Ring Flip of Proline Residue via the Transition State with an Envelope Conformation

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The ring flip of proline residue in *N*-acetyl-L-proline-*N*′-methylamide (Ac-Pro-NHMe) with trans and cis peptide bonds was studied by adiabatic optimizations along the torsion angle χ^1 of the prolyl ring at the HF/6-31+G(d) level. By analyzing the potential energy surface and local minima, it is observed that the prolyl ring flips from a down-puckered conformation to an up-puckered one through the transition state with an envelope form having the N atom at the top of envelope and not a planar one for both trans and cis conformers. At the B3LYP/6-311++G(d,p) level, the barriers to ring flip $\Delta G^{\ddagger}_{\text{down}\rightarrow\text{up}}$ are estimated to be 2.58 and 3.00 kcal/mol for trans and cis conformers at room temperature, respectively.

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$^{\alpha}$ 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 been known to be almost equally probable from analysis of X-ray structures of peptides[3−5] and proteins.[6−8] The down- and up-puckered conformations are defined as those where the C$^{\gamma}$ 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$^{\delta}$, N, and C$^{\alpha}$ (Figure 1).

^{13}C NMR relaxation measurements have provided abundant evidence for the interconversion between two puckerings in peptides in solution and in the solid state, whose relaxation time was estimated to be about 1−30 ps[9−11] and the apparent barrier to be about 1.3 kcal/mol for DL-proline in the solid state.[10] Although considerable empirical and quantum mechanical calculations have been carried out on various model compounds of proline residue, only a few works have focused on the transition of prolyl puckering. From empirical force field calculations on *N*-acetylproline methyl ester (Ac-Pro-OMe)[4] and *N*-acetyl-2-methylpyrrolidine (Ac-MePyr),[5] the conversion of ring puckering was found to proceed through a flat saddle with a barrier of 3.6 kcal/mol and a planar transition state with a barrier of 2.0 kcal/mol above the global minima, respectively. Pyrrolidine, the simplest model of proline residue, was known to flip via a planar transition state with a barrier of about 3−5 kcal/mol at various levels of quantum mechanical theory.[12,13] The HF and B3LYP calculations on proline (H-Pro-OH) indicated that there are two pairs of preferred conformers, depending on the N···H−O and N−H···O hydrogen bonds, whose barriers to ring flip were estimated to be 4.1 and 8.2 kcal/mol at the HF/6-311++G(d,p) level[14] and 0.8 and 3.3 kcal/mol at the B3LYP/aug-cc-pVDZ level,[15] respectively. In particular, it should be noted that pyrrolidine and proline cannot be good models for the Pro residue in peptide and protein, because the bonding character of the ring N atom for all conformers is pyramidal instead of trigonal.[12−15] From B3LYP/6-31G(d) calculations, it was reported that the barrier to ring flip from the up-puckered conformation to the down-puckered one is 2.1 kcal/mol for *N*-formyl-*trans*-proline amide (For-Pro-NH$_2$).[16] The barrier to ring flip from the down-puckered conformation to the up-puckered one was estimated to be 2.7 and 3.3 kcal/mol for trans and cis conformers of *N*-acetylproline amide (Ac-Pro-NH$_2$) at the HF/6-31G(d) level, respectively.[17] *N*-Acetyl-L-proline-*N*′-methylamide (Ac-Pro-NHMe) has been quantum-mechanically studied as a model for the Pro residue in peptide and protein, especially for the cis−trans isomerization of the X−Pro bond.[18−26] 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.[24,26] However, no structural information and thermodynamic properties of the transition state for the prolyl ring flip were available up to now for For-Pro-NH$_2$, Ac-Pro-NH$_2$, and Ac-Pro-NHMe.

