Theoretical Study of the Hydration Effects on the Conformation of N-pivaloyl-L-prolyl-N-methyl-N'-isopropyl-L-alaninamide

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To investigate the hydration effects on the conformational changes of N-pivalolyl-L-prolyl-N-methyl-N'-isopropyl-L-alaninamide (PPMIA), the conformational free energy changes have been calculated by using an empirical potential function varying all the independent degrees of freedom of PPMIA backbones. It is found that *cis* conformers are folded by a strong intramolecular hydrogen bond involving both terminal CO and NH groups whereas *trans* conformers accommodate the open conformation. Conformers in the free state are proved to be less stable than in the hydrated state. The free energy changes of *cis* and *trans* PPMIA due to the hydration are −50.5 and −39.8 kcal/mole, their conformational energy changes are −52.3 and −41.0 kcal/mole, and their conformational entropy changes are −5.9 and −4.0 e.u., respectively. The free energy changes of *cis* PPMIA to *trans* PPMIA in the free and hydrated states are 5.3 and 16.0 kcal/mole, their conformational energy changes are 7.6 and 18.8 kcal/mole, and the entropy changes due to the conformational transitions correspond to 7.5 and 9.4 e.u., respectively. From these results, it is found that the bound water molecules play an important role in stabilizing the conformation of PPMIA.

Introduction

N–methylated peptides deserve to be considered because many peptides of well known biological importance contain N–methylated amino acid residues.[1,2] Moreover the introduction of tertiary amide function in a peptide chain results in the two following point:[2] (1) suppression of a proton donating NH group able to participate in hydrogen bonding systems; (2) occurrence of nearly equally probable *cis* and *trans* conformations of the amide link. For a peptide N–pivaloyl-L-prolyl-N-Methyl-N'-Isopropyl-L-Alaninamide (PPMIA) in the free and hydrated states, the conformational free energy changes are calculated by using an empirical potential function.[3-8] To recognize the factor involved in the stability of a given conformation and in the change of one conformation to another, the configurational entropies of *cis* and *trans* conformers in the both states, and the water molecules bound to them are evaluated. In addition, the conformational changes of *cis* and *trans* conformers due to the hydrogen bonding effects and a ring opening of hydrogen bond are investigated.

A. Potential Fucntions

Interaction energies have been calculated using the empirical potential function by Kang and Jhon.[3-8] These are composed of the electrostatic energy E_{el}, polarization energy E_{pol}, nonbonded energy E_{nb}, torsional energy E_{tor}, and hydrogen bond energy E_{hb}; The total energy is given by the following expression:

$$E_{tot} = \sum_{nb\ pair} (E_{el} + E_{pol} + E_{nb}) + \sum_{torsion} E_{tor} + \sum_{hb\ pair} E_{hb} \qquad (1)$$

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Atomic partial charges have been used for the calculation of the electrostatic and polarization energies using the Del-Re method[9~11] for σ charges and the Hückel method[12] for π charges. In the case of the free (vacuum) state, a dielectric constant of 1.0 was used. For the hydrated state, a distance–dependent dielectric constant, $\varepsilon(r_{ij})$, was used as follows;[6-8]

$$\varepsilon(r_{ij}) = (\varepsilon_0 - 1)[(r_{ij} - r_{con})/(r_{eff} - r_{con})]^n + 1 \qquad (2)$$

Where at the contact distance r_{con}, is $\varepsilon(r_{ij}) = 1.0$ and at some particular interaction distance r_{eff}, the dielectric constant $\varepsilon(r_{ij}) = \varepsilon_0$, the bulk dielectric constant of the system. Only in the region $r_{con} < r_{ij} < r_{eff}$, $\varepsilon(r_{ij})$ is considered to be a function of r_{ij}.

Boundary conditions such as $\varepsilon(3\text{Å}) = 1.0$ and $\varepsilon(7\text{Å}) = 4.0$ were taken from the work of Hopfinger.[12] The nonbonded energy was approximated using the Lennard-Jones type, in which the dispersion coefficient was calculated by the London formula[13] and the equilibrium distance was assigned to be a distance approximately 0.2 Å greater than the sum of the van der waals radii of the interacting atom.[14]

The atomic static polarizabilities used in the polarization and nonbonded terms were taken from the work of Kang and Jhon.[15] The torsional potential was composed of usual periodic function.[16] Potential parameters of hydrogen bond were obtained, which was fitted to the results of ab initio computation. Details of the nature of potential energy functions and various parameters used are in ref. 6.

B. Conformational Entropy

(1) *Conformational Energy of Biomolecule.* If the joint probability distribution function for each set of torsion angles of biomolecules satisfies a normalized multivariate Gaussian distribution function,[17] the conformational entropy at the i th minimum is given by

$$S_q^{(i)} = \frac{1}{2}nR + \frac{1}{2}R \ln[(2\pi)^n \sigma^{(i)}] \qquad (3)$$

where n is the number of degree of freedom in a set of torsion angles and $\sigma^{(i)}$ is the determinant of the covariance matrix at ith minimum.

Hence the entropy difference associated with the conformational change of biomolecules is given by

$$\varDelta S_{q(i \to j)} = \frac{R}{2} \ln (\sigma^{(i)}/\sigma^{(j)}) \qquad (4)$$

where $\sigma^{(i)}$ and $\sigma^{(j)}$ are the determinants of the covariance matrix at the ith and jth minima, respectively. Karplus *et al.*[18,19]. Obtained the elements of the covariance matrix from the harmonic analysis results for each conformation at the local mininum. Here, we used a new method[6-8] for the evaluation of the conformational entropy of biomolecules with the covariance matrix by analyzing the potential energy surface.

