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Catalysis by enzyme conformational change as illustrated by orotidine 5′-monophosphate decarboxylase

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An energy decomposition scheme has been used to elucidate the importance of the changes of enzyme conformational substates to the reduction of the activation barrier in enzyme-catalyzed reactions. This analysis may be illustrated by the reaction of orotidine 5′-monophosphate decarboxylase, which exhibits a remarkable rate enhancement of over 17 orders of magnitude compared to the uncatalyzed process. The mechanism shows that the enzyme conformation is more distorted in the reactant state than in the transition state. The energy released from protein conformation relaxation provides the predominant contribution to the rate enhancement of orotidine 5′-monophosphate decarboxylase. The proposed mechanism is consistent with results from site-directed mutagenesis experiments, in which mutations distant from the reactive center can have significant effects on the catalytic rate enhancement (k_{cat}), but rather a small influence on the binding affinity for the substrate (K_M).

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Current Opinion in Structural Biology 2003, **13**:184–192

This review comes from a themed issue on
Theory and simulation
Edited by Charles L Brooks III and David A Case

0959-440X/03/$ – see front matter

DOI 10.1016/S0959-440X(03)00041-1

Abbreviations

EVB	empirical valence bond
FEP	free energy perturbation
MM	molecular mechanics
ODCase	orotidine 5′-monophosphate decarboxylase
OMP	orotidine 5′-monophosphate
PMF	potential of mean force
QM	quantum mechanics
TS	transition state

Introduction

Dynamic fluctuations and internal motions of proteins are an integral part of their biological function and activity [1]. This view of proteins as dynamic systems is fundamental to our understanding of protein function; however, the specific role of protein internal motions in enzyme catalysis is still not fully understood. Numerous experimental and theoretical studies have indicated that there is a direct correlation between the internal motions of an enzyme and its activity. For example, the catalytic activities of many enzymes are closely associated with loop motions that open and close the active site, and position key residues in contact with the substrate [2,3]. The fluctuations of enzymes are also required for substrate binding and product release [4]. X-ray crystal structures of ligand-bound and free enzymes show that substantial conformational changes can be induced by ligand binding and by chemical transformation during an enzymatic reaction [5,6]. Enzyme activity exhibits a remarkable nonadditive effect both from the enzyme, whereby double mutations can have effects greater than the sum of two single mutations [7], and from the substrate itself, whereby the sum of the binding affinities of two separate functional groups of the substrate can be much smaller than the binding affinity of the entire substrate [8•]. These examples raise fundamental questions concerning changes of enzyme conformational substates. Of particular interest is whether the change in enzyme conformation governs the rate of the enzymatic reaction and, if so, to what magnitude.

This review focuses on a discussion of the energy of enzyme conformational change during an enzymatic reaction, which lowers the free energy of activation of the chemical process. This model, which is similar in spirit to the Circe effect proposed by Jencks [9], is illustrated, as an example, by the reaction of orotidine 5′-monophosphate (OMP) decarboxylase (ODCase). It is also of interest to compare the conclusions from two different groups on the mechanism of ODCase, a remarkable enzyme that accelerates the spontaneous decarboxylation of the substrate in water by more than 17 orders (k_{cat}/k_{aq}) of magnitude [10].

There have been extensive studies of the ODCase reaction. Crystal structures of the enzyme from four different species complexed with several inhibitors show remarkable similarity of the active site [11•,12••,13,14]. The active site consists of a network of charged residues (Lys42-Asp70-Lys72-Asp75b; *M. thermoautotrophicum* ODCase numbering) that are strictly conserved over 80 species [15]. Site-directed mutagenesis experiments by Wolfenden and co-workers [16] showed that replacement of any of these charged residues essentially abolishes the enzyme activity. More intriguingly, mutations of residues that are far from the active site also significantly reduce the catalytic activity [8•]. On the theoretical side, several mechanisms that were proposed before the determination of the enzyme structure, including formation of a zwitterion by protonation at the C2 carbonyl group [17], a

carbene intermediate as a result of protonation at the C4 carbonyl group [18] and protonation or nucleophilic addition to C5 of the pyrimidine ring [19,20], are now considered to be unlikely because of a lack of acidic residues as a potential proton donor or nucleophilic residues near C5 [21•]. This review will focus on recent studies based on structures of ODCase.

