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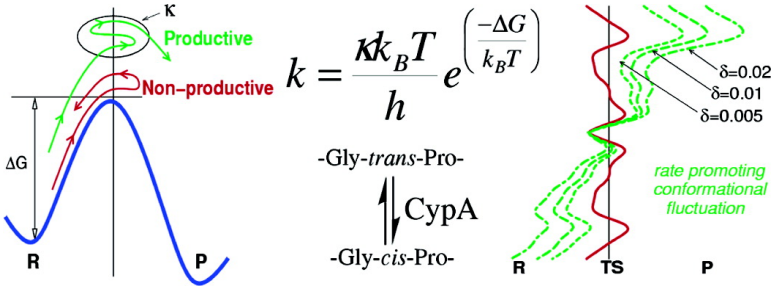

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Role of Protein Dynamics in Reaction Rate Enhancement by Enzymes

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Abstract: An integrated view of protein structure, dynamics, and function is emerging, where proteins are considered as dynamically active assemblies and internal motions are closely linked to function such as enzyme catalysis. Further, the motion of solvent bound to external regions of protein impacts internal motions and, therefore, protein function. Recently, we discovered a network of protein vibrations in enzyme cyclophilin A, coupled to its catalytic activity of peptidyl-prolyl cis−trans isomerization. Detailed studies suggest that this network, extending from surface regions to active site, is a conserved part of enzyme structure and has a role in promoting catalysis. In this report, theoretical investigations of concerted conformational fluctuations occurring on microsecond and longer time scales within the discovered network are presented. Using a new technique, kinetic energy was added to protein vibrational modes corresponding to conformational fluctuations in the network. The results reveal that protein dynamics promotes catalysis by altering transition state barrier crossing behavior of reaction trajectories. An increase in transmission coefficient and number of productive trajectories with increasing amounts of kinetic energy in vibrational modes is observed. Variations in active site enzyme−substrate interactions near transition state are found to be correlated with barrier recrossings. Simulations also showed that energy transferred from first solvation shell to surface residues impacts catalysis through network fluctuations. The detailed characterization of network presented here indicates that protein dynamics plays a role in rate enhancement by enzymes. Therefore, coupled networks in enzymes have wide implications in understanding allostericity and cooperative effects, as well as protein engineering and drug design.

Introduction

Enzymes are highly efficient catalysts;[1] a number of theories have been proposed to explain the reaction rate enhancement achieved by enzymes.[2,3] For more than a century, enzymologists have known the importance of structure; the "lock-and-key" and "induced-fit" models suggested that function depends on direct structural interaction between the enzyme and the substrate.[4,5] Increasing evidence, however, continues to reveal that proteins are dynamically active assemblies with a link between structure and dynamics. The range of time scales of dynamical events that occur in enzyme complexes and the time scales for substrate turnover steps catalyzed by enzymes are similar, raising the question of whether they are interrelated or not.[6] Protein dynamics[7] has been implicated in many aspects of enzyme function including substrate/cofactor binding and release. Evidence linking protein dynamics and the substrate turnover step also continues to grow.[6,8−12] Moreover, studies have also linked dynamical motions of solvent molecules on the exterior of a protein complex with enzyme function.[13−15]

A variety of internal protein dynamics events occur on the femtosecond to second time scales;[6,9,16] experimental investigations have already revealed a wealth of information for events occurring at select time scales. Techniques including neutron and X-ray scattering,[17,18] NMR spectroscopy,[8,19,20] X-ray crystallography,[21] and hydrogen−deuterium exchange[22] continue to provide fascinating details about movement of different parts of proteins. Enzyme kinetics and mutation studies have provided compelling evidence linking protein dynamics to the substrate

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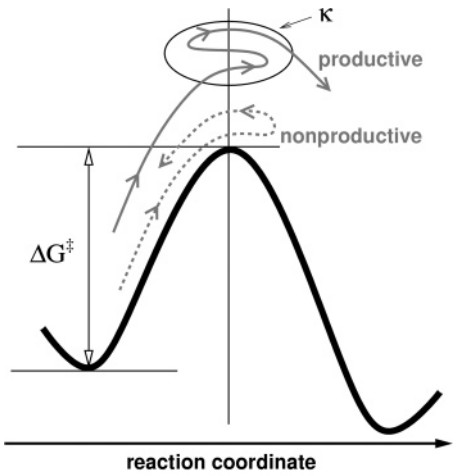

$$k_{TST} = \left(\frac{k_B T}{h}\right) \exp\left(\frac{-\Delta G^{\ddagger}}{k_B T}\right)$$

$$k = \kappa k_{TST}$$

Figure 1. Schematic illustration of free energy profile for an enzymatic reaction. Protein dynamics can influence reaction rates in two possible ways; by altering the height of the activation free energy barrier ($\Delta G^{\ddagger}$) and transmission coefficient (κ).

turnover step.[10,11,23] Further, Mössbauer and neutron scattering experiments have also indicated that bulk-solvent and hydration-shell fluctuations control protein dynamics and function.[13−15] Obtaining much needed collective information on multiple time scales, using experimental techniques, however, still remains a challenge.

