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Intra- and Intermolecular Interaction Inducing Pyramidalization on Both Sides of a Proline Dipeptide during Isomerization: An Ab Initio QM/MM Molecular Dynamics Simulation Study in Explicit Water

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Abstract: The cis−trans isomerization of the peptide bond preceding a proline plays important roles in protein folding and biological function. Although many experimental and theoretical studies have been done, the mechanism has not yet been clearly elucidated. We studied the cis−trans isomerization of the proline dipeptide (Ace−Pro−NMe) in explicit water by molecular dynamics simulations using a combined potential derived from ab initio quantum mechanics and empirical molecular mechanics. We obtained the free energy landscape during the isomerization by using the umbrella sampling method. The free energy landscape is in good accordance with previous experimental and theoretical values. We observed that in the middle of the isomerization, the prolyl nitrogen transiently takes pyramidal conformations in two polarized directions and that, simultaneously, the prolyl C−N bond extends. We show that these geometrical changes cooperatively transform the prolyl nitrogen from a sp^2-hybridized electronic state into a sp^3-hybridized one, and thus realize a transition state that reduces the rotational barriers separating the cis- and trans-states. We also found that the hydration of the prolyl nitrogen stabilizes the negative pyramidal conformation, while an intramolecular interaction mainly stabilizes the positive one. Fluctuations in the polarity and magnitude of the pyramidal conformation during the isomerization are interpreted as a competition between the hydrogen-bonding partners for the prolyl nitrogen between different sides of the pyrrolidine ring.

Introduction

The cis−trans isomerization of a peptide bond is a very slow process under normal conditions due to the resonance condition that results in a partial double bond character.[1] There are two stable conformations that correspond to energy minima when the C(=O)−N dihedral angle ω is about 0° corresponding to the cis-state or 180° corresponding to the trans-state, with rotational barriers of approximately 20 kcal/mol at $\omega = 90°$ and 270°, respectively. Moreover, due to the resonance condition, the C−N bond is rather short as compared to normal sp^3-hybridized bonds. Almost all of the peptide bonds are in the trans-state in folded proteins as shown from vast X-ray and NMR atomic level structural data.[2] However, about 5% of peptide bonds preceding proline are in the cis-state.[3] The cyclic nature of the side chain of proline means that cis- and trans-conformations are closer in energy than in other amino acids, because steric hindrance between the δ-CH_2 group of the pyrrolidine ring and the α-CH group of the preceding residue destabilizes the trans form and results in a relatively more stable cis form.[4]

The cis−trans isomerization process of a prolyl peptidyl bond plays an important role in the rate-limiting step of folding and denaturing of proteins, as well as in the function of enzymes.[5] In the past decade, proline cis−trans isomerization has been shown to play a critical role in such biological processes as RNA splicing, cell signaling, and trans-membrane active transport.[6] Accordingly, cis−trans isomerization has been extensively studied experimentally and theoretically. Beausoleil and Lubell studied the relative populations of the amide cis- and trans-isomers as well as the energy barriers for amide isomerization using NMR experiments.[7] Cox et al. reported NMR and FTIR

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experimental studies of intramolecular catalysis of prolyl amide bond isomerization associated with the hydrogen bond between the side chain and the prolyl nitrogen and estimated the intramolecular interaction effect.[8,9] Fisher et al. analyzed the cis−trans imide isomerization of the proline dipeptide in vacuo by empirical energy functions and ab initio calculations, and concluded that an essential element is the presence of a dipole moment on the imide nitrogen.[10] Kang studied the cis−trans isomerization of Ace−Pro−NMe and its derivatives by extensive ab initio molecular orbital and density functional methods employing the self-consistent reaction field method to include solvent effects, and obtained energy surfaces and solvation free energies of several conformations in chloroform and in water.[11]

QM/MM simulations are now widely used in biomolecular and nanomaterial simulations, because conventional force fields based on classical mechanics do not take into account the effect of electronic state changes in molecular reactions such as chemical bond breaking and formation, and electronic excitations.[12−14]One cannot apply quantum mechanics to whole dynamic molecular systems of proteins or even peptides because the computational cost exceeds current resources. In contrast to a full quantum mechanics calculation, QM/MM treats a limited region, where electronic state changes are important, quantum mechanically. The rest of the system, where such effects are less important, is treated classically using an empirical molecular force field. In this study, a quantum mechanical treatment of the peptide is indispensable for modeling cis−trans isomerization because the change of or perturbation to the electronic states significantly influences the energy minima and rotational barriers of the molecule.

