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Structure-specific model of hemoglobin cooperativity

(allosteric protein/stereochemical mechanism/statistical thermodynamics model/Bohr effect)

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ABSTRACT **A generalization of the Szabo–Karplus statistical mechanical model for hemoglobin cooperativity is formulated. The model fits the available thermodynamic and spectroscopic data with assumptions that are consistent with structural results and empirical energy function calculations. It provides a mechanism of hemoglobin cooperativity that is a generalization of the proposals of Monod, Wyman, and Changeux and of Perutz. The role of nonsalt-bridge related sources of constraints on ligand affinity and the mode of salt-bridge coupling to tertiary–quaternary structural changes are examined within the framework of the model. Analysis of proton release data for a range of pH values indicates that a pH-*independent* part of cooperativity must be present. The pH dependence of the first and last Adair constants point to partial linkage of salt bridges to ligation in the deoxy state and to a destabilized intra-β-chain salt bridge in the unliganded oxy state.**

Hemoglobin has long been regarded as the prototype system for the investigation of cooperativity in macromolecules. The essential aim of such studies is to determine the detailed relationship between structural changes induced by ligation and the thermodynamic and kinetic manifestation of cooperativity. The x-ray structures of various hemoglobins solved by Perutz and his collaborators (1) have demonstrated that there exist two quaternary structures (deoxy and oxy) for the tetramer and two tertiary structures for each individual chain (liganded and unliganded). Based on the structural results and other data, Perutz (2, 3) proposed a stereochemical mechanism for cooperativity, in which salt bridges (some with ionizable protons in the neutral pH range) provide the link between ligand-induced tertiary structural changes and the relative stability of the two quaternary forms. To examine the thermodynamic correlates of the Perutz mechanism, its elements were incorporated into a statistical-mechanical model (4) (referred to as SK in what follows) that utilizes a partition function describing the influence of homotropic (oxygen) and heterotropic (protons and 2,3-bisphosphoglycerate) effectors on the set of contributing structures. It was found that the model was able to provide a satisfactory description of the cooperativity in ligand binding, the alkaline Bohr effect, the effect of 2,3-bisphosphoglycerate on ligand affinity, and the influence of chemical modifications and certain mutations on these properties (4–6). Further, the values of the model parameters, which correspond to physically meaningful quantities, were in the range suggested by independent estimates, thus providing support for the basic postulates of the Perutz mechanism.

The structural, spectroscopic, and thermodynamic data on hemoglobin that have become available since the model was proposed call for its reevaluation at this time. Of particular importance are the structural results concerning the nature of tertiary–quaternary coupling. It is now clear from comparisons of crystal structures of unliganded deoxy (2, 3) and liganded oxy tetramers (2, 3, 7) with liganded deoxy tetramers (8, 9) and unliganded oxy tetramers (10) that the quaternary structure has an important influence on the tertiary conformation of a subunit. Further, empirical energy calculations (11, 12) have suggested that the low affinity of the deoxy quaternary structure arises not only from the perturbation of salt bridges but also from steric constraints imposed by contacts at the $\alpha_1\beta_2$ and $\alpha_2\beta_1$ interfaces that prevent the dissipation of ligation-induced strain in the "allosteric core" (the heme, proximal histidine, F helix, and the FG corner of each subunit). A corollary of this finding is the observation (8, 9, 13) that formation of the salt bridges is determined not only by the ligation state of the subunits.

Spectroscopic studies of probes sensitive to specific changes are providing valuable information for testing models of cooperativity; an example is the attempt to distinguish between α- and β-chain oxygenation by NMR (14, 15) and by optical measurements (16). Highly accurate equilibrium measurements for oxygen binding in hemoglobin have been made under carefully controlled conditions (17, 18); this removes some of the uncertainties present in the Roughton–Lyster data (19) on which the original fits were based. Also, direct determinations of the pH dependence of the binding curve at very low and high oxygen pressures now exist (ref. 20; C. Poyart, personal communication).

In this paper we present a generalization of the SK model. An important element is the inclusion of two different tertiary structures for each of the two quaternary structures, in accord with the data cited above. This leads directly to the possibility of constraints on ligand binding independent of the salt bridges and also permits a general formulation of the salt bridge coupling to tertiary and quaternary structural change. We demonstrate that the present treatment, which reduces to the SK model as a limiting case, provides a satisfactory description of the available experimental results. We focus particularly on equilibrium measurements that are related to specific aspects of the model and analyze the data concerning them.

