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Protein Motions Promote Catalysis

Minireview

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A relationship between molecular dynamics motions of noncatalytic residues and enzyme activity has recently been proposed. We present examples where mutations either near or distal from the active site residues modify internal enzyme motion with resulting modification of catalysis. A better understanding of internal protein motions correlated to catalysis will lead to a greater insight into enzyme function.

How does enzyme structure contribute to catalytic power [1]? A special interest has recently emerged in the area of internal protein motions, stemming from studies suggesting an intimate relation between molecular motion and enzyme activity. The majority of studies regarding the influence of internal enzyme motion on reactivity, independent from the event of ligand binding, have been undertaken by direct modification of catalytic residues within the active site to study short-range effects. The "distal environment," which we will consider to be outside of the immediate area defined by the catalytic residues, is generally neglected, although a growing body of evidence indicates that it can be required in defining the body of molecular dynamics that governs catalysis. This new outlook on the impact of the distal environment toward enzymatic reactivity has been made possible as a result of more efficient technologies, particularly molecular dynamics simulations.

The enzyme active site generally corresponds to a fairly restricted location in the protein. Its catalytic residues can create new intermolecular interactions when in proximity with the substrate. Mutating catalytic residues generally results in a dramatic loss of activity, as classically illustrated by mutation of any residue constituting a catalytic triad [2]. Mutation of catalytic residues is more frequently practiced than mutation of noncatalytic residues because it is statistically easier to identify a residue of importance to catalysis among the small number present at the active site than in the large number that constitute the distal environment. The catalytic residues may directly contact the reactive substrate atoms or may participate in reactivity without direct contact. For example, the proton-relay mechanism of catalytic triads relies on residues (Asp, His) that are a few angstroms distant from the reactive substrate atoms. However, is there a catalytic role for residues that are not directly implicated in the chemical transformation, belonging to what we will refer to as the "distal environment?" Can residues that are located too far from the site of chemical transformation to participate directly in catalysis modulate catalysis otherwise?

Some recent studies on unrelated enzymes have revealed mutations of distal residues (i.e., residues that are not directly implicated in the catalytic mechanism) that have effects on enzymatic catalysis without interacting directly with the substrate. Instead, their effect is a consequence of modified internal dynamics of the enzymes in question. The internal motions were modified by specific mutations, producing different subsets of conformations that can be attained by the mutants relative to the native enzymes. These conformations are sampled through time until those resembling the transition state are achieved, allowing catalysis to proceed. Thus, varying internal protein motions by mutation can affect the probability of attaining the transition state. Although the mutations were of noncatalytic residues, they affect catalysis because they result in conformational and dynamic changes that have repercussions on the active site.

Time scale must be considered when studying internal enzyme motions, as different types of motions occur on time scales that may or may not be relevant to the reaction of interest. We will demonstrate, providing specific examples from four systems, that motions of certain noncatalytic residues can help define the preorganization of enzyme active sites and the transition-state stabilization [3]. These initial studies suggest that we must explore a large ensemble of internal fluctuations that participate in global enzyme molecular dynamics to comprehend the influence of dynamics on catalysis.

"Molecular dynamics" is a combination of all three-dimensional motions in a molecule throughout time. Protein motions occur on a time scale ranging between 10^{-15} to 10^{4} seconds and can cover amplitudes ranging between 0.01 and 100 Å with an energy variation of 0.1 to 100 kcal/mol [4, 5]. Three classes of internal motions have been identified in proteins. The smallest, local motions (10^{-15} to 10^{-1}s), include atomic fluctuations, side chain motions, loop motions and terminal arm motions [4]. Rigid-body motions (10^{-9} to 1s) constitute the second type of internal motions, where a small part of the protein moves in relation to another [4]. These motions are responsible for altering the height of the activation free energy barrier at the transition state [6]. They include helix motions, domain motions, and subunit motions. Finally, large-scale motions are similar to rigid-body motions but occur on a greater time scale (10^{-7} to 10^{4}s) [4, 5]. Helix-coil transitions, dissociation/association, coupled structural changes, opening/distortional fluctuations, and folding/unfolding transitions are examples of large-scale motions. All of these internal motions influence protein organization.

