Minireview

Solvation, Reorganization Energy, and Biological Catalysis*

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The question of how enzymes greatly enhance the rate of reactions has been discussed for years but remains a vigorously debated issue. Rapid progress has been made on the mechanism of individual enzymes by a combination of kinetic, chemical, and structural approaches. The push and pull of electrons and the resulting bond changes are well understood for many enzymes. However, the larger question of general features that enzymes use to produce rate accelerations of $10^{6}$–$10^{15}$ has remained a contentious issue. We believe that such rate accelerations can be readily explained by reasonable physical principles (1). Though much of this understanding stems from research done in the 1970s and 1980s, these insights are often underappreciated or even completely neglected when examining enzymic rate accelerations. Perhaps this is because of our intense focus on enzymes themselves and relative neglect of the solvent as being a major determinant of the rate of the uncatalyzed reaction. In the latter, the majority of the work in determining the free energy barrier to the reaction occurs as a result of the binding process and not unusually strong interactions between the enzyme and substrate in the transition state configuration than in the enzymic ground state configuration. In this case, as the enzymic reaction proceeds along the lower path in Scheme 1, the binding of the reactant to the enzyme increases significantly. As an example, it has been proposed that short, strong hydrogen bonds could account for much of the “missing” energy required for the differential stabilization of the ES complex compared with the ground state ES complex (6) (see the first minireview in this series by Cleland et al. (48) for details).

The second possibility is that the free energy of interaction between the solvent environment and S is much less favorable than between the solvent and S. Then, as the solution reaction proceeds toward the transition state along the upper path in Scheme 1, the free energy change includes a large and significant contribution from solvation. Although it is clear that the correct answer is a combination of the two scenarios, it is important for catalyst design purposes to determine their relative contributions toward enzymic rate accelerations. In the limiting cases, the former perspective incorporates all of the catalytic prowess into the interactions that develop exclusively within the ES complex, whereas the latter perspective regards the solvent as being a major determinant of the rate of the uncatalyzed reaction. In the latter, the majority of the work in lowering the free energy barrier to the reaction occurs as a result of the binding process and not unusually strong interactions between the enzyme and substrate in the ES complex (see Refs. 3 and 7 for earlier views on this subject).

Do Unusually Strong Interactions Exist in ES?

A useful exercise is to plot $K_{TS}$ against $k_{cat}$ and $K_{non}$ for a series of reactions where the rates of both the enzymic reaction and an appropriate model solution reaction are known. If enzymes predominantly function by interacting with the transition state configuration of the reactant dramatically more strongly than with the ground state configuration of the reactant, then we might expect that the most “powerful” enzymes, as determined by the lowest values of $K_{TS}$, would also be the enzymes that have the fastest turnover rates, $k_{cat}$.

In contrast, if large rate accelerations (small $K_{TS}$) are due in large part to retardation of the solution reaction by the solvent environment, then such enzymes would be associated with a very slow solution reaction.

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1 The abbreviation used is: TSA, transition state analog.
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the rate for a model decarboxylase reaction by 10^3. Moreover, both experimental and theoretical studies have shown that reactions in solution are often retarded by the solvent when compared with a similar gas phase reaction (13–16). Table I compares rates for the $S_n2$ displacement reaction, CH$_3$Br + Cl$^-$ → CH$_3$Cl + Br$^-$, in solvents of differing polarity and dielectric response, including a “null” solvent, the gas phase environment. The effect of the solvent is to retard the rate relative to what would be observed for this reaction under the same conditions in the gas phase. The solvent must reorganize to obtain a configuration that will allow the reaction to proceed (1, 15, 17–22). The fast rate of reactions in the gas phase led Dewar and Storch (23) to propose that enzymes act through a desolvation mechanism, but it was pointed out by Warshel et al. (24) that the effect is more correctly described as solvent replacement. Early on, Perutz (25) suggested such a scenario, and Krishtalik (17) and Warshel (18) offered detailed explanations. Krishtalik (17) examined enzyme reactions with a simple yet insightful model that considered the enzyme to be a low dielectric, low polarity medium, whereas Warshel (18) made a further step in the model development by including a preorganized polar framework (see the third minireview in this series by Warshel (49) for more details).