Theoretical and computational modeling of protein dynamics and enzyme kinetics is complementing the knowledge gained from experimental studies;[24−36] two recent reviews overview numerous theoretical investigations that continue to provide valuable insights into the detailed mechanisms of reactions catalyzed by enzymes.[6,37] Theoretical investigations of enzyme catalyzed reactions are based on some variation of the generalized transition state theory (TST);[6,33,37] like other condensed phase reactions, an enzyme catalyzed reaction can be expressed in terms of a free energy profile (FEP). In the TST framework, enzymes can influence reaction rates by decreasing the activation energy barrier (see Figure 1). It has also been suggested that enzymes, which are dynamical systems, can have an impact on reaction rates by altering the active site environment such that more trajectories become productive after successful barrier crossing.[33,37] Figure 1 illustrates the behavior of two reaction trajectories close to the transition state (TS). The first trajectory crosses the TS but returns to the reactant side (nonproductive), while the second trajectory crosses the barrier several times before reaching the product state (productive). The transmission coefficient (κ) is a prefactor which corrects the TST reaction rate for the number of barrier recrossings. Theoretical modeling by Truhlar, Gao, and co-workers[32,37,38] and Hammes−Schiffer and co-workers,[6,25,30,33] and others have estimated transmission coefficients for a number of enzyme reactions.

How do dynamical events within an enzyme complex alter reaction trajectories and reaction rates? The relationship between dynamics and the transmission coefficient has been investigated in the interesting work of Kramers and other supporting analyses.[39,40] These investigations describing the dynamical effect as "friction", indicated that at low friction the transmission coefficient is proportional and at high friction it is inversely proportional to the friction coefficient.[41] For a more direct link, important clues are available from recent theoretical studies of reactions in small chemical systems; addition of small amounts of kinetic energy (KE) into select vibrational modes increases TS barrier recrossings.[42,43] This approach, known as the dynamic reaction path (DRP) method,[44,45] indicated that if sufficient energy is added to vibrational modes coupled to the reaction, trajectories quickly become productive. For enzymes, it has been suggested that correlated motions may be involved in increasing the frequency of barrier crossing.[46] It has also been noted that in some enzymes the contribution of transmission coefficient to overall rate enhancement may be small.[37]

With the aim of investigating the link between protein dynamics and reaction rate enhancement by enzymes, we recently identified a network of protein vibrations in enzyme cyclophilin A (CypA) coupled to its activity of catalyzing peptidyl-prolyl cis−trans isomerization (see Figure 2).[26] Extending from surface loop residues of the enzyme all the way to the active site, this network was first discovered during catalysis of small peptide substrates by CypA and later confirmed during the catalysis of the N-terminal domain of capsid protein (CA^N) from human immunodefiency virus type 1 (HIV-1), a biologically relevant substrate.[26,27] The dynamical events in the network occur on time scales ranging from picosecond to microsecond−millisecond (time scale of the reaction). Genomic and structural analyses indicated that the residues and hydrogen bonds forming crucial points in the network are conserved in several cyclophilin structures of species ranging from bacteria to human. NMR relaxation studies have observed motions in some of these residues coupled to the substrate turnover step.[19] The proposed importance of the dynamical events in this network lies in their ability to impact catalysis by changing the crucial interactions between the CypA residues and substrate.[26] Previously, another network of coupled motions promoting hydride transfer in

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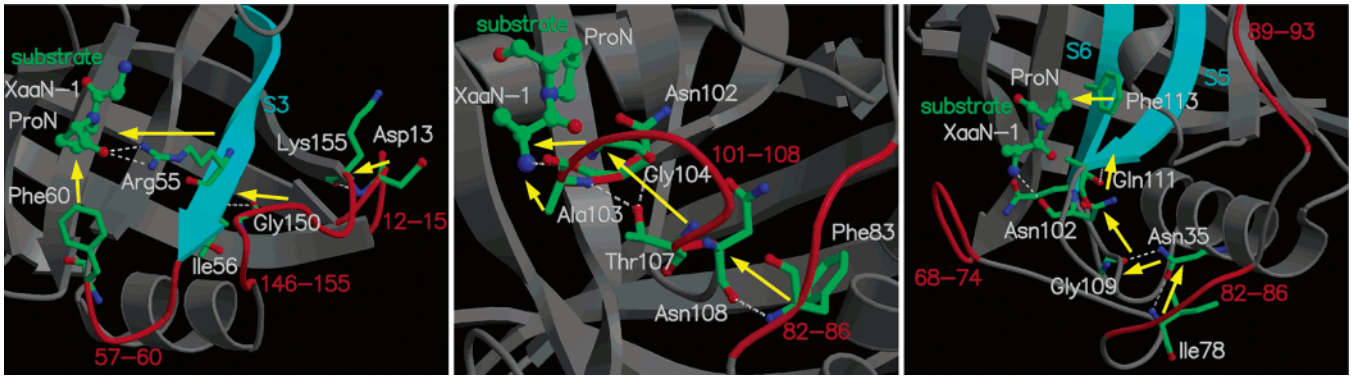

Figure 2. A network of coupled protein vibrations promoting catalysis in cyclophilin A.[26] Loops colored in red and residues indicated by ball-and-stick show largest displacements in vibrational modes coupled to the substrate turnover step. The yellow arrows represent the network pathway from outside of the enzyme to the active site. Reprinted with permission from Agarwal et al. *Biochemistry 2004, 43*, 10605−10618. Copyright 2004 American Chemical Society.

dihydrofolate reductase has been identified by Agarwal et al.[24] However, the detailed understanding of the role these networks play in rate enhancement during enzyme catalysis, specifically the effect on activation free energy barrier ($\Delta G^{\ddagger}$) and TS barrier crossings (κ), remains limited. Obtaining estimates of transmission coefficient for an enzyme-catalyzed reaction from experimental techniques still remains a difficult task. Further, experimental investigations of the link between protein dynamics and dynamical barrier crossing are even more challenging.