Formulation of the model

In this section we describe the generalized structure-specific model (the SKL model) for the hemoglobin tetramer. Although tetramer dissociation (i.e., the monomer–dimer–tetramer equilibrium) has been included in the model, it is not essential for the present analysis of cooperativity in hemoglobin. Because the oxygen binding by the monomer and dimer are noncooperative (17), it is necessary only to correct the measured binding curves for dissociation so as to be able to extract results for the cooperative tetramer.

As in the original SK model (4), we use a generating function, Ξ, based on the binding potential introduced by Hill (21) and by Wyman (22); from the generating function, the fractional saturation by any ligand is obtained by differentiation. Includ-

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ing oxygen and hydrogen ion binding and writing Ξ as the sum of Ξ_D and Ξ_O, the generating functions for the deoxy and oxy quaternary states, respectively, we have

$$\Xi_D(\lambda,\mu) = \frac{QS^6}{(r')^2}[1 + \mu H^\alpha/S]^2[1 + \mu H^\beta/S]^2 \times \left\{1 + \lambda K_D^\alpha r^2 \frac{[1 + \mu H^\alpha/(rS)]}{[1 + \mu H^\alpha/S]}\right\}^2 \times \left\{1 + \lambda K_D^\beta r^2 \frac{[1 + \mu H^\beta/(rS)]}{[1 + \mu H^\beta/S]}\right\}^2 \quad [1a]$$

$$\Xi_O(\lambda,\mu) = [1 + \mu H^\alpha]^2[1 + \mu H^\beta/(r'S)]^2 \times \{1 + \lambda K_O^\alpha\}^2 \left\{1 + \lambda \frac{K_O^\beta}{(r'S)} \frac{[1 + \mu H^\beta]}{[1 + \mu H^\beta/(r'S)]}\right\}^2$$

and the fractional saturation by oxygen and hydroxyl ion is

$$\langle y\rangle_{O_2} = {}^1/_4 \frac{\partial \ln \Xi}{\partial \ln \lambda}; \qquad \langle y\rangle_{OH^-} = {}^1/_4 \frac{\partial \ln \Xi}{\partial \ln \mu}. \quad [1b]$$

Here λ is the partial pressure of oxygen (mm of Hg) and μ is the hydroxyl ion concentration (M). H^α and H^β are the hydroxyl ion binding constants [$pK_{\alpha(\beta)} = 14 - \log H^{\alpha(\beta)}$] for the α- and β-chain Bohr groups, respectively; S is the strength of the ionic interactions (salt bridges) of the type $NH_n^+ \ldots COO^-$ for a given set of solution conditions in the unliganded deoxy tetramer; (rS) is the effective strength in liganded chains of the deoxy tetramer, and $(r'S)$, that of the internal salt bridge of unliganded β chains in the oxy tetramers; K_D^α, K_D^β, K_O^α, and K_O^β are the intrinsic ligand affinities for the α and β chains in the deoxy (D) and oxy (O) quaternary structures; and Q determines the relative stability of the two quaternary states in the absence of ligands or salt bridges. The SK model is obtained for $r = 1/S$, $r' = 1$, and $K_D^\alpha = K_O^\alpha = K^\alpha$, $K_D^\beta = K_O^\beta = K^\beta$; $r = 1$, $r' = 1/S$ corresponds to pure quaternary coupling and requires $K_D^\alpha < K_O^\alpha$, $K_D^\beta < K_O^\beta$. An extension of the SK model that allows different strengths for the salt bridges that are pH dependent (S) and pH independent (S_1) in the alkaline physiological range is obtained from Eq. 1 with QS^6 replaced by $QS_1^4S^2$, $r = 1/S_1$, $r' = 1$, $K_D^\alpha = K_O^\alpha = K^\beta$, and $K_D^\beta = K_O^\beta = K^\beta$.

