The Magnitude of Enzyme Transition State Analog Binding Constants[1]

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ABSTRACT

Transition state binding theory utilizes non-enzymic and enzymic rate ratios to predict the ratio of transition state analog dissociation constants to substrate dissociation constants. In this paper we show that enzyme rate accelerations due solely to lessened entropy requirements, arising from the juxtaposition of a catalytic group and a substrate binding site at an enzyme active site, will result in a ratio of transition state and substrate dissociation constants which is different, in general, from the ratio of non-enzymic and enzymic rate constants. The arguments presented in this paper provide a possible explanation for the frequently observed large discrepancy between the measured and predicted values for transition state analog dissociation constants.

A considerable effort has recently been expended in the search for compounds which resemble in structure, charge, etc. the presumed transition states of enzyme-catalyzed reactions (1,2,3). Such transition state analogs have been predicted to have dissociation constants much smaller than enzyme substrates (4). Experimentally, a large number of analogs have been found which do indeed have lower dissociation constants than substrates (1,2,3). However, the observed decreases in dissociation constants do not, in general, exceed 10^{-3}-10^{-4}. The reason these values do not equal the predicted values, 10^{-8}-10^{-14}, is clearly related to the impossibility of synthesizing true transition state analogs, that is, compounds containing partial bonds. However, in this paper we propose an additional explanation why the large postulated decreases in dissociation constant may not be expected.

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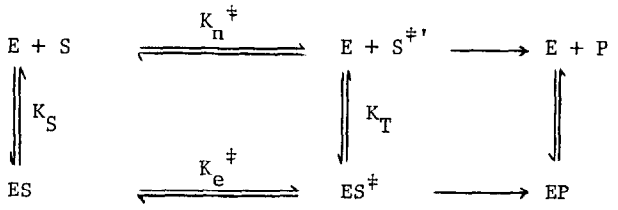
The theory of enhanced transition state binding is generally depicted for a unimolecular process as in Scheme I:



SCHEME I

K_S and K_T are dissociation constants for the formation of free enzyme, E, and either substrate, S, or transition state, $S^{\ddagger'}$, from ES and $ES^{\ddagger}$, respectively. $K_n^{\ddagger}$ and $K_e^{\ddagger}$ are equilibrium constants for the formation of $E + S^{\ddagger'}$ from E + S and $ES^{\ddagger}$ from ES. From transition state theory, rate constants for the formation of product, P, from E + S, k_n, and for the formation of product from ES, k_e, are directly proportional to $K_n^{\ddagger}$ and $K_e^{\ddagger}$. The interrelationship of these constants is expressed in equation (1).

$$\frac{K_n^{\ddagger}}{K_e^{\ddagger}} = \frac{k_n}{k_e} = \frac{K_T}{K_S} \qquad (1)$$

Since $k_n/k_e \cong 10^{-8}$-10^{-14}, K_T should be lower than K_S by this factor[2].

The following thermodynamic expression relates the free energy of activation of a reaction, $\Delta F^{\ddagger}$, to $K^{\ddagger}$:

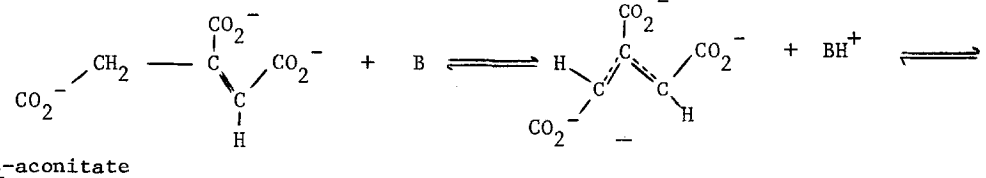
$$\Delta F^{\ddagger} = \Delta H^{\ddagger} - T\Delta S^{\ddagger} = -RT\ln K^{\ddagger} \qquad (2)$$

Thus, the difference in the free energy of activation for an enzymic vs. a non-enzymic reaction will be reflected in the ratio K_T/K_S. The factors which may give rise to a decreased free energy of activation for a single substrate enzyme reaction have been reviewed elsewhere (5,6,7), and include (i) strain or distortion of enzyme-bound substrate, (ii) changes in the environment of a reaction when reaction occurs from an ES complex (this

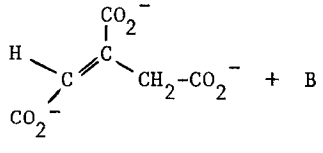
[2] The reader is referred to recent reviews by Lienhard (3) and Wolfenden (1) for a more thorough treatment of this theory.

effect will include any influence of the enzyme on the changes in solvation which accompany the conversion of substrate to transition state), and (iii) the positioning of amino acid side chains at the enzyme active site which can participate in nucleophilic or general acid-base catalysis.

