Cis−Trans Isomerization and Puckering of Pseudoproline Dipeptides

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We report here the results on *N*-acetyl-*N*′-methylamides of oxazolidine and thiazolidine (Ac-Oxa-NHMe and Ac-Thz-NHMe) calculated using the ab initio and density functional computations with the reaction field theory at the HF, MP2, and B3LYP levels of theory with the 6-31+G(d) basis set. The displacement of the γ-CH_2 group in proline ring by oxygen and sulfur atoms has affected the structure of proline, cis−trans equilibrium, and rotational barrier. The up-puckered structure is found to be prevalent for the trans conformers of pseudoproline amides. The higher cis populations of pseudoproline amides can be interpreted due to the longer distance between the acetyl methyl group and the 5-methylene group of the ring for the trans conformer of pseudoproline amides than that of the proline amide. The changes in charge of the prolyl nitrogen and the decrease in electron overlap of the C−N bond for transition state (TS) structures seem to play a role in lowering rotational barriers of Oxa and Thz amides compared to that of the proline (Pro) amide. The calculated preferences for cis conformers in the order of Oxa > Thz > Pro amides and for trans-to-cis rotational barriers in the order of Pro > Oxa > Thz amides in water are consistent with experimental results on pseudoproline-containing peptides. The pertinent distance between the prolyl nitrogen and the N−H amide group to form a hydrogen bond might indicate that this intramolecular hydrogen bond could contribute in stabilizing the TS structures of pseudoproline and proline amides and play a role in prolyl isomerization.

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 to not form a hydrogen bond and the N−C^{α} rotation to be rigid.[1] The pyrrolidine of the Pro residue is a five-membered ring, which may adopt two distinct down- and up-puckered conformations[2] that are almost equally favorable.[3−8] 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 C^{δ}, N, and C^{α} atoms. Pro residue has a relatively high intrinsic probability of having the cis peptide bond preceding proline as compared with other amino acids,[9,10] since the configurations of atoms of the Pro residue adjacent to the α-carbon of the preceding residue are quite similar in either trans or cis conformation.

The cis−trans isomerization of the X-Pro peptide bond has been suggested to be often involved in the rate-determining steps for folding and refolding of various proteins.[11] However, the heterogeneity of the X-Pro bonds of proteins in the unfolded state has been known to obscure the folding process and cause its interpretation to be difficult.[12] Many experiments[13] and computation[14] studies have been carried out on the cis−trans isomerization of proline-containing peptides. In particular, *N*-acetyl-*N*′-methylprolineamide (Ac-Pro-NHMe) has been widely used as a model for experimental[15−17] and theoretical[5,6,8,18−22] studies on the conformational preferences and transition states for X-Pro isomerization.

A number of attempts have been made to constrain the X-Pro bond to either trans or cis conformations by alkylation,[12,17,23−26] cyclization,[27−29] and heteroatomic susbstitution.[30−34] In particular, constrained proline derivatives have been used to enhance the activity of bioactive peptides by restricting the attack of proteases.[35,36] The alkylations at positions 2, 3, and 5 of proline have been shown to have a subtle influence on the backbone conformation of proline.[8,12,17,23−26] In particular, the cis−trans isomer equilibrium of the X-Pro peptide bond has been significantly affected by the alkylation at position 5 of proline.[8,12,17,24−26] This is mainly ascribed to the steric interactions of the alkyl groups with the preceding residue.

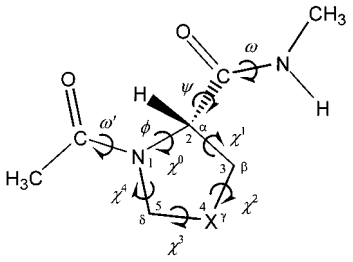

Figure 1. Definition of torsion angles and structural parameters for Ac-ΨPro-NHMe: X = CH_2 for Pro; X = O for Oxa; X = S for Thz.

Recently, it has been reported that Ser/Thr-derived (*S*)-oxazolidine-4-carboxylic acid (Oxa) and Cys-derived (*R*)-thiazolidine-4-carboxylic acid (Thz), denoted as pseudoprolines (ΨPro's, Figure 1), exert a pronounced effect on the peptide backbone, preventing peptide aggregation and self-association, thus improving the solvation and coupling kinetics in peptide assembly.[37] NMR studies of a series of ΨPro-containing peptides have revealed a considerable effect of 2-C substitution[38] on the cis−trans equilibrium of the X-ΨPro peptide bond in solution.[30−33] In general, ΨPro-containing peptides exhibit enhanced rate constants for cis−trans isomerization compared to their proline analogues depending on stereochemistry and the degree of substitution at the 2-C position.[31−33] The enhanced isomerization rates of the X-ΨPro peptide bond are ascribed to

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TABLE 1: Backbone Torsion Angles, Endocyclic Torsion Angles, and Puckering Amplitudes Optimized at the HF/6-31+G(d) Level

		backbone[a]				endocyclic[a]					puckering amplitude		
dipeptide	conf	ω'	ϕ	ψ	ω	χ^0	χ^1	χ^2	χ^3	χ^4	q_α[b]	q_z[c]	χ_m[d]
Pro[e]	trans	−172.8	−86.2	74.9	−176.5	−14.7	31.8	−37.5	28.2	−8.5	10.5	0.366	37.4
	cis	9.2	−89.5	−9.1	−179.7	−13.4	31.1	−37.4	29.0	−9.8	10.5	0.366	37.4
	TS	116.4	−106.2	−9.9	−178.5	4.6	20.6	−37.1	39.9	−28.0	11.5	0.396	43.0
Oxa	trans	−168.9	−87.3	75.2	−177.0	−5.2	−17.7	35.8	−39.1	26.8	10.7	0.344	40.1
	cis	12.8	−91.4	−9.3	−176.0	−10.3	29.2	−39.5	32.5	−12.2	10.6	0.345	37.7
	TS	119.5	−114.3	−2.6	−176.5	−1.3	23.9	−39.7	39.3	−22.3	11.2	0.360	37.7
Thz	trans	−171.6	−88.2	84.8	174.5	−12.4	−17.7	32.8	−39.4	36.7	12.1	0.468	44.8
	cis	6.7	−90.1	−2.5	−176.2	−19.0	37.9	−38.0	27.3	−8.9	11.6	0.450	41.5
	TS	115.5	−115.5	2.5	−176.7	−7.1	33.6	−40.3	36.9	−23.0	12.7	0.496	46.1

[a] Defined in Figure 1; units in degrees. [b] Units in degrees; calculated by the method of Han and Kang in ref 46. [c] Units in angstroms; calculated by the method of Cremer and Pople in ref 47. [d] Units in degrees; calculated by the method of Altona and Sundaralingam in ref 48. [e] Taken from ref 22.

the lowered transition state barriers.[32,33] It has been suggested that increased cis populations and enhanced isomerization rates of the X-ΨPro peptide bonds might be attributed to the reduced electron density of the peptide bond[31] and the different ring puckerings[32] imposed by substitution of heteroatoms.