In this study, we report the results of molecular dynamics simulations of *N*-acetyl-*N*′-methylprolineamide, which is referred to as Ace−Pro−NMe or proline dipeptide, in explicit water with an ab initio QM/MM potential. The Ace−Pro−NMe system has been widely used as a minimal and standard model for experimental[7−9,15] and theoretical[10,11a−c] studies on the isomerization of peptide bonds including proline. In addition, the importance of the role of the hydration on protein dynamics was emphasized by McCammon et al.[16] They compared molecular dynamics simulations in explicit solvent to simulations using an implicit solvent model for a peptide including proline and showed that there are significant differences in the dynamics and roughness of the energy landscape of the peptide in implicit and explicit models. Accordingly, we treated the peptide by quantum mechanics, while the surrounding solvent molecules were modeled explicitly using a classical molecular mechanics force field. We defined a reaction coordinate using the isomerization degree of freedom and computed the free energy, including solvent effects, as a function of this coordinate.

As expected, we identified a transition state that adopts a pyramidal conformation during the isomerization. Surprisingly, the sign of the polarity of the pyramidal conformation fluctuated, as intra- and intermolecular hydrogen-bond partners competed for the prolyl nitrogen.

Method

QM/MM Scheme. Our combined quantum mechanics (QM) and molecular mechanics (MM) hybrid potential employed the electronic embedding scheme[12] as shown in eq 1.

$$E_{\mathrm{tot}} = E_{\mathrm{QM}} + E_{\mathrm{MM}} + E_{\mathrm{QM/MM}} \quad (1)$$

E_{tot}, E_{QM}, E_{MM}, and $E_{\mathrm{QM/MM}}$ are the total energy of the system, the energy of the QM part, the energy of the MM part, and the interaction energy between the QM part and MM part, respectively. $E_{\mathrm{QM/MM}}$ is the contribution from two different interactions, as presented in eq 2.

$$E_{\mathrm{QM/MM}} = E_{\mathrm{vdW}} + E_{\mathrm{ele}} \quad (2)$$

Here, E_{vdW} and E_{ele} are the van der Waals interactions and the electrostatic interactions between the QM atoms and the MM atoms. Therefore, the potential energy of the QM atoms is calculated by ab initio molecular orbital theory, while the electrostatic potential energy arising in the QM part from the MM atoms is calculated quantum mechanically by using the point charges of the empirical force field.

Molecular Dynamics Simulation. We modeled the simulation system by immersing the *N*-acetyl-*N*′-methylprolineamide (Ace−Pro−NMe) in the center of a pre-equilibrated TIP3P water[17] sphere of radius 19 Å and removing overlapping water molecules within 3.0 Å from the peptide. The number of water molecules in the system was 1354. An harmonic restraint potential with a force constant of 150 kcal/mol/Å^2 was applied to the center-of-mass of the water molecules over the sphere boundary, while the center-of-mass of the Ace−Pro−NMe was fixed to avoid drift during the simulations.

