Binding Energy and Enzymatic Catalysis

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For nearly 100 years, the extraordinary catalytic power of enzymes has fascinated biochemists (*1*). In a recent article in *this Journal*, Splittgerber (*2*) analyzed several of the factors by which enzymes are thought to effect catalysis, including proximity and orientation effects, substrate strain, acid–base catalysis, and nucleophilic catalysis. Here we will discuss the fundamental role that the favorable free energy of binding of the rate-determining transition state plays in catalysis and will review the principle that all of the catalytic factors mentioned above, as well as numerous others (*3*), are realized by the use of this binding energy. At the most basic level, enthalpically favorable binding interactions between an enzyme and the transition state of the reaction being catalyzed—regardless of the nature of these interactions—lower the free energy of activation and thus lead to catalysis. Indeed, as Jencks has recently reiterated (*4*), transition state stabilization "is required by the definitions of catalysis and of the transition state."

As early as 1930, Haldane (*5*) recognized the importance of binding energy in catalysis when he suggested that an enzyme acts by distorting the structures of the substrates toward those of the products. Eighteen years later, Pauling, in a seminal statement (*6*), modified Haldane's suggestion and postulated that "enzymes are molecules that are complementary in structure to the [transition states] of the reactions that they catalyze." In 1975, Jencks (*7*), concisely defined binding energy, calling it

> the currency to pay for substrate destabilization through distortion, electrostatic interactions, and desolvation, for bringing about the necessary loss of entropy by freezing the substrates in the proper position for reaction, and for binding to the transition state. *The maximum binding energy is then not realized directly in the binding of the substrate, but is more completely realized in the transition state* (italics added).

Jencks continued, stating that the importance of binding energy in enzymatic catalysis

> immediately provides a qualitative rationale for the large size of enzymes, coenzymes, and some substrates. Energy from the specific binding interactions between an enzyme and a substrate or coenzyme is required in order to bring about the (highly improbable) positioning of reacting groups in the optimum manner [to react] and such binding requires . . . a large interaction area.

Similarly, Warshel (*8*) has suggested that enzymes are large so that sufficient folding energy is available to hold active-site residues in a relatively high-energy conformation. These active-site residues can then act to stabilize the transition state maximally. However, before fully assessing the amount of binding energy required to achieve the enormous catalytic accelerations observed for many enzymes, we must first discuss the magnitude of these rate accelerations.

Catalytic Enhancements

Since many enzymes catalyze reactions that do not proceed at a measurable rate in the absence of a catalyst, the quantification of catalytic rate enhancements is often im-

possible. Furthermore, even for those reactions that do go at a measurable rate, the chemical participation of active-site amino acids usually makes the choice of appropriate reaction conditions for the uncatalyzed process unclear. Competing side reactions can also complicate the kinetic analysis. Nevertheless, a few rate constants for uncatalyzed reactions have been measured and compared with the analogous enzymic rate constants (*9*). (In addition, see Schowen (*10*) for an incisive comment on the comparison of enzymic and nonenzymic rate constants.) For example, the k_{uncat} for the deamination of adenosine has been calculated to be 1.8×10^{-10} s^{-1} at 310 K and pH 7, while k_{cat} for the adenosine deaminase catalyzed reaction is 3.75×10^2 s^{-1}. The ratio of k_{cat} to k_{uncat} is thus 2×10^{12}, requiring that the rate-determining transition state be stabilized upon binding to the enzyme by at least 17.5 kcal/mol at 310 K—17.5 kcal/mol is the minimum value as the enzymic and nonenzymic reactions may proceed by different mechanisms. As the first-order rate constant k_{cat} is a measure of the free energy difference between the enzyme–substrate *complex* and the enzyme-bound transition state, this value of 17.5 kcal/mol does not include the energy required simply to hold the substrate in the active site, and thus the total binding energy of adenosine deaminase for the transition state is even greater. Wolfenden's group has calculated it to be at least 22.8 kcal/mol (*9*), which corresponds to an association constant of 1.25×10^{16} M^{-1} at 310 K. Of course, this number cannot be directly measured, but we may ask whether there exists independent evidence for association constants of such enormous magnitude.

