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Entropic and Surprisingly Small Intramolecular Polarization Effects in the Mechanism of Cyclophilin A

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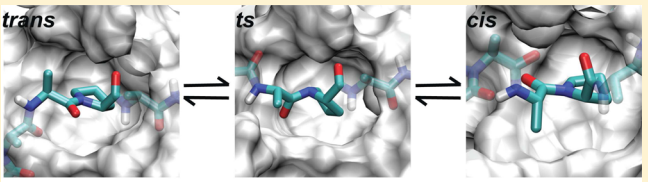
ABSTRACT: The precise catalytic mechanism of peptidyl−prolyl *cis*−*trans* isomerases (PPIases) has been elusive, despite many experimental and computational studies. The more than 5 orders of magnitude speedup achieved in catalysis by cyclophilin A (CypA) has been attributed to several factors, including substrate desolvation, enzyme dynamics, and preferential binding of the transition state. Here, we explore the conformational space of a substrate analogue of CypA using accelerated molecular dynamics, free in solution and in the active site of CypA, in order to probe its conformational interconversion during catalysis. We show that the undemanding exchange of the free substrate between β- and α-helical regions is lost in the active site of the enzyme, where it is mainly in the β-region. Our results suggest that the loss in conformational entropy at the transition state relative to the *cis* and *trans* states in the free substrate is decreased in the complex. This relative change in conformational entropy contributes favorable to the free energy of stabilizing the transition state by CypA. We also show that the ensuing intramolecular polarization, as a result of the loss in pseudo double bond character of the peptide bond at the transition state, contributes only about −1.0 kcal/mol to stabilizing the transition state. This relatively small contribution demonstrates that routinely used fixed charge classical force fields can reasonably describe these types of biological systems. Our results provide further insights into the mechanism of CypA, a member of a poorly understood family of enzymes that are central to many biological processes.



INTRODUCTION

Although nonproline *cis*-peptide bonds (ω-bonds) have been observed in numerous protein crystal structures,[1] proline is the most distinguishable amino acid that can readily adopt both *cis* and *trans* isomers due to the small free energy difference between the two configurations.[2] This unique feature confers proline-containing proteins the possibility of attaining different conformational states, which can regulate biologically important processes, including protein folding,[3] ion channel gating,[4] and signal transduction.[5] However, peptidyl prolyl *cis*−*trans* isomerization is inherently slow, due to a high free energy barrier separating the *cis* and *trans* states. The rate of these processes are therefore enhanced by a group of ubiquitous and evolutionarily conserved enzymes called peptidyl−prolyl isomerases (PPIases)[6] that can increase the rate of *cis*−*trans* isomerization by more than 5 orders of magnitude.[2]

Four structurally unrelated families of PPIases include cyclophilins (Cyps), FK506-binding proteins (FKBPs), parvulins, and Ser/Thr phosphatase 2A (PP2A) activator PTPA.[2,7] Cyclophilins and FKBPs are of special interest because they are considered as cellular targets for immunosuppressive drugs cyclosporin A (CsA) and FK506, respectively.[8,9] Also, Pin1, a homologue of the parvulin family, has been shown to play an important role in Alzheimer disease[10] and cancer.[11] Human cyclophilin A (CypA) is also being considered as a possible drug target in treating HIV infection because of its transient interaction with a −Gly−Pro− motif on the loop of the HIV capsid monomer.[12] Although CypA is suggested to regulate assembly and disassembly of the viral capsid core,[12−14] the exact catalytic role is not clear.[15−17] Therefore, understanding the detailed mechanism of CypA and other families of related enzymes will aid in the treatment of many diseases.

Cyclophilin A has been shown to considerably accelerate the isomerization rate of peptidyl-prolyl bond that occurs in hundreds of seconds to a more biologically relevant millisecond time scale.[18] Several different factors have been attributed to this tremendous rate enhancement, including transition state stabilization,[19] a possible nucleophilic attack on the carbonyl carbon of the amide bond, and the effect of substrate desolvation.[20−23] Furthermore, the role of enzyme dynamics in enhancing the catalytic rate has been studied extensively.[24−31] For example, Fraser et al.[30] used ambient temperature X-ray crystallography and NMR relaxation experiments to study a less active CypA mutant and concluded that the reduction in catalytic rate was due to interruption of the motions involved in the interconversion of the substrate. However, our recent study[31] pointed to the importance of enzyme dynamics for the reorganization of the active site residues for better stabilization of the transition state.[32]