Serum albumin is well known to act as a weak catalyst by a medium effect. Hollfelder et al. (26) have shown that this transport protein can compete with the catalytic antibody 34E4 with respect to rate enhancements ($k_{cat}/k_{non}$) for an elimination reaction initiated by proton removal. This result is reminiscent of work with subtilisin in which the catalytic triad was replaced by three alanine residues, yet the modified enzyme was still capable of a rate enhancement of 10^5–10^7 for anilide hydrolysis even without forming the acyl enzyme intermediate (27).

The effect of the medium on the reaction will depend on both the dielectric response and the polarity of the medium. For example, enzyme active sites generally have a low dielectric response but are highly polar and thus can generate intense electric fields. An active site will retard a reaction rate unless it is organized to be electrostatically complementary to the transition state configuration of the reactant (21, 22). If this is not the case, the reactant can become "locked in" to a nonreactive configuration, that is, enzymes provide more than a simple medium effect.

As in all generalizations, the concept that biochemically relevant reactions will be slower in aqueous solvent than an organic solvent such as dimethyl formamide (e.g. Table I) has exceptions. For example, the reaction catalyzed by chorismate mutase, the Claisen rearrangement of chorismate to prephenate, is actually 100-fold faster in water than in methanol. However, as one might anticipate, the rate acceleration that the enzyme provides (10^9) is modest and is on the lower end of the scale of Fig. 1. In this case, the enzyme increases the reaction rate to a large extent by organizing the reactant relative to the configurations found in aqueous or nonaqueous solution. In such cases as this, a catalytic antibody may provide for reaction rates approaching those seen in their enzymatic counterparts (28). However, because catalytic antibodies do not contain deep active site clefts and hence will not shield the reaction

**Biological Reactions Are Slow in Absence of a Catalyst**

The stability of biomolecules in solution and in the absence of a specific catalyst is essential for the existence of metabolic pathways. Are these solution reactions intrinsically slow or does the solvent environment act to retard the rate that would be observed in the absence of such an environment? Experimental (10) and theoretical (11) studies suggested that the rates of biomolecular reactions in solution are slow in large part because of the entropic penalty involved in bringing the two reactants together into a bimolecular complex; three translational and three rotational degrees of freedom are lost in the association reaction and are apparently uncompensated for by the six new internal rotations and vibrations of the bimolecular complex (10, 11). However, the majority of reactions in Fig. 1 are either unimolecular decomposition reactions or pseudo-first order reactions. Although loss of intrinsic entropy during an association process may be an important aspect of enzyme catalysis for bimolecular reactions, it cannot explain the trend in rate accelerations shown in Fig. 1.

However, a change in the nature of the solvent can increase reaction rates dramatically. Crosby et al. (12) showed that simply changing the solvent from water to ethanol can increase


The time scales of various dynamic events that can occur in an enzyme complex (29)

| Motion                          | Time scale |
|---------------------------------|------------|
| Bond vibration                  | \( \log_{10} t_s / s \) |
| \( k_{Th} \)                    | \(-14 \) to \(-13 \) |
| Elastic vibration of globular region | \(-13 \) |
| Sugar repacking                 | \(-12 \) to \(-11 \) |
| Rotation of side chains at surface | \(-11 \) to \(-10 \) |
| Torsional libration of buried groups | \(-11 \) to \(-9 \) |
| Stern-Volmer energy transfer of DHFR\(^{\ast}\) cofactor (46) | \(-9 \) |
| Hinge bending                   | \(-11 \) to \(-7 \) |
| Protein breathing motions       | \(-4 \) to \(0 \) |
| Rotation of medium sized side chains in interior | \(-5 \) to \(0 \) |
| Alloteric transitions           | \(-5 \) to \(0 \) |
| Local denaturation              | \(-5 \) to \(1 \) |
| \( E. \, coli \) DHFR catalysis (1/\( k_{cat} \)values) (47) | \(-3 \) |

\( \ast \) DHFR, dihydrofolate reductase.

