# A MATHEMATICAL MODEL FOR STRUCTURE-FUNCTION RELATIONSHIPS IN HEMOGLOBIN*

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SUMMARY. A mathematical model is outlined for the cooperative binding of oxygen by hemoglobin. The model, which is based on the Perutz stereochemical mechanism, involves parameters that correspond to well-defined chemical processes. Illustrative results are presented for the oxygenation curve, the Bohr effect, the variation of the Hill constant with pH, and the behavior of the des-hist (146β) species.

INTRODUCTION. Recently Perutz (1) has proposed a stereochemical mechanism for the cooperative binding of oxygen by hemoglobin. The essential elements are oxy and deoxy quarternary conformations of different stability for the hemoglobin tetramer, liganded and unliganded tertiary structures for each of the chains, and interchain salt bridges which couple the tertiary and quarternary structure. In this communication, we outline some results obtained from the formulation of a detailed mathematical model based on the Perutz mechanism. We demonstrate that the model, with values for its parameters chosen in accord with the limits set by their physical significance, is able to account for certain aspects of the available data for hemoglobin oxygenation. A more detailed evaluation of the model and its applications will be published elsewhere (2).

OUTLINE OF MATHEMATICAL MODEL. The model uses a generating function (3) to determine the equilibrium properties of hemoglobin. For the binding of $O_2$ and $OH^-$ (i.e., release of $H^+$), we write the generating function $\Xi$ in the form

$$\Xi = \sum_{i=0}^{N_1} \sum_{j=0}^{N_2} \lambda^i \mu^j q_{ij}^0 + \sum_{i=0}^{N_1} \sum_{j=0}^{N_2} \lambda^i \mu^j q_{ij}^D \qquad (1)$$

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Here $\lambda$ and $\mu$ are the activities of $O_2$ and $OH^-$ with $N_1$ and $N_2$ binding sites, respectively, and $q^O_{ij}$ and $q^D_{ij}$ are the equilibrium constants of the reaction $Hb + iO_2 + jOH^- \rightleftarrows Hb(O_2)_i(OH^-)_j$ for the oxy and deoxy quarternary conformation. From Eq. (1), the fractional saturation $\langle y \rangle$ by $O_2$ and $OH^-$ is obtained directly; e.g., $\langle y_{O_2} \rangle = (\lambda/N_1)(\partial[\ell n \Xi(\lambda,\mu)]/\partial\lambda)_\mu$.

The specific assumptions of the model are introduced in the evaluation of $q^O_{ij}$ and $q^D_{ij}$. To obtain these quantities, it is necessary to enumerate the possible configurations of the hemoglobin tetramer and to determine their relative probabilities. This is done by utilizing diagrams, analogous to those of Perutz (1), and developing a simple set of rules for finding the relative probabilities from them. The elements involved are illustrated in Fig. 1.

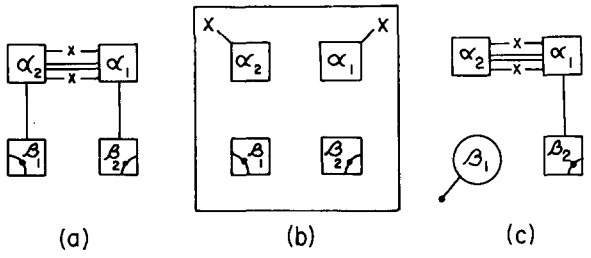


Fig. 1. Elements of Diagrams (see text for details).

Figure 1a represents the deoxy conformer with all chains having the unliganded structure (□) and all salt bridges intact. There are two pairs of α-α' salt bridges; a pair of α-β salt bridges, and a pair of internal β chain salt bridges; two of the α-α' salt bridges (—✱—) and the internal β-chain salt bridge (—●—) have protonated nitrogens that are expected to ionize in the alkaline pH range. In Fig. 1b, the oxy conformer with unliganded chains is shown; here only the internal β-chain salt bridges remain and the free —✱ lines are included to indicate the ionizable protons. Figure 1c corresponds to the deoxy conformer with one liganded β chain (○) (chain $\beta_1$); the salt bridges from that chain ($\beta_1$-$\alpha_2$, $\beta_1$ internal) are broken, but the other salt bridges are intact.