We minimized the energy of the system by 300 steps of conjugate gradient and 100 steps of steepest decent minimization. Next, we gradually equilibrated the system from a low temperature of 3 K to a high temperature of 300 K by NVT molecular dynamics simulations with the Hoover−Evans method[18] over 10 ps. The SHAKE algorithm[19] was applied to all hydrogen atoms. The Amber force field (parm96)[20] was used to model the atomic interactions. A cutoff of 14 Å was used for treatment of the nonbonded interactions. We integrated the equations of motion by the velocity Verlet integration algorithm with a time step of 2 fs. The final conformation of the peptide was near to the trans-state with $\omega' = 180°$, where ω' is the dihedral angle of O1−C1−N−C_δ as shown in Figure 1. Note that our definition of ω' is different from the standard dihedral angle ω of a peptide backbone. Fischer et al. used the angle ζ for isomerization, instead of the ω, so as to uncouple the change of the isomerization from that of the pyramidalization.[10] Similarly, we found that our ω' values employed carry out the uncoupling and suit the reaction coordinate of the umbrella sampling simulations.

After this first equilibration step, we applied an harmonic umbrella potential centered at 180° on the rotation angle ω' and

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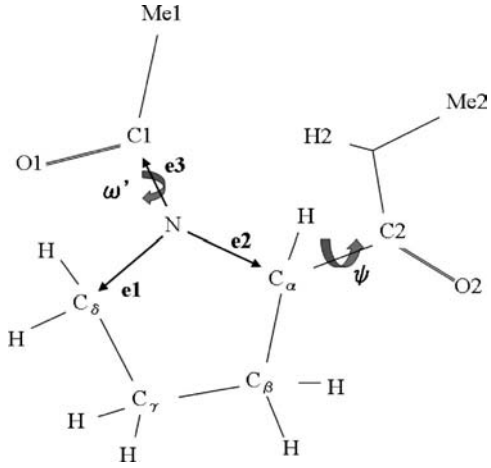

Figure 1. Definition of torsion angle, atoms, and angles for the calculation of the pyramidalization of the Ace−Pro−NMe.

then equilibrated the system for 1 ns. To create new systems with different umbrella potential centers, we moved the center of the umbrella potential by 10° with respect to the previous ω' and then equilibrated the new system for 1 ns after minimization. We repeated the process until we reached a center of approximately 360°. We consequently made 36 simulation systems (hereafter referred to as "windows") with different umbrella potential centers. We found that a force constant of 0.1 kcal/mol/deg^2 for the umbrella potential guaranteed well overlapped density of states among the windows.

After 1 ns of equilibration at 300 K for all of the 36 systems, we exchanged the potential applied to Ace−Pro−NMe from the empirical force field to the ab initio QM potential calculated by using the 4-31G basis set with the restricted Hartree−Fock level of molecular orbital theory, and we continued the molecular dynamics simulations of all systems. We selected the 4-31G basis set out of consideration of its computational abilities. To evaluate the quality of the basis set, we compared the 4-31G with a larger basis set of 6-31G(d) over several isomerization conformations of the Ace−Pro−NMe in vacuo and did not observe significant differences in the potential energies (see Figure S2 and the descriptions of section 2 in the Supporting Information). Indeed, Kang has evaluated the several conformational Ace−Pro−NMe energies by MP2, B3LYP, and HF and observed no intrinsic differences with respect to the level of the molecular orbital theory in water.[11] Moreover, Kang concluded that the electron correlations to the conformational energies were 1 kcal/mol or less at both MP2 and B3LYP levels. Here, MM point charges within 12 Å from the center-of-mass of the proline were taken into account for the molecular orbital calculations as one-electron integrals. We took trajectories of all system coordinates every 20 fs. In the QM/MM simulations, the initial 20 ps from the potential exchanges was regarded as QM/MM equilibration, which we omitted. For analysis, we used the following 100 ps of each window. We calculated the potential of mean force (PMF) of the trajectories and combined them into a continuous free energy landscape using the Umbrella integration method developed by Kastner and Thiel.[21]

Fischer et al. have reported a strong dependence of the activation barrier on the dihedral angle ψ (see Figure 1) of the Ace−Pro−NMe during isomerization.[10] According to their potential surface as calculated by ab initio methods, it is suggested that the amide hydrogen tends to point toward the prolyl nitrogen ($\psi \approx 0°$) during isomerization to reduce the rotational barriers. In our simulations, we utilized several conformations of the Ace−Pro−NMe. Indeed, after equilibrium we observed that ψ has a tendency to orient the amide hydrogen to point toward the prolyl nitrogen whichever initial conformation was selected. Thus, in this Article, our Ace−Pro−NMe is limited to the vicinity of the $\psi \approx 0°$ conformation.

