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Mechanistic Insight into the Role of Transition-State Stabilization in Cyclophilin A

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Abstract: Peptidyl prolyl *cis*−*trans* isomerases (PPIases) are ubiquitous enzymes in biology that catalyze the *cis*−*trans* isomerization of the proline imide peptide bond in many cell signaling pathways. The local change of the isomeric state of the prolyl peptide bond acts as a switching mechanism in altering the conformation of proteins. A complete understanding of the mechanism of PPIases is still lacking, and current experimental techniques have not been able to provide a detailed atomistic picture. Here we have carried out several accelerated molecular dynamics simulations with explicit solvent, and we have provided a detailed description of *cis*−*trans* isomerization of the free and cyclophilin A-catalyzed process. We show that the catalytic mechanism of cyclophilin is due mainly to the stabilization and preferential binding of the transition state that is achieved by a favorable hydrogen bond interaction with a backbone NH group. We also show that the substrate in the transition state interacts more favorably with the enzyme than the *cis* isomer, which in turn interacts more favorably than the *trans* isomer. The stability of the enzyme−substrate complex is directly correlated with the interaction the substrate makes with a highly conserved arginine residue. Finally, we show that catalysis is achieved through the rotation of the carbonyl oxygen on the N-terminal of the prolyl peptide bond in a predominately unidirectional fashion.

Introduction

Peptidyl prolyl *cis*−*trans* isomerases regulate many biological processes by interacting with molecular switches whose conformations are modulated via *cis*−*trans* isomerization of the prolyl peptide (ω) bond.[1] Many molecular switches are involved in cell signaling pathways, and deregulation of these pathways could trigger cellular transformation, oncogenesis, and other diseases.[2] There are three structurally unrelated classes of PPIases that are known to date: the cyclophilins that bind cyclosporine, the FK506 binding proteins (FKBP), and the parvulins, of which Pin1 is a member. Undoubtedly, *cis*−*trans* isomerization of the peptide bond is one of the slowest conformational transitions found in proteins. The detailed atomistic understanding of the mechanism of PPIases is still lacking, and the bits and pieces that are known do not always form a coherent story.

The local changes of the isomeric state of the prolyl peptide bond act as a switching mechanism in altering the overall conformation of proteins. Protein signaling processes utilize the additional conformational variability that arises due to the resulting *cis* and *trans* isomers of peptide bonds. Several molecular switches that are regulated by *cis*−*trans* isomerization have been discovered over the years.[2] The role of *cis*−*trans* isomerization of the prolyl peptide bond in interleukin tyrosine kinase (Itk) SH2 domain[3,4] that is regulated by cyclophilin, and ligand-gated 5HT3 ion channel,[5] are two recognizable examples. The binding site of the SH2 domain of Itk discriminates between two different ligands, depending on the isomeric state of a distal prolyl peptide bond. Similarly, the conformation of five prolyl peptide bonds, one in each subunit, was shown to determine the state of the ligand-gated 5HT3 ion channel. When the prolyl peptide bonds adopt the *trans* conformation, the channel is closed; when they are in the *cis* isomeric state, the channel is open, allowing ions to flow through. Importantly, the HIV virus has also been shown to use the human cyclophilin[6,7] during its final stages of viral replication, which has rekindled interest in this enzyme especially for drug design purposes. Human cyclophilin catalyzes *cis*−*trans* isomerization of a prolyl peptide bond of the HIV capsid in order to trigger a conformational change necessary for viral packaging.

cis−*trans* Isomerization of prolyl peptide bonds is characterized by a very high activation energy barrier of around 16−22 kcal/mol, and the rate is in the order of tens to hundreds of

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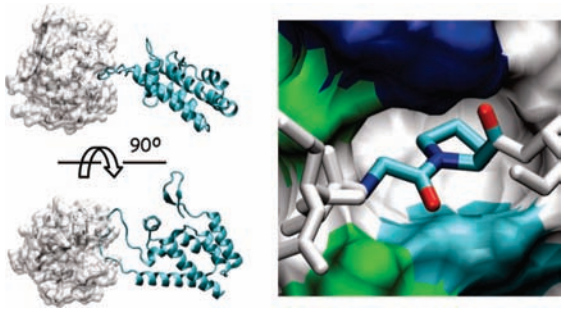

