Y-ligated form of the macromolecule. Symmetry of $\ln K$ in this case is subject to a set of conditions among the coefficients of the partition functions for ligand X binding to the unligated and Y-ligated forms of the macromolecule. If α_j and β_j are the coefficients entering the definition of ${}^0\bar{X}(x)$ and ${}^1\bar{X}(x)$, K_j is the value of K in the absence of ligand X, and x_s is the center of symmetry of $\ln K$, then the conditions for symmetry are given by Eq. **23**. Summations in this case run from zero to the number of binding sites for ligand X. Analogous expressions arise in the analysis of linkage effects in steady-state kinetics (15), where K represents either K_m or k_{cat} as a function of a control ligand.

This work was supported by National Science Foundation Grant DMB91-04963 (E.D.C.).

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