Enzymatic Catalysis and Transition-State Theory

Transition-state analogs show that catalysis is due to tighter binding of transition states than of substrates.

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In this article I show how the application of the transition-state theory of reaction rates to enzymatic catalysis leads to the conclusion that the problem of enzymatic catalysis can be perceived as a special case of the general problem of the interaction of ligand molecules with proteins. I describe the factors that may account for the tighter binding of the transition state than of the substrate(s) and so account for catalysis. I define and give examples of a special class of potent enzyme inhibitors, known as transition-state analogs. These analogs have provided evidence in support of the theory and offer a way of elucidating the interactions between the enzyme and the substrate in the transition state of the enzymatic reaction. Moreover, the rational basis upon which these analogs are designed provides a new approach for the preparation of powerful enzyme inhibitors. Most of the ideas that I present are not my own. Pauling briefly and eloquently stated the consequences of the application of transition-state theory to enzymatic catalysis in 1948 (*1*). More recently, Jencks (*2*), Wolfenden (*3, 4*), and Jencks and Page (*5*) have developed the subject in different ways.

Theory for One-Substrate Reactions

The unimolecular conversion of a reactant, S, to a product, P, will occur by the reaction pathway with the lowest energy barrier and requires that the reactant have sufficient energy to overcome this energy barrier (see dashed curve in Fig. 1). The structure of highest energy on this pathway of lowest energy is designated the transition state, $S^{\ddagger}$. According to transition-state theory (*6*), the transition state is in equilibrium with the reactant (Eq. 1):

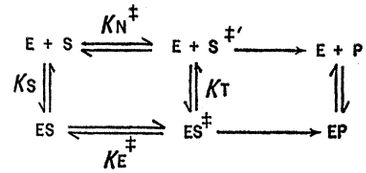
$$K^{\ddagger} = [S^{\ddagger}]/[S] \qquad (1)$$

where $K^{\ddagger}$ is the equilibrium constant for the formation of the transition state. Moreover, the rate of reaction is proportional to the concentration of the transition state; and the proportionality constant is the Boltzmann constant k times the absolute temperature T, divided by Planck's constant h (Eq. 2).

$$d[P]/dt = \frac{kT}{h}[S^{\ddagger}] = \frac{kTK^{\ddagger}}{h}[S] = k_x[S] \qquad (2)$$

Consequently, $K^{\ddagger}$ is equal to the measurable first-order rate constant for the reaction, k_x, times the factor h/kT (Eq. 2). The difference between the free energy of the reactant and the transition state, $\Delta F^{\ddagger}$, is related to the equilibrium constant, $K^{\ddagger}$, by the usual thermodynamic equation, $\Delta F^{\ddagger} = -RT \ln K^{\ddagger}$, and thus is also calculable from k_x.

The application of transition-state theory to a single-substrate enzymatic reaction and to the corresponding nonenzymatic reaction is shown in scheme 1:



In this scheme, K_S is the equilibrium constant for association of the substrate, S, with the enzyme, E; $K_N^{\ddagger}$ and $K_E^{\ddagger}$ are equilibrium constants for the formation of the transition states of the nonenzymatic and enzymatic reactions, $S^{\ddagger\prime}$ and $ES^{\ddagger}$, respectively; K_T is the equilibrium constant for the binding of $S^{\ddagger\prime}$ to E to form $ES^{\ddagger}$. The expressions for these four equilibrium constants show that they are related by Eq. 3.

$$\frac{K_T}{K_S} = \frac{K_E^{\ddagger}}{K_N^{\ddagger}} = \frac{k_E}{k_N} \qquad (3)$$

Moreover, according to the simplest version of transition-state theory, $K_E^{\ddagger}$ is related to k_E, the first-order rate constant for the conversion of ES to EP, by the same factor (h/kT) that relates $K_N^{\ddagger}$ to k_N, the first-order rate constant for the corresponding nonenzymatic reaction. Thus, transition-state theory yields the important conclusion that enzymatic catalysis, expressed by the ratio k_E/k_N, is equivalent to tighter binding of the transition state than the substrate to the enzyme, expressed by the ratio, K_T/K_S (Eq. 3) (*4*). The values of k_E/k_N that have been tabulated suggest that the value for a typical enzymatic reaction will fall in the range of 10^8 to 10^{14} (*4; 7; 8*, part 1, p. 4). Since K_S is usually in the range of 10^3 to $10^5 M^{-1}$, the values expected for K_T are extremely large, of the order of $10^{15} M^{-1}$. These relationships are presented in Fig. 1 in terms of a free energy–reaction pathway profile for a hypothetical single-substrate enzymatic reaction and the corresponding nonenzymatic reaction.

