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# Mechanism of Cooperative Oxygen Binding to Hemoglobin: Equilibrium Aspects†

Teresa Ree Chay* and David K. Brillhart

ABSTRACT: The sequential theory of D. E. Koshland, G. Nemethy, and D. Filmer [(1966), *Biochemistry 8,* 2580] is extended to explain the influence of 2,3-diphosphoglycerate and inositol hexaphosphate on the oxygen-binding properties of hemoglobin. The modification of the theory is made on the basis that organic phosphates can bind to hemoglobin molecules in intermediate stages of oxygenation and that the ligand affinities of the $\alpha$ and $\beta$ chains differ from each other when the hemoglobin molecule forms a complex with the organic phosphate. This model is applied to equilibrium oxygenation studies of hemoglobin and is seen to accurately represent the published data with a minimum of parameters.

The molecular mechanism for the cooperative oxygenation of hemoglobin (Hb) has been a subject of intense research during the past 70 years and has also been used as a model for understanding the actions of regulatory enzymes. There are enormous amounts of experimental data in the literature on both equilibrium and kinetic studies of this protein (Huisman and Schroeder, 1971; Antonini and Brunori, 1971). Human adult hemoglobin is a protein molecule consisting of four subunits, namely two $\alpha$ chains with 141 amino acid residues each and two $\beta$ chains with 146 amino acid residues each (Huisman and Schroeder, 1971). In 1910, Hill (1910) fitted the experimental data on the oxygenation of hemoglobin by using the empirical relations

$$\mathrm{Hb} + n\mathrm{O_2} \overset{k}{\rightleftharpoons} \mathrm{Hb(O_2)_n}$$

$$Y_{O_2} = kp^n/(1 + kp^n) \quad (1)$$

where $Y_{O_2}$ is the fraction of oxygenation, $k$ is the association constant, $p$ is the partial pressure of oxygen, and $n$ is the Hill coefficient which is an empirical measure of the cooperativity of the oxygenation of hemoglobin. For a protein molecule consisting of four subunits, the maximum value of $n$ is 4. The Hill coefficient in eq 1 would be unity if the four subunits were identical and noninteracting and if there were no quaternary structural change in the protein molecule. The Hill coefficient for the oxygenation of hemoglobin has been found to be approximately 3 (Antonini and

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Brunori, 1971). This suggests that the oxygenation of hemoglobin occurs in a cooperative manner. In 1925, Adair (1925a,b) obtained a better fit to the experimental oxygenation curve of Hb with four parameters, which are the successive affinity constants of oxygen molecules to the four heme groups in a hemoglobin molecule.

In the past, two phenomenological models have been proposed to account for the oxygenation of Hb: (i) the *allosteric transition model* proposed by Monod *et al.* (1965) and (ii) the *sequential model* proposed by Koshland *et al.* (1966). In the allosteric transition model, it is assumed that the binding of a ligand to one subunit does not affect the reactivity of the neighboring subunits and that the protein exists in two conformational states or quaternary structures (namely T and R) which differ in their ligand affinities. This model postulates the existence of an equilibrium between the two states of the protein even in the absence of a ligand. The sequential model, on the other hand, assumes that the ligand binding to one subunit in an oligomeric protein induces a conformational change in that subunit which in turn affects the interactions and the ligand affinities of the neighboring subunits. Thus, in an oligomeric protein, each subunit exists in one of the two conformations, A or B, where A and B are in equilibrium and the conformational state B is induced by binding of the ligand L to the state A. In the sequential theory (Koshland *et al.*, 1966), the interactions between the subunits may be represented by a set of three equilibrium constants; namely, $K_{AA} = 1$ (chosen as an arbitrary reference), $K_{AB} = (AB)(A)/(AA)(B)$, and $K_{BB} = (BB)(A)(A)/(AA)(B)(B)$. Here, the concentration terms with double letters indicate interacting subunits and those with single letters indicate noninteracting subunits. In addition, $K_L = (BL)/(B)(L)$ is the ligand binding constant, and $K_t = (B)/(A)$ is the equilibrium constant for changing the subunit in A conformation to that in B conformation. Attempts to distinguish these two models on the basis of the available information have not been very successful, although a number of illuminating papers relating certain experimental results to one model or the other have been published (Ogawa and McConnell, 1967; Ogawa *et al.*, 1968; Ogawa and Shulman, 1971; Edelstein, 1971; Minton, 1971).

