The Depth of Chemical Time and the Power of Enzymes as Catalysts

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ABSTRACT

The fastest known reactions include reactions catalyzed by enzymes, but the rate enhancements that enzymes produce had not been fully appreciated until recently. In the absence of enzymes, these same reactions are among the slowest that have ever been measured, some with half-times approaching the age of the Earth. This difference provides a measure of the proficiencies of enzymes as catalysts and their relative susceptibilities to inhibition by transition-state analogue inhibitors. Thermodynamic comparisons between spontaneous and enzyme-catalyzed reactions, coupled with structural information, suggest that in addition to electrostatic and H-bonding interactions, the liberation of water molecules from an enzyme's active site into bulk solvent sometimes plays a prominent role in determining the relative binding affinities of the altered substrate in the ground state and transition state. These comparisons also indicate a high level of synergism in the action of binding determinants of both the substrate and the enzyme, that are not directly involved in the chemical transformation of the substrate but contribute to the rate of its transformation at an enzyme's active site.

Enzymes allow organisms to channel the flow of matter to their own advantage, allowing some reactions to proceed rapidly compared with other reactions that offer no selective advantage. After a substrate is bound at an enzyme's active site, its half-life is usually a small fraction of 1 s. Rapid turnover is necessary if any enzyme is to produce a significant rate of reaction at the limited concentration ($<10^{-5}$ M) at which enzymes are present within the cell. Many enzymes are known to have evolved to work nearly as efficiently[1] as is physically possible, with second-order rate constants that approach their rates of encounter ($\sim 10^9$ s^{-1} M^{-1}) with the substrate in solution (Figure 1).[2]

How rapidly would biological reactions occur if an enzyme were not present? Until recently, some reactions were known to require several years,[3] and everyday experience suggests that some reactions are slower still. The survival of paper documents and ancient ships for long periods under water implies that the glycosidic bonds of cellulose, for example, are very resistant to hydrolysis in the absence of cellulases that catalyze their hydrolysis.

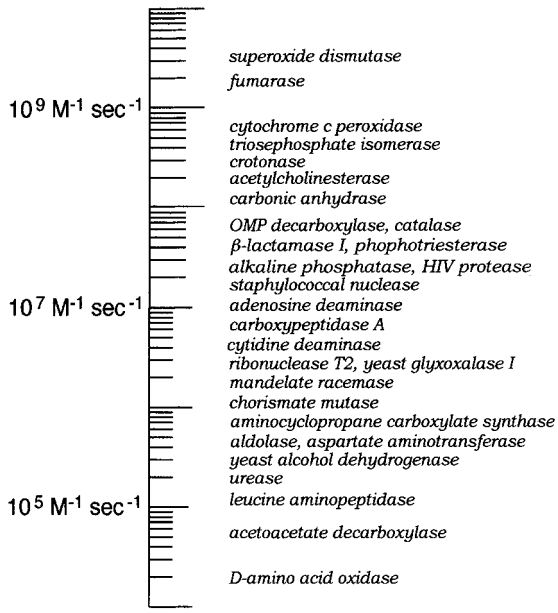

FIGURE 1. Representative values of k_{cat}/K_m at 25 °C compiled from the literature.

Why would one wish to know the rate of a biological reaction in the absence of an enzyme? That information would allow biologists to appreciate what natural selection has accomplished in the evolution of enzymes as proficient catalysts and would enable chemists to compare enzymes with artificial catalysts produced in the laboratory. Such information might also be of value in considering the design of enzyme antagonists: the greater the rate enhancement that an enzyme produces, the greater is its affinity for the altered substrate in the transition state compared with its relatively modest affinity for the substrate in the ground state (Figure 2).[4−6] That principle has furnished a basis for the design of transition-state analogues, stable molecules exploiting that special affinity. Examples have now been discovered for enzymes of every class, including agents that are currently used to control hypertension, the spread of HIV, the maturation of insects, and the growth of weeds.[7] By allowing "snapshots" of enzymes in action, transition-state analogues have also provided valuable tools for investigating enzyme structures and mechanisms, most recently that of the peptide bond-forming center of the ribosome.[8] Those enzymes that produce the largest rate enhancements and transition-state affinities should offer the most sensitive targets for inhibitor design.

This Account describes experiments exploring the ability of enzymes to enhance the rate of a reaction above the rate at which it would occur spontaneously in neutral aqueous solution. This work has uncovered several of the slowest reactions whose progress has ever been determined in water, with rate constants spanning a range of at least 16 orders of magnitude. The energetic terrain of biological chemistry in the absence of enzymes is much more rugged than had been appreciated, and the catalytic power of some enzymes is remarkable. The methods used