Figure 1. Interaction of cyclophilin A with full-length HIV capsid. Cyclophilin catalyzes *cis*−*trans* isomerization of a -Gly-Pro- motif on the exposed loop structure of the HIV capsid (left). The binding site of cyclophilin (right) has a very hydrophobic pocket with the nonpolar residues shown as white, an arginine residue at the entrance of the pocket shown as blue surface, a histidine shown as cyan surface, and two asparagines shown as green. The side-chain ring of the proline residue fits very nicely into the hydrophobic pocket of the binding site.

seconds.[8,9] Therefore, *cis*−*trans* isomerization is involved in slow conformational changes, including the rate-limiting step in protein folding.[10−12] Nature has provided the PPIase enzymes to circumvent this very slow kinetics by catalyzing the *cis*−*trans* isomerization and decreasing the time scale from seconds to the more biologically relevant millisecond time scale. The mechanism of the PPIases is still not well understood and is controversial, and has been the subject of many experimental and computational studies.[1,13−15] For example, it was earlier thought that the remarkable speedup is achieved by a nucleophilic attack to the carbonyl carbon atom of the preceding residue that would result in the loss of the pseudo-double-bond character of the peptide bond. This possible loss in pseudo-double-bond character could then result in a lower activation energy barrier and therefore lead to a faster rate of isomerization. However, this mechanism was shown to be implausible due to the retention of catalytic activity of cyclophilin after mutagenesis studies that were carried out on all the residues that have the ability to act as the nucleophile.[16]

Therefore, PPIases are one of the rare enzymes in biology that carry out their function in the absence of any actual bond formation and cleavage. How do the PPIases then achieve this remarkable speedup of more than 5 orders of magnitude? Several hypotheses have been proposed over the years that include the effect of substrate desolvation and the idea of preferential transition-state binding in the active site.[1] It was shown that the effect of removing the substrate from aqueous solution to the hydrophobic pocket of the PPIases, as shown in Figure 1, could result in a speedup of *cis*−*trans* isomerization. This effect is partly due to the weakening of the pseudo-double-bond character of C−N in nonaqueous environment, resulting in a small reduction of the transition barrier height by about 1.3 kcal/mol.[17] Similarly, a speedup of up to about 20-fold of the rate of *cis*−*trans* isomerization was later observed in micelles that also resulted in a small decrease in the barrier height by about 1.8 kcal/mol, assuming that the speedup is purely due to barrier reduction.[18] Likewise, we have previously observed a speedup in the rate of *cis*−*trans* isomerization using molecular dynamics simulations in the absence of explicit water molecules around the prolyl peptide bond due to a reduction in the effective roughness on the energy landscape that results in a change in the kinetic prefactor.[19] The kinetic prefactor depends on the diffusion coefficient on the landscape, which in turn depends on the effective roughness of the landscape. Also, the speedup can simply be a consequence of the change in the frictional drag experienced by the substrate in moving from an aqueous environment to the dry hydrophobic cavity of the binding site of the PPIases. However, these prefactor effects and slight reduction in barrier height due to the lack of aqueous medium could not account for the more than 5 orders of magnitude increase in the observed rate of *cis*−*trans* isomerization due to PPIases. Also, it has previously been shown that an increase in the rate of *cis*−*trans* isomerization of the ω angle can be achieved by constraining the peptide bond in a loop conformation,[20,21] but the extent of the role of this phenomenon in the catalysis of *cis*−*trans* isomerization of the peptide bond by PPIases is not known.