In recent years proton nuclear magnetic resonance (nmr) studies have shown that the $\alpha$ and $\beta$ chains in the human adult Hb are nonequivalent (Davis *et al.*, 1969, 1971; Lindstrom and Ho, 1972; Ogawa and Shulman, 1972). Very recently, Johnson and Ho (1974) have found that $O_2$ exhibits a very slight preferential binding to the $\alpha$ chain in the absence of phosphate, marked preferential binding to the $\alpha$ chain in the presence of 2,3-diphosphoglycerate (DPG),[1] and almost exclusive binding to the $\alpha$ chain in the presence of inositol hexaphosphate (IHP). In contrast, CO exhibits a possible, but very slight preference to the $\alpha$ chain in the presence of DPG and a small but definite preferential binding to the $\alpha$ chain in the presence of IHP. The allosteric transition theory of Monod *et al.* (1965) and the sequential theory of Koshland *et al.* (1966) both assume that the four subunits in Hb are equivalent. Consequently, a ligand shows no preferential binding to the $\alpha$ or $\beta$ chain. Thus, these experimental findings imply that a simple version of the allosteric transition model and of the sequential model is quite unsuited to represent the oxygenation process of Hb.

Earlier, Ogata and McConnell (1972) proposed the generalized concerted transition model, which is essentially the same as the allosteric transition model, except that the $\alpha$ and $\beta$ chains are treated as nonequivalent. This theory contains the five parameters, $k_\alpha^T$, $k_\beta^T$, $k_\alpha^R$, and $k_\beta^R$ (the ligand dissociation constants of the $\alpha$ and $\beta$ chains in the T and R states), and $L = [T]/[R]$ (the allosteric transition equilibrium constant). The Hill plot for nonstripped Hb obtained from this theory, however, does not fit the experimental data of Tyuma *et al.* (1971a,b). A similar model has been proposed by Szabo and Karplus (1972a,b), in which they have considered the Bohr effect explicitly according to the stereochemical mechanism proposed by Perutz (1970). Janssen and DeBruin (1973) have modified the sequential theory of Koshland *et al.* (1966) to include the fact that the $\beta$ chains are constrained to the A form by bound DPG, and thus in the presence of DPG the interaction constant $K_{AA}$ between two $\beta$ chains is different from that between two $\alpha$ chains or between $\alpha$ and $\beta$ chains. The four Adair constants obtained from their theory are in agreement with experimental work of Tyuma *et al.* (1971a,b). However, this theory implies that the $\alpha$ chains will be liganded preferential to the $\beta$ chain whether the ligand is $O_2$ or CO, and thus is in contradiction to the experimental finding of Johnson and Ho (1974).

In this paper, we propose a new model, which accounts for the observed DPG and IHP effects. In the absence of organic phosphates (*i.e.*, stripped Hb), our formulation follows exactly that of the sequential theory (Koshland *et al.*, 1966; Chay and Ho, 1973). In the presence of organic phosphate (hereafter referred to as OP), we modify the sequential theory according to the following two assumptions. We assume that (i) an equilibrium exists between $Hb(O_2)_n$ and $Hb(O_2)_n \cdot OP$ (OP–Hb complex), where the index $n$ runs from 0 to 4; and (ii) the ligand affinity of the $\beta$ chain in OP–Hb complex is different from that of the $\alpha$ chain in the complex and both are different from that of DPG-free Hb.[2] In the latter part of our work, we apply our theory to the equilibrium oxygenation studies of hemoglobin.

## Model

Using the lattice statistical argument, Chay and Ho (1973) have shown that for hemoglobin (which is tetragonally arranged) the generating function (Wyman, 1968; Szabo and Karplus, 1972a,b) of the sequential model can be written as

$$\Xi = \sum_{i=0}^{4} H_i \lambda^i \tag{2}$$

where $\lambda$ is the activity of oxygen, and $H_i$ is the canonical ensemble partition function having I B units and may be expressed as

$$\begin{aligned} H_1 &= 4H_0 Z^3 Q \\ H_2 &= 6H_0 Z^4 Q^2 \\ H_3 &= 4H_0 Z^3 Q^3 \\ H_4 &= H_0 Q^4 \end{aligned} \tag{3}$$

Here, $Q$ measures the relative stability between the conformations A and B and is related to the parameters in the se-

[1] Abbreviations used are: DPG, 2,3-dephosphoglycerate; IHP, inositol hexaphosphate; OP, organic phosphate; Hb, hemoglobin.

[2] This assumption may be restated as follows: we introduce the two new parameters, $Q_\alpha$ and $Q_\beta$, which measure the ligand affinities of the $\alpha$ and $\beta$ chains in the OP–Hb complex.

