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Cis–trans isomerization and puckering of proline residue

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Abstract

We report here the results on *N*-acetyl-L-proline-*N*′-methylamide (Ac-Pro-NHMe) calculated at the HF/6-31+G(d) level with the conductor-like polarizable continuum model (CPCM) of self-consistent reaction field methods to investigate the changes of backbone and prolyl ring along the *cis–trans* isomerization of the prolyl peptide bond. From the potential energy surface, the barrier to ring flip from the down-puckered conformation to the up-puckered one is estimated to be 2.5 and 3.2 kcal/mol for *trans* and *cis* conformers of Ac-Pro-NHMe, respectively. In particular, the ring flip seems to be inaccessible in the intermediate regions between *trans* and *cis* conformations, because of higher barriers (~ 13–19 kcal/mol) to rotation of the prolyl peptide bond. The torsion angles for backbone and prolyl ring vary largely around the transition states at $\omega' \approx 120°$ and $-70°$ for the prolyl peptide bond. Three kinds of puckering amplitudes show the same trend of puckering along the *cis–trans* isomerization although their absolute values are different. In particular, *trans* and *cis* conformations have the almost same degree of puckering. The *cis* populations and barriers to rotation of the prolyl peptide bond for Ac-Pro-NHMe are increased with the increase of solvent polarity, which is mainly ascribed to the decreases of relative free energies for *cis* conformations and the increase of relative free energies for transition states.

Keywords: Ab initio calculations; *Cis–trans* isomerization; Puckering; Solvation; Proline residue

1. Introduction

The proline (Pro) residue is unique in that its side chain is covalently bonded to the nitrogen atom of the peptide backbone. This leads the backbone not to form a hydrogen bond and the N–C^{α} rotation (ϕ) to be restrained to about $-60°$. Because of these conformational restrictions, Pro occurs in turns, in nonrepetitive structure, and at the ends of strands and helices of proteins [1]. In particular, the pyrrolidine of Pro residue is a five-membered ring that may adopt two distinct down- and up-puckered conformations [2], which have known to be almost equally probable from the analysis of X-ray structures of peptides [3–5] and proteins [6–8]. The down- and up-puckered conformations are defined as those of which the C^{γ} atom and the C=O group of Pro residue lie on the same and opposite sides, respectively, of the plane defined by three atoms C^{δ}, N, and C^{α} (Fig. 1).

Pro residue has a relatively high intrinsic probability 5.2% of having the *cis* peptide bond preceding proline as compared with other amino acids (0.03%) from the analysis of a non-redundant set of 571 X-ray protein structures [9]. It has been reported that the *cis–trans* isomerization of the X-Pro bond is often involved in the rate-determining steps for folding and refolding of various proteins [10–13]. Several enzymes so-called peptidyl prolyl *cis–trans* isomerases (PPIases) have been identified, which significantly accelerate the isomerization of peptides and denatured proteins [10,11]. In particular, it has been reported that PPIases are involved in cell signaling and replication, and implicated in several diseases such as cancer, AIDS, and Alzheimer's disease [13].

The correlations of the *cis*/*trans* population and the prolyl puckering were reported by analyzing X-ray structures of proteins and peptides [6–8,14] and by the empirical free energy calculations for X-Pro dipeptides [15]. Considerable experiments [16] and computations [15] have been carried out on the *cis–trans* isomerization of proline-containing peptides. In particular, *N*-acetyl-L-proline-*N*′-methylamide (Ac-Pro-NHMe) has been widely used as a model for

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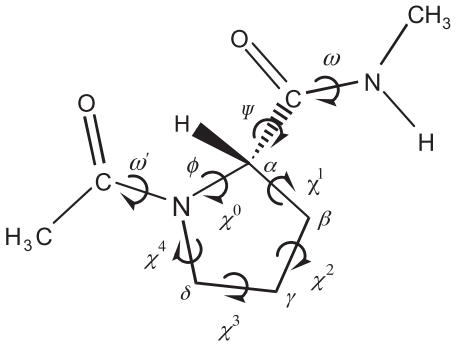

Fig. 1. Definition of torsion angles and structural parameters for Ac-Pro-NHMe.

experimental [17–22] and theoretical [15,23–34] studies on the conformational preferences and transition states for the X-Pro isomerization. Recently, the *cis–trans* isomerization and backbone population of Ac-Pro-NHMe in chloroform and water were reasonably described by us at the HF/6-31 + G(d) level with the self-consistent reaction field method [32,34].