This report describes investigations of the relationship between dynamical modes in proteins to enzyme rate enhancement, using the CypA−CA^N complex as a prototypical system. A new approach is designed that allows addition of kinetic energy to a specific dynamical mode of protein. The behavior of trajectories in real time near TS was monitored after KE was added to protein vibrational modes from the network of protein vibrations. Results provide insights into how dynamical fluctuations in the active site environment cause reaction trajectories to surmount the barrier, therefore, leading to productive trajectories. The transfer of energy between the solvent and enzyme at the surface has also been investigated. Together these results provide new insights into role of internal protein dynamics and solvent fluctuations in promoting enzyme catalysis. Experimental techniques, such as neutron scattering, continue to make significant progress in investigating protein dynamics and protein−solvent interface properties. Collaborative investigations, using experimental techniques and the new theoretical method approach described here, will provide means for a better understanding of protein dynamical events, energy flow within a protein, between protein and solvent, and enzyme catalysis at multiple time scales.

Methods

The model for the solvated CypA−CA^N complex from our previous study was used for investigations of reaction trajectories.[27] Based on the crystallographic structure from Howard et al. (PDB code 1M9C),[47] this model consists of 165 CypA and 146 residues from HIV-1 capsid protein and explicit solvent. The system consisted of 2503 CypA atoms, 2273 CA^N atoms, and 51 240 solvent atoms. In our previous study, a potential of mean force (PMF) representation for the FEP of cis−trans isomerization of the Gly89-Pro90 peptide bond (counterclockwise rotation) was obtained using the amide bond dihedral angle, ω, as the reaction coordinate. The TS was determined as the highest point on FEP, $\omega = 107.6°$. A network of protein vibrations coupled to the reaction was identified based on analysis of CypA−CA^N and CypA−peptide complexes with a variety of computational techniques including quasi-harmonic analysis, dynamic cross-correlated motions, monitoring key inter-residue distances over the reaction, and structural analysis of the cyclophilin structure.[26,27] Protein vibrational modes relevant at the time scale of the reaction were identified using quasi-harmonic analysis of system snapshots traversing the entire reaction path. Note that a protein vibrational mode, indicated by φ, contains vector displacements for atoms in the enzyme−substrate complex; solvent is excluded from the calculation of φ. Three reaction coupled protein vibrational modes (mode A, B, and C) were found to impact the reaction significantly. Several conserved and semiconserved residues, including surface residues, show large displacements in these modes. See refs 26 and 27 for illustrations and animation movies of these modes.

For the present study, system snapshots close to the TS were selected from the FEP of cis−trans isomerization of the Gly89-Pro90 peptide bond. Regions R* ($116° < \omega < 117°$) and P* ($97° < \omega < 98°$), respectively, indicate a small section of the reactant side and product side in the vicinity of the TS (~10° away). 40 system snapshots separately from the R* region and the P* region were selected for investigations. Atomic velocities associated with system coordinates were also selected. System snapshots were selected from four different umbrella sampling runs, to avoid any sampling bias.

A new approach is used in this study to investigate the effect of protein vibrations on reaction trajectories by adding kinetic energy to specific parts of the enzyme−substrate complex or the hydration-shell solvent. This approach is analogous to the DRP method previously used to investigate small chemical systems.[42−45] KE was added to select protein vibrational modes by scaling velocities proportional to atomic displacements indicated in protein vibrational mode. The total system energy was kept unchanged by scaling down velocities of the entire system (enzyme, substrate, and solvent), according to the following equation:

$$\frac{1}{2}\sum_{i=1}^{N_{enz-subs}}\sum_{\alpha} m_i[(1-\delta)^{1/2}v_{i\alpha} + \eta\varphi_{i\alpha}]^2 + \frac{1}{2}\sum_{i=1}^{N_{sol}}\sum_{\alpha} m_i(1-\delta){v_{i\alpha}}^2 = \frac{1}{2}\sum_{i=1}^{N_{total}}\sum_{\alpha} m_i {v_{i\alpha}}^2 \quad (1)$$

where v represents component of velocity for atom i; m_i is the mass of the atom; $N_{enz-subs}$ is the number of solute atoms; N_{sol} is the number of solvent atoms; N_{total} are total atoms in the system; α represents summation over axes x, y, z; parameter δ represents the fraction of system KE transferred into the protein vibrational mode φ; and η is a

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Table 1. Rate-Enhancing Protein Vibrational Modes Investigated in This Study[a]

protein vibrational mode	protein regions with large displacements	active site motions
A	CypA: 12−18, 66−75, 78−84, 118−124, 136−152 CA^N: 16−31, 35−44, 59−63, 88−95,110−120	CypA residues 60, 102, 103, 122 and CA^N residues 89, 90 show large displacements
B	CypA: 12−15, 68−76, 80−85, 90−94, 101−106, 118−125, 144−156 CA^N: 35−44, 88−95, 110−124	motions of CypA residues 101−104, concerted with those of substrate
C	CypA: 12−15, 66−74, 90−94, 101−107, 118−125, 136−156 CA^N: 1−15, 88−95	fluctuations in CypA 102-CA^N 89 and CypA 55-CA^N 90 hydrogen bonds

[a] These modes are coupled to the cis−trans isomerization in the CypA-CA^N complex. Pictorial representation and animation movies of these modes are available with refs 26 and 27.

variable calculated based on the above equation. Scaled velocities (v^n) for atoms were assigned according to following expressions:

enzyme−substrate complex: $$v^n_{i\alpha} = (1-\delta)^{1/2} v_{i\alpha} + \eta\varphi_{i\alpha} \quad (2)$$

solvent: $$v^n_{i\alpha} = (1-\delta)^{1/2} v_{i\alpha} \quad (3)$$

Note, system coordinates were not manipulated. In this paper, φ from quasi-harmonic analysis based on system snapshots from the entire reaction were used; however, this new approach can also be used with φ obtained from normal-mode analysis as well. A similar methodology was used for adding KE to the solvent molecules. Atomic velocities of solvent molecules in the first solvation (or select molecules of the first solvation shell) were scaled to increase KE (total system KE was unchanged). In this study, the following values of δ were used for investigations: 0.0002, 0.001, 0.002, 0.005, 0.01, 0.02, and 0.05.

