BRIEF COMMUNICATION

A SIMPLE TENSION-DISPLACEMENT MODEL FOR HEMOGLOBIN COOPERATIVITY

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ABSTRACT Based on the Perutz view of hemoglobin cooperativity and the methodology of statistical physics, a molecular model for heme-heme interactions is proposed. The motion of the iron atom with respect to the heme plane is assumed to be the important feature of the oxygenation step, and results in an expression for hemoglobin saturation as an explicit function of the internal tension of the hemoglobin molecule. Closure of the equation is obtained with the assumption of linearity between the internal tension and the displacement of the iron atom above the heme plane. All model parameters are physically realizable and are characteristic of the hemoglobin molecule. Finally, the model is capable of discriminating between positive and negative cooperativity.

INTRODUCTION

Based on extensive studies of the dependence of the oxygen-carrying capacity of hemoglobin on such variables as temperature, pH, and the concentration of other chemical species (e.g., 2,3-diphosphoglycerate), numerous models describing the oxygen-hemoglobin equilibrium have been proposed. Although the models and data are suggestive of the interrelation between structure and function of the hemoglobin molecule, only recently, through X-ray diffraction and nuclear magnetic resonance (NMR) studies, has this cooperative effect been clearly established. When coupled with the techniques of statistical physics (1, 2), the experimental evidence for the cooperativity displayed by hemoglobin has resulted in somewhat more realistic models for the binding of hemoglobin with oxygen.

The purpose of this paper is to demonstrate how specific structural assumptions can be used to formulate a unique molecular model for the equilibrium properties of hemoglobin. We utilize the elements of the Perutz view of hemoglobin cooperativity, and express hemoglobin saturation as an explicit function of the hemoglobin internal tension. Although many details concerning the electronic and geometric changes that occur during oxygenation have been omitted, the structural features included make it possible to attach a physical significance to each model parameter. This approach offers the prospect of relating the macroscopic properties (e.g., the fractional saturation of hemoglobin with oxygen) to a single set of molecular parameters in a logical and consistent manner. It is anticipated that models of this type will lead to a quantitative description of the oxygen exchange process, thereby providing a guide to the understanding of certain pathological states of the hemoglobin molecule.

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MODEL DEVELOPMENT

To formulate a suitable model for the oxygenation process, it is first necessary to identify the various mechanisms involved. Perutz (3) described the quaternary structure of hemoglobin as four heme groups ($\alpha_1, \alpha_2, \beta_1, \beta_2$) located roughly at the corners of an irregular tetrahedron, the distance between the groups ≥ 25 Å. Heme-heme interactions may be defined as the dependence of the oxygen binding properties of a particular subunit heme on the state of other subunit hemes of the same hemoglobin molecule. Minton and Libby (4) concluded that the possibility of cooperative effects due to direct interaction between heme groups is slight, since long-range forces acting over this distance (25 Å) are unlikely. Heme-heme interactions arising from steric hindrance, as implied by the "buried heme" hypothesis suggested by St. George and Pauling (5), may also be ruled out: the overall arrangement of heme groups in the molecule does not change when cooperative binding is eliminated as a result of chemical modifications.

Perutz (6) attributes heme-heme interactions to structural changes accompanying the reversible transition between the oxygenated and deoxygenated states of the hemoglobin molecule. The differences in both the ternary structures of the α and β subunits and in their quaternary arrangements within the tetramer are brought about through simultaneous changes in the spin state of the iron atom and length of the iron-nitrogen bonds. Experimental evidence suggests that the transition between the quaternary structures alters the oxygen affinity of the molecule through changes in the spin states of the subunit hemes. When oxygenated (R), the hemes are in a low spin state and are as relaxed as if free subunits. However, the deoxygenated (T) structure is literally tense, in that constraints are placed on the hemes. These constraints raise the spin state of hemes, thereby diminishing their oxygen affinity by opposing the change to the low spin state.

THEORETICAL DEVELOPMENT

The cooperative effect arising from the heme-heme interaction are extremely complex. Any model purporting to describe such phenomena would, out of necessity, be an oversimplification. The best one could hope to achieve is a model both simple and realistic, and whose parameters have a physical meaning. It is in this context that the following model is presented.