We report here the results on Ac-Pro-NHMe calculated using the ab initio computations with the self-consistent reaction field (SCRF) method to investigate the changes of backbone and prolyl ring along the *cis–trans* isomerization of the prolyl peptide bond.

2. Computational methods

The torsion angles and structural parameters for Ac-Pro-NHMe are defined in Fig. 1. All ab initio calculations were carried out using the Gaussian 98 package [35]. Here, each backbone conformation of Ac-Pro-NHMe is represented by a capital letter depending on its ϕ and ψ values (Fig. 1) [23]. Conformations A, C, and F are defined by the backbone torsion angle ψ with the backbone torsion angle ϕ in the range of $-110° < \phi < -40°$: the conformation A, $-90° < \psi < -10°$; the conformation C, $50° < \psi < 130°$; the conformation F, $130° < \psi < 180°$ or $-180° < \psi < -140°$. *Trans* and *cis* conformations for the Ac-Pro peptide bond are denoted by t and c, respectively. Down and up puckering of proline ring are represented by d and u, respectively.

The 3-D potential energy surfaces (PES) of Ac-Pro-NHMe with *trans*/*cis* peptide bonds and down/up puckerings were calculated along the torsion ω' for the Ac-Pro bond and the endocyclic torsion angle χ^1 at the HF/6-31 + G(d) level (Fig. 1). The prolyl puckering was successively described by the torsion angle χ^1 (i.e. positive and negative χ^1 for the down- and up-puckered structures, respectively) [3]. The adiabatic optimization was performed at each pair of ω' and χ^1, which was generated by combining the values of ω' for $-180° \le \omega' \le 180°$ with the interval of 15° and χ^1 for $-60° \le \chi^1 \le 60°$ with the interval of 5°. All χ^1 values of local minima and transition states for Ac-Pro-NHMe optimized at the HF/6-31 + G(d) level [34] lie within the range considered here. The local minima tCd, tCu, cAd, and cAu for Ac-Pro-NHMe optimized at the HF/6-31 + G(d) level [32,34] were used as initial structures for adiabatic optimizations. For the ranges of $-180° \le \omega' \le -60°$ and $60° \le \omega' \le 180°$, conformations tCd and tCu were used as starting points for down- and up-puckered structures, respectively. Conformations cAd and cAu were used as initial points for optimizations of down- and up-puckered conformations, respectively, for the range of $-120° \le \omega' \le 120°$. The lower conformational energies were chosen in the overlapped regions of $-120° \le \omega' \le -60°$ and $60° \le \omega' \le 120°$.

In order to investigate the changes of backbone and prolyl ring along the *cis–trans* isomerization, the lowest energy conformations for down- and up-puckered structures at each value of ω' on the 3-D PES were reoptimized at the HF/6-31 + G(d) level. The degree of puckering for prolyl ring was calculated using three kinds of puckering amplitudes, i.e. q_α of Han and Kang [36], q_z of Cremer and Pople [37], and χ_m of Altona and Sundaralingam [38]. q_α is the maximum angle between the mean plane and the line joining the center of mass and each atom of the ring. q_z corresponds to the maximum z-displacement perpendicular to the mean plane of the ring. χ_m is the maximum value attainable by endocyclic torsion angles of the ring.

We employed the conductor-like polarizable continuum model (CPCM) SCRF method [39], implemented in the Gaussian 98 package, to compute solvation free energies at the HF/6-31 + G(d) level. Solvents considered here are chloroform and water, whose dielectric constants are 4.9 and 78.4 at 25 °C, respectively. Solvation free energies were calculated for each minimum of down- and up-puckered structures optimized along the torsion angle ω' in the gas phase.