Energy consideration

Considering the following thermodynamic cycle:

$$\begin{array}{ccc} E+S & \xrightarrow{\Delta G^{\ddagger}_{aq}} & E+S^{\ddagger} \\ \Delta G_b(E'S)\ \downarrow & & \downarrow\ \Delta G_b(E''S^{\ddagger}) \\ E'S & \xrightarrow{\Delta G^{\ddagger}_{cat}} & E''S^{\ddagger} \end{array}$$

the change in the free energy of activation from water to the enzyme active site is related to the binding free energies of the substrate and its distorted structure in the transition state (TS) according to the following equation:

$$\Delta\Delta G^{\neq} = \Delta G^{\neq}_{cat} - \Delta G^{\neq}_{aq} = \Delta G_b(E''S^{\neq}) - \Delta G_b(E'S) \qquad (1)$$

where $\Delta G^{\neq}_{cat}$ and $\Delta G^{\neq}_{aq}$ are, respectively, the free energies of activation of the catalyzed and uncatalyzed reactions, the binding free energy of the substrate $[\Delta G_b(E'S)]$ is related to K_M by $\Delta G_b(E'S) = -RT\ln(1/K_M)$ and $\Delta G_b(E''S^{\neq})$ is the apparent binding free energy of the species corresponding to the TS of the enzymatic reaction. Because $\Delta\Delta G^{\neq} \leq 0$ for catalyzed reactions, Equation 1 can always be interpreted in terms of TS stabilization by electrostatic interactions [22], giving rise to a greater binding affinity of the enzyme for the TS than the substrate. However, this does not tell us the means by which such stabilization is achieved. Of particular interest are the changes of the enzyme conformational substates during the chemical transformation [6,23••].

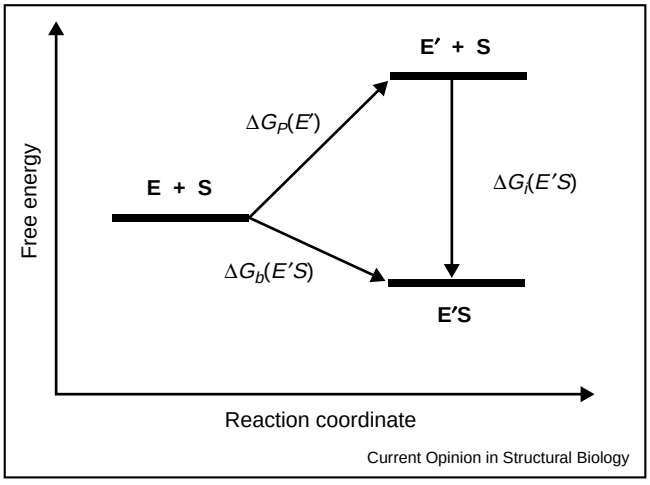
To understand this effect, the free energy difference in Equation 1 must be separated into specific components. We decompose the binding free energy of the substrate $[\Delta G_b(E'S)]$ into two terms, as depicted in Figure 1. First, substrate binding induces conformational change of the enzyme to a distorted substate, E'. The associated free energy change is the protein distortion energy, $\Delta G_P(E')$, which is positive because the relaxed apo enzyme is the more stable conformation in water. Then, we define the intrinsic binding free energy of the substrate $[\Delta G_i(E'S)]$ as the interaction between the substrate and the distorted enzyme. The observed binding free energy is the sum of the two contributions (Figure 1):

$$\Delta G_b(E'S) = \Delta G_P(E') + \Delta G_i(E'S) \qquad (2)$$

A similar decomposition can be made for the apparent binding free energy of the TS species:

$$\Delta G_b(E''S^{\neq}) = \Delta G_P(E'') + \Delta G_i(E''S^{\neq}) \qquad (3)$$

Figure 1



Schematic representation of the decomposition of the total binding free energy into the enzyme distortion free energy and the intrinsic binding free energy of the distorted enzyme for the substrate.

where the notation E'' specifies the enzyme conformational substate at the TS of the substrate–protein complex. Consequently, Equation 1 can be rewritten as follows and the change in the free energy of activation of the enzyme originates from two important factors:

$$\Delta\Delta G^{\neq} = \Delta\Delta G^{\neq}_{ES} + \Delta\Delta G^{\neq}_{PP} \qquad (4)$$

where $\Delta\Delta G^{\neq}_{ES} = \Delta G_i(E''S^{\neq}) - \Delta G_i(E'S)$ is the difference in intrinsic binding free energy between the TS and the reactant state, and $\Delta\Delta G^{\neq}_{PP} = \Delta G_P(E'') - \Delta G_P(E')$ is the difference in protein distortion energy.

At this point, we must consider what energy terms are specifically responsible for TS stabilization in a catalyzed reaction. This requires comparison of the barriers to the catalyzed and uncatalyzed reactions; this is, in turn, affected by the choice of the reference, uncatalyzed process in water. For bimolecular reactions, especially if an active site residue is one of the reactant species, this comparison can be complicated because the reference reaction must include a component that is part of the enzyme. This difficulty can be circumvented by considering a bimolecular complex in a solvent cage [24]. For unimolecular processes, such as the ODCase reaction, the comparison can be rigorously defined by the ratio k_{cat}/k_{aq}, which gives $\Delta\Delta G^{\neq}$. In this case, both solvent and enzyme can be considered as the environment, and TS stabilization (or destabilization) specifies the change in the chemical reactivity of the substrate due to interactions in the two environments. Clearly, the term $\Delta\Delta G^{\neq}_{ES}$ in Equation 4 is an important contributor to TS stabilization because it represents the change in substrate–solvent and substrate–enzyme interactions during the chemical process. On the other hand, although $\Delta\Delta G^{\neq}_{PP}$ contributes to the overall

change in the activation barrier, it represents the change in the environment, in particular, protein internal energy, accompanying the catalyzed reaction and does not directly 'stabilize' the substrate. Thus, the term $\Delta\Delta G^{\neq}_{PP}$ does not contribute to TS stabilization.

