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Application of linear free energy relations to protein conformational changes: The quaternary structural change of hemoglobin

(kinetics/dynamics/transition state theory/reaction path/allostery)

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ABSTRACT **The transition state for the R ⇋ T quaternary conformational change of hemoglobin has thermodynamic properties much closer to those of the R conformation than to those of the T conformation. This finding is based on a comparison of activation and equilibrium enthalpy and entropy changes and on the observation of a linear free energy relationship between quaternary rate and equilibrium constants. A previous theoretical study [Janin, J. & Wodak, S. J. (1985) *Biopolymers* 24, 509–526], using a highly simplified energy function, suggests that the R-like transition state is the result of a reaction pathway with the maximum buried surface area between $\alpha\beta$ dimers.**

Conformational changes play a critical role in the function of many proteins. In multisubunit proteins, the rearrangement of the packing of subunits, the quaternary conformational change, is responsible for cooperative behavior (1). In spite of the importance of quaternary conformational changes in protein chemistry, very little is known about their kinetics or molecular mechanisms. Hemoglobin remains the only protein for which there is a substantial body of kinetic data for a quaternary conformational change, beginning with the studies of Gibson performed over 30 years ago (2).

An essential element in the investigation of the molecular mechanism of a quaternary conformational change is the experimental characterization of the transition state. Knowledge of the transition state can explain systematic changes in rates and provides an important test of theoretical descriptions of reaction paths. In this paper, we describe results on hemoglobin which indicate that the transition state has properties much closer to those of the R quaternary structure than to those of the T quaternary structure. We also point out that an R-like transition state may be explained by a reaction path similar to the one Janin and Wodak (3) generated by maximizing the buried surface area at the subunit interfaces between $\alpha\beta$ dimers.

In a recent study on trout hemoglobin, time-resolved absorption spectroscopy was used to determine the activation parameters for the R → T transition of the molecule with no ligands bound (4). At 20°C, the conformational change was found to occur with a rate constant of 5×10^4 sec^{-1}, and with an activation energy, E_a, of 8.0 kcal/mol (1 cal = 4.184 J). Using the equation from transition state theory (TST),

$$k = \kappa k^{TST} = \kappa(k_B T/h)e^{-\Delta G^{\ddagger}/RT} = \kappa(k_B T/h)e^{\Delta S^{\ddagger}/R}e^{-\Delta H^{\ddagger}/RT}, \quad [1]$$

with $\Delta H^{\ddagger} = E_a - RT$, together with the equilibrium enthalpy and entropy changes (5, 6), the activation enthalpies and entropies were calculated for both the $R_0 \rightarrow T_0$ and $T_0 \rightarrow R_0$ quaternary structural changes (Fig. 1). The data in Fig. 1 show that the activation enthalpies and entropies for the $T_0 \rightarrow R_0$ transition are much more similar to the equilibrium enthalpies and entropies than the corresponding values for the $R_0 \rightarrow T_0$ transition. That is, the thermodynamic properties of the transition state are much more similar to those of the R quaternary conformation than to those of the T quaternary conformation (4).†

An R-like transition state for the unliganded molecule has important and interesting consequences. Adding ligands, or changing solution variables such as pH, is expected to change the free energy of the transition state by nearly the same amount as the R state. The free energy barrier for the R → T transition (and hence its rate) is therefore expected to change much less than the free energy barrier for the T → R transition (Fig. 2). This prediction is borne out by the published results summarized in Table 1 on the kinetics and thermodynamics of human hemoglobin analyzed in terms of the two-state allosteric model of Monod *et al.* (20).§ The results show that decreasing the allosteric equilibrium constant, $L_i = [T_i]/[R_i] = L_0 c^i = k(R_i \rightarrow T_i)/k(T_i \rightarrow R_i)$, by adding ligands such as carbon monoxide or oxygen to ferrous hemes, or cyanate and azide to ferric hemes, changes the R → T rates much less than the T → R rates (12, 18). Also, increasing the pH, which decreases L_0, changes the $R_0 \rightarrow T_0$ rate much less than the $T_0 \rightarrow R_0$ rate. Thus, changes in the R ⇋ T conformational equilibrium are manifested mainly as changes in the T → R rates, as predicted for an R-like transition state (Fig. 2).

