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An analytic model for kinetics of hemoglobin reacting with ligand

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The saturation function $Y(t)$ descriptive of the kinetics of ligand binding by a biological macromolecule such as hemoglobin can be represented by $Y(t) = Y_{eq}\{[1+\epsilon][1-\exp-(\sigma_1 t)]\}/\{[1+\epsilon]-\epsilon\exp-(\sigma_1 t)\}$, where Y_{eq} is the fraction of sites bound at equilibrium, and σ_1 and ϵ are parameters which can be determined by kinetics measurements. If the sites bind independently, fixed functional relations hold between the quantities (Y_{eq}, σ_1, ϵ). These relations do not hold for cooperative ligand binding. The departures of these quantities from that required by the independent sites relations provide a measure of cooperativity. The present formulation, which includes the approximation that the multiplicity of chemical relaxation processes are dominated by a single one, can be extended for more refined applications.

1. Introduction

Extensive evidence for cooperativity of ligand binding by a biological macromolecule such as hemoglobin has been provided by measurements of the equilibrium binding curve. Kinetic studies involving the approach to equilibrium also indicate cooperativity [1–6]. The purpose of the present study is to present a simple analytic scheme for extracting evidence for cooperative behaviours from kinetics data.

2. Theory

Consider a biological macromolecule M which binds ligand X at N sites according to the sequential kinetic scheme

$$\mathrm{MX}_n + \mathrm{X} \underset{k_{\mathrm{off}}^{(n+1)}}{\overset{k_{\mathrm{on}}'^{(n)}}{\rightleftharpoons}} \mathrm{MX}_{n+1}; \quad \left[n = 0, 1 \ldots N;\ k_{\mathrm{off}}^{(0)} = k_{\mathrm{on}}'^{(N)} = 0\right] \tag{1}$$

If C is the total concentration of the macromolecule in all its forms and $[\mathrm{MX}_n]_t$ the concentration of the n-liganded species at time t, then the kinetic equations are expressed as

$$\frac{\mathrm{d}P_n}{\mathrm{d}t} = k_{\mathrm{on}}^{(n-1)}(t)P_{n-1}(t) - \left[k_{\mathrm{on}}^{(n)}(t) + k_{\mathrm{off}}^{(n)}\right]P_n(t) + k_{\mathrm{off}}^{(n+1)}P_{n+1}(t)$$

$$P_n(t) = \frac{[\mathrm{MX}_n]_t}{C}, \quad \sum_{n=0}^{N} P_n(t) = 1;$$

$$k_{\mathrm{on}}^{(n)}(t) = k_{\mathrm{on}}'^{(n)}X(t), \quad k_{\mathrm{off}}^{(0)} = k_{\mathrm{on}}'^{(N)} = 0 \tag{2}$$

where $P_n(t)$ and $X(t)$ are, respectively, the probability of occurence of the n-liganded species and the ligand activity at time t. The saturation function $Y(t)$, which is the fraction of sites bound, is

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the normalized first moment of the discrete distribution $P_n(t)$

$$Y(t) = \frac{\bar{n}(t)}{N} \equiv \frac{1}{N} \sum_{n=0}^{N} nP_n(t) \tag{3}$$

and the ligand activity is subject to the conservation condition

$$X(t) + C\bar{n}(t) = X(\infty) + C\bar{n}(\infty) \tag{4}$$

Substitution of eq. 4 into eq. 2 produces coupled nonlinear differential equations for P_n that are not amenable to analytical solution for arbitrary values of the on- and off-rate parameters. On the other hand, eq. 2 can be solved by standard eigenvalue techniques if the ligand is buffered. Phillipson and Wyman [7] developed approximate analytic solutions to the linearized MWC model [8] and found that, in the case of hemoglobin, cooperativity appears to enhance the importance of one relaxation parameter at the expense of others. In the more realistic situation that the amount of ligand is held constant, then if this were also the case, it would suggest that the coupled nonlinear kinetic scheme could be replaced by a simple analytic kinetic model for the approach to equilibrium by an allosteric macromolecule characterized by a single relaxation time. We will initially assume that the rate parameters are statistical, which means they have values as if the sites are independent: $k_{on}^{\prime(n)} = (N-n)k'_{on}$, $k_{off}^{(n)} = nk_{off}$. Then, substitution into eq. 2 combined with eq. 4 gives exactly

$$\frac{d\bar{n}}{dt} = Nk_{on}(t) - \left[k_{on}(t) + k_{off}\right]\bar{n} \tag{5a}$$

$$k_{on}(t) = k'_{on}\{X(\infty) + C\left[\bar{n}(\infty) - \bar{n}(t)\right]\} \tag{5b}$$