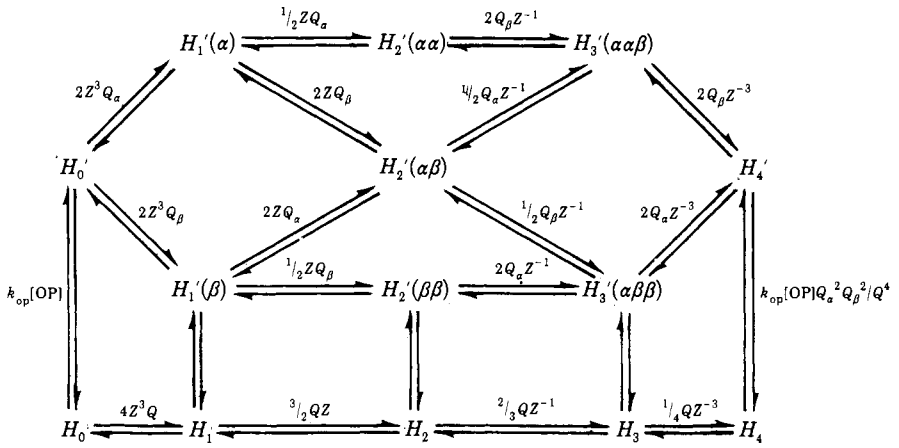


FIGURE 1: Diagrammatic sketch of the oxygen-binding mechanism of hemoglobin in the presence of organic phosphate. The equilibrium constants for each step are shown in this figure. The equilibrium constants between the organic phosphate and the partially oxygenated species are given by eq 9 and 10.

quential theory by

$$Q = K_L K_t (K_{BB}/K_{AA})^{3/2} \tag{4a}$$

In eq 3, $Z$ is a measure for $K_{AB}$ relative to $K_{BB}$ and is, therefore

$$Z = K_{AB}/(K_{AA}K_{BB})^{1/2} \tag{4b}$$

From this generating function, one may obtain the fraction of ligand bound to hemoglobin by the relation

$$Y_L = \frac{1}{4}\left[\partial \ln \Xi/\partial \ln \lambda\right] \tag{5}$$

It is implicitly assumed in eq 3 that all four subunits are equivalent; hence, eq 3 is applicable only to stripped hemoglobin.

The generating function for nonstripped hemoglobin (*i.e.*, hemoglobin in the presence of OP) can be formulated with the following plausible assumptions: (i) 1 mol of OP (such as DPG and IHP) can combine with 1 mol of hemoglobin tetramer in any oxygenation stage in a reversible manner; (ii) the ligand affinity of the $\beta$ chain of OP–Hb complex is different from that of the $\alpha$ chain of the complex and both are different from that of OP free Hb;[2] (iii) the strength of the subunit interactions, $Z$, of OP–Hb complex is the same as that of OP free Hb. (This assumption is made to simplify the mathematics.) The oxygenation process of hemoglobin in the presence of organic phosphate is depicted in Figure 1.

According to assumptions (ii) and (iii), the canonical ensemble partition functions for OP–Hb complexes in various oxygenation stages may be written as

$$\begin{gathered} H_0' = H_0 K_{op}[OP] \\ H_1'(\alpha) = 2H_0'Z^3Q_\alpha, \quad H_1'(\beta) = 2H_0'Z^3Q_\beta \\ H_2'(\alpha\alpha) = H_0'Z^4Q_\alpha^2, \\ H_2'(\alpha\beta) = 4H_0'Z^4Q_\alpha Q_\beta, H_2'(\beta\beta) = H_0'Z^4Q_\beta^2 \\ H_3'(\alpha\alpha\beta) = 2H_0'Z^3Q_\alpha^2Q_\beta, \quad H_3'(\alpha\alpha\beta) = 2H_0'Z^3Q_\alpha Q_\beta^2 \\ H_4' = 4H_0'Q_\alpha^2Q_\beta^2 \end{gathered} \tag{6}$$

In the above equations, $H_1'(\alpha)$, for example, is the partition function of one $\alpha$-liganded hemoglobin which formed a complex with OP, $k_{op}$ is the binding constant of deoxyhemoglobin–organic phosphate complex, and [OP] is the concentration of organic phosphate.

The generating function for nonstripped hemoglobin then is written as

$$\Xi = \sum_{i=0}^{4} H_i \lambda^i + \sum_{i=0}^{4} H_i' \lambda^i \tag{7}$$

From this generating function, one may obtain the fractional saturations of ligand and of OP by relations 8a and b, respectively:

$$Y_L = \{\partial \ln \Xi/\partial \ln \lambda\}_{op} \tag{8a}$$

$$Y_{op} = \{\partial \ln \Xi/\partial \ln [OP]\}_\lambda \tag{8b}$$

The binding constant of organic phosphate to hemoglobin in various oxygenation stages can be obtained from eq 3 and 6. As an example, the binding constant of organic phosphate to one $\alpha$-liganded Hb species can be found from the relation, $H_1'(\alpha)\{H_1(\alpha)[OP]\}^{-1} = k_{op}Q_\alpha/Q$. Thus, in general, for the following chemical reaction (eq 9), the binding

$$\alpha_2(O_2)_i\beta_2(O_2)_j + OP \rightleftharpoons \alpha_2(O_2)_i\beta_2(O_2)_j{}'OP \quad i,j = 0,1,2 \tag{9}$$

constant can be written as

$$M_{ij} = k_{op}(Q_\alpha/Q)^i(Q_\beta/Q)^j \tag{10}$$

where $M_{00} = k_{op}$.