The enzymatic reaction rate is directly influenced by the height of the activation free energy barrier and by the transmission coefficient (Figure 1). Internal enzyme motions can modify the catalytic rate in two distinct ways. First, by influencing the height of the activation free energy barrier (Figure 1A), which implies a modification of the equilibrium between the transition state and the reactants and products. Second, by influencing the capacity of recrossing the barrier (Figure 1B), which

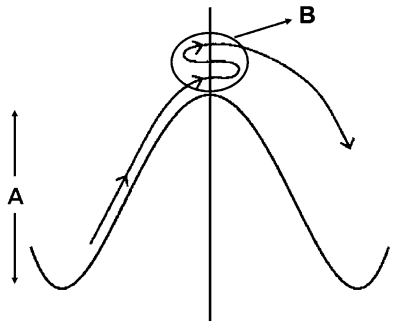

Figure 1. The Free Energy Profile of an Enzymatic Reaction

"A" represents the height of the activation free energy barrier, and "B" represents barrier recrossing events, which are characterized by the transmission coefficient (κ). (Adapted from [6].)

is characterized by the transmission coefficient (κ) [6]. These concepts have been thoroughly discussed in a recent review [6], the details of which are beyond the scope of this review. We briefly present them below so as to introduce the concepts required for the following discussion.

Promoting motions, which represent conformational changes occurring on the time scale of the overall reaction along the reaction coordinate, modify the height of the activation free energy barrier. Dynamical motions, on the other hand, occur on the femtosecond time scale and specifically influence the transmission coefficient (κ). These motions composed of local, rigid-body, and large-scale motions contribute in different ways to the catalytic rate. The reaction rate is defined as $k_{dyn} = \kappa k_{TST}$, where k_{dyn} is the overall rate constant, k_{TST} is the equilibrium transition-state theory rate constant (which accounts only for crossing of the free energy barrier), and κ is the transmission coefficient (which accounts for barrier recrossing). k_{TST} is defined by

$$k_{TST} = \left(\frac{k_B T}{h}\right)^{(-\Delta G‡/k_B T)},$$

where h is Planck's constant, k_B is Boltzmann's constant, $\Delta G‡$ is the activation free energy barrier obtained from energetic profiles, and T is the temperature of the system [6].

From these equations, we observe that a variation of free energy at the transition state $\Delta G‡$ affects the reaction rate exponentially, while the transmission coefficient κ, which is responsible for barrier recrossing, has an effect only as a prefactor. Therefore, promoting motions (occurring on the time scale of the reaction) should have a greater impact than dynamical motions (fs time scale) on enzymatic activity [6]. However, all of these types of motions, whether of small or large amplitude or frequency and whether involving few noncatalytic residues or entire segments of the enzyme, may influence catalysis. We present recent studies on the influence of motions of noncatalytic residues on catalysis that have been performed on four enzymes: dihydrofolate reductase (DHFR), triosephosphate isomerase (TIM), liver alcohol dehydrogenase (LADH), and β-lactamase (TEM-1), which are all structurally and functionally unrelated. While a wealth of research focuses on these well-characterized enzymes, we have identified a restricted number of research papers that specifically relate motions of noncatalytic residues to catalysis in each of these enzymes. We now examine them as being the first examples in a new approach to a more detailed understanding of catalysis, which are likely to serve as precursors to further similar observations in many other enzymes in the future.

Dihydrofolate Reductase

Dihydrofolate reductase is one of the best-characterized enzymes from a structural and a functional point of view. Crystallographic analyses have demonstrated that *E. coli* DHFR adopts different conformations during catalysis, which result from a subtle internal protein motion implicating an important fraction of the enzyme [7].