Substitution of eq. 5b into eq. 5a results in an elementary first-order differential equation whose solution, assuming the macromolecule is unbound at zero time, is

$$Y(t) = \frac{Y_{eq}\{[1+\epsilon][1-\exp-(\sigma_1 t)]\}}{\{[1+\epsilon] - \epsilon \exp-(\sigma_1 t)\}} \tag{6}$$

where

$$Y_{eq} = \frac{k}{1+k},\ k = \frac{k'_{on}X(\infty)}{k_{off}} \tag{7a}$$

$$\sigma_1 = \left\{k_{off} + k'_{on}\left[X(\infty) + NC(1 - Y_{eq})\right]\right\} \tag{7b}$$

$$\epsilon = \frac{NCk'_{on}Y_{eq}}{\sigma_1} \tag{7c}$$

Y_{eq} is the equilibrium binding function characteristic of independent sites, σ_1 is a bimolecular rate parameter whose functional form has been used to analyze the kinetics of hemocyanins [4–6], and ϵ is a measure of nonlinearity. In the approximation of constant ligand activity, $NC/X(\infty) \to 0$ which implies $\epsilon \to 0$ and $\sigma_1 \to k_{off} + k'_{on}X(\infty)$. This limiting case is appropriate to a linear unimolecular process characterized by a single relaxation time σ_1^{-1}. By making Y_{eq} of eq. 7a the independent

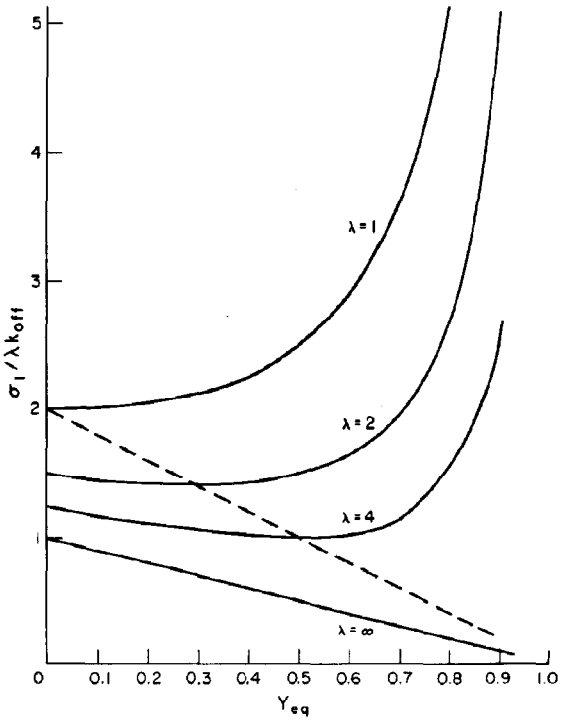

Fig. 1. Curves of the bimolecular rate parameter vs saturation for independent binding sites according to eq. 8. Dashed line indicates envelope of the minima.