In order to fully understand the catalytic mechanism at the atomistic detail, one has to be able to observe the *cis*−*trans* isomerization of the peptide bond. In this regard, all-atom molecular dynamics simulation has proven invaluable as a complementary technique to existing experimental results in fully understanding protein function.[22] However, normal molecular dynamics simulation has not been able to provide a complete picture of the catalytic mechanism of the PPIases because of the time scale limitation, and therefore the *cis*−*trans* isomerization cannot be simulated directly. The time scale of *cis*−*trans* isomerization and even the time scale of the catalyzed process are beyond the submicrosecond time scale of normal molecular dynamics (MD). Therefore, earlier computational studies of this system have used umbrella sampling and restrained molecular dynamics in order to traverse the isomerization path.[23−25] A limitation of these types of techniques is the reliance on *a priori* decisions about the transition path that could potentially bias the outcome.

Previously, we developed an accelerated MD method[26] that was used to simulate for the first time the *cis*−*trans* isomerization of the prolyl peptide bond,[27] and we were able to

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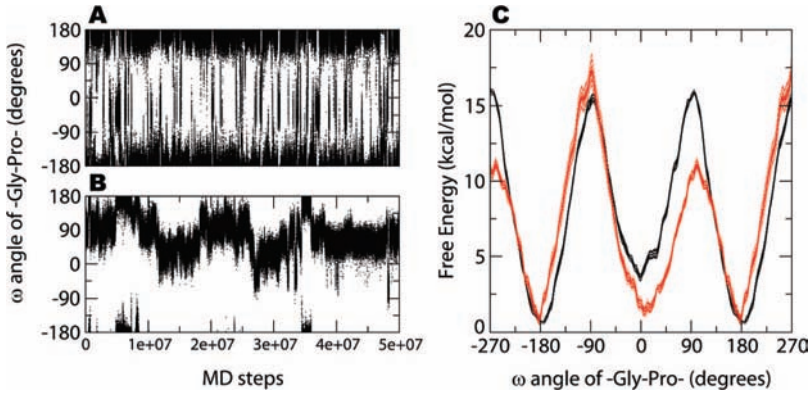

Figure 2. *cis*−*trans* Isomerization of the ω bond of the -Gly-Pro- motif of the free substrate (A) and enzyme−substrate bound complex (B) and the corresponding free energy profile after reweighting of the distribution (C) for the free substrate (black) and enzyme−substrate complex (red).

calculate the free energy barrier and rate constants in both implicit and explicit solvent.[19,28] This method speeds up the transition over energetic barriers with little or no prior knowledge of the energy landscape. In this work, we have used the accelerated MD method to fully study the catalytic mechanism of the cyclophilin A enzyme by simulating the *cis*−*trans* isomerization of the free substrate taken from the HIV capsid and that of the enzyme−substrate complex, both in explicit solvent.

Results and Discussions

Simulation of the Catalytic Process of *cis*−*trans* Isomerization. The catalytic process of cyclophilin does not involve any bond formation or cleavage. Therefore, we have used classical molecular mechanics to study the catalytic mechanism of this enzyme. We observed *cis*−*trans* isomerization of the -Gly-Pro- ω bond of the free substrate Ace-His-Ala-Gly-Pro-Ile-Ala-Nme from the accelerated molecular dynamics simulations in explicit water as shown in Figure 2A. The substrate is derived from the loop region of the HIV capsid (Figure 1) that is regulated by cyclophilin A. In addition to the crystal structures of cyclophilin complexed with the whole HIV capsid, cyclophilin has also been cocrystallized with the short piece taken from the full-length capsid.[29,30] The free energy profile along the ω bond was estimated as shown in Figure 2C, after reweighting the distribution of the peptide ω angle of -Gly-Pro-. The free energy barriers are similar regardless of the direction of rotation, with a barrier height of about 16.5 ± 1.2 kcal/mol going from the *trans* to *cis* isomer and about 12.8 ± 1.5 kcal/mol going from the *cis* to *trans* isomer. The similarity of the free energy profile in both directions can be attributed to the lack of the side chain in the preceding glycine residue, thus allowing for almost equal probability of undergoing clockwise and anticlockwise rotations. This result contrasts with our previous study of the -Ser-Pro- motif that has an asymmetric free energy profile, which could be attributed to the side chain of serine hindering the *trans*-to-*cis* clockwise rotation.[27]

