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Ab initio conformational study of *N*-acetyl-L-proline-*N*′,*N*′-dimethylamide: a model for polyproline

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Abstract

We report here the results on *N*-acetyl-L-proline-*N*′,*N*′-dimethylamide (Ac–Pro-NMe_2) as a model for polyproline at the HF/6-31+G(d) level with the conductor-like polarizable continuum model of self-consistent reaction field methods to figure out the conformational preference and cis–trans isomerization of polyproline in the gas phase, chloroform, methanol, and water. The second methyl substitution at the carboxyl amide end results in different backbone structures and their populations from those of *N*-acetyl-L-proline-*N*-methylamide (Ac–Pro-NHMe). In particular, all conformations with the C_7 hydrogen bond between acetyl and amide ends, which is the most probable conformations of Ac–Pro-NHMe in the gas phase and in nonpolar solvents, disappeared for Ac–Pro-NMe_2 even in the gas phase due to the lack of amide hydrogen. The dominant conformation for Ac–Pro-NMe_2 is the polyproline II structure with the trans prolyl peptide bond in the gas phase and in solutions. In methanol, the population of the polyproline I structure with the cis prolyl peptide bond is calculated to be larger than that in water, which is consistent with experiments. It should be noted that Ac–Pro-NMe_2 has higher rotational barriers for the cis–trans isomerization of the Ac–Pro peptide bond than Ac–Pro-NHMe in the gas phase and in solutions, which could be due to the lack of the intramolecular hydrogen bond between prolyl nitrogen and carboxyl N–H group for the transition state of Ac–Pro-NMe_2. The rotational barriers for Ac–Pro-NMe_2 are increased with the increase of solvent polarity, as seen for Ac–Pro-NHMe.

Keywords: Polyproline; Ac–Pro-NMe_2; Ab initio calculations; Conformational analysis; Prolyl cis–trans isomerization; Solvent effects

1. Introduction

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]. 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 nonredundant set of 571 X-ray protein structures [9]. It has been shown 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].

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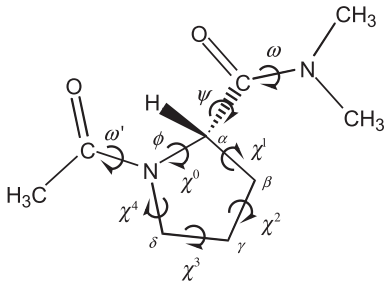

Fig. 1. Definition of torsion angles and structural parameters for Ac–Pro-NMe_2.

Poly-L-proline can form two regular conformations known as poly(Pro) I (PPI) and poly(Pro) II (PPII) in the solid state and in solution [14]. Although both conformations have similar values of ϕ and ψ, i.e., (ϕ, ψ)= (-83°, 158°) and (-77°, 146°) for PPI and PPII conformations, respectively, PPI is a right-handed helix with all cis peptide bonds having 3.33 residues per turn, whereas PPII is a left-handed helix with all trans peptide bonds having 3.00 residues per turn [15]. The PPI conformation is stable in aliphatic alcohol solutions and the PPII conformation predominates in aqueous and organic acid solutions [14]. In aqueous or acid solutions, PPI converts to PPII in a few hours. From NMR [16] and hydrolysis kinetics [17,18] experiments on poly(Pro), it is known that the conversion of PPI into PPII initiates at the amino end of the chain and proceeds to the carboxyl end.

N-Acetyl-L-proline-*N*′,*N*′-dimethylamide (Ac–Pro-NMe_2) is the simplest model for poly(Pro), on which ORD, CD, and NMR experiments [19], empirical energy calculations [5,20], and ab initio MP2 computations [21] have been carried out. These experiments show that an equilibrium mixture of the cis (PPI) and trans (PPII) isomers of Ac–Pro-NMe_2 is present in solution. The trans isomer predominates in aqueous solution, but the equilibrium shifts to favor the cis isomer in nonpolar organic solvent such as cyclohexane. From empirical energy calculations for Ac–Pro-NMe_2 with the fixed ϕ=-75.0° and down-puckering [20], the α-helical minima at $\psi \approx -40^\circ$ were not observed, but only PPI and PPII conformations at $\psi \approx 160^\circ$ appeared; the PPII conformation was more stable by about 1.5 kcal/mol than the PPI conformation. By flexible conformational energy calculations [5], the most probable conformation for Ac–Pro-NMe_2 was known to be the α-helical conformation at (ϕ, ψ)=(-58°, 55°) with the cis peptide bond and up puckering, followed by the up-puckered PPII conformation at (ϕ, ψ)=(-56°, 120°) with a relative energy of 0.4 kcal/mol. The dominant prolyl ring structure was reported to be up-puckered for both PPI and PPII conformations. The barrier for the transition from α-helical conformation to PPII one was estimated to be 10 kcal/mol. Recently, the potential of mean force for rotation about C^α–C^α virtual bonds was studied on Ac–trans-Pro-NMe_2 at the ab initio MP2 level of theory with the 6-31G(d,p) basis set, which was implemented in the united-residue force field for the Pro–Pro sequence [21].