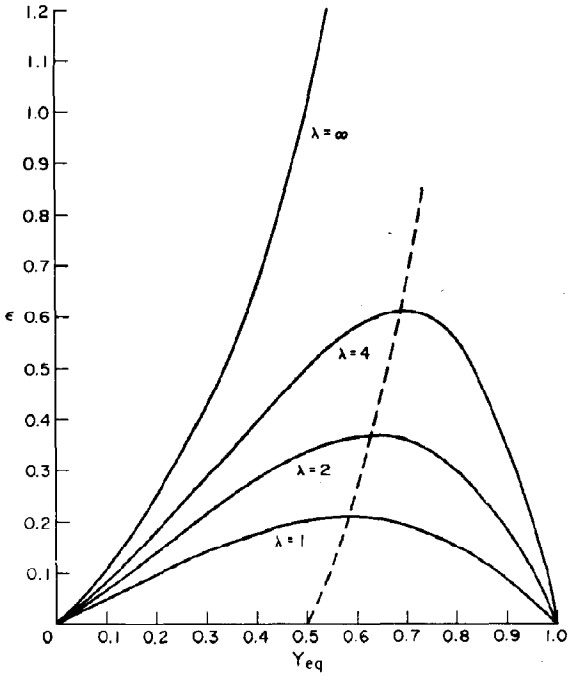

Fig. 2. Curves of the nonlinear parameter vs saturation for independent binding sites according to eq. 8. Dashed line indicates envelope of the maxima.

variable, then σ_1 and ϵ of eqs 7b and 7c are given by

$$\frac{\sigma_1}{\lambda k_{off}} = \frac{1+\lambda(1-Y_{eq})^2}{\lambda(1-Y_{eq})}, \quad \epsilon = \frac{\lambda Y_{eq}(1-Y_{eq})}{1+\lambda(1-Y_{eq})^2}$$

$$\left(\lambda = \frac{NCk'_{on}}{k_{off}}\right) \tag{8}$$

The behaviours of these functions, parameterized by λ, are shown in figs 1 and 2. If $\lambda > 1$, σ_1 has a minimum at a value of saturation $1-\lambda^{-1/2}$, at which point $\sigma_1/\lambda k_{off} = 2\lambda^{-1/2}$ (fig. 1). The parameter ϵ is always positive and has a single maximum at the value of saturation $[(\lambda+1)-(\lambda+1)^{1/2}]/\lambda$ at which point $\epsilon = [(\lambda+1)^{1/2}-1]/2$ (fig. 2). This maximum is bounded between half-saturation and unity over the entire range of λ. These behaviours of σ_1 and ϵ appropriate to independent sites are interrelated, since from eq. 8, $\sigma_1\epsilon/\lambda k_{off} = Y_{eq}$.

The extension of these considerations to cooperative kinetics is based upon the postulate that the saturation as a function of time will be taken to be given by eq. 6 but the parameters (Y_{eq}, σ_1, ϵ) will be fixed *independently* by experiment, unconstrained by eqs 7 and 8. Y_{eq} is presumed known from equilibrium studies and the conditions of experiment. In principle, σ_1 and ϵ are determined from the asymptotic behaviours of the experimental $Y(t)$ curve, since from eq. 6

$$\frac{-\ln\left[\dfrac{Y_{eq}-Y(t)}{Y_{eq}}\right]}{t} = \begin{bmatrix} (1+\epsilon)\sigma_1 & \text{as} & t\to 0 \\ \sigma_1 & \text{as} & t\to\infty \end{bmatrix} \tag{9}$$

In practice, two data points for a given experimental measurement of $Y(t)$ are sufficient to fix these parameters.

We consider first the binding of carbon monoxide by sheep hemoglobin [1]. Since the binding is assumed to be irreversible, $Y_{eq} = 1$, and if the process is noncooperative $k_{off} = 0$ implies $\epsilon = NC/X(\infty) > 0$ from eq. 7. Comparison of theory