The meticulous work of Benkovic and Hammes-Schiffer has related this important internal protein motion to catalysis [3, 7]. Mutagenesis studies showed that two major loops (Met-20 loop and βF-βG loop; Figure 2) containing conserved but noncatalytic residues are the most affected by this internal motion. Their studies reveal a network of coupled promoting motions, each of small amplitude (0.5 Å), existing throughout the enzyme, which altogether modify the height of the activation free energy barrier [7]. Their effect on catalysis is the consequence of a network of hydrogen bonds, present between the loops, that induce a motion throughout the enzyme. The motion leads to the formation of many structural conformations including those most similar to the transition state conformation. Consequently, these distal motions of noncatalytic residues influence catalysis in DHFR by long-range structural perturbations whose effect is transmitted to the active site [8]. Let us review the evidence that allowed the link between distal motions and catalysis to be established.

DHFR catalyzes the reduction of 7,8-dihydrofolate (DHF) to 5,6,7,8-tetrahydrofolate (THF) using nicotinamide adenine dinucleotide phosphate (NADPH) as the reducing cofactor. NMR analyses of *E. coli* DHFR have demonstrated that the Met-20 loop, constituted by residues 9 to 24 (Figure 2), can adopt three different conformations: open, occluded, or closed, depending on the nature of the bound substrate [8]. When DHF and NADPH are bound, the Met-20 loop adopts a closed conformation, resulting in a strong interaction between the loop and NADPH, allowing hydride transfer to occur [7]. In this form, a more rapid (ns to ps time scale) dynamic motion in the βF-βG loop (residues 117–131; Figure 2) is proposed to be important for hydride transfer (ms time scale), although it contains no catalytic residues [3]. Its importance was demonstrated by mutagenesis of the conserved Gly121, located 12 Å from the site of hydride transfer, to Val. The mutation decreased the rate of hydride transfer by a factor of 163 because of an increase in the activation free energy barrier [9].

Further investigation by molecular dynamic (MD) simulations identified important hydrogen bonds. A hydrogen bond between Gly15 (Met-20 loop) and Asp122 (βF-βG loop) has been observed only in the closed conformation, leading to the suggestion that the closed conformation, stabilized by the Gly15-Asp122 hydrogen bond, favorably positions DHF and NADPH proximal to

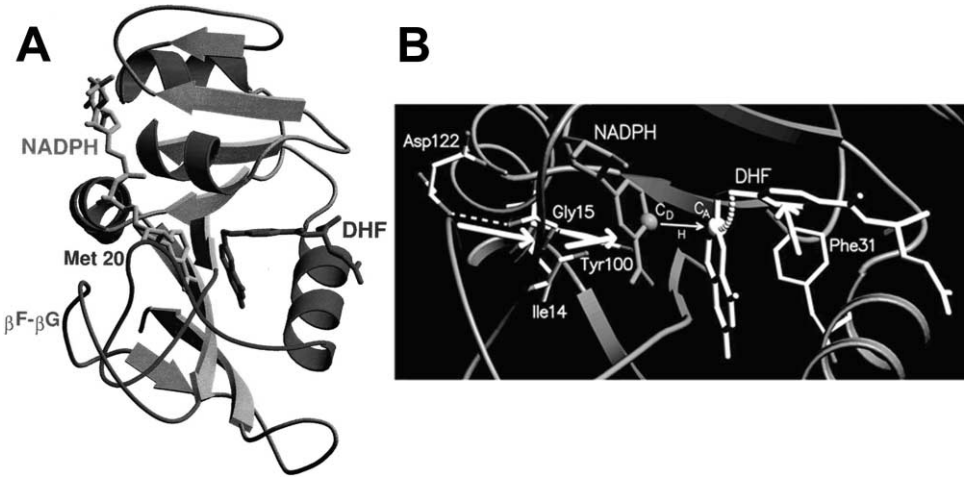

Figure 2. Internal Protein Motions in Dihydrofolate Reductase

(A) Three-dimensional structure of *E. coli* DHFR [9].

(B) A portion of a network of coupled promoting motions in DHFR. Only the active site region is shown, although the proposed network extends throughout the enzyme. Arrows and a dotted arc indicate one potential motion of DHFR that may be conducive to catalysis of hydride transfer. [27].

each other to initiate the reaction [7]. This hypothesis was verified by NMR, demonstrating that conformational changes of the Met-20 loop control substrate positioning in a manner that is critical for hydride transfer. It thus appears that the juxtaposition of DHF and NADPH in a catalytically productive arrangement is modulated by a change in protein motion provoked by contacts distal from the active site.