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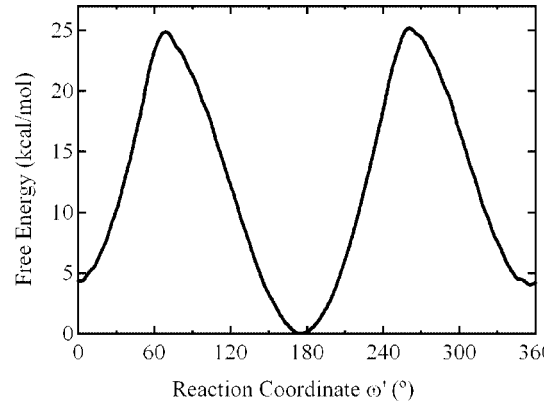

Figure 2. The free energy landscape of the cis−trans isomerization as a function of the reaction coordinate ω'.

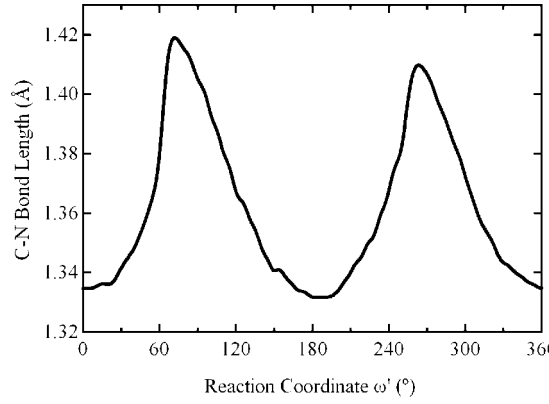

Figure 3. The length of the prolyl C−N bond as a function of the reaction coordinate ω'.

In this study, we used the program presto[22] for minimization and molecular dynamics simulations and AMOSS[23] for ab initio molecular orbital calculations.

Results and Discussion

The Free Energy Landscape. We constructed a potential of mean force (PMF) for each simulation window and combined them into a continuous free energy landscape along the reaction coordinate as shown in Figure 2. We could see that the trans-state is more stable than the cis-state by 4 kcal/mol and that the two states are separated by two barriers corresponding to free energy maxima of 24.9 and 25.2 kcal/mol at 69° and 261°, respectively. These values are in accord with the reported experimental values of 20.4 kcal/mol[7] and theoretical values of 21.1 kcal/mol[11a] including solvent effects.

The C−N Bond Length. We further analyzed the rotational angle dependence of the prolyl bond length C−N. The averaged C−N bond length of the simulations is shown in Figure 3 as a function of the rotational angle of the ω'. The length was 1.33 Å at both 0°, corresponding to the cis-state, and 180°, corresponding to the trans-state. It extends to 1.42 Å at 72° and to 1.41 Å at 263°. The extension was clear evidence for the change in character of the prolyl bond from a sp^2-hybridized to a sp^3-

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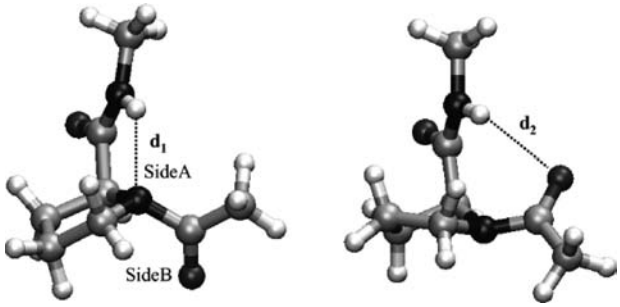

Figure 4. Typical positive (a) and negative (b) pyramidal conformations in the current simulations. The ω' values of the positive and negative pyramidal conformations are, respectively, 70° and 250°. Side A and side B are also shown. The N−HN and O−HN interactions are represented by dotted lines referred to as d_1 and d_2, respectively.

hybridized electronic state, which reduces the rotational barrier of the isomerization to some extent.[11a,24] It should be noted that the ω' dependence of the C−N bond length and that of the free energy landscape are well correlated.