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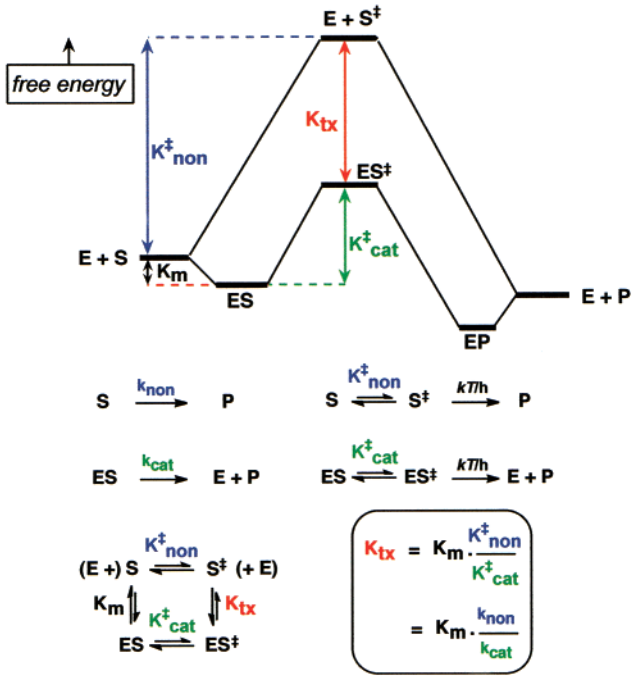

FIGURE 2. If equilibrium is maintained between the ground state and the transition state in dilute solution, the formal dissociation constant of the altered substrate in the transition state (K_{tx}) is expected to be less than that of the substrate in the ground state (K_m), by a factor matching the factor by which the rate constant of the catalyzed reaction (k_{cat}) exceeds that of the uncatalyzed reaction (k_{non}). Effects of desolvation, charge separation, or proximity in multisubstrate reactions can be considered to involve subpopulations of ES that depart from the mean in the usual statistical description of molecules in the ground state. At equilibrium, any of these subpopulations can be more reactive than ES but can do so only to the extent that it is rare. Transition-state affinity may be underestimated if the mechanism of reaction in solution differs fundamentally from the mechanism of reaction at the enzyme's active site, or $k_{cat}K_m$ is limited by enzyme−substrate encounter.[5] This relationship is not applicable to reactions involving tunneling and requires modification for reactions proceeding through covalent intermediates.[6]

to follow these very slow reactions, involving observations at elevated temperatures, also generate some of the information that is needed to analyze the catalytic power of enzymes in terms of their constituent parts.

Methods for Following the Rates of Very Slow Reactions

The characterization of very slow reactions presents unusual demands. It is necessary at the outset to choose a model reaction that is uncomplicated by competing side reactions, such as glycoside cleavage in a nucleotide whose rate of decarboxylation one wishes to measure. If a reaction follows simple first-order kinetics, its rate constant can sometimes be obtained from observations at room temperature, using a sensitive method for detecting reaction products in very small amounts.[9−11]

An alternative approach, adopted in the present work, is to measure reaction rate at series of elevated temperatures, following them to completion. The resulting Arrhenius plot, if linear, can then be extrapolated to 25 °C. How slow a reaction can be measured by this method? According to a "rule of thumb", traceable to some early experiments by Harcourt, reaction rates tend to double with a 10 °C rise of temperature[12] so that "$Q_{10} = 2$". One would then expect a 65-fold increase in rate if the temperature rose from 25 to 100 °C. However, reactions can be conducted at higher temperatures in sealed tubes, and (see below) very slow reactions tend to have larger heats of activation. The rate of a reaction with an E_{act} of 24 kcal/mol ($Q_{10} = 4$), such as the deamination of cytidine, increases 3×10^6-fold as the temperature is raised from 25 to 200 °C, and 1 month of observation can be compressed into a single second.

In much of this work, reaction mixtures were sealed in quartz, heated over various intervals, and then opened for analysis by proton NMR. The vapor pressure of water does not change enough to affect reaction rates significantly, but aqueous samples tend to burst at temperatures above 250 °C (40 atm). Explosions are avoided by placing reaction tubes in water inside a steel bomb, equalizing pressure across the wall of each tube. PTFE vessels must be used for alkaline solutions which attack quartz.

To establish the rate of an uncatalyzed reaction in water in the absence of acids, bases, or other catalysts, it is necessary to find conditions under which the rate of reaction does not change with changing pH (Figure 3).[13] Complications are thus avoided that might otherwise arise from differing heats of ionization of substrates or buffers. Most simple reactions yield linear Arrhenius plots over a sufficient range (60−100 °C) to allow estimation of the rate constant at ambient temperature with reasonable accuracy.

Degrees of Difficulty of Biological Reactions in Water

When this work began, little information was available about the rate of spontaneous hydrolysis of bonds that join biological polymers together or the catalytic proficiencies of the large number of enzymes that cleave these bonds.

