Imide Cis−Trans Isomerization of *N*-Acetyl-*N*′-methylprolineamide and Solvent Effects

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We report here the results on conformational free energies of *N*-acetyl-*N*′-methylprolineamide (Ac-Pro-NHMe) at the HF, MP2, and B3LYP levels of theory with the 6-31+G(d) basis set to see how well the ab initio and density functional computations with the self-consistent reaction field (SCRF) method would reproduce the observed solvent effects on the cis−trans isomerization of imide bond. The augmentation of the prolyl amide cis-isomer population with increased solvent polarity was reproduced by the MP2 or B3LYP free energies using the SCRF solvation free energies. Furthermore, the energy barriers for amide isomerization that were calculated for Ac-Pro-NHMe in different solvents reflected the experimentally observed trend for dimethylacetamide in which the energy for rotation augmented with increased solvent polarity. However, it should be noted that neither the isodensity polarizable continuum model (IPCM) nor the self-consistent isodensity polarizable continuum model (SCI-PCM) predicted properly both solvent effects. Our results indicate the need for improved solvation theory.

Introduction

The cis−trans isomerization of the imide bond preceding proline has been suggested to be often involved in the rate-limiting slow steps for protein folding.[1−3] Many experimental[4] and computational[5] studies have been carried out on the cis−trans isomerization of the imide bond of proline-containing peptides. In particular, considerable effort has been made on *N*-acetyl-*N*′-methylprolineamide (Ac-Pro-NHMe) experimentally[6] and theoretically[7] to model the conformational preferences and transition states of imide isomerization.

Solvent effects for the isomerization of tertiary amides have been examined experimentally[8] and computationally[8c,9] on *N*,*N*′-dimethylacetamide (DMA), which is the simplest model for the imide bond of proline. The experimental results on DMA clearly show that the rotational barrier of amide bond is increased as the polarity or hydrogen-bonding ability of the solvent is increased.[8] This observation has been attributed to stabilization of the larger dipole moment of the ground state relative to the less polar transition state. Recent calculations on DMA at the G2(MP2) level with the reaction field theory support this interpretation.[8c]

CD and NMR measurements on Ac-Pro-NHMe indicated that the intramolecularly hydrogen-bonded trans conformer becomes less populated as solvent polarity increases.[6d] The electrostatic interaction between the carbonyl carbon of proline and the carbonyl oxygen of the preceding residue was shown by molecular mechanics calculations to be the major factor influencing the X-Pro amide cis-trans isomer equilibrium.[10]

We report here the results on Ac-Pro-NHMe at higher levels of theory to see how well the ab initio and density functional computations with the reaction field theory would reproduce the observed solvent effects on the cis−trans isomerization of imide bond.

Computational Methods

All ab initio and density functional calculations were carried out using the Gaussian 94 package[11] run on the Cray-XMP C90 supercomputer and the Silicon Graphics Indigo 2 workstation. Geometry optimizations for the trans and cis conformers and the transition state (TS) of Ac-Pro-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. Lowest-energy conformations with trans and cis imide bonds optimized at the HF/6-31G(d,p) level[7f] were used as starting points for reoptimizations at the HF/6-31+G(d) level. The cis conformation optimized at the HF/6-31+G(d) level with $\omega_0 = 90°$ for the imide torsion was chosen as a starting point for the optimization of the transition state.[12]

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 imide 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.[13] Single-point MP2 and B3LYP energy calculations were also performed for the HF/6-31+G(d) optimized geometries using the same basis set.

Two self-consistent reaction field (SCRF) methods, the isodensity polarizable continuum model (IPCM) and the self-consistent isodensity polarizable continuum model (SCI-PCM),[14,15] were employed at the HF/6-31+G(d) level to determine solvent effects using Gaussian 94. Solvation free energies were calculated for optimized conformations in a series of solvents to represent a wide range of polarity. Solvents considered here are carbon tetrachloride, chloroform, dichloromethane, acetone, methanol, acetonitrile, and water, whose dielectric constants (ϵ) used are 2.2, 4.7, 8.9, 20.7, 32.6, 35.7, and 78.4 at 298.15 K.[16]