Understanding Catalysis in One-Substrate Reactions

The above development leads to the conclusion that enzymatic catalysis can be understood by describing the factors that determine the relative magnitudes of the binding constants, K_T and K_S. These factors can be considered in terms of five categories: (i) changes in the basic structure of the transition state; (ii) entropy changes; (iii) interactions with the solvent water; (iv) interactions with the enzyme; and (v) conformational changes of the enzyme. I will consider each of these categories in turn.

Transition-state theory gives no information about the extent of similarity in structure between the transition state of the nonenzymatic reaction, $S^{\ddagger\prime}$, and the substrate portion of the transition

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state of the enzymatic reaction ($S^{\ddagger}$ part of $ES^{\ddagger}$). Regardless of the extent of similarity, Eq. 3 is valid. In considering the relationship between the two, it is useful to divide the equilibrium for the association of $S^{\ddagger\prime}$ with the enzyme into two equilibria (*9*). In the first, $S^{\ddagger\prime}$ rearranges to $S^{\ddagger}$, a structure that is identical to the $S^{\ddagger}$ part of $ES^{\ddagger}$; and in the second, $S^{\ddagger}$ binds to the enzyme (Eq. 4).

$$E + S^{\ddagger\prime} \rightleftharpoons E + S^{\ddagger} \rightleftharpoons ES^{\ddagger} \qquad (4)$$

Our present knowledge of the mechanisms of enzymatic reactions and of the corresponding nonenzymatic reactions suggests that in most cases there is a basic similarity in the bond-making and bond-breaking processes that S undergoes, so that $S^{\ddagger\prime}$ and $S^{\ddagger}$, are similar in structure and energy (*8*, part 1; *10*). Consequently, the value of K_T is not largely determined by a structural change in the transition state that alters its intrinsic energy. However, there may be exceptions to this conclusion, in which the mechanism of the enzymatic reaction is fundamentally different from the corresponding nonenzymatic reaction.

These exceptions will generally be ones for which the energy of $S^{\ddagger}$ is greater than that of $S^{\ddagger\prime}$: if the energy of $S^{\ddagger}$ were lower than that of $S^{\ddagger\prime}$, the corresponding nonenzymatic reaction would proceed by this alternative, enzyme-type mechanism. Thus, the effect of a fundamental change in mechanism is to decrease the magnitude of K_T and thus of the catalytic ratio, K_T/K_S, relative to the case in which there is no change in mechanism. This effect may be taken to be the reason why there is usually a basic similarity between the mechanism of an enzymatic reaction and the corresponding nonenzymatic reaction. In the description given below of factors (ii) through (v), I consider the binding of $S^{\ddagger}$ rather than $S^{\ddagger\prime}$, because after separation of the binding of $S^{\ddagger\prime}$ into two equilibria according to Eq. 4, these factors refer to the second equilibrium only.

The category of entropy change refers largely to the loss of entropy of S and $S^{\ddagger}$ upon their binding to the enzyme. Both species lose translational and overall rotational entropy, and this loss makes an unfavorable contribution to binding for both. In addition, the defined geometries of the ES and $ES^{\ddagger}$ complexes require some loss of the entropy of internal rotations. For some reactions it seems likely that the loss of internal rotational entropy upon binding will be less for $S^{\ddagger}$ than for S. The reason is that for some nonenzymatic reactions the internal rotational freedom of the substrate has already been restricted upon going to the transition state of the nonenzymatic reaction and, consequently, $S^{\ddagger}$ has less internal rotational entropy to lose upon binding than has *S*. Where this difference in loss of internal rotational entropy exists, it is a factor that contributes to tighter binding of $S^{\ddagger}$ than of S and so contributes to catalysis. The complete freezing in $S^{\ddagger}$ of a single free internal rotation in S is accompanied by the loss of 7 entropy units, which corresponds to a factor of 34 in the ratio of K_T to K_S (from the relationship $\Delta F_T - \Delta F_S = -RT\ln(K_T/K_S) = -T\Delta\Delta S$, where $\Delta\Delta S$ is + 7 entropy units) (*11*). Additional entropy changes that contribute to the ratio of K_T to K_S may occur as the result of interactions with water (*5*). For example, if more molecules of solvating water are released upon binding of $S^{\ddagger}$ to E than upon binding of S to E, then there is a substantial entropic advantage for the formation of $ES^{\ddagger}$.