We report here the results on *N*-acetyl-*N*′-methylamides of oxazolidine and thiazolidine (Ac-Oxa-NHMe and Ac-Thz-NHMe) calculated using the ab initio and density functional computations with the reaction field theory at higher levels of theory to investigate the influence of displacement of the γ-CH_2 group in proline ring by oxygen and sulfur atoms on the structure of proline, cis−trans equilibrium, and rotational barrier in the gas phase, chloroform, and water.

Computational Methods

All ab initio and density functional calculations were carried out using the Gaussian 94[39] and Gaussian 98[40] packages. Geometry optimizations for the trans and cis conformers and the transition states (TS) of pseudoprolineamides Ac-Oxa-NHMe and Ac-Thz-NHMe were carried out at the HF/6-31+G(d) level of theory, in which the diffuse functions were included for the proper representation of the lone pairs. Optimized structures of Ac-Pro-NHMe at the HF/6-31+G(d) level with trans and cis peptide bonds[22] were used as starting points for optimization of pseudoprolineamides. As noted in our previous optimization of the transition state for Ac-Pro-NHMe,[22] cis conformations of pseudoprolineamides with $\omega' = 90°$ for the X-ΨPro bond were employed as initial structures for minimization of their transition states (Figure 1).[41] In addition, the TS structure of Ac-Pro-NHMe[22] was used as another starting point for optimization of the TS states of pseudoprolineamides.

Although the down-puckering was found to be favored for Ac-Pro-NHMe,[6,8,22] geometry optimizations were also carried out on pseudoprolineamides with the up-puckering. Two conformations whose backbone torsion angles (ϕ, ψ) are (−83°, 84°) and (−77°, −20°) were chosen as starting points for optimization of the up-puckered pseudoprolineamides with the trans and cis peptide bonds, respectively. These two conformations have known to be feasible for Ac-Pro-NHMe from ab initio calculations at the HF/6-31G(d,p) level.[6]

Vibrational frequencies were calculated for fully optimized conformations at the HF/6-31+G(d) level using the same basis set, which were used to compute the enthalpy changes and the Gibbs free energy changes for the X-ΨPro isomerization at 298.15 K in the gas phase. A scale factor of 0.890 was used for vibrational frequencies in computing thermodynamic quantities, which was chosen to reproduce experimental frequencies for the amide I band of *N*-methylacetamide in Ar and N_2 matrixes.[42] Single-point MP2 and B3LYP energy calculations were also performed for the HF/6-31+G(d) optimized geometries using the same basis set.

We have employed two self-consistent reaction field (SCRF) methods at the HF/6-31+G(d) level to include solvent effects, implemented in Gaussian 94, which are the isodensity polarizable continuum model (IPCM) and the self-consistent isodensity polarizable continuum model (SCI-PCM).[43] Solvation free energies were calculated for optimized conformations in chloroform and water, whose dielectric constants used are 4.7 and 78.4 at 298.15 K.[44]

For Ac-Pro-NHMe, the IPCM calculations predict satisfactorily the increase of rotational barriers as the augmentation of solvent polarity,[22] which is consistent with observed results on *N*,*N*-dimethylacetamide.[45] On the other hand, the lessening stability of the trans conformer is predicted from the SCI-PCM calculations as the solvent becomes more polar,[22] which is accord with CD and NMR measurements on Ac-Pro-NHMe.[16] Therefore, the IPCM and SCI-PCM methods were employed to see the solvent effects on the isomerization barrier and the cis population of pseudoprolineamides, respectively. Although the SCI-PCM method can satisfactorily predict the favored solvation of the cis conformer for Ac-Pro-NHMe, the relative solvation free energy (ΔG_s) of each cis conformer from the SCI-PCM calculations has been scaled, in order to reproduce the observed cis population (27%) of Ac-Pro-NHMe in water.[17] Scale factors used here are 0.334 and 0.587, derived from relative free energies (ΔG_g) at MP2 and B3LYP levels, respectively.[22] The 0.0004 electron per cubic Bohr (e/B^3) electron density surface was used for the IPCM and SCI-PCM calculations to define the boundary of solute, which reproduces the observed molar volumes of *N*,*N*-dimethylamides.[45]

Results

Structures of Backbone and Proline Ring. The torsion angles and structural parameters for *N*-acetyl-*N*′-methylamide of ΨPro are defined in Figure 1. Table 1 lists the backbone torsion angles, endocyclic torsion angles, and puckering amplitudes for *N*-acetyl-*N*′-methylamides of Pro, Oxa, and Thz optimized at the HF/6-31+G(d) level. The optimized trans, cis, and TS structures of two pseudoprolineamides are shown in Figures 2 and 3. To investigate the degree of puckering of proline ring, three kinds of puckering amplitudes, i.e., q_α of Han and Kang,[46] q_z of Cremer and Pople,[47] and χ_m of Altona and Sundaralingam,[48] were calculated. The parameter q_α is the maximum angle between the mean plane and the line joining the geometrical center and each atom of the ring.[46] The parameter q_z corresponds to the maximum *z*-displacement

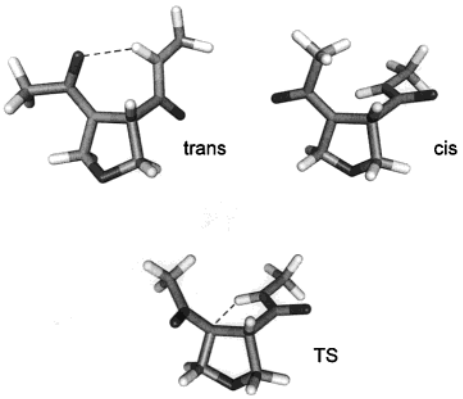

Figure 2. Optimized trans, cis, and TS structures of Ac-Oxa-NHMe. Hydrogen bonds are represented by dotted lines.