The Proline Conformational Change during the Isomerization. Rankin and Boyd studied the Ace−Pro−NMe by density functional theory in vacuo and emphasized the importance of the hydrogen bond between the prolyl nitrogen and amide hydrogen subsequent to the proline. Thus, the N−HN intramolecular interaction in the transition state stabilizes and enhances the pyramidal conformation of the prolyl nitrogen.[24] To elucidate the effects, we analyzed the pyramidalization and the N−HN intramolecular hydrogen-bond properties.

We defined the degree of pyramidalization, hereafter referred to as pyramidality, in terms of the prolyl nitrogen as shown in eq 3.

$$\text{pyramidality} = \det |\mathbf{e1}, \mathbf{e2}, \mathbf{e3}| \qquad (3)$$

where **e1**, **e2**, and **e3** are unit vectors, whose directions are from N to C_δ, C_α, and C1, respectively, as illustrated in Figure 1. The determinant corresponds to the volume of the tetrahedron formed by the above four atoms, and thus it can be a good estimate for the degree of the pyramidalization. The prolyl nitrogen was defined as the center of the pyramidal conformation. According to eq 3, the sign of the determinant indicates the direction of the polarization. The positive pyramidality is defined as a conformation in which the prolyl nitrogen moves upward to the plane of the pyrrolidine ring (see Figure 1), and the negative one is the inverse. Typical positive and negative pyramidal conformations are represented in Figure 4.

In Figure 5a, the free energy landscape of the Ace−Pro−NMe as a function of the pyramidality and the reaction coordinate ω' is shown. In this two-dimensional landscape, there are clearly two clusters whose energy minima correspond to the cis- and trans-states. Between the two clusters, the transition states are located near $\omega' = 70°$ and 260°, respectively. At those transition states, varieties of pyramidality can be assumed, suggesting a large fluctuation occurs. The average pyramidality of the prolyl nitrogen is shown in Figure 5b as a function of ω'. As ω' increases, the average pyramidality gradually decreases from zero at 17° to a small negative peak at 61° and then sharply increases to a large positive peak at 85°. It again then gradually decreases to a negative value at 252° followed by a sharp increase at 269°.

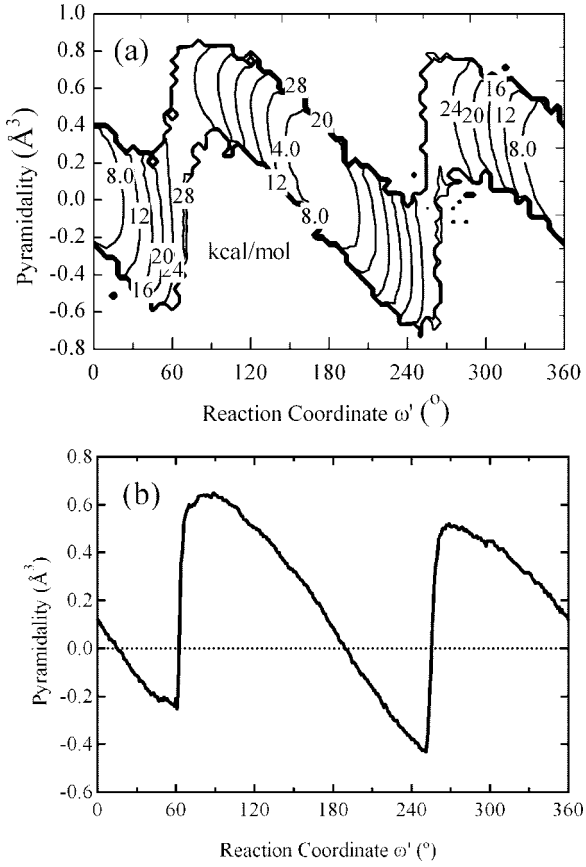

Figure 5. The free energy landscape of the Ace−Pro−NMe as a function of the pyramidality and the reaction coordinate ω' (a), and the averaged pyramidality of the Ace−Pro−NMe as a function of the reaction coordinate ω' (b).