We begin by considering the hemoglobin molecule to consist of M[1] identical binding sites, which interact only through the aforementioned tension changes. Each binding site is assumed to be in one of three states: state (σ) of tension before oxygenation, a state (ξ) of reduced tension due to oxygenation of neighboring sites, and a state (γ) of direct oxygenation. Associated with each state is a length l, where $l_\sigma > l_\xi > l_\gamma$. It is useful to consider l_i (for $i = \sigma, \xi, \gamma$) to be proportional to the distance between the iron atom and the plane formed below by the four central nitrogen atoms.[2] As suggested by Perutz (6), the motion of the iron atom with respect to the heme plane is the important feature of the oxygenation

[1] $M = 4$ for hemoglobin in its tetrameric configuration.

[2] The present estimated location (l_σ) of the iron atom above the plane is 0.75 Å in T structure deoxyhemoglobin. In the oxygenated state the heme group is approximately planar, i.e., $l_\gamma = 0$. No information is available on the intermediate length, l_ξ.

step. The tension change, $d\tau$, is then the result of the transition $l_\sigma \rightarrow l_\xi \rightarrow l_\gamma$ upon oxygenation.

We now view the hemoglobin molecule as being subdivided into immobile components (the heme groups) forming a stable one-dimensional network that provides the molecule with elastic properties, and a mobile component (oxygen) traveling within the network (7). Since the heme groups cannot move freely inside the hemoglobin molecule, diffusional equilibrium with respect to these groups is, in principle, impossible, thus making the definition of their chemical potentials somewhat nebulous. Nevertheless, we assume here that the structural changes that occur during binding are such that all binding sites (states) are in a state of equilibrium; i.e., $\mu_\gamma = \mu_\xi = \mu_\sigma \equiv \mu_s$, where μ_i is the chemical potential of a site in state i. Denoting M_i as the number of sites in state i, where

$$M = M_\sigma + M_\xi + M_\gamma, \tag{1}$$

the first law of thermodynamics takes the form

$$dU = TdS + \tau(l_\xi - l_\sigma)dM_\xi + \tau(l_\gamma - l_\sigma)dM_\gamma + (\mu_s + \tau l_\sigma)dM + \mu dN. \tag{2}$$

Here τ is the internal tension of the hemoglobin molecule, N represents the number of bound oxygen molecules, and μ is the corresponding chemical potential.

If we denote j_i as the single site partition function for state i, then the canonical ensemble partition function, Q, is (8)

$$Q(\{M\}, T) = \left[\frac{M!}{M_\sigma! M_\xi! M_\gamma!}\right] j_\sigma^{M_\sigma}(T) j_\xi^{M_\xi}(T) j_\gamma^{M_\gamma}(T). \tag{3}$$

Note that Eq. 3 assumes there are no long-range forces of interaction between the various heme groups, an assumption consistent with the findings of Minton and Libby (4). To relate the degree of saturation of the hemoglobin molecule with oxygen to the partition functions j_i (for $i = \sigma, \xi, \gamma$), we consider the partition function

$$Y(\lambda, T) \equiv \sum_N \sum_{\{M\}} Q(\{M\}, T) \eta_\xi^{M_\xi} \eta_\gamma^{M_\gamma} \lambda^N,$$

where $\sum_{\{M\}}$ implies a summation over all binding sites, such that Eq. 1 is satisfied,

$$\eta_\xi \equiv \exp\{\tau(l_\xi - l_\sigma)/kT\}, \qquad \eta_\gamma \equiv \exp\{\tau(l_\gamma - l_\sigma)/kT\},$$

and

$$\lambda \equiv \exp\{\mu/kT\}. \tag{4}$$

Since the number of oxygen molecules bound to a hemoglobin molecule is simply M_γ, Eq. 4 reduces to

$$Y(\lambda, T) = [j_\sigma + j_\xi \eta_\xi + j_\gamma \eta_\gamma \lambda]^M. \tag{5}$$