In solution, the relative total free energy (ΔG_{tot}) of a peptide can be calculated by the sum of the relative conformational free energy (ΔG) and the relative solvation free energy (ΔG_s). The relative ab initio conformational free energy can be expressed as the sum of the relative electronic energy (ΔE) with zero-point and thermal corrections, and the relative entropic contribution [40]. The relative conformational free energy can be approximated to be the relative electronic energy if other contributions are assumed to be constant. Relative total free energies computed by the sum of ΔE and ΔG_s at the HF/6-31 + G(d) level with the CPCM method predicted better the experimental populations of backbone and *cis* conformations for Ac-Pro-NHMe in chloroform and water than those by the sum of ΔG and ΔG_s [34]. The former free energy also reproduced satisfactorily experimental rotational barriers about the C–N bond of amides in chloroform and water [41]. However, these results may indicate that the need for improvement in the CPCM method, as pointed out previously [42]. In this work, the relative total free energy (ΔG_{tot}) was computed by the sum of ΔE and ΔG_s in solutions.

According to the PES of Ac-Pro-NHMe along the backbone torsion angle ψ, conformations tCd and tCu are known to be most probable in the gas phase because of the hydrogen bond between two terminal acetyl and –NHMe groups, but they are not feasible in chloroform and water [34]. Instead, the polyproline-like conformations tFd and tFu are found to be stable in solutions, to which the values of $\psi = 142.1°$ and 137.6°, respectively, optimized for Ac-Pro-NMe_2 were assigned [34]. For $-180° \le \omega' \le -150°$ and $150° \le \omega' \le 180°$, additional adiabatic optimizations were carried out at these fixed ψ values. The lower value of the two free energies for conformations tCd and tFd, or tCu and tFu at each of these ω' domains in chloroform and water was used to plot the PESs in solutions.

3. Results and discussion

3.1. Potential energy surface along torsion angles ω' and χ^1

Fig. 2 shows the 3-D PES of Ac-Pro-NHMe with *trans*/*cis* peptide bonds and down/up puckerings calculated along the torsion angle ω' for the Ac-Pro bond and the torsion angle χ^1 of prolyl ring at the HF/6-31+G(d) level. Four local minima (i.e. tCd, tCu, cAu, and cAd) and four transition states (i.e. ts1 to ts4) are denoted by + and × on the PES, respectively.

From the PES, the electronic barrier ($\Delta E_e^{\ddagger}$) to ring flip from the down-puckered conformation to the up-puckered one is estimated to be 2.5 and 3.2 kcal/mol for *trans* and *cis* conformers of Ac-Pro-NHMe at the HF/6-31+G(d) level, i.e. for the paths tCd → tCu and cAd → cAu, respectively. Recently, each transition state was identified as an envelope form having the N atom at the top of envelope and not a planar one for both *trans* and *cis* conformers [43]. At the B3LYP/6-311++G(d,p) level, the corresponding electronic barriers to ring flip are estimated to be 2.1 and 2.4 kcal/mol for *trans* and *cis* conformers, respectively, whereas the free energy barriers ($\Delta G^{\ddagger}$) to ring flip are estimated to be 2.6 and 3.0 kcal/mol at room temperature, respectively [43]. On the other hand, the ring flip seems to be inaccessible in the intermediate regions between *trans* and *cis* conformations, because the barriers to rotation of the Ac-Pro peptide bond are calculated to be 13.1–19.3 kcal/mol at the HF/6-31+G(d) level, as described below.

3.2. Conformational energy change along the cis–trans isomerization

The relative conformational energies of down- and up-puckered Ac-Pro-NHMe along the torsion angle ω' are plotted in Fig. 3. Using the relative conformational energies of local minima and transition states [34] as well as the PESs shown in Fig. 3, the barriers to rotation $\Delta E^{\ddagger}_{trans \to cis}$ and $\Delta E^{\ddagger}_{cis \to trans}$ for the Ac-Pro peptide bond can be calculated. Transition states ts1 and ts2 correspond to the *syn*/*exo* structures with down and up puckerings, respectively, whereas transition states ts3 and ts4 are the *anti*/*exo* structures with down and up puckerings, respectively, according to the definition of Fischer et al. [26]. Transition states ts1 to ts4 are depicted on the PES in Fig. 2.