The parameters corresponding to the structural elements are the intrinsic free energy difference (-RT&nQ) between the oxy and deoxy quarter-

nary conformation of the tetramer, the free energy of oxygen binding and change in tertiary structure ($-RT\ell nK^{\alpha}$ and $-RT\ell nK^{\beta}$) of the individual chains in the tetramer, the free energy of salt bridge formation ($-RT\ell nS$), and the free energies of ionization ($-RT\ell nK_H^{\alpha}$ and $-RT\ell nK_H^{\beta}$) of the protonated nitrogens involved in salt bridges. All of these quantitite, except $RT\ell nQ$, are related to processes for which independent information is available so that possible values are limited in range. In what follows, we indicate how the parameters were determined and describe some applications of the model.

RESULTS AND DISCUSSION. The model parameters were determined by fitting the high-accuracy Roughton and Lyster (4) results for pH 7 and 9.1; $pK_H^{\beta}$ was set equal to 6.2, the free imidazole group value, and the other parameter were found to be $Q = 4.9\times10^{-5}$, $S = 39.3$, $K^{\alpha} = 4.69$ mmHg$^{-1}$, $K^{\beta} = 5.33$ mmHg$^{-1}$, $pK_H^{\alpha} = 7$. The resulting curves are shown in Fig. 2 with the experimental values. The

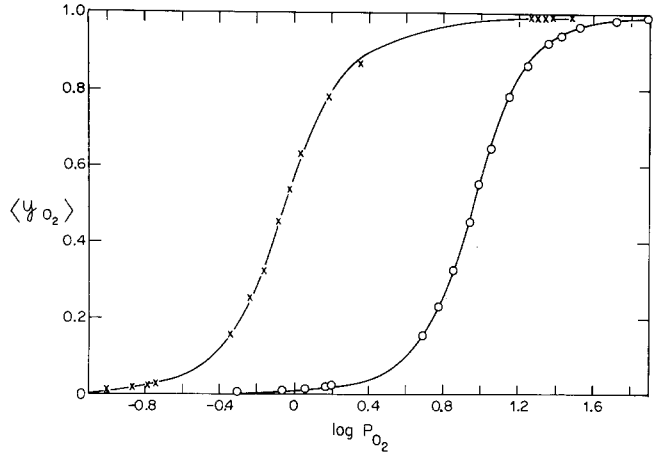


Fig. 2. Fractional Saturation versus $O_2$ partial pressure at pH 7 and pH 9.1. Curves are from model; experiments at pH 7 (ooo) and 9.1 (xxx).

important point is not that the agreement, per se, but rather that it is achieved with a satisfactory set of the physically meaningful parameters. The free energy change corresponding to S is 2.2 kcal/mole, a value in the range of data for model systems. (5) The binding constants $K^{\alpha}$ and $K^{\beta}$ are similar to the free chain values ($K \simeq 2$ mm Hg$^{-1}$); that the constants are

somewhat larger in the tetramer (which follows from the Roughton-Lyster data at high oxygen pressures) suggests that incorporation of the chains leads to structural alterations, in agreement with independent data (6). The $pK_H^\alpha$ value is in the range for the amino group of the N-terminal valine of the α-chain for the amino acid in proteins ($pK_H^\alpha = 7.5 - 8.3$), though it is significantly lower than the free valine value of 9.6. No direct estimate for the parameter Q is possible since detailed structural changes involved are not known. However, if Q and the interchain salt bridges were to account for the large difference between the dissociation constant of deoxy and oxy hemoglobin, one finds a value of $QS^6 \approx 2 \times 10^5$ in the first approximation; this is in satisfactory order of magnitude agreement with experiment (7).

The mathematical model provides a detailed picture of the contribution of various structures as a function of $O_2$ pressure, pH, and other variables. It is found that structures with two or three oxygens bound make only a small contribution and that at low oxygen pressures, the first β chain is oxygenated preferentially to an α chain, due to the salt bridges present in the deoxy conformer. Recently some experiments related to this point have been reported (8), but unequivocal results are not available.