We report here the results on Ac–Pro-NMe_2 calculated using the ab initio computations with the self-consistent reaction field (SCRF) method to figure out the conformational preference and cis–trans isomerization of poly(Pro) in the gas phase, chloroform, methanol, and water.

2. Computational methods

The torsion angles and structural parameters for Ac–Pro-NMe_2 are defined in Fig. 1. All ab initio calculations were carried out using the Gaussian 98 package [22]. The values of ϕ and ψ for local minima of *N*-acetyl-L-proline-*N*′-methylamide (Ac–Pro-NHMe) optimized at HF/6-31G(d,p) [23] and HF/6-31+G(d) [24] levels as well as those of X-ray structures of PPI and PPII [15] were used as starting points for the empirical energy optimization of Ac–Pro-NMe_2 using the ECEPP/3 force field [25]. These minimized conformations from empirical energy calculations were used as initial structures for optimization of Ac–Pro-NMe_2 at the HF/6-31+G(d) level. Here, each backbone conformation is represented by a capital letter depending on its values of ϕ and ψ for backbone (Fig. 1) [26]. Conformations A, C, and F are defined by the backbone torsion angle ψ with the backbone torsion angle ϕ in the range of $-110^\circ<\phi<-40^\circ$: the conformation A, $-90^\circ<\psi<-10^\circ$; the conformation C, $50^\circ<\psi<130^\circ$; the conformation F, $130^\circ<\psi<180^\circ$ or $-180^\circ<\psi<-140^\circ$. Trans and cis conformations for the Ac–Pro peptide bond are defined by the orientation of the methyl carbon of acetyl group and the C^α of Pro residue, which are denoted by t and c, respectively. Down- and up-puckered conformations are defined as those of which the C^γ atom and the C═O group of the 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), which are represented by d and u, respectively.

Transition states ts1 and ts2 of Ac–Pro-NMe_2 were found by optimizing the conformations obtained from the adiabatic optimization of the conformations cAd and cAu with ω' =+116° for the Ac–Pro bond, respectively, as done for Ac–Pro-NHMe [24]. Transition states ts3 and ts4 were located starting from the ts1 and ts2 with $\omega'=-70^\circ$ and -60°, respectively. Fischer et al. [27] proposed four possible transition states of the twisted imide bond depending on the twist orientation (i.e., syn and anti clockwise) and the configuration of two carbonyl carbons preceded and followed by the Pro residue (i.e., exo and endo configuration). Our transition states ts1 and ts2 are similar to the syn/exo structures with down- and up-puckerings, respectively, whereas ts3 and ts4 resemble the anti/exo structures with down- and up-puckerings, respectively.

The 2-D potential energy surfaces (PESs) of the down- and up-puckered conformations with trans and cis peptide bonds for Ac–Pro-NMe_2 were calculated along the backbone torsion angle ψ at the HF/6-31+G(d) level, in which

adiabatic optimizations were performed at each value of ψ with an interval of 30° for $-180° \le \psi \le 180°$ and the conformations tFd, tFu, cFd, and cFu (Table 1) were used as initial structures for optimizations.

We employed the conductor-like polarizable continuum model (CPCM) SCRF method [28], implemented in the Gaussian 98 package, to compute solvation free energies at the HF/6-31+G(d) level. Solvation free energies of Ac–Pro-NMe_2 were calculated for its local minima and PESs optimized in the gas phase. Solvents considered here are chloroform, methanol, and water, whose dielectric constants are 4.9, 32.6, and 78.4 at 25 °C, respectively.

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_e) with zero-point and thermal corrections, and the relative entropic contribution [29]. The relative conformational free energy can be approximated to be the relative electronic energy if the other contributions are assumed to be constant. Relative total free energies computed by the sum of ΔE_e 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 [24]. The former free energy also reproduced satisfactorily experimental rotational barriers about the C–N bond of amides in chloroform and water [30]. However, these results may indicate that the need for improvement in the CPCM method, as pointed out previously [31]. In this work, the relative total free energy (ΔG_{tot}) was computed by the sum of ΔE_e and ΔG_s in solutions.

3. Results and discussion

3.1. Conformations in the gas phase

The 2-D potential energy surfaces (PESs) of Ac–Pro-NMe_2 with trans/cis peptide bonds and down/up-puckerings were calculated along the backbone torsion angle ψ at the HF/6-31+G(d) level, which are shown in Fig. 2.