In the model (Eq. 1) there are four salt bridges per $\alpha\beta$ dimer (2–4, 23), of which two titrate in the physiologic pH range. Supporting evidence for the identification of the Bohr groups has come from NMR (24, 25), from hydrogen exchange experiments (26), from chemical reactivity studies (27, 28), and from studies of mutant and modified hemoglobins (29–32). Measurements indicate that the α_1-α_2 and intra-β-chain Bohr groups contribute 60–80% of the total alkaline Bohr effect at normal (e.g., 0.1 M Cl^-) salt concentrations (20, 29–34). The remainder is thought to come from other chloride-linked sites (27, 35, 36).

As the parameters are physically meaningful, estimates of allowed ranges for their magnitudes can be given (4); the ranges are -8.5 ± 1.5 kcal/mol for $-RT \ln K_O^\alpha$ and $-RT \ln K_O^\beta$, -2.5 ± 2 kcal/mol for $-RT \ln S$ and $-RT \ln S_1$, <10 kcal/mol for $-RT \ln Q$, and 7.5 ± 1.0 and 6.5 ± 1.0 for pK_α and pK_β, respectively. The ranges for r and r' are $1/S \leq (r,r') \leq 1$ and $K_D^{\alpha(\beta)}$ is less than or equal to $K_O^{\alpha(\beta)}/(rS)^2$. We restrict the present calculations to normal salt concentrations and include only the original alkaline Bohr groups, which means that their contribution will be somewhat overestimated.

In formulating the current model, we have reviewed the literature to determine if assumptions other than those modified in the generalized model require revision. The possibility of more than two quaternary structures has been introduced (37, 38). There is no structural evidence for this and the data on which it was based are consistent with the SK model, which involves only two quaternary structures but an equilibrium of many species at each state of oxygenation (e.g., figures 4 and 5 of ref. 4). Also, it has been suggested that cooperativity can occur in the absence of a change in quaternary structure (14, 39). Experimental results indicate that such a contribution to the cooperative oxygen binding, if it exists, is not important. It has been proposed that the Bohr effect may involve a number of titrating groups, in addition to the salt bridges included in the Perutz model (40–43). The evidence for this is not unequivocal—e.g., the most recent NMR measurements (40) have not located all of the histidine resonances in deoxy- and oxyhemoglobin. However, variations in ionic strength and the types of anions present clearly do influence the properties of hemoglobin.

Extension of the model to include the different possibilities summarized here is straightforward (unpublished data); in this paper we restrict the model to the form given above (Eq. 1), which includes the most important effects and permits us to delineate its attributes without unduly complicating the analysis.

Application and results

We first apply the model to the oxygen binding measurements at one pH. The results of Mills *et al.* (18) are analyzed and it is shown that an excellent fit is obtained with the SK model when parameters in the physically reasonable range are used. We also examine measurements by Viggiano and Ho (14) and Nasuda-Kouyama *et al.* (16) on α,β chain inequivalence. Because of the limitation inherent in comparisons at one or two pH values, we then treat data for the Bohr effect (proton release) at a series of pH values obtained by Antonini *et al.* (44). The results demonstrate the importance of such an analysis for evaluating the relative contribution to cooperativity of steric effects and of the salt bridges in the extended model. Finally, we indicate how the parameters r and r' (see Eq. 1) can be estimated from the pH dependence of the first and fourth tetramer Adair constant, as measured by Poyart *et al.* (ref. 20; C. Poyart, personal communication).

Oxygenation Binding Data at One pH. At a single pH, the SK and SKL models, as well as the Monod–Wyman–Changeux model (46) for inequivalent chains (4, 6, 47, 48), are isomorphous. Thus, we restrict the analysis to the SK model and apply it to the Mills *et al.* data (18) at pH 7.4 (21.5°C, 0.1 M Cl^-, 5.35–382 μM heme). A significant fraction of the hemoglobin molecules is expected to have dissociated into dimers at the lower concentrations used by Mills *et al.*; e.g., at 5.35 μM, 36% of the heme exist in dimeric form in oxyhemoglobin. Consequently, it is necessary to take into account both dimers and tetramers. Because insufficient information is available for a detailed treatment of the dimer–tetramer system, we have fitted the SK model to data generated from tetramer Adair constants reported by Mills *et al.* (18).