Previously, accelerated molecular dynamics was used to investigate the catalytic mechanism of cyclophilin A.[19] The *cis*−*trans* isomerization of a substrate analogue with a −Gly−Pro−

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motif derived from the HIV-1 capid monomer was simulated directly, without any conformational constraint, free in explicit water and in the active site of cyclophilin A. The resulting reweighted free energy profiles suggested that catalysis occurs mainly through the stabilization of the transition state in the active site due to a combination of favorable hydrophobic and hydrogen bonding interactions (Figure 1). However, in addition to these specific interactions that are mainly enthalpic in nature, little is known about possible entropic effects on the free energy of stabilizing the transition state. In trying to fully understand the mechanism of CypA, we have further examined the conformational phase space of a well-studied substrate analogue, Ace−Ala−Ala−Pro−Phe−Nme, with improved dihedral force field parameters for the ω-bond angle,[33] free in solution and in the active site of CypA. The conformational preferences of the substrate have entropic implications for the free energy of stabilizing the transition state since the relative change in the conformational entropy of binding can affect the free energy.

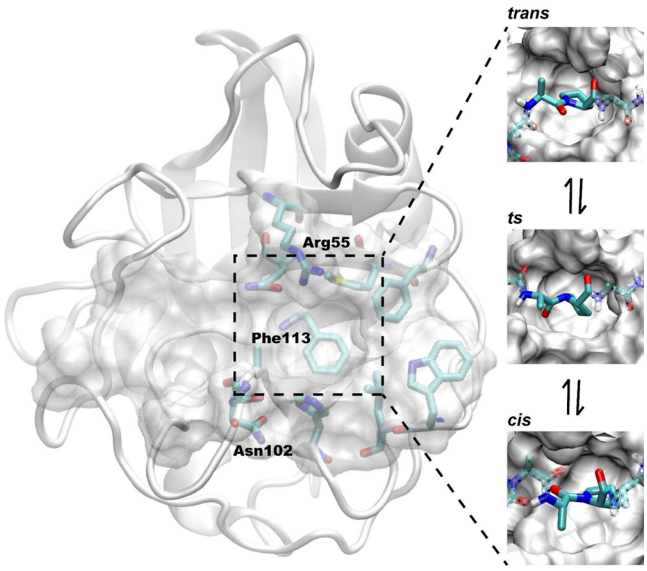

Figure 1. *Cis*−*trans* isomerization of the peptidyl−prolyl bond of a substrate analogue with the −Ala−Pro− motif by CypA. (Left) The binding site of CypA with the hydrophobic pocket and some of the key residues highlighted. (Right) The relatively planar configurations of the *cis* and *trans* isomers separated by a pyramidal transition state in active site of CypA.

As indicated in studies by Warshel and co-workers,[34,35] electrostatic effects can provide important enthalpic contributions to enzyme catalysis, due to better stabilization of possible charge buildup at the transition state in the active site than in water. Therefore, the importance of electrostatics in enzyme catalysis calls for precise treatments of the electron distribution, which is modeled as partial charges, and effects of polarizability during catalysis. It is also well-known that the electron distribution is influenced by the geometrical changes in the molecular structure.[36−39] Intramolecular polarizability describes redistribution of the electron cloud in response to alteration of the internal electric field arising from changes in nuclei positions. Methods are being developed to incorporate both inter- and intramolecular polarization effects in polarizable force fields.[40−43] However, pure classical molecular dynamics simulations would not accurately capture intramolecular polarization resulting from peptidyl−prolyl *cis*−*trans* isomerization. Therefore, considerable error may be associated with the computed conformational energies.[44] In isomerization of the peptidyl−prolyl bond (Figure 1), the transition state partially loses the pseudo double bond character of the ω-bond angle that leads to redistribution of the electrons, especially on the nitrogen. What is the effect of charge redistribution at the transition state on the mechanism of CypA? The answer to this question is important for two reasons: one, it will allow us to understand the suitability of the fixed charge model in studying the catalytic mechanism of cyclophilin A, and two, it will provide the free energy contribution of the charge redistribution to the stabilization of the transition state by CypA. Therefore, in this work, we have also determined the effect of intramolecular polarizability (redistribution of the partial charges at the transition state) on the binding free energy of the substrate in the transition state by CypA.