For Ac-trans-Pro-NMe_2, the PESs show two minima tAd and tFd at $\psi = -18°$ and 142°, respectively, for the down-puckered conformation, and two minima tAu and tFu at $\psi = -37°$ and 138°, respectively, for the up-puckered conformation (see Table 1). Because of no amide hydrogen of the carboxyl end, conformations tCd and tCu are inaccessible to Ac–Pro-NMe_2, of which the former is the lowest energy conformation for *N*-acetyl-*N*′-methylamides of proline [23,24,32–35], pseudoprolines [36], and 5-methylated prolines [37] with a C_7 hydrogen bond between C═O of the amino end and N–H of the carboxyl end. Instead, PPII-like conformations tFd and tFu are significantly stabilized, which are not local minima for Ac–Pro-NHMe [23,24]. The barriers for the conformational transition between the α-helical conformation and the PPII structure, i.e., tAu→tFu and tFu→tAu, for up-puckered structures are estimated to be 5.4 and 11.0 kcal/mol, respectively, whereas the barriers for the transitions tAd→tFd and tFd→tAd for down-puckered structures are estimated to be 0.3 and 7.1 kcal/mol, respectively. This may indicates that the down-puckered conformations are likely interchangeable between these two structures. From flexible conformational energy calculations [5], the barrier for the transition of the α-helical conformation to the PPII structure was overestimated to be 10 kcal/mol.

On the PESs of Ac-cis-Pro-NMe_2, there are two minima cAd and cFd at $\psi = -19°$ and 166°, respectively, for the down-puckered conformation, and two minima

Table 1
Backbone torsion angles, endocyclic torsion angles, and puckering amplitudes of Ac–Pro-NMe_2 optimized at the HF/6-31+G(d) level

Conformer	Backbone[a]				Endocyclic[a]					Puckering amplitude		
	ω'	ϕ	ψ	ω	χ^0	χ^1	χ^2	χ^3	χ^4	q_α[b]	q_z[c]	χ_m[d]
tFd	176.8	−70.0	142.1	−178.5	−15.8	30.4	−34.2	24.5	−5.4	9.6	0.337	34.5
tFu	175.7	−61.0	137.6	−178.1	−5.4	−18.0	33.7	−36.2	26.4	10.4	0.362	37.5
cFd	−2.6	−75.4	166.4	−176.6	−19.7	33.8	−36.0	24.2	−2.7	10.4	0.363	37.1
cFu	−5.7	−60.8	160.6	−177.7	−4.5	−18.8	34.1	−36.1	25.8	10.4	0.362	37.6
cAu	6.3	−61.1	−38.0	−178.0	9.2	−28.7	37.4	−31.6	14.0	10.5	0.367	37.4
tAu	−173.4	−58.1	−37.0	173.8	10.2	−29.5	37.7	−31.3	13.2	10.6	0.369	37.7
cAd	8.3	−77.9	−18.6	177.9	−8.4	26.6	−34.7	29.2	−13.2	9.7	0.340	34.7
tAd	−171.3	−76.7	−17.9	173.4	−8.2	27.5	−36.5	31.0	−14.5	10.2	0.358	36.5
ts1	120.8	−73.7	−41.8	−178.1	36.3	−13.7	−12.6	34.4	−44.6	12.2	0.409	44.3
ts2	119.2	−84.8	−37.9	−178.2	20.7	−36.8	38.7	−26.9	3.9	11.3	0.392	40.3
ts3	−65.9	−59.1	−58.5	−179.5	40.7	−19.6	−6.8	30.8	−45.1	12.5	0.415	45.6
ts4	−71.2	−74.1	−41.6	174.6	21.8	−36.4	37.4	−24.7	1.8	11.0	0.382	39.2

[a] Defined in Fig. 1; units in degrees.
[b] Units in degrees; calculated by the method of Han and Kang [39].
[c] Units in Angstrom (Å); calculated by the method of Cremer and Pople [40].
[d] Units in degrees; calculated by the method of Altona and Sundaralingam [41].