The selected snapshots with increased KE in the protein vibrational mode or the surface solvent molecules were propagated using molecular dynamics (MD). The AMBER suite of programs with parm98 force-field was used for MD runs and analysis.[48,49] A time step of 1 fs was used, and other conditions were similar to those of our previous studies.[26,27] The interaction energy for the enzyme−substrate complex was calculated as a sum of electrostatic and van der Waals energies between all atom pairs:

$$E_{\text{enz-subs}} = \sum\left[\left(\frac{q_i q_j}{\epsilon(r) r_{ij}}\right) + \left(\frac{A_{ij}}{r_{ij}^{12}} - \frac{B_{ij}}{r_{ij}^{6}}\right)\right] \quad (4)$$

where the first term is the electrostatic contribution and the second term is for van der Waals interactions, q_i are partial charges, and A_{ij}, B_{ij} are the Lennard−Jones parameter and the summation runs over all atom pairs for the enzyme−substrate complex. The charges and Lennard−Jones parameters were obtained from the AMBER parm98 force field. A distance-dependent dielectric function was used:

$$\epsilon(r_{ij}) = A + \frac{B}{1 + k\exp(-\lambda B r_{ij})} \quad (5)$$

where $B = \epsilon_o - A$; $\epsilon_o = 78.4$ for water; $A = -8.5525$; $\lambda = 0.003\,627$ and $k = 7.7839$.[50,51]

Results and Discussion

Theoretical and experimental studies of CypA indicate that internal protein dynamics events are linked to its catalytic activity of peptidyl-prolyl cis−trans isomerization.[19,20,26,27] The discovered network of protein vibrations, coupled to the substrate turnover step, plays a role in promoting catalysis. The dynamical events in this network range from picosecond to microsecond−millisecond time scales (note that the substrate turnover step catalyzed by CypA occurs on the microsecond−millisecond time scale). On one side of this range, the network consists of fast and harmonic motions known as vibrations, occurring on femtosecond−picosecond time scales. These vibrations, involving mainly the movement of bonds, angles, and a few atoms, alter the enzyme−substrate interaction in the active site. On the other side, the network also consists of collective conformational fluctuations (low-frequency modes) spanning larger areas of protein and occurring on microsecond and longer time scales (see Table 1). Note these coherent, collective, and repeated movements of many residues spanning different parts of the protein are different from random conformational fluctuations observed in proteins. For the discovered network, several residues participating in rate enhancing conformational fluctuations are conserved due to their role in enzyme function, even though some of these residues are several angstroms away from the active site.[26] NMR relaxation studies of CypA provide experimental evidence for the existence of concerted fluctuations coupled to the reaction; motions in several conserved residues forming crucial points in the network (Arg55, Asn102, Ala103, and Gly109) were only observed during the presence of substrate.[19,20] Further, large temperature factors (β-factors)[52−54] associated with the conserved network residues also provide supporting evidence for the existence of vibrations. The role of these vibrations, proposed on the basis of theoretical studies, in altering the active site enzyme−substrate interactions agrees with the detailed reaction mechanism based on recent crystallographic structures.[47]

Detailed investigations of dynamical reaction trajectories indicate that certain protein dynamics events (conformational fluctuations) coupled to the catalytic step play a role in reaction rate enhancement achieved by enzymes. For these investigations, a new method was designed to add kinetic energy to a specific protein vibrational mode (see Methods section for details). This method is analogous to the DRP method, which has been used to investigate vibrational modes coupled to reactions in small chemical systems.[42,43] Investigations of CypA with the new method reveals that protein vibrational modes, discovered as a part of the network of protein vibrations, and corresponding to the time scale of substrate turnover, impact reaction trajectories by influencing the TS barrier crossings (Figure 3). Figure 3a shows the behavior of a representative trajectory, in the vicinity of the TS, attempting to proceed from the trans (reactant) to the cis (product) state. The original trajectory, indicated by solid

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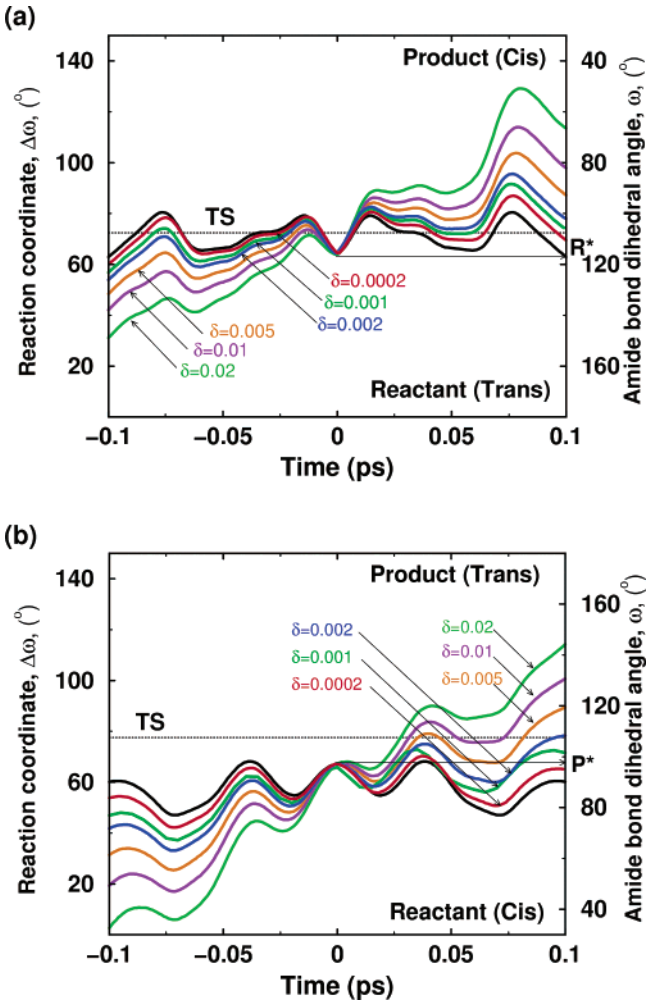