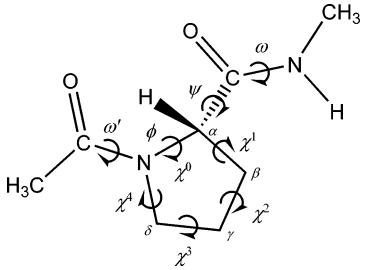

Figure 1. Definition of torsion angles and structural parameters for Ac-Pro-NHMe. The torsion angle χ^1 is defined for the N−C$^{\alpha}$−C$^{\beta}$−C$^{\gamma}$ sequence.

We report here the results on the ring flip of proline residue in Ac-Pro-NHMe with trans and cis peptide bonds studied by adiabatic optimizations along the torsion angle χ^1 of the prolyl ring at the HF/6-31+G(d) level. The barriers to ring flip $\Delta G^{\ddagger}_{\text{down}\rightarrow\text{up}}$ are estimated at HF/6-31+G(d) and B3LYP/6-311++G(d,p) levels.

Computational Methods

Torsional parameters for the backbone and ring of prolyl residue are defined in Figure 1. Because the prolyl puckering

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TABLE 1: Torsion Angles and Thermodynamic Properties of Local Minima and Transition States for Ac-Pro-NHMe Optimized at HF/6-31+G(d) and B3LYP/6-311++G(d,p) Levels[a]

conformer	ω'	ϕ	ψ	ω	χ^0	χ^1	χ^2	χ^3	χ^4	ΔE_e	ΔH	ΔG
						HF/6-31+G(d)[b]						
tCd	−172.8	−86.2	74.9	−176.5	−14.7	31.8	−37.5	28.2	−8.5	0.00	0.00	0.00
tCu	−174.7	−82.6	84.5	−174.4	−9.7	−14.3	31.9	−37.1	29.7	1.69	1.76	1.73
ts1	−178.6	−85.1	82.1	−174.0	−28.9	14.5	3.4	−20.0	31.1	2.48	2.03	2.87
cBd	9.2	−89.5	−9.1	−179.7	−13.4	31.1	−37.4	29.0	−9.8	2.37 (0.00)	2.21 (0.00)	1.81 (0.00)
cAu	5.7	−76.6	−21.9	−177.2	0.9	−23.1	36.1	−35.0	21.6	3.45 (1.07)	3.24 (1.03)	2.60 (0.79)
ts2	−2.4	−85.6	−7.3	179.7	−24.5	13.3	1.1	−15.0	25.2	5.53 (3.16)	4.91 (2.71)	4.97 (3.16)
						B3LYP/6-311++G(d,p)						
tCd	−172.6	−83.6	71.2	−177.2	−13.7	31.3	−37.6	28.8	−9.5	0.00	0.00	0.00
tCu	−173.9	−81.7	78.0	−175.6	−10.3	−13.3	30.9	−36.5	29.7	1.03	1.14	1.21
ts1	−177.7	−83.3	75.4	−175.8	−28.2	17.3	−1.8	−14.5	27.1	2.12	1.68	2.58
cBd	10.0	−91.7	−5.0	179.3	−14.6	31.8	−37.5	28.4	−8.6	3.31 (0.00)	3.17 (0.00)	2.16 (0.00)
cAu	8.2	−79.3	−18.9	−177.3	0.0	−22.2	35.6	−35.1	22.2	4.13 (0.81)	3.98 (0.81)	3.70 (1.55)
ts2	5.1	−97.7	5.6	178.8	−27.9	15.7	0.7	−16.8	28.4	5.74 (2.43)	5.13 (1.96)	5.16 (3.00)

[a] Angles are in deg and energies in kcal/mol. For transition states, the values of ΔG correspond to barriers to ring flip ($\Delta G^{\ddagger}_{down\rightarrow up}$), and the values of ΔE_e and ΔH are electronic and enthalpic contributions to $\Delta G^{\ddagger}$. [b] Data for local minima taken from refs 24 and 26.