Experimental predictions regarding the magnitude of K_T/K_S will vary depending on the nonenzymatic reaction chosen for comparison with the enzyme reaction of interest. Mechanistic studies on a very large number of single substrate enzymes have indicated the general importance of active site residues in nucleophilic and acid-base catalysis (8,9). As Lienhard has pointed out (3), evidence for such catalysis by active site residues in enzyme reactions will influence the choice of an appropriate model reaction. For example, a considerable number of enzyme-catalyzed isomerizations (e.g., the reactions catalyzed by triosephosphate isomerase, glucose-6-P isomerase, xylose isomerase and aconitate isomerase) have been described by mechanisms involving general base catalysis by an active site residue (8). Thus, a base catalyzed isomerization of cis- to trans-aconitate would be the model reaction which best approximates the mechanism of action of aconitate isomerase (10):



cis-aconitate

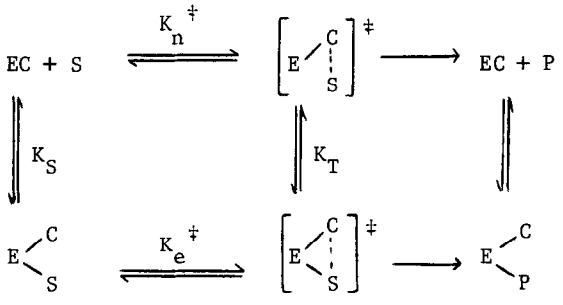


trans-aconitate

A second order rate constant for the hydroxide ion catalyzed isomerization of cis- to trans-aconitate was found to be 4×10^{-6} M^{-1} sec^{-1} at 25° (11). From the turnover number of aconitate isomerase, at 25° an enzymatic rate acceleration of approximately 5×10^{8} M is calculated (10). Several authors

have estimated the rate accelerations for the conversion of a second order reaction to a first order process (6,12,13). Recent calculations by Page and Jencks (12) indicate that this rate acceleration may be as large as 10^8-10^{10} M corresponding to a reduction in the entropy of activation of 35-45 e.u. Thus, it is possible that catalysis in the aconitate isomerase reaction may result exclusively from the entropic advantage of having a catalytic residue incorporated into the primary sequence of the enzyme. In this paper we consider the relationship between enzyme catalysis which results from such a reduction in the entropy requirements of a reaction and the theory of enhanced transition state binding.

Anchoring of a Catalytic Residue at an Enzyme Active Site. A diagram which incorporates the important role played by active site residues for single substrate enzyme reactions is illustrated in Scheme II-A:

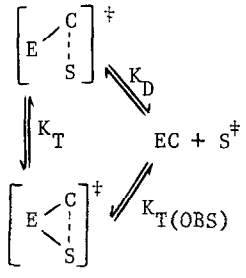


SCHEME II-A

According to Scheme II-A both the non-enzymic and enzymic reactions proceed by identical mechanisms involving the participation of a catalytic residue, C. The role of E-C for the non-enzymatic reaction is solely to provide a catalytic residue, that is the reaction of E-C and S is a second order process. In $[E\!-\!\overset{C}{\underset{S}{\vdots}}]^{\ddagger}$ the C portion of E-C is interacting with S; however, there is no binding between S and the enzyme portion of E-C. In analogy with Scheme I, equation (1) will relate the rate constants k_n and k_e to the constants K_T and K_S. However, K_T does not describe a dissociation of the transition state from the enzyme; rather, K_T is an equilibrium constant describing an isomeri-

zation of $[E\!<\!\overset{C}{\underset{S}{\vdots}}\,]^{\ddagger}$ to $[E\!-\!\overset{C}{\underset{S}{\vdots}}\,]^{\ddagger}$. This isomerization occurs by disrupting the forces of interaction between the substrate and enzyme. Lienhard has constructed a scheme similar to Scheme II-A above to illustrate enzyme reactions which are characterized by covalent intermediates, and by extension acid-base catalysis; he has pointed out that for such schemes the binding of substrate to enzyme is accompanied by entropic losses which do not occur upon transition state binding (3).

A consideration of the constants described in Scheme II-A reveals that while K_S, k_n, and k_e are obtained experimentally, K_T is not. That is, one measures a dissociation constant for the release of a transition state from an enzyme, $K_{T(OBS)}$: such a constant reflects both the forces of interaction between the transition state and the enzyme (K_T) and the dissociation of $[EC\cdots S]^{\ddagger}$ to give $EC + S^{\ddagger}$ (K_D).



SCHEME II-B

As is illustrated in Scheme II-B, $K_{T(OBS)} = K_T \times K_D$; substitution of $K_{T(OBS)}/K_D$ for K_T in equation (1) leads to equation (3):

$$K_D \times \frac{k_n}{k_e} = \frac{K_{T(OBS)}}{K_S} \qquad (3)$$

According to equation (3), the ratio of $K_{T(OBS)}$ to K_S will be equal to the product of K_D and k_n/k_e. Except where $K_D = 1M$, the absolute magnitude of K_D will determine whether k_n/k_e is an underestimate ($K_D < 1M$) or overestimate ($K_D > 1M$) of the weaker dissociation of a transition state relative to substrate.