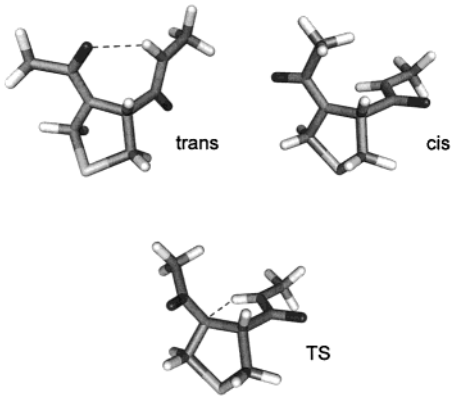

Figure 3. Optimized trans, cis, and TS structures of Ac-Thz-NHMe. Hydrogen bonds are represented by dotted lines.

perpendicular to the mean plane of the ring.[47] The parameter χ_m is the maximum value attainable by endocyclic torsion angles of the ring.[48]

As seen in Table 1, heteroatomic substitution results in large changes in backbone torsion angles ω', ϕ, ψ, and ω as well as in endocyclic torsion angles $\chi^0-\chi^4$ (see Figure 1 for definition of structural parameters). In particular, the up-puckered structures of Ac-Pro-NHMe with trans and cis X-Pro bonds were computed to be higher by 1.69 and 1.07 kcal/mol in electronic energy than the corresponding down-puckered structures, respectively.[8,49] On the other hand, the up-puckered conformations of Ac-Oxa-NHMe and Ac-Thz-NHMe with the trans X-ΨPro bond are found to have lower HF electronic energies by 0.36 and 0.66 kcal/mol than the corresponding down-puckered conformations, respectively.[51] The down-puckering, however, is still dominated for pseudoprolineamides with the cis X-ΨPro bond. It should be noted that the degree of puckering (i.e., measured by puckering amplitudes) for Ac-Oxa-NHMe is quite similar to that of Ac-Pro-NHMe, whereas Ac-Thz-NHMe has the more puckered structure than Ac-Pro-NHMe.

In the trans Oxa amide, there are small changes in backbone structure except for $\Delta\omega' = +4°$, despite the alteration in puckering. The changes of 4° in backbone torsion angles ω' and ω are found for the cis Oxa amide. The shifts of backbone torsion angles ϕ and ψ by −8° and 7°, respectively, are found for the TS structure of the Oxa amide. There are changes of −2° to 4° and −6° to 6° in endocyclic torsion angles for cis and TS conformers of the Oxa amide, respectively.

For the Thz amide with the trans peptide bond, there are shifts of 10° in backbone torsion angles ψ and ω as well as the alteration in puckering. The changes of 7° and 4° in backbone torsion angles ψ and ω, respectively, are calculated for the cis Thx amide, which has changes of −6° to 7° in endocyclic torsion angles. The large changes of −9° and 12° are calculated for backbone torsion angles ϕ and ψ, respectively, for the TS structure of the Thz amide, which has changes of −12° to 13° in endocyclic torsion angles.

Geometrical Parameters. Optimized bond lengths and bond angles of Oxa and Thz amides are shown in Table 2. Although there are increases of 0.001−0.01 Å in the C−N peptide bond lengths for trans, cis, and TS conformers of two pseudoproline amides compared to those of Ac-Pro-NHMe, the relative C−N bond lengths of TS structures to trans and cis conformers for pseudoproline amides are almost the same as for the proline amide. The bond angles $C^\alpha-N-C^\delta$, $C-N-C^\alpha$, and $C-N-C^\delta$ are computed to be 112.0°−114.3° for TS structures of Oxa and Thz amides, similar to those of the proline amide, except for 106.3° for the bond angle $C^\alpha-N-C^\delta$ of the Oxa amide. These calculated bond lengths and bond angles around the C−N peptide bond for TS structures of Oxa and Thz amides as well as the Pro amide are quite similar to those of triethylamine determined by gas electron diffraction.[52] These results may indicate that the prolyl nitrogen for the TS structure for Oxa and Thz amides is in the sp^3 hybridization, whereas that for the trans and cis conformers is in the sp^2 state.

The lengths of the N−H bond of NHMe group for trans conformers of Oxa and Thz amides are optimized to be longer by 0.004 Å than those of the cis and TS conformers, respectively, which results from the hydrogen bond between the N−H of NHMe group and the O═C of acetyl group, as found for the Pro amide.

As might be expected, the displacement of the γ-CH_2 group in proline ring by oxygen and sulfur atoms has significantly affected the bond lengths and bond angles of proline ring. The bond lengths $C^\beta-X$ and $X-C^\delta$ of trans, cis, and TS conformers are decreased by 0.12−0.15 Å for the Oxa amide and increased by 0.28−0.29 Å for the Thz amide compared to those of the Pro amide. The bond angles $C^\alpha-N-C^\delta$ and $N-C^\alpha-C^\beta$ are decreased by 1.1°−4.4° for conformers of the Oxa amide and increased by 0.8°−5.0° for conformers of the Thz amide compared to those of the Pro amide. On the other hand, the bond angle $C^\beta-X-C^\delta$ for Oxa amides is increased by 3.1°−4.5° and is significantly decreased by 13.0°−14.7° for Thz amides compared to those of the Pro amide. Our calculated results indicate that the larger changes in bond lengths and bond angles of proline ring are brought about by the substitution of sulfur atom rather than oxygen atom. The HF/6-31+G(d) optimized values of the bond length $C^\beta-X$ and the bond angles $C^\alpha-C^\beta-X$ and $C^\beta-X-C^\delta$ for Oxa and Thz amides are reasonably consistent with those of electron diffraction measurements for tetrahydrofuran[53] and tetrahydrothiophene.[54]

Relative Stability of Cis Conformers. Relative electronic energies, enthalpies, and free energies of cis conformers to trans conformers in the gas phase computed at HF, MP2, and B3LYP levels with the 6-31+G(d) basis set are listed in Table 3. Heteroatomic substitution has stabilized the cis conformers by 2.0 and 1.8 kcal/mol for Oxa and Thz amides over the Pro amide at the HF level, respectively. The electron correlation has increased the relative stability of cis conformers for Oxa and Thz amides by 1.6 and 1.4 kcal/mol at the MP2 level and by 1.4 and 1.0 kcal/mol at the B3LYP level. Heteroatomic substitution has contributed to the relative enthalpies (ΔH_g) by −2.1 and −1.9 kcal/mol at the HF level, −1.7 and −1.6 kcal/mol at the MP2 level, and −1.5 and −1.2 kcal/mol at the B3LYP level for Oxa and Thz amides, respectively. Thus, the

