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Perspective on "The energetics of enzymatic reactions"

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Arieh Warshel

Department of Chemistry, University of Southern California, Los Angeles, CA 90089, USA

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Abstract. The origin of the catalytic power of enzymes has been one of the most important open problems in molecular biology. Our early computer modeling studies [Warshel A, Levitt M (1976) *J Mol Biol* 103: 227 indicated that electrostatic effects give the largest contributions to enzyme catalysis; however, it was not clear how enzymes can provide more electrostatic stabilization to their transition states than water does. This fundamental problem has been solved by the title paper. The paper pointed out that in reactions in water the solvent must pay significant electrostatic energy for orienting its permanent dipoles toward the transition states. It was then demonstrated that in enzymes the active site dipoles are already partially preoriented in the optimum direction and so much less electrostatic energy is lost in the reorganization process. It was further demonstrated that ion pairs and related transition states are less stable in water than in preoriented dipolar environments in general and in the active sites of real enzymes in particular. Thus, it was concluded that enzymes stabilize their transition states by preoriented dipoles and that the catalytic energy is already stored in the preorientation of these dipoles during the folding process rather than in the enzyme substrate interaction.

Key words: Preorganized sites – Catalysts – Electrostatic energies – Folding energy

Enzymatic reactions are involved in most biological processes; thus, there is a major practical and fundamental interest in elucidating the origin of the catalytic power of enzymes. Despite enormous progress in biochemical and structural studies [1] we still do not understand what energy contributions make enzymes so efficient.

Many prominent proposals have been put forward to explain the action of enzymes (for reviews see Refs. [1–3]). Unfortunately, it is hard to use the available experimental information to determine in a unique way which proposal is correct (see discussion in Refs. [3, 4]). It is possible, however, to use energy considerations and computer simulations to exclude different proposals [4]. For example, many of the early proposals involve ground-state destabilization rather than transition-state (TS) stabilization and this appears to be inconsistent with conceptual studies and mutation experiments [3].

Among the few proposals that can account for TS stabilization, the proposal of electrostatic stabilization [2, 5, 6] of the TS charges is probably the most reasonable proposal. This proposal has been supported by early microscopic calculations of the catalytic reaction of lysozyme [2]. Further support has been obtained from additional theoretical studies and analysis of mutation experiments.[4, 7, 8]; however, the proposal of a large electrostatic stabilization was hard to rationalize, i.e. this proposal requires that the enzyme stabilize the TS charges of the substrate more than water does (the reference reaction occurs in water). On the other hand, simulation studies have indicated that the average electrostatic interactions between the protein dipoles and the TS charges are similar in magnitude to, rather than larger than, the corresponding interactions in water. Furthermore, enzyme active sites do not provide more polar groups (e.g. hydrogen bonds) than a typical first solvation shell in water. In view of these considerations it was hard to see how enzymes could stabilize their TSs more than water does.

The 1978 paper [9] identified and solved the abovementioned fundamental problem. The paper demonstrated that preorganized dipoles can stabilize ("solvate") ion pairs and other charge distributions more than water does. The reason for this remarkable effect is that in water about half of the energy gain from charge–dipole (solute–solvent) interactions is spent on changing the dipole–dipole (solvent–solvent) interactions. Thus the free energy of solvation is given by

$$\Delta G^{w}_{sol} \cong \Delta G^{w}_{Q\mu} - \Delta G^{w}_{\mu\mu} \cong \Delta G^{w}_{Q\mu} - \tfrac{1}{2}\Delta G^{w}_{Q\mu} = \tfrac{1}{2}\Delta G^{w}_{Q\mu} \ , \quad (1)$$

where $Q\mu$ and $\mu\mu$ designate charge–dipole and dipole–dipole, respectively. In proteins, however, the active site dipoles associated with polar groups (e.g. hydrogen bonds and C=O dipoles), the internal water molecules, and ionized residues are already partially oriented toward the TS charges. Thus, the loss in $\Delta G_{\mu\mu}$ is smaller than in water, and less free energy is spent on orienting the dipoles of the protein toward the TS of the substrate. The free-energy term $\Delta G_{\mu\mu}$ is closely related to the so-called "reorganization energy" of the given reaction [4, 10]. For example, in water we have to break water–water interactions to form good hydrogen bonds to the TS. In the enzyme, on the other hand, the hydrogen bonds are already partially oriented toward the TS charges [4, 11].