Figure 3. The effect of increasing KE in a network protein vibrational mode (mode A) on reaction trajectories. (a) Representative trajectories starting in the R* region. (b) Representative trajectories starting in the P* region (reverse reaction). The dashed horizontal line indicates the TS, the solid black curve represents the native trajectory, and the parameter δ indicates the fraction of system KE added to the protein vibrational mode (see eq 1).

black curve, shows several barrier recrossings but is not productive. Addition of KE into a network vibrational mode coupled to the reaction alters the behavior of the trajectory. Even a small amount of energy, such as 0.02% of system KE (δ = 0.0002), indicates deviation from the original trajectory. Increasing the amount of KE added to the mode leads to reduction in the number of barrier recrossings, and with 2% system KE (δ = 0.02) added, trajectory quickly becomes productive, on the picosecond time scale in investigation, with just a single crossing of the barrier. The reverse reaction (cis to trans) also shows similar behavior as depicted in Figure 3b; a nonproductive trajectory with no barrier crossings starts to cross the TS barrier and becomes productive with only one crossing. The decrease in the number of barrier recrossings and increase in the number of trajectories with successful barrier crossings lead to a larger value of κ, therefore, rate enhancement. Note that the addition of a large amount of energy illustrates successful barrier crossing at the picosecond time scale in investigations; furthermore the trend also indicates that smaller amounts of energy in the protein vibrational mode will cause barrier crossings at longer time scales (nanosecond and microsecond). For computational reasons, the detailed investigations described below used only trajectories with δ = 0.02. The results from trajectories with the addition of smaller amounts of energy, however, are also expected to be qualitatively similar at longer time scales.

Table 2. The Effect of Increasing KE in Protein Vibrational Mode and First Solvation Shell on TS Barrier Recrossings and Productive Nature of Reaction Trajectories[a]

	total number of trajectories	trajectories with forward crossings			productive/ nonproductive trajectories
		0	1	>=2	
regular	40[b]	33	1	6	1/39
2% system KE added in protein vibrational mode (δ = 0.02)					
mode A	40[b]	8	24	8	25/15
mode B	40[b]	10	20	10	23/17
mode C	40[b]	17	16	7	19/21
mode NP1	40[b]	32	1	7	1/39
mode NP2	40[b]	31	1	8	1/39
regular	20[c]	11	0	9	9/11
KE in first solvation shell[d]					
δ = 0.05 (5%)	20[c]	4	1	15	11/9

[a] NP1 and NP2 are nonpromoting modes. [b] Trajectory duration: 0.1 ps. [c] Trajectory duration: 1.0 ps. [d] KE was only added to solvent molecules around surface residues which are a part of network.

Multiple low frequency conformational fluctuations coupled to reaction possibly provide a way for enzymes to achieve large rate enhancements by promoting trajectories to successfully surmount the activation energy barrier. All three protein vibrational modes, identified as a part of the network of protein vibration in CypA, show changes in barrier recrossings with the addition of KE leading to productive trajectories. Table 2 presents a summary of observations for a variety of trajectories with additional KE in the protein vibrational modes. Only 1 out of 40 regular trajectories (without addition of KE) was productive, and the majority of trajectories did not have any forward crossings. However, redistribution of 2% system KE (δ = 0.02) into the three protein vibrational modes from the network makes the majority of trajectories recross the barrier several times. Further, around 50% of trajectories also became productive within 0.1 ps. It is important to note that not every low frequency conformational fluctuation promotes the reaction, as is indicated by results from two nonpromoting modes; only slight increases in barrier crossings were observed for these modes and no change in the number of productive trajectories. Figure 4 shows representative trajectories indicating the effect of increasing KE in modes A, B, and C forming the network of protein vibrations and also a nonpromoting mode. These figures indicate that the presence of energy in a reaction promoting conformational fluctuations alters the dynamical behavior of trajectories, which can lead to successful barrier crossing.

The biophysical impact of the discovered network on the reaction can be understood by inspecting changes in the active site environment that are introduced by reaction promoting conformational fluctuations. Detailed analysis reveals that TS barrier recrossing behavior is correlated with the fluctuations in the enzyme−substrate interactions as a result of increased energy in protein vibrational mode. The insights into the reaction mechanism from previous theoretical and crystallographic studies indicate that the interaction between target proline residue of the substrate (Pro90) and active site hydrophilic and hydrophobic residues of enzyme play a crucial role during the reaction.[26,47] Rate-enhancing conformational fluctuations impact