TABLE 2: Geometric Parameters Optimized at the HF/6-31+G(d) Level[a]

	Pro[b]			Oxa			Thz			Oxa	Thz	
	trans	cis	TS	trans	cis	TS	trans	cis	TS	X-ray[c]	X-ray[d]	X-ray[e]
Bond Length, Å												
Xaa												
N−C$^{\alpha}$	1.469	1.458	1.478	1.465	1.453	1.473	1.469	1.456	1.476	1.448	1.455	1.448
N−C$^{\delta}$	1.467	1.468	1.476	1.457	1.464	1.478	1.451	1.463	1.473	1.480	1.422	1.467
C$^{\alpha}$−C$^{\beta}$	1.528	1.539	1.544	1.539	1.537	1.537	1.540	1.531	1.533	1.542	1.526	1.515
C$^{\beta}$−X	1.530	1.530	1.528	1.409	1.402	1.403	1.817	1.813	1.811	1.434	1.812	1.809
X−C$^{\delta}$	1.530	1.529	1.522	1.384	1.389	1.382	1.812	1.812	1.807	1.383	1.840	1.800
Ac-Xaa												
C−N	1.349	1.361	1.434	1.356	1.362	1.437	1.359	1.367	1.440	1.335	1.357	1.339
C═O	1.212	1.204	1.189	1.208	1.203	1.188	1.209	1.203	1.188	1.244	1.248	1.223
C−C	1.512	1.514	1.505	1.512	1.512	1.504	1.513	1.513	1.504	1.513	1.499	
Xaa-NHMe												
C$^{\alpha}$−C	1.538	1.532	1.528	1.537	1.529	1.527	1.540	1.535	1.533	1.532	1.536	1.535
C═O	1.207	1.206	1.207	1.206	1.206	1.206	1.206	1.205	1.206	1.211		1.190
C−N	1.341	1.342	1.341	1.339	1.339	1.339	1.341	1.340	1.339	1.354	1.308	
N−C	1.447	1.449	1.447	1.448	1.450	1.448	1.448	1.450	1.448	1.442		
N−H	0.998	0.993	0.994	0.998	0.994	0.994	0.998	0.994	0.994	0.884		
Bond Angle, deg												
Xaa												
C$^{\alpha}$−N−C$^{\delta}$	112.4	112.7	109.1	108.0	110.1	106.3	113.2	116.3	112.5	109.8	115.9	115.9
N−C$^{\alpha}$−C$^{\beta}$	103.0	103.0	105.2	101.9	100.4	102.9	108.0	106.0	108.4	101.2	106.7	105.7
C$^{\alpha}$−C$^{\beta}$−X	103.6	103.4	103.7	104.7	103.7	103.4	107.1	104.7	104.3	102.9	102.8	103.5
C$^{\beta}$−X−C$^{\delta}$	103.5	103.4	102.1	106.6	107.7	106.6	88.8	90.4	88.8	106.6	88.6	91.1
N−C$^{\delta}$−X	103.8	103.6	103.4	104.1	103.8	104.8	104.9	106.0	106.4	103.3	105.3	105.5
Ac-Xaa												
C$^{\alpha}$−N−C	121.2	126.0	114.2	120.9	127.3	114.3	119.0	125.4	113.4	121.4	121.0	124.3
C$^{\delta}$−N−C	125.2	119.1	113.2	126.2	119.3	113.6	125.2	117.0	112.0	126.8	121.3	119.3
N−C═O	121.8	120.9	122.2	121.3	120.3	122.0	121.0	120.5	122.0	119.7	119.6	123.6
N−C−C	117.3	117.9	115.0	117.8	117.7	114.7	118.4	118.2	114.8	119.7	118.4	
Xaa-NHMe												
N−C$^{\alpha}$−C	111.3	115.1	113.3	112.7	115.9	114.1	110.1	115.5	113.4	110.4	115.3	
C$^{\beta}$−C$^{\alpha}$−C	112.5	110.9	111.0	112.9	111.1	111.4	111.3	110.5	110.6	109.7	112.9	
C$^{\alpha}$−C═O	121.8	119.2	119.6	121.6	118.6	118.9	121.7	118.5	119.0	122.1	118.6	
C$^{\alpha}$−C−N	115.1	117.7	117.3	115.0	117.8	117.4	114.8	117.9	117.4	112.7	117.4	
C−N−C	121.5	121.7	121.9	121.5	121.6	121.7	121.3	121.4	121.5	121.6		

[a] See Figure 1 for definition of structural parameters. Only structural parameters for non-hydrogen atoms are included, except for N−H. [b] Calculated from the structures reported in ref 22. [c] Ac-Ala-Oxa-NHMe; taken from ref 57. [d] Ac-Thz-NH_2; taken from ref 58. [e] *t*-Boc-Thz-OH; taken from ref 59.

TABLE 3: Relative Electronic Energies, Enthalpies, and Free Energies at HF, MP2, and B3LYP Levels with the 6-31+G(d) Basis Set in the Gas Phase[a]

		HF			MP2			B3LYP		
dipeptide	conf	ΔE_e[b]	ΔH_g[c]	ΔG_g[c]	ΔE_e[b]	ΔH_g[c]	ΔG_g[c]	ΔE_e[b]	ΔH_g[c]	ΔG_g[c]
Pro[d]	trans	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	cis	2.37	2.21	1.81	2.28	2.11	1.71	3.17	3.00	2.61
	TS	17.62	16.52	16.97	18.54	17.44	17.88	20.04	18.94	19.39
Oxa	trans	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	cis	0.36	0.08	−0.29	0.72	0.44	0.07	1.79	1.51	1.14
	TS	15.22	14.10	14.77	15.99	14.87	15.53	17.64	16.53	17.19
Thz	trans	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
	cis	0.59	0.28	0.01	0.86	0.55	0.29	2.15	1.84	1.58
	TS	15.54	14.28	14.86	16.41	15.16	15.74	18.58	17.33	17.91

[a] Energies in kcal/mol. [b] Relative electronic energy at $T = 0$ K. [c] Calculated with a scale factor of 0.890 for vibrational frequencies at $T = 298.15$ K. ΔH_g and ΔG_g for each TS conformation correspond to the activation enthalpy ($\Delta H^{\ddagger}_g$) and activation free energy ($\Delta G^{\ddagger}_g$ or $\Delta G^{\ddagger}_{tc}$) for the trans-to-cis rotation of the X-Pro (or X-ΨPro) bond in the gas phase, respectively. [d] Taken from ref 22.

contribution of the thermal correction appears to be negligible (∼−0.3 kcal/mol).