## Results and Discussion

In Figures 2 and 3, we compare the oxygen binding curves obtained from the present theory with the experimental data (Gibson, 1970; Tyuma *et al.*, 1971a,b). Gibson's experiment on stripped Hb was with 53 $\mu$M in heme at 20° in 0.05 M bis-tris buffer (pH 7.0), and his experiment on nonstripped Hb was with 41.5 $\mu$M in heme at 21.5° in 0.1 M phosphate buffer (pH 7.0). The Tyuma experiment used 15 $\mu$M human hemoglobin in either 0.01 M Tris-HCl or 0.05 M bis-Tris-HCl buffer (pH 7.40) at 25°, where both buffers gave the same results.

We note that, in the case of stripped hemoglobin, the theory has been fitted to experimental data with just the two parameters, $Q$ and $Z$. In the case of nonstripped hemoglobin, the three parameters, $Q_\alpha$, $Q_\beta$, and $k_{op}$[Op], have been used to fit the data with $Q$ and $Z$ fixed from stripped Hb data. $K_{op}$[OP] could also be fixed from experimental results (*e.g.*, Deal, 1973), so that there are only two independent parameters, $Q_\alpha$ and $Q_\beta$. The model parameters obtained from the oxygenation curve of Figures 2 and 3 are given in Table I. The parameters are obtained by a nonlinear least-squares fit (Bevington, 1969). Uncertainties quot-

TABLE I: Hemoglobin Model Parameters.

| Parameters | Gibson (1970) | | Tyuma *et al.* (1973) | | | | |
|---|---|---|---|---|---|---|---|
| | | | No Salt | | | 0.1 M NaCl | |
| | Stripped | Nonstripped | Stripped | 2 mM DPG | 1.7 mM IHP | Stripped | 2 mM DPG |
| $Z$ | 0.573 ± 0.015 | | 0.5388 ± 0.0132 | | | 0.443 ± 0.011 | |
| $Q$ | 0.566 $Torr^{-1}$ (0.325 ± 0.018 $\mu M^{-1}$) | | 0.5954 ± 0.0319 $Torr^{-1}$ | | | 0.176 ± 0.0014 $Torr^{-1}$ | |
| $Q_\alpha$ | | $0.157Q$ | | 0.1005 ± 0.0088 | 0.0513 ± 0.0341 | | 0.1435 ± 0.0075 |
| $Q_\beta$ | | $0.0628Q$ | | 0.0102 ± 0.0025 | 0.00120 ± 0.0006 | | 0.0167 ± 0.0005 |
| $k_{op}[OP]$ | | $1.01 \times 10^3$ | | $2.229 \times 10^6 \times$ [2 mM] | $1.356 \times 10^9 \times$ [1.7 mM] | | $(3.41 \pm 0.02) \times 10^4$[2mM] |

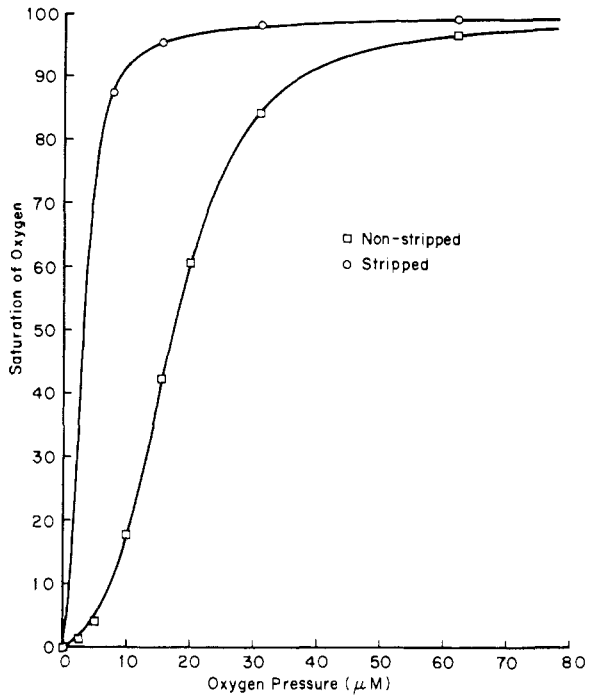


FIGURE 2: A comparison of the oxygen-binding curve between the experiment [dots; Gibson (1970)] and the theory (solid line).