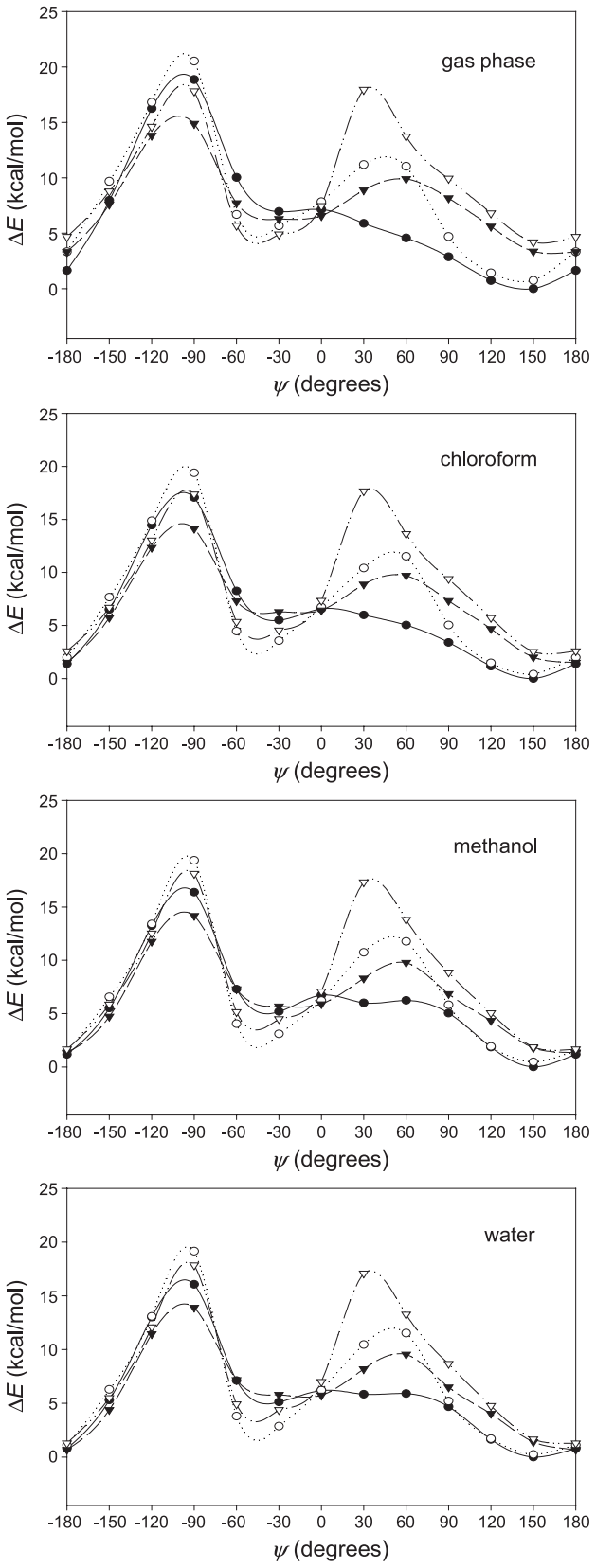

Fig. 2. Potential energy surfaces of Ac–Pro-NMe_2 at the HF/6-31+G(d) level with the CPCM method along the backbone torsion ψ in the gas phase, chloroform, methanol, and water: trans-down (●), trans-up (○), cis-down (▼), and cis-up (▽).

cAu and cFu at $\psi = -38^\circ$ and 161°, respectively, for the up-puckered conformation (see Table 1). In particular, the higher barriers appear at $\psi \approx 30^\circ$ and 60° for up- and down-puckered structures, respectively, with the backbone conformation C, which may be ascribed by the unfavorable nonbonded interaction between the methyl carbon of acetyl group and the C^α of Pro residue and by the weak electrostatic interaction between the carbonyl oxygen of acetyl group and the carbonyl carbon of Pro residue [33,38]. The lack of the C_7 hydrogen bond between two terminal groups seems to allow the PPI-like conformations cFd and cFu to be more feasible. Although the overall shapes of PESs of Ac-cis-Pro-NMe_2 are similar to those of Ac-cis-Pro-NHMe [24], the barriers at $\psi \approx -105^\circ$ for down-and up-puckered structures and at $\psi \approx 30^\circ$ for the up-puckered structure of Ac-cis-Pro-NMe_2 are elevated by about 6 kcal/mol than Ac-cis-Pro-NHMe. The conformation cAu and PPI-like conformations cFd and cFu are more stabilized by 1.4, 3.2, and 2.2 kcal/mol than the conformation cAd, respectively, which is the lowest energy conformation for Ac-cis-Pro-NHMe [24]. For Ac-cis-Pro-NMe_2, the barriers for the transitions A→F (or F→A) are computed to be about 4 (or 7) kcal/mol and 13 (or 14) kcal/mol for down- and up-puckered conformations, respectively, which are higher than those of Ac-trans-Pro-NMe_2.