After simulating the cyclophilin−substrate complex with accelerated MD simulations in explicit water, we also observed *cis*−*trans* isomerization of the ω bond of -Gly-Pro- in the catalytic pocket of the enzyme that allowed us to monitor and study the catalytic mechanism of cyclophilin (Figure 2B). An immediate observation of the time series of the ω angle is the directionality of the *cis*−*trans* isomerization. The transition from *trans* to *cis*, and vice versa, is mainly unidirectional, and the directionality of the transitions is also obvious from the estimated free energy profile along the ω bond, also shown in Figure 2C. The barrier height of the transition from the *trans* to *cis* isomer is lower for the anticlockwise direction, and that for the *cis* to *trans* transition is lower along the clockwise direction. Also, the trajectory of *cis*−*trans* isomerization of the enzyme-bound substrate is noticeably different from that of the free substrate as can be seen in Figure 2, A and B. An ensemble of conformations around 90° of the ω angle of -Gly-Pro- is stabilized as compare to the free substrate.

Therefore, after reweighting the distributions, two other main observations from the free energy profile are the stabilization of the *cis* isomer against the *trans* isomer as compared to the free substrate and the lowering of the barrier height as we go from the *trans* isomer to the *cis* isomer (anticlockwise). The *cis* and *trans* isomers of the substrate in the binding site now have almost equal probability of occurrence, with the transition state at a higher free energy. The barrier height from *trans* to *cis* is now the same as that from *cis* to *trans*. Even though the barrier height of the transition state is lowered in the enzyme−substrate complex as compared to that of the free substrate, the barrier reduction is not enough to make the transition-state complex more stable than the complex with the *cis* or *trans* isomer. Consequently, crystal structures of the enzyme−substrate complexes are expected to be found either in the *cis* or *trans* conformation, as is the case. The barrier height from the *trans* to *cis* isomer in the enzyme−substrate complex is now about 10.2 kcal/mol. The reduction in the barrier height is therefore estimated to be around 6.3 kcal/mol. As a result, the magnitude of the reduction of the barrier height coupled with a possible order of magnitude speedup[28,31] due to the kinetic prefactor effect is enough to increase the rate of the isomerization by more than a factor of 10^5. Since the time scale of the *cis*−*trans* isomerization of the free substrate occurs in 1−1000 s, this speedup is enough to put the catalyzed process in the biologically relevant millisecond time scale.

Stabilization of the Transition State. We can see from Figure 2 that the transition state in the complex has lower free energy than that in the free substrate, relative to the *trans* isomer. The

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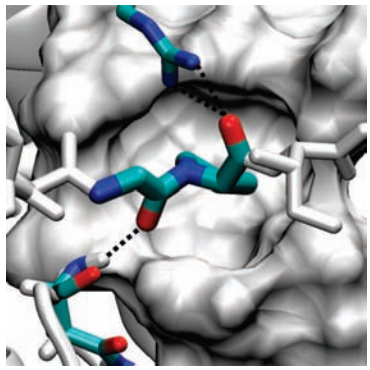

Figure 3. The enzyme−transition-state complex. The enzyme-bound substrate is stabilized by three main interactions: the nonpolar interaction proline makes with the hydrophobic pocket, the hydrogen-bonding interaction between the guanidinium moiety of the conserved arginine (top) and the carbonyl oxygen of proline, and the hydrogen bond between the carbonyl oxygen of glycine and the backbone hydrogen (below) of asparagine.