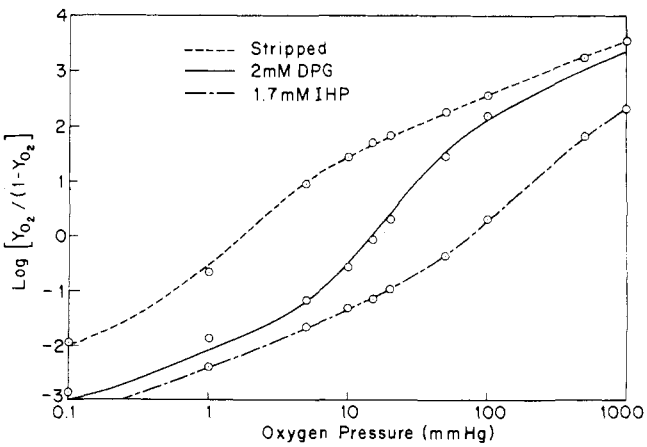


FIGURE 3: A comparison of the Hill plot between the experiment (Tyuma *et al.*, 1971) and the theory (the solid line).

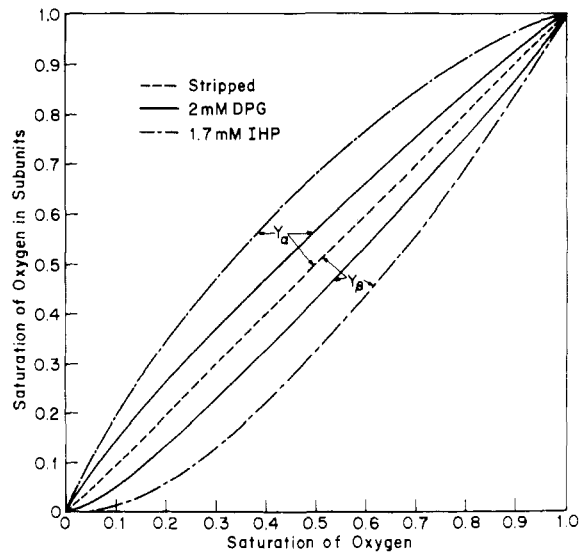


FIGURE 4: Fractional $\alpha$ and $\beta$ subunit saturations $Y_\alpha$ and $Y_\beta$ as a function of the oxygen saturation. The parameters used to calculate the curves are given by the third column of Table I.

ed for parameters are defined as the amount of change in one parameter required to increase the $\chi^2$ of the fit (a measure of goodness of fit) by one (after optimizing other parameters), assuming that all parameters are independent (Bevington, 1969). The parameters $Q_\alpha$ and $Q_\beta$ in Table I may not be entirely unique, as they sometimes can be made to fit another set of values, though $Q_\alpha$ is always greater than $Q_\beta$. As an example, the Hill plot of Tyuma *et al.* (2 mM DPG) can also be fitted with the parameters: $k_{DPG} = 2.525 \times 10^6$ $M^{-1}$, $Q_\alpha = 0.1428$ Torr, and $Q_\beta = 0.00013$ Torr. The parameters, $Q_\alpha = 0.1428$ and $Q_\beta = 0.00013$, suggest that oxygen binds exclusively to the $\alpha$ chain of DPG–Hb complex and that the $\beta$ chain in the complex hardly binds oxygen. This is consistent with Perutz stereomechanism (Perutz, 1970, 1972). However, other experiments (Johnson and Ho, 1974; Gibson, 1973) suggest that the $\beta$ chain of DPG–Hb complex can also be oxygenated during the oxygenation process.

In Figure 4, we show the preferential binding of oxygen by the $\alpha$ and $\beta$ chains by using the parameters in the third column of Table I. According to the present model, the difference in saturation between the $\alpha$ and $\beta$ chains in the presence of 2 mM of DPG is 13% at 50% of total saturations and in the presence of 1.7 mM of IHP is 38% at 40% of total

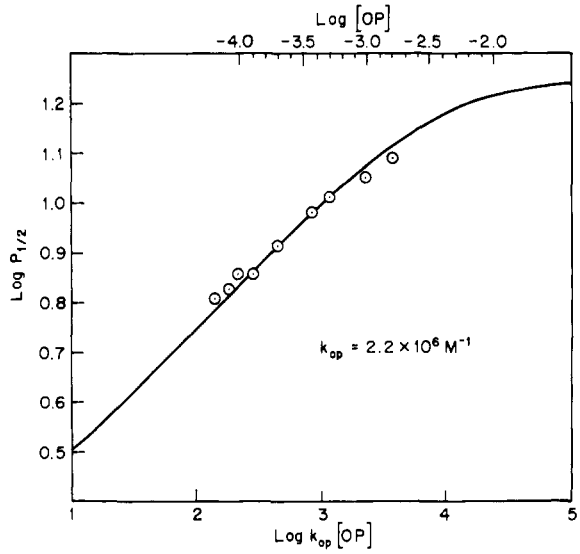


FIGURE 5: Relation between $P_{1/2}$ and DPG concentration. The circles are experimental [Benesch *et al.* (1971)] and the lines are calculated from the theory with the parameters given by the second column of Table I.

