EFFECTS OF HEMOGLOBIN SYMMETRY IN A STATISTICAL EQUILIBRIUM MODEL FOR OXYGEN BINDING

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ABSTRACT The effect of hemoglobin symmetry on the statistical mechanics of its motions is considered. Hemoglobin binding equilibrium constants are presented in which symmetry factors appear that differ from one binding step to another. Inclusion of the symmetry factors improves the fit of a symmetry-modified Koshland, Némethy, Filmer expression (1966, *Biochemistry*, 5:365–385) with tetrahedral oxy-oxyhemoglobin subunit interactions to the high-ionic-strength binding curve of Rossi-Fanelli, Antonini and Caputo (1961, *J. Biol. Chem.*, 236:397–400).

INTRODUCTION

Hemoglobin is a protein that binds oxygen in the alveoli and distributes it throughout the body. Therefore it must bind oxygen at the partial pressure of ~100 torr prevailing in the lungs and release it in the tissues where the partial pressure is lower (1). Essentially all hemoglobin binding sites are occupied in the lungs, but a substantial amount is released in the tissues (2). This property of hemoglobin makes it well-suited for its role of oxygen carrier.

The hemoglobin molecule contains two alpha subunits and two beta subunits. The subunits are arranged such that the hemoglobin molecule possesses a dyad, or twofold, axis of symmetry. The alpha and beta subunits differ primarily in the number and sequence of their amino acid residues (3).

The site of oxygenation in hemoglobin is a substituted porphyrin ring embedded in a pocket formed by the protein subunit to which it is attached at the *N* atom of the proximal histidine. There are four such pockets each located close enough to the surface of the molecule (4) for easy oxygenation. The porphyrin ring contains the central iron to which oxygen attaches. The pressure dependent ability of hemoglobin to deliver oxygen in the living system is thought to be caused by subunit interactions which facilitate oxygen uptake at high pressures and release at low pressures (5, 6). These characteristics of hemoglobin are known as cooperative effects (5, 6).

Concerning the precise nature of the physical cause of cooperativity, not a great deal is known definitively. However, stereochemical changes in structure are observed in going from deoxy- to oxy-hemoglobin by means of x-ray analysis of the crystal structures of oxy- and deoxy-hemoglobin (5). Specifically, there is a change in the relative orientations of the four subunits in which the distances between certain iron atoms of the subunits change measurably. These stereochemical effects are accompanied by physiologically important secondary shifts in chemical equilibria involving salt bridges, Bohr protons, phosphate binding and carbon dioxide solubility (5, 6). A great deal of evidence has accumulated to support Perutz's suggestion (5) that the triggering of the changes is very closely associated with the fact that the distance of the iron atom from the heme plane changes upon oxygenation. Perutz offered a provocative mechanical model (5) of how this heme geometry change in one subunit is transmitted to other heme subunits. Presumably the movements are transmitted via a series of electronic interactions between iron and histidine, histidine and protein, protein and solvent.

We are not aware of considerations of the entropic effects of the symmetry making and breaking when successive molecules of oxygen are bound to hemoglobin. However, various models have been proposed to explain cooperativity, such as those of Monod, Wyman, and Changeux (MWC) (7), Koshland, Némethy, and Filmer (KNF) (8), and Perutz (5). Each of these models makes certain assumptions. The MWC model assumes that there is no difference between the alpha and beta subunits, that hemoglobin has two quaternary conformations and that these conformations have different affinities for oxygen. The KNF model allows for different deoxy-deoxyhemoglobin, oxy-oxyhemoglobin, and oxy-deoxyhemoglobin subunit interactions. Both of these models have been fit to the oxygen binding curve. Whereas the MWC and KNF models deal with hemoglobin mainly on the phenomenological level, the Perutz (5) model considers hemoglobin at the molecular level and seeks to explain the cooperative effect using the stereochemical interactions of the subunits.