Relative free energies (ΔG_g) in the gas phase are computed to be −0.29 and 0.01 kcal/mol at the HF level, 0.07 and 0.29 kcal/mol at the MP2 level and 1.14 and 1.58 kcal/mol at the B3LYP level for Oxa and Thz amides, respectively. This indicates the order of stability of cis conformers to be Pro < Thz < Oxa, which is the same as in ΔE_e and ΔH_g, as seen above. Because the entropic contributions ($-T\Delta S_g$) to ΔG_g are calculated to be −0.4 and −0.3 kcal/mol at the HF level for Oxa and Thz amides, respectively, they seem to be negligible. Therefore, it can be said that the stability of the cis conformer for pseudoproline amides in the gas phase predominantly depends on the relative electronic energy, as found for the proline amide (Table 3).

Table 4 summarizes total free energies (ΔG) of cis conformers relative to trans conformers in the gas phase, chloroform, and water. In chloroform, there are gains in free energy (ΔG_s) by solvation for Oxa and Thz amides by −0.60 and −0.53 kcal/mol at the MP2 level and by −1.07 and −0.95 kcal/mol at the B3LYP level, respectively. The different values of ΔG_s for the same amide are ascribed to the different scale factors used for

TABLE 4: Total Free Energies (ΔG) and Rotational Barriers ($\Delta G^{\ddagger}_{tc}$) of Cis and TS Conformers Relative to Trans Conformers at the 6-31+G(d) Basis Set in the Gas Phase, Chloroform, and Water[a,b]

		gas phase		chloroform		water	
dipeptide	method	ΔG	$\Delta G^{\ddagger}_{tc}$	ΔG	$\Delta G^{\ddagger}_{tc}$	ΔG	$\Delta G^{\ddagger}_{tc}$
Pro[c]	MP2	1.71	17.88	0.95	19.09	0.59	19.57
	B3LYP	2.61	19.39	1.23	20.60	0.59	21.08
Oxa	MP2	0.07	15.53	−0.53	15.94	−0.83	15.97
	B3LYP	1.14	17.19	0.07	17.60	−0.48	17.62
Thz	MP2	0.29	15.74	−0.24	14.59	−0.53	13.96
	B3LYP	1.58	17.91	0.63	16.76	0.12	16.12

[a] $\Delta G = \Delta G_g + f\Delta G_s$(SCI-PCM); scale factors f of 0.334 and 0.599 were derived using the values of ΔG_g for Ac-Pro-NHMe at MP2 and B3LYP levels, respectively; see ref 22. [b] $\Delta G^{\ddagger}_{tc} = \Delta G^{\ddagger}_{g} + \Delta G^{\ddagger}_{s}$ (IPCM). [c] Taken from ref 22.

ΔG_g at the MP2 and B3LYP levels, as described under Computational Methods. However, heteroatomic substitution has induced the unfavored solvation of cis conformers for Oxa and Thz amides by 0.2 and 0.2 kcal/mol at the MP2 level and by 0.3 and 0.4 kcal/mol at the B3LYP level, respectively. This indicates that the preference of solvation for cis conformers in chloroform is found to be in the order of Pro > Oxa > Thz, although the differences in ΔG_s are not profound.

In water, the changes in relative free energy (ΔG_s) for Oxa and Thz amides by solvation are computed to be −0.90 and −0.82 kcal/mol at the MP2 level and −1.62 and −1.46 kcal/mol at the B3LYP level, respectively. These values indicate that the cis conformers are a little more stabilized in water than in chloroform. The increase of cis population as the augmentation of solvent polarity is in accord with CD and NMR measurements on Ac-Pro-NHMe[16] as well as with NMR observations on 5-alkylated Pro amides.[17,24] Heteroatomic substitution results in unfavored solvation free energies of 0.2 and 0.3 kcal/mol at the MP2 level and 0.4 and 0.6 kcal/mol at the B3LYP level for Oxa and Thz amides, respectively. This implies that the unfavored solvation for the cis conformers of Oxa and Thz amides in water is somewhat induced by heteroatomic substitution rather than in chloroform, although the differences in ΔG_s are not significant.

Rotational Barriers. Relative electronic energies, enthalpies, and free energies for the trans-to-cis rotation in the gas phase computed at HF, MP2, and B3LYP levels with the 6-31+G(d) basis set are listed in Table 3. The electronic trans-to-cis barriers to rotation ($\Delta E^{\ddagger}_{e}$) of the C−N peptide bond in the gas phase are calculated to be in the order of Oxa < Thz < Pro at all HF, MP2, and B3LYP levels, and they increase in the order of HF < MP2 < B3LYP levels. Those values of $\Delta E^{\ddagger}_{e}$ for Oxa and Thz amides are 15.22 and 15.54 kcal/mol at the HF level, 15.99 and 16.41 kcal/mol at the MP2 level, and 17.64 and 18.58 kcal/mol at the B3LYP level, respectively. Heteroatomic substitution results in the change of $\Delta E^{\ddagger}_{e}$ by −2.4 and −2.1 kcal/mol at the HF level for Oxa and Thz amides, respectively. This implies that the TS structures of pseudoproline amides are stabilized by heteroatomic substitution. The electron correlations are computed to be ∼0.1 kcal/mol for Oxa and Thz amides at both MP2 and B3LYP levels, except for the Thz amide at the B3LYP level.

The enthalpy changes for the rotation ($\Delta H^{\ddagger}_{g}$) in the gas phase are computed to be 14.10 and 14.28 kcal/mol at the HF level for Oxa and Thz amides, respectively. The thermal contributions are found to be −1.1 and −1.3 kcal/mol for Oxa and Thz amides, respectively. Because of less contribution by thermal corrections, the order of magnitudes in $\Delta H^{\ddagger}_{g}$ is the same as in $\Delta E^{\ddagger}_{e}$.

The calculated trans-to-cis rotational barriers ($\Delta G^{\ddagger}_{g}$) in the gas phase are 14.77 and 14.86 kcal/mol at the HF level for Oxa and Thz amides, respectively. The contributions of entropic terms ($-T\Delta S^{\ddagger}_{g}$) to $\Delta G^{\ddagger}_{g}$ are 0.7 and 0.6 kcal/mol for Oxa and Thz amides, respectively. This indicates that the electronic term is dominant for the barrier to rotation of pseudoproline amides in the gas phase, as found for the proline amide (Table 3).