**Enzymes Are Preorganized for Reaction**

In contrast to reactions in solution, the enzymic environment is preorganized to be complementary to the transition state configuration of the reactants, and as a result the reorganization penalty is relatively small.

As an example consider the possible events that can occur while a reactant is bound to the active site of an enzyme. Protein conformational changes can occur on a wide range of time scales as shown in Table II (29). Neglecting electron dynamics, the fastest motions are the bond vibrations occurring on the order of tens to hundreds of femtoseconds \((10^{-14} - 10^{-13} \text{ s})\). The universal frequency factor, \( k_{Th} \), which is commonly used in transition state theory analyses, likewise occurs on the subpicosecond time scale.

In contrast, the rotation of a tyrosine ring in the interior of a protein occurs on the second to submillisecond time scale (30). During this time reactive motions associated with \( k_{Th} \) can occur up to \( 10^{13}\) times, although not all such motions would result in a reaction. If enzyme catalysis were dependent on a conformational change such as ring flipping, then the rate-limiting step would be the motion of the ring; dynamical effects resulting from bond vibrations would be masked by the conformational change (1).

For *Escherichia coli* dihydrofolate reductase, the chemical reaction occurs on the millisecond time scale, which is sufficient time for many conformational changes to occur that could induce the reaction. If this were the case, then the triggering conformational change should mask any effects from faster degrees of freedom such as bond vibrations. However, the reaction rate measured under presteady-state conditions \((1000 \text{ s}^{-1})\). Table II exhibits a deuterium isotope effect of 3, which stems from changes in the nature of a bond vibration. We conclude that no kinetically significant reorganization of the enzyme-substrate cofactor Michaelis complex is necessary for catalysis to occur.

Slow reorganization of the reaction environment can modulate the efficiency of a reaction (20). In this regard, a significant amount of catalysis can be accounted for by a preorganized and electrically stable (low dielectric) reaction environment when compared with reactions in solution. For example, the reactions catalyzed by staphylococcal nuclease and orotidine-5-monophosphate decarboxylase have the largest rate accelerations \((1/\langle k_{cat} / K_{m} \rangle)\) measured to date (5) \((-\log(1/\langle k_{cat} / K_{m} \rangle / k_{non}) = 21 \text{ and } 23 \text{ in Fig. 1})\). In both cases studies have shown that the model solution reaction rate can be increased 17 or more orders of magnitude by simply preorganizing the active site by supplying strategically located weak acids and/or bases and replacing the high dielectric solvent environment with a low dielectric one that is complementary to the proposed transition state of the reactants (31, 32). (However, in the case of orotidine 5-monophosphate it has recently been determined that the carbanion-stabilizing group is likely a previously undiscovered zinc atom and not an essential lysine as thought (33).) The rate increase for the model reaction is obtained without unusually strong or dramatic interactions between the transition state of the reactants and the enzyme. For the slowest uncatalyzed reactions of biological relevance, a simple change in the nature of the environment (as occurs during the binding process) can account for dramatic rate enhancements.

A chemical reaction is an inherently dynamical process, and as such it might be tempting to propose that the reaction dynamics within the enzyme active site are fundamentally different from those in solution. Nonequilibrium dynamical effects are accounted for in transition state theory by the use of a transmission coefficient \((\kappa_{TS})\), which will be much less than unity if such effects are strong. However, for both relevant solution reactions and enzyme reactions studied to date, no significant deviations from ideal transition state theory \((\kappa = 1)\) have been seen (34–36). Of course, dynamical motion along the reaction coordinate does occur for every reaction; for example, as the distances between the reacting atoms decrease and increase with the low frequency motions of domain vibrations, the instantaneous barrier for the reaction also decreases and increases. However, such instantaneous modulations of the barrier height are taken into account in transition state theory by the use of a free energy barrier in which the barrier height is a thermal average. An excellent perspective on applications of transition state theory to biochemical phenomena can be found in Ref. 37. At this point it is not clear how mutations in enzymes affect motions along the reaction coordinate.