Perutz's model is somewhat qualitative in specifying exactly what the physical sources of the heme subunit interactions are but the possibility of direct electronic

interactions between the hemes is discounted (5). Disappointingly little progress has been made in pinpointing the actual physical means by which oxygen binding triggers these changes. One interesting attempt is Rousseau's electron donor-acceptor model (9). In view of recently discovered low-lying heme electronic states, perhaps the matter deserves further attention. In this paper we consider the statistical mechanical effects of symmetry changes in successive oxygen binding steps.

Because hemoglobin binds oxygen reversibly with up to four oxygen molecules per hemoglobin molecule, Adair (10) proposed a sequence of binding steps with equilibria.

$$\mathrm{Hb(O_2)_{n-1}} + \mathrm{O_2} \underset{k_{-n}}{\overset{k_n}{\rightleftharpoons}} \mathrm{Hb(O_2)_n} \qquad n = 1, 2, 3, 4. \tag{1}$$

The tetrameric hemoglobin molecule is represented by Hb.

Adair's model views the binding of oxygen in a sequential way. It involves two simplifying assumptions in its application to hemoglobin equilibria: (*a*) Each stage can be treated as a single reaction step with an "on" and "off" rate constant; (*b*) all forms that contain equal numbers of oxygenated hemes are equivalent (10).

From the equilibrium constants defined in Eqs. 2 and 3, Adair showed that the average number, N, of oxygens

$$K_n = \frac{k_n}{k_{-n}} \tag{2}$$

$$K_n = \frac{[\mathrm{Hb(O_2)_n}]}{[\mathrm{Hb(O_2)_{n-1}}][\mathrm{O_2}]} \tag{3}$$

bound at a given oxygen concentration is given by Eq. 4:

$$N = \frac{\{K_1[\mathrm{O_2}] + 2K_1K_2[\mathrm{O_2}]^2 + 3K_1K_2K_3[\mathrm{O_2}]^3 + 4K_1K_2K_3K_4[\mathrm{O_2}]^4\}}{\{1 + K_1[\mathrm{O_2}] + K_1K_2[\mathrm{O_2}]^2 + K_1K_2K_3[\mathrm{O_2}]^3 + K_1K_2K_3K_4[\mathrm{O_2}]^4\}}. \tag{4}$$

Equilibrium constants for successive binding steps have been used in Eq. 4 rather than the affinity constants often employed.

The fact that Adair's expression gave a sigmoidal shape for the oxygen binding curve with K_n that differed by as much as their statistical error pointed to the existence of some cooperative phenomenon between the hemoglobin subunits (2).

The KNF model formulates the equilibrium in a way similar to Adair's model but incorporates equilibrium constants for the subunit interactions. The model gave a satisfactory fit to experimental binding curves (8).

In this paper, a statistical mechanical formulation of binding is considered that accounts for the symmetry of hemoglobin, but otherwise uses Adair's assumptions. A symmetry modified KNF expression for the binding curve is developed from the statistical equilibrium constants. The symmetry adapted binding expression gives an improved fit to the experimental oxygen binding curve.

STATISTICAL EQUILIBRIUM MODEL

The equilibrium constants in the Adair model may also be expressed in statistical mechanical terms (11) by beginning with Eq. 5:

$$\frac{N_{\mathrm{Hb(O_2)_n}}}{N_{\mathrm{Hb(O_2)_{n-1}}} N_{\mathrm{O_2}}} = e^{-\Delta E_n/kT} \frac{q_{\mathrm{Hb(O_2)_n}}}{q_{\mathrm{Hb(O_2)_{n-1}}} q_{\mathrm{O_2}}} W_n \tag{5}$$

where N is the number of each species, q is the partition function of each species relative to its ground state energy, and ΔE_n is the difference in the ground state energies between the product and the reactants in Eq. 1. W_ns are statistical factors that are the ratios of the numbers of ways of binding n and $n-1$ oxygens. The W_ns do not take the symmetries of $\mathrm{Hb(O_2)_n}$ and $\mathrm{Hb(O_2)_{n-1}}$ into account.