The barriers to rotation $\Delta E^{\ddagger}_{trans \to cis}$ for the down-puckered conformation are calculated to be 17.6 and 19.3 kcal/mol for the paths tCd → ts1 → cAd and tCd→ts3→cAd, respectively. In addition, the barriers $\Delta E^{\ddagger}_{cis \to trans}$ are estimated to be 15.3 and 16.9 kcal/mol for the paths cAd → ts1 → tCd and cAd → ts3 → tCd, respectively. These results may indicate that the *cis*–*trans* isomerization for the down-puckered conformation follows the path by way of the transition state ts1.

For the up-puckered Ac-Pro-NHMe, the barriers to rotation $\Delta E^{\ddagger}_{trans \to cis}$ are calculated to be 16.5 and 18.1 kcal/mol for the paths tCu → ts2 → cAu and tCu → ts4 → cAu, respectively. The barriers $\Delta E^{\ddagger}_{cis \to trans}$ are known to be 13.1 and 14.7 kcal/mol for the paths cAu → ts2 → tCu and cAu → ts4 → tCu, respectively. From these results, the *cis*–*trans*

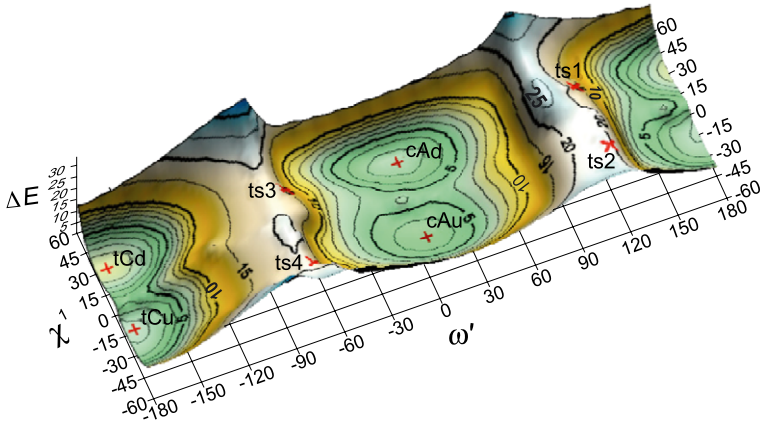

Fig. 2. Potential energy surface of Ac-Pro-NHMe optimized adiabatically at the HF/6-31+G(d) level along the backbone torsion angle ω' and the endocyclic torsion angle χ^1 in the gas phase. Local minima and transition states are denoted by + and ×, respectively.