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TABLE 1: Torsion Angles, Relative Energies, Enthalpies, and Free Energies of Three Conformers for Ac-Pro-NHMe at HF, MP2, and B3LYP Levels with the 6-31+G(d) Basis Set[a]

		torsion angles[c]				puckering amplitudes[d]			energies		
conf.	level of theory[b]	ω_0	ϕ_1	ψ_1	ω_1	q_α[e]	q_z[f]	χ_m[g]	ΔE_e[h]	ΔH_g[i]	ΔG_g[i]
trans	HF	−172.8	−86.2	74.9	−176.5	10.5	0.366	37.4	0.00	0.00	0.00
	MP2								0.00	0.00	0.00
	B3LYP								0.00	0.00	0.00
cis	HF	9.2	−89.5	−9.1	−179.7	10.5	0.366	37.4	2.37	2.21	1.81
	MP2								2.28	2.11	1.71
	B3LYP								3.17	3.01	2.61
TS	HF	116.4	−106.2	−9.9	−178.5	11.5	0.396	43.0	17.62	16.52[j]	16.97[j]
	MP2								18.54	17.44[j]	17.88[j]
	B3LYP								20.04	18.94[j]	19.39[j]

[a] Energies in kcal/mol; angles in degrees. [b] HF = HF/6-31+G(d)//HF/6-31+G(d); MP2 = MP2/6-31+G(d)//HF/6-31+G(d); B3LYP = B3LYP/6-31+G(d)//HF/6-31+G(d). [c] Optimized at the HF/6-31+G(d) level. The torsion angle ω_0 is defined for the Ac−Pro bond and the torsion angles ϕ_1, ψ_1, and ω_1 for the Pro residue. [d] See ref 7f. [e] Units in degrees; ref 17a. [f] Units in Å; ref 17b. [g] Units in degrees; ref 17c. [h] Relative electronic energy at $T = 0$ K. HF zero of energy = −569.775 251 2 hartrees; MP2 zero of energy = −571.502 322 2 hartrees; B3LYP zero of energy = −573.289 714 3 hartrees, calculated with the option Int = FineGrid. [i] Calculated with vibrational frequencies scaled by 0.890 at the HF/6-31+G(d) level at $T = 298.15$ K. [j] Activation enthalpy ($\Delta H_g^{\ddagger}$) and free energy ($\Delta G_g^{\ddagger}$) for the trans to cis isomerization of the Ac−Pro peptide bond in the gas phase, respectively.

Results and Discussion

Table 1 lists structural parameters and thermodynamic quantities of Ac-Pro-NHMe obtained at the HF, MP2, and B3LYP levels with the 6-31+G(d) basis set. Calculated backbone torsion angles of trans and cis conformations are quite similar to those obtained at the HF/6-31+G(d) level by McDonald and Still[7e] and the HF/6-31G(d,p) level by Kang.[7f] To investigate the degree of puckering of pyrrolidine ring,[7f] three kinds of puckering amplitudes (i.e., q_α,[17a] q_z,[17b] and χ_m[17c]) were calculated. It was found that trans and cis conformers have a similar degree of puckering, whereas the transition state is more puckered. The higher puckering of the transition state can be attributed to the $sp^2 \rightarrow sp^3$ hybridization of the nitrogen atom of pyrrolidine ring.

The electronic energy difference (ΔE_e) between cis and trans conformers was calculated to be 2.37 kcal/mol at the HF/6-31+G(d) level in favor of the trans conformation, which is lower by 0.65 kcal/mol than the values obtained at the HF/6-31G(d)[7d] and HF/6-31G(d,p)[7f] levels. This energy becomes 2.28 kcal/mol by introducing the electron correlation (MP2) at the same basis set. The earlier computed value of ∼2 kcal/mol for the cis conformer relative to the trans conformer from the ECEPP force field[7a,7b] accords with these ab initio values. However, at the B3LYP level with the same basis set the calculated ΔE_e is 3.17 kcal/mol, which is higher by 0.80 and 0.89 kcal/mol than the values at HF and MP2 levels, respectively.