To confirm the importance of hydrogen bonds between the Met-20 and βF-βG loops, catalytic parameters were determined. Replacement of residues 16–19 in the Met-20 loop by only one Gly decreased substrate binding only 10-fold but decreased the rate of hydride transfer 400-fold due to a global alteration of the motion of both loops as detected by NMR [3]. Also, mutagenesis of Asp122 revealed a strong correlation between the presence of hydrogen bonds and the rate of hydride transfer [10]. These results demonstrate that the motion of a noncatalytic residue such as Asp122, which is located far from the active site (~8 Å) (Figure 2), can influence enzymatic activity by affecting protein motion. It is interesting to note that several of these distal residues are highly conserved, just as are catalytic residues.

As a result of these studies, it was suggested that a network of coupled promoting motions is responsible for the internal dynamics of *E. coli* DHFR. The network includes the Met-20 and the βF-βG loops, but extends yet farther from the active site and is comprised of many small amplitude motions (~0.5 Å). Kinetic analysis of double mutants of other distal, noncatalytic residues has shown a nonadditive effect on activity, further supporting the hypothesis of coupled promoting motions in catalysis. This is the case for Gly121-Met42 double mutants that are 20 Å apart and Gly67-Gly121 double mutants that are approximately 30 Å apart. These mutations are spatially separated and distal to the active site yet enhance each other's effect on activity relative to the effects of the individual mutations [7–9] More recently, MD simulations were undertaken by Rod, Rodkiewicz, and Brooks, III, to visualize the effect of the proposed coupled motions on catalysis. Mutants M42F, G121S, G121V, and M42F/G121S were constructed in silico. Correlated motions, conformational changes, hydrogen bonds, and nonadditive effects were specifically studied. Their work confirms that mutation of noncatalytic residues can affect correlated motions and coupling of many structural elements throughout DHFR by long-range interactions and further points to internal motions implicating noncatalytic residues that can have an important effect in enzyme catalysis. This body of work reveals the importance of understanding the dynamic makeup of an enzyme to better understand enzymatic catalysis.

Triosephosphate Isomerase

Triosephosphate isomerase catalyzes the isomerization of dihydroxyacetone phosphate (DHAP) to D-glyceraldehyde 3-phosphate (GAP). TIM (from chicken, yeast, and trypanosome sources) controls active site access via the large-scale motion of a loop [11]. This conserved loop of 11 residues (residues 166–176) behaves like a "lid" to the active site (Figure 3A). TIM thus has two forms, an open and a closed form, which differ by an ~12 kcal/mol energy difference and by a local internal movement of 7 Å of loop 166–176 [12, 13]. The amplitude of movement can be deduced by comparing the crystal structures of the two forms: the distance between Gly173 and Ser211 is 9.77 Å in the open form and 2.7 Å in the closed form, while the distance between Ala176 and Tyr208 is 5.05 Å in the open form and 2.9 Å in the closed form.

The potential role of noncatalytic residues of TIM in catalysis was examined by NMR following the observation that the time scale for the conformational transition of loop 166–176 correlated well with the measured rate of transformation of DHAP to GAP [12]. Transition between the open and the closed conformations occurred at a rate in the range of 10^3 to 10^4 s^{-1}, consistent with the turnover rate of 0.9×10^4 s^{-1} [12, 14]. Further, more