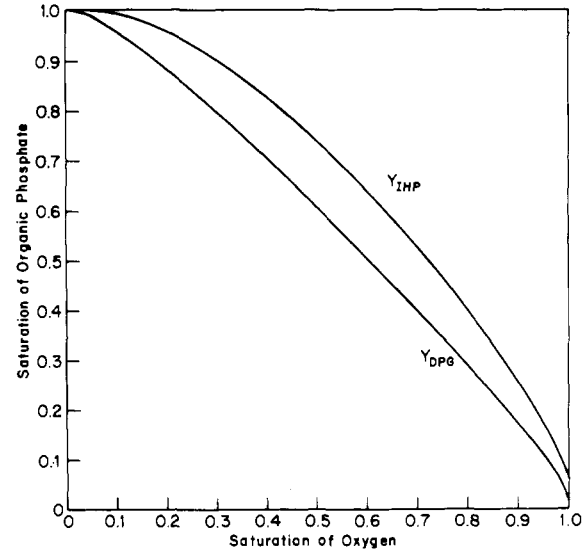


FIGURE 6: Relation between the organic phosphate saturation and the oxygen saturation. The curves are calculated with the parameters given by the third column of Table I.

saturations. Based on their nmr work, Johnson and Ho (1974) have found that in 32–35 mM of DPG and 11–17 mM of IHP, the differences in the partial fractional saturations for the $\alpha$ and $\beta$ chains are, respectively, 15.0 ± 4.2% at 50% of total saturation and 60.2 ± 4.4% at 40% of total saturation.

The values of $k_{DPG}$ in Table I seem to be consistent with experimental values. The theoretical $k_{DPG} \simeq 2.2 \times 10^6$ $M^{-1}$ in the fourth column agrees exactly with that obtained by Deal (1973), and $k_{DPG} \simeq 3.4 \times 10^4$ $M^{-1}$ in the last column also agrees with $3.1 \times 10^4$ $M^{-1}$ obtained by Tyuma *et al.* (1973). The large binding constant for IHP in the third column of Table I is as expected, since IHP binds very strongly to deoxy-Hb (Gray and Gibson, 1971; Janig *et al.*, 1971; Tyuma *et al.*, 1971a,b).

Figure 5 shows the partial pressure of oxygen at the half-oxygen saturation point, $P_{1/2}$, *vs.* the DPG concentration in the absence of salt. Here, the free DPG concentration is approximated as the total DPG concentration. Since experiments done in the absence of salt are not available in literature, the present theory is compared with data of Benesch *et al.* (1971), which was done in the presence of 0.1 M of $Cl^{-1}$. The value of $k_{op}$ obtained from this figure is $2.2 \times 10^6$. The experimental and theoretical $P_{1/2}$'s, in the presence of salt, are compared in Table II. In the same table, we also compare the theoretical and experimental maximum Hill coefficients, $n_{max}$'s. We find a fair agreement between the theory and experiment for both $P_{1/2}$ and $n_{max}$.

Figure 6 shows the plots of the fractional saturations of DPG and of IHP *vs.* the oxygen saturation by using the parameters given in the third column of Table I. We note that the plot deviates considerably from linearity and that nearly all organic phosphates have been freed from OP–Hb complex during the oxygenation process.

Figure 7 illustrates the fractions of $Hb(O_2)_n$ ($n$ = 0, . . . 4) as a function of oxygenation. As shown in this figure, the dominant contribution comes from the deoxy and/or fully liganded hemoglobins for all values of $Y_{O_2}$. The partially oxygenated species are relatively small for all $Y_{O_2}$, except that one liganded species are important in the low $Y_{O_2}$. For stripped hemoglobin, the three liganded species are also important for the high $Y_{O_2}$. It is clear that DPG has

TABLE II: Partial Pressure of Oxygen at Half-Saturation Point and Maximum Hill Coefficient in the Presence of 0.1 M NaCl.[a]

| DPG Concn (mM) | $P_{1/2}$ (mm) | | $n_{max}$ | |
|---|---|---|---|---|
| | Exptl | Theoretical | Exptl | Theoretical |
| 0.0 | 5.8 | 5.7 | 2.98 | 2.97 |
| 0.2 | 8.9 | 9.4 | 3.14 | 3.14 |
| 0.5 | 12.6 | 11.5 | 3.05 | 3.08 |
| 1.0 | 15.1 | 13.4 | 3.18 | 3.00 |
| 2.0 | 15.5 | 15.2 | 3.05 | 2.89 |

[a] Parameters used are given in Table I under Tyuma *et al.* (0.1 M NaCl).