The significance of enzyme conformational change in enzyme catalysis has long been recognized [25]. In the Circe effect mechanism [9], Jencks proposed that part of the intrinsic binding energy can be used to destabilize the reactive part of the substrate so that the observed free energy of activation is reduced. The analysis presented here is an extension of this proposal in that a fraction of the intrinsic binding energy is stored in the form of enzyme conformational energy in the Michaelis complex. This energy is then released at the TS of the enzyme–substrate complex, resulting in reduction of the activation barrier. This mechanism is different from the induced-fit proposal in that the latter is concerned with changes of the enzyme conformation to better fit and bind the substrate so that protein–substrate interactions are enhanced [25].

Computational approach

It would be very difficult to determine experimentally the free energy terms in Equation 4, but this can be done by free energy calculations. In this regard, both the description of the potential energy surface and the simulation methods are critical to the accuracy of the computational results.

Potential energy surface

A prerequisite to computational study of enzymatic reactions is an accurate description of the potential energy surface that includes the breaking and forming of chemical bonds. In principle, the most accurate approach is quantum mechanics (QM). A computationally feasible method is the combination of QM with molecular mechanics (MM) [26,27], in which the active site is represented by QM and the remainder of the protein/solvent system is approximated by MM. The combined QM/MM method has the advantage of computational accuracy by treating the reactive part of the system with QM and computational efficiency by approximating the much larger part of the system with a force-field. Furthermore, the electronic polarization of the active site is naturally included in electronic structural calculations. In fact, combined QM/MM methods have emerged as the best approach for studying enzymatic reactions [28].

Another technique that has been extensively explored is the empirical valence bond (EVB) approach [24]. In this case, empirical potentials are used to approximate the potential energy surfaces of the reactant state and the product state, which are also called effective diabatic states. The potential energy surface of the chemical reaction is evaluated by an analytical function corresponding to the lowest eigenstate of the mixture of the diabatic states. As this mixture of diabatic states, together with the diagonalization of the Hamiltonian, is analogous to the QM treatment of the H_2 problem by valence bond theory, the method has the flavor of QM. It should be noted that electronic structure is not explicitly treated (i.e. no wave functions). Although empirical, the EVB method strives for accuracy by fitting three parameters in the Hamiltonian to three experimental observables for the reaction in water, corresponding to the dissociation energies of the reactant (ε_1) and product (ε_2) states, and the free energy of activation (ε_{12}). Thus, the EVB model is used to study the enzyme reaction [24].

Umbrella sampling yields the potential of mean force

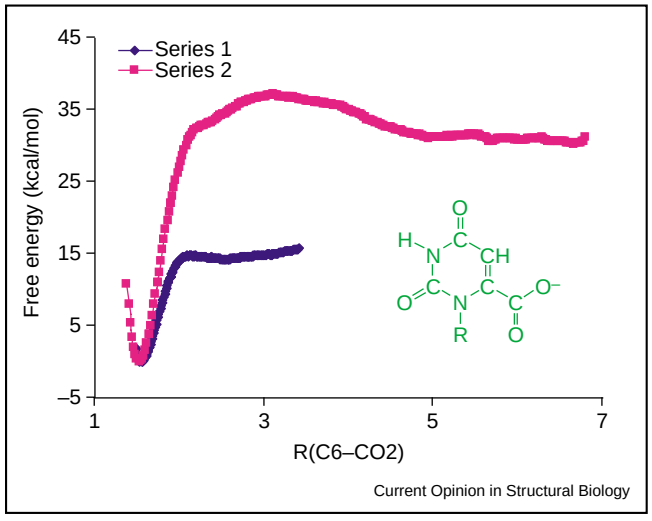
The free energies of activation for the catalyzed and uncatalyzed reactions can be obtained from the corresponding potential of mean force (PMF) along the reaction coordinate. For OMP decarboxylation by ODCase, the reaction coordinate is conveniently defined as the distance between the C6 atom of the pyrimidine ring and the carbon of the carboxylate group of OMP [12••]. The PMF is computed by the umbrella sampling technique, which yields the probability density $\rho(R_c)$ at a given value of the reaction coordinate, R_c:

$$W(R_c) = -RT\ln\rho(R_c) + C \qquad (5)$$

where C is an arbitrary normalization constant, R is the gas constant and T is temperature. In practice, the umbrella sampling calculation is carried out by modifying the potential energy surface with the addition of a biasing potential, which ideally is the negative of the PMF, $W(R_c)$. Therefore, enzyme conformations at high-energy regions, including the TS, can be adequately sampled in molecular dynamics simulations. It is important to note that $W(R_c)$ is the free energy of the system along the reaction coordinate and includes both protein internal energy and protein–substrate interaction energy.