COMPUTATIONAL METHODS

MD Simulations of Catalytic Process of *cis*−*trans* Isomerization. The simulations were carried out with the well-studied substrate analogue, Ace−Ala−Ala−Pro−Phe−Nme, and the 1.58 Å resolution crystal structure complex of CypA with PDB ID 1AWR. The substrate analogue in the complex was modified from HAGPIA to Ace−AAPF−Nme. The coordinates of the backbone atoms and the common atoms of the side chain residues were kept the same. The *xleap* module in AMBER was used to add the missing atoms and solvate the free peptide and enzyme-bound substrates with explicit TIP3P water model[45] in a cubic and octahedron box, respectively. All simulations were run using the AMBER 10 suite of programs[46] with the modified version[47] of the parm99(ff99SB) all-atom force field[48] and the reoptimized dihedral parameters for peptide ω-bond.[33] The systems were equilibrated in the NTP ensemble with pressure of 1 bar and temperature of 300 K followed by MD simulations in the NVT ensemble at 300 K. During all simulations, the temperature was regulated with a Langevin thermostat with a collision frequency of 1 ps^{-1}. A cutoff of 10.0 Å was applied for nonbonded short-range interaction, while the long-range electrostatic interactions were treated by the particle mesh Ewald method.[49] All bonds involving hydrogen atoms were maintained using the SHAKE algorithm.[50] A time step of 2 fs was used to numerically integrate Newton's equation of motions.

The simulations were carried out with accelerated molecular dynamics,[51] in order to accelerate the rate of the slow *cis*−*trans* isomerization of the peptidyl−prolyl bond. A modified version of pmemd in AMBER 10 suite of programs was used to run all accelerated molecular dynamics simulations. For the free substrate in water, five independent accelerated MD simulations were carried out for a total time of 1.3 μs using a boost energy E of 60 kcal/mol above the average total dihedral energy calculated after equilibration and α of 10 kcal/mol. For the CypA complex in water, we carried out eight independent accelerated MD simulations (a total time of 1.19 μs) with the ligand selectively boosted,[19,52] using the same boost parameters as the free substrate. The configurations were then reweighted by the strength of the Boltzmann factor of the bias potential energy, $\exp[\beta\Delta V(r)]$, in order to calculate the probability distributions. The Boltzmann reweighting is exact, and the related statistical analysis on the precision has been previously discussed.[53]

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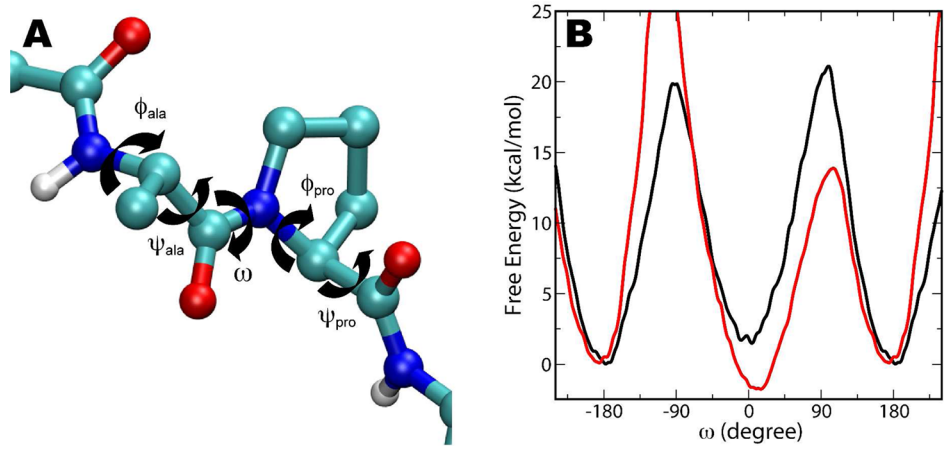

Figure 2. Motif of −Ala−Pro− of the substrate analogue (Ace−Ala−Ala−Pro−Phe−Nme) with the (A) backbone dihedral angles, ω, φ, and ψ, defined and (B) the free energy profiles along the ω-bond angle of the −Ala−Pro− motif, for the (black) free substrate in solution and (red) in the enzyme−substrate complex.