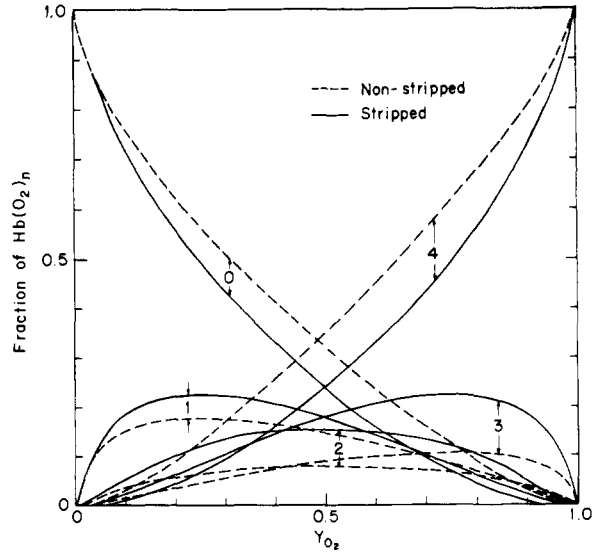


FIGURE 7: Fractional contribution of species $Hb(O_2)_n$ ($n$ = 0, 1 . . . 4) as a function of oxygen saturation for both stripped and nonstripped hemoglobins. The parameters used to calculate the curves are given by the second column of Table I.

TABLE III: Concentration of All the Intermediate Species as a Function of Oxygen Saturation.[a]

| $Y_{O_2}$ | $H_0'$ | $H_0$ | $H_1'$ ($\alpha$) | $H_1'$ ($\beta$) | $H_1$ | $H_2'$ ($\alpha\alpha$) | $H_2'$ ($\alpha\beta$) | $H_2'$ ($\beta\beta$) | $H_2$ | $H_3'$ ($\alpha\alpha\beta$) | $H_3'$ ($\alpha\beta\beta$) | $H_3$ | $H_4'$ | $H_4$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0.0 | 1.0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 0.1 | 0.76 | 0.00 | 0.11 | 0.04 | 0.00 | 0.01 | 0.02 | 0.00 | 0.00 | 0.01 | 0.00 | 0.01 | 0.00 | 0.03 |
| 0.2 | 0.62 | 0.00 | 0.13 | 0.05 | 0.00 | 0.02 | 0.03 | 0.00 | 0.00 | 0.01 | 0.00 | 0.02 | 0.01 | 0.09 |
| 0.3 | 0.51 | 0.00 | 0.13 | 0.05 | 0.00 | 0.02 | 0.04 | 0.00 | 0.01 | 0.02 | 0.00 | 0.03 | 0.02 | 0.16 |
| 0.4 | 0.41 | 0.00 | 0.12 | 0.05 | 0.00 | 0.03 | 0.04 | 0.00 | 0.01 | 0.03 | 0.00 | 0.04 | 0.02 | 0.24 |
| 0.5 | 0.32 | 0.00 | 0.11 | 0.04 | 0.00 | 0.03 | 0.04 | 0.00 | 0.01 | 0.03 | 0.00 | 0.04 | 0.03 | 0.33 |
| 0.6 | 0.24 | 0.00 | 0.09 | 0.04 | 0.00 | 0.03 | 0.04 | 0.00 | 0.01 | 0.04 | 0.01 | 0.05 | 0.04 | 0.42 |
| 0.7 | 0.16 | 0.00 | 0.07 | 0.03 | 0.00 | 0.02 | 0.04 | 0.00 | 0.01 | 0.04 | 0.01 | 0.05 | 0.05 | 0.51 |
| 0.8 | 0.09 | 0.00 | 0.05 | 0.02 | 0.00 | 0.02 | 0.03 | 0.00 | 0.00 | 0.04 | 0.01 | 0.05 | 0.06 | 0.62 |
| 0.9 | 0.03 | 0.00 | 0.02 | 0.01 | 0.00 | 0.01 | 0.02 | 0.00 | 0.00 | 0.03 | 0.01 | 0.04 | 0.07 | 0.73 |
| 1.0 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.09 | 0.91 |

[a] Parameters used are given in Table I under Gibson (Nonstripped).

the effect of lowering the concentrations of the partially oxygenated species, while it increases those of deoxy and full liganded hemoglobins. In Table III, we list the concentrations of each species, *i.e.*, the DPG–Hb complexes or DPG free species in each oxygenation stage. The dominant contributions are due to the deoxyhemoglobin which formed complex with DPG and/or a fully liganded DPG free Hb hemoglobin. At low oxygen saturation, one $\alpha$ oxygenated DPG–Hb complexes are present in significant amounts.