Defining the fractional saturation, ψ, as

$$\psi \equiv <N> / M. \tag{6}$$

where

$$<N> = \sum_N N \sum_{\{M\}} Q(\{M\}, T)\eta_\xi^{M_\xi}\eta_\gamma^{M_\gamma}\lambda^N \Big/ Y(\lambda, T), \tag{7}$$

leads to the desired result:

$$\psi = \eta_\gamma\lambda/[(j_\sigma/j_\gamma) + (j_\xi/j_\gamma)\eta_\xi + \eta_\gamma\lambda]. \tag{8}$$

Since the explicit form used for j_i depends on the particular system of interest, it is necessary to make some assumptions concerning the nature of the binding sites. Note, however, that the ratios j_σ/j_ξ and j_ξ/j_γ reflect the relative stability of three states (σ, ξ, γ).

An assumption regarding the nature of the tension-displacement interaction must be made to render Eq. 8 tractable to numerical evaluation. The response of atoms in a molecule to an applied force may be considered linear, as long as the force is sufficiently small (9). Of course, the criterion for smallness depends on the bond type: a very weak bond can store only energies less than kT in a linear fashion, whereas a covalent bond will still be responding linearly when it has stored several kilocalories of strain energy. For this reason, the assumption of linearity is somewhat ambiguous. However, since it does simplify the treatment considerably, we make that assumption here:

$$d\tau = E d<l> . \tag{9}$$

In Eq. 9 the proportionality constant E is the elastic modulus and $<\ldots>$ denotes an ensemble average similar to Eq. 7. Since the tension decreases upon oxygenation, we require $E > 0$. Defining

$$l \equiv M_\sigma l_\sigma + M_\xi l_\xi + M_\gamma l_\gamma, \tag{10}$$

we find

$$<l> = M[l_\sigma + (l_\xi - l_\sigma)\phi + (l_\gamma - l_\sigma)\psi], \tag{11}$$

where ϕ is the fraction of sites in state ξ,

$$\phi = <M_\xi>/M, \tag{12}$$

and is given by

$$\phi = \eta_\xi/[(j_\sigma/j_\xi) + \eta_\xi + (j_\gamma/j_\xi)\eta_\gamma\lambda]. \tag{13}$$

Note that the assumption of linearity implied by Eq. 9 eliminates the direct functional dependence of ψ on τ and therefore provides a closed equation for the fractional saturation. Also note that it is possible to construct a nonlinear model for τ at the expense of introducing additional parameters whose physical significance is uncertain.

The final step in the reduction of Eq. 8 is the evaluation of the chemical potential of the bound oxygen, μ. Since equilibrium exists between the bound oxygen and free oxygen in solution, we have $\mu = \mu^*$, where μ^* is the free oxygen chemical potential in solution:

$$\mu^* = \mu_0 + kY \ln [P_{o_2}]. \tag{14}$$

Here μ_0 is the reference state chemical potential. It is apparent from Eq. 14 that λ is proportional to the oxygen partial pressure, P_{o_2}.

MODEL BEHAVIOR

Based on specific structural assumptions regarding the nature of heme-heme interactions, a molecular model for the fractional saturation of hemoglobin with oxygen has been developed in terms of the single-site partition functions, j_i (for $i = \sigma, \xi, \gamma$), and the elastic modulus, E. In this section we examine the functional dependence of ψ on these parameters; a complete data regression will appear later.

Many details of the binding process are contained in the single-site partition functions, j_i. To obtain an explicit form for the partition functions, it is first necessary to make some assumptions regarding the nature of the binding sites. For simplicity, we consider a lumped-parameter (with respect to j_i) analysis of the fractional saturation, and examine the dependence of ψ on the elastic modulus. As suggested by Cornish-Bowden and Koshland (10), a functional analysis of this type provides substantial information concerning the binding process. For example, if the sites interact in such a way that binding at any one site impedes (negative cooperativity) binding at the remaining sites, we require the constraint $E < 0$. Also, there may be no cooperativity at all, in which case the internal tension remains constant and $E = 0$. Cornish-Bowden et al. (10) provide a detailed analysis of the various combinations of the "intrinsic association constants" appearing in the Adair model; we present only the three cases $E > 0$ (positive cooperativity), $E = 0$ (noncooperative), and $E < 0$ (negative cooperativity) to illustrate the functional behavior suggested by Eq. 8.