Table 1 lists the backbone torsion angles, endocyclic torsion angles, and puckering amplitudes of the local minima and transition states for Ac–Pro-NMe_2 optimized at the HF/6-31+G(d) level. Four representative conformations tFd, tFu, cFd, cFu, and ts1 are presented in Fig. 3. To investigate the degree of puckering of proline ring, three kinds of puckering amplitudes, i.e., q_α of Han and Kang [39], q_z of Cremer and Pople [40], and χ_m of Altona and Sundaralingam [41], were calculated. 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. Recently, Hudáky et al. [42] proposed an alternative method to get pseudorotational amplitude and phase angle for endocyclic torsion angles in terms of a cosine function. However, their amplitudes are not included in this work because their expression is similar to that proposed by Altona and Sundaralingam [41].

Conformations tFd and cAd become less puckered than other conformations. As discussed above, Ac–Pro-NMe_2 has PPII-like conformations tFd and tFu, which are not local minima for Ac–Pro-NHMe, and conformations tCd and tCu feasible for Ac–Pro-NHMe are not preferred for Ac–Pro-NMe_2. The common conformations for these two proline amides are cFd and cFu, of which the torsion angles ϕ and prolyl ring puckerings are similar to each other. However, the torsion angle ψ shifts by $+11^\circ$ and $+7^\circ$ for conformations cFd and cFu, respectively, by introducing the second methyl group into the carboxyl end.

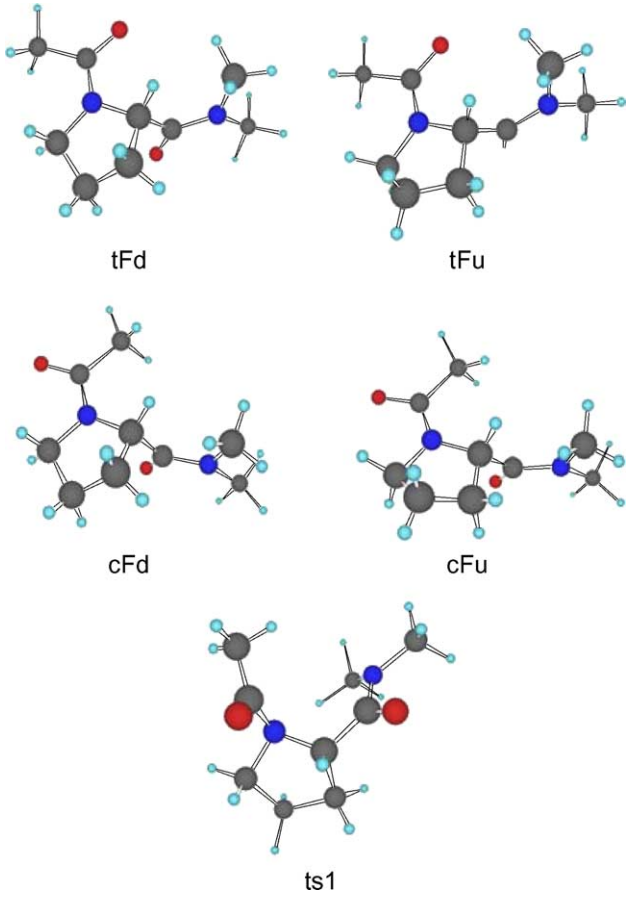

Fig. 3. Optimized tFd, tFu, cFd, cFu, and ts1 conformations of Ac–Pro-NMe_2 at the HF/6-31+G(d) level in the gas phase.

Because of no X-ray structure for Ac–Pro-NMe_2 is available, X-ray structures for the first Pro residue in *tert*-Boc-Pro-Pro-OH [43,44] and *tert*-Boc-Pro-Pro-NHMe [45] are compared with our optimized conformations. Our calculated values of (ϕ, ψ) for the tFd conformation are ($-70°$, $142°$), which are consistent with the mean X-ray values of ($-66°$, $146°$) in *tert*-Boc-Pro-Pro-OH. Our values of (ϕ, ψ) for the tFu conformation are calculated to be ($-61°$, $138°$), which accord with the mean X-ray values of ($-62°$, $140°$) in *tert*-Boc-Pro-Pro-OH and *tert*-Boc-Pro-Pro-NHMe. The mean puckering amplitudes χ_m for X-ray structures of *tert*-Boc-Pro-Pro-OH and *tert*-Boc-Pro-Pro-NHMe are 32.8° and 39.6° for conformations tFd and tFu, respectively, whereas our calculated values at the HF/6-31+G(d) level are 34.5° and 37.5°, respectively (Table 1). The differences in puckering amplitudes may be ascribed to the packing and intermolecular hydrogen bonds in crystal, which cannot be considered in the isolated proline amide.