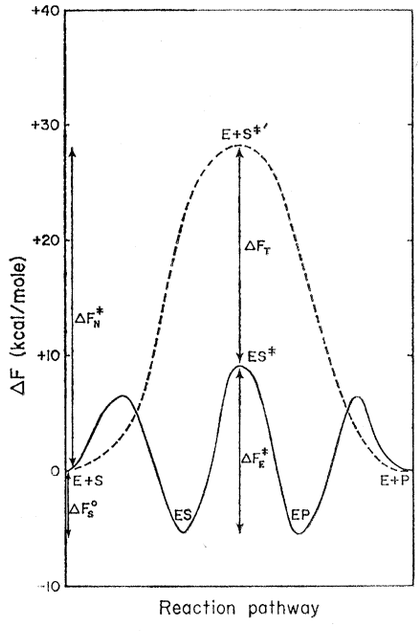


Fig. 1. Free energy-reaction pathway profiles for a hypothetical single-substrate enzymatic reaction and the corresponding nonenzymatic reaction. The free energy changes (ΔF's) refer to the following reactions (see scheme 1): ΔF_S°, formation of ES from E and S; ΔF_T, formation of $ES^{\ddagger}$ from E and $S^{\ddagger\prime}$; $\Delta F_N^{\ddagger}$, formation of $S^{\ddagger}$ from S; $\Delta F_E^{\ddagger}$, formation of $ES^{\ddagger}$ from ES. The values that were used to calculate the free energy changes were: K_S and K_P (the association constant for the product), $10^4 M^{-1}$; k_d (the first-order rate constant for dissociation of ES to E and S and of EP to E and P), 10^4 sec^{-1}; k_E, 10^2 sec^{-1}; k_N, 10^{-8} sec^{-1}; the concentrations of S and of P, 1.0*M*; the equilibrium constant for the formation of P from S, 1.0.

The associations of S and of $S^{\ddagger}$ with the enzyme are accompanied by the disruption of some of the interactions that occur between water molecules and functional groups of S, $S^{\ddagger}$, and the enzyme (Eq. 5). The water that is released interacts with itself:

$$\begin{array}{ccccc} E + S \text{ or } S^{\ddagger} & \rightleftharpoons & ES \text{ or } ES^{\ddagger} & + & H_2O \\ \vdots & & \vdots & & \vdots \\ H_2O \quad H_2O & & & & H_2O \end{array} \qquad (5)$$

The interactions with water are noncovalent ones in which hydrogen bonds, van der Waals forces, and electrostatic interactions are participating (*8*, part 2). Even though all the interactions with water contribute to the absolute magnitude of K_S and K_T, the effect of water interactions on the relative magnitude of K_S and K_T will be determined largely by the relative strengths of interaction of S and $S^{\ddagger}$ with water (Eq. 5). If S interacts with water more strongly than $S^{\ddagger}$, then water interactions favor tighter binding of $S^{\ddagger}$ than S and so contribute to catalysis. If the reverse is true, water interactions are hindering catalysis. The relative strengths of interaction of S and $S^{\ddagger}$ with water will vary with the reaction in question.

The fourth category of factors that account for the ratio K_T/K_S is the relative strengths of the noncovalent interactions between the enzyme and S and $S^{\ddagger}$ in the ES and $ES^{\ddagger}$ complexes, respectively (Eq. 5). These interactions fall into the same classes as those with water: hydrogen bonding, electrostatic interactions, and van der Waals forces. It is important to realize that if the active site of the enzyme possesses an optimal complementarity to the transition-state structure of the reacting functional group of the substrate, it cannot also make optimal interactions with the ground-state structure. Thus, although the interactions between the enzyme and the substrate in the ES complex are, on balance, attractive ones, the interactions between the enzyme and the functional group of the substrate that will undergo reaction are likely to be weak or even repulsive. In fact, there is considerable evidence that a repulsive steric interaction in the ES complex makes a substantial contribu-

tion to catalysis by the enzyme lysozyme (*12, 13*).

Eventually, it may be possible to identify and to estimate the relative strength of the specific interactions of an enzyme with S and S‡ by making use of the structures determined by x-ray crystallography. Structures of complexes between enzymes and stable substrate analogs or slowly reacting substrates are now available (*14*). The structures of ES‡ complexes are being deduced by model building based upon these known structures (*12, 15*). They may also be deduced by determining the crystal structures of the complexes between enzymes and stable analogs of S‡ (*16*).