Unfortunately, the calculation of an enzyme–transition state association constant in terms of the specific thermodynamic forces important in ligand binding—electrostatic and van der Waals attractions, hydrogen bonding, and hydrophobic interactions (*11, 12*)—is problematic. Large free energy changes in solvent water often obscure those due to the binding of the ligand itself. In addition, the dielectric constant of a protein is anisotropic, thus complicating calculations of electrostatic interactions between proteins and ligands (*13*). (Fersht and co-workers (*14*), however, have made progress in determining experimentally the net free energy change for the formation of a hydrogen bond between a ligand and protein, and Kati and Wolfenden (*15*) have recently reported additional data.) Finally, even if such analyses were straightforward, uncertainties about the precise structures of transition states would render the results suspect. Given these difficulties, we may ask how tightly can a protein bind a ligand in a system for which maximal binding of the ground state is presumed to be advantageous. For example, antigens and antibodies combine with association constants of up to 10^{11} M^{-1} (*16*), but this value is far lower than that discussed above. It is not believed, however, that antibodies have evolved to bind specific antigens as tightly as possible. In contrast, the egg white protein avidin apparently has evolved specifically to bind the cofactor biotin (*17*). Here, the association constant is 10^{15} M^{-1}, slightly lower

than that required for transition state binding in the adenosine deaminase catalyzed reaction. (Recently, the structure of biotin bound to streptavidin, which is homologous to avidin, was determined, and the molecular basis for this extraordinarily tight binding was analyzed (18).) Obviously, the binding energy of a protein for a given molecule will depend on the size and structure of the molecule, but interestingly, the binding energy observed for the association of biotin with avidin approaches that required for the largest of enzymatic accelerations.

Energetics of Catalysis

The catalysis that results from the use of binding energy can be divided into two parts, as is shown in Figure 1. The first part is that gained from the equal stabilization of all enzyme-bound species, including the rate-determining transition state. Albery and Knowles (19) have termed this use of binding energy "uniform binding". The second part, which Albery and Knowles have termed "catalysis of an elementary step", is that gained from the stabilization of the rate-determining enzyme-bound transition state relative to all other bound species. (See below for a discussion of the original Albery–Knowles analysis.) The profiles in Figure 1 illustrate the reaction of a substrate with a nonenzymic chemical catalyst that cannot bind to the substrate, as compared with the interaction of a substrate with an enzymic catalyst that is able to utilize binding energy. Profile 1 illustrates the reaction of the substrate with the nonenzymic catalyst. (The local free energy minimum shown in profile 1 for the nonenzymic catalyst and substrate entering the same solvent cage is chosen arbitrarily to simplify the discussion below; Jencks (20) has discussed reactions for which evidence for the formation of such "preassociative complexes" exists.) Profile 2 illustrates the reaction of a substrate with an enzyme that effects only uniform binding, and profile 3 illustrates the reaction with an enzyme that also selectively stabilizes the rate-determining transition state. We will now discuss evidence for both types of transition state stabilization in turn.

Uniform Binding

Uniform binding was first analyzed theoretically by Westheimer (21), who in 1962 proposed that enzymes can act as "entropy traps". Westheimer stated that "an enzyme catalyzes a reaction in part by overcoming the unfavorable entropy of activation usually inherent in a chemical reaction." That is, upon substrate binding, a reaction of high kinetic order (especially when considering the chemical participation of enzyme active-site acids and bases) is converted

into a first-order process. Westheimer continued, "In order to constrain a molecule onto an enzyme surface, some strong forces must be involved; van der Waals and electrostatic attractions must produce free-energy changes to compensate for the translational entropy lost in forming a complex." Catalysis results because binding energy is utilized to overcome the unfavorable entropic requirements of bringing the catalyst and reactants together in the proper orientation to react. Thus, as is indicated in profile 2, the enzyme–substrate complex $A \cdot E$ and the transition state are stabilized with respect to their counterparts in profile 1.

Experimental evidence that overcoming the entropy of activation results in catalysis comes primarily from the comparison of intermolecular reactions with their intramolecular counterparts. As Kirby (22) stated in 1980:

> [Intramolecular reactions] are generally faster than the corresponding intermolecular processes, and are frequently so much faster that it is possible to observe those types of reaction involved in enzyme catalysis... Bimolecular reactions in water ... are frequently too slow to detect even under vigorous conditions. But when the catalytic and substrate groups are brought together in the same molecule such otherwise unreactive compounds may [react] under quite mild conditions.