We can take the analysis one step further and ask whether there is any simple quantitative relationship between the rate and equilibrium constants. The simplest of such relationships is a linear free energy relationship. A linear free energy relationship describes the situation in which the transition state has properties that are intermediate between reactants and products and in which, upon changing some variable, the change in the free energy of the transition state is a linear combination of the changes in free energy of the reactants and products (22, 23). Given this assumption, the variation in the

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†There are two reasons why the entropy of activation for $T_0 \rightarrow R_0$ is expected to be less than the equilibrium value even in the limit of virtually identical structures for the transition state and the R_0 state (4). One reason is that the transition state is missing one vibrational degree of freedom, corresponding to motion along the reaction coordinate. The entropy of the transition state is therefore lowered by $R \ln(k_B T/h\nu)$, where ν is the frequency of the missing vibration. The second reason is that in calculating the activation parameters the transmission coefficent, κ, in Eq. 1 was assigned a value of 1, which assumes that there are no recrossings of the barrier (8). For values of $\kappa < 1$, the calculated entropy of activation would be more positive.

§The interface between $\alpha\beta$ dimers (7, 21), which is the interface that changes with the quaternary transition, is sufficiently similar for trout I and human hemoglobin that it is reasonable to assume that the transition states are very similar for the two molecules (4).

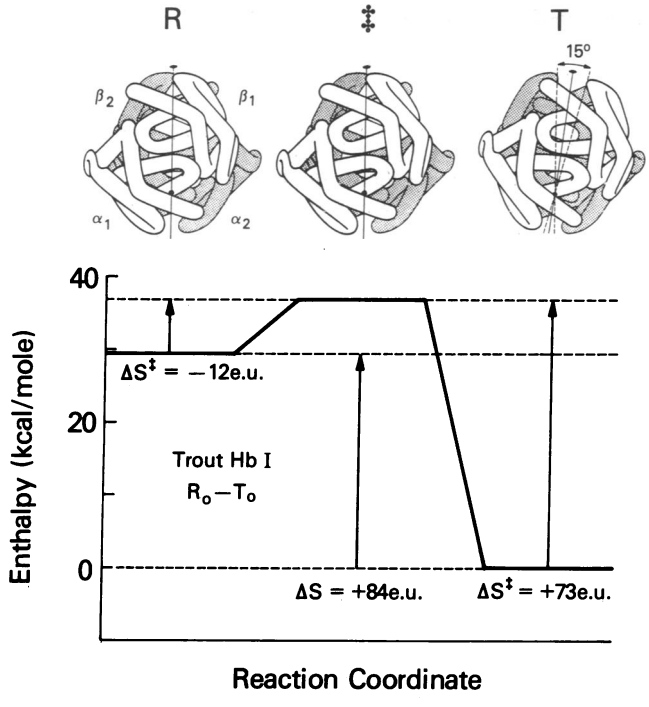

FIG. 1. Activation and thermodynamic parameters for $R_0 \rightarrow T_0$ quaternary conformational change of trout I hemoglobin (4). The quaternary change consists of a rotation of symmetrically related $\alpha\beta$ dimers by ≈15° relative to each other with a translation of ≈0.1 nm along the rotation axis (3, 7). The actual reaction pathway may be much more complex (see text) (3).

activation free energy change is proportional to the variation in the equilibrium free energy change (22–24)—i.e.,

$$\delta\Delta G^{\ddagger} = \alpha\delta\Delta G \quad \text{or}$$

$$\Delta G^{\ddagger} = \alpha\Delta G + \text{constant}. \qquad [2]$$

The proportionality constant, α, may be interpreted as a measure of the position of the transition state along the reaction path. It takes on values between 0 and 1. For $\alpha = 0$, the transition state is completely reactant-like, while for $\alpha = 1$ the transition state is completely product-like (Fig. 2). Linear free energy relations and the concept of the position