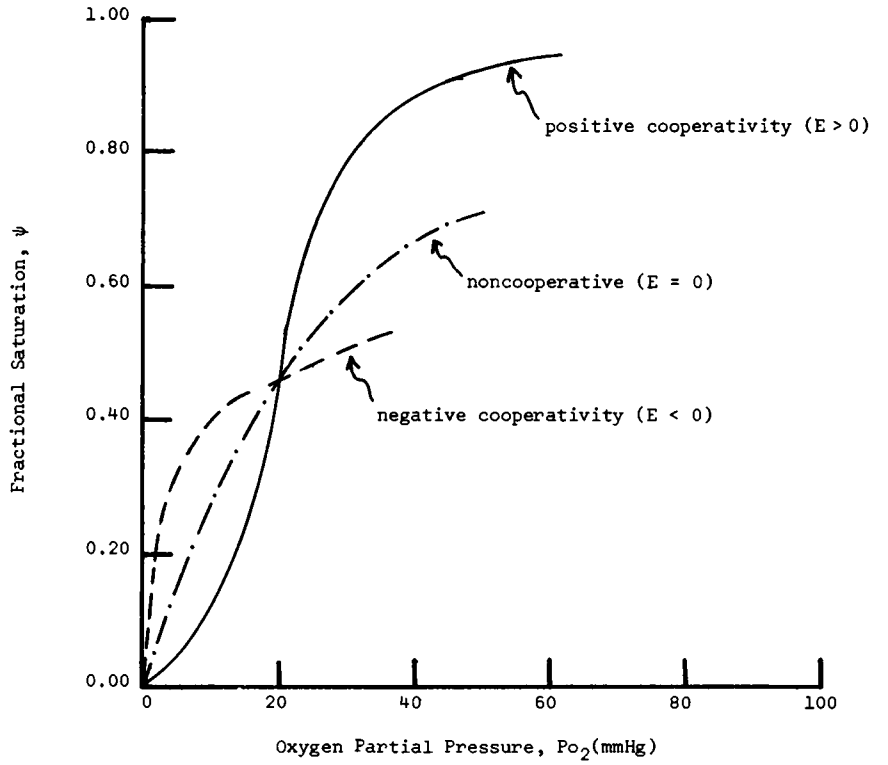

FIGURE 1 Functional dependence of the fractional saturation of hemoglobin with oxygen on the elastic modulus, E.

TABLE I
PARAMETERS USED IN MODEL SIMULATION

Parameter	Case		
	Negative cooperativity	Noncooperative	Positive cooperativity
E	−2.27	0.0	1.67
$e^{-\mu_0/kT\left(\frac{j_\sigma}{j_\gamma}+\frac{j_\xi}{j_\gamma}\right)}$	257.20	20.00	3.05

$M = 4; l_\gamma = 0; l_\xi = l_\sigma = 0.75$.

That this model is consistent with the assumed molecular behavior is clearly illustrated in Fig. 1. For comparison, the various values (see Table I) for the model parameters were chosen such that all three curves intersect at the point (P_{O_2} = 20 mm Hg, ψ = 0.45). The effects of the model parameters will be discussed in detail and compared with oxygen saturation data in a subsequent communication.[3]

CONCLUDING REMARKS

The tension-displacement model proposed here is based on the Perutz view of hemoglobin cooperativity, and provides an expression for the hemoglobin saturation as an explicit function of the internal tension of the hemoglobin molecule. Because of the molecular framework of the model, every parameter has a unique physical significance. Furthermore, all thermodynamic variables, e.g., the Gibbs free energy change on binding,[3] are expressible as functions of the same set of parameters. Of course, extensions of the above model proceed in a logical and well-defined manner. It is felt that a unified treatment of this kind offers a comprehensive examination of hemoglobin cooperativity and provides for the prospect of interpreting certain pathological states of the hemoglobin molecule in terms of parameters characteristic of the molecule.

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[3] In preparation.