Although the meaningful comparison of a first order rate constant (in units of s^{-1}) with its second order counterpart (in units of $M^{-1} s^{-1}$) is impossible, the "effective molarity", which is defined as the rate constant for the unimolecular reaction divided by the rate constant for the bimolecular reaction (and thus has units of M), is a useful parameter. Effective molarities may be interpreted as "the concentration of the catalytic group required to make the intermolecular reaction go at the observed rate of the intramolecular process" (22). Effective molarities of 10^5 M are not atypical as in, for example, the unimolecular carboxylate ion catalyzed hydrolysis of a succinate ester as compared with the bimolecular acetate ion catalyzed counterpart (Fig. 2). Thus, an (unattainable!) concentration of acetate ion of 10^5 M would be required to yield the hydrolysis products from the bimolecular reaction at a rate equal to that for the unimolecular reaction; alternatively, at 1 M standard state the unimolecular reaction would proceed 10^5 times faster than the bimolecular reaction, at 1 mM standard state 10^8 times faster, etc. The unimolecular reaction is, of course, analogous to the enzyme-catalyzed process and proceeds faster than the bimolecular reaction because unfavorable entropic constraints have been removed by the covalent linking of the two reactants. Amazingly, effective molarities as high as 10^{16} M have been measured, but their precise interpretation can
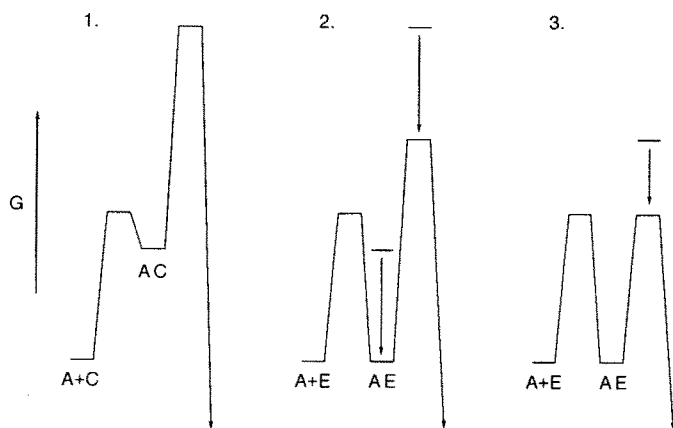

Figure 1. 1. Free energy profile for the reaction of a substrate A with nonenzymic catalyst C. 2. Free energy profile for the reaction of a substrate A with an enzyme E that effects uniform binding. 3. Free energy profile for the reaction of a substrate A with an enzyme E that also effects the selective stabilization of the transition state.
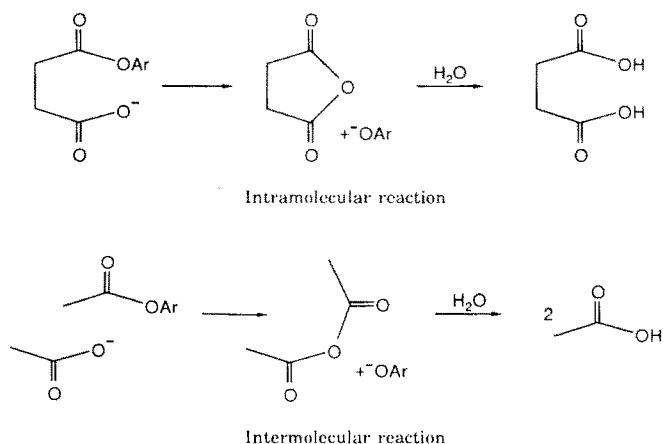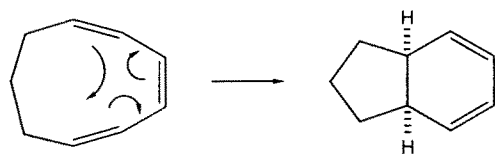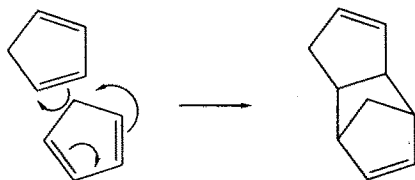

Figure 2. Intramolecular verses intermolecular catalysis of ester hydrolysis.

Intramolecular reaction

Intermolecular reaction

Figure 3. Intramolecular and intermolecular reactions for the Page–Jencks analysis.

Figure 4. Transition state analogues and substrates for proline racemase (I–III) and triosephosphate isomerase (IV–VI).

be complicated by mechanistic differences between the unimolecular and bimolecular reactions, and especially by the presence of ground-state strain in the unimolecular systems (22). Recently, Menger and Ladika (23) have presented a spectacular example of an intramolecular amide hydrolysis reaction with an effective molarity of at least 10^{14} M.