Two choices were used for the pKs of the Bohr groups: $pK_\alpha = 7.5$ and $pK_\beta = 6.2$ from Szabo and Karplus (4) and $pK_\alpha = 7.7$ and $pK_\beta = 5.93$ from Johnson and Ackers (45). To determine the four remaining model parameters (Q, S, K^α, K^β) from the data, a Newton–Raphson procedure with analytic first and second derivatives was employed to locate minima on the multidimensional parameter function space. The model results for the tetramer are shown in Table 1. All of the fits are in good agreement with experiment and yield parameter values in the

Table 1. SK model results for Mills *et al.* measurements (18)*

Parameter values†				
pK_α, pK_β	$-RT \ln Q$	$-RT \ln S$	$-RT \ln K^\alpha$, $-RT \ln K^\beta$	Variance
7.5, 6.2	6.16	−2.61	−9.68, −9.44	8.3×10^{-12}
7.7, 5.93	6.24	−2.60	−9.70, −9.15	8.4×10^{-12}
7.7, 5.93‡	5.52	−3.04	−11.44, −9.47	2.1×10^{-9}

*The Adair constants (corrected for statistical factors) are: $\Delta G_{41} = -5.46$ kcal/mol; $\Delta G_{42} = -5.432$ kcal/mol; $\Delta G_{43} = -7.658$ kcal/mol; $\Delta G_{44} = -8.71$ kcal/mol.

†All values, except pKs and variances, are in kcal/mol.

‡Fit obtained with the model parameters reported by Johnson and Ackers (45) as initial values.

allowed ranges (see above); it is evident that the information from such equilibrium measurements is not sufficient to determine a unique parameter set. We have included a fit obtained starting with the parameter set of Johnson and Ackers (45), which has the special property noted by them that K^α has an unreasonably high value ($-RT \ln K^\alpha = -11.3$ kcal/mol). It is clear from a comparison with the other results in Table 1 that such a high K^α is not required to fit the Mills *et al.* data. Application of the tetramer parameters from Table 1 and the experimental dimer–tetramer equilibrium constants to the Mills *et al.* data at five heme concentrations (18) yields excellent fits that are indistinguishable within experimental error, confirming the validity of the analysis.

Another test of the SK model is provided by the calculated Bohr effect at pH 7.4. With the parameter set for $pK_\alpha = 7.5$ and $pK_\beta = 6.2$ given in Table 1, there is a release of 2.4 protons per tetramer, in reasonable agreement with independent measurements (20, 34); results for the alkaline Bohr curve over a range of pH values are considered below. Finally, the predictions for the relative fractional saturation of the α and β hemes ($\langle y \rangle^\alpha$ versus $\langle y \rangle^\beta$) as a function of the total saturation are examined. The calculated results are shown in Fig. 1; the maximal difference between the two curves with $\langle y \rangle^\beta$ larger than $\langle y \rangle^\alpha$ is <5% and occurs between 10 and 20% oxygenation. This

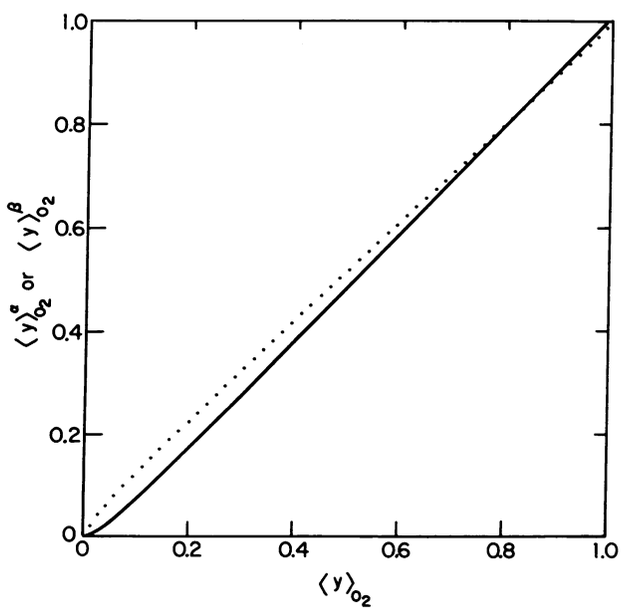

FIG. 1. Computed relative fractional saturation of α- and β-chain hemes as a function of oxygenation. —, $\langle y \rangle^\alpha$; ·····, $\langle y \rangle^\beta$; parameters for the SK model ($pK_\alpha = 7.5$, $pK_\beta = 6.2$ set of Table 1).