The PMFs for the decarboxylation of an *N*-methyl orotate ion in water and OMP substrate in ODCase have been computed using the combined QM/MM method in Monte Carlo and molecular dynamics simulations (Figure 2) by Wu *et al.* [12••]. A separate calculation has been performed for the reaction of OMP in the gas phase, which yielded a reaction profile similar to that of *N*-methyl orotate in the gas phase, indicating that there is no self-catalysis by the auxiliary phosphoribosyl group, consistent with experiment [29]. The QM method used in these calculations is the semi-empirical Austin Model 1 (AM1) [30], which yields an energy of reaction of 35.5 kcal/mol, in excellent agreement with the value of 36.4 kcal/mol from *ab initio* QM calculations at the second order Moller–Plessett perturbation theory using the 6-31+G(d) basis set [18]. In water, the calculated $\Delta G^{\neq}_{aq}$ is 37.2 kcal/mol, compared with the experimental value of 38.5 kcal/mol [10]. Interestingly, there is little solvent effect on the decarboxylation reaction, which was also

Figure 2



Computed PMF for the decarboxylation of OMP in water and in the enzyme ODCase. The structure shown is the orotate substrate: R = methyl group (*N*-methyl orotate in water; series 2). R = phosphoribosyl (OMP in ODCase; series 1). R(C6–CO2) is the distance between the C6 carbon of the pyrimidine ring and the carboxylate carbon of orotic acid.

noticed by Lee and Houk [18] using a continuum solvation model in *ab initio* calculations. The free energy of activation of the decarboxylation of OMP in ODCase was estimated to be 14.8 kcal/mol, a reduction of 22.4 kcal/mol relative to the uncatalyzed reaction, and is also consistent with experiment (15.2 kcal/mol from the k_{cat}) [10]. This corresponds to a computed rate enhancement of $k_{cat}/k_{aq} = 2.6 \times 10^{16}$, which may be compared with the experimental value of 1.7×10^{17}. Thus, combined QM/MM simulations yield results in agreement with *ab initio* or experimental data for the decarboxylation reaction in the gas phase, in water and in the enzyme ODCase using exactly the same potential energy function [12••].

Using the EVB approach, Warshel *et al.* [31••] also reproduced the experimental free energies of activation for the catalyzed (OMP) and uncatalyzed (orotate ion) reactions. In this work, they made an important contribution to the understanding of the catalytic mechanism of ODCase by analyzing the ionization states of various charged residues in and near the active site. In particular, it was found that the substrate OMP, Asp20, Glu25, Lys42, Asp70, Lys72, Asp75b, Glu78b, Lys82b, His98 and Arg203 are all ionized, which is exactly the same ionization state used in the study by Wu *et al.* [12••].

In analyzing the origin of catalysis, Warshel *et al.* emphasized the importance of comparing the calculated free energy of activation of the enzymatic reaction with that in water. Although this can be difficult in cases in which such a direct comparison is not possible because of differences in the reaction mechanism or because of the formation of a covalent intermediate with active site residues, there is no complication for OMP decarboxylation, which is a unimolecular process. Thus, it is possible to compare the free energies of activation of the reaction in water and in the enzyme, as has been done in the experimental work of Wolfenden and co-workers [10], and the computational study of Wu *et al.* [12••]. Surprisingly, however, Warshel *et al.* [31••] suggested that the lysine residue (Lys72) that is the proton donor to the carbanion intermediate is part of the reacting system and thus should be included as an ion pair in the reference state in water. With this reference state, they obtained ''a different conceptual picture'' of TS stabilization to that when the reacting system is the substrate alone. Although this is an interesting analysis, the unimolecular decarboxylation is the rate-limiting step before the protonation step by Lys72 [32] and the effect of moving one charged residue out of the enzyme on the analysis of the origin of catalysis is arbitrary and not clear. Even when this analysis is adopted, the stabilization energy gained from the interaction between the ammonium ion (i.e. Lys72) and the protein environment would actually be part of the change in the internal conformational energy of the enzyme, not the stabilization energy of the TS of the OMP substrate.