In particular, we could locate four transition states for the cis–trans isomerization of the Ac–Pro peptide bond in this work; ts1 and ts2 are similar to syn/exo structures with down and up puckerings, respectively, whereas ts3 and ts4 resemble anti/exo structures with down and up puckerings, respectively, according to the definition of Fischer et al. [27]. Comparing with Ac–Pro-NHMe [24], there are large shifts in backbone torsion angles ϕ and ψ, i.e., the increase of 10° to 36° in the angle ϕ and the decrease of 25° to 58° in the angle ψ by introducing the second methyl group into the carboxyl end. In particular, the prolyl puckering is remarkably affected for transition states ts1 and ts3 with a large change in the endocyclic torsion angle χ^1 by about $-35°$.

Relative electronic energies of local minima and transition state structures to the trans conformer tFd optimized at the HF/6-31+G(d) level are listed in Table 2. The relative electronic energies of the trans-up (tFu) conformation to the trans-down (tFd) one as well as the cis conformations (cFd and cFu) to the trans conformations (tFd and tFu) are decreased compared to those of Ac–Pro-NHMe [24]. This may be ascribed to the shift in the torsion angle ψ, and the no intramolecular hydrogen bond between the amino end and carboxyl end that plays a role in stabilizing the lowest

Table 2
Relative solvation free energies, relative total free energies, and dipole moments of Ac–Pro-NMe_2 in the gas phase and solutions

Conformer	Gas phase		Chloroform			Methanol			Water		
	ΔE_e^a	μ^b	ΔG_s^c	ΔG_{tot}^d	μ^b	ΔG_s^c	ΔG_{tot}^d	μ^b	ΔG_s^c	ΔG_{tot}^d	μ^b
tFd	0.00	3.31	0.00	0.00	4.07	0.04	0.04	4.70	0.07	0.07	4.74
tFu	0.52	3.65	−0.22	0.30	4.49	−0.52	0.00	5.14	−0.52	0.00	5.19
cFd	2.95	7.50	−1.69	1.26	8.96	−2.22	0.73	9.69	−0.96	1.99	9.45
cFu	4.02	7.56	−1.97	2.05	9.04	−3.37	0.65	10.21	−3.38	0.64	10.28
cAu	4.77	3.49	−0.56	4.21	4.08	−1.29	3.48	4.46	−1.17	3.60	4.49
tAu	5.61	7.08	−2.29	3.32	8.52	−3.38	2.23	9.51	−3.36	2.25	9.58
cAd	6.15	2.74	−0.54	5.61	3.17	−1.94	4.21	3.39	−1.82	4.33	3.40
tAd	6.87	6.72	−1.56	5.31	8.05	−2.60	4.27	9.00	−2.49	4.38	9.07
ts1	20.09	5.77	0.59	20.68	6.88	0.47	20.56	7.54	0.72	20.81	7.59
ts2	20.64	5.59	0.76	21.40	6.67	0.51	21.15	7.34	0.81	21.45	7.39
ts3	22.91	4.12	0.63	23.54	4.91	0.75	23.66	5.44	0.93	23.84	5.48
ts4	23.45	4.54	0.43	23.88	5.37	0.17	23.62	5.92	0.35	23.80	5.96

[a] Relative electronic energies in kcal/mol.
[b] Dipole moments in Debye.
[c] Relative solvation free energies in kcal/mol.
[d] Relative total free energies in kcal/mol. In solutions, ΔG_{tot} is calculated as the sum of ΔE_e and ΔG_s; see the section of Computational methods.

conformations tCd and tCu for Ac–Pro-NHMe, as described above.

Although previous empirical energy calculations for Ac–Pro-NMe_2 with the fixed ϕ=−75.0° and down-puckering predicted well the existence of PPI and PPII conformations at $\psi \approx 160°$ [20], the value of ψ is larger by about 20° than our HF value and the relative energy of the PPI conformation to the PPII one was underestimated by about 1.5 kcal/mol than our HF values. From flexible conformational energy calculations on Ac–Pro-NMe_2, it was concluded that the most probable conformation is the α-helical conformation with the cis peptide bond and up puckering, followed by the up-puckered PPII conformation at (ϕ, ψ)= (−56°, 120°) with a relative energy of 0.4 kcal/mol [5]. In addition, the dominant prolyl ring structure was reported to be up-puckered for both PPI and PPII conformations [5]. However, these results are quite different from our HF results reported here.

The relative electronic energies of transition states (i.e., rotational barriers for the trans-to-cis isomerization of the Ac–Pro peptide bond) to the lowest energy conformation are more increased by 2.4–3.6 kcal/mol than those of Ac–Pro-NHMe. This could be due to the lack of the intramolecular hydrogen bond between prolyl nitrogen and carboxyl N–H group for the transition states of Ac–Pro-NMe_2, which was suggested as an important factor to stabilize the transition states of *N*-acetyl amides of proline [24,27,33,35,46], pseudoprolines [36], and 5-methylated prolines [37]. Transition states ts1 and ts2 were proven to have lower energies than the transition states ts3 and ts4, and the transition state ts1 appears to be the saddle point for the cis–trans isomerization of the Ac–Pro peptide bond in the gas phase, as similar to Ac–Pro-NHMe [24].