**Conclusion**

When a variety of enzymic systems are compared, the observed rate enhancements and the calculated dissociation constant \( K_{TS} \) can vary over many orders of magnitude. However, this does not imply that those systems with the highest magnitude of \( K_{TS} \) have stronger interactions between the respective enzyme and the transition state configuration of the reactant. Instead, the large rate enhancements result from large solvent reorganization energies required for the reference solution reaction to occur. This is manifest in \( K_{TS} \) by an unfavorable solvation free energy of the reactant in its transition state geometry. This is not to say that various aspects of enzyme mechanics, such as proximity and orientation effects (38–40), hydrogen bond energetics (41, 42), tunneling (43), and metallochemistry are not important, nor does it mean that interactions between the substrate and the enzyme are not somewhat stronger in \( ES \) than in \( ES\). However, as was pointed out 20 years ago (18), we cannot fully understand enzymic rate enhancements until we understand solution reactions.

Furthermore, the correlation of \( K_{TS} \) with large solvent effects should be taken into account in the design of transition state analog inhibitors. That is, if transition state analogs are to capture all of the binding affinity calculated in \( K_{TS} \), then unfavorable solvation properties will have to be incorporated into the design of the transition state analogue. This is a double edged sword, of course, because lower solubility of the transition state analogue inhibitor can have other consequences as well.

At the heart of the matter of rate enhancements seen when comparing enzyme reactions and solution reactions is the ability of a biological system to control metabolic processes. That is,
in order for a given reaction to serve as a metabolic control point or switch, the reaction in solution must be slow to provide for the stability of the reactants and hence the stability of the corresponding metabolic signal. From the development of conceptual models of biological catalysis in recent years, we can list several catalytic principles that allow enzymes to act as metabolic switches.

**Screening from Bulk Solvent**—The reactants must be sufficiently removed from solvent. It has been demonstrated that the effective dielectric constant remains rather high within a region or switch, the reaction in solution must be slow to provide for the stability of the reactants and hence the stability of the corresponding metabolic signal. From the development of conceptual models of biological catalysis in recent years, we can list several catalytic principles that allow enzymes to act as metabolic switches.

**Preorganization**—The catalyst must sufficiently organize the reactants such that they are properly oriented with respect to each other. No significant reorganization within the active site should be necessary for the chemical step to take place. In other words, the manner in which the reactants bind to the enzyme should provide for their activation.

Appropriate general acids and/or general bases should be provided by the catalysts. As these enzyme groups actively participate in the reaction, they too must be preorganized for reaction by the binding of the reactants.

**Catalyst Stability**—In order for metabolic control to be realized, the catalyst itself must be thermally stable and not degrade easily. This thermal or kinetic stability can be achieved if the catalyst has a high heat capacity. For peptide polymers this requires stable scaffolds containing α-helices and β-sheets. Additionally, this (along with item 1) may explain why enzymes are large molecules; greater specific heat is contained in larger proteins than in smaller ones.

**Future Directions**

Although our general understanding of enzyme catalysis has increased significantly in the last 30 years, catalyst design is still problematic with many unanswered questions. For instance, how can we design catalysts that meet the dual requirements of having glasslike dynamics to provide a stable reaction environment and yet be fluid enough to allow uptake of the reactants into an environment well shielded from the effects of bulk solvent and likewise provide for the release of the products? The exploitation of existing enzyme scaffolds is one answer (44, 45). In this regard, do protein scaffolds belonging to the same class have similar low frequency motions? Are these low frequency motions characteristic for particular classes of reactions? What interactions on the protein surface regulate the opening and closing of active site loops which in turn regulate substrate binding? How well do we understand which residues of the enzyme scaffold are essential for chemistry and

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2 When a series of catalytic antibody reactions is analyzed in the manner of Fig. 1, there is very little correlation between $K_{D}$ and $K_{m}$ for the antibody nor is a correlation observed between $K_{m}$ and $k_{cat}$. Furthermore, the dissociation process for a transition state analog inhibitor (dissociation constant $K_{D}^{TS}$) is thought to be a model for the dissociation of the transition state from the catalyt ($K_{cat}$). However, plotting $K_{D}^{TS}$ versus $K_{m}$ for a series of catalytic antibodies does not indicate any correlation (J. Stewart, personal communication; data from Ref. 50).