For some enzymes it is probable that changes in the conformation of the protein accompany the binding of the substrate to the enzyme. Such a change in conformation in itself must be energetically unfavorable (*5*). If this were not so, the free enzyme would exist in the altered conformation that it adopts in the *ES* complex. A scheme of these energetic relationships is shown in Eq. 6,

$$E_1 + S \underset{}{\overset{K_I < 1}{\rightleftharpoons}} E_2 + S \overset{K'_S \gg 1}{\rightleftharpoons} E_2S \quad (6)$$

and

$$K_S = [E_2S]/[E_1][S] = K_IK'_S > 1$$

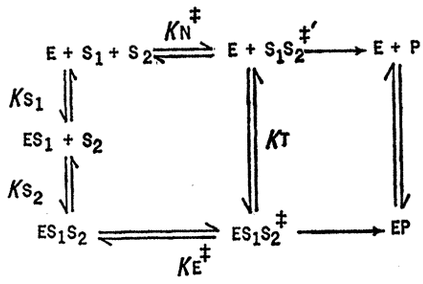
where E_2 is the conformation of the enzyme in the ES complex and K_I is the equilibrium constant for the conformational change from E_1 to E_2. If the enzyme in the ES‡ complex has the E_1 conformation, so that binding of S‡ to the enzyme is accompanied by no conformational change, then effect of the conformational change upon substrate binding is to contribute to the tighter binding of S‡ by the factor $1/K_I$. Alternatively, the enzyme may adopt a third conformation in the ES‡ complex. The conformational change that accompanies the binding of S‡ will also be energetically unfavorable in itself (Eq. 7).

$$E_1 + S^\ddagger \overset{K_I^\ddagger < 1}{\rightleftharpoons} E_3 + S^\ddagger \rightleftharpoons E_3S^\ddagger \quad (7)$$

In this case, the conformational changes will contribute the factor $K_I^\ddagger/K_I$ to the ratio K_T/K_S. Crystallographic analysis of the appropriate complexes appears to be the best way to determine exactly what conformational changes do occur, and in the future such structures may serve as the basis for calculation of K_I and $K_I^\ddagger$ values.

Theory for Two-Substrate Reactions

The application of the transition-state theory to a two-substrate enzymatic reaction that proceeds by way of a ternary complex of the enzyme and both substrates, ES_1S_2, is shown in scheme 2:



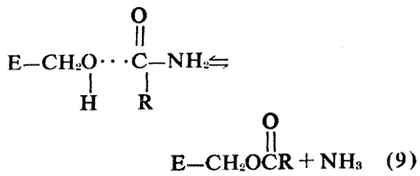
This scheme yields the relationship:

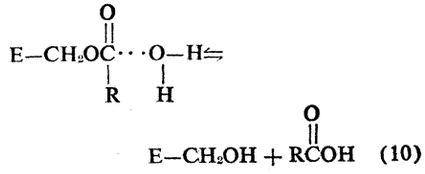
$$\frac{K_T}{K_{S_1}K_{S_2}} = \frac{K_E^\ddagger}{K_N^\ddagger} = \frac{k_E}{k_N} \quad (8)$$

which equates the ratio between the binding constants for the transition state and the substrates to the ratio between the catalytic constant, k_E, and the second-order constant for the nonenzymatic reaction, k_N (*4*). The factors that must be taken into account in order to explain catalysis (k_E/k_N) in terms of the binding ratio $K_T/K_{S_1}K_{S_2}$ are the same ones that have been described above. In the case of two-substrate reactions the contribution of the entropy factor may be much greater. The formation of the species ES_1S_2 is accompanied by the loss of translational and overall rotational entropy of both S_1 and S_2, whereas the formation of $ES_1S_2^\ddagger$ is accompanied by the loss of translational and overall rotational entropy of only one species, $S_1S_2^{\ddagger\prime}$. This difference in the entropy changes has been estimated to be the source of a factor of as large as 10^8M in the value of $K_T/K_{S_1}K_{S_2}$ (*11*).

Covalent Intermediates

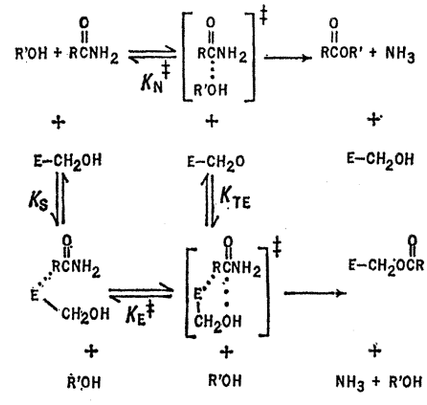
Many enzymatic reactions proceed by way of intermediates in which a covalent bond is formed between the enzyme and the substrate or a portion of the substrate (*17*). The covalent intermediate subsequently reacts to yield product(s) and in so doing regenerates the free enzyme. The enzyme chymotrypsin (E.C. 3.4.4.5), which catalyzes the hydrolysis of acyl compounds, such as amides, is an example of this type of reaction sequence (*18*). In the first step of the reaction the acyl group of the substrate reacts with the hydroxyl oxygen of a specific seryl residue of the enzyme (E–CH_2OH) to yield an acyl-enzyme intermediate (Eq. 9).