The magnitude of K_D will depend on the relative entropic and enthalpic

contributions to the free energy change characteristic of this equilibrium constant. On the basis of the calculations of Page and Jencks (12) upper and lower limits can be assigned to K_D and k_n/k_e, respectively: the conversion of $[E\overset{C}{\underset{S}{\diagup\!\!\diagdown}}]^{\ddagger}$ to EC + $S^{\ddagger}$, which involves a 1 → 2 process, can be assigned an upper limit[3] of 10^{8}-10^{10} M; the non-enzymic and enzymic reactions represented in Scheme II-A have been defined so as to differ only in the molecularity of the two reactions so that a lower limit of 10^{-8}-10^{-10} M^{-1} can be assigned to k_n/k_e. It can be seen that in the absence of electronic interactions between EC and $S^{\ddagger}$, the product of K_D and k_n/k_e approximates one and $K_{T(OBS)}$ equals K_S.[4] Clearly, since the non-enzymatic reaction illustrated in Scheme II-A is a catalyzed process, attractive forces between C in EC and $S^{\ddagger}$ will occur: it is these forces which give rise to enhanced transition state binding. Without a detailed understanding of the transition state structure of reactions, however, it is not possible to evaluate quantitatively this enthalpic contribution to K_D. For reactions characterized by transition state structures in which a great deal of atom rearrangement has occurred, attractive forces between $S^{\ddagger}$ and C in EC might considerably reduce the magnitude of K_D. It is important to note that the state EC + $S^{\ddagger}$, Scheme II-B, does not necessarily represent the state E + $S^{\ddagger'}$, Scheme I; that is, $S^{\ddagger}$ represents the transition state structure of the substrate in a catalyzed process. The faster rate of a catalyzed vs. an uncatalyzed non-enzymatic reaction is expected to reflect both a change in the transition state structure of the substrate, and the forces of attraction between $S^{\ddagger}$ and C; these considerations suggest that the rate enhance-

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[3] The dissociation of $[E\overset{C}{\underset{S}{\diagup\!\!\diagdown}}]^{\ddagger}$ to EC + $S^{\ddagger}$ involves the initial formation of $EC^{\ddagger}$ + $S^{\ddagger}$ followed by a relaxation of $EC^{\ddagger}$ to EC. Since $EC^{\dagger}$ is of higher energy than EC the effect of such a relaxation would be to further increase the magnitude of K_D.

[4] It should be noted that an experimental determination of k_n/k_e will reflect entropy losses for the non-enzymatic reaction due to the bringing together of two small molecules, whereas K_D reflects an increase in entropy upon the release of a small molecule, $S^{\ddagger}$, from a larger molecule, EC. As the result of these mass differences the entropic contribution to K_D may exceed that to k_n/k_e, i.e., $K_D \times k_n/k_e > 1$.

ment for the catalyzed process cannot be used to evaluate the enthalpic contribution to K_D.

Catalysis by two or more catalytic residues is a logical extension of the above treatment, with corresponding entropic rate factor increases. A difficulty arises in that the appropriate non-enzymic reaction is seldom observed, and therefore an evaluation of k_n/k_e is not possible.

An extension of Schemes IIA and B can be constructed in which catalysis is the result of both entropic factors and additional enthalpic factors, e.g., the distortion of enzyme bound substrate, the stabilization of enzyme bound transition state, and environmental effects. For such a scheme $K_{T(OBS)}$ is predicted to be smaller than K_S, even when $K_D \times k_n/k_e = 1$. Such an enhanced binding of the transition state is dependent on the extent to which enzyme catalysis results from a decrease in the enthalpy of activation.

CONCLUSION

Previous authors have pointed out that the observed rate enhancements for single substrate enzyme reactions may be largely due to the lower entropic requirements of the enzymic reaction. For single substrate enzyme reactions in which the catalytic role of the enzyme is simply to reduce the entropic requirements of a reaction by juxtaposing a catalytic group and a substrate binding site at an enzyme active site, we conclude that $K_{T(OBS)}/K_S$ does not necessarily equal k_n/k_e. Whereas the ratio K_T/K_S, which equals k_n/k_e, is a measure of the entropic advantage of the enzymatic reaction, the ratio $K_{T(OBS)}/K_S$ is a measure of an electronic interaction between $S^{\ddagger}$ and C in EC which occurs upon the formation $[E\diagup \begin{matrix} C \\ \vdots \\ S \end{matrix}]^{\ddagger}$ from $EC + S^{\ddagger}$. Depending on the magnitude of this interaction, k_n/k_e may be either an underestimate or an overestimate of $K_{T(OBS)}/K_S$. Aside from considerations of the impossibility of constructing perfect transition state analogs, the arguments presented in this paper provide a possible explanation for the number of cases where transition state analog dissociation constants are much larger than the predicted values; it is suggested that these constants may be much closer to the attainable values, i.e.,

these transition state analogs are better analogs than previously believed.

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