Free energy perturbation gives the intrinsic electrostatic binding free energy

The intrinsic binding free energy of the substrate corresponds to the free energy of transfer from water into the enzyme active site, which is related to the free energies of 'solvation' of the substrate in these two environments. Here, we use the generic term solvation to describe the free energy of transfer from the gas phase into a specific environment. Computationally, the free energy perturbation (FEP) method has been established as an ideal approach for these calculations [33].

$$\Delta G_{sol}^{0\rightarrow 1} = -RT \ln \langle e^{-(V_1 - V_0)/RT} \rangle_{V_0} \quad (6)$$

where $\Delta G_{sol}^{0\rightarrow 1}$ is the free energy difference between state 0 and 1, V_0 and V_1 are the potential energies of the two states, and the brackets $\langle \ldots \rangle_{V_0}$ indicate the ensemble average over the potential surface of state 0. Typically, the calculation of solvation free energy is divided into two steps: firstly, electrostatic charge annihilation, followed by a 'mutation' of the van der Waals spheres of atoms into nothing [33]. The latter term is often related to the free energy of creating a solvent cavity and it makes relatively small contributions to the total free energy of solvation for an ionic molecule. The difference in van der Waals contribution for the substrate in water and in the enzyme would be even smaller, and is generally considered to have minor effects on enzyme catalysis.

The electrostatic component of $\Delta\Delta G_{E.S}^{\neq}$ was obtained by computing the electrostatic free energies of solvation for the substrate and its corresponding TS species in water

Table 1

Computed electrostatic free energies (kcal/mol) for orotate ion in water (w) and in ODCase (p) relative to that in the gas phase (g).

	Reactant state	Transition state
$\Delta G_{sol}^{g\rightarrow w}$	−66.7	−57.5
$\Delta G_{sol}^{g\rightarrow p}$	−48.9	−41.9
$\Delta G_{i}^{w\rightarrow p}$	17.8	15.6
$\Delta\Delta G_{ES}^{\neq}$	0.0	−2.2
$\Delta\Delta G^{\neq}$	0.0	−22.4
$\Delta\Delta G_{P}^{\neq}$	0.0	−20.2

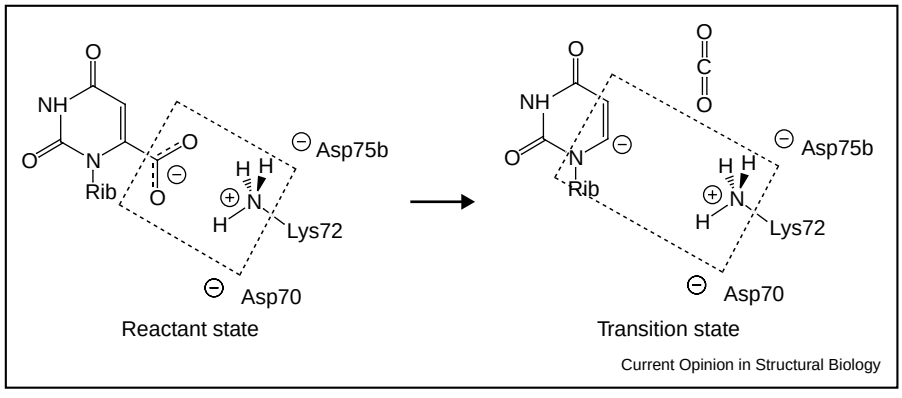
and in the enzyme through FEP simulations [12••]. The calculation for the TS was started using the coordinates from the umbrella sampling simulations, corresponding to the highest point of the PMF. Table 1 summarizes the results, which reveal that the $\Delta\Delta G_{ES}^{\neq}$ term lowers the overall free energy barrier by only 2.2 kcal/mol for the enzymatic reaction compared to the uncatalyzed reaction in water. Therefore, the largest contribution to the reduction of the free energy barrier in the enzyme is not due to enhanced electrostatic interactions between the substrate and the enzyme at the TS. Instead, it originates from the change of enzyme conformation. By rearranging Equation 4, we obtain a $\Delta\Delta G_{PP}^{\neq}$ term of about 20 (22.4–20.2) kcal/mol. We shall return to this point later.

Using the FEP method, Warshel *et al.* [31••] also computed the electrostatic component of binding free energies for the substrate orotate and its distorted TS in water and in ODCase, or $\Delta\Delta G_{sol}^{w\rightarrow p}$. They obtained a binding free energy of −3 kcal/mol for the orotate part of the substrate OMP and −23 kcal/mol for the TS. In addition, the orotate–ammonium ion pair model has a binding free energy of −30 kcal/mol and −47 kcal/mol for its corresponding TS. It was concluded that the difference between their 'binding' results (−17 to −20 kcal/mol) reproduced the catalytic effect of the enzyme [31••], in contrast to the work of Wu *et al.* [12••]. Although this comparison is interesting, there is no particular reason to expect that the reduction in the activation barrier in the enzyme computed from umbrella sampling will be the same as the difference from the computed 'binding free energies' using the FEP method. This is because the change in enzyme conformational energy is included in the PMF in the umbrella sampling simulations, whereas the FEP calculations were performed at the two specific conformation substates, corresponding to the E′S complex for the reactant state and E″S for the TS. The free energy difference between the E′ and E″ conformations is not included in the computed binding free energies. If the difference is significant, the estimated TS stabilization from FEP calculations will be much smaller than the barrier reduction determined from the computed PMFs.