lower barrier height of the transition state is therefore partly responsible for the speedup of the rate of *cis*−*trans* isomerization. Also, the free energy of the *cis* isomer is similar to that of the *trans* isomer, thermodynamically increasing the population of the *cis* isomer relative to that in the free substrate. Mechanistically, how does cyclophilin speed up the *cis*−*trans* isomerization of the peptide bond? We analyzed the ensemble of structures of the transition state, and we observed that the transition state is formed when the carbonyl oxygen of Gly forms a hydrogen bond with a backbone NH group of Asn 102, as shown in Figure 3. This favorable hydrogen-bonding interaction between the carbonyl oxygen and the backbone hydrogen of Asn 102 is formed when the N-terminal of the peptide bond rotates clockwise (looking from the N-terminal to the C-terminal along the ω bond). The C-terminal of the peptide bond, which comprises the proline ring, never rotates for this particular substrate and stays snuggled in the hydrophobic pocket, as is depicted in Figures 1 and 3. The rotation of the N-terminal of the peptide bond during catalysis has also been suggested from crystal structure analyses,[32] contradicting previously reported C-terminal rotation of the peptide bond.[33] However, the rotating end could be dependent on the sequence of the substrate or the family of PPIase.

The results above suggest a possible stabilization of the transition state by cyclophilin. Therefore, in order to further probe the extent of transition-state stabilization, we have constructed a thermodynamic cycle as shown in Figure 4. The thermodynamic cycle links the free energies of binding between the *trans*, transition state, and *cis* isomers of the substrate and cyclophilin to the free energies of *cis*−*trans* isomerization. It is clear from this analysis that the transition state binds more strongly to cyclophilin followed by the *cis* isomer, with the enzyme having the least affinity for the *trans* isomer. This result agrees with previous NMR experiments[34] which showed that the *cis* isomer binds 4 times stronger than the *trans* isomer to cyclophilin, which correlates with the fact that many of the cyclophilin−substrate structures adopt the *cis* form. Our suggestion that the transition state interacts more favorably with

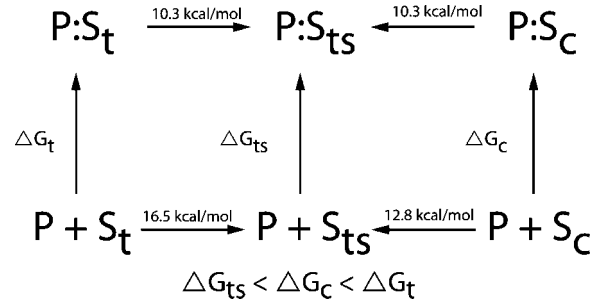

Figure 4. Thermodynamic cycle connecting the free energy of binding and free energy of *cis*−*trans* isomerization. P and S represent the enzyme and substrate, respectively. **t**, **ts**, and **c** represent the *trans*, transition state, and *cis* conformations, respectively.

cyclophilin does not contradict the fact that all of the structures of the cyclophilin−substrate complexes are either in the *cis* or *trans* conformation. Despite the fact that the transition state interacts more favorably with the enzyme, the total free energy of the enzyme−transition-state complex is higher than that of the ground state *cis* and *trans* isomers as can be seen in Figure 2C, due mainly to the high penalty of activation.

Furthermore, stabilization of the transition state is due not only to the favorable interaction made by the carbonyl oxygen of the Gly with the backbone hydrogen of Asn and by the favorable nonpolar interaction of the proline residue with the hydrophibic pocket but also to a favorable interaction of the carbonyl oxygen of Pro with the guanidinium moiety of the highly conserved Arg 55, also shown in Figure 3. Arginine 55 is shown to interact quite differently with the *trans*, transition state, and *cis* isomers, and therefore partly responsible for the differences in binding affinity.

In this regard, a catalytic antibody, abzyme, optimized to recognize and bind a transition-state mimic, accelerates the rate of *cis*/*trans* isomerization of the peptide bond, but to a much lesser extent than cyclophilin.[35] Why would the optimized abzyme have a much smaller increase in the rate of *cis*−*trans* isomerization of the peptide bond than cyclophilin? The answer, we believe, lies in the nature of the peptide bond mimic, an α-ketoamide, that looks like a distorted peptide bond. The chemistry of the hapten (the distorted peptide bond mimic) on the N-terminal side of the proline residue is rather different from that of typical substrates. The peptide bond connecting the preceding residue and the proline is replaced by a dicarbonyl moiety in the hapten. This moiety allows the angle of the amide bond to be around 90°, similar to that of the transition state of the peptide bond. However, the additional carbonyl group slightly alters the conformation of the remaining backbone. Therefore, this small change from the real substrate could compromise the activity of the abzyme on the real substrate, since the specificity of action of the abzyme was optimized for a substrate with a slightly different chemistry.