Our first experiments concerned the hydrolysis of peptide bonds in proteins and peptides. These include C-terminal peptide bonds such as those cleaved by a carboxypeptidase, internal peptide bonds that are cleaved by endopeptidases, and the bonds in simple dipeptides. Each of these reactions, modeled by a suitable derivative of glycylglycine, could be followed by proton NMR in the temperature range near 150 °C. In each case, the linear Arrhenius plot yielded a half-time of approximately 400 years at 25 °C,[14] comparable with those for hydrolysis of the exocyclic amino groups of cytidine and adenosine.[3] Similar results were obtained by a sensitive colorimetric method to monitor the release of free amino groups at 25 °C.[11] Thus, polypeptides are reasonably stable in the pH range near neutrality. When the amino group of a polypeptide is exposed, however, diketopiperazines are formed so rapidly ($t_{1/2} \approx 3$ weeks at 37 °C) that a 100-amino acid peptide with a free amino terminus would undergo complete degradation to diketopiperazines before

Table 1. Cleavage of Polymers at 25 and 100 °C

reaction	bond $t_{1/2}$ 25 °C	bond $t_{1/2}$ 100 °C	no. of bonds per polymer	$t_{1/2}$ per cleavage event 25 °C	$t_{1/2}$ per cleavage event 100 °C
protein hydrolysis	400 years	5.5 weeks	123 (RNase A)	4 years	7 hours
polysaccharide hydrolysis	4.7×10^6 years	160 years	10^5 residues (glycogen)	50 years	12 hours
RNA hydrolysis	4 years	9 days	70 residues (tRNA)	20 days	3 hours
DNA hydrolysis	140 000 years	22 years	10^9 residues (human DNA)	1 month	2 hours

the half-time for hydrolysis of a single peptide bond.[14,15] That may explain the fact that proteins in higher organisms, which must survive for days or weeks, are usually *N*-acetylated, whereas *N*-acetylation is seldom observed in proteins from microorganisms with short generation times.[14]

Next, we considered the hydrolysis of the internucleotide linkages in nucleic acids. The phosphodiester linkages of RNA, destabilized by the neighboring 2′-hydroxyl group, are cleaved with an estimated half-time of 4 years.[16] When we examined the hydrolysis of dimethyl phosphate, as a model for the cleavage of phosphodiester linkages in DNA, we observed a half-time, pH-independent in the range near neutrality, of 140 000 years.[17] Even at that level of stability, it can be inferred that a single DNA molecule from the human chromosome (with $\sim 3 \times 10^9$ linkages of this kind) undergoes one backbone cleavage in 20 min. Accordingly, the genetic material would be highly unstable without enzymes to bring about DNA repair.

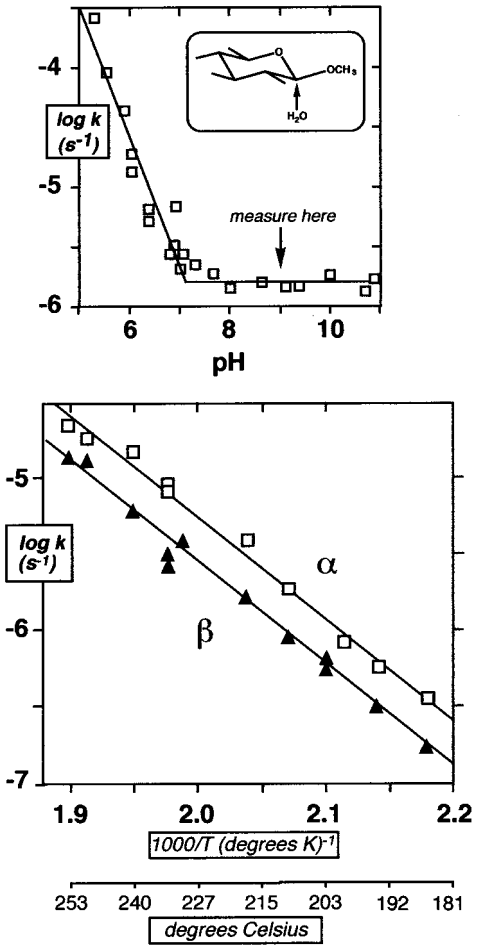

FIGURE 3. Arrhenius plot and pH profile for the uncatalyzed hydrolysis of α- and β-1-methylglucopyranosides.[13]

In view of the fact that cellulose and chitin constitute much of the carbon in the biosphere, it was also of interest to examine the uncatalyzed hydrolysis of the α- and β-glycosidic bonds that join polysaccharides. Somewhat surprisingly (in view of their susceptibility to acid hydrolysis[18]), *O*-glycosides are even more stable than proteins or phosphodiesters. In neutral solution, where glycoside hydrolysis becomes pH-independent (Figure 3), Arrhenius plots yield half-times at 25 °C of 5 million and 8 million years for hydrolysis of β- and α-methylglucosides, respectively.

Because of their differing sizes, rates, and heats of activation, the half-times for a single polymer-cleaving event are very different for a typical protein, DNA, or polysaccharide molecule. Interestingly, each of these half-times amounts to only a few hours at 100 °C (Table 1). Without some means of protection or repair, the survival of any of these polymers might constitute a problem for a thermophilic organism with a long division time.