The mechanism of the orotidine 5′-monophosphate decarboxylase reaction

Can a ground state destabilization mechanism be possible for the ODCase-catalyzed reaction? To answer this question, we first examine the possibility of TS stabilization, which can be achieved in many enzymes by changing the electrostatic or hydrogen-bonding interactions between the substrate and the enzyme along the reaction path [34]. In the case of OMP decarboxylation, there is a shift of the anionic charge from the C6 carboxylate group to the C6 carbanion, a change of roughly 2 Å. In order to stabilize the TS, a cationic residue must somehow specifically interact more preferentially with the C6 carbanion than with the carboxylate ion. This would lead to a large

Figure 3



TS stabilization mechanism involving the assistance of Lys72 proposed by Warshel *et al.* [31••]. The authors used the figure to illustrate that the dipole moment of the reacting system is significantly increased in the TS compared to the reactant state, leading to dipolar stabilization by the protein environment [31••].

negative value in the $\Delta\Delta G^{\neq}_{ES}$ term (Equation 4). However, a value of −2.2 kcal/mol was found in FEP calculations, far from its capability to account for the observed total barrier reduction of 22.4 kcal/mol. Therefore, specific electrostatic stabilization of the TS is not supported by FEP calculations.

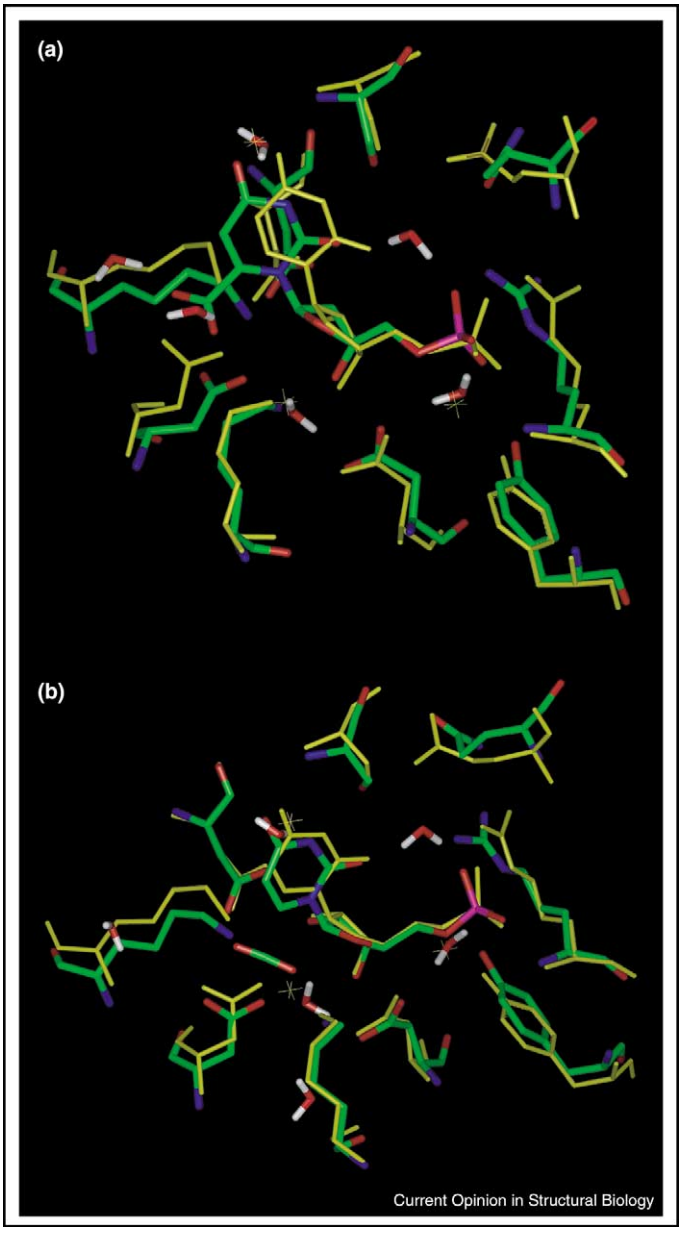
An alternative scenario was proposed by Warshel *et al.* [31••]. They considered that the reactant state has a small dipole moment because the C6 carboxylate charge is neutralized by Lys72, whereas this dipole increases in the TS because the anionic charge is shifted to the C6 atom, further away from Lys72, resulting in stabilization by the environment (Figure 3). Although this appears to be a very attractive mechanism, it would not work if the energy penalty for creating a greater charge separation is included. Assuming that the OMP carboxylate and Lys72 ions are 4 Å apart in the reactant state and 6 Å in the TS, the energy penalty would be about 28 kcal/mol, far greater than the stabilization that can be gained from a dipolar solvation model with any dielectric constant for the active site.