We compared the pyramidality defined in the current work with the nonplanarity defined by Kang.[11c] Kang estimated the degree of nonplanarity of the imide nitrogen defined by $S - 360$, where S is the sum of the angles C1−N−C_γ, C_γ−N−C_α, and C1−N−C_α (see Figure 1). We then found they show the same trend for representing the absolute degree of pyramidal conformation. However, their measure of the nonplanarity does not reflect the polarity of the pyramidal conformations, which our definition in eq 3 can identify as the positive or negative volume of the tetrahedron around the prolyl nitrogen.

The averaged ω values in the simulations are in the vicinity of the angles such that the amide hydrogen following the proline points toward the prolyl nitrogen.

The solid line in Figure 6 indicates the average N−HN intramolecular hydrogen-bond length as a function of ω'. The hydrogen-bond length is 2.44 Å at 0° in the cis-state and 2.46 Å at 180° in the trans-state. When ω' increases, the hydrogen-bond length decreases to 2.30 Å at 70° and 2.27 Å at 267°; the length increases to 2.61 Å at 58° and to 2.72 Å at 253°, showing that the rotational angle dependence correlates well with the pyramidality in Figure 5a and b.

These results reveal that in the region where the pyramidality is positive, the N−HN hydrogen bonding is strengthened and stabilizes the pyramidal conformation as described by the Rankin and Boyd model.[24] In contrast, in the region where the pyramidality is negative, the N−HN hydrogen bond is weakened so that the N−HN hydrogen bond does not contribute to stabilization of the pyramidal conformation.

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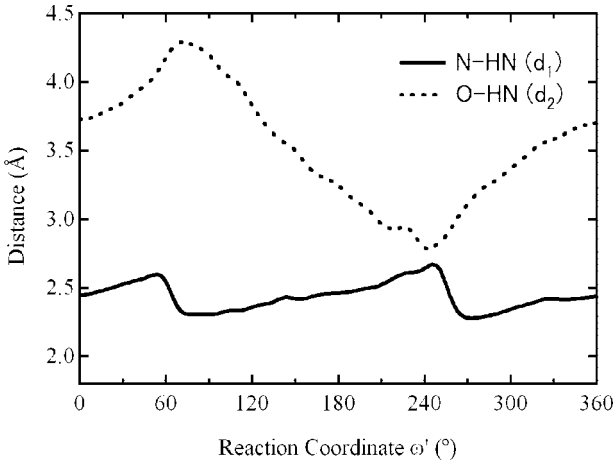

Figure 6. The distance between the prolyl nitrogen and the amide hydrogen following the proline as a function of the reaction coordinate ω' (d_1: solid line), and the distance between the amide oxygen preceding the proline and the amide hydrogen as a function of the ω' (d_2: dotted line).

We also represent the average O−HN intramolecular hydrogen-bond length as a function of ω' indicated by dotted line in Figure 6, where O is the amide oxygen preceding the proline and HN is the amide hydrogen subsequent of the proline. Here, O−HN length has the maximum value of 4.31 Å at 70° and the minimum value of 2.73 Å at 241°. Thus, this hydrogen bond also contributes to stabilization of the trans-form of the proline dipeptide. This stabilization due to the weak O−HN hydrogen bond supports the previous experimental study, suggesting that the O−HN bond contributes to stabilization of the trans-state.[26]

Moreover, we examined the averaged endocyclic torsions and averaged puckering ω_m defined by Altona and Sundaralingam[25] in terms of the proline ring (see Figure S1 and the descriptions of section 1 in the Supporting Information).