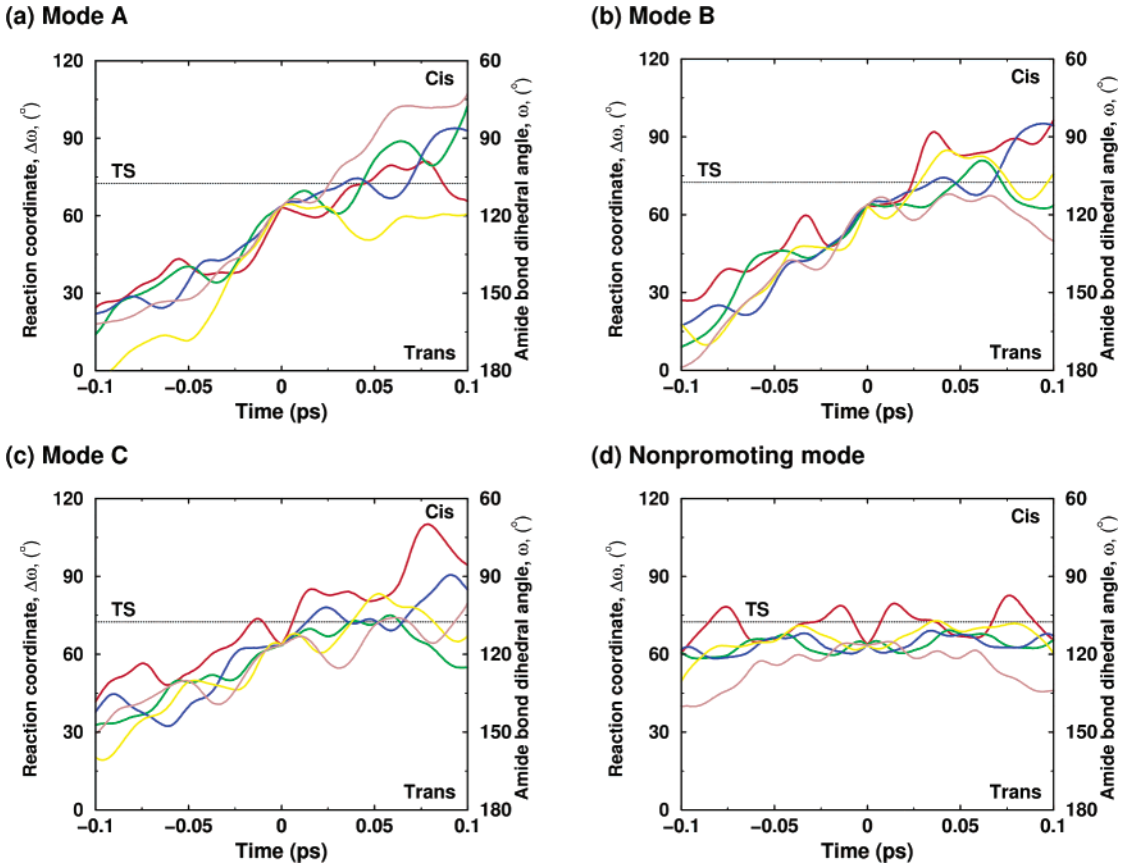

Figure 4. Representative trajectories from simulations with increased KE in network protein vibrational modes (modes A−C) and a nonpromoting mode. 2% of system KE ($\delta = 0.02$) was added to protein vibration modes. Five representative trajectories from each mode are shown in different colors. Not all protein vibrational modes show increased barrier recrossing; much less effect on the barrier crossing is seen in a mode not coupled to the reaction.

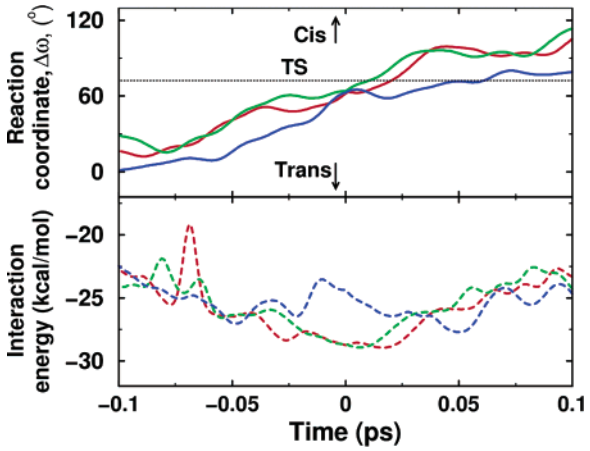

Figure 5. Correlation between TS barrier recrossings (top) and enzyme−substrate interactions (bottom). The interaction energy shown was calculated for the CA^N residues Gly89-Pro90 and all the residues of enzyme CypA. Results from three representative trajectories with 2% KE ($\delta = 0.02$) added to mode A are shown.

these key interactions in the active site as indicated by the enzyme−substrate interaction energy profile in Figure 5. An interesting observation is that the maximum enzyme stabilization of the substrate occurs close to TS (consistent with the TS stabilization theory for enzyme catalysis). The role of the reaction promoting low frequency conformational fluctuations could, therefore, be interpreted as concerted internal protein movements, which facilitate in stabilization of the TS. The conservation of the network residues and hydrogen bonds as a part of cyclophilin structure, or the conservation of protein "fold", indicates a link among protein structure, dynamics, and function. Previous investigations of hydride transfer reaction catalyzed by enzyme dihydrofolate reductase provides evidence that changing the structure leads to changed dynamics and, therefore, a change in function.[23,55,56] Mutation of a single surface residue in dihydrofolate reductase, more than 12 Å away from the active site, changes the dynamics and leads to a rate reduction by a factor of 163.

The impact of rate-enhancing conformational fluctuation on the reaction trajectory is related to the amount of energy associated with the vibrational mode (see Figure 3). Estimates for the amount of energy associated with the conformational fluctuations in the discovered network are currently not available. The DRP method based investigations of a small chemical system had indicated that addition of 13−16 kcal/mol of energy in a mode promotes the reaction.[42] For the system investigated in this study, 0.02% of total system KE which is about 6 kcal/mol shows changes in the dynamical behavior of trajectories. Further, in a previous theoretical study, Kidera et al. have investigated the transfer of energy between protein vibrational modes by addition of 1 kcal/mol in a vibrational mode of myoglobin.[57] In that investigation of myoglobin, the energy added to the vibrational mode was not lost by dissipation but