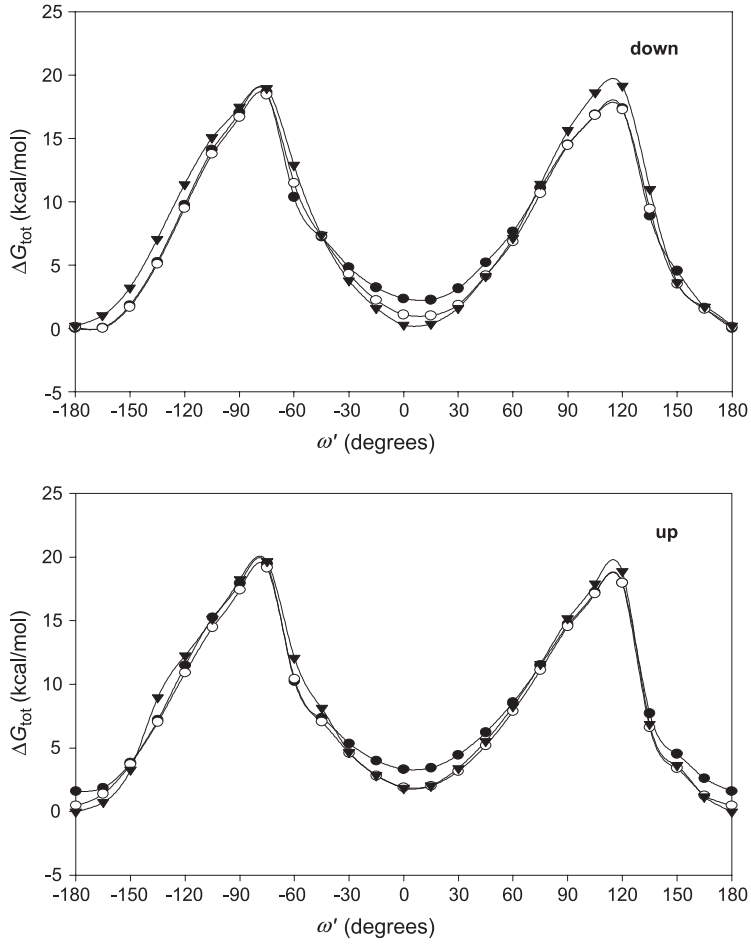

Fig. 3. Relative energies (ΔE) and relative total free energies (ΔG_{tot}) of down- and up-puckered Ac-Pro-NHMe at the HF/6-31+G(d) level along the backbone torsion angle ω' in the gas phase (●), chloroform (○), and water (▼); $\Delta G_{tot} = \Delta E$ in the gas phase.

isomerization for the up-puckered conformation appears to pass through the transition state ts2.

Therefore, the transition states ts1 and ts2 at $\omega' \approx 120°$ seem to be more feasible than the transition states ts3 and ts4 at $\omega' \approx -70°$ for down- and up-puckered conformations, respectively. It should be noted that the barriers to rotation are lower for the up puckering than the down puckering, although the relative energies of conformations tCu and cAu are higher than those of conformations tCd and cAd, respectively.

3.3. Changes of backbone torsion angles along the cis–trans isomerization

The optimized values of backbone torsion angles ϕ and ψ for down- and up-puckered conformations along the torsion angle ω' are plotted in Fig. 4. The torsion angles ϕ and ψ vary largely around the transition states at $\omega' \approx 120°$ and $-70°$ and the changes are remarkable for the up-puckered conformations than the down-puckered conformations. The values of ϕ and ψ lie between $-111°$ and $-58°$, and between $-12°$ and 95°, respectively, for the down-puckered conformations. In the case of the up-puckered conformations, ϕ and ψ span over $-113°$ to $-40°$ and $-5°$ to 139°, respectively. In particular, the polyproline II structures are found to be stable in the domain $135° \leq \omega' \leq 165°$ for the up-puckered conformations.

In particular, the average values of ϕ for local minima of Ac-Pro-NHMe optimized at the HF/6-31+G(d) level are $-86.2°$, $-82.4°$, $-78.1°$, and $-67.9°$ for *trans*-down, *cis*-down, *trans*-up, *cis*-up conformers, respectively [34]. This indicates that the backbone torsion angle ϕ is more negative for down-puckered conformers than up-puckered ones for both *trans* and *cis* peptide bonds, which is consistent with the statistical analyses on X-ray structures of proteins [8] and peptides [25]. This is also found to be true during the prolyl *cis–trans* isomerization except for $75° \leq \omega' \leq 90°$, as shown in Fig. 4.

3.4. Changes of prolyl ring conformations along the cis–trans isomerization

The optimized values of endocyclic torsion angles χ^0, χ^1, χ^2, χ^3, and χ^4 for down- and up-puckered conformations along the torsion angle ω' are plotted in Fig. 5. As seen for

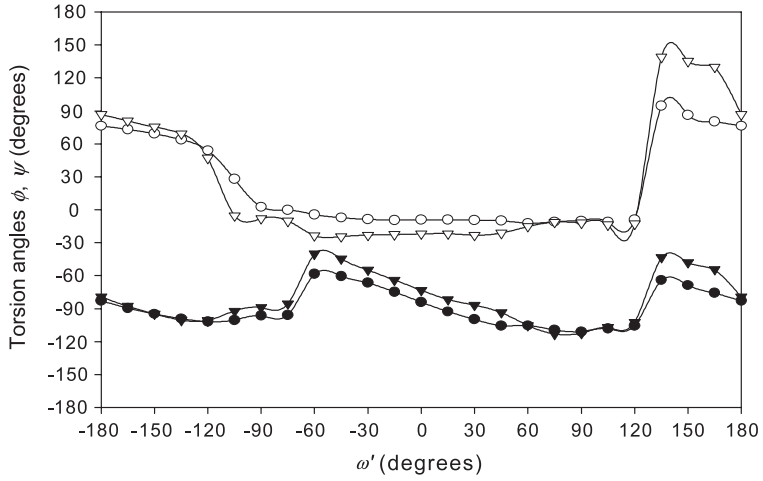

Fig. 4. Backbone torsion angles ϕ and ψ of Ac-Pro-NHMe optimized at the HF/6-31 + G(d) level along the backbone torsion angle ω' in the gas phase: ●, ϕ for the down puckering; ○, ψ for the down puckering; ▼, ϕ for the up puckering; ▽, ψ for the up puckering.