Let us express the partition functions of the system with regard to the modes of motion of the hemoglobin and the oxygen in solution. In particular, let us distinguish between translational diffusion (diff) and all other modes of motion around the center of mass (cm). Then it is assumed that the partition functions of the species $\mathrm{Hb(O_2)_n}$, $\mathrm{O_2}$ and $\mathrm{Hb(O_2)_{n-1}}$ can be factored into products of partition functions of the diffusional and center-of-mass motions given in Eqs. 6–8:

$$q_{\mathrm{Hb(O_2)_n}} = q^{\mathrm{diff}}_{\mathrm{Hb(O_2)_n}}\, q^{\mathrm{cm}}_{\mathrm{Hb(O_2)_n}} \tag{6}$$

$$q_{\mathrm{Hb(O_2)_{n-1}}} = q^{\mathrm{diff}}_{\mathrm{Hb(O_2)_{n-1}}}\, q^{\mathrm{cm}}_{\mathrm{Hb(O_2)_{n-1}}} \tag{7}$$

$$q_{\mathrm{O_2}} = q^{\mathrm{diff}}_{\mathrm{O_2}}\, q^{\mathrm{cm}}_{\mathrm{O_2}} \tag{8}$$

If the partition functions are substituted into Eq. 5 and the factors that involve the diffusion and center-of-mass motions are lumped together, Eq. 9 results:

$$\frac{N_{\mathrm{Hb(O_2)_n}}}{N_{\mathrm{Hb(O_2)_{n-1}}} N_{\mathrm{O_2}}} = e^{-\Delta E_n/kT} \left(\frac{q^{\mathrm{diff}}_{\mathrm{Hb(O_2)_n}}}{q^{\mathrm{diff}}_{\mathrm{Hb(O_2)_{n-1}}}\, q^{\mathrm{diff}}_{\mathrm{O_2}}}\right) \cdot \left(\frac{q^{\mathrm{cm}}_{\mathrm{Hb(O_2)_n}}}{q^{\mathrm{cm}}_{\mathrm{Hb(O_2)_{n-1}}}\, q^{\mathrm{cm}}_{\mathrm{O_2}}}\right) W_n. \tag{9}$$

Next, the diffusion partition functions are expressed in the form of the translational partition function in Eq. 10.

$$q^{\mathrm{diff}} = \left(\frac{2\pi m^{\mathrm{diff}} kT}{h^2}\right)^{3/2} V \tag{10}$$

where m^{diff} represents the effective diffusional mass of the species, T represents the temperature, and V the free volume of the system. The ability to be factored and the specific form of the diffusion partition function will not be of great further importance except for the general principal that q be proportional to the sample volume. The factor in Eq. 9 for the diffusion is given separately in Eq. 11:

$$\frac{q^{\mathrm{diff}}_{\mathrm{Hb(O_2)_n}}}{q^{\mathrm{diff}}_{\mathrm{Hb(O_2)_{n-1}}}\, q^{\mathrm{diff}}_{\mathrm{O_2}}} = \left(\frac{h^2}{2\pi kT}\right)^{3/2} \left(\frac{m^{\mathrm{diff}}_{\mathrm{HB(O_2)_n}}}{m^{\mathrm{diff}}_{\mathrm{Hb(O_2)_{n-1}}}\, m^{\mathrm{diff}}_{\mathrm{O_2}}}\right)^{3/2} V^{-1}. \tag{11}$$