In the second step of the reaction, the acyl group is transferred from the seryl oxygen to water (Eq. 10).

The application of transition-state theory to these reactions can best be made by considering the enzyme to be both a catalyst and a substrate. The catalytic effect of the enzyme is then estimated by comparing the enzymatic reaction to the nonenzymatic reaction between the substrate and the amino acid residue that undergoes the covalent reaction. Thus, for the acylation of chymotrypsin scheme 3, in which R'OH is *N*-acetyl-L-serineamide, is applicable:



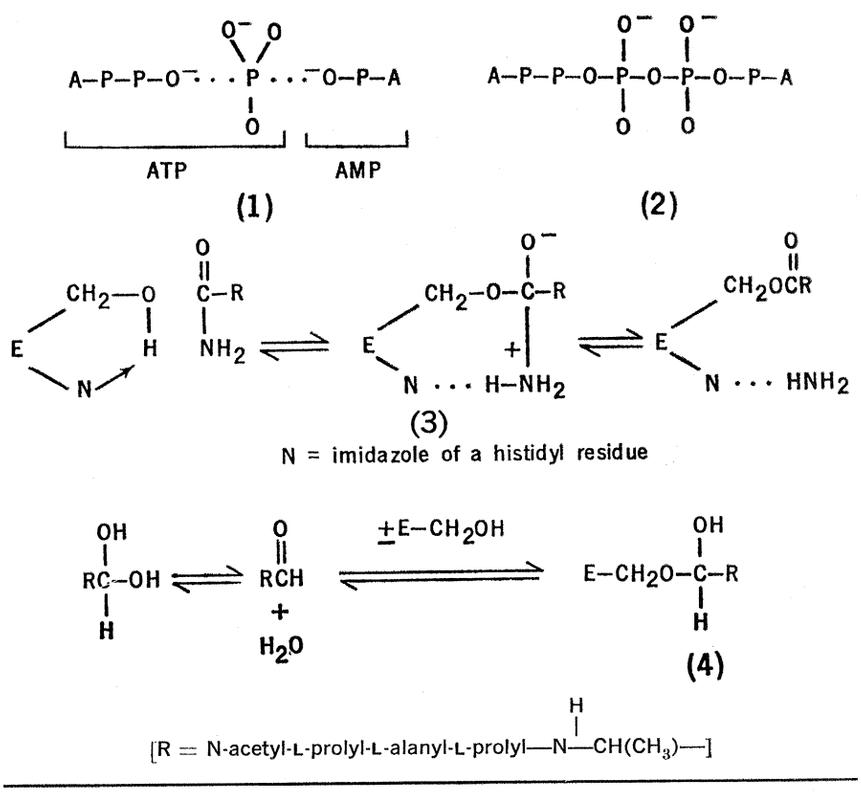
R'OH = N-acetyl-L-serinamide

The equilibrium constant K_{TE} in this scheme refers to a transition-state interchange in which the enzyme replaces *N*-acetylserineamide. The ratio K_{TE}/K_S equals $K_E^\ddagger/K_N^\ddagger$ and k_E/k_N and thus is the measure of catalysis in the acylation step relative to the corresponding nonenzymatic reaction. The catalysis of the deacylation step can be considered in a similar way by a comparison of the rate of the hydrolysis of the acyl enzyme to the rate of hydrolysis of the *O*-acyl derivative of *N*-acetylserineamide.

The advantage of this approach to enzymatic reactions with covalent intermediates is simply that a comparison is made with the corresponding nonenzymatic reaction for each step. The ratio K_{TE}/K_S will be determined by the same factors that have been described above for single-substrate reactions. Note that in the case of the acylation of chymotrypsin, the binding of the substrate to the enzyme is accompanied by the loss of translational and overall rotational entropy of the substrate, whereas the transition-state interchange, on balance, does not fix a species. Thus, in similarity with the two-substrate, ternary-complex enzymatic reaction, the entropy changes greatly favor the transition-state interchange.