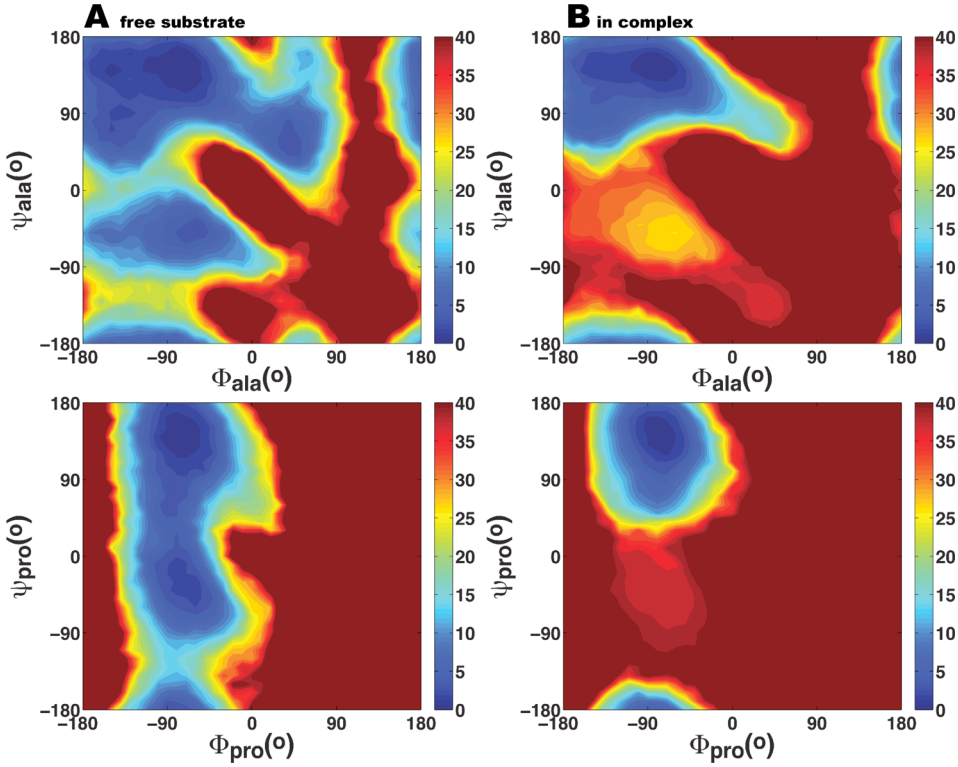

Figure 3. Two-dimensional contour plots in kcal/mol of the conformational space of Ala and Pro in the free substrate and enzyme−substrate complex. (A) φ−ψ or Ramachandran plot of Ala (top) and Pro (bottom) of the −Ala−Pro− motif of the free substrate. (B) φ−ψ or Ramachandran plot of Ala (top) and Pro (bottom) of the substrate in the CypA−substrate complex.

Calculating Atomic Partial Charges at the Transition State. We created the initial configuration of the Ace−Ala−Pro−Nme, with the peptidyl−prolyl bond of Ala−Pro in the transition state, i.e., $\omega \approx 90°$, using the *xleap* module in AMBER. The transition state configuration of the Ace−Ala−Pro−Nme was then minimized using the Gaussian03 program,[54] and the electrostatic potential was calculated at the HF/6-31G* level of theory. The calculated electrostatic potential was then used to obtain partial charges using the standard two-step RESP method.[55,56] The partial charges of Ace and Nme were set to those in the standard AMBER force field. We therefore only allowed the partial charges of the central −Ala−Pro− motif to vary in the transition state of the substrate analogue, Ace−Ala−Ala−Pro−Phe−Nme.

Free Energy Calculations. Thermodynamic integration[57] was used to calculate the relative binding free energy of the substrate in the transition state by CypA between the two states: the initial state has the standard partial charges from the AMBER force field and the final state has the recomputed partial charges specific to the transition state configuration. In this regard, free energy is defined as a function of a developing, coupling parameter, λ, which represents the progress of the partial charges of the transition state from an initial state (with AMBER standard charges) to the final state (with redistributed charges). The configuration of the peptidyl−prolyl bond of the