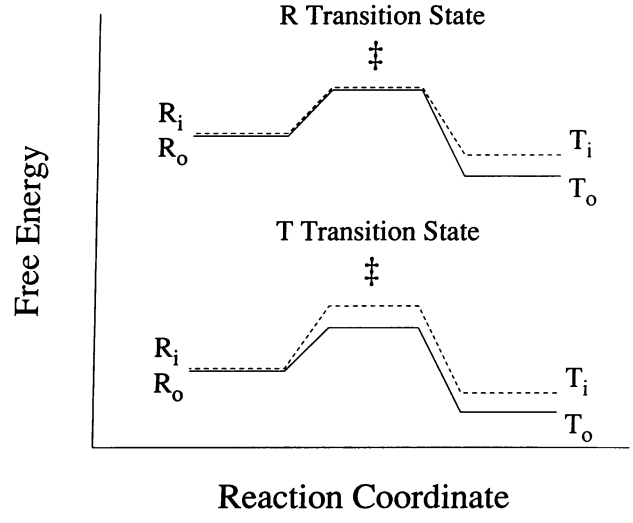

FIG. 2. Schematic diagram of consequences of R-like and T-like transition states on relationship between quaternary rates and equilibria. The continuous and dashed lines represent the free energy for a different number of bound ligands or different solution conditions. The free energy of the R state is used as a reference state. For a completely R-like transition state, all of the change in the equilibrium free energy change appears in the $T \rightarrow R$ rate with no change in the $R \rightarrow T$ rate, while for a completely T-like transition state all of the change appears in the $R \rightarrow T$ rate with no change in the $T \rightarrow R$ rate.

along the reaction coordinate have been used by Szabo to explain the differences in kinetic cooperativity between oxygen and carbon monoxide binding to hemoglobin (25–27).

For the $R \rightarrow T$ quaternary conformational change Eq. 2 becomes

$$k(R_i \rightarrow T_i) = \gamma(L_0 c^i)^{\alpha}, \qquad [3]$$

and

$$k(T_i \rightarrow R_i) = \gamma(L_0 c^i)^{\alpha-1}, \qquad [4]$$

where γ is a scale factor. In this case $\alpha = 0$ corresponds to a completely R-like transition state and $\alpha = 1$ corresponds to a completely T-like transition state (Fig. 2). Fig. 3 shows a plot of the logarithm of the $R \rightarrow T$ rate constants versus the logarithm of the allosteric equilibrium constant, L_i, at various

Table 1. Rate and equilibrium constants for $R \rightarrow T$ quaternary structural changes in human hemoglobin

Ligation state (ligand)	pH (buffer)	Rate constant			Equilibrium constant		
		t, °C	$k(R_i \rightarrow T_i)$, $\times 10^4$ sec^{-1}	Ref.	t, °C	L_i	Ref.
$R_0 \rightarrow T_0$	7.0 (A)	22.5	4.0	9	20	1.8×10^7*	10
$R_0 \rightarrow T_0$	7.0 (A)	21	3.8	11	20	1.8×10^7*	10
$R_0 \rightarrow T_0$	9.0 (B)	20	0.64	12	20	3×10^3†	13
$R_0 \rightarrow T_0$	7.0 (C)	20	0.44	14	25	3.7×10^3‡	15
$R_1 \rightarrow T_1$ (N_3^-)	7.0 (A)	22.5	1.7§	9	20	6.1×10^4*	10
$R_1 \rightarrow T_1$ (CNO^-)	7.0 (A)	22.5	1.0¶	9	20	6.1×10^4*	10
$R_1 \rightarrow T_1$ (CO)	9.0 (B)	20	0.28	12	20	23†	13
$R_3 \rightarrow T_3$ (CO)	7.0 (C)	21	0.34	16	21	0.16	16
$R_3 \rightarrow T_3$ (O_2)	7.0 (D)	25	0.3	17	25	1.5	17
$R_3 \rightarrow T_3$ (CO)	7.0 (D)	25	0.16	17	25	0.38	17
$R_3 \rightarrow T_3$ (CO)	6.5 (D)	19	0.12	18	19	0.34	18
$R_3 \rightarrow T_3$ (CO)	7.0 (D)	19	0.10	18	19	0.33	18

Buffers: A, 0.1 M potassium phosphate; B, 0.05 M borate; C, 0.05 M Bistris/9 mM Cl^-; D, 0.15 M potassium phosphate.
*Determined by fitting the saturation versus oxygen pressure curve (with logarithmic spacing of the pressures and no weights) generated from the Adair constants of Imai and Yonetani (10).
†Calculated by Sawicki and Gibson (19) from Adair oxygen binding constants of Roughton and Lyster (13).
‡Buffer was 0.05 M Bistris/7 mM Cl^-, pH 7.4.
§Hemoglobin with one oxidized heme liganded with azide.
¶Hemoglobin with one oxidized heme liganded with cyanate.