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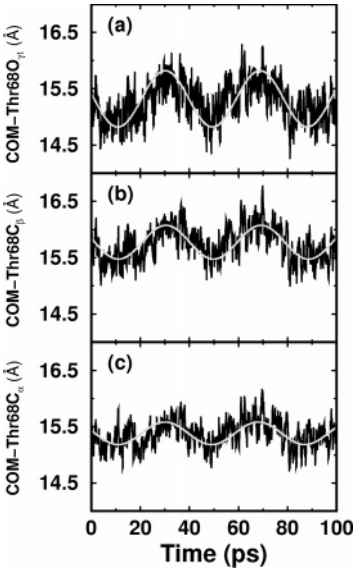

Figure 6. Real-time fluctuations of Thr68, a surface residue, indicate vibrations. The gray curve indicated a fitted curve indicating vibration of this protein residue. The distances between center-of-mass of the enzyme−substrate complex (COM) and selected atoms on the residue are shown.

instead was found to be transferred to other modes. Our theoretical investigations provide insights into how increasing amounts of energy in a reaction promoting conformational fluctuation have a positive effect on reaction trajectories on a picosecond time scale. In enzyme systems, smaller amounts of KE, which are expected to be more realistic, will cause barrier crossings on microsecond and longer time scales. Note, in our simulations, KE was added to only one protein vibrational mode; however, in real enzymes, energy is expected to be present in several promoting modes simultaneously. Our observations, therefore, support the suggestion that protein dynamics in enzyme complexes changes the barrier crossings such that it increases reaction rate by several orders of magnitude.[46]

What is the source of energy for rate-enhancing conformational fluctuations? In cases where enzymes work without any other energy source, bulk solvent is expected to be the source of thermal energy for surmounting the activation energy barrier. Recent investigations have demonstrated that the bulk solvent and hydration-shell fluctuations control internal protein dynamics.[13,14,58] Our investigations indicate that the dynamical coupling between hydration-shell solvent and surface enzyme residues provides a mechanism for the transfer of energy from the solvent to protein. In CypA, several surface residues, including Val29, Thr68, and Lys82, extend out into the solvent and show large displacements in the protein vibrational modes of the discovered network. Note these surface residues are semiconserved or are neighbors to conserved residues. Investigations of the real-time trajectories reveal the presence of fast vibrations in surface residues; for example, Figure 6 shows the presence of vibrations on the picosecond−nanosecond time scale in surface residue Thr68. To understand the role of hydration-shell solvent in enzyme function, we investigated the transfer of KE from the first hydration shell to external regions of protein and its effect on the reaction trajectories (Figure 7 and Table 2). Figure 7a shows results from two representative trajectories propagated

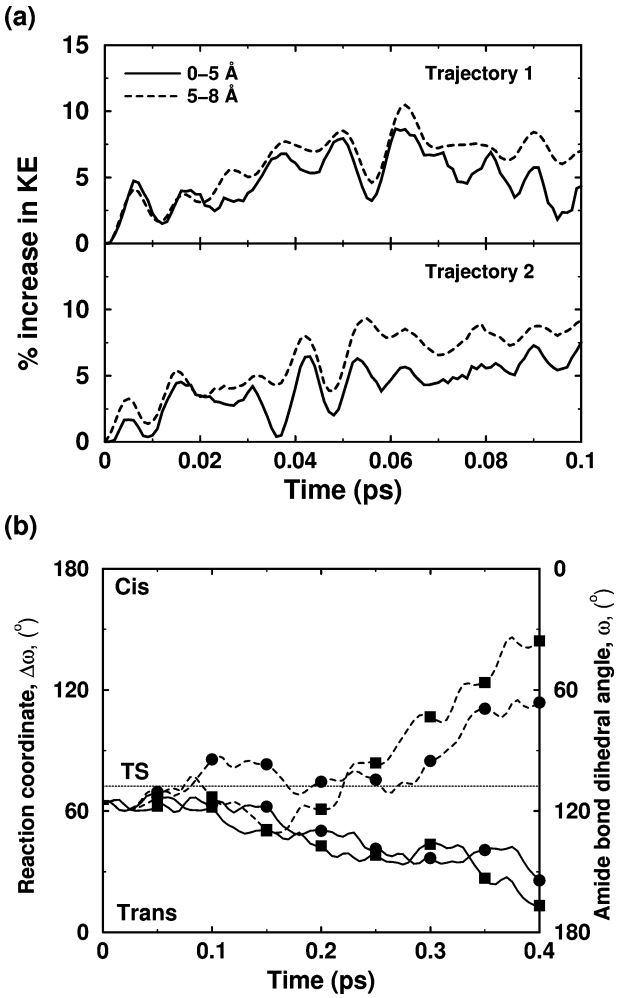

Figure 7. Effect of additional KE in first solvation shell. (a) The KE is transferred from the solvent to the protein residues, as indicated by increasing energy in the protein regions (up to 5 Å from protein surface, and between 5 Å and 8 Å from the surface). (b) Two otherwise nonproductive regular trajectories (solid lines) become productive (broken lines) due to transfer of energy from the solvent to residues forming parts of the protein vibrations network. The corresponding trajectories are indicated by squares and circles.

after increasing the KE of the first solvation shell by 5%; within 0.1 ps an increase in KE of external protein regions is observed. These results indicate the transfer of energy from solvent to protein residues, with an increase in energy of protein residues more than 8 Å away from the surface occurring within a very short time. This transfer of energy into the protein residues impacts the barrier crossing behavior of reaction trajectories; as depicted in Figure 7b, certain trajectories that are otherwise nonproductive cross barrier within a short time period to become productive trajectories. Note, to obtain insights within a reasonable duration of simulation for investigations indicated in Figure 7b, KE was only added to solvent molecules around surface residues that are a part of the network. However, the distribution of energy in the entire first solvation shell is expected to provide similar results in longer simulations.