This treatment of covalent intermediates leaves out a contribution to the overall catalysis that is made by the very fact that an enzymatic reaction proceeds by way of a covalent intermediate rather than by direct reaction. This factor can be estimated separately by comparison of the rates of appropriate model reactions. Thus, for example, in the case of chymotrypsin one can determine whether the second-order rate constant for the direct reaction of water with a substrate amide is larger or smaller than the second-order rate constants for the reaction of *N*-acetylserineamide with the substrate amide and for the hydrolysis of the *O*-acyl-*N*-acetylserineamide.

It seems reasonable to include within the general classification of reactions with covalent intermediates that large number of enzymatic reactions in which a proton is transferring to the enzyme from the substrate, or vice versa, in the transition state of the enzymatic reaction. Consequently, such acidic or basic catalysis by an enzyme can be considered in a similar way to that described above for the formation and breakdown of the acyl-enzyme intermediate. An alternative description of such catalysis by proton transfer is that there is a stronger hydrogen bond in the $ES^{\ddagger}$ complex than in the ES complex (*4*). However, this description is possibly misleading, since there are reasons to suspect that in the transition state leading to an intermediate with complete proton transfer, the proton is at an energy maximum rather than at the minimum that is expected for a typical strong hydrogen bond (*19*). Also, the consideration of such proton transfers as examples of covalent catalysis emphasizes the fact that there are two reasons for catalysis: one is the substitution of a stronger general acid or general base than water for water and the other is the large entropy advantage arising from the transition state interchange.



Transition-State Analogs

The prediction from transition-state theory that an enzyme binds the transition state of the corresponding nonenzymatic reaction much more tightly than the substrate cannot be tested directly, since the transition state is by definition the species which is most unstable and therefore present in the lowest concentration. However, it has been tested indirectly through the use of transition-state analogs. A transition-state analog for an enzyme is a stable compound that resembles in structure the substrate portion of the transition state of the enzymatic reaction. A substantial number of such compounds have now been investigated (*4, 20*).

Most of the values of the ratios of the binding constant for the analog to that for the substrate(s) fall into the range beween 10^2 and 10^5. The fact that these values are lower than the values of 10^8 to 10^{14}, which are expected for K_T/K_S, $K_T/K_{S_1}K_{S_2}$, and K_{TE}/K_S, is probably largely due to the imperfect nature of the analogs rather than to the incorrectness of the theory. Because a complete compilation of transition-state analogs has recently appeared (*20*), I will include here a description of only a few new examples. I must emphasize that in most of these examples the proposed transition state and proposed similarity of the analog to the transition state are chemically reasonable hypotheses rather than verities established by extensive experimentation.

An example of a two-substrate enzymatic reaction that proceeds by way of a ternary complex is the transfer of the terminal phosphoryl group from adenosine triphosphate (ATP) to adenosine monophosphate (AMP), which is catalyzed by the enzyme adenylate kinase (E.C. 2.7.4.3) (*21*). The transition state for this reaction probably resembles **1**, in which there is a pentavalent phosphorus atom (*22*).

This enzyme is potently inhibited by compound **2**, P^1,P^5-di(adenosine-5′) pentaphosphate (association constant, $4 \times 10^8 M^{-1}$ at 25°C and *p*H 8) (*23*). Even though the pyrophosphate linkage itself is not a good analog of the pentacovalent phosphorus and even though the ratio of the association constant for **2** to the product $K_{S_1}K_{S_2}$ is near unity, this compound might be considered to be a crude transition-state analog by virtue of the fact that both the transition state and compound **2** incorporate in one species two aden-