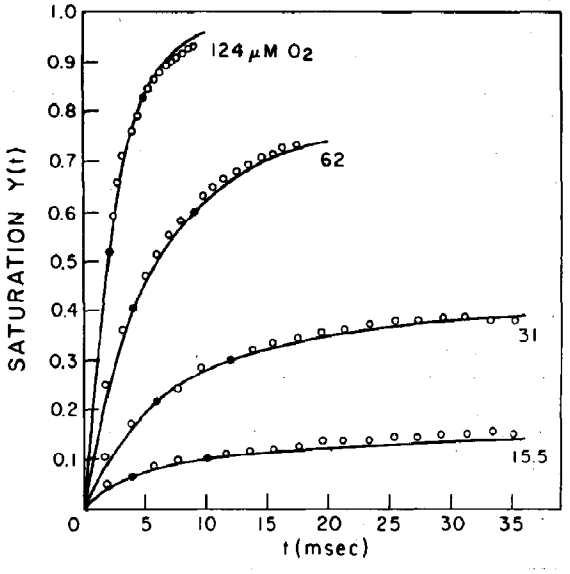

Fig. 3. Saturation vs time for the binding of oxygen by hemoglobin. The curves are computed from eq. 6 with the parameters listed in table 2 for buffered hemoglobin. (○) Data points reported by Gibson (fig. 1 of ref. 2). (●) Experimental points chosen to fix the parameters (cf. table 2[b]).

Table 1

Saturation Y at various times for the binding of carbon monoxide by sheep hemoglobin

Time (ms)	Experiment [a]	Theory [b]	Time (s)	Experiment [c]	Theory [d]
35	0.157	0.157	10.4	0.118	0.118
65	0.305	0.291	17.4	0.187	0.191
95	0.445	0.417	24.4	0.257	0.259
125	0.562	0.532	31.4	0.316	0.322
155	0.656	0.631	38.4	0.379	0.381
185	0.732	0.713	45.4	0.432	0.435
215	0.792	0.781	52.4	–	0.485
245	0.839	0.834	59.4	0.530	0.531
275	0.875	0.875	66.4	–	0.573
			73.4	0.612	0.612
			80.4	–	0.647
			87.4	0.680	0.680

[a] From ref. 1, table 1. pH = 7.1, 23.6° C.
[b] From eq. 6 with $\sigma_1 = 10.43\ s^{-1}$, $\epsilon = -0.5770$. Fixed such that theory and experiment agree at 35 and 275 ms.
[c] From ref. 1, tables 3 and 4. pH = 9.1, 20.3° C.
[d] From eq. 6 with $\sigma_1 = 14.75\ s^{-1}$, $\epsilon = -0.1928$. Fixed such that theory and experiment agree at 10.4 and 87.4 ms.

and experiment is shown in table 1, where it is seen that the values are in agreement within 5%. The cooperative nature of the binding is indicated in the present context by the fact that the values of ϵ are here negative. Secondly, we consider application of eq. 6 to stopped-flow studies [2] of the reversible binding of oxygen by hemoglobin with the parameters listed in table 2. Agreement between theory and experiment is within 2%. A graphical comparison is shown in fig. 3. Table 2 indicates that cooperativity in this case is reflected by either (1) the behaviours of σ_1 as a function of saturation (stripped case) going through a maximum instead of a minimum implied by independent sites (fig. 1), or (2) the fact that ϵ either oscillates or goes through a minimum as a function of saturation, but not the maximum required in the absence of cooperativity (fig. 2).

3. Discussion

The present model constitutes a reduction of complicated relaxation processes characteristic of allosteric proteins to a contracted description which assumes one dominant relaxation parame-

Table 2

Parameter fit to hemoglobin data from ref. 2

Buffered human hemoglobin [b]				Stripped human hemoglobin [c]			
$[O_2]$ (μM) [a]	Y_{eq}	σ_1 (s^{-1})	ϵ	$[O_2]$ (μM) [a]	Y_{eq}	σ_1 (s^{-1})	ϵ
124	0.98	370	0.030	62	0.94	190	1.68
62	0.80	100	1.05	31	0.53	410	−0.345
31	0.40	90	0.55	15.5	0.26	190	0.58
15.5	0.15	34	4.0	7.8	0.13	130	0.80

[a] Free oxygen concentration.
[b] Parameters σ_1, ϵ of eq. 6 fixed from the data points at the following times (in ms) from the corresponding curves of ref. 2, fig. 1: 124 μM O_2, $t = 2$, 5: 62 μM O_2, $t = 2$, 9; 31 μM O_2, $t = 6$, 12; 15.5 μM O_2, $t = 4$, 10.
[c] Parameters σ_1, ϵ of eq. 6 fixed from the data points at the following times (in ms) from the corresponding curves of ref. 2, fig. 3: 62 μM O_2, $t = 2$, 6; 31 μM O_2, $t = 3$, 8; 15.5 μM O_2, $t = 2$, 6; 7.8 μM O_2, $t = 2$, 8.