The electronic activation energy ($\Delta E_e^{\ddagger}$) for the imide rotation is calculated to be 17.62, 18.54, and 20.04 kcal/mol at the HF, MP2, and B3LYP levels with the 6-31+G(d) basis set, respectively. The HF/6-31+G(d) value is lower by 0.3 kcal/mol than the value obtained at the HF/6-31G(d) level.[7d] Our calculated results indicate that the electron correlation (MP2) stabilizes slightly the cis conformation and destabilizes the transition state by 0.92 kcal/mol. In particular, the $\Delta E_e^{\ddagger}$ at the B3LYP/6-31+G(d) level is found be higher by 2.42 and 1.50 kcal/mol than corresponding values at the HF and MP2 levels, respectively. Hence, the B3LYP functionals appear to enhance the strength of an intramolecular hydrogen bond of the trans conformer.

The free energy of the cis conformer (ΔG_g) relative to the trans conformer in the gas phase is calculated to be 1.81, 1.71, and 2.61 kcal/mol at the HF, MP2, and B3LYP levels, respectively, which are lower by 0.56 kcal/mol than the corresponding values of ΔE_e. The calculated activation free energies ($\Delta G_g^{\ddagger}$) are 16.97, 17.88, and 19.39 kcal/mol at the HF, MP2, and B3LYP levels, respectively, which are lower by 0.65 kcal/mol than the corresponding values of $\Delta E_e^{\ddagger}$. By comparing $\Delta G_g^{\ddagger}$ and $\Delta H_g^{\ddagger}$, it is known that the cis−trans isomerization is entirely enthalpy-driven in the gas phase and that the entropic contribution ($-T\Delta S_g^{\ddagger}$) is 0.45 kcal/mol. The values of $\Delta G_g^{\ddagger}$ for Ac-Pro-NHMe at the MP2 and B3LYP levels are higher by about 3−4 kcal/mol than the observed value of 15.3 ± 0.1 kcal/mol for DMA.[8b]

TABLE 2: Relative Solvation Free Energies of Cis and TS Conformers at the HF/6-31+G(d) Level in Different Solvents[a]

	IPCM		SCI-PCM	
solvents	ΔG_s	$\Delta G_s^{\ddagger}$	ΔG_s	$\Delta G_s^{\ddagger}$
carbon tetrachloride	0.34	0.74	−1.38	−0.60
chloroform	0.71	1.21	−2.29	−1.04
dichloromethane	0.94	1.45	−2.78	−1.29
acetone	1.11	1.61	−3.14	−1.49
methanol	1.17	1.65	−3.25	−1.55
acetonitrile	1.18	1.67	−3.26	−1.56
water	1.22	1.69	−3.37	−1.61

[a] Units are in kcal/mol. ΔG_s and $\Delta G_s^{\ddagger}$ are solvation free energies of cis and TS conformers relative to the trans conformer, respectively.

In Table 2, computed solvation free energies (ΔG_s and $\Delta G_s^{\ddagger}$) of cis and TS conformers relative to the trans conformer with IPCM and SCI-PCM methods at the HF/6-31+G(d) level in different solvents are shown. The IPCM method produces uniformly the increase in the values of ΔG_s and $\Delta G_s^{\ddagger}$ as the solvent polarity is increased, which indicates the trans conformer being more stabilized than cis and TS conformers in polar solvents. The results on $\Delta G_s^{\ddagger}$ from the IPCM method are consistent with experimental results on DMA.[8] On the other hand, the SCI-PCM calculations predict the trans conformer to be destabilized as the solvent becomes more polar. Therefore, the SCI-PCM results on ΔG_s are in accord with those of CD and NMR measurements on Ac-Pro-NHMe which show the population of the trans conformer to decrease as the solvent polarity is increased.[6d]

Although the SCI-PCM method can satisfactorily predict the favored solvation of the cis conformer, relative solvation free energies of cis conformers appear to be overestimated, e.g., −3.37 kcal/mol in water. To reproduce the observed cis population (27%) of Ac-Pro-NHMe in water,[6f] the value of ΔG_s

TABLE 3: Total Free Energies (ΔG) and Activation Free Energies ($\Delta G^{\ddagger}$) of Cis and TS Conformers Relative to the Trans Conformer at the 6-31+G(d) Basis Set[a]