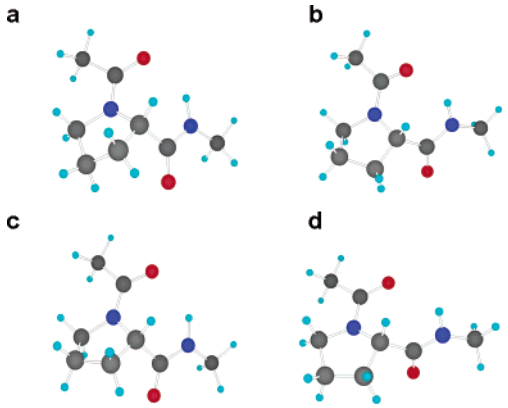

Figure 2. Structures for Ac-*trans*-Pro-NHMe optimized at the HF/6-31+G(d) level: (a) the down-puckered structure (tCd); (b) the up-puckered structure (tCu); (c) the transition state ts1 structure; (d) the peak t* structure. The backbone notation C corresponds to the conformation with a C_7 intramolecular hydrogen bond between C═O of the acetyl group and H−N of the NHMe group.

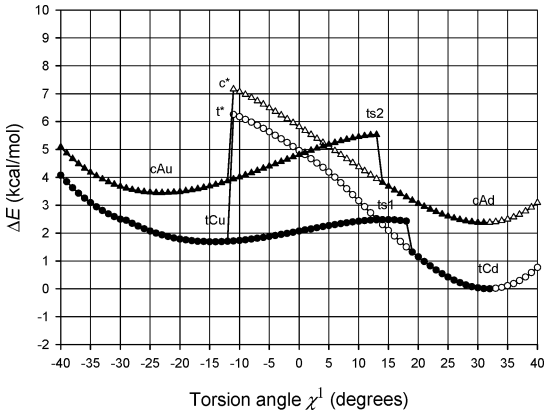

Figure 3. Relative potential energies along the torsion angle χ^1 for Ac-Pro-NHMe. Full geometry was adiabatically optimized for each χ^1 between −40 and +40° with a step of 1° at the HF/6-31+G(d) level. The conversions for trans-up to trans-down, trans-down to trans-up, cis-up to cis-down, and cis-down to cis-up are represented by the symbols ●, ○, ▲, and △, respectively.

was successively described by an endocyclic torsion angle χ^1 (i.e., positive and negative χ^1 for the down- and up-puckered structures, respectively),[3] the HF/6-31+G(d) energies were adiabatically optimized by varying the χ^1 value. Because of the higher barrier to rotation of the peptide bond (~20 kcal/mol) than the barrier to ring flip, calculations were carried out only for trans and cis conformers.

All ab initio and density functional calculations were carried out using the Gaussian 98 package.[27] Four conformations tCd, tCu, cBd, and cAu for Ac-Pro-NHMe optimized at the HF/6-31+G(d) level[24,26] were used as starting points for adiabatic optimizations, which are shown in Figure 2. Here, each backbone conformation of Ac-Pro-NHMe is represented by a capital letter depending on its values of ϕ and ψ for backbone, whose values are listed in Table 1. Trans and cis conformations for the Ac−Pro peptide bond are denoted by t and c, respectively. Down and up puckerings of proline ring are represented by d and u, respectively.

The structures were adiabatically optimized along the torsion angle χ^1 starting from the down-puckered minimum to the up-puckered one and vice versa for each χ^1 between −40 and +40° with a step of 1° for both trans and cis conformers at the HF/6-31+G(d) level. For the conversions of trans-up to trans-down, trans-down to trans-up, cis-up to cis-down, and cis-down to cis-up, the χ^1 value spans over $-40 \le \chi^1 \le +32°$, $-14 \le \chi^1 \le +40°$, $-40 \le \chi^1 \le +31°$, and $-23 \le \chi^1 \le +40°$, respectively. Local minima and transition states optimized at the HF/6-31+G(d) level were used as initial points for optimizations at the B3LYP/6-311++G(d,p) level. Vibrational frequencies were calculated for all stationary points at both levels, which were used to compute enthalpies and Gibbs free energies with scale factors of 0.89[28] and 0.98[29] at HF and B3LYP levels, respectively, at 25 °C and 1 atm. A scale factor of 0.89 at the HF/6-31+G(d) level was chosen to reproduce experimental frequencies for the amide I band of *N*-methylacetamide in Ar and N_2 matrices.[28] A scale factor of 0.98 at the B3LYP/6-311++G(d,p) level reproduced well some experimental frequencies of proline in an Ar matrx.[29]