ter. Why the model might be expected to give reasonable agreement with experiment as in the cases considered above can be understood by returning to the exact eqs 2. These equations can be expressed in terms of normal coordinates Q_ν ($\nu = 0, 1, 2, \ldots N-1$) according to the following transformation

$$P_n(t) = P_n(\infty) + f_n(t),\ f_n = \sum_{\nu=1}^{N} A_{n\nu} Q_\nu \qquad (10a)$$

$$\frac{dQ_\nu}{dt} = -\sigma_\nu Q_\nu + \sum_{\mu_1}\sum_{\mu_2} C^{(\nu)}_{\mu_1\mu_2} Q_{\mu_1} Q_{\mu_2} \qquad (10b)$$

$$P_n(\infty) = \frac{K_n}{\sum_{n=0}^{N} K_n},\ K_n = \frac{k_{\text{off}}^{(n+1)}}{k_{\text{on}}^{(n)}(\infty)} \prod_{m=0}^{n} \frac{k_{\text{on}}^{(m)}(\infty)}{k_{\text{off}}^{(m+1)}} \qquad (10c)$$

Eq. 10a expresses $P_n(t)$ with reference to the equilibrium probabilities given by eq. 10c. The latter follow from eq. 2 with $dP_n/dt = 0$ and the principle of detailed balance. The functions $f_n(t)$ are expanded in terms of normal modes Q_ν with $A_{n\nu}$ the transformation coefficients. Eq. 10b are the rate equations, eq. 2, in terms of the normal modes. In the linear, buffered, approximation, σ_ν are the eigenvalues which guide the approach to equilibrium as a linear combination of exponentials $\exp-(\sigma_\nu t)$, $\nu = 1, 2 \ldots N-1$ ($\sigma_0 = 0$). In the general case, they function as relaxation parameters shifted to include the constraint of conservation of the total amount of ligand. The C coefficients multiplying the nonlinear quadratic terms in Q are complicated functions of the transformation coefficients $A_{n\nu}$, rate parameters, equilibrium probabilities, C and $X(\infty)$. Assume now that σ_ν are nondegenerate and ordered such that $\sigma_\nu < \sigma_{\nu+1}$. The discussion in ref. 7 suggested that the effect of cooperativity is to enhance the dominance of the smallest relaxation parameter relative to the others. Then $\sigma_\nu \gg \sigma_1$ ($\nu \neq 0, 1$) so that as a first approximation $Q_\nu(t) = 0$, $\nu > 1$, which leaves only one survivor in eq. 10b: $dQ_1/dt = -\sigma_1 Q_1 + C^{(1)}_{11} Q_1^2$. Solution of this equation combined with eq. 10a yields an analytic solution for $P_n(t)$ in the single-relaxation-time approximation. Insertion of this solution into eq. 3 results in the starting point of the analysis, eq. 6, with the identification $\epsilon = NC^{(1)}_{11} Y_{\text{eq}}/\sigma_1(\sum_n A_{n1})$. ϵ is a complicated function of the on- and off-rate parameters. There is no reason in principle why it should be either positive or negative, nor why it should be very large or very small. These tendencies are shown in table 1 and 2. In situations where the discrepancy between experiment and the present single-relaxation-time model is unacceptable, one can extend the scheme to include two relaxation parameters, σ_1 and σ_2, which would parameterize the nonlinear coupling between two normal modes, Q_1 and Q_2. Eq. 10b then reduces to two coupled nonlinear equations for which exact solutions do not exist. However, assuming $\sigma_1 \ll \sigma_2$, it is possible to develop iterative solutions to any desired degree of approximation.

Theoretical analyses have often centered on either the two-state MWC model [8] or extensions of it [3]. However, the parameter assignments are not unique [7]. This suggests that, independent of the model, the present scheme may provide a simple quantitative basis for interpretation of the kinetics in terms of specific mechanisms.

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