backbone torsion angles ϕ and ψ in Fig. 4, there are larger changes in torsion angles χ^0 to χ^4 around the transition states at $\omega' \approx 120°$ and $-70°$. The largest changes are found in χ^0 and χ^4, followed by χ^1 and χ^3 for both down- and up-puckered conformations. There are small variations of χ^2 for both puckered conformations.

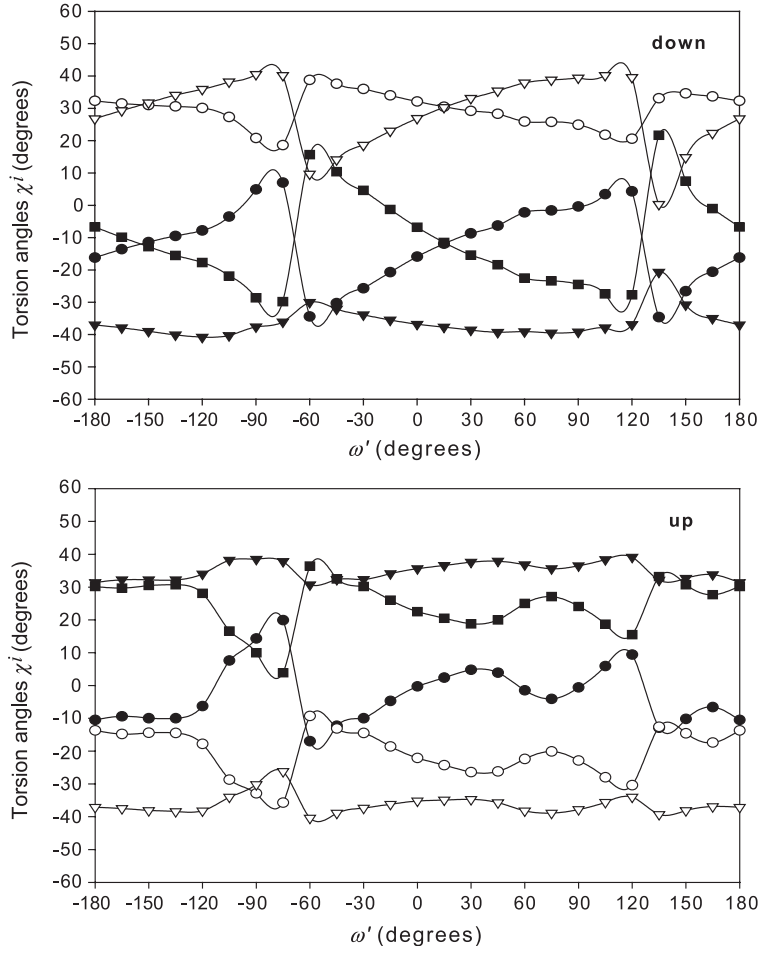

Fig. 5. Endocyclic torsion angles χ^0 to χ^4 of Ac-Pro-NHMe optimized at the HF/6-31 + G(d) level along the backbone torsion angle ω' in the gas phase: ●, χ^0; ○, χ^1; ▼, χ^2; ▽, χ^3; ■, χ^4.

The three puckering amplitudes q_α, q_z, and χ_m for down- and up-puckered conformations along the torsion angle ω' are shown in Fig. 6. As expected from the changes of endocyclic torsion angles in Fig. 5, there are larger changes of puckering amplitudes around the transition states. The $sp^2 \rightarrow sp^3$ hybridization of the prolyl nitrogen may play a role in making the puckering amplitudes higher around the transition states. However, three amplitudes show the same trend of puckering along the *cis–trans* isomerization although their absolute values are different. In particular, *trans* and *cis* conformations have the almost same degree of puckering for both down- and up-puckered conformers, whereas there are more variations of puckering amplitude for down-puckered conformers than up-puckered ones in the intermediate regions between *trans* and *cis* conformations.