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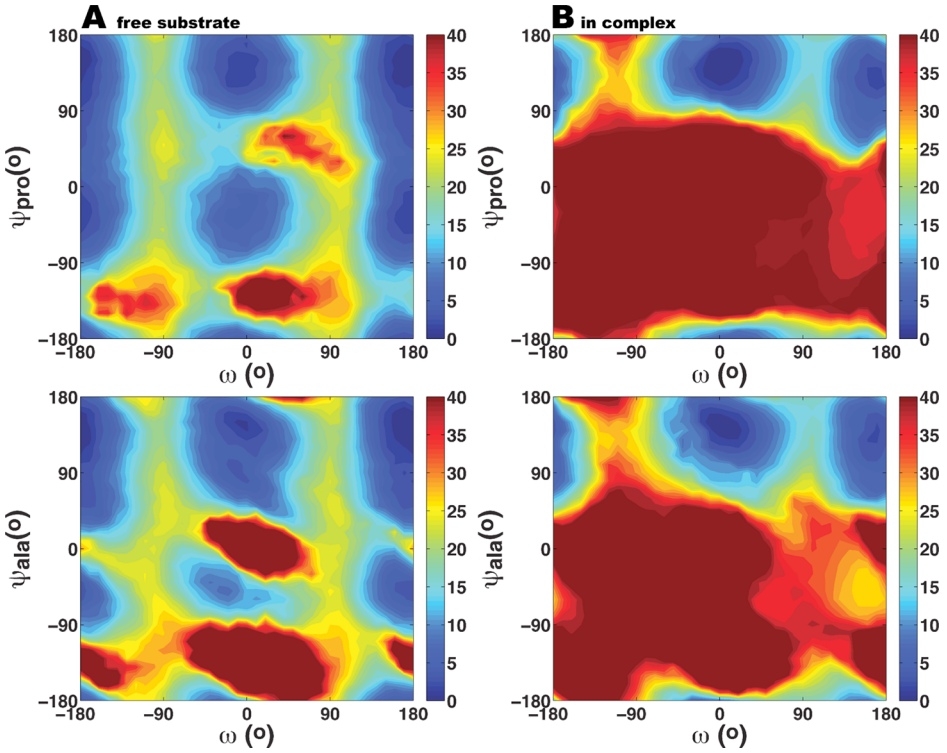

Figure 4. Two-dimensional contour plots in kcal/mol of the $\omega-\psi$ dependency for Pro (top) and Ala (bottom) of the −Ala−Pro− motif of the substrate analogue (A) free in solution and (B) in the CypA−substrate complex.

Ala−Pro motif of the substrate was maintained at the transition state by restraining the ω-bond angle around 90° using a flat-bottomed well potential with a force constant of 1000 kcal/mol/rad^2. A seven point Gaussian quadrature was used, with λ values corresponding to 0.02544, 0.12923, 0.29707, 0.50000, 0.70292, 0.87076, and 0.97455, to approximate the integral of the total free energy. Three separate runs with different initial random seeds were carried out for each window.

RESULTS AND DISCUSSION

Accelerated Molecular Dynamics Simulations of the Catalytic Process of CypA. Catalysis of *cis*−*trans* isomerization of the −Ala−Pro− ω-bond (Figure 2A) of a substrate analogue (Ace−Ala−Ala−Pro−Phe−Nme) by CypA was studied using accelerated molecular dynamics. The reweighted free energy profiles along the ω-bond angle were computed with the *trans* state as the reference state (Figure 2B). The free energy profiles show approximately 9 kcal/mol stabilization of the transition state by CypA, relative to the uncatalyzed reaction. Unlike the relatively planar configuration of the −Ala−Pro− peptide bond in the *cis* and *trans* states, isomerization goes through a pyramidal transition state (Figure 1). The main factors in transition state stabilization by CypA have been attributed to interactions of the proline side chain with the hydrophobic cavity (Figure 1) and hydrogen bonds (mainly between the substrate and Arg55 and Asn 102 of CypA) in the active site of CypA.[19] These interactions are best achieved through an N-terminal rotation around the peptide bond, suggested by crystallographic studies and MD simulations.[19,58,59]