agrees with the NMR experiments of Viggiano and Ho (14) for stripped hemoglobin, who found $\langle y \rangle^\alpha$ and $\langle y \rangle^\beta$ to be equal within experimental error, and with the optical absorbance measurements of Nasuda-Kouyama *et al.* (16), who found $\langle y \rangle^\beta$ to be slightly larger than $\langle y \rangle^\alpha$ throughout the entire oxygenation curve. We conclude that the SK model *can* explain the single pH binding data of Mills *et al.* (18), the Bohr proton release at pH 7.4, and the NMR, as well as optical measurements, on chain inequivalence. This disagrees with the conclusion of Johnson and Ackers (45), who claimed that these data disproved the SK model.

The parameters (4) that fit the Roughton and Lyster data (19) at pH 7 are in the same range but differ somewhat from those for the Mills *et al.* data (18), demonstrating that differences in solution conditions can perturb the model results. Because inorganic phosphates and 2,3-bisphosphoglycerate (6), which may have been present in the Roughton–Lyster study (19) and were absent in that of Mills *et al.* (18), were not treated explicitly, parameter changes are expected, though the data are not sufficient for a quantitative analysis.

Proton Release as a Function of pH. Because oxygenation data at one or even two pH values (4) can be adequately described by the SK model, it is necessary to introduce additional information to apply its generalization (SKL model) in a meaningful way. Here we examine the experimentally determined differential titration curves for the total proton release on oxygenation as a function of pH in the range 7.0 to 9.2 at 20°C and 0.25 M Cl^- obtained by Antonini *et al.* (44).

To make clear why the pH data are important, we examine the behavior of the proton release curve. We use the Wyman relation (22) ($\Delta H^+ = d \ln K_{Tot}/d\text{pH}$, where ΔH^+ is the total proton release on ligation and K_{Tot} is the overall oxygen binding constant). With this relationship and the SKL model under conditions valid for the range pH 7 to 9 (unliganded and fully liganded tetramers essentially all in the deoxy and oxy quaternary structures, respectively), ΔH^+ can be accurately approximated by

$$\Delta H^+ \cong 2\frac{(S-1)}{S}\left\{\frac{\mu H^\alpha}{(1+\mu H^\alpha)(1+\mu H^\alpha/S)} + \frac{\mu H^\beta}{(1+\mu H^\beta)(1+\mu H^\beta/S)}\right\}, \quad [2]$$

from which it follows that the effective pK value of a Bohr group involved in a salt bridge is given by $pK^S_{eff} = pK + \log S$; this makes it possible to determine from the model (Eq. 1) exactly how the effective pK is altered on ligation or quaternary structural change (or both). Eq. 2 shows that the proton release depends on only the three parameters S, pK_α (or H^α), and pK_β (or H^β); all of the other model parameters cancel in the expression for ΔH^+. In fact, for each Bohr group the pH profile can be defined by the expression

$$pH_{max} = pK + {}^1/_2 \log S; \qquad \Delta H^+_{max} = \frac{(S-1)}{(1+\sqrt{S})^2}, \quad [3]$$

where pH_{max} is the pH at which the maximal proton release, ΔH^+_{max}, occurs. From Eq. 3 it follows for a single Bohr group that ΔH^+_{max} determines the salt-bridge strength and that the observed pH_{max} then fixes the intrinsic pK value. Because two types of Bohr group are present in the model, the pKs are not unique and a fit to the variation of ΔH^+ with pH is required to evaluate them.