In 1971 Page and Jencks (24) attempted to quantify theoretically the rate increase expected due to the freezing out of entropy upon substrate binding, stating, "we would like to know the nature and magnitude of the maximum increase in rate that may be brought about by bringing together two properly oriented reactants in the active site of an enzyme without invoking strain or desolvation." They compared the entropic requirements for the Diels–Alder dimerization of cyclopentadiene with those for a unimolecular counterpart, the disrotatory ring closure of 1,3,5-cyclononatriene (Fig. 3). The formation of the transition state for a bimolecular reaction requires the net loss of three translational and three rotational degrees of freedom with the concomitant gain of six internal motion degrees of freedom. A typical value for the three translational modes in the gas phase for a standard state of 1 M is 30 eu (which corresponds to 9 kcal/mol at 298 K), for the three rotational modes 25 eu (7.5 kcal/mol), and for the six internal modes 12 eu (3.5 kcal/mol). For a bimolecular reaction, a total of 43 eu are therefore lost upon forming the "unimolecular" transition state. On transferring the reaction from the gas phase into a nonpolar solvent, this value is reduced by approximately 5 eu to 38 eu, which corresponds to a factor of 10^8 M. (The experimentally measured difference in the entropies of activation for the dimerization of cyclopentadiene and the intramolecular ring-closure reaction of 1,3,5-cyclononatriene corresponds to a factor of 10^6 M at 298 K.) These calculations suggest that the mere binding of two substrates in an enzyme site (in the proper orientation to react) may yield an acceleration of up to 10^8 M. Comparison of a unimolecular reaction with its termolecular counterpart yields the huge advantage of 10^{16} M².

Soon after the Page–Jencks analysis of cyclopentadiene dimerization, Dafforn and Koshland (25) analyzed the entropic factors involved in the dimerization of two bromine atoms to form molecular bromine and concluded that an advantage of only 10^2 M is obtained for a unimolecular reaction. As these authors pointed out, the geometric constraints in the transition state for bromine recombination are far less than those for cyclopentadiene dimerization (as for example, bromine atoms have no rotational entropy that
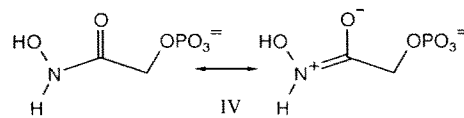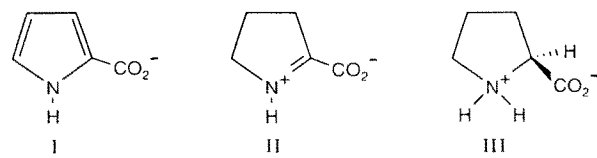
need be lost upon formation of the transition state), and this difference may well be the cause of the discrepancy. It is important to note, however, that the actual unimolecular advantage is of little importance in determining catalytic efficiency. If the entropy frozen out upon substrate binding is small (and, presumably therefore, the entropy lost upon formation of the transition state in the uncatalyzed reaction is also small), little acceleration will be gained from uniform binding. Only a small amount of the available enzymic binding energy will need to be expended, however, and the rest may be utilized to stabilize the transition state selectively. If, on the other hand, the entropy frozen out upon substrate binding is large (and thus a considerable expenditure of binding energy is required), a large catalytic acceleration will be gained. (In 1973 Larsen (26) calculated the enthalpic and entropic contributions for cyclopentadiene dimerization in aqueous solution and concluded that enthalpy, and not entropy, may play the decisive role in the rate enhancements calculated by Page and Jencks. Nevertheless, the intramolecular reaction is still greatly favored.)

The Selective Stabilization of the Transition State

The second basic manifestation of binding energy is in the selective stabilization of the bound transition state relative to all other bound species, as is illustrated in profile 3 of Figure 1. Evidence for this selectivity has come from a number of sources (27), two of which we will discuss here. The first is that transition state analogues—stable molecules that resemble the transition state of a reaction—often bind to enzymes more tightly than do the actual substrates (28, 29). For example, the affinity of proline racemase for both pyrrole-2-carboxylic acid (I, Fig. 4) and Δ-1-pyrolline-2-carboxylic acid (II) is 160 times greater than is the enzyme's affinity for either of the substrates, L-proline (III) or D-proline (30, 31). In the transition state for the enzyme-catalyzed racemization reaction, the α-carbon atom of proline and the substituents surrounding it are thought to become coplanar, and I and II each mimic this geometry. Similarly, the transition state analogue 2-phosphoglycolohydroxamate (IV) binds to triosephosphate isomerase approximately 250 times more tightly than does the substrate dihydroxyacetone 3-phosphate (V) (32). Because of the delocalized structure of the amide bond (Fig. 4), III is thought to mimic the transition state leading from dihydroxyacetone 3-phosphate to the enediol phosphate intermediate (VI). To date, nearly 100 examples of transition state analogues have been reported, and they are among the best inhibitors of many enzymes