Experimental investigations are required to obtain further details of the role of protein dynamical events and solvent fluctuations in enzyme catalysis. Significant progress has recently been made in Mössbauer, neutron scattering, and NMR relaxation techniques, instrumentation, and analysis relevant to investigations of fluctuations in proteins and solvent surrounding

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the protein.[17,59−62] Individual residues can now be investigated using deuterium labeling, and using instruments with different resolutions, it will be possible to differentiate between the motions and energy associated with protein and solvent at the interface.[62] Collaborative experimental and theoretical investigations will be key to understanding the role of protein dynamics in function;[10] theoretical techniques, such as the one described in this paper, will guide the experimental investigations by highlighting the areas of proteins which can make the most impact on catalysis.

In our past reports and this present paper, we have used the term protein dynamics to describe internal protein motions occurring at all time scales. Note, this does not conflict with the previous terminology used by Hammes-Schiffer and coworkers; who have used "dynamics" for motions that influence the transmission coefficient, κ.[6,33] Our present investigations indicate that not only the fast motions but also conformational fluctuations on the time scale of reaction impact the barrier recrossing behavior of trajectories leading to a significant increase in transmission coefficient. Therefore, in addition to averaged distances, dynamical fluctuations on all time scales are also expected to impact enzyme catalysis.

In addition to making an impact on TS barrier recrossings, an alternate way in which slower conformational fluctuations and fast vibrations can impact catalysis in the TST framework is by changing the height of the free energy activation barrier. Some insights into the effect of protein vibrational modes on the free energy profile have been obtained in our previous studies of CypA.[26] Early indication obtained from quantum-mechanical calculations of the enzyme active site model are that the system (potential) energy close to the TS decreases as the system is displaced along the protein vibrational modes. Recently, reports from other research groups have also indicated that vibrations and correlated motions play a role in the fluctuation of the activation energy barrier.[63,64] Note, it is possible that the effect of protein dynamics on the activation energy barrier height and transmission coefficient may be interrelated. Further investigations are required for understanding the role of conformational fluctuations coupled to the reaction in modulating the height of the barrier region. Concerted conformational fluctuation in the enzyme−substrate complex may possibly be the mechanism, in part, for decreasing the activation energy barrier for an enzyme-catalyzed reaction as compared to a reaction in solution (in the absence of enzyme).

Conclusions and Summary

In this report, we present theoretical investigations of the link between internal protein motions and rate enhancement by enzymes. Recently, we discovered a network of protein vibrations coupled to the peptidyl-prolyl cis−trans isomerization catalyzed by CypA.[26,27] This network extends from surface regions of the enzyme to the active site. The fast motions (vibrations) in this network impact the hydrophobic and hydrophilic interactions between the enzyme and substrate. The concerted conformational fluctuations (low-frequency modes) spanning conserved residues impact the dynamical behavior of reaction trajectories. Using a method analogous to DRP, reaction trajectories were propagated after the addition of KE to protein vibrational modes. Results indicate that certain conformational fluctuations alter transition state barrier recrossings. An increase in productive trajectories was observed with increasing KE in select protein vibrational modes corresponding to low-frequency network fluctuations, suggesting the role of these protein dynamics in enzyme rate enhancement by increasing the transmission coefficient. Our simulations also show that energy transfer from the first solvation shell to surface protein residues impacts catalysis through conformational fluctuations in the discovered network.

Taken together, recent experimental observations and our theoretical studies provide insights into the emerging integrated view of solvated protein structure, dynamics, and function. Several experimental techniques (including NMR and neutron scattering) indicate that proteins are not rigid but are dynamical assemblies with a close link between structure and dynamics.[17] Mössbauer and neutron scattering experiments indicate that bulk solvent and hydration-shell fluctuations control protein dynamics.[13−15] Our theoretical investigations of CypA indicated the transfer of energy from solvent molecules in the first solvation shell to external protein regions through dynamical coupling; this energy is then transferred to a network of protein vibrations, which promote the reaction by changing the dynamical behavior of the reaction trajectories. Note that crucial parts of the network are found to be conserved across several species[26] and experimental evidence for the existence of this network comes from previous NMR relaxation studies, where motions in several residues forming parts of this network were detected only during substrate turnover.[19] High-temperature factors (from X-ray crystal structures) associated with the network residues in CypA crystal structures provide further evidence.[26,52−54] The link between atomic fluctuations visible in high resolution crystal structures and enzyme function has previously been noted for other enzymes.[65,66] Understanding reaction-coupled protein dynamics has interesting implications in manipulating enzyme-catalyzed reactions. For example, laser pulse has already been used to initiate an enzyme reaction involving thermally excited protein dynamics (molecular motions on a picosecond time scale).[67] Recent progress made in experimental techniques, instrumentation, and analysis for investigating protein and solvent dynamics will lead to more detailed investigations.[59,62]

The characterized network of vibrations promoting enzyme catalysis in CypA and also the one previously characterized in the enzyme dihydrofolate reductase have wide implications in understanding enzyme rate enhancement, allostericity, and cooperative effects, as well as protein engineering and drug design. These networks also provide some insights into the conservation of protein "folds"; enzymes catalyzing similar reactions often belong to the same fold family, and enzymes catalyzing mechanistically similar reactions belong to same protein superfamily.[68,69] Sequence analyses with thermodynamic

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mapping have indicated long-range energetic coupling in proteins;[70] low frequency conformational fluctuations could possibly be the mechanism of energy transfer over long ranges in protein structure and, therefore, provide insights into understanding allostericity. Simulations have already revealed that energy can be transferred between specific vibrational modes in a protein.[57,71] Emerging evidence continues to link protein structure, dynamics, and function. In this report, we have described the role of protein dynamics in rate enhancement by enzymes. Further insights from our ongoing investigations also suggest that protein vibrations may also have a role in biomolecular recognition and in overcoming local energy barriers in protein folding.

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