Although the Bohr proton release at pH 7.4 was found to be in agreement with experiment for the SK model (see above), calculation of the Bohr effect curve shows that, relative to the

Antonini *et al.* results (44), it is shifted too far into the alkaline range. When the first parameter set for the Mills *et al.* data in Table 1 is used, ΔH^+_{max} is found at pH 7.8 instead of 7.5 and ΔH^+_{max} is overestimated (2.6 instead of 2.1 protons released). The essential point is that to obtain the smaller ΔH^+_{max} value found experimentally, the salt-bridge strength must be reduced, in accord with Eq. 3. With this restriction on S provided by the proton release curve and the assumption of the same strength for pH-independent and pH-dependent salt bridges, the SK model cannot fit the oxygenation data. Because the reduced ligand affinity in the deoxy structure is determined by S (see Ξ_D in Eq. 1), a small value of S means that the ligand affinity is too large. Consequently, use of a generalized model is required with $K^\alpha_D < K^\alpha_O$ and $K^\beta_D < K^\beta_O$. Fits to the Mills *et al.* data (18) with two different choices for the pK values are given in Table 2; because the results are not sensitive to r and r' we use the SK values ($r = 1/S$, $r' = 1$). Calculated and experimental proton release curves are shown in Fig. 2; they agree within experimental error over most of the range, with larger deviations for pH 7 to 7.5 as expected, due to the neglect of acid Bohr groups. As to the α,β chain inequivalence, the maximal difference in fractional saturation ($\langle y\rangle^\beta > \langle y\rangle^\alpha$) is 6% at an overall saturation of 25%.

For the SK model with unequal salt bridge strengths, the parameters pK_α and pK_β can be determined from the proton release results as above (Table 2). A fit of the remaining parameters to the Mills *et al.* data (18) yields the values $-RT \ln Q = 6.393$ kcal/mol, $-RT \ln S_1 = -3.031$ kcal/mol, $-RT \ln K^\alpha = -8.675$ kcal/mol, and $-RT \ln K^\beta = -9.643$ kcal/mol, all within the allowed ranges.

From the above analysis, it is clear that the intrinsic affinities in the deoxy quaternary structure have to be reduced relative to those in the oxy structure and/or the pH-independent salt bridge strength has to be greater than the pH-dependent salt bridge strength to fit both the oxygenation and proton release data. From the structural and energy minimization data, it seems likely that in addition to energy expended in the weakening of salt bridges, as in the SK model, there are unfavorable steric interactions arising from constraints on the allosteric core due to intersubunit contacts (11, 12).

Other Data. An important feature of the SKL model is its ability to describe different tertiary–quaternary coupling schemes within one framework by changing the values of r and r' (see above). As r controls the coupling of proton release to ligation in the deoxy state, it is expected that the pH dependence of the first Adair constant, K_1, will be most sensitive to this parameter; correspondingly, the pH dependence of K_4 is most sensitive to r'. Recently, Poyart *et al.* (20) have made precise measurements of the pH dependence of the oxygenation curve in the limiting region of low oxygen concentration from which r can be estimated. Under the simplifying assumption that singly liganded tetramers exist primarily in the deoxy quaternary structure (valid for pH $\lesssim$ 8.5), it can be shown that the value of r in the SKL model is coupled to the parameters S, pK_α, and pK_β and the ratio K^α_D/K^β_D. Because the overall proton release determines S, pK_α, and pK_β (see above), only r and K^α_D/K^β_D need to be fitted. Utilizing measurements of the pH dependence of the first Adair constant in the range pH 6.5 to 9 at 20°C and 0.1 M Cl^- and the fits from Table 2, we find the allowed r values to satisfy $0 \le \ln rS/\ln S \le 0.3$. Such a relationship implies that the salt bridges are weakened but not necessarily broken ($r = 1/S$) on ligation in the deoxy quaternary structure.

Table 2. SKL model results*

Parameter values†				
pK_α, pK_β	$-RT \ln Q$	$-RT \ln S$	$-RT \ln K^\alpha_D$, $-RT \ln K^\beta_D$	$-RT \ln K^\alpha_O$, $-RT \ln K^\beta_O$
7.12, 6.05	2.51	−1.89	−7.79, −8.10	−8.85, −9.03
6.96, 6.75	0.99	−1.67	−7.28, −8.30	−8.94, −9.38

* Fitted to Mills *et al.* data (18) and Antonini *et al.* data (44); the SK values for r and r' were used ($r = 1/S$, $r' = 1$).
† All values, except pKs, are in kcal/mol.