At this point, it is interesting to compare the structures obtained from molecular dynamics QM/MM simulations with the X-ray structure of ODCase complexed with 6-aza-OMP (a TS analog) [12••]. In Figure 4, the computed structures have been superimposed with the X-ray structure by matching the backbone atom positions of residues 30–200 in the first monomer. Clearly, the TS structure from the molecular dynamics simulation closely resembles the X-ray structure, whereas there is a large conformational distortion in the enzyme configuration in the reactant state (Michaelis complex). Most significant are changes of the sidechain positions of residues Lys72, Asp70 and Asp75b, among others, in the range 1.5–2 Å. Notice that the phosphoribosyl group is found essentially in the same position in the X-ray structure for both the reactant state and TS, whereas the orotate ring extrudes forward to minimize electrostatic repulsion with Asp70. Lys72 forms hydrogen bonds with Asp70, Asp75b and the 2′-hydroxyl group in the reactant state. The last hydrogen bond is replaced by interactions with the C6 carbanion in the TS as a result of a 2 Å conformation change. The distance between the sidechain nitrogen atom of Lys72 and the carbonyl group of the substrate is about 4.5 Å in the reactant state, and 2.8 Å to the C6 anion in the TS. This is similar to the change observed by a separate molecular dynamics simulation. This trend (shorter distance between Lys72 and the C6 carbanion at the TS than the distance between Lys72 and OMP carboxylate at the reactant state) is opposite to the ideal, static picture proposed by Warshel *et al.* (Figure 3). Hur *et al.* [21•] also noted that the conformational change of a loop consisting of residues 203–218 may play a catalytic role.

The change of enzyme conformation during the catalyzed reaction process described above has important implications for the reduction of the observed free energy barrier. In fact, it is key to understanding the mechanism of the ODCase reaction. Figure 5 reveals the origin of the extraordinary catalytic power of ODCase. In this figure, the free energy of the apo enzyme and substrate in water has been used as the reference (zero energy), and the internal energy of the substrate has been subtracted because we are concerned only with contributing factors that lower the reaction barrier. The formation of the Michaelis complex has an observed binding free energy of about 8 kcal/mol (from the experimental K_M) [10], which has two contributing factors: the intrinsic binding free energy and the protein conformational distortion energy (red lines). At the TS, the E''–$S^{\neq}$ interaction is enhanced by about 2.2 kcal/mol relative to E'–S (Table 1). Concomitantly, the enzyme becomes more relaxed, with a much smaller distortion free energy (about 20 kcal/mol) than in the E' state. This results in a markedly increased apparent binding affinity for the TS and a net lowering of the observed reaction barrier. Recall that the value of 20 kcal/mol protein distortion energy was obtained from the difference between the computed $\Delta\Delta G^{\neq}$ and $\Delta\Delta G^{\neq}_{ES}$ by rearranging Equation 4. In Figure 5, the reactant state of the enzyme is destabilized in the Michaelis complex and the destabilization energy is subsequently released during the chemical reaction through protein conformational change.

An important criterion for the validity of a catalytic mechanism is testing if it is consistent with and can explain the results of site-directed mutagenesis experiments. The effect of mutating any one of the four charged residues Asp70, Asp75b, Lys42 and Lys72 can be understood because these changes affect the stability and interactions of the active site so dramatically that the enzyme's activity is essentially lost [16]. In an unpublished study, the decarboxylation of OMP in the Asp70Ala mutant was found to have a barrier of 30 kcal/mol, an increase of about 14 kcal/mol compared to the wild-type enzyme (K Byun, J Gao, unpublished data). What is intriguing is that mutations in the phosphate-binding pocket have a remarkable effect on the k_{cat} value, but produce only a marginal change in K_M. For example, the Tyr217Ala mutation (yeast ODCase numbering in the mutation study) increases the barrier height by 4.7 kcal/mol, but the change in binding affinity is about 1 kcal/mol [8•]. This mutation experiment is particularly interesting because Tyr217 is not hydrogen bonded to the orotate group and is not expected to provide a specific stabilization or destabilization contribution to the TS. In fact, Tyr217 is at least 8 Å away from the reactive group. If anything, Gln215 would have a much greater effect on the k_{cat} value because it was found to hydrogen bond to the O2 carbonyl group of the orotate ring. Yet, there is essentially little effect on k_{cat}/K_M (0.8 kcal/mol) [16]. Wu *et al.* predicted that the catalytic rate would be significantly decreased if the OMP substrate was replaced