	ΔG^{b}		$\Delta G^{\ddagger c}$	
solvents	MP2	B3LYP	MP2	B3LYP
gas phase	1.71 (5.3)	2.61 (1.2)	17.88	19.39
carbon tetrachloride	1.25 (10.8)	1.78 (4.7)	18.62	20.13
chloroform	0.95 (16.8)	1.23 (11.1)	19.09	20.60
dichloromethane	0.79 (21.0)	0.94 (16.9)	19.34	20.84
acetone	0.66 (24.6)	0.73 (22.7)	19.49	21.00
methanol	0.63 (25.7)	0.66 (24.7)	19.54	21.04
acetonitrile	0.62 (25.9)	0.65 (25.0)	19.55	21.06
water	0.59 (27.0)	0.59 (27.0)	19.57	21.08

[a] Free energies are in kcal/mol. [b] $\Delta G = \Delta G_g + f\Delta G_s$(SCI-PCM); scale factors f of 0.334 and 0.599 were derived using the values of ΔG_g at MP2 and B3LYP levels, respectively, in Table 1. See ref 18 for details. Each value in parentheses is the cis population (%) computed from the corresponding ΔG at 298.15 K. [c] $\Delta G^{\ddagger} = \Delta G_g^{\ddagger} + \Delta G_s^{\ddagger}$(IPCM).

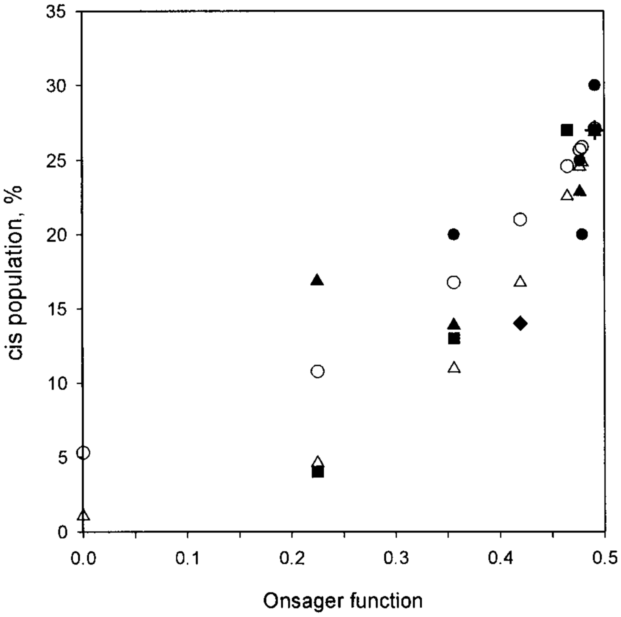

Figure 1. Comparison of calculated and observed solvent effects on cis populations for Ac-Pro-NHMe against the Onsager function ($(\epsilon - 1)/(2\epsilon + 1)$). The calculated values with MP2 and B3LYP free energies are shown as open circles and open triangles, respectively. The observed values are represented as ▲ (ref 6a), ■ (ref 6b), ● (ref 6d), ◆ (ref 6e), and + (ref 6f). The experimental ones from left to right correspond to the gas phase, carbon tetrachloride, chloroform, dichloromethane, acetone, methanol, acetonitrile, and water.

by the SCI-PCM method was scaled using factors of 0.334 and 0.599 derived from the values of ΔG_g at MP2 and B3LYP levels, respectively.[18] The total free energy (ΔG) of the cis conformer in each solvent was computed by a sum of the ΔG_g at the MP2 or B3LYP levels and the scaled ΔG_s with the SCI-PCM method (Table 3).

Figure 1 shows a comparison of the calculated and observed solvent effects on cis populations for Ac-Pro-NHMe against the Onsager dielectric constant function,[8c] $(\epsilon - 1)/(2\epsilon + 1)$. The calculated trend in cis populations is consistent with the observed one. The calculated cis population varies from 5.3% with the MP2 ΔG_g or 1.2% with the B3LYP ΔG_g in the gas phase to 27.0% in water as the increase in the solvent polarity (Table 3). It should be noted that cis populations calculated using the ΔG_g at the MP2 level (open circles in Figure 1) are higher by 1−5% than those at the B3LYP level (open triangles in Figure 1), to which the higher value of ΔG_g at the B3LYP level appears to contribute dominantly (see Table 1).