Role of the Highly Conserved Arginine 55. The effect of Arg 55 is visually evident from the crystal structure of the cyclophilin−substrate complexes in which the guanidinium moiety forms hydrogen bonds with the carbonyl oxygen of proline, similar to the interaction shown in Figure 3. Arg 55 is highly conserved, and its replacement has been shown to decrease the catalytic activity of cyclophilin.[36] The catalytic efficiency (k_{cat}/K_m) of the wild-type cyclophilin was estimated

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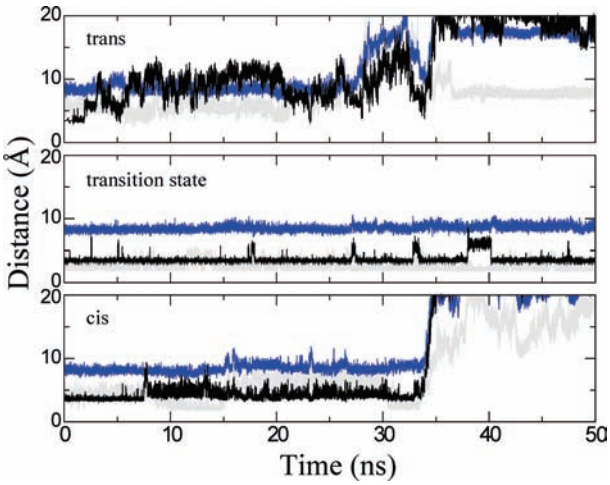

Figure 5. Hydrogen-bonding interactions between the binding site of cyclophilin and the substrate in the *trans*, transition state, and *cis* conformations. The black line represents the distance between the carbon atom of the guanidinium moiety of the conserved Arg 55 residue in the binding site of cyclophilin and the carbonyl oxygen of the proline of the substrate. The gray line depicts the distance between the backbone NH group of Asn 102 in the binding site of cyclophilin and the carbonyl oxygen of Gly of the substrate. The blue line monitors the substrate in the binding site of cyclophilin and represents the distance between the C-α atom of proline of the substrate and the C-α atom of phenylalanine, one of the residues in the hydrophobic pocket of the binding site.

to be ~16 μM^{-1} s^{-1}, and that of the Arg55Ala mutant was estimated to be ~0.016 μM^{-1} s^{-1}, about 0.1% of the catalytic efficiency of the wild-type. Therefore, these results suggest that Arg 55 might be important for recognition, since some catalytic activity is retained for the Arg55Ala mutant. The binding affinity of cyclophilin for its substrates is very low, about 20−40 μM,[34] and therefore, this single arginine residue could potentially be critical for both recognition and stability. It was previously shown that Arg 55 was stable during one nanosecond of MD simulation, and elimination of its overall charge destabilized the transition state and the *cis* isomer complexes.[24] We therefore decided to explore the role of Arg 55 in the catalytic process over a much longer time scale and the effect this residue might have on the stability of the transition-state complex.

We carried out three normal MD simulations, 50 ns each, on the enzyme−substrate complex with the substrate -Gly-Pro- ω bond in the *trans*, transition state, and *cis* state, bound separately to the enzyme. The ω angle of only the transition state was held at 90° by applying a 1000 kcal/mol/rad^2 angle restraint on only that degree of freedom. The distance between the carbon of the guanidinium moiety of the arginine residue to the carbonyl oxygen of the substrate proline residue was monitored for the three simulations as summarized in Figure 5 (black lines). It can be seen in Figure 5 that this hydrogen-bond contact is not stable in the complex with the *trans* isomer. Also, the hydrogen-bond formation is reproducibly correlated with the stability of the complex. At around 35 ns in this simulation of the enzyme−substrate complex of the *trans* isomer, the arginine residue disengages the carbonyl oxygen of the proline residue one more time, and this event is followed by the diffusion of the substrate out of the binding site (Figure 5; blue lines). The blue line is the distance between the C-α of the proline residue of the substrate and the C-α of the phenylalanine residue in the hydrophobic pocket.