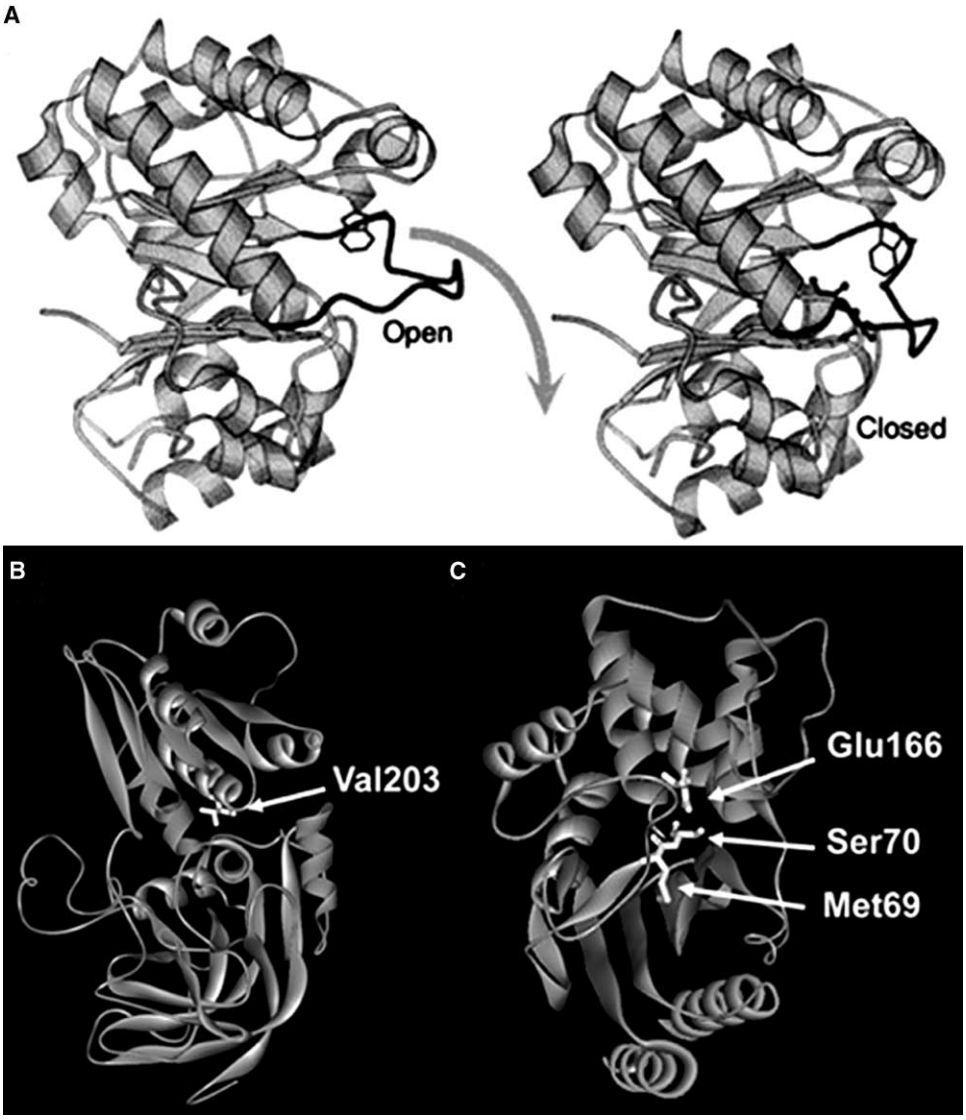

Figure 3. Noncatalytic Residues Proposed to Promote Catalysis via Protein Motions in Three Further Enzymes

(A) TIM active site accessibility is controlled by movement of loop 166–176. The open and closed forms of TIM are illustrated [12].

(B) Three-dimensional structure of LADH showing the central active-site cleft and position 203 [28].

(C) Three-dimensional structure of *E. coli* TEM-1 β-lactamase [29].

specific MD analyses have demonstrated that noncatalytic residues present at the active site (Ala176 and Tyr208) are essential for enzyme reactivity [11]. The hydroxyl group of Tyr208 forms an essential hydrogen bond with the amide nitrogen of Ala176 during loop closure. Closure appears to stabilize the charged intermediate by preventing water molecules from accessing the active site and also prevents elimination of phosphate [11, 15].