(33); indeed, the design of these analogues has become increasingly important in the pharmaceutical industry (34–36).

Given the huge rate accelerations effected by enzymes, one might expect transition state analogues to bind with greater affinities than are actually observed. For example, proline racemase (37) and triosephosphate isomerase (19, 38) are far more efficient catalysts than is suggested by the 160- and 250-fold differences in binding discussed above. Of course, transition state analogues are only analogues, and the true transition states presumably bind more tightly. Furthermore, as Schray and Klinman have discussed (39), if, for a given enzyme, uniform binding contributes greatly to catalysis, then large association constants for transition state analogues and that enzyme will not be observed. If, at the extreme, catalysis is due exclusively to uniform binding, then the association constants for the substrate and for the transition state will be equal. (See below for a discussion of the relative contributions of uniform binding and of selective stabilization of the transition state in the triosephosphate isomerase catalyzed reaction.)

To address the question of whether a transition state analogue is in fact being recognized by an enzyme as such, one may, as was first suggested by Thompson (40) and by Westerik and Wolfenden (41), study a series of related inhibitors. An excellent example of this approach is that of Bartlett and Marlowe (42), who synthesized six inhibitors of the protease thermolysin. The inhibitors each contain a negatively charged, tetrahedral phosphonamidate functionality that mimics the presumed transition state in a peptide bond hydrolysis reaction, but each has a different C-terminal residue (Fig. 5). (Note that these phosphonamidate inhibitors are formally analogous to the high-energy, tetrahedral *intermediates* involved in peptide bond hydrolysis; by the Hammond postulate (43), however, the structure of the highest energy transition state will closely resemble that of these intermediates.) Bartlett and Marlowe observed a linear correlation between the measured dissociation constants (K_i) of these phosphonamidates and the second-order rate constants (k_{cat}/K_m) of the corresponding peptide substrates (Fig. 5); they found no correlation, however, between the dissociation constants of the inhibitors and the Michaelis constants (K_m) of the substrates. Since the rate constant k_{cat}/K_m is a measure of an enzyme's affinity for the transition state, these data suggest that the tetrahedral phosphonamidate inhibitors are indeed recognized by thermolysin as transition state analogues, and not merely as substrate analogues.

Crystallographic data of a transition state analogue bound to an enzyme can provide further evidence that the transition state is being mimicked. For example, recent data obtained by Agard and co-workers (44) demonstrated that the structure of α-lytic protease is little changed upon binding of a boronic acid transition state analogue. The authors concluded that "α-lytic protease in its native structure is largely complementary either to the high-energy tetrahedral intermediate that is formed during the substrate hydrolysis or to the nearly tetrahedral transition state for the reaction." (It is important to note, however, that the existence of "slow-binding inhibitors" suggests that many enzymes are not simply ridgid frameworks that are complementary to their transition states (45).)

Work on mutant enzymes that have been created by the recently developed site-directed mutagenesis techniques provides the second, and better, area of evidence for selective transition state stabilization. For example, Fersht and co-workers (46) constructed several tyrosyl-tRNA synthetase mutants in which histidine₄₅ and threonine₄₀ were changed to glycine and alanine, respectively. Kinetic data for these mutants are shown in the table. For each of the mutants, the binding constants for the two substrates are altered by at most a factor of three, while the rate constant k_3

for interconversion of substrates and products is reduced by a factor of up to 3.2×10^5. These results indicate that histidine₄₅ and threonine₄₀ strongly interact with and stabilize the transition state and that they interact only marginally with the bound substrates, tyrosine and ATP. The authors concluded, "it is possible that the γ-phosphate of substrate ATP does not bind between histidine₄₅ and threonine₄₀ but just remains solvated by water. During the reaction, [the γ-phosphate group] swings into its binding site and releases the solvated water."