Similarly, but to a lesser extent, the distance between the enzyme−substrate complex with the substrate in the *cis* isomer is not stable and also correlated with the stability of the complex. The substrate of the *cis* isomer diffuses out of the binding site immediately as the arginine residue disengages with carbonyl oxygen of the proline residue. However, the hydrogen-bond contact that is formed between the arginine residue and the carbonyl oxygen of proline of the *cis* isomer is more stable than that of the *trans* isomer (Figure 5). In contrast to the enzyme−substrate complexes of the *trans* and *cis* isomers, the hydrogen bond between the guanidinium moiety of the arginine residue and the carbonyl oxygen of proline of the transition-state complex is very stable and stays in the binding site during the course of the 50 ns simulation, as also shown in Figure 5.

Arginine 55 therefore acts as an anchor for the substrate in the binding site by preferentially stabilizing the transition state over the *trans* and the *cis* isomers. Consequently, mutation of Arg 55 to Ala would result in reduction of the catalytic efficiency, as was previously shown.[36] The transition state also makes an additional favorable contact (Figure 3) due to the long-lasting hydrogen-bond interaction between the carbonyl oxygen of the Gly residue and the backbone hydrogen of Asn 102, as also shown in Figure 5 (gray line). The Gly of the *trans* isomer in the complex never forms a hydrogen bond with the backbone NH group of Asn 102 as also shown in Figure 5. The carbonyl oxygen of Gly of the *cis* isomer in the complex does form a hydrogen bond with the backbone NH group of Asn 102 (Figure 5) as in the transition-state complex, but to a much lesser extent. Therefore, the stabilizing role of Arg 55 and the Asn 102 qualitatively agrees with the predicted trend in binding energies; that is, the transition state binds stronger than the *cis* isomer, which in turn binds stronger than the *trans* isomer.

Conclusions

cis−*trans* Isomerization of peptide ω bonds of proteins is a very important switching mechanism in biology that is involved in many cell signaling pathways. However, this process is notoriously slow. Even with the help of peptidyl prolyl isomerases, such as cyclophilin, the resulting time scale is beyond that of normal molecular dynamics. It is clear from previous experiments that there are no bond formation and cleavage events occurring during catalysis; therefore, we have used classical molecular mechanics coupled with the accelerated molecular dynamics methodology to shed some light on this very important catalytic mechanism. Using the accelerated molecular dynamics method that allows us to overcome the submicrosecond time scale limitation of normal molecular dynamics simulations, we have studied the catalytic mechanism of cyclophilin in full-atomistic detail in explicit water. Aside from a possible small electronic contribution that has not been captured by the classical mechanics empirical force field, we are able to fully describe the catalytic mechanism of cyclophilin and provide quantitative estimates of the free energies associated with the process. The catalysis is shown to occur mainly through the stabilization of the transition state in the binding site due to a combination of favorable hydrophobic and very long-lasting hydrogen bonding interactions. Cyclophilin decreases the barrier height of the *trans* to *cis* transition by 6.3 kcal/mol, which when coupled with other factors, such as a possible change in the kinetic prefactor, could speed up the isomerization process by as much as 10^6. A possible effect that the classical force field

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could not be able to fully capture is some of the small reduction in the barrier height of 1.3 kcal/mol[17] due to a small electronic effect of desolvation of the substrate. If we added this small correction to the calculated barrier height, the overall barrier reduction would be around 7.6 kcal/mol, which would further increase the estimated rate of isomerization. Also, since the guanidinium moiety of the conserved arginine residue stabilizes the transition state through interactions with the carbonyl oxygen of proline, this also puts it somewhat close to the nitrogen of the proline. The closeness of the guanidinium moiety to the proline nitrogen could weaken the delocalization of the electron cloud along the pseudo-double peptide bond. This could also possibly contribute a small amount to the lowering of the barrier. However, as we can see, majority of the speedup has been captured using classical accelerated molecular dynamics.