Comparison of the backbone $\varphi-\psi$ conformational phase space of Ala and Pro in the free and enzyme−substrate complex (Figure 3A,B) shows that the β- and polyproline II (PPII)-region are predominantly populated in the complex, whereas the α-helical region is hardly sampled. Despite an apparent preference for the β-region by the free substrate over the α-helical region, the energetic barriers between α- and β-regions is still low enough to allow for easy interconversion. The effect of confinement of the substrate in the β/PPII-region upon binding to the enzyme is also clearly seen in the 2D $\omega-\psi$ free energy landscape of Pro in Figure 4. As previously observed,[60] there is coupling between the ψ of proline and the ω-bond angle. Free in solution (Figure 4), the substrate prefers to be in the β-region when in the *cis* configuration and can easily interconvert between the β-region and the α-helical region, when in the *trans* configuration. However, this easy backbone interconversion is restricted in the active site of the enzyme (Figure 4B). In the active site, the substrate is mainly in the β-region for both the *cis* and *trans* configurations of the prolyl peptide bond with a much lower free energy barrier separating the two isomers, compared to the free substrate. The enzyme limits the flexibility of the substrate and creates a low energetic valley up to the transition state that connects the *cis* and *trans* isomers (Figure 4B). Similar $\omega-\psi$ coupling is observed for the preceding Ala residue (Figure 4, bottom). Unlike the Pro residue, the free energy barriers separating the α- and β-regions for Ala are slightly higher in both the *cis* and *trans* states of the free substrate. Also, Ala is almost exclusively in the β-region when in the *cis* state, as compared to Pro.

A previous study[61] of the catalytic mechanism of CypA using metadynamics showed that the free substrate with a −Gly−Pro− motif preferred being in the α-helical region to the β-region, contrary to our present results. We believe that this discrepancy is not due to differences in the sequence but due to the choice of the force field since the present results are similar to earlier studies of *cis*−*trans* isomerization in different sequences.[62] The older AMBER parm99 force field used in their study is known to overstabilize the α-helical conformation

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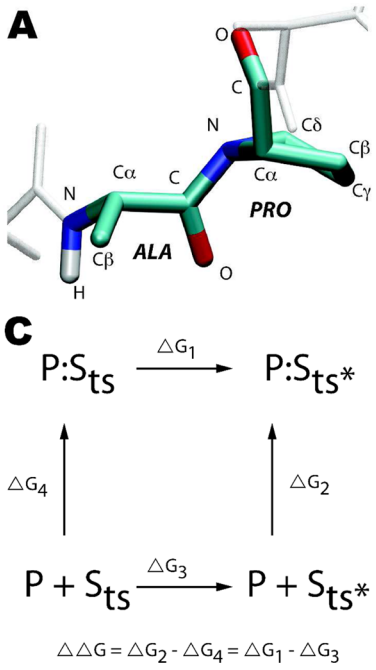

B

	Atoms	ts	ts*
ALA	N	-0.4157	-0.3884
	H	0.2719	0.2516
	Cα	0.0337	-0.0793
	Cβ	-0.1825	-0.2286
	C	0.5973	0.8344
	O	-0.5819	-0.5284
PRO	N	-0.2548	-0.5922
	Cα	-0.0266	-0.0142
	Cβ	-0.0070	-0.1962
	Cγ	0.0189	0.0094
	Cδ	0.0192	-0.0595
	C	0.5896	0.7003
	O	-0.5748	-0.6374

Figure 5. (A) Motif of −Ala−Pro− of the substrate analogue in the transition state with the atoms defined. (B) Partial atomic charges of the −Ala−Pro− motif as defined in the AMBER force field (ts) and the recomputed partial charges in the transition state configuration (ts*). (C) Thermodynamic cycle connecting the free energy of binding and free energy of charge redistribution of the substrate free in solution and in the active site of CypA−substrate complex. P and S represent the enzyme and substrate, respectively; ts and ts* represent the transition state with the AMBER force field partial charges and the recomputed partial charge for the transition state configuration, respectively.

of protein backbone and has since been modified to parm99SB in order to correct for this problem. We are using the updated parm99SB force field, with corrections for the dihedral parameters of the ω-bond angle.[33] However, despite over stabilization of the α-helical region of the free substrate in their study, the β-sheet and PPII conformations of the substrate were found to be more stable in the complex, inline with our present findings, emphasizing the preference for the backbone of the substrate in the β and PPII conformations by CypA.