To change the number ratio in Eq. 9 to the form of the concentration equilibrium constant K_n in Eq. 3, Eq. 11 is substituted into Eq. 9, and both sides are multiplied by $L \times V$ where L is Avogadro's number. The result is Eq. 12:

$$K_n = L\left(\frac{h^2}{2\pi kT}\right)^{3/2} e^{-\Delta E_n/kT}\left(\frac{m^{\text{diff}}_{\text{Hb}(O_2)_n}}{m^{\text{diff}}_{\text{Hb}(O_2)_{n-1}}\, m^{\text{diff}}_{O_2}}\right)^{3/2} \left(\frac{q^{\text{cm}}_{\text{Hb}(O_2)_n}}{q^{\text{cm}}_{\text{Hb}(O_2)_{n-1}}\, q_{O_2}}\right) W_n. \quad (12)$$

In the following development, two aspects will be considered: First, the extent to which the equilibrium constant, K_n, is dependent on the particular binding step n is relevant to the cooperativity of binding. For example, if the effective diffusional masses of the hemoglobin species are essentially the same, as given in Eq. 13, the translational diffusion degrees of freedom make no n-dependent contribution to Eqs. 11 or 12:

$$m^{\text{diff}}_{\text{Hb}(O_2)_n} = m^{\text{diff}}_{\text{Hb}(O_2)_{n-1}} \quad (13)$$

The second aspect is the symmetry of the hemoglobin molecule at various stages of binding. Fully oxygenated or deoxygenated hemoglobin posesses a twofold dyad axis. Hemoglobin bound to one or three oxygen molecules has no symmetry. Two possibilities exist when two oxygens are bound. If one alpha and one beta subunit are bound there is no symmetry, but if both alpha or both beta are bound there is again a twofold axis of symmetry. These symmetries influence the q^{cm} in the equilibrium constant K_n in Eq. 12 through the so-called symmetry numbers. This will be discussed next.

In most elementary presentations, the symmetry number is introduced in connection with the rotational partition function q^{rot}. The rotational partition function exists for independent rotational motion decoupled from other modes such as is found in the gas phase. In general, Eq. 14 gives the rotational partition function at ambient temperatures for a polyatomic molecule whose principal moments of inertia are I^a, I^b, and I^c (11):

$$q^{\text{rot}} = \frac{\pi^{1/2}}{\sigma}\left(\frac{2kT}{\hbar^2}\right)^{3/2}(I^a I^b I^c)^{1/2}. \quad (14)$$

The symmetry number σ appears in the denominator. Its effect is to divide the integral over all the molecular coordinates that yield the remainder of the expression by the number of equivalent and quantum mechanically redundant orientations of the molecule.

It could be argued persuasively that oxygenation of hemoglobin with a molecular weight of 64,000 with an oxygen molecule of molecular weight 32 will give no appreciable change in its principal moments of inertia. Even the known heme-heme distance changes (3) between oxy- and deoxy-hemoglobin account for at most 15% changes in the moments of inertia over four separate oxygenation steps. By comparison, the symmetry number changes from 1 (no symmetry) to 2 (twofold symmetry) are large. Thus the symmetry number would make a major n-dependent contribution to an equilibrium constant K_n expression in which rotational motion was factored from other motions.

However illuminating the inspection of the gas phase q^{rot} may be, it is questionable that rotation of the hemoglobin molecule or even the oxygen molecule can be completely decoupled from other center-of-mass or solvent motions. Moreover, it is also unlikely that vibrational or electronic motions can be completely decoupled. Fortunately the symmetry number concept can be applied to the whole center-of-mass partition function.

Consider q^{cm} for a molecule with σ symmetry equivalent regions. From a classical point of view q^{cm} is a phase space integral over just one of the σ symmetry equivalent regions of the molecular coordinates. Let us define Q^{cm} as the corresponding phase space integral over the whole molecular phase space. With this definition the $Q^{\text{cm}}_{\text{Hb}(O_2)_n}$ and $Q^{\text{cm}}_{\text{Hb}(O_2)_{n-1}}$ integrals would be over comparable regions of phase space. The center-of-mass partition function q^{cm} may now be expressed in terms of Q and the symmetry number σ as given in Eq. 15:

$$q^{\text{cm}} = Q^{\text{cm}}/\sigma. \quad (15)$$

If the q^{cm} in Eq. 15 is substituted into the K_n expression, Eq. 12, the expression for K_n in Eq. 16 is found:

$$K_n = \frac{L}{\sigma_{O_2}}\left(\frac{h^2}{2\pi kT}\right)^{3/2} e^{-\Delta E_n/kT}\left(\frac{m^{\text{diff}}_{\text{Hb}(O_2)_n}}{m^{\text{diff}}_{\text{Hb}(O_2)_{n-1}}\, m^{\text{diff}}_{O_2}}\right)^{3/2} \cdot \left(\frac{Q^{\text{cm}}_{\text{Hb}(O_2)_n}}{Q^{\text{cm}}_{\text{Hb}(O_2)_{n-1}}\, Q^{\text{cm}}_{O_2}}\right)\left(\frac{\sigma_{\text{Hb}(O_2)_{n-1}}}{\sigma_{\text{Hb}(O_2)_n}}\right) W_n. \quad (16)$$

The K_n expression may be further simplified to the form in Eq. 17:

$$K_n = W_n K'_n \frac{\sigma_{\text{Hb}(O_2)_{n-1}}}{\phi_{\text{Hb}(O_2)_n}}. \quad (17)$$

The constant K'_n is defined in Eq. 18 and is independent of hemoglobin symmetry and statistical factors:

$$K'_n = \frac{L}{\sigma_{O_2}}\left(\frac{h^2}{2\pi kT}\right)^{3/2} e^{-\Delta E_n/kT}\left(\frac{m^{\text{diff}}_{\text{Hb}(O_2)_n}}{m^{\text{diff}}_{\text{Hb}(O_2)_{n-1}}\, m^{\text{diff}}_{O_2}}\right)^{3/2} \cdot \left(\frac{Q^{\text{cm}}_{\text{Hb}(O_2)_n}}{Q^{\text{cm}}_{\text{Hb}(O_2)_{n-1}}\, Q^{\text{cm}}_{O_2}}\right). \quad (18)$$

Therefore K'_n contains all of the energy- and interaction-dependent contributions to cooperative behavior. If the ground state energy differences ΔE_n, the effective diffusional masses and the ratio of the center-of-mass partition functions are the same for every $\text{Hb}(O_2)_n$ and $\text{Hb}(O_2)_{n-1}$, then K'_n will be independent of the particular binding step n. Thus, even when the energetics and interactions at each binding step are equal, K_n may still depend on the particular binding step, not only through the well-recognized

statistical factor W_n, but also through the ratio of symmetry numbers. To our knowledge this has not been accounted for in previous treatments. In the next section of this paper, the effect of symmetry on the oxygen binding curve is considered.

SYMMETRY-MODIFIED BINDING

The symmetry factor in the equilibrium constant K_n in Eq. 18 depends on the particular binding step n. The presence of this factor introduces an apparent cooperativity into the oxygen binding. It does not depend on any physical interaction and is entirely entropic in nature. Therefore, it is natural to ask if symmetry modified binding expressions can make realistic predictions about the oxygen binding curve.

The first question to be asked is whether the symmetry factor alone, without any energy or interaction dependence on binding step n can predict the observed oxygen binding dependence on pressure. If it cannot, the final question to be asked is whether the symmetry modification can improve the fit of binding expressions that do include energy or interaction dependence on n.