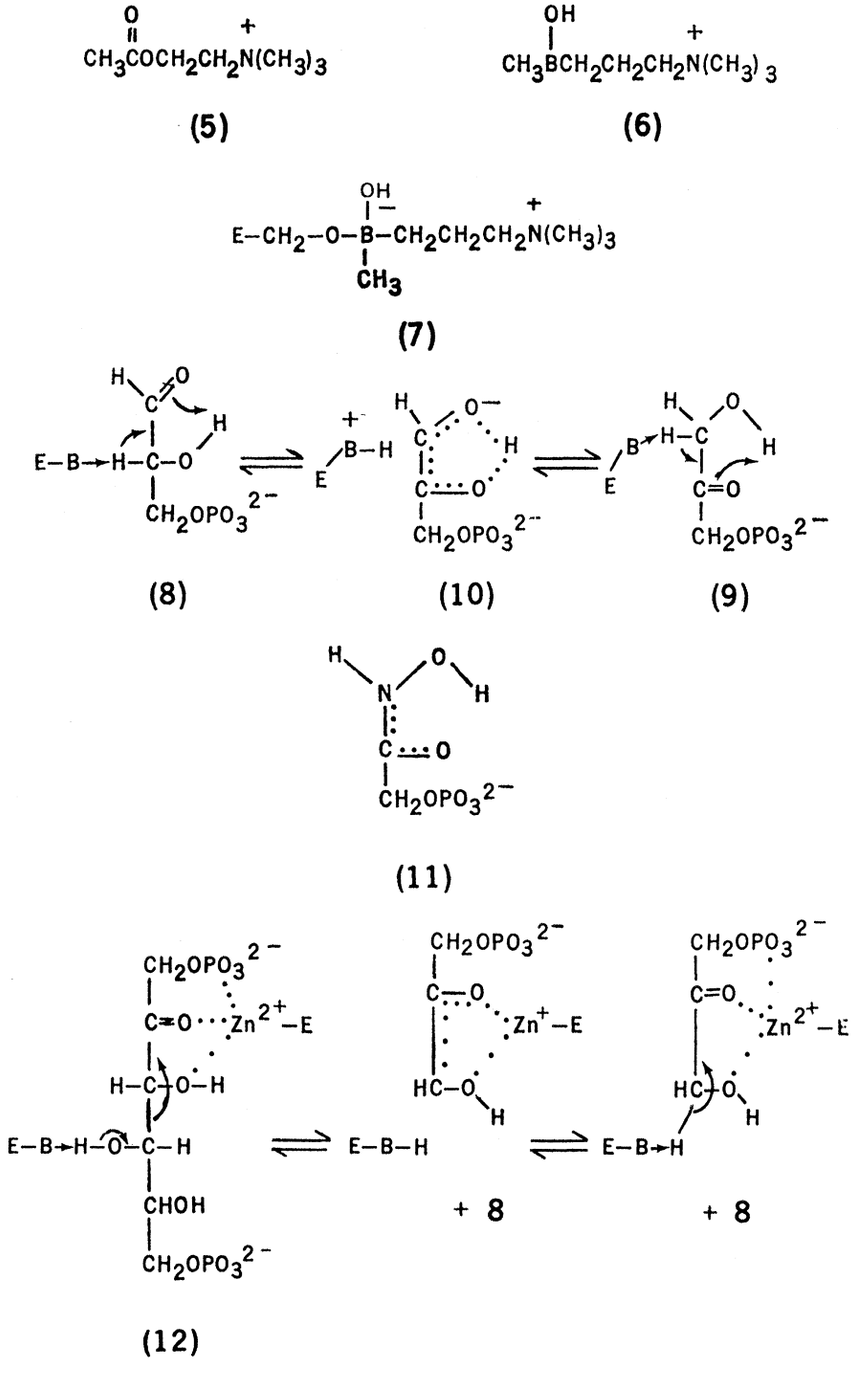
osines that are in an appropriate relationship for tight binding. An alternative, and perhaps better, name for a compound that binds only as tightly as the product of the association constants of the two substrates is "multisubstrate analog" (*4*).

The enzyme elastase (E.C. 3.4.4.7) provides an example of an enzymatic reaction with a covalent intermediate for which a potential transition-state analog has been prepared. Elastase, like chymotrypsin, catalyzes the hydrolysis of esters and amides by way of an acyl-enzyme intermediate with a specific seryl residue (*24*). The transition states for acylation and deacylation of the enzyme probably resemble metastable tetrahedral adducts to the acyl function (*8*, part 3), such as compound **3** shown for the acylation of the enzyme by an amide. It has recently been shown that the aldehyde derived from a specific substrate for elastase is a powerful competitive inhibitor of the enzyme, and this effect has been reasonably explained by assuming that the aldehyde binds to elastase as a hemiacetal with the active site serine, as shown in compound **4** (*25*).

The similarity between **3** and **4** are evident, and the ratio of value of the equilibrium constant for the interchange with the aldehyde hydrate (similar to K_{TE} above) to the value of K_S for the corresponding amide substrate is about $2.0 \times 10^5 M^{-1}$. Several naturally occurring extremely potent inhibitors of the proteolytic enzymes, plasmin, trypsin, and papain, have been shown to be short peptides that terminate in the aldehyde formed from the reduction of arginine (*26*). In addition, Westerik and Wolfenden have found that *N*-acetyl-L-phenylalanylaminoacetaldehyde is a very effective inhibitor of papain (association constant $2 \times 10^7 M^{-1}$ at *p*H 5.5 and 25°C) (*27*). These compounds probably function in the same way as the elastase inhibitor.