Some of the reactions of intermediary metabolism proceed spontaneously with relative ease. These include the isomerization of chorismic acid ($t_{1/2} = 38$ h)[19] and of glyceraldehyde 3-phosphate ($t_{1/2} = 2$ months).[5,20] Other biological reactions occur much more slowly in the absence of enzymes, including the reversible hydration of fumarate ($t_{1/2} = 730\,000$ years)[21] and the racemization of mandelate ($t_{1/2} = 100\,000$ years).[22] The last step in pyrimidine biosynthesis is the decarboxylation of orotidine 5′-phosphate to form UMP. The rate of the spontaneous reaction, modeled with 1-methylorotic acid, is 2.8×10^{-16} s^{-1} at 25 °C, corresponding to a half-time of 78 million years.[23]

The Catalytic Powers of Enzymes

The spontaneous rates of biological reactions examined in this way span a range of more than 16 orders of magnitude (Figure 4), with half-times ranging from 5 s for the hydration of CO_2[24] to 1.1 billion years for the decarboxylation of amino acids.[25] The active sites of enzymes have evolved to allow these same reactions to proceed at rates that fall within a relatively narrow range, with most k_{cat} values in the neighborhood of 100−1000 s^{-1}. Thus, enzymes differ enormously in the rate enhancements (k_{cat}/k_{non}) that they produce, ranging from 10^7-fold to 10^{19}-fold (Figure 5).

As a measure of catalytic proficiency and a benchmark for the design of potential transition-state analogue inhibitors, the value of K_{tx} (obtained by dividing the rate constant of the uncatalyzed reaction (k_{non}) by k_{cat}/K_m; see Figure 2) is of special interest. In Figure 6, the length of each vertical bar represents the value of K_{tx}. These values range from 10^{-8} to 10^{-23} M, setting an upper limit (Figure

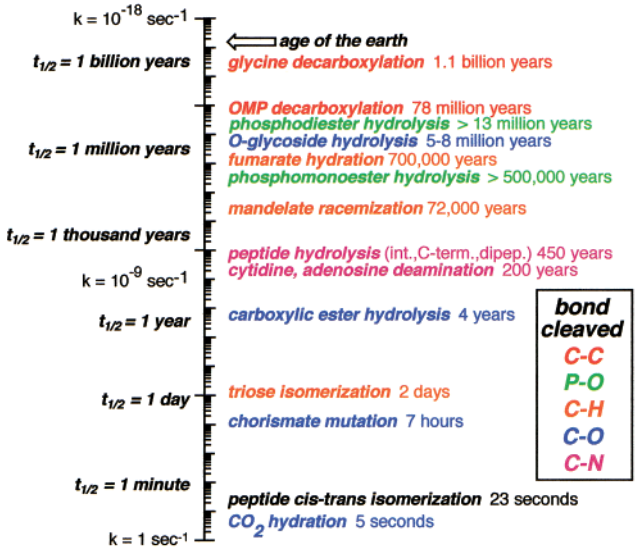

FIGURE 4. Natural half-times of some biological reactions in neutral solution at 25 °C.

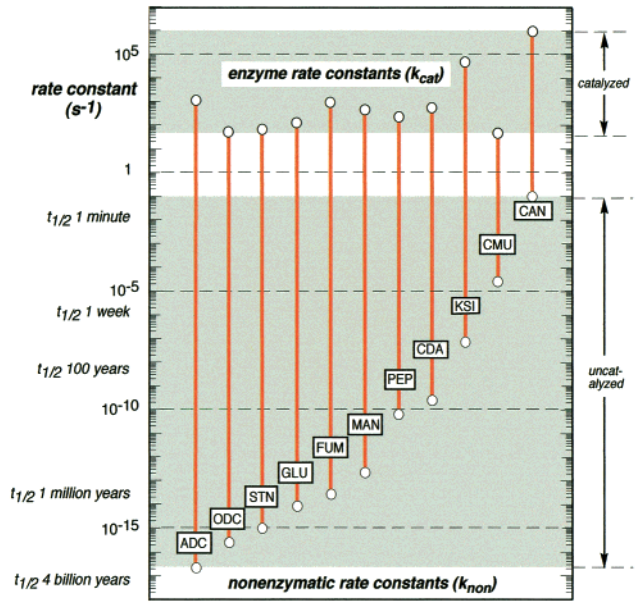

FIGURE 5. Logarithmic scale of k_{cat} and k_{non} values for some representative reactions at 25 °C. The length of each vertical bar represents the rate enhancement by ADC = arginine decarboxylase;[25] ODC = orotidine 5′-phosphate decarboxylase;[23] STN = staphylococcal nuclease;[17] GLU = sweet potato β-amylase;[13] FUM = fumarase;[21] MAN = mandelate racemase;[22] PEP = carboxypeptidase B;[14] CDA = *E. coli* cytidine deaminase;[30] KSI = ketosteroid isomerase;[23] CMU = chorismate mutase;[19] CAN = carbonic anhydrase.[23]

2) on the dissociation constant of the enzyme's complex with the altered substrate in the transition state.

Thermodynamic Origins of Transition-State Affinity

Do the catalytic effects of enzymes usually arise from a reduction in the heat of activation or from an increase in the entropy of activation? In an early collection of rate constants of spontaneous reactions in neutral solution,[26] $\Delta H^{\ddagger}$ values were found to vary from 4 kcal/mol for trimethyl phosphate hydrolysis to 24 kcal/mol for methyl iodide hydrolysis, with a median value of 14 kcal/mol corresponding to a Q_{10} value of 2.2. An early survey of the temperature dependence of k_{cat} for enzyme reactions found typical $\Delta H^{\ddagger}$ values in the neighborhood of 8−10 kcal/mol, corresponding to a Q_{10} of roughly 1.7,[27] although a few higher values had been recorded at the time of a more recent review.[28] Considered together, these findings seemed to suggest that enzymatic and nonenzymatic reactions differ only slightly in their response to changing temperature, implying that the catalytic effect of enzymes is mainly entropic in origin.