The most important catalytic residue is Glu165, which promotes proton transfer to produce GAP. MD studies have demonstrated that Glu165 follows the motion of loop 166–176. When the enzyme is in its free form, Glu165 hydrogen bonds with Ser96; the bond is broken upon substrate binding [12]. Hence, the loop-closing mechanism may be induced by substrate binding, breaking the hydrogen bond and allowing Glu165 to move 2 Å to interact with the substrate. In addition, the hydrogen bond formed between Ala176 and Tyr208 further stabilizes this closed conformation. Recent studies concerning loop motions in TIM also propose that the open conformation compensates for the loss of intramolecular hydrogen bonds (Ala176-Tyr208, Glu129-Trp168, and Ser211-Gly173) by forming new intramolecular (Glu165-Ser96) and intermolecular hydrogen bonds with solvent molecules. The solvent/enzyme hydrogen bonds considerably lower the barrier to transition and thus the energetic difference to transit from the closed to the open form [11]. A more comprehensive model of TIM-mediated catalysis at an atomic level has recently been published by Guallar et al. [16]. The relation between motion and catalysis documented in TIM may represent a model for similar large-scale, hinge-type motions in other enzymes that have been suggested,

generally on the basis of crystallographic information, to be related to catalysis, although the correlation of motion to catalysis has not been established [17, 18]. TIM is therefore a further example demonstrating that protein dynamics generated by noncatalytic residues enhance the probability of attaining the transition state [11, 12].

Liver Alcohol Dehydrogenase

Horse liver alcohol dehydrogenase represents a further example of an enzyme where the motion of a noncatalytic residue, Val203, has a significant influence on reactivity. LADH is a Zn^{2+}-dependent metallo-enzyme that catalyzes the reversible oxidation of various alcohols to their corresponding aldehydes or ketones, using NAD^+ as the hydride acceptor. Hammes-Schiffer has reported on the motion of Val203, which is located 5 Å from the reactive center and the hydride-acceptor C_4 of NAD^+ [6], within the active-site cleft yet on the face opposite the catalytic residues (Figure 3B). By dynamics simulations, the distance between Val203 $C\gamma$ and C_4 of NAD^+ was shown to increase as the transition state was approached. This is the result of a thermally averaged promoting motion, proposed to significantly decrease the activation free energy barrier to the reaction. The motion of Val203 appears to favor the approach of the hydride-donating carbon (on the alcohol being oxidized) and the acceptor (C_4 of NAD^+) via steric interactions. This proposed role correlates well with the decrease in reaction rate observed upon mutation of Val203 to the smaller alanine. The local motion of Val203 is of small amplitude ($\sim$0.6 Å), and thus its investigation may be limited essentially to molecular modeling approaches. The case of LADH further illustrates how enzyme motions by noncatalytic residues may promote catalysis.

β-*Lactamase*

β-lactamases protect bacteria from the lethal effects of β-lactam antibiotics by hydrolyzing the amide bond of their β-lactam ring. Class A β-lactamases are active-site serine peptidases [19, 20]. The mechanism of β-lactamase turnover, which is not fully understood, comprises a first step of enzyme acylation via the nucleophilic attack by the active-site serine hydroxyl group (Ser70; numbering according to *E. coli* TEM-1 β-lactamase). In the deacylation step, an oxygen atom of Glu166 deprotonates a water molecule to provide a free hydroxyl group that is positioned to attack the carbonyl of the cleaved β-lactam, resulting in product release. To return to the fully active state, the Glu166 proton appears to be transferred to the oxygen of Ser70 [19]. Hence Glu166, which is located on the Ω-loop (residues 163–178) appears to act as the general base catalyst in deacylation [21]. Clavulanate, sulbactam, and tazobactam are three mechanism-based inhibitors of TEM-1 β-lactamase. They react with the active site serine, creating long-lived acyl-enzyme intermediates and thus inactivate the enzyme.