3.2. Conformations in solutions

The PESs of Ac–Pro-NMe_2 along the angle ψ at the HF/6-31+G(d) level with the CPCM method in chloroform, methanol, and water are shown in Fig. 2. In chloroform, there is no significant change in PES compared with that in the gas phase, except for the small increase of relative stability for up-puckered conformations tFu and cFu. The relative stabilities of down- and up-puckered conformations for PPII and PPI structures become almost equally probable and a shoulder at ψ=30° appears in methanol solution. The PES in water is quite similar to that in methanol solution. Less significant changes in PES with the increase of solvent polarity or hydrogen bonding ability may be attributed to the lack of intramolecular hydrogen bonds to stabilize the conformations even in the gas phase as seen for Ac–Pro-NHMe. However, it should be noted that the relative stabilities of local minima and transition states are affected with the increase of solvent polarity or hydrogen bonding ability. In particular, the α-helical conformation tAd becomes more feasible in solutions and the relative free energy of the conformation tAu decreases with the increase of solvent polarity.

With the increase of solvent polarity, the barriers for the transition of the α-helical conformation to the PPII structure (i.e., A→F) for Ac-trans-Pro-NMe_2 are somewhat increased, whereas the barriers for the transition F→A are nearly constant. This is because conformations tAd and tAu become more stable but the hill heights between conformations A and F are nearly constant with the increase of solvent polarity. In the case of Ac-cis-Pro-NMe_2 with down-and up-puckerings, the barriers for the transition A→F and F→A are nearly constant and somewhat increased, respectively, because only polyproline conformations cFd and cFu become more stabilized with the increase of solvent polarity.

Relative solvation free energies, relative total free energies, and dipole moments of local minima and transition state structures in solutions are summarized in Table 2. It was found that the solvation became more favorable and the dipole moment is increased with the increase of solvent polarity. In chloroform, the order of stabilities for conformations tFd, tFu, cFd, and cFu are the same as those in the gas phase, although the relative stabilities of up-puckered conformations tFu and cFu are increased. However, in methanol and water solutions, the up-puckered conformations tFu and cFu become more stable than the down-puckered conformations tFd and cFd, respectively.

3.3. Population of backbone conformations and cis–trans isomerization

Calculated populations of backbone and cis conformations for Ac–Pro-NMe_2 at the HF/6-31+G(d) level in the gas phase, chloroform, methanol, and water are shown in Table 3. In the gas phase, the populations of polyproline conformations tF (PPII) and cF (PPI) are found to be dominant. Populations for PPII and PPI structures are computed to be 99.4% and 0.6%, respectively. The cis population is calculated to be 0.6% due to PPI structures.

In solutions, the populations of polyproline structures are still dominant, although there are some increases of the populations for α-helical conformation A with the increase of solvent polarity, as seen on the PESs in Fig. 2. The conformation tF (PPII) is found to be the most preferred in solutions as well as in the gas phase. However, the populations of cF (PPI) structures are increased with the increase of solvent polarity and the maximum value is obtained in methanol solution. In particular, the larger population of PPI in methanol than in water is consistent with experiments [19]. The calculated cis populations of 24.4% and 16.6% in methanol and water solutions, respectively, are consistent with experimental values of 22%, and 15% [19], respectively. However, the cis population in chloroform is calculated to be 8.5%, which is considerably low than experimental values of 43% and

Table 3
Populations of backbone conformations, rotational barriers, and relative energies of cis conformers for Ac–Pro-NMe_2 and Ac–Pro-NHMe in the gas phase and solutions