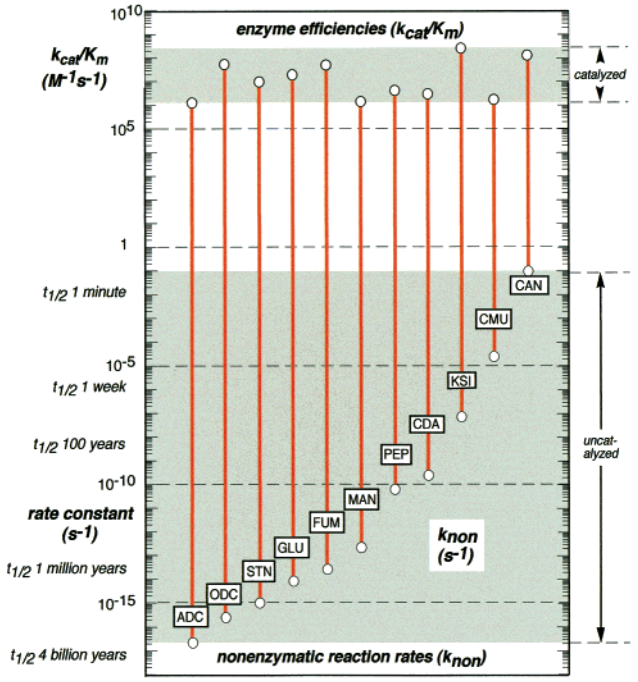

FIGURE 6. Logarithmic scale of k_{cat}/K_m and k_{non} values for some representative reactions at 25 °C. The length of each vertical bar represents transition-state affinity or catalytic proficiency (its reciprocal). For abbreviations and references, see Figure 5.

That impression is misleading, as shown by a recent comparison[29] of the temperature dependences of uncatalyzed single-substrate and hydrolytic reactions with those of the corresponding enzyme reactions. The results, summarized in Figure 7, indicate that the entropy of activation is sometimes raised and sometimes lowered by these enzymes. Without exception, however, the rate enhancement is accompanied by a major reduction in the enthalpy of activation.

That tendency is not surprising if we consider the limited amount of entropy that is "available" to be lost in passing from the ground state to the transition state for reactions involving simple molecules. Figure 7 shows that $T\Delta S^{\ddagger}$ is often small and negative for hydrolytic reactions, or small and positive for single-substrate reactions such as orotate decarboxylation, triose isomerization, and hydrolysis of methyl phosphate. Heats of activation, in contrast, are large and positive for the slow reactions that are catalyzed by enzymes. *It would be virtually impossible to increase the rates of these reactions much without reducing their heats of activation*. For enzyme reactions,

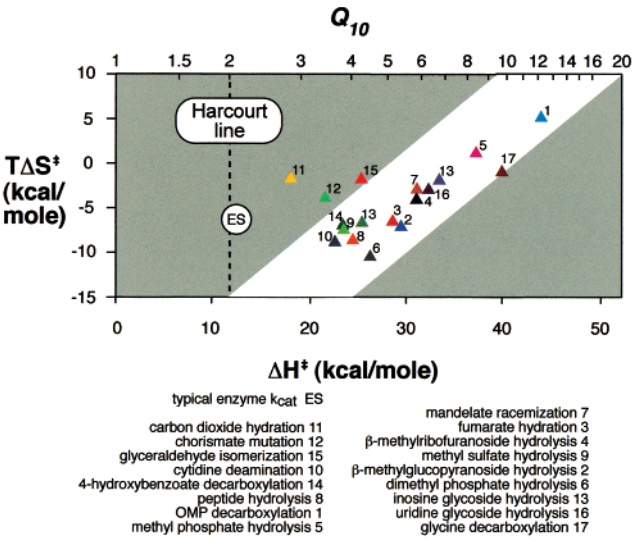

FIGURE 7. Heats and entropies of activation of spontaneous biological reactions, compared with k_{cat} for a typical ES complex.[29]

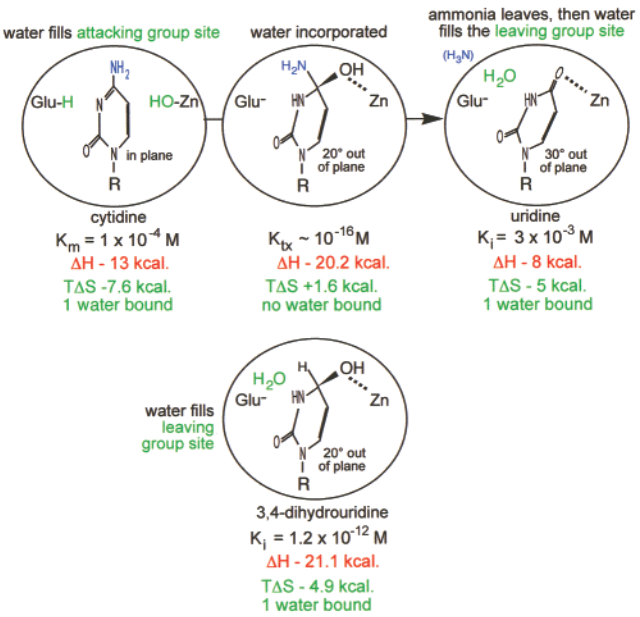

FIGURE 8. Heats and entropies of ligand binding by cytidine deaminase.[40]