Boron acids are another type of potential transition-state analog for acyl-transferring enzymes that function by way of acyl-enzyme intermediates (*9*). The enzyme acetylcholinesterase, which catalyzes the hydrolysis of the ester function of **5**, acetylcholine, is potently inhibited by **6**, a borinic acid analog of acetylcholine (*28*). The association constant for the binding of this compound to the enzyme at *p*H 7.5 and 25°C is approximately 10^4 times larger than the kinetically estimated association constant for acetylcholine. Since oxygen anions are known to form stable tetrahedral adducts with borinic acids, the most probable structure for the complex between the enzyme and **6** is one in which the hydroxyl group of the seryl residue at the active site has added to the boron, as shown in compound **7**. This structure is similar to the tetrahedral-like transition state that is expected for the acetylation of the serine hydroxyl of the enzyme by acetylcholine (*29*).

The isomerization of glyceraldehyde 3-phosphate (see compound **8**) to dihydroxyacetone phosphate (**9**), which is catalyzed by the enzyme troisephosphate isomerase (E.C. 5.3.1.1), provides an example of a proton transfer reaction with which a new type transition-state analog has been studied. The essential mechanism of the enzymatic reaction is proton transfer from carbon-2 of the substrate to a basic group of the enzyme (E–B) to form **10**, a metastable *cis*-enediol intermediate which is then protonated by the enzyme on carbon-1 (*30*). It is not known whether



the enzyme participates in proton transfers to and from the oxygen atoms; I have depicted the proton transfer between the oxygen atoms as a direct one only for the sake of simplicity. The transition states for the conversion of **8** to **10** and of **9** to **10** are structures in which the proton is partially transferred between E–B and a carbon atom of the substrate. Since the enediol is known to be a higher energy species than either of the substrates (*31*), it seems likely that the substrate portion of the transition states will resemble the enediol intermediate more closely than the substrates (*32*). The analog (**11**) that has recently been prepared is the hydroxamate of phosphoglycolic acid (*33*).

Compound **11** is a good analog of **10** by virtue of the fact that the atoms of the hydroxamate function and of the enediol function lie in one plane, which differs from the tetrahedral geometry of the α-hydroxycarbonyl function in the substrates. To the extent that the transition states resemble **10**, **11** is also a transition-state analog. The value of the association constant for the binding of **11** to rabbit muscle triosephosphate isomerase is $3 \times 10^5 M^{-1}$ at 25°C and *p*H 7.0. The values of the association constants for the binding of the substrates to the enzyme are not accurately known, but they are probably close to $10^4 M^{-1}$ for both substrates (*30, 31, 34*). Thus, the analog binds approximately 30 times more tightly.

Another enzymatic reaction that involves the enediol species derived from dihydroxyacetone phosphate as a high energy intermediate is the dealdolization of **12**, fructose 1,6-diphosphate, to dihydroxyacetone phosphate and glyceraldehyde 3-phosphate, which is catalyzed by the so-called class II fructose 1,6-diphosphate aldolases (E.C. 4.1.-2.13). These enzymes contain divalent zinc, which participates in catalysis by acting as an electron sink (*35*).

The hydroxamate of phosphoglycolic acid is an extremely potent inhibitor of the class II aldolase from yeast; its association constant at *p*H 7.5 and 25°C is $2 \times 10^7 M^{-1}$, a value that is about 5000 times larger than the kinetically estimated association constant for **12** and 50,000 times larger than that for **9** (*33*). It is interesting to note that **11** binds less tightly to rabbit muscle fructose 1,6-diphosphate aldolase than does the substrate, compound **12** (*33*). This finding is explained by the fact that rabbit muscle aldolase is one of the metal-free class I aldolases which catalyze the reaction by way of an alternative mechanism in which **10** is not the reactive intermediate (*36*). Since animals have class I aldolases whereas bacteria and fungi have class II aldolases (*36*), **11** at low concentrations is a potential antibacterial and antifungal agent.

Summary

The application of transition-state theory to enzymatic catalysis provides an approach to understanding enzymatic catalysis in terms of the factors that determine the strength of binding of ligands to proteins. The prediction that the transition state should bind to the enzyme much more tightly than the substrate is supported by the experimental results with stable analogs of transition states. Transition-state analogs have great potential for use in understanding enzymatic catalysis and in inhibiting enzymes. Because of their potency and specificity as enzyme inhibitors, some of them may become very useful chemotherapeutic agents.

References and Notes

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