Results and Discussion

Relative potential energies along the torsion angle χ^1 for trans and cis conformers are plotted in Figure 3. The optimized conformations around local minima are found to be identical for the down-to-up and up-to-down flips for both trans and cis conformers. However, the different peaks are found for the down-to-up and up-to-down flips. The peaks for the up-to-down flips are identified as transition states (represented by ts1 and ts2 for trans and cis conformers, respectively, in Figure 3), because each of them has one imaginary frequency after the transition state optimization, whereas the peaks for the down-to-up flips (represented by t* and c* for trans and cis

conformers, respectively) are conversed to the transition states after the transition state optimization. The optimized structures for the transition state ts1 and the structure for the peak t* obtained from energy scanning are shown in Figure 2. Because the ts2 and c* structures are similarly puckered to the ts1 and t* ones, they are not shown separately. The peak structures t* and c* are higher in energy by 3.8 and 1.6 kcal/mol than transition states ts1 and ts2, respectively. The most interesting thing is that the ts1 and t* structures are both enveloped forms with the N atom at the top of envelope, not planar ones. However, two atoms C^{β} and C^{γ} are syn and anti to the C−N prolyl peptide bond in the ts1 and t* structures, respectively, which can be denoted by ${}^{N}E$ and ${}_{N}E$, respectively. Therefore, the path for the prolyl ring flip can be represented by down ↔ ${}^{N}E$ ↔ up for both trans and cis conformers. In particular, the optimized mean values for the $C^{\delta}-N-C^{\alpha}$ bond angle are 112.1 and 111.5° at the HF/6-31+G(d) level for local minima and transition states, respectively. The corresponding values are 111.7 and 110.8° at the B3LYP/6-311++G(d,p) level. This indicates that the bonding character of the prolyl N atom for all conformers of Ac-Pro-NHMe is trigonal, whereas it is pyramidal for pyrrolidine and proline, as noted above.

Table 1 lists torsion angles and thermodynamic properties of local minima and transition states for Ac-Pro-NHMe optimized at HF/6-31+G(d) and B3LYP/6-311++G(d,p) levels. The difference in backbone torsion angles optimized at two levels is less than 3°, except for the ts2 structure. The relatively larger change is found in the torsion angle ψ than other ones. There is only a small change less than 1° for endocyclic torsion angles χ^0 to χ^4, except for transition states ts1 and ts2. The barriers to ring flip from the down-puckered conformation to the up-puckered one ($\Delta G^{\ddagger}_{down\rightarrow up}$) are estimated to be 2.87 and 3.16 kcal/mol for trans and cis conformers of Ac-Pro-NHMe at the HF level, respectively, and the corresponding values are 2.58 and 3.00 kcal/mol at the B3LYP level. By analyzing the electronic and enthalpic contributions to $\Delta G^{\ddagger}$, the ring flip is dominantly electronically driven. In a recent work on proline,[30] the B3LYP/6-311++G(d,p) level provide the relative energies very close to those obtained at the extrapolated CBS CCSD(T) level and the rotational constants in good agreement with experiments.

Conclusions

We identified that the prolyl ring flip proceeds from down-puckered conformation to up-puckered one through the transition state with an envelope form having the N atom at the top of envelope and not a planar one for both trans and cis conformers. At the B3LYP/6-311++G(d,p) level, the barriers to ring flip $\Delta G^{\ddagger}_{down\rightarrow up}$ are estimated to be 2.58 and 3.00 kcal/mol for trans and cis conformers at room temperature, respectively. The ab initio conformational studies on the cis−trans isomerization and puckering of proline derivatives are now in progress.

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