k_{cat} is less temperature-sensitive than k_{non}, and k_{cat}/K_m is almost completely temperature-independent in some cases.[30] As a result, rate enhancement tends to increase with decreasing temperature, and enzyme affinities for transition-state analogue inhibitors are expected to increase with decreasing temperature, more sharply than do enzyme affinities for the corresponding substrates in the ground state. That tendency has been confirmed in the case of adenosine deaminase[31] and has been analyzed in more detail for substrates and inhibitors of cytidine deaminase, as described below.

The tendency of enthalpy to dominate the thermodynamics of enzyme rate enhancements has been shown to apply to reactions involving single substrates and to hydrolytic reactions in which water (the second substrate) is already present in abundance. That generalization seems less likely to apply to two-substrate reactions (including reactions involving coenzymes or other cofactors) which often require that the two substrates be bound in a configuration conducive to reaction. Indeed, a recent comparison of the enzymatic with the spontaneous phosphorylation of glucose by ATP, in the presence and absence of hexokinase, suggests that enthalpic and entropic contributions to transition-state affinity are of comparable magnitude,[32] and the enhancement of the rate of reduction of an imino acid by glutamate dehydrogenase appears to be entirely entropic in origin.[33]

Structural Origins of Transition-State Affinity

The ability of enzymes to reduce the enthalpy of activation of the reactions that they catalyze would seem understandable if strong electrostatic and H-bonding interactions developed in the transition state that were not present in the ground-state enzyme−substrate complex.[34] Considerable experimental evidence supports the view that polar interactions are commonly responsible for transition-state affinity. For example, both triosephosphate isomerase[35] and carboxypeptidase A[36] have been shown by NMR to bind transition-state analogue inhibitors in highly charged forms that are rare in solution at physiological pH.

Escherichia coli cytidine deaminase offered an attractive opportunity to explore the hypothesis that disruption of polar interactions in the transition state might be responsible for the increase the enthalpy of activation of the enzyme reaction. In the reaction catalyzed by this enzyme, K_m appears to be a true enzyme−substrate dissociation constant, and k_{cat} describes the chemical transformation of the substrate rather than product release. Both the enzymatic and the nonenzymatic reactions are insensitive to changing pH, simplifying analysis of the thermodynamics of activation.[31] Furthermore, crystal structures have been solved for cytidine deaminase complexes with substrate,[37] transition-state,[38] and product[39] analogue inhibitors.

Kinetic experiments indicate that in this reaction (Figure 8) the enthalpy of binding of the altered substrate in the transition state amounts to −20 kcal/mol,[30] almost identical with the enthalpy of binding of the transition-state analogue inhibitor 3,4-dihydrouridine (−21 kcal/mol).[40] In these complexes, several new H-bonds (particularly involving Glu-104) and electrostatic interactions (particularly with an active site Zn) arise that were not present in the E−S complex (modeled by 3-deazacytidine)[36] or enzyme−product[38] complexes for which the enthalpy of binding is much less favorable.

During enzymatic deamination, the substrate's ribofuranosyl group serves as a kind of "anchor" for the substrate in the transition state. An H-bond between the substrate's 3′-OH group and Glu-91 contributes 5.5 kcal/mol to the free energy of transition-state stabilization.[41] When that H-bond is broken by mutating Glu-91 to alanine, or by replacing the substrate's 3′-OH group by a hydrogen atom, the ΔH of transition-state binding becomes less favorable by ~8 kcal/mol, as measured by

the temperature dependence of the decrease in k_{cat}/K_m. Thus, the anchor appears to have lost its grip. With either of these "mutations", the entropy of transition-state binding shows a compensating increase, as would be expected if mutation resulted in a loosening of interactions governing the range of motion of the ribofuranosyl anchor within the active site.[30]

We were surprised to find that the virtual binding of the altered substrate in the transition state by cytidine deaminase is accompanied by a *positive* change of entropy (Figure 8). That behavior, also observed for fumarase,[21] seems surprising at first glance for a process that involves the combination of two molecules to form a single complex. Upon closer inspection, that behavior seems understandable in terms of the role of substrate water in this reaction. During the action of cytidine deaminase, the activated substrate in the transition state incorporates a covalently bound water molecule, so that the virtual equilibrium in which this (already hydrated) species is bound from free solution involves displacement of a substrate water molecule from the native enzyme. It seems reasonable to ascribe the more favorable entropy change that accompanies the binding of the activated substrate in the transition state for hydrolytic deamination, compared with the binding of cytidine in the ES complex, to the release (by the activated form of the substrate) of bound water into bulk solvent. Similar water displacement, although less complete, is observed for the equilibrium binding by cytidine deaminase of the transition-state analogue 3,4-dihydrouridine, an inhibitor whose structure also incorporates a water molecule.[39] No such displacement occurs when cytidine is bound in the E−S complex or when uridine is bound in the E−P complex (Figure 8).[30,38,42] The entropic cost of incomplete displacement of water from the active site appears to explain, almost in full, the shortcomings in binding affinity of 3,4-dihydrouridine as an ideal transition-state analogue.[39] If that cost could be avoided, it might be possible to approach the binding affinity expected in the ideal case.