One major implication of the restriction of the conformational space of the substrate in the active site is entropic in nature. The entropic contribution to the free energy of binding could come from the solvent,[63] the ligand,[64] and the receptor.[65] Here, we focus on the conformational changes of the substrate upon binding and during catalysis, in order to assess its contribution to stabilizing the transition state. As previously shown by Chang et al.,[64] binding of drug-like molecules to proteins results in a loss in configurational entropy, which is unfavorable to the overall attractive forces. Also, the evolution of the conformational state of the substrate from *trans* or *cis* to the transition state involves inherent negative conformational entropy change. In the enzyme−substrate complex, there is a less negative change in the conformational entropy to reach the transition state from the *cis* or *trans* state as compared to that in free substrate. The consequence is a positive relative conformational change in entropy ($\Delta\Delta S$) that contributes favorably to the free energy of binding ($\Delta\Delta G$) the transition state relative to the *cis* or *trans* state. Considering the free energy of binding. the *cis* or *trans* ($\Delta G_{cis/trans}$) and transition states (ΔG_{ts}), then $\Delta G_{cis/trans} = \Delta H_{cis/trans} - T\Delta S_{cis/trans}$ and $\Delta G_{ts} = \Delta H_{ts} - T\Delta S_{ts}$. The relative free energy of binding the transition state, $\Delta\Delta G = \Delta G_{ts} - \Delta G_{cis/trans} = \Delta\Delta H - T\Delta\Delta S$. Therefore, a positive $\Delta\Delta S$ change in the conformational entropy will result in a more negative $\Delta\Delta G$, leading to a lower free energy barrier of isomerization in the active site of the enzyme.

Effect of Intramolecular Polarizability in Stabilizing the Transition State. The conformational dependence of atomic partial charges has long been considered for accurate calculation of intermolecular energies.[38,44,66,67] Reynolds et al.[44] examined the errors in free energy calculations as a result of neglecting variations in atomic charges during conformational changes and concluded that these errors could dominate over all other errors. Conformational charge redistribution may also have implications for the mechanism of CypA. As mentioned earlier, during *cis*−*trans* isomerization, the relatively planar geometry of the peptidyl−prolyl amide bond of the *cis* and *trans* isomers becomes pyramidal in the transition state, as shown in Figure 1. The nitrogen atom of proline is pushed out of plane to form a tetrahedral-like configuration, and its lone pair of electrons is more localized instead of being delocalized along the pseudo double C−N bond. This configuration at the transition state therefore leads to a partial loss of the pseudo double bond character of the C−N bond; resulting in a new distribution of partial charges, especially on atoms around the prolyl−peptide bond. The question now is how much does this charge redistribution on the atoms, especially on the nitrogen, affect the binding free energy of the transition state of the substrate by CypA? Also, it has been suggested that Arg55 could form an essential hydrogen bond with the lone-pair of electrons on the nitrogen of proline to stabilize the transition state.[68,69] However, this interaction does not seem mandatory for catalytic activity as mutation of Arg55Ala retains some activity in CypA.[70] Also, an Arg55Lys mutation is not as active as the wild type, even though the possibility of the hydrogen bond is preserved.[71] As seen in Figure 2B, the reduction of 9−10 kcal/mol in the free energy barrier height (which can potentially bring about more than 10^5 speedup, not considering

the prefactor effects) in enzyme-catalyzed reaction is achieved already with a pure classical description of the system with a fixed charge model, that is, without accounting for conformational dependence of the partial charges and the charge redistribution in the transition state (intramolecular polarizability). Nonetheless, does the enzyme take additional advantage of charge redistribution to further stabilize the transition state? To answer this question, we carried out free energy calculations using thermodynamics integration to estimate the relative change in free energy of binding the transition state by CypA with two different sets of partial charges: the normal fixed AMBER partial charges that correspond to the substrate in the *trans* state and that of the redistributed partial charges recomputed using the transition state configuration. The transition state has been shown to bind to the enzyme better than the *cis* and *trans* isomers with the maximum conformational distortion from the ground states.[19]

Quantum mechanical calculations were carried out on the Ace−Ala−Pro−Nme motif (Figure 5A) to compute the electrostatic potential at the HF/6-31G* level of theory, fixing the configuration of the prolyl−peptide bond at the transition state. The RESP method[55,56] was then used to calculate the partial charges on atoms in the Ala−Pro motif at the transition state (Figure 5B). According to the thermodynamic cycle shown in Figure 5C, the binding free energies corresponding to the two sets of charges (ΔG_2 and ΔG_4) are connected to the changes in free energies when the substrate undergoes charge redistribution free in solution and in the active site of CypA (ΔG_3 and ΔG_1, respectively). Therefore, the relative binding free energy of the substrate in the transition state with the redistributed partial charges, as compared to the regular AMBER partial charges, is $\Delta\Delta G = \Delta G_2 - \Delta G_4 = \Delta G_1 - \Delta G_3$. This relative free energy difference ($\Delta\Delta G$) was calculated to be $\sim -0.6 \pm 1.2$ kcal/mol. This surprisingly small change in the relative binding free energy due to intramolecular polarizability demonstrates that routinely used fixed charge classical force fields can reasonably describe these types of biological systems.