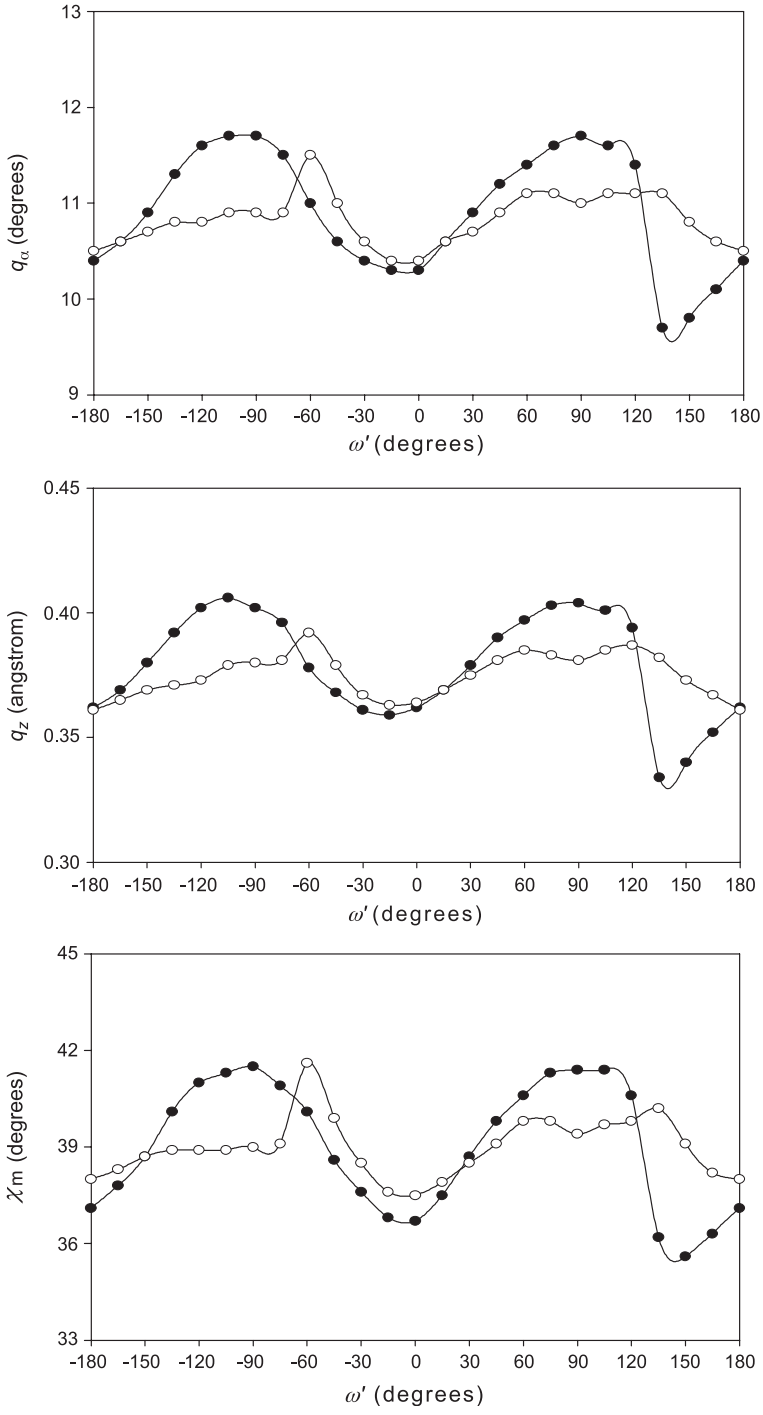

Fig. 6. Puckering amplitudes of Ac-Pro-NHMe optimized at the HF/6-31+G(d) level along the backbone torsion angle ω' in the gas phase: ●, the down puckering; ○, the up puckering.