When the subunits are energy and interaction equivalent, all of the K_n's given by Eq. 18 become equal. If these values are called K, the products $K_1, K_1K_2, \ldots$ that appear in the Adair Eq. 4 (10) may be found from Eq. 17. The general expression for these products is given in Eq. 19.

$$\prod_{i=1}^{n} K_i = \left(\prod_{i=1}^{n} W_i\right) K^n \frac{\sigma_{Hb(O_2)_0}}{\sigma_{Hb(O_2)_n}}, \qquad n = 1, 2, \ldots 4. \quad (19)$$

Here $\sigma_{Hb(O_2)_0}$ designates the symmetry number of deoxyhemoglobin (n = 0). The statistical constants W_n and symmetry constants $\sigma_{Hb(O_2)_n}$ are given in Table I for hemoglobin with various numbers n of bound oxygen molecules. Similar W_ns have been given by KNF for a tetrahedron of four equivalent subunits (8). These values are substituted into Eq. 19 and the resulting K_i products are substituted into the Adair (10) Eq. 4. The coefficients for the doubly oxygenated species of both symmetries are simply added together in the $[O_2]^2$ term: The result is given in Eq. 20.

TABLE I
THE STATISTICAL CONSTANTS W AND SYMMETRY CONSTANTS σ FOR HEMOGLOBIN WITH VARIOUS NUMBERS n OF BOUND OXYGEN MOLECULES

n	W_n	$\sigma_{Hb(O_2)_n}$
0	1	2
1	4	1
2 ($\alpha\alpha$ or $\beta\beta$)	2	2
2 ($\alpha\beta$)	4	1
3	4	1
4	1	2

$$N = \frac{8K[O_2] + 20K^2[O_2]^2 + 24K^3[O_2]^3 + 4K^4[O_2]^4}{1 + 8K[O_2] + 10K^2[O_2]^2 + 8K^3[O_2]^3 + K^4[O_2]^4} \quad (20)$$

The corresponding KNF expression (8) for noninteracting energy-equivalent subunits without the symmetry modification is given in Eq. 21 for comparison:

$$N = \frac{4K[O_2] + 12K^2[O_2]^2 + 12K^3[O_2]^3 + 4K^4[O_2]^4}{1 + 4K[O_2] + 6K^2[O_2]^2 + 4K^3[O_2]^3 + K^4[O_2]^4}. \quad (21)$$

When the subunits are assumed to interact but are otherwise energy-equivalent and the KNF derivation (8) is modified in a completely analogous way for symmetry, the binding expression in Eq. 22 is found

$$N = \frac{\{8K_{AB}^3K_{st}[O_2] + 20K_{AB}^4K_{BB}K_{st}^2[O_2]^2 + 24K_{AB}^3K_{BB}^3K_{st}^3[O_2]^3 + 4K_{BB}^6K_{st}^4[O_2]^4\}}{\{1 + 8K_{AB}^3K_{st}[O_2] + 10K_{AB}^4K_{BB}K_{st}^2[O_2]^2 + 8K_{AB}^3K_{BB}^3K_{st}^3[O_2]^3 + K_{BB}^6K_{st}^4[O_2]^4\}} \quad (22)$$

Here K_{st} is the product of the KNF substrate binding and transformation equilibrium constants, K_sK_t(8). The K_{AB} and K_{BB} are KNF interaction equilibrium constants between oxy- and deoxy-subunits and between oxy- and oxy-subunits, respectively, relative to the interaction between deoxy-subunits (8). The corresponding KNF expression without the symmetry modification is given in Eq. 23.

$$N = \frac{\{4K_{AB}^3K_{st}[O_2] + 12K_{AB}^4K_{BB}K_{st}^2[O_2]^2 + 12K_{AB}^3K_{BB}^3K_{st}^3[O_2]^3 + 4K_{BB}^6K_{st}^4[O_2]^4\}}{\{1 + 4K_{AB}^3K_{st}[O_2] + 6K_{AB}^4K_{BB}K_{st}^2[O_2]^2 + 4K_{AB}^3K_{BB}^3K_{st}^3[O_2]^3 + K_{BB}^6K_{st}^4[O_2]^4\}} \quad (23)$$

To answer the first question about the utility of the symmetry modification without subunit interactions, Eq. 20 was least-squares fitted to the high ionic strength binding data of Rossi-Fanelli et al. (12). This fit is compared in Fig. 1 with the fit of the KNF expression (reference 8, Eq. 21) to the same data. The symmetry adapted model ($\sigma = 0.4334$) does not fit the data as well as the KNF model ($\sigma = 0.3991$) but neither model appears to be very satisfactory without subunit interactions. Interestingly, both fits give the same constant K. It is clear that the symmetry modification alone is insufficient to fit the binding data. It is clearly necessary to include subunit interactions even when the symmetry modification is included.