Our theory can easily explain why the fourth Adair constant, $k_4$, is about the same for both stripped and nonstripped hemoglobins. The fourth Adair constants of our theory may be expressed as

$$k_4 = Q/4Z^3 \tag{11a}$$

for stripped Hb and

$$k_4 = \frac{Q^4 + k_{op}[OP]Q_\alpha^2 Q_\beta^2}{2Z^3\{2Q^3 + k_{op}[OP]Q_\alpha Q_\beta(Q_\alpha + Q_\beta)\}} \simeq Q/4Z^3 \tag{11b}$$

for nonstripped Hb, where the approximation follows from the fact that $k_{op}[Op]Q_\beta$ is relatively small.

## Comparison with Other Theories

A comparison of the present theory with other theories is of interest. The present formulation, in the absence of OP, is based on the sequential theory of Koshland *et al.* (1966). In the presence of OP, however, our theory is somewhat similar to the generalized concerted transition model (GCT) of Ogata and McConnell (1972). Although in our theory the conformational changes occur in a sequential manner, our DPG–Hb complexes and DPG free species seem to correspond to the species in the T and R states in GCT, respectively. With this correspondence, the following similarities and differences between the two theories are noted. (i) The strength of the subunit interactions $Z$ (or $K_{AA}$, $K_{AB}$, $K_{BB}$) is taken to be unity in GCT but less than unity in our theory. (ii) In GCT the allosteric transition constant $L$ is taken to be $L_0(1 + k_{op}[OP])$. In the present theory, the constant corresponding to $L$ is simply taken to be $k_{op}[OP]$. (iii) We distinguish, as in GCT, the ligand affinity constants of the $\alpha$ and $\beta$ chains in the DPG–Hb complexes (or the T state). Unlike GCT, however, the ligand affinity constants in the DPG free species (or the R state) are the same for the $\alpha$ and $\beta$ chains.

The present theory is also consistent with some aspect in Perutz' model (1970, 1972). Perutz, from his X-ray diffraction studies, has suggested that the deoxy- and oxyhemoglobins are predominantly in the DPG–Hb complexes and DPG free species, respectively. The values listed in Table III are in agreement in this respect. Perutz has also suggested that only after two $\alpha$ chains in DPG–Hb complex are oxygenated, DPG will be expelled and the two $\beta$ chains will be oxygenated. The second set of parameters we have found by fitting the data of Tyuma *et al.* (1971a,b), $Q_\alpha = 0.1428$ and $Q_\beta = 0.00013$, indicate that this is so in equilibrium.

The advantage that the present theory has is that it uses fewer parameters than other theories to completely describe the system. For example, in the case of stripped Hb, the allosteric transition model (Monod *et al.*, 1965) has three parameters, $L$, $K_R$, and $K_T$, whereas the present approach uses two parameters, $Q$ and $Z$. It should be noted that these two parameters contain the same information as the parameters in the sequential theory, since only two out of the three parameters, $K_s K_t$, $K_{AB}$, and $K_{BB}$, in the sequential theory, are independent. In the case of nonstripped Hb, the present theory also has fewer parameters than other theories (Ogata and McConnell, 1972; Szabo and Karplus, 1972). That is, the present theory has two adjustable parameters, $Q_\alpha$ and $Q_\beta$, and three fixed parameters, $Q$, $Z$, and $k_{op}$. The present theory also has the advantage that it is based on the sequential theory of Koshland *et al.* (1966), which gives a good physical picture of the mechanism of the hemoglobin molecule, and it also incorporates some useful ideas from the allosteric transition model.

In summary, our present theory predicts that in the case of stripped Hb, the positive cooperativity in the Hill plot (see Figure 3) is due to $Z < 1$ (Chay and Ho, 1973) and that in the case of nonstripped Hb the positive cooperativity is due not only to $Z < 1$ but also the differences in $Q_\alpha$ and $Q_\beta$ from $Q$.[3]

[3] This can be explained by taking the simple case where $Z = 1$. For this case, our generating function becomes $\Xi = (1 + Q\lambda)^4 + L'(1 + Q_\alpha\lambda)^2(1 + Q_\beta\lambda)^2$, where $L' = k_{op}[OP]$. One can see that, like in the allosteric transition theory, the Hill plot exhibits no cooperativity when $Q = Q_\alpha = Q_\beta$. On the other hand, if $Q \neq Q_\alpha$ and/or $Q \neq Q_\beta$, then the Hill plot shows positive cooperativity. Hence, in the presence of OP, the positive cooperativity is due to $Z < 1$ and $Q \neq Q_\alpha$ and $Q_\beta$.

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