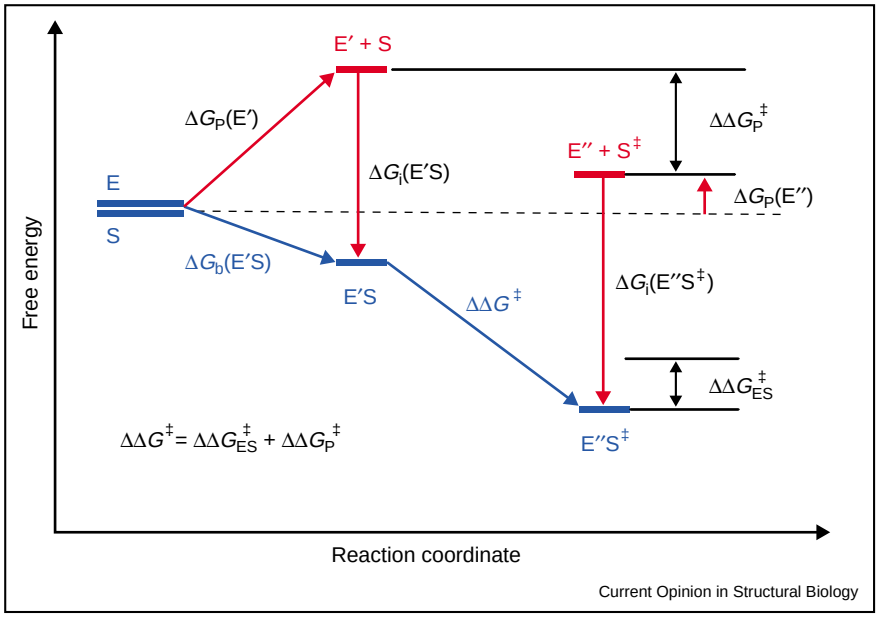
Figure 4



Comparison of the structures of **(a)** the Michaelis complex and **(b)** the TS complex from molecular dynamics QM/MM simulations by Wu *et al*. [12••] with the X-ray structure (yellow) of ODCase complexed with 6-aza-OMP. Both simulation structures, which were a snapshot of the system after at least 200 ps molecular dynamics simulation, were superimposed with the X-ray structure on the basis of the optimal fit of backbone atoms for residues 30–200. (a) The reactant state shows significant sidechain conformational change, distorted away from the X-ray structure, while the substrate also experiences conformational stress. The distortion is widespread, away from and close to the reactive orotate group. Two water molecules were found to form hydrogen bonds with the carboxylate group of OMP in the simulation. (b) The simulated structure of the TS is in remarkable agreement with the X-ray configuration, for both the enzyme and the substrate, suggesting that the protein is less stressed. The two water molecules noted in (a) diffuse away by more than 5 Å. The color scheme for atom representation is as follows: blue, nitrogen; red, oxygen; green, carbon; pink, phosphorus; and white, hydrogen.

by 2′-deoxy-OMP substrate because the 2′-hydroxyl group plays an important role in interacting with Lys72 in the distorted reactant state, according to molecular dynamics QM/MM simulations [12••]. This was confirmed by Miller *et al*. [35••], who showed that the decarboxylation barrier for 2′-deoxy-OMP is 4.6 kcal/mol higher than for OMP, with small binding contributions. According to the TS stabilization mechanism, these mutations are expected to significantly increase the K_M value, but to have little effect on k_{cat}, because the loss of hydrogen-bonding interactions

Figure 5



Schematic illustration of the origin of catalysis by the enzyme ODCase. Red lines indicate the free energy decomposition of Equations 2 and 3 that separates the binding free energy into a protein distortion term and an intrinsic substrate–protein interaction component. The figure shows that the enzyme has a smaller distortion energy than in the reactant state, so the change in enzyme conformational energy provides the predominant contribution to the lowering of the activation free energy of the catalyzed reaction.

remains the same in both the reactant state and TS of the reaction. The expectation is inconsistent with experimental observation.

The mechanism illustrated in Figure 5 is fully consistent with the mutation experiments. Although both amino acid and substrate mutations result in loss of hydrogen bonds, the weakened substrate–enzyme interactions lead to smaller enzyme distortion. These two effects compensate each other, resulting in a small net effect on the observed binding energy. However, because the enzyme is less distorted in the reactant state of the mutant, the amount of energy released during the chemical reaction is smaller. Therefore, the k_{cat} value is significantly reduced compared to the K_M.

Conclusions

In summary, we propose that substrate binding induces an electrostatic stress in the enzyme, which is compensated by intrinsic enzyme–substrate interactions. Large protein distortions can result from unfavorable electrostatic interactions between the enzyme and a small part of the substrate, although the overall binding interaction is still favorable. As the decarboxylation of OMP occurs in the enzyme ODCase, the protein conformation becomes less distorted at the TS in response to the charge reorganization of the substrate, releasing the reactant state electrostatic stress. Although the substrate is not particularly more stabilized by the enzyme at the TS than at the reactant state, the change of enzyme conformational energy helps to reduce the overall free energy of activation. This mechanism is consistent with the experimental findings that mutations distant from the active site can have large effects on k_{cat}, but rather a small influence on K_M because of the compensating factors of protein distortion and substrate intrinsic binding interactions.

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