Knowles, Gilbert, and co-workers (47), again using site-directed mutagenesis, altered the enzyme triosephosphate isomerase by changing glutamate₁₆₅, the active-site base that participates in catalysis (Fig. 6), to aspartate. Kinetic analysis of the mutant enzyme showed that while the binding constant for the substrate dihydroxyacetone 3-phosphate is altered only slightly, the rate of reaction of this substrate decreases by approximately 1,000-fold. As with the amino acids histidine₄₅ and threonine₄₀ in tyrosyl-tRNA synthetase, glutamate₁₆₅ interacts almost exclusively with the transition state—in this case to accept the abstracted substrate proton.

Catalytic Antibodies

In late 1986 two groups, one led by Schultz (48) and the other by Tramontano and Lerner (49), provided an elegant demonstration of the central role of binding energy in catalysis when they reported that antibodies that bind transition state analogues can have catalytic activity. Their results came almost 40 years after Pauling (50) first noted that:

... an enzyme has a structure closely similar to that found for antibodies, but with one important difference, namely, that the surface configuration of the enzyme is not so closely [complemen-
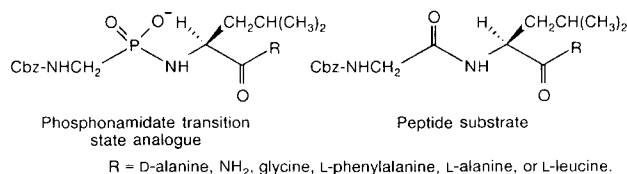

Phosphonamidate transition state analogue

Peptide substrate

R = D-alanine, NH₂, glycine, L-phenylalanine, L-alanine, or L-leucine.

Figure 5. Inhibitors and substrates used in the Bartlett and Marlowe analysis.

Tyrosyl-tRNA Synthetase Mutants

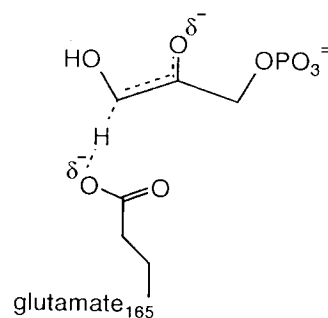| enzyme	k_3 (s^{-1})	K_s (tyrosine) (mM)	K_s (ATP) (mM)
wildtype	38	12	4.7
histidine₄₅ → glycine	0.16	10	1.2
threonine₄₀ → alanine	0.0055	8.0	3.8
histidine₄₅ → glycine and threonine₄₀ → alanine	0.00012	4.5	1.1

glutamate₁₆₅

Figure 6. Transition state for the triosephosphate isomerase catalyzed isomerization of dihydroxyacetone 3-phosphate.

tary] to its specific substrate as is that of an antibody to its homologous antigen, but is instead complementary to an unstable molecule with only transient existence—namely, the "activated complex" for the reaction that is catalyzed by enzyme,

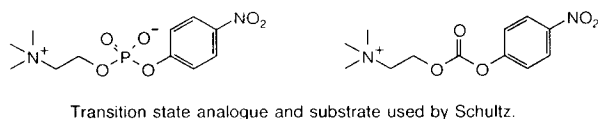and almost 20 years after Jencks stated (51):

> If complementarity between the active site and transition state contributes significantly to enzymatic catalysis, it should be possible to synthesize an enzyme by constructing such an active site. One way to do this is to prepare an antibody to a haptenic group which resembles the transition state of a given reaction. The combining sites of such antibodies should be complementary to the transition state and should cause an acceleration by forcing bound substrate to resemble the transition state.

Both groups studied antibodies that bind phosphate esters. Phosphate esters mimic the presumed transition state in the base-catalyzed hydrolysis of carboxylate esters (cf. the thermolysin transition state analogues discussed above). The Schultz group reported that the pre-existing mouse monoclonal antibody MOPC167, which binds nitrophenyl phosphorylcholine, catalyzes the hydrolysis of the analogous carbonate, and the Tramontano and Lerner group demonstrated that a monoclonal antibody elicited against a synthetic phosphonate ester catalyzes the hydrolysis of the analogous carboxylate ester (Fig. 7). Both groups measured rate accelerations of approximately 1,000-fold and noted that their antibodies have many of the same characteristics as enzymes: each shows substrate specificity, exhibits saturation kinetics, and is subject to competitive inhibition. Although the detailed chemical mechanisms of these catalytic antibodies have yet to be determined, it is reasonable to suppose that the planar, sp^2-hybridized ester or carbonate is strained toward a tetrahedral geometry upon binding, thus facilitating the attack of hydroxide ion. More recently, a number of additional reactions have been catalyzed by antibodies elicited against transition state analogues and large rate accelerations have been reported (52).
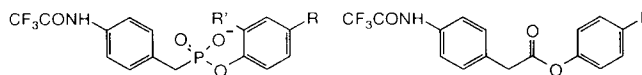
The Evolution of Enzyme Efficiency