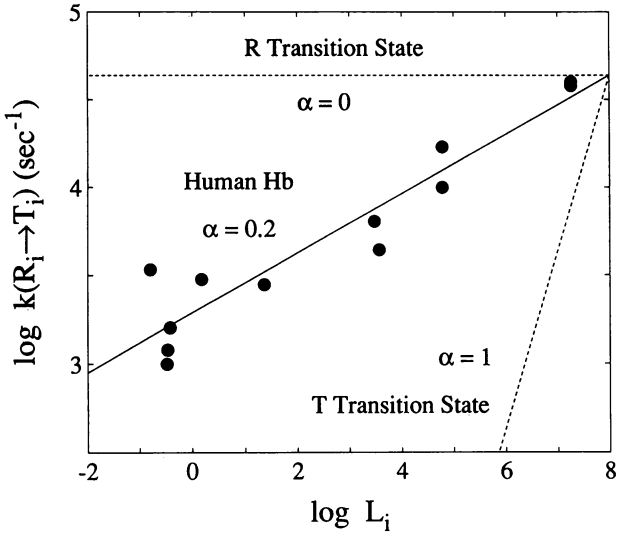

FIG. 3. Correlation between measured R → T rates [$k(R_i \rightarrow T_i)$] for human hemoglobin and the allosteric equilibrium constant L_i. The points are the data in Table 1, and the line through the points results from a least-squares fit with a slope of 0.17 ± 0.02 and an intercept of 3.29 ± 0.07. Dashed lines represent the predicted relationship between the rates and equilibrium constants for the R transition state and for the T transition state. The absolute values of the predicted rates are arbitrary.

ligation states and pH values. For several cases, the values of L_i correspond to solution conditions that are very similar, but not identical, to the solution conditions of the kinetic experiments. Furthermore, the use of the value of the allosteric equilibrium constant obtained from oxygen binding curves assumes that any additional processes, such as proton or anion binding, equilibrate rapidly compared with the rate of the conformational change. In spite of these limitations, the results in Fig. 3 clearly show that there is a positive correlation between the logarithm of the R → T rate constant and the logarithm of the allosteric equilibrium constant. They also indicate that the correlation is approximately linear with a slope α, from Eq. 3, of about 0.2.¶ The linear relationship produces the useful practical result that all 10 quaternary rates (two for each of five ligation states) can be estimated from Eqs. 3 and 4 with just four parameters—L_0, c, α, and γ. It should be noted in this regard that the two-state allosteric model (20) places no restrictions on the quaternary rates, apart from those imposed by the equilibrium constants.

Both the similarity of the activation and equilibrium parameters for the $T_0 \rightarrow R_0$ transition (Fig. 1) and the linear free energy relation with a small slope (Fig. 3) are consistent with the conclusion that the transition state has thermodynamic properties that are much more R-like than T-like. A structural interpretation of this result is not straightforward. Not only is the description of the transition state for a system with so many atoms a very complex problem, but even the most fundamental aspects of the relationship between structure and energetics in hemoglobin are still unsettled. The most obvious approach is to assume that the resemblance of the transition state to the R-state is structural as well as energetic. This would suggest that in the path from T_0 to R_0, the subunits have already almost completely rearranged into the packing corresponding to the R configuration upon reaching the transition state, while in the reverse direction the transition state is reached with very little change in the subunit packing (Fig. 1). A value of 0.2 for α further suggests that in the quaternary transition from R to T the molecule has reached the transition state after proceeding ≈20% of the way along the reaction coordinate. If it is naively assumed that the reaction coordinate is the angle of rotation of the $\alpha\beta$ dimers (Fig. 1), then the dimers rotate ≈3° in proceeding from the R state to the transition state and ≈12° in proceeding from the T state to the transition state.

Another approach is to consider the simplest model for the relationship between structure and energetics, which has been used to explain the equilibrium binding properties of hemoglobin. This model is a synthesis of the two-state allosteric model of Monod, Wyman, and Changeux (3, 28, 29), the stereochemical model of Perutz (30, 31), and the statistical mechanical formulation of the Perutz model by Szabo and Karplus (32–35). According to this MWC–PSK model, intersubunit salt bridges are responsible for stabilizing the T quaternary structure relative to the R quaternary structure and act as the constraints that lower the oxygen affinity of subunits in the T quaternary structure. Since the affinity of the transition state for ligands is very similar to that of the R state (Fig. 2), this model predicts that most of the salt bridges are broken in the transition state. The model by itself does not predict the spatial relationship between $\alpha\beta$ dimers in the transition state, but it does suggest that the transition state may not be as close to the R configuration of the subunits as suggested by the simple geometric model discussed above.