Synergistic Group Contributions to Transition-State Affinity

Very tight binding of the altered substrate in the transition state would be expected to require several strong binding interactions, and numerous polar and electrostatic interactions are usually present in enzyme complexes with transition-state analogue inhibitors. Nonetheless, even with a substantial number of such interactions at work in the transition state, the sub-yoktomolar values of K_{tx} that have been derived for the reactions catalyzed by mandelate racemase, fumarase, β-amylase, staphylococcal nuclease, and OMP decarboxylase seem startling and difficult to understand.

It was once believed that the free energy of binding of a substrate or inhibitor should tend to be equivalent to the sum of the free energies of binding of its constituent parts. It is now understood that departures from additivity are to be expected when protein binding of one part of a ligand limits the freedom of movement of another part,

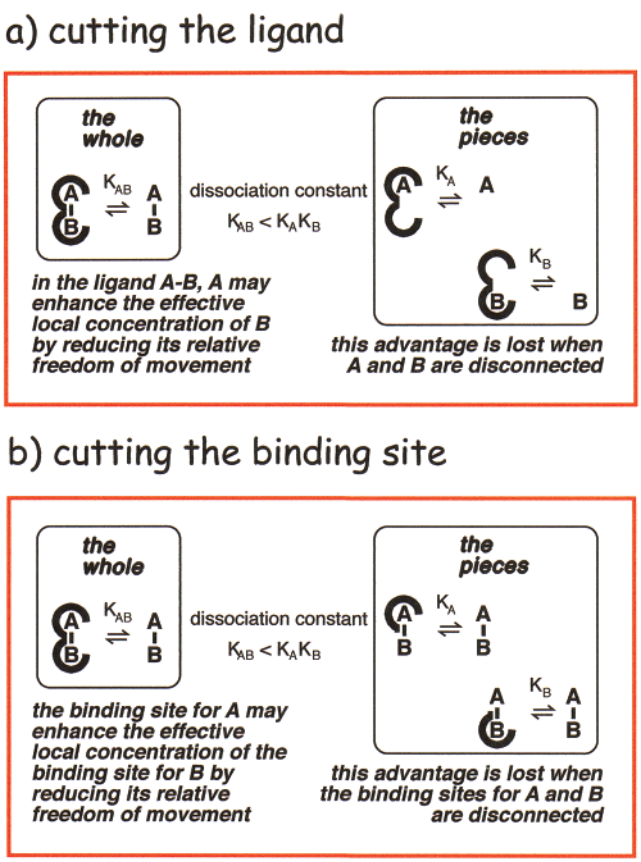

FIGURE 9. Effects on binding affinity of cutting a ligand or binding site into two pieces.

so as to assist its binding and enhance its effective "local concentration" (Figure 9).[43] Such connectivity effects, familiar in the behavior of chelating agents, can be expressed in terms of the concentration of the component that would be needed to render the other component as reactive as the whole molecule. The importance of appropriate spatial relationships is evident in model systems,[44] some of which exhibit "effective concentrations" as high as 10^6 M,[45] and rate enhancements as high as 10^{10} M have been observed in some examples of intramolecular catalysis.[46]

The extent to which such effects contribute to the catalytic action of enzymes, and to their transition-state affinities, can be assessed experimentally by cutting a substrate or transition-state analogue into two "pieces" and then comparing an enzyme's affinity for the pieces with its affinity for the whole molecule (Figure 9a). Recent work applying that criterion has uncovered several cases in which the effective concentration of one part of a substrate in the transition state is as large as 10^9 M relative to another part (Figure 10a). That is the case for the binding by cytidine deaminase of cytidine in the transition state for its deamination ($K_{tx} = 1 \times 10^{-16}$ M) compared with its "pieces": (a) cytosine in the transition state for its deamination ($K_{tx} = 7 \times 10^{-8}$ M) and (b) ribose ($K_i = 1.2 \times 10^{-2}$ M).[47] That is also the case for the binding by yeast orotidine 5′-phosphate decarboxylase of the substrate in the transition state for its decarboxylation ($K_{tx} = 4.4 \times 10^{-24}$ M) compared with its pieces: (a) orotate in