The idea that enzymes use preorganized dipoles should not be confused with proposals which are based on oversimplified macroscopic considerations, i.e. the leading term of the activation energy of charge-transfer reactions is the Marcus expression [10]

$$\Delta g^{\neq} = (\Delta G^0 + \lambda)^2/4\lambda + w \ , \qquad (2)$$

where ΔG^0 is the free energy of the reaction, λ is the solvent reorganization energy, and w is the work of bringing the reactants to the optimum interaction distance. With this expression in mind, one may ask what factors can reduce $\Delta g^{\neq}$. A seemingly obvious suggestion is that enzymes reduce $\Delta g^{\neq}$ by having low dielectric (and presumably nonpolar) active sites where λ would be reduced. The problem with such an oversimplified macroscopic view is that low dielectric and relatively nonpolar active sites would increase ΔG^0 (relative to ΔG^0 in water) for reactions that involve the formation of ionic products (or intermediates) from neutral reactants. In cases of reaction with ionic reactants the low dielectric will increase the w term. Both cases will result in an overall anticatalytic effect (i.e. an increase in $\Delta g^{\neq}$ relative to the corresponding $\Delta g^{\neq}$ in water), i.e. $\Delta g^{\neq}$ will have to reflect the desolvation effect associated with bringing charges from water to nonpolar regions (see discussion in Refs. [12–14]). Thus, what is missing in the above model is the idea that enzymes reduce both λ and ΔG^0 by a preorganized polar (rather than nonpolar) environment.

The fact that the enzymes are polar and that this might be important for catalysis was realized by Krishtalik [15], who unfortunately described enzymes by using macroscopic models of a structureless sphere with low dielectric constant. This was done without noting (at least in early works [15]) that such a model leads to anticatalytic effects. Subsequent attempts [16] to include the effect of the field from the protein polar part resulted in a nonquantitative model which could not be used to explore the origin of the catalytic power of enzymes.[1] In fact, representing the protein consistently as a partially fixed polar environment, while using continuum models with a well-defined dielectric constant, is a challenge that has not been met even today (see discussion in Ref. 17). On the other hand, our early microscopic studies [2] have overcome the continuum problems and traps by avoiding the concept of a dielectric constant altogether. This has allowed us to demonstrate that enzyme catalysis is indeed due to preoriented dipoles [9].

The idea that the active site dipoles are preoriented raises the question of the source of the energy for this catalytic effect. The 1978 paper proposed that the preorganization free energy is already invested in the folding process and therefore should not be invested during the reaction.[2] The prediction that the folding energy is used to preorient the enzyme dipoles is supported by the finding that mutations which increase the activation barrier, $\Delta g^{\neq}_{\text{cat}}$, also increase the protein stability [18]. Thus, the protein ordered structure that was optimized by evolution for catalysis cannot be optimized simultaneously for stability.

If enzymes really use a preoriented polar environment to stabilize the TS, then we understand why it was so difficult to elucidate and quantify the origin of enzyme catalysis. First, the catalysis reflects the fact that the reference reaction in water involves a large investment of reorganization energy. As argued above, a significant part of this energy is not invested in the enzymatic reaction. Thus, those who did not consider the reference reaction in water overlooked the major catalytic effect. Second, the catalytic energy appears to be stored in the enzyme itself and not in the enzyme–substrate interaction. Realizing this point is important since the tendency is to search for an especially strong interaction between the enzyme and the substrate rather than to look for the energetics stored in the enzyme; however, when we find, for example, a large catalytic contribution from a given hydrogen bond donor we have to realize that this contribution is due to the preorientation of the donor group. Similarly, the popular entropy proposal [20] attributes the catalytic effect to the loss of entropy of assembling the substrate fragments upon moving them to the active site. This entropic effect is usually quite small and the catalytic effect appears to be associated with fixing the environment (rather than the substrate).

The somewhat complex concept of a preorganized active site is starting to gain wider recognition [21, 22] and will probably emerge as one of the most important factors in enzyme catalysis. The quantitative establishment of the importance of this effect will probably continue into the next century and will involve more calculations of reorganization free energy of the type described in Refs. [12, 23] and studies of the effect of mutations on folding and catalysis [18]; however, the 1978 paper was the first to demonstrate in a semiquan-

[1] Krishtalik's attempt to include the field of the protein polar part in a spherical model of the protein [16] has not reproduced any catalytic effects. All the intraglobular field in chymotrypsin reported in Ref. [16] is extremely small except for the field from Asp102, which is a part of the reactant system rather than a source for a field on this system. Hence the resultant large $Asp^{-}ImH^{+}$ ion-pair stabilization in a low dielectric medium is an artifact of neglecting the Born energy of transferring the ions from water to the hypothetical low dielectric protein [19]

[2] This preorganization energy is not the λ of Eq. (2), which is related to the actual reaction coordinate, but the free energy of orienting the dipoles to stabilize the TS charges. Thus, for example, an enzyme can evolve to mainly reduce ΔG^0 by its fixed dipoles and in this case it will have reduced preorganization energy without a reduction in λ

titative way the catalytic effect of preorganized enzyme dipoles and to provide a rationale to the result of our early electrostatic calculations [2].

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