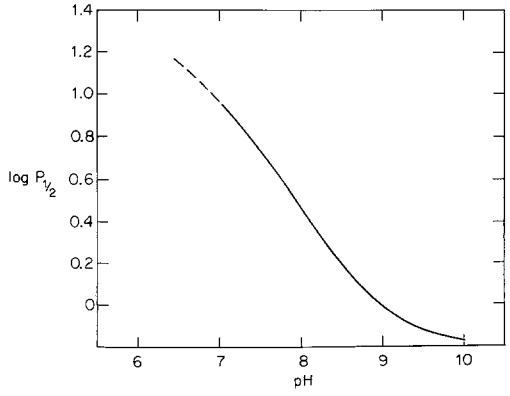


Fig. 3. Calculated Bohr effect: oxygen pressure at half saturation versus pH.

In Fig. 3, the calculated Bohr effect is shown by a plot of the logarithm of the oxygen partial pressure at half saturation versus pH. The Bohr effect is an intrinsic part of the present model; that is, it results

from the fact that the salt bridges involving nitrogens with ionizable protons can form only if the protons are present. Hence, there is no need for ad hoc assumptions such as have been used in the past to obtain the Bohr effect; e.g., different pK values for the oxy and deoxy forms (9), or arbitrary variation of the allosteric constant (e.g., L of the Monod-Wyman-Changeux model) with pH (10).

A consequence of the dependence of salt bridge formation on pH is that the Hill constant n varies with pH (see Fig. 4). The variation is rather weak, however, because of the six interchain salt bridges only two $\alpha$-$\alpha'$ bridges have ionizable protons (see Fig. 1a). The experimental resuls **for** n versus pH are not clear. The work of Antonini and coworkers (11) indicates that n is constant within experimental error between pH 5 to pH 10, while the Roughton and Lyster results have n equal to 3 and 2.6 at pH 7 and 9.1, respectively. Since the latter measurements are complicated by a difference in ionic strength, it would be helpful to have additional data for comparison.

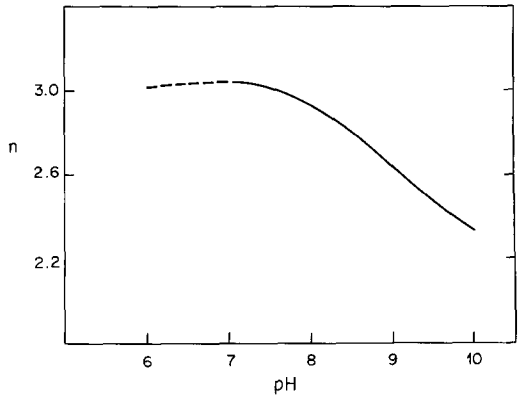


Fig. 4. Calculated variation of Hill constant n with pH.

Also, the stereochemical details of the $\alpha$-$\alpha'$ salt bridges involving the ionizable proton are somewhat uncertain; the coupling of n with pH would be reduced if allowance were made for the possibility that these interchain salt bridges have intrachain components.

As another application of the model, we consider the modified hemoglobins in which the C-terminal residues have been removed from the chains.

This case can be treated approximately by altering the parameter for the salt bridges in which the deleted residue was involved. In the des-hist (146β) molecules, for example, the internal β salt bridges are absent and the β-α salt bridges are weak. Introducing $\bar{S} = \frac{1}{2} S$ for the weak bridges, we find at pH 7, $n = 2.4$ and $p_{1/2} = 2.87$ mmHg, as compared with the experimental values of $n = 2.5$ and $p_{1/2} = 2.5$ mmHg (12).

It is clear from the above that a mathematical model based on the Perutz stereochemical mechanism can reproduce some of the data concerning hemoglobin oxygenation. A subsequent publication (2) presents a detailed description of the model; included are applications to the behavior of mutant and mixed-state hemoglobins and to the effect of 2,3-diphosphoglycerate, ionic strength, and dissociation on hemoglobin oxygenation.

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