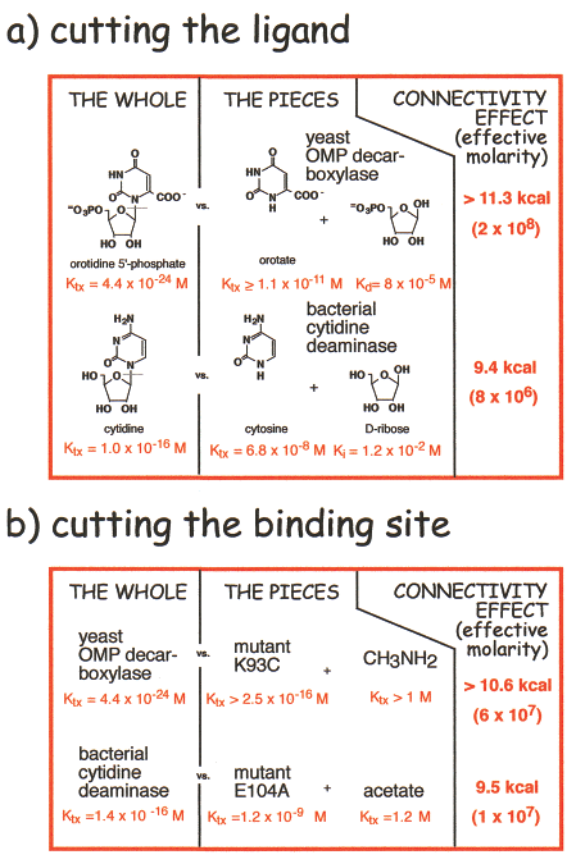

FIGURE 10. Effects on transition-state binding affinity (K_{tx}) of cutting (a) the substrate or (b) the enzyme's active site in two pieces. In the latter case, the value of K_{tx} for catalysis by the native enzyme is compared with the K_{tx} values observed for catalysis by each of the two pieces.

the transition state for its decarboxylation ($K_{tx} = 1 \times 10^{-11}$ M) and (b) ribose 5-phosphate ($K_i = 8 \times 10^{-5}$ M).[48] Earlier, adenosine deaminase was found to exhibit a similarly large connectivity effect when the enzyme's affinity for the altered substrate in the transition state, or for the transition-state analogue 1,6-dihydroinosine, was compared with those of the pieces obtained by cutting the substrate in two.[49]

The magnitude of connectivity effects can be evaluated in a different way by "cutting" the enzyme, instead of the substrate, into two pieces and then comparing their catalytic activities with that of the native enzyme (Figure 9b). In that way, one can estimate the effective concentration of that group at the enzyme's active site. For example, Glu-317 of mandelate racemase acts as a general acid, and this action is mimicked by simple carboxylic acids in solution. When the value of K_{tx} for catalysis by the native enzyme is compared with the K_{tx} values observed for *catalysis by each of the two pieces*, (a) a mutant enzyme in which the carboxylate group has been neutralized by conversion to an amide and (b) acetate, representing the "missing piece", the carboxylate group is found to exhibit an effective molarity of 3×10^5 M.[50] A similar analysis of the contribution of Glu-104 of cytidine deaminase indicated an effective molarity of 8×10^6 M,[51] and a larger value is associated with cutting a critical lysine reside in the transition state for the reaction catalyzed by yeast orotidine 5′-phosphate decarboxylase (Figure 10b).[52]

The magnitude of these connectivity effects is larger than those that have been observed for most chelating agents, but the principles appear to be the same. Such effects have sometimes been described as "entropic",[53] to suggest that they depend on the integrity of the binding site of the enzyme (or the chelating agent) in space, bringing two or more binding determinants to bear on the guest. However, the chelate effect is sometimes expressed entirely as a gain in entropy of binding[54] and sometimes entirely as a decrease in the enthalpy of binding.[55] There appear to be at least two important reasons why that effect need not be expressed entirely, or even partly, in terms of entropy. First, compensating changes in solvation by water may play an important role in determining the thermodynamics observed for ligand binding as suggested in the previous section. Second, when two monodentate ligands of like charge are brought together in a complex, work must be done against their electrostatic repulsion. That is less true of a chelating agent or the active site of an enzyme, in which some of that repulsive energy can be considered to have been "built in", contributing to the enthalpy of synthesis (or biosynthesis) of the host molecule.[34,56]

Conclusions

The fastest known reactions include reactions catalyzed by enzymes, but the rate enhancements that enzymes produce had not been fully appreciated until recently. In the absence of enzymes, these same reactions are among the slowest that have ever been measured, some with half-times approaching the age of the Earth. This difference provides a measure of the proficiencies of enzymes as catalysts and their relative susceptibilities to inhibition by transition-state analogue inhibitors. Thermodynamic comparisons between spontaneous and enzyme-catalyzed reactions, coupled with structural information, suggest that in addition to electrostatic and H-bonding interactions, the liberation of water molecules from an enzyme's active site into bulk solvent sometimes plays a prominent role in determining the relative binding affinities of the altered substrate in the ground state and transition state. These comparisons also indicate a high level of synergism in the action of binding determinants of both the substrate and the enzyme, that are not directly involved in the chemical transformation of the substrate but contribute to the rate of its transformation at an enzyme's active site.

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(1) In this survey, "efficiency" denotes k_{cat}/K_m; "rate enhancement" denotes k_{cat}/k_{non}; and "proficiency" is used to denote $(k_{cat}/K_m)/k_{non}$, equivalent to the reciprocal of K_{tx} in Figure 2. Note that an enzyme may approach perfect efficiency if k_{cat}/K_m approaches the diffusion limit, yet may produce a smaller rate enhancement than another enzyme that is more proficient.

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