The rotational barriers ($\Delta G^{\ddagger}_{tc}$) of TS conformers relative to trans conformers in the gas phase, chloroform, and water are summarized in Table 4. In chloroform, the rotational barrier is increased by 0.41 kcal/mol for the Oxa amide and lowered by 1.15 kcal/mol for the Thz amide, whereas the rotational barrier of the Pro amide is increased by 1.21 kcal/mol. In water, the computed changes in relative free energies by the solvation are 0.43 and −1.78 kcal/mol for Oxa and Thz amides, respectively, whereas the rotational barrier of the Pro amide is increased by 1.69 kcal/mol. As a result, the calculated rotational barriers are decreased in the order of Pro > Oxa > Thz amides in chloroform and water at both MP2 and B3LPY levels.

Discussion

Structural Changes. As described under Results, the displacement of the γ-CH_2 group in proline ring by oxygen and sulfur atoms results in large changes in structures of the backbone and proline ring. Heteroatomic substitution has significantly affected the bond lengths and bond angles of proline ring, especially for the bond lengths of C^{β}−X and X−C^{δ} and bond angles of C^{α}−N−C^{δ}, N−C^{α}−C^{β}, and C^{β}−X−C^{δ}.

In particular, the up-puckered conformations of Ac-Oxa-NHMe and Ac-Thz-NHMe with the trans X-ΨPro bond are found to have lower electronic energies than the corresponding down-puckered conformations, although the down-puckering is still dominated for pseudoprolineamides with the cis X-ΨPro bond. The preference of the up-puckered conformation for pseudoproline amides with the trans peptide bond is similar to that of proline in proteins,[50] but is quite different from that of Ac-Pro-NHMe and its 5-methylated derivatives.[8,22] Our calculated results indicate that the degree of puckering for Ac-Oxa-NHMe is quite similar to that of Ac-Pro-NHMe, whereas Ac-Thz-NHMe has the more puckered structure than Ac-Pro-NHMe.

Despite the different puckering, the distances for the hydrogen bond between the C═O of acetyl group and the N−H of NHMe group for pseudoproline amides with the trans peptide bond are computed to be quite similar to that of the Pro amide; the values are 2.11, 2.10, and 2.16 Å for Pro, Oxa, and Thz amides, respectively. However, there are shifts of +4° for backbone torsion angle ω' of the Oxa amide and of +10° for backbone torsion angles ψ and ω of the Oxa amide, compared to those of the Pro amide.

To figure out the steric effects imposed by heteroatomic substitution, the distance $d(C_{Ac}\cdots C_{NHMe})$ between the methyl carbon of acetyl group and the methyl carbon of NHMe group is monitored. The optimized distances are 5.89, 4.94, and 5.11

TABLE 5: Atomic Electronic Charges of Pro, Oxa, and Thz Amides[a]

	Pro[c]			Oxa			Thz		
atoms[b]	trans	cis	TS	trans	cis	TS	trans	cis	TS
C	0.798	0.713	0.498	0.772	0.675	0.422	0.754	0.713	0.452
O	−0.647	−0.556	−0.442	−0.634	−0.552	−0.443	−0.627	−0.542	−0.438
N	−0.343	−0.482	−0.312	−0.429	−0.586	−0.405	−0.266	−0.449	−0.264
C^{α}	0.102	−0.004	0.033	0.087	0.152	0.191	0.138	0.024	0.167
C^{β}	−0.551	−0.441	−0.399	−0.390	−0.237	−0.143	−0.835	−0.612	−0.590
X	−0.115[d]	−0.109[d]	−0.065[d]	−0.437	−0.474	−0.465	0.147	0.069	0.093
C^{δ}	−0.178	−0.059	−0.177	0.025	0.153	0.041	−0.501	−0.301	−0.435

[a] Units are in electronic charge. [b] See Figure 1 for definition of structural parameters. C and O are carbon and oxygen of the C=O group preceding the C−N prolyl peptide bond. [c] Calculated for optimized conformations of ref 22. [d] Calculated for the γ-CH_2 of the Pro amide.

Å for trans, cis, and TS structures of the Oxa amide and 5.83, 4.91, and 5.03 Å for trans, cis, and TS structures of the Thz amide, respectively; they are longer by 0.04−0.21 Å than those of the Pro amide. In particular, the longer distances for TS structures of pseudoproline amides than the that of proline amide appear to contribute in stabilizing the TS structures and in lowering the rotational barriers.

Changes in Electron Populations. As described under Geometrical Parameters, there are increases of 0.001−0.01 Å in the C−N peptide bond lengths for trans, cis, and TS conformers of two pseudoproline amides over those of the proline amide. Despite small increases in length, the electron overlaps of the C−N bond calculated at the HF level are decreased by 0.06 and 0.11 e for cis and TS structures of Oxa and Thz amides compared to that of the Pro amide, respectively, whereas there is an increase of 0.02 e and a decrease of 0.01 e for trans conformations of Oxa and Thz amides, respectively.[55]

To figure out the influence of heteroatomic substitution on electron populations, atomic Mulliken charges of Pro, Oxa, and Thz amides are calculated for the conformations optimized at the HF level. The large changes in atomic charge (i.e., greater than 0.1 e) are held only at the atoms of proline ring compared to those of the Pro amide, which are listed in Table 5. The atomic charge of the prolyl nitrogen becomes more negative for conformers of the Oxa amide and more positive for conformers of the Thz amide. The atomic charge of the X atom at the C^{γ} position becomes more negative and those of the C^{β} and C^{δ} atoms become more positive for the Oxa amide, whereas the opposite results are obtained for the Thz amide. The changes in charge of the prolyl nitrogen may be interpreted as that the C−N bond becomes more ionic for the Oxa amide and more covalent for the Thz amide. Therefore, the changes in charge of the prolyl nitrogen and the decrease in electron overlap of the C−N bond for TS structures seem to play a role in lowering rotational barriers of Oxa and Thz amides compared to that of the Pro amide in the gas phase (Table 3). According to the results on the changes that occur during the rotation of the C−N bond of formamide, the oxygen population was little affected by the rotation and the main charge shift was between carbon and nitrogen of the C−N bond,[56] which is consistent with our results for Pro, Oxa, and Thz amides.