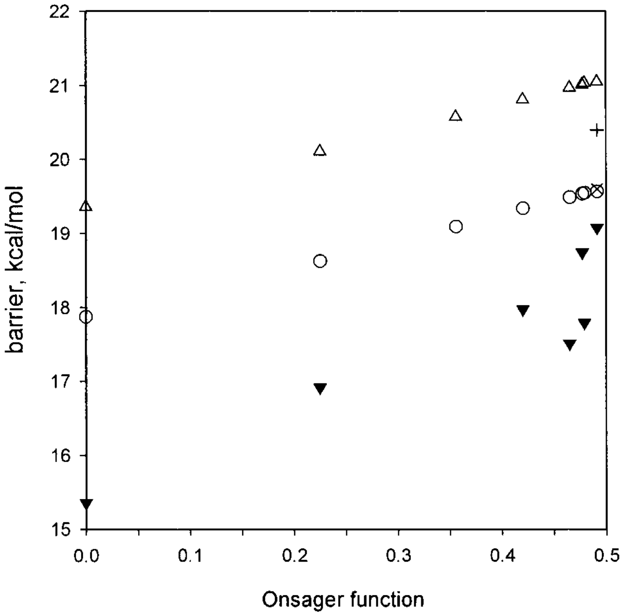

Figure 2. Comparison of calculated solvent effects on barriers ($\Delta G^{\ddagger}$) for imide bond rotation of Ac-Pro-NHMe with relevant experimental data against the Onsager function. The calculated values with MP2 and B3LYP free energies for Ac-Pro-NHMe are shown as open circles and open triangles, respectively. The observed values for DMA are represented as ▼ (ref 8c). The experimental ones for Ac-Pro and Ac-Pro-NHMe in water are shown as × (ref 6c) and + (ref 6f), respectively. See the caption of Figure 1.

The calculated activation free energies ($\Delta G^{\ddagger}$) for the isomerization of the imide bond of Ac-Pro-NHMe, each of which is given by a sum of $\Delta G_g^{\ddagger}$ and $\Delta G_s^{\ddagger}$(IPCM), are listed in Table 3. They vary from 17.9 or 19.4 kcal/mol in the gas phase to 19.6 or 21.1 kcal/mol in water for the values of $\Delta E_g^{\ddagger}$ obtained at MP2 or B3LYP levels, respectively. The computed values of $\Delta G^{\ddagger}$ in water are consistent with the observed one of 20.4 kcal/mol from NMR experiments on Ac-Pro-NHMe in D_2O at 25 °C[6f] as well as the experimental value of 19.6 kcal/mol for Ac-Pro in water.[6c]

Because there are only experimental data available for the barrier of isomerization of Ac-Pro-NHMe in water,[6f] we compared the computed trend with experimental values obtained for DMA in different solvents. A comparison of the calculated solvent effects on rotational barriers for Ac-Pro-NHMe and the observed ones for DMA is plotted against the Onsager function in Figure 2. In general, the calculated trend is reasonably consistent with the observed one. Our calculations suggest that the trans conformation is more stabilized by polar solvents than the transition state, leading to an increase in the barrier of imide rotation.[8a,c] Experimental data for Ac-Pro-NHMe in various solvents is now required to check the validity of our calculations. As seen in the gas phase, the imide cis−trans isomerization is calculated to be enthalpy-driven in solution, which is also consistent with observations for proline-containing peptides.[4]

In summary, ab initio HF and MP2, and density functional B3LYP calculations at higher levels of theory with SCRF methods can reproduce solvent effects on the equilibrium of prolyl amides and the barrier of tertiary amide bonds. The present versions of the SCRF methods were only partially successful in reproducing the observed solvent effects, and the theory behind these calculations requires further improvement.

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(18) The ΔG_s for the cis conformer was given by the scaled ΔG_s(SCI-PCM). For example, the ΔG for the cis conformer of Ac-Pro-NHMe in water is given by ΔG_g(MP2 or B3LYP) + 0.334 (or 0.599) × ΔG_s(SCI-PCM) = 1.713 (or 2.607) kcal/mol + 0.334 (or 0.599) × (−3.370 kcal/mol) = 0.588 kcal/mol, which estimates the cis population to be 27% at 298.15 K.