Hydration of the Prolyl Nitrogen. Many experimental studies have reported that solvent has significant effects on prolyl isomerization. The amide bond has a dipole moment that results in a net transfer of charge from nitrogen to the carbon and oxygen. Thus, the origin of the solvent effect comes from the interaction between the dipole moments of the amide bond and solvent.[1] Higashijima et al. showed that NMR spectra of Ace−Pro−NMe greatly depended on the polarity of solvent they used.[26] Eberhardt et al., using ^{13}C NMR and IR spectroscopy, reported that the isomerization rate had strong dependence on the ability of a solvent to donate a hydrogen bond.[27] During the isomerization or in the pyramidal conformation, the existence of an intramolecular hydrogen bond to the prolyl nitrogen, stabilizing the pyramidal conformation, was confirmed by using IR and NMR spectroscopy[8,9] and using crystal structure data.[28] When the prolyl nitrogen is hydrated by neighbor waters, the hydration should stabilize the pyramidal conformation as well as the intramolecular hydrogen bond does.

To elucidate the hydration effect in the pyramidalization, we investigated the hydration of the prolyl nitrogen by evaluating the probability density of hydrated water on the prolyl nitrogen

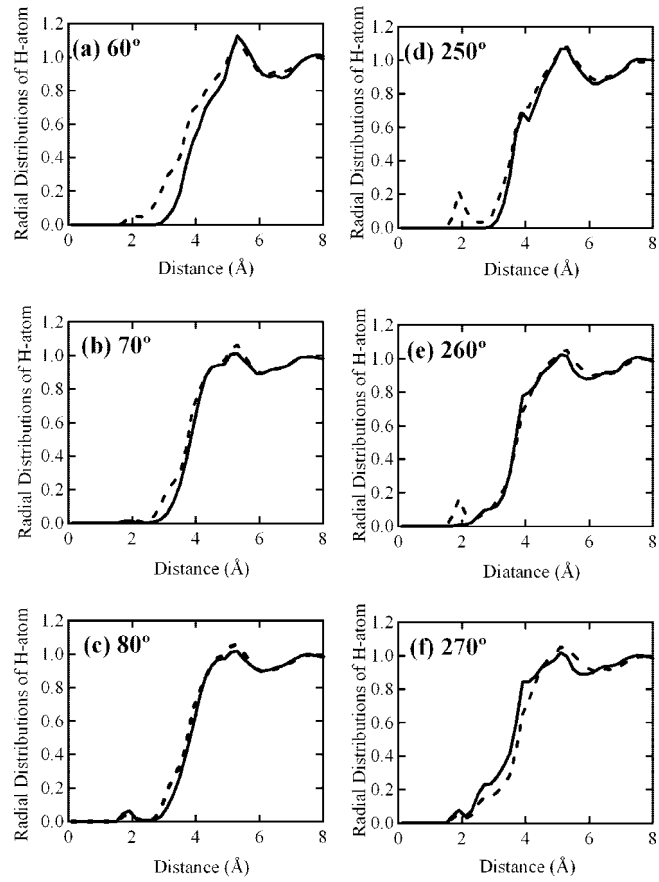

Figure 7. The radial distributions of the hydrogen atom of water to the prolyl nitrogen on the same side as the amide hydrogen subsequent to the proline (side A: solid line) and the opposite side (side B: dotted line), $\omega' = 60°$ (a), $\omega' = 70°$ (b), and $\omega' = 80°$ (c), $\omega' = 250°$ (d), $\omega' = 260°$ (e), and $\omega' = 270°$ (f).

with respect to two different sides of the pyrrolidine ring. We defined side A as that facing the amide hydrogen downstream from the proline; the opposing face was defined as side B (see Figure 4a). Side A and side B correspond, respectively, to the positive and negative directions of the pyramidalization. We calculated the radial distribution of the hydration probability density $g(r, \omega)$ using eq 4:

$$g(r, \omega') = \frac{\langle N(r, \omega')_{\delta r} \rangle}{\frac{4}{3}\pi[(r + \delta r)^3 - r^3]} \tag{4}$$

where $N(r, \omega')_{\delta r}$ is the number of the hydrogen atoms of water between r and $r + \delta r$ at ω', and the angular brackets denote the ensemble average.