Cis−Trans Isomerization. As seen in Table 3, there are negligible contributions from thermal corrections and entropic terms to relative free energies of cis conformers to trans conformers for Oxa and Thz amides as well as the Pro amide. The increase in relative stabilities of cis conformers by heteroatomic substitution could be interpreted due to the unfavored distances between the methyl carbon of acetyl group and the methylene carbon at position 5 (i.e., C^{δ}) of proline ring for trans conformers. These distances are computed to be 2.90, 2.95, and 2.93 Å for Pro, Oxa, and Thz amides, respectively. The relative instability of trans conformers are predicted in the order of Oxa < Thz < Pro amides at both MP2 and B3LYP levels. Although the relative stabilities of cis conformers for Oxa and Thz amides have increased by solvation in chloroform and water, heteroatomic substitution has induced the unfavored solvation for their cis conformers compared to that of the Pro amide. The calculated preference for cis conformers in the order of Oxa > Thz > Pro amides in water is consistent with experimental results on pseudoproline-containing peptides.[31,34]

By analyzing the contributions to rotational barriers for the trans-to-cis isomerization of the C−N bond for pseudoproline amides, the isomerization has proved to be entirely enthalpy driven in the gas phase, chloroform, and water, to which the electronic energies have considerably contributed. This is consistent with the experimental results on pseudoproline-containing peptides[31,32] and proline-containing peptides,[13] determined kinetically as a function of temperature.

The increase in rotational barrier of the proline amide is attributed to the greater stability of the trans conformation with increasing solvent polarity.[22] For the Oxa amide, the relative stability of the trans conformation is decreased, although the trans conformation is still preferred to the TS structure. However, the TS structure of the Thz amide has a greater preference for solvation than the trans conformation. As a result, the rotational barriers of the Thz amide in chloroform and water are calculated to be lower than those of the Oxa amide, although they show an opposite trend in the gas phase (Table 4). The calculated rotational barriers are decreased in the order of Pro > Oxa > Thz amides in chloroform and water at both MP2 and B3LPY levels, whereas the order is Pro > Thz > Oxa amides in the gas phase. The calculated order of rotational barriers in water is consistent with the experimental result for Ala-Gly-Xaa-Phe-*p*NA in 10 mM sodium phosphate buffer, pH 6.0.[31]

Comparison with X-ray Diffractions. The X-ray diffraction on Ac-Ala-Oxa-NHMe[57] has indicated that the backbone torsion angles ω', ϕ, and ψ of Oxa residue are 171°, −56°, and 148° with the down-puckering, respectively, whereas the corresponding optimized values are −168.9°, −87.3°, and 75.2°, respectively, with the up-puckering. According to an X-ray diffraction study on Ac-Pro-NH_2 and Ac-Thz-NH_2,[58] their geometries and backbone conformations are quite similar, except for different geometries around the C^{γ}-position of the ring and different degrees of puckering. Our backbone conformations and geometries for Ac-Pro-NHMe and Ac-Thz-NHMe optimized at the HF/6-31+G(d) level are consistent with these X-ray results. In particular, the backbone torsion angle ψ is estimated to be −14° and −9° for Ac-Pro-NH_2 and Ac-Thz-NH_2, respectively, from X-ray diffraction;[58] they are close to our calculated values of −9.1° and −2.5° for cis conformers (Table 1), although the trans conformation prevails for X-ray structures. On the other hand, the X-ray structure of *t*-Boc-Thz-OH[59] is known to have the cis *t*-Boc-Thz bond with $\omega' = -6.0°$ and backbone torsion

TABLE 6: Comparison of Cis Populations and Rotational Barriers in Chloroform and Water

		cis population (%)			$\Delta G^{\ddagger}_{tc}$ (kcal/mol)		
dipeptide	solvent	MP2	B3LYP	exptl	MP2	B3LYP	exptl
Pro[a]	chloroform	16.8	11.1	15 ± 4[b]	19.1	20.6	
	water	27.0	27.0	27 ± 3,[c] 23[d]	19.6	21.1	20.4,[c] 20.3,[d,e] 21.3[f]
Oxa	chloroform	70.9	47.0		15.9	17.6	
	water	80.3	69.1	38[d]	16.0	17.6	17.1,[d,e] 18.4[e,f]
Thz	chloroform	60.1	25.8	30[g]	14.6	16.8	
	water	70.8	45.0	28,[d] 54[h]	14.0	16.1	17.3[d,e]

[a] Taken from ref 22. [b] Taken from ref 23. [c] Taken from ref 17. [d] Ala-Gly-Xaa-Phe-*p*NA in 10 mM sodium phosphate buffer, pH 6.0; taken from ref 31. [e] Estimated using the cis content and $\Delta G^{\ddagger}_{ct}$. [f] Suc-Val-Xaa-Phe-*p*NA in DMSO; taken from ref 32. [g] Ac-Thz-OMe; taken from ref 61. [h] Ac-Thz-OH; taken from ref 61.

angle ψ equal to $-74.7°$, which is close to the value of X-ray structure of Ac-Thx-NH_2[58] and our calculated value of Ac-Thz-NHMe, despite different end groups.

The HF optimized geometrical parameters for trans and cis conformers of Ac-Oxa-NHMe and Ac-Thz-NHMe shown in Table 2 are consistent within ± 0.03 Å in bond length and $\pm 4°$ in bond angle with those of X-ray structures for Ac-Ala-Oxa-NHMe,[57] Ac-Thz-NH_2,[58] and *t*-Boc-Thz-OH.[59] Our optimized ring structures of Ac-Oxa-NHMe and Ac-Thz-NHMe are up-puckering, whereas the down-puckering is preferred for X-ray structures. The X-ray structures indicate that the degree of puckering for the Oxa ring is quite similar to that of the Pro ring, whereas the Thz ring has a more puckered structure than the Pro ring.[57−59] This is confirmed by our calculations (Table 1). In particular, the optimized endocyclic torsion angles of Oxa and Thz amides with the cis peptide bond are consistent with those of X-ray structures.[60] The differences in backbone conformations and puckerings between our optimized results and X-ray structures might be ascribed to the different end groups and the crystal packing that is governed by van der Waals contacts and strong intermolecular hydrogen bonds.[58,59]