There is currently only one detailed theoretical investigation of the reaction pathway for the R ⇋ T quaternary conformational change. This study was carried out by Janin and Wodak (3) using a highly simplified description of the energetics of the interaction between $\alpha\beta$ dimers. In this description, repulsive interactions were represented by a "soft"-sphere potential with no attractive term, in which each residue was replaced by a single sphere. In contrast to the MWC–PSK model, attractive interactions were assumed to arise solely from the hydrophobic contribution as estimated by the buried surface area. A large number of intermediate quaternary structures were generated by reconstituting tetramers from dimers having a T tertiary structure (i.e., the tertiary structure found in deoxyhemoglobin) or the R tertiary structure (i.e., the tertiary structure found in carbonmonoxyhemoglobin). With use of only these R and T tertiary structures, a reaction pathway was constructed by considering the intermediate quaternary structures to be those with the largest buried surface area. The reaction pathway was found to be more complex than the relative rotation of $\alpha\beta$ dimers about the single axis that is depicted in Fig. 1. The interesting finding was that buried surface areas comparable in magnitude to those found in deoxyhemoglobin could be generated by associating dimers with the T tertiary structure into tetramers having a range of quaternary structures from T to near R. In contrast, dimers with the R tertiary structure could only be associated into tetramers with a large buried surface area having structures close to R. These results require that the tertiary conformational change occurs at a position along this reaction pathway that is closer to the R than to the T quaternary structure.

Although the authors (3) did not discuss these calculations in relation to transition state theory, their results can rationalize an R-like transition state. Within the limitations of their model that there are only two tertiary structures and that the free energy differences among the tetramers are primarily determined by the buried surface area, the minimum in the

¶At higher and lower values of L_i, deviations from a linear relationship are expected. At values of $L_i << 1$, such that the R state becomes much more stable than the T state, the position of the transition state must move toward the T quaternary structure to preserve a barrier for the T → R transition (Fig. 2). Also, there is an upper limit to the rate of the quaternary transition, which is $\approx 10^8$ sec^{-1}, the inverse of the correlation time (τ_R) for the free rotational diffusion of an $\alpha\beta$ dimer in water (calculated from the relation $\tau_R = \eta V/kT$). This upper limit can be considered analogous to the diffusion limited rate for a bimolecular reaction.

buried surface area along the hypothetical reaction pathway corresponds to a maximum in the free energy. This free energy maximum is, therefore, a saddle point on the free energy surface and defines the position of the transition state. The position of the transition state is predicted to be much closer to the R quaternary structure than to the T quaternary structure. Furthermore, the theoretical free energy barrier is roughly 10 kcal/mol, compared with 11–12 kcal/mol calculated from the experimental data for the $R_0 \rightarrow T_0$ transition (Table 1) using Eq. 1.[‖] If the change in the angle (χ) of rotation about the line joining the centers of mass of the two $\alpha\beta$ dimers (3) is chosen as a measure, then the transition state is located ≈20% of the distance along the reaction path from R to T. The agreement with the simplest interpretation of the experimental results is striking and suggests that maximizing the number of interatomic contacts between $\alpha\beta$ dimers is an important element in the reaction pathway. It will clearly be worthwhile to investigate the Janin and Wodak pathway further by using a more realistic treatment of the interatomic interactions.

[‖]We can make a crude estimate of the magnitude of the free energy barrier within the framework of the Janin and Wodak approach (3). In their reaction path for the (alchemical) transition from R_4 to T_0, the buried surface area of the transition state is less than the R_4 ground state by ≈400 Å^2. With the value of 25 cal·mol^{-1} for each Å^2 of buried surface area (36), the predicted activation free energy is therefore ≈10 kcal/mol. There are, however, two caveats. First, this number cannot strictly be compared with experimental values for the $R_0 \rightarrow T_0$ transition, since there are tertiary conformational changes upon removing the 4 ligands from the R_4 molecule, which might affect the buried surface area (30, 31, 37) (as well as the position of the transition state). Second, the value of 25 cal·mol^{-1}·Å^{-2} could be in significant error (38).

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