The radial distributions for ω' are 60°, 70°, 80°, 250°, 260°, and 270° and are shown in Figure 7a−f. Those ω' values correspond to the ranges where the pyramidalization changes the polarity. In the case where ω' is 60°, the hydrated water of side B is closer to the prolyl nitrogen than that of side A, and, when ω' is 70° and 80°, the hydration distance of both sides is comparable. When ω' is 250° and 260°, the hydration of side B is positioned closer to the prolyl nitrogen than that of side A. The closer hydration side of the prolyl nitrogen changes from side B to side A when ω' is 270°. The results show the neighbor hydration shell of the prolyl nitrogen changes as the polarity of the pyramidalization changes. When ω' is 250° and 260°, the hydration shows a remarkable peak at 2 Å, where the negative

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pyramidalizations are induced, which clearly indicates that the hydration stabilizes the pyramidal conformation. In the same way, when ω' is 60°, the hydration on side B stabilizes the pyramidal conformation.

It is shown that the negative pyramidalization is mainly stabilized by the hydration from side B, while the positive pyramidalization is mostly stabilized by the intramolecular N−HN interaction and by the hydration from side A. (See section 4 in the Supporting Information.) In the case where ω' is around 70° and 260°, the two stabilization factors are equivalent, and the pyramidality largely fluctuates with the zero average pyramidality value. Thus, the large fluctuation could be attributed to competition of the hydrogen-bonding partners for the prolyl nitrogen between the two different sides, sides A and B in Figure 4a, of the pyrrolidine ring.

The catalysis of amide isomerization by strong Brönsted acids is a well-known process that proceeds through a putative N-protonated intermediate present at low-pH conditions. In the absence of Brönsted acidic media, our study shows that the prolyl isomerization in water can utilize the intra- and intermolecular hydrogen bonds as a proton donor, gaining stability for the pyramidalized transition state. The strength of the hydrogen bond is then correlated with the degree of pyramidalization of the proline. Cyclophiline, FKBP, and Pin1 are prolyl peptidyl isomerases (PPIases), which reduce the rotational barriers to accelerate isomerization. Their enzymatic mechanisms have been extensively studied but have yet to be clearly understood. Those enzymatic mechanisms should be analyzed in a way similar to that of the current simulation method.

Conclusions

To elucidate the cis−trans isomerization mechanism of proline in water, we carried out a molecular dynamics simulation with the hybrid potential combining quantum mechanics and molecular mechanics for the cis−trans isomerization process of the prolyl peptide bond of the Ace−Pro−NMe in explicit solvent. We used an umbrella sampling method to obtain the free energy landscape along the reaction coordinate. The free energy obtained by the umbrella integration method showed that the trans-state is more stable than the cis-state by 4 kcal/mol and the high energy barrier separating the two states is about 20 kcal/mol, in good accordance with experimental values. The shortening of the C−N bond preceding the proline clearly shows that the C−N bond switches from a sp^2-hybridized to a sp^3-hybridized state, thus reflecting the reduction of the partial resonance character of the electronic state. It is shown that the hydration of the prolyl nitrogen stabilizes the negative pyramidal conformation, while an intramolecular interaction mainly stabilizes the intramolecular hydrogen-bonding interaction. We also found that the sharp change between the negative pyramidalization and the positive pyramidalization observed during the isomerization can be explained by competition by intra- and intermolecular hydrogen-bonding partners for the prolyl nitrogen between different sides of the pyrrolidine ring.

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Supporting Information Available: (1) Endocyclic torsions and puckering amplitude of the proline ring. (2) Energy surfaces of the proline dipeptide using basis sets 4-31G, 6-31G, and 6-31G(d). (3) Mulliken charges of the proline dipeptide from the 4-31G and MM charges from the Amber force field. (4) Evaluating the solvent and intramolecular hydrogen-bond energies associated with the prolyl nitrogen. This material is available free of charge via the Internet at http://pubs.acs.org.

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