Solvent	Backbone[a]							Rotational barrier[b,c]		Relative energy[b,d]
	tF[e]	cF[e]	F[e]	A	C	Cis[f]	Experimental cis[f]	$\Delta G_{tc}^{\ddagger}$	$\Delta G_{ct}^{\ddagger}$	$\Delta G_{c/t}$
Ac–Pro-NMe_2										
Gas phase	99.4	0.6	100.0	0.0	0.0	0.6		20.09	17.14	2.95
Chloroform	91.3	8.4	99.7	0.3	0.0	8.5	43,[g] 55[g]	20.68	19.42	1.26
Methanol	74.7	24.2	98.9	1.1	0.0	24.4	22[g]	20.56	19.91	0.65
Water	82.4	16.4	98.8	1.2	0.0	16.6	15[g]	20.81	20.17	0.64
Ac–Pro-NHMe[h]										
Gas phase	0.0	0.0	0.0	2.1	97.8	2.0		17.62	15.25	2.37
Chloroform	31.7	2.2	33.8	26.4	39.7	11.0	14,[i] 15±4[j]	17.51	16.52	0.99
Methanol	41.5	11.4	52.9	46.3	0.9	38.1	23,[i] 25[k]	18.74	18.82	−0.08
Water	39.2	12.3	51.6	47.6	0.8	32.9	24±4,[j] 30,[k] 27±3[l]	18.96 (20.4)[l]	18.84 (19.8)[l]	0.13 (0.57)[l]

[a] Units in %. Computed from relative total energies or free energies of Table 2 by using the Boltzmann statistical weights at 25°C.
[b] Energies in kcal/mol. The lowest total energy for each of trans, cis, and transition state conformations was used for these calculations. Experimental values are listed in parentheses.
[c] $\Delta G_{tc}^{\ddagger}$ and $\Delta G_{ct}^{\ddagger}$ represent the barriers for the trans-to-cis and cis-to-trans rotations for the Ac–Pro peptide bond, respectively.
[d] $\Delta G_{c/t}$ is the relative total energy of the cis conformer to the trans conformer.
[e] tF and cF conformers correspond to polyproline II and I structures, respectively. The population of F conformer is a sum of those of tF and cF conformers.
[f] Cis Ac–Pro peptide bond.
[g] Taken from Ref. [19].
[h] The calculated values for Ac–Pro-NHMe in the gas phase, chloroform, and water are taken from Ref. [24]. The values in methanol are additionally calculated in this work.
[i] Taken from Ref. [47].
[j] Taken from Ref. [48].
[k] Taken from Ref. [49].
[l] Taken from Ref. [50].

55% [19]. This could be in part ascribed to that the CPCM method used here is weaker in describing solvation free energies in less polar solvent such as carbon tetrachloride [31]. Nevertheless the CPCM method reproduced satisfactorily the experimental cis populations of Ac–Pro-NHMe in chloroform, methanol, and water solutions [47–50], as shown in Table 3.

The transition state ts1 appears to be the saddle point for the cis–trans isomerization of the Ac–Pro peptide bond in solutions, as found in the gas phase. Although the order of barriers ($\Delta G_{tc}^{\ddagger}$ and $\Delta G_{ct}^{\ddagger}$) for the trans-to-cis and cis-to-trans isomerization, respectively, of the Ac–Pro peptide bond in solutions is the same as that in the gas phase, the barriers are increased with the increase of solvent polarity (Tables 2 and 3), as seen for Ac–Pro-NHMe [24,32,33]. It should be noted that Ac–Pro-NMe_2 has higher rotational barriers than Ac–Pro-NHMe in all solutions considered here. This could be due to the lack of the intramolecular hydrogen bond between prolyl nitrogen and carboxyl N–H group for the transition state of Ac–Pro-NMe_2, which was suggested as an important factor to stabilize the transition state of Ac–Pro-NHMe, as discussed above. The increase of rotational barriers was also confirmed by experiments on *N*,*N*-dimethylacetamide [51]. In addition, the relative energies of cis conformations were reduced with the increase of solvent polarity, as seen for the cis populations above.

4. Conclusions

The second methyl substitution at the carboxyl amide end results in different backbone structures and their populations from those of Ac–Pro-NHMe. In particular, all conformations with the C_7 hydrogen bond between acetyl and amide ends, which is the most probable conformations of Ac–Pro-NHMe in the gas phase and in nonpolar solvents, disappeared for Ac–Pro-NMe_2 even in the gas phase due to the lack of amide hydrogen. The dominant conformation for Ac–Pro-NMe_2 is the polyproline II structure with the trans prolyl peptide bond in the gas phase and in solutions. In methanol, the population of the polyproline I structure with the cis prolyl peptide bond is calculated to be larger than that in water, which is consistent with experiments. It should be noted that Ac–Pro-NMe_2 has higher rotational barriers for the cis–trans isomerization of the Ac–Pro peptide bond than Ac–Pro-NHMe in the gas phase and in solutions, which could be due to the lack of the intramolecular hydrogen bond between prolyl nitrogen and carboxyl N–H group for the transition state of Ac–Pro-NMe_2. The rotational barriers for Ac–Pro-NMe_2 are increased with the increase of solvent polarity, as seen for Ac–Pro-NHMe. The ab initio conformational studies on *N*-acetyl-*N*′,*N*′-dimethylamides of oligoprolines as the polyproline model are now in progress.

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