In 1976 Albery and Knowles (19) attempted to quantify the effect of binding energy on catalysis by defining an efficiency function, which has a value of 1.0 for a perfect catalyst and becomes smaller as catalytic efficiency decreases. These authors then analyzed the enzyme triosephosphate isomerase. The free energy profiles for the interconversion of dihydroxyacetone 3-phosphate (DHAP) and D-glyceraldehyde 3-phosphate (GAP) as catalyzed by chicken muscle triosephosphate isomerase and by acetate ion (which contains the same functionality as the active-site glutamate$_{165}$ of triosephosphate isomerase) are shown in Figure 8.[1] (The interconversion catalyzed by both triosephosphate isomerase and by acetate ion apparently proceeds via the enediol phosphate intermediate shown in the energy profiles.)

The value of the efficiency function for triosephosphate isomerase is 0.6 and that for acetate ion is 2.5×10^{-11}. Albery and Knowles asked, "How has the enzyme achieved this remarkable improvement?" and postulated that triosephosphate isomerase evolved by the blending of three processes, each of which involves the use of binding energy. In the first and least difficult to achieve and for which Albery and Knowles originally proposed the term "uniform binding",

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[1] In 1984 Richard (38) demonstrated that during the nonenzymic isomerization of DHAP, the enediol phosphate intermediate decomposes to form inorganic phosphate and methylglyoxal, in addition to reacting to form GAP. The free energy profile for the acetate ion catalyzed reaction utilized in the Albery and Knowles analysis is therefore incorrect. Nevertheless, the correction for this decomposition of the enediol phosphate is relatively small (no greater than a factor of 10 in rate), and for this discussion the original calculations will be used.

Transition state analogue and substrate used by Schultz.

Transition state analogue and substrate used by Tramontano and Lerner.

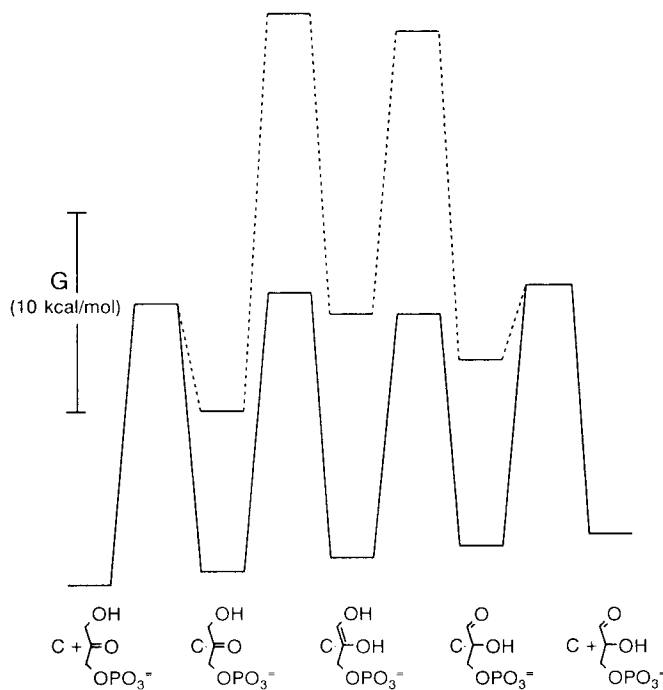Figure 7. Analogues and substrates used in the study of catalytic antibodies.