Computational Methods

All of the simulations were carried out in explicit TIP3P[37] water using the AMBER 8 suite of programs[38] and the all-atom parm99SB force field parameters.[39] The simulation of the free substrate was done in a cubic periodic box, while that of the enzyme−substrate complex was carried out in a periodic truncated octahedron. During the simulations, an integration time step of 0.002 ps was used to numerically solve Newton's equations of motion. After equilibrating the systems in the NTP ensemble to a pressure of 1 bar (1 bar = 100 kPa) and a temperature of 300 K, the rest of the simulations were carried out in the NVT ensemble at 300 K. Long-range electrostatic interactions were evaluated using the particle mesh Ewald method, with the remaining nonbonded interactions subjected to a 9 Å cutoff. Also, the SHAKE algorithm[40] was used to restrain all bonds involving a hydrogen atom. Snapshots were saved every 500 steps for further analyses. In addition, the simulation code was modified to output the ω dihedral angle of the -Gly-Pro- motif at every step, in order to improve the statistics of the estimated free energies after reweighting.[41]

All of the accelerated molecular dynamics simulations were carried out using a modified version of the sander module in AMBER 8. The accelerated molecular dynamics (aMD) method has been previously described[26] and is based on earlier approaches by Grubmuller,[42] Voter,[43] and others[44−47] to promote sampling of infrequent events of biomolecular systems without prior knowledge of the locations of wells and barriers on the potential energy surface. In our implementation, a continuous non-negative boost potential function $\Delta V(r)$ is defined such that when the true potential $V(r)$ is below a chosen threshold boost energy E, the simulation is performed on the modified potential $V^*(r) = V(r) + \Delta V(r)$, and when $V(r)$ is greater than E, the simulation is performed on the true potential $V^*(r) = V(r)$. This leads to an enhanced escape rate for $V^*(r)$. The bias potential $\Delta V(r)$, as shown in eq 1, is chosen such that the derivative of $V^*(r)$ has no discontinuity, and the modified potential reflects the shape of the minima, as long as α is positive and nonzero.

$$\Delta V(r) = \begin{cases} 0, V(r) \geq E \\ \dfrac{(E - V(r))^2}{\alpha + (E - V(r))}, V(r) < E \end{cases} \quad (1)$$

The corrected canonical ensemble averages on the unmodified potential for the system are obtained by simply reweighting each point of the distribution of the ensemble obtained on the modified potential by the strength of the Boltzmann factor of the bias potential energy, $\exp[\beta \Delta V(r)]$, as shown in eq 2. When the system is on the normal potential, the boost potential $\Delta V(r) = 0$.

$$\begin{aligned} \langle A \rangle &= \int A(r) e^{-\beta V(r)} dr / \int e^{-\beta V(r)} dr \\ &= \int A(r) e^{-\beta V^*(r)} e^{\beta \Delta V(r)} dr / \int e^{-\beta V^*(r)} e^{\beta \Delta V(r)} dr \\ &= \langle A(r) e^{\beta \Delta V(r)} \rangle^* / \langle e^{\beta \Delta V(r)} \rangle^* \end{aligned} \quad (2)$$

Because the barriers in the torisonal potential largely govern the rate of sampling biomolecular rotameric states, as in the study of the *cis*−*trans* isomerization, the boost potential was applied to the total torsional term of the potential energy function of the substrate. For all of the simulations the boost energy E was set to 60 kcal/mol above the average total torsional energy on the unmodified potential, and α was set to 17 kcal/mol. For the free substrate in water, one very long accelerated MD simulation was carried out for 5×10^8 steps, which is 1 μs of simulation time if it were a normal MD simulation. The error was estimated by splitting the simulation into five equal blocks. We carried out seven independent accelerated MD simulations of the cyclophilin−substrate complex in explicit water. Each simulation was carried out for at least 5×10^7 steps, which is 0.1 μs each if it were just a normal MD simulation. The error was estimated by averaging over all the seven independent simulations.

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