Why is the binding energy of the transition state not significantly affected by intramolecular polarizability? The answer may be found in the suggested mechanism of isomerization of the prolyl−peptide bond in the active site of CypA. As was earlier shown, CypA binds the transition state more favorably than the *cis* isomer, with the enzyme having the least binding affinity for the *trans* isomer. Catalysis occurs mainly through the stabilization of the transition state in the binding site due to a combination of favorable hydrophobic and long-lasting hydrogen bonding interactions. The C-terminal of the peptide bond comprises the proline ring that is clutched in the hydrophobic pocket of the enzyme. Hydrogen bonds involving interactions between the carbonyl oxygen of the amide bond and the backbone NH group of Asn 102, as well as between the guanidinium moiety of Arg 55 and the carbonyl oxygen of proline, help to stabilize the transition state. As shown in Figure 5B, partial charges of the atoms involved in these hydrogen bonds (carbonyl oxygen of both proline and its preceding residue) only change slightly, with no change in the overall net charge. The nitrogen atom of proline, which shows a maximal increase in the magnitude of the partial negative charge, has little contribution to hydrogen bonding due to its average distance of ∼4 Å from the nitrogen atom of the guandinitum moiety of Arg 55. This distance is considered to be too long to form any energetically meaningful hydrogen bonding interaction.

In addition to studying the effects of charge redistribution on the relative free energy of binding the transition state, we also took into account changes in several bond lengths, especially the C−N peptide bond during the free energy calculations. Conformational dependence of bond lengths[72] is not as influential on the free energy as that of dihedral angles, but the evidence of such effects have been previously studied.[73] Here, we looked for possible effects of such bond length changes on the relative binding free energy of the transition state by CypA. The bonds that are around and directly involved in the peptidyl−prolyl amide ω-bond were allowed to change during the free energy calculations, in addition to the partial charges. Comparison of QM optimized bond lengths at the transition state and those obtained from the AMBER force field parameters shows only small differences. As expected, the bond lengths that changed the most include the C−N (peptide), as well as the C═O, N−Cα, and N−Cδ bonds. The changes observed for C−N and C═O bonds are especially in accordance with expected changes in bond lengths due to the loss of the pseudo double bond character of the peptide bond in the transition state. The electronic rearrangement in the transition state led to an increase in the C−N bond length from 1.33 to 1.41 Å, similar to previous observation,[74] and a small decrease (about 0.04 Å) in the bond length for the C═O bond. Allowing these bond lengths to also vary during the free energy calculations, in addition to the partial charges, did not make any noticeable difference in the calculated relative binding free energy.

■ CONCLUSIONS

Molecular dynamics simulations were used to investigate the uncatalyzed and catalyzed *cis*−*trans* interconversion and the role of intramolecular polarizability of the substrate in the catalytic mechanism of CypA. As previously observed, preferential binding of the transition state of the substrate by CypA is the dominant contribution to catalysis. We show that the conformational space of the substrate is more restricted in the active site of the enzyme than when it is free in solution. The results suggest that the relative change in conformational entropy of binding the transition state by CypA is positive, contributing favorably to the relative free energy of binding. Therefore, the overall effect will lead to a reduction in free energy barrier of *cis*−*trans* isomerization in the active site of the enzyme, as compared to the free substrate in solution.

In addition, our results suggest that intramolecular polarizability due to the loss of the pseudo double bond character of the C−N bond contributes only about −1.0 kcal/mol to the binding free energy of the transition state. This surprisingly small contribution from intramolecular polarizability to the free energy barrier amounts to a fraction of the overall expected barrier reduction. The results therefore validate the use of molecular mechanics force fields with fixed partial charges to reliably describe these types of systems in which the active site of the enzyme is essentially hydrophobic.

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Notes

The authors declare no competing financial interest.

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