Next, the second question about the utility of the symmetry modification in the presence of subunit interactions is addressed. Eq. 22 with $K_{AB} = 1$ was least-squares fitted to the same binding data (12) and compared with the fit of the unmodified KNF expression Eq. 23. Setting $K_{AB} = 1$ is purely for numerical convenience and is discussed by Koshland et al. (8). (See Fig. 1.) Again, both fits give identical binding constants K_{st} and interaction constants K_{BB}. The improved agreement in Fig. 1 leaves no doubt that including the subunit interactions is necessary even when symmetry is taken into account.

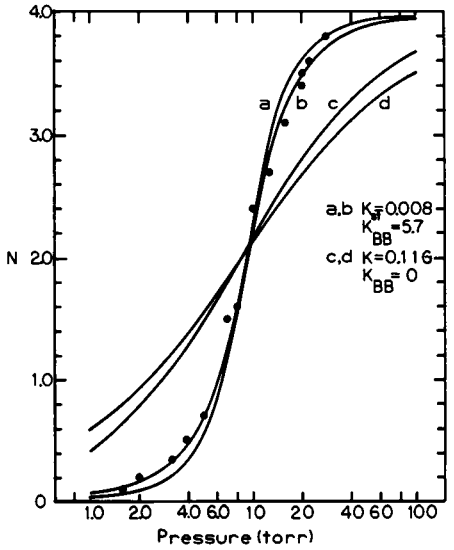

FIGURE 1 A comparison of four theoretical binding curves least-squares fitted to the experimental data (●) for human hemoglobin at high ionic strength. (*a*) The KNF model with subunit interactions. (*b*) The symmetry modified model with subunit interactions. (*c*) The KNF model with no subunit interactions. (*d*) The symmetry model with no subunit interactions.

However, the noteworthy point for the present discussion is that the symmetry modification gives a better fit ($\sigma = 0.1479$) than the KNF expression ($\sigma = 0.2006$). The fit is better particularly at the low and high pressure end of the binding curve. Precisely these discrepancies for the tetrahedral model led Koshland et al. (8) to prefer the structurally less attractive square model. The present work suggests that it is also necessary to include the symmetry of hemoglobin at various stages of binding. Thus, both subunit interactions and the symmetry modification appear to be necessary to describe oxygen binding but neither is sufficient alone.

SUMMARY

A quantum statistical mechanical formulation of the oxygen-binding equilibrium constants for hemoglobin has been developed. The reduction of the problem to types of factors that depend on the particular binding step has useful predictive value. The statistical, symmetry, and energy or subunit interaction factors were shown to depend on the particular binding step.

The equivalences with the binding curve constants of Adair (10) and Koshland et al. (8) were found. It was found necessary to modify the KNF binding expression for the symmetry factors for hemoglobin at its various stages of oxygen binding.

The possible significance of the symmetry factor was explored. Fits of the binding expressions with and without subunit interactions and symmetry modification were made to experimental oxygen binding data. The conclusion is that both subunit interactions and hemoglobin symmetry changes are in effect.

The symmetry effect is a purely entropic effect that contributes to the cooperativity of binding. It stabilizes the asymmetric singly- and triply-oxygenated species relative to the other forms. The symmetric doubly oxygenated form also tends to destabilize the doubly oxygenated forms slightly. The magnitude of the entropy effects of the symmetry changes in hemoglobin of the order of ln 2 entropy units is apparently significant to binding cooperativity. It is possible that the entropy effect of symmetry changes may also be relevant to the operation of other symmetric enzymes.

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