The correlation between function and motion in noncatalytic residues in β-lactamase is more subtle than in the previous examples because the noncatalytic residue of interest (M69) is the immediate neighbor of the catalytic Ser70 (Figure 3C). Furthermore, the role of motion in the neighboring residue has been implicated in ligand discrimination (binding function) rather than in the catalytic mechanism. The mutation Met69Leu in TEM-1 gives rise to TEM-33 β-lactamase, an inhibitor-resistant form of TEM-1. The dissociation constant for the acylation complex is slightly greater in TEM-33 than in the native TEM-1 [22]. Therefore, the probability of forming the preacylation complex between an inhibitor and the enzyme is reduced, resulting in resistance. The contribution of the mutation M69L to the loss of affinity toward inhibitors is not intuitive because residue 69 does not directly interact with the bound inhibitors but points away from bound ligand. Only small changes in van der Waals and electrostatic energies were observed as a result of this mutation [22]. The protein backbone and the side chain conformations are positioned almost identically in TEM-1 and TEM-33 forms (the rmsd for the main chain is 0.57 Å and for all protein atoms is 0.72 Å). Nonetheless, the activities of both enzymes diverge importantly with respect to inhibitor specificity.

Because residue 69 is not directly implicated in the substrate binding, it was suggested that it could influence ligand specificity by provoking active site fluctuations (correlated motions) via other residues or by provoking a general alteration of the protein motion [22]. Consequently, MD simulations of both TEM-1 and TEM-33 were performed. Mobashery and coworkers identified two regions where the molecular dynamics of TEM-33 differ from the wild-type TEM-1: the Ω-loop (residues 163–178) and the L1 loop (residues 96–108). In both enzymes, large fluctuations are observed for the first nanosecond of simulation, but after 500 ps a greater conformational change is observed in the Ω-loop of TEM-33 than TEM-1. A more pronounced deviation was also observed in loop 96–108. The different conformations result in a small energetic increase (1.9 ± 0.2 kcal/mol) for the formation of the preacylation complex in TEM-33 relative to TEM-1, which appears to be responsible for the difference in ligand discrimination [22].

Since the side chain of residue 69 points away from the active site, β-lactamase constitutes a further example where a noncatalytic residue that is not directly involved in ligand binding affects protein motion with ensuing effects on enzyme function [22].

In DHFR, TIM, LADH, and β-lactamase, the internal motions demonstrated to be implicated in enzyme function are either of the local or of the large-scale type. Different amplitudes of motions are observed in the four models, the greatest being observed in TIM. Hydrogen bonds between loops or domains were recurrently found to be an important factor in induction of motion and result in accessing many different structural conformations, which may stabilize the transition state or may result in a conformational sampling that facilitates reaching a conformation that is similar to the transition state. Hence, the enzymatic organization defined by noncatalytic residues can be an important contributor to catalysis. Although the correlation between the motions and catalytic activity in β-lactamase and LADH is not yet as well established as in DHFR and TIM, it is becoming increasingly clear that protein motions other than by active-site residues influence protein conformation and dynamics, which may then have important effects in catalysis. In the above examples, specific noncatalytic residues of the Met-20 and the βF-βG loops for DHFR,

of loop 166–176 for TIM, or of residues near the active site (Val203 for LADH and M69 for β-lactamase) all appear to contribute to catalysis by their contribution to protein dynamics. These noncatalytic residues, like the catalytic ones, are also generally evolutionarily conserved. Therefore, by comparing homologous enzymes, it may be possible to propose noncatalytic residues for investigation with respect to their potential for promoting catalysis via protein motions. New methodologies continue to promote advances in the field, including NMR methodologies identifying correlated motions and a computational method to identify correlated motions that create promoting vibrations and may be important for catalysis [23, 24].

Significance

The objective of this review is to illustrate the importance of residues other than catalytic ones in enzyme catalysis. In all cases presented, the first indications of the coupling effect were made evident by mutagenesis studies. Recent advances in molecular modeling now also allow for better characterization of internal motions independent from a conformational change triggered by ligand binding in these enzymes. This approach is very useful to replicate various conditions, mutations, and ligands to evaluate the dynamic behavior of enzymes. While an experimental approach is necessary to confirm hypothetical models created by modeling, the information obtained by modeling is proving to be instrumental in achieving a greater understanding of enzyme catalysis. Although domain motions in proteins are progressively better characterized, there are still few enzymes where correlated motion promoting catalysis has been formally demonstrated. Human lactate dehydrogenase and soybean lipoxygenase-1 are two further well-characterized models [25, 26]. As more enzymes become better characterized by these approaches, the important contribution of the dynamics of the entire protein toward catalysis will be increasingly better understood.

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