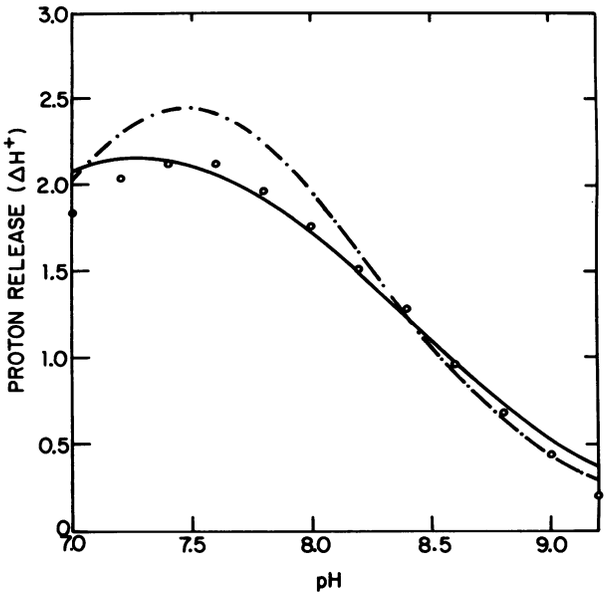

FIG. 2. Proton release (ΔH^+) on oxygenation. —, SKL model with $pK_\alpha = 7.12$, $pK_\beta = 6.05$; –·–·–, SKL model with $pK_\alpha = 6.96$, $pK_\beta = 6.75$; ○○○, data of Antonini *et al.* (44).

For r' and K_4, the situation is complicated, as there are conflicting data on the pH dependence of K_4 (49, 50). However, recent measurements by Poyart *et al.* (20) show that K_4 is pH dependent, with the largest change occurring between pH 6 and 7. A preliminary analysis favors a model with r' somewhat larger than $1/S$. More quantitative results will have to await additional information on the pH dependence of K_4 and on the Bohr groups contributing in the pH range between 6 and 7.

Related information concerning the salt-bridge coupling to tertiary and quaternary structure is obtained from the dependence on pH of the Hill "constant," n. With a parameter set for the SKL model (Table 2), n decreases by 0.2 between pH 7 and 9; for the original SK model fit (4), n decreases by 0.5 between pH 7 and pH 9. This is due to the pH-independent contributions to constraints in the deoxy state, made apparent by the present study.

Conclusion

A refined structure-specific model for hemoglobin function is presented. It is an extension of the original formulation by Szabo and Karplus (4) and incorporates the results of structural and thermodynamic studies that have accumulated over the last 10 years. Key features of the model are a flexible formulation of the coupling of salt bridges to the tertiary and quaternary structures and the introduction of constraints on the subunit ligand affinities that are independent of the salt bridges. Experimental results for the total proton release data over a wide pH range and the pH dependence of the first and fourth Adair constant are examined. Although the results obtained here involve fitting the model parameters to equilibrium measurements, the primary importance of the model lies in the formulation of the

relationship of hemoglobin function to specific aspects of its structure.

The significance of the present formulation is best considered in light of its relation to the stereochemical mechanism for hemoglobin cooperativity developed by Perutz in 1970 on the basis of structural information. The main features of the statistical mechanical model follow the Perutz mechanism—i.e., there are two quaternary structures (deoxy and oxy) for the tetramer, two tertiary structures (liganded and unliganded) for the subunits in each form of the tetramer, and structural elements that couple the stabilities of the tertiary and quaternary structures. In the original Perutz mechanism and the statistical mechanical model based on it, it was assumed for simplicity that the tertiary–quaternary coupling was due entirely to a set of salt bridges of equal strength. The present analysis, in conjunction with structural and empirical energy function results, indicates that the salt bridges may be of unequal strengths and that steric constraints, not dependent on pH, are involved in the coupling and make a contribution to reducing the ligand affinity in the deoxy tetramer. To explain the approximately linear dependence of proton release on oxygenation, the original model assumed that the salt bridges were broken when a subunit bound oxygen. The present analysis shows that the salt bridge coupling is more complicated—i.e., the salt bridge strengths are a function of both the tertiary structure of a subunit and the quaternary structure of the tetramer.

The generalized model proposed here and its success in describing the currently available thermodynamic data with assumptions that are based on structural and spectroscopic data provide strong support for the fundamental ideas of the Perutz mechanism. It is not surprising that the increased information that now exists makes it possible to introduce refinements and specific details that were not included in the original formulation.

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