Figure 8. Free energy profiles for the interconversion of DHAP and GAP as catalyzed by triosephosphate isomerase (solid line) and acetate ion (dahsed line). "C" is the catalyst.

the enzyme binds the ground states and transition states of the acetate ion catalyzed pathway equally well. When uniform binding is optimized, the value of the efficiency function rises to 3×10^{-6}. In the second process, which they termed "differential binding", the enzyme discriminates between bound intermediates so as to equalize the rate of turnover of each intermediate (53). (Differential binding, which has only a small effect on the catalytic efficiency of triosephosphate isomerase, has no direct analogy to either type of transition state stabilization discussed above.) After differential binding is optimized and uniform binding is readjusted, the value of the efficiency function increases to 1.5×10^{-4}. In the third and most difficult process, which Albery and Knowles originally termed "catalysis of an elementary step", and for which we have been using the term "selective stabilization of the transition state", the free energy of the kinetically significant transition states are lowered with respect to the kinetically significant inetermediates. Ultimately, after uniform and differential binding are further readjusted, the free energy of all the enzyme-bound transition states becomes lower than that of the higher diffusive transition state, and the free energy of all the enzyme-bound ground states becomes higher than that of the more

stable unbound species, which in this case is DHAP. These two conditions are met for the interconversion of DHAP and GAP as catalyzed by triosephosphate isomerase (see Fig. 8), and the value of the efficiency function for present day triosephosphate isomerase is 0.6. Although a value of 1.0 is deemed "perfect", little advantage is gained by further catalyzing the chemical steps once a diffusive barrier has become rate limiting. By the use of binding energy then, the efficiency of the triosephosphate isomerase catalyzed reaction has been increased by over 10^{10}-fold relative to the acetate ion catalyzed reaction. Interestingly, uniform binding and catalysis of an elementary step each contribute approximately one-half the overall increase in catalytic efficiency.

To achieve its enormous rate enhancement, triosephosphate isomerase apparently also makes use of electrophilic catalysis not available in the acetate ion catalyzed reaction (54). Lysine$_{13}$ and/or histidine$_{95}$, each of which is located in the active site, appear to participate in catalysis either by polarizing or protonating the substrate carbonyl group and thereby acidifying the proton that is to be abstracted by glutamate$_{165}$. However, this electrophilic catalysis is again a manifestation of binding energy (just as is the participation of glutamate$_{165}$). In the transition state for enolization, negative charge develops on the carbonyl oxygen, and lysine$_{13}$ and histidine$_{95}$ can act to stabilize this charge selectively (Fig. 9) relative to the less charged ground state. (When histidine$_{95}$ of triosephosphate isomerase is changed to glutamine (55), not only does the catalytic activity drop by approximately 400-fold, but, remarkably, the mechanism of catalysis is also changed.)

Finally, one may further pursue the Albery and Knowles analysis to ask whether enzymes have actually evolved via the step-by-step process outlined above. For example, Fierke and Benkovic (56) have demonstrated that when the conserved residue threonine$_{113}$ of dihydrofolate reductase is changed to valine, complicated changes in the binding of both ground states and transition states are observed (unlike the tyrosyl-tRNA synthetase (46) and triosephosphate isomerase (47) mutants discussed above). They state:

[Our mutant enzyme, shows] no clean separation of uniform, differential, or transition-state binding processes. It appears likely that strictly conserved active-site residues can be expected to manifest multiple effects on key ground and transition states, so that in an evolutionary sense all the energetic aspects of turnover may be sampled simultaneously.

Clearly then, some amino acid residues play more than one role in catalysis, and thus, in an evolutionary sense, the optimal time for their incorporation into an enzyme is unclear.

Although in the extensive literature on enzymatic catalysis, many concepts such as approximation, orientation,
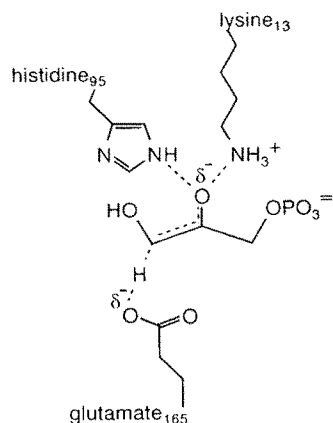
strain, stereopopulation control, ground state destabilization, and desolvation have been invoked to account for huge accelerations (3), all of these catalytic strategies, as well as many others, are merely manifestations of binding energy. Distinguishing among the possible causes for acceleration (for example, approximation versus orientation or ground state destabilization verses transition state stabilization) often becomes a matter of semantics. The binding energy of an enzyme for a transition state is the force that drives enzymatic catalysis, regardless of the actual manifestation of that force.[2]

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Figure 9. Transition state stabilization effected by lysine$_{13}$ and histidine$_{95}$ of triosephosphate isomerase.

[2] In addition to this discussion, the reader is referred to an article by Kraut (57) for a summary of the theoretical basis for binding energy and catalysis and for a discussion of some additional experimental findings.

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