Comparison with NMR Experiments. The calculated cis populations for Ac-Oxa-NHMe and Ac-Thz-NHMe are listed in Table 6. The cis populations in chloroform and water have been calculated and increase in the order of Pro < Thz < Oxa amides. The calculated order in water is consistent with the observed one for Ala-Gly-Xaa-Phe-*p*NA in 10 mM sodium phosphate buffer, pH 6.0.[31] The cis populations of 69% for the Oxa amide and 45% for the Thz amide in water calculated using the B3LYP electronic energies seem to be overestimated compared to experimental values.[31] The disagreement between calculated and experimental cis populations of Oxa and Thz amides in water might be ascribed to the deficiency in the continuum SCI-PCM method employed for solvation calculations and to different systems of peptides.[22] It has been suggested that the cis population is strongly dependent on the surrounding residues based on the different cis populations for pseudoprolines incorporated into two different sequences of Ala-Xaa-*p*NA and Ala-Gly-Xaa-Phe-*p*NA.[31] Nonetheless, the calculated cis populations of 26% and 45% for Ac-Thz-NHMe in chloroform and water agree with the experimental estimates of 30% for Ac-Thz-OMe in $CDCl_3$ and 54% for Ac-Thz-OH in water.[61]

The trans-to-cis rotational barriers have been calculated to be in the order of Pro > Oxa > Thz amides at both MP2 and B3LYP levels. The results at the B3LYP level seem to be better than those at the MP2 level. The rotational barrier for Oxa and Thz amides are computed to be 17.6 and 16.1 kcal/mol, respectively, in water at the B3LYP level, which is consistent with experimental estimates of 17.1 and 17.3 kcal/mol for Ala-Gly-Xaa-Phe-*p*NA in 10 mM sodium phosphate buffer[31] and of 18.4 kcal/mol for Suc-Val-Oxa-Phe-*p*NA in DMSO.[32] For Oxa and Thz amides, there are decreases in rotational barriers compared to that of the Pro amide. The pseudoprolines themselves seem to play a role in determining this propensity and seem not to be affected by the surrounding residues, because similar values of barriers are obtained for two sequences of pseudoproline-containing peptides[31] as well as for the Pro amide and proline-containing peptides, as shown in Table 6. For comparison of rotational barriers for pseudoproline amides in chloroform, further experimental data are needed.

Intramolecular Catalysis of Prolyl Isomerization. Recently, a comparative study on rotational barriers has been undertaken for two sterically similar prolines in organic and in aqueous/organic solutions; one proline contains the catalytic N−H amide group and the other has the ester end group.[62] The kinetic and spectroscopic results have been interpreted as evidence that indicates the existence of an intramolecular hydrogen bond between the prolyl nitrogen and the following amide N−H group, which is capable of catalyzing the prolyl isomerization by up to 260-fold in model peptides.[62] The existence of this hydrogen bond and its role in prolyl isomerization has been also proposed based on the adiabatic energy map of proline dipeptide at the HF/6-31G(d)//HF/3-21G level.[20] Recently, the role of this hydrogen bond in prolyl isomerization of 5-methylated proline amides has also been suggested by us.[8]

The distance between the prolyl nitrogen and the following hydrogen of NHMe group is calculated here to be 2.27, 2.27, and 2.26 Å for the TS structures of Pro, Oxa, and Thz amides, respectively. They are shorter by 0.08−0.10 Å than those of corresponding cis conformers, but longer by 0.10−0.17 Å than the distance between the carbonyl oxygen of acetyl group and the amide hydrogen of NHMe group of corresponding trans conformers. The sp^3 hybridization of the prolyl nitrogen for the TS structures confirmed by analyzing geometrical parameters (Table 2) and the pertinent distance to form a hydrogen bond might indicate that this intramolecular hydrogen bond could contribute in stabilizing the TS structures of proline amide and pseudoproline amides as well as 5-methylated proline amides[8] and play a role in prolyl isomerization, although it does not seem to be very strong. In addition, the change in the backbone torsion angle ψ from $\psi = 75°-85°$ for trans conformers to $\psi = -9°-+3°$ for cis and TS conformers for each proline amide (Table 1) can be attributed to these favorable interactions between the prolyl nitrogen and the H−N of NHMe group, as pointed out previously for proline dipeptide[20] and 5-methylated proline dipeptides.[8]

Conclusions

The displacement of the γ-CH_2 group in proline ring by oxygen and sulfur atoms has resulted in remarkable changes in structures of backbone and proline ring. The up-puckered conformations of Ac-Oxa-NHMe and Ac-Thz-NHMe with the trans X-ΨPro bond are found to have electronic energies lower

than the corresponding down-puckered conformations, although the down-puckering is still dominated for pseudoprolineamides with the cis X-ΨPro bond. Our calculated results indicate that the degree of puckering for Ac-Oxa-NHMe is quite similar to that of Ac-Pro-NHMe, whereas Ac-Thz-NHMe has more puckered structures than Ac-Pro-NHMe.

The higher cis populations of pseudoproline amides can be interpreted as due to the longer distance between the acetyl methyl group and the 5-methylene group of the ring for the trans conformer of pseudoproline amides than that of the proline amide. By analyzing the contributions to rotational barriers for the trans-to-cis isomerization of the C−N bond for pseudoproline amides, the isomerization has proved to be entirely enthalpy driven in the gas phase, chloroform, and water, to which electronic energies have significantly contributed. The changes in charge of the prolyl nitrogen and the decrease in electron overlap of the C−N bond for TS structures seem to play a role in lowering rotational barriers of Oxa and Thz amides compared to that of the Pro amide.

Although the relative stabilities of cis conformers for Oxa and Thz amides have increased by solvation in chloroform and water, heteroatomic substitution has induced the unfavored solvation for their cis conformers compared to that of the Pro amide. Due to the differing propensity of solvation for pseudoproline amides in chloroform and water, the rotational barriers of the Thz amide are calculated to be lower than those of the Oxa amide, although they show an opposite trend in the gas phase. The calculated preferences for cis conformers in the order of Oxa > Thz > Pro amides and for trans-to-cis rotational barriers in the order of Pro > Oxa > Thz amides in water are consistent with experimental results on pseudoproline-containing peptides. The cis populations and rotational barriers calculated using the B3LYP electronic energies show better agreement with experiments than the MP2 values.

In particular, the sp^3 hybridization of the prolyl nitrogen for the TS structures confirmed by analyzing geometrical parameters as well as the pertinent distance between the prolyl nitrogen and the N−H amide group to form a hydrogen bond might indicate that this intramolecular hydrogen bond could contribute in stabilizing the TS structures of pseudoproline and proline amides and play a role in prolyl isomerization. Related to this work, the study on the influence of the methylation at the 2-C position of the pseudoproline ring on the cis−trans equilibrium and isomerization is now in progress.

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Supporting Information Available: Cartesian coordinates and electronic energies of trans, cis, and TS conformers for Pro, Oxa, and Thz amides optimized at the HF/6-31+G(d) level. This information is available free of charge via the Internet at http://pubs.acs.org.

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