According to the pseudorotational model of Cremer and Pople [37], the average phase angles of local minima for Ac-Pro-NHMe optimized at the HF/6-31+G(d) level [34] can be calculated to be 85° and 289° for down- and up-puckered conformers, respectively, which are similar to the values of 93° and 282° obtained from the analysis of X-ray structures of proteins [8]. The average value for transition states ts1 and ts3 is 113°, which is close to that of down-puckered conformers, whereas the average value for transition states ts2 and ts4 is 268°, which is close to that of up-puckered conformers. In the intermediate regions between *trans* and *cis* conformations, the phase angles are found to oscillate between the values of local minima and transition states for both down- and up-puckered conformations.

3.5. Solvation effects on the cis–trans isomerization

The relative energies and relative total free energies for down- and up-puckered Ac-Pro-NHMe in the gas phase, chloroform, and water are plotted in Fig. 3. The relative total free energies of *cis* conformations to *trans* conformations are decreased with the increase of solvent polarity. This results in the increase of *cis* populations, which is consistent with NMR experiments [20].

In the gas phase → chloroform transfer, there is the increase of rotational barrier $\Delta G^{\ddagger}_{cis \rightarrow trans}$ for both down- and up-puckered conformations due to the decrease of relative total free energies for *cis* conformations in chloroform. On the other hand, the rotational barrier $\Delta G^{\ddagger}_{trans \rightarrow cis}$ is nearly constant and increased for down- and up-puckered conformations, respectively. This is because relative total free energies of transition states in chloroform are almost the same as those in the gas phase and because the *trans* up-puckered conformation becomes more stable in chloroform. In chloroform, the *cis–trans* isomerization passes through the transition states ts1 and ts2 for down- and up-puckered conformations, respectively, as the same as in the gas phase.

In the chloroform → water transfer, relative total free energies are increased for all transition states, and decreased for all *cis* conformations and the *trans* up-puckered conformations, which yields the increase of both rotational barriers $\Delta G^{\ddagger}_{trans \rightarrow cis}$ and $\Delta G^{\ddagger}_{cis \rightarrow trans}$. Using relative total free energies for local minima of Ac-Pro-NHMe, the values of $\Delta G^{\ddagger}_{trans \rightarrow cis}$ and $\Delta G^{\ddagger}_{cis \rightarrow trans}$ are calculated to be 19.0 and 18.8 kcal/mol, respectively [34], which accord with experimental values of 20.4 and 19.8 kcal/mol, respectively [22]. It should be noted that the *cis–trans* isomerization for down-puckered conformations in water goes through the transition state ts3 instead of the transition state ts1 because the relative total free energy of the ts1 is higher than that of the ts3. However, the isomerization for up-puckered conformations proceeds via the transition state ts2 as the same as in the gas phase and chloroform.

Overall, the barriers to rotation of the prolyl peptide bond for Ac-Pro-NHMe are increased with the increase of solvent polarity, which is consistent with experimental barriers of *N,N*-dimethylacetamide in solutions [44].

4. Conclusions

From the PES, the barrier to ring flip from the down-puckered conformation to the up-puckered one is estimated to be 2.5 and 3.2 kcal/mol for *trans* and *cis* conformers of Ac-Pro-NHMe, respectively at the HF/6-31+G(d) level. In particular, the ring flip seems to be inaccessible in the intermediate regions between *trans* and *cis* conformations, because of higher barriers (~ 13–19 kcal/mol) to rotation of the prolyl peptide bond.

The torsion angles for backbone and prolyl ring vary largely around the transition states at $\omega' \approx 120°$ and $-70°$ for the prolyl peptide bond. Three kinds of puckering amplitudes show the same trend of puckering along the *cis–trans* isomerization although their absolute values are different. In particular, *trans* and *cis* conformations have the almost same degree of puckering.

The *cis* populations and barriers to rotation of the prolyl peptide bond for Ac-Pro-NHMe are increased with the increase of solvent polarity, which is mainly ascribed to the decreases of relative free energies for *cis* conformations and the increase of relative free energies for transition states. The ab initio conformational studies on the *cis–trans* isomerization and puckering of proline derivatives are now in progress.

Acknowledgements

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