The element of the covariance matrix at the ith minimum is

$$\sigma_{kl}^{(i)} = \rho_{kl}^{(i)} \sigma_k^{(i)} \sigma_l^{(i)} \qquad (5)$$

where $\sigma_k^{(i)}$ and $\sigma_l^{(i)}$ are standard deviations of the kth and lth torsion angles, respectively, and ρ_{kl} is the correlation coefficient between them. Each standard deviation for each torsion angle was obtained while the other torsion angles were fixed at the minimum value. The detailed procedure for the evaluation of the covariance is in ref. 6.

(2) *Configurational Entropy of Bound Water Molecules.* The coordinates of water molecule bound to the biomolecule can be expressed with six external variables in Figure 1. If it is assumed that the joint probability distribution function for the external variables of a water molecule follows the multivariated Gaussian distribution function, the configurational entropy of a water molecule is approximately given by

$$S_w = \frac{1}{2} nR + \frac{1}{2} R \ln [(2\pi)^n \sigma_w^*] \qquad (6)$$

where n is the external degree of freedom of a water molecule and σ_w^* is the determinant of the covariance matrix which is a dimensionless quantity; standard deviations of each external variable must be reduced approximately. The potential barriers of the external variables can be approximately classified into the following three cases; the very high, low, and intermediate barrier. For very high barrier, the motion eventually approximates to a harmonic oscillation of small amplitude.

For the very low barrier, the concept of the free rotation is adopted and the torsional motion with the intermediate potential barrier can be treated approximately as the hindered rotation.

To reduce elements of the determinant for various potential barriers of the external variables, harmonic oscillator and hindered rotation approximations were used. Theoretical derivations of the two methods are minutely presented in refs. 6 and 8.

C. Model Compounds

We have considered the PPMIA (*cis* PPMIA and *trans* PPMIA) which consists of four amino acid residues: pivaloyl, prolyl, isopropyl, and L-alaninamide: (the geometrical definition of PPMIA refer to ref. 2). Especialy, the molecules are folded by a short and almost linear intramolecular hy-

drogen bond involving the NH[43] and CO[15] site.

The geometrical definitions are shown in Figures 2 and 3. Comparing experimental values with calculated ones in this work, it appears that the N-Methylation of the middle amide function introduces considerable conformational changes concerning the internal rotational angles, ω_2, ϕ_2, and ψ_2 (see Table 1).

Both the model molecule in this work and experimental molecule are hence folded by an intramolecular N⋯H[44]⋯O[15]= C[14] hydrogen bond; the N⋯O distance 2.90 Å in the theoretical calculation is in good agreement with the experimental value.[2] Since our attention was centered on the conformational changes of PPMIA due to the hydration and a ring opening of hydrogen bond, the computations are carried out on the backbone of PPMIA; the proyl region is frozen and the conformational changes of side chain is only considered.

D. Determination of Conformational Energy and Entropy

The sum of the total conformational energy is

$$E_{tot} = E_{intra} + E_{inter} \qquad (7)$$

where E_{intra} is the internal conformational energy of PPMIA and E_{inter} is the interaction energy between PPMIA and water molecules. For the hydrated system, a hydrogen bond energy was additionally included in E_{inter}.

The torsion angles of PPMIA were allowed to move during minimization by using a Quasi Newton method developed by Fletcher[20] with a convergence criterion of 0.005 kcal/mole and with a step length of 2 degrees for all the torsion angles. The number of iterations was limited to 100 cycles.

In order to generate the coordinates of the *cis* and *trans* PPMIA, seven independent torsion angles are needed for *cis* and *trans* PPMIA, respectively. Crystallographic results of *cis* PPMIA were used as the preliminary geometries of minimization. This starting points for minimization of the total energy of each conformation were then determinated by the refinement of those geometries varying each torsion angle of the backbond one after another.

For the hydrated system, the hydration schemes were obtained from the optimization of water molecules bound to the system, and then the conformations of *cis* and *trans* **PPMIA** were minimized once more.

This procedure was repeated until the difference of each torsion angles obtained from the previous and later iterations remains 1 degree. Each water molecule bound to the system was described by the six external variables, d, θ_1, θ_2, ϕ_1, ϕ_2 and ϕ_3 depicted in Figure 1. This optimization step lengths were all 0.01Å for distances and 2 degrees for all the angles. The total entropy of the compound through the hydration was estimated as

$$\varDelta S_{tot} = \varDelta S_{sub} + \varDelta S_w \qquad (8)$$

where $\varDelta S_{sub}$ is the conformational entropy change of the substrate and $\varDelta S_w$ is the cdnfigurational entropy change of water molecules bound to the *cis* and *trans* PPMIA.

It is assumed that $\varDelta S_w$ is the difference between the entropy of liquid water at room temperature and the conformational entropy of the optimized water molecules. Hence, $\varDelta S_w$ is

represented as

$$\Delta S_w = S_{sw} - nS_w{}^\circ \qquad (9)$$

where S_{sw} is the sum of the configurational entropy S_w given by eq. (6), n is the number of bound water molecules, and $S_w{}^\circ$ is the entropy of liquid water at the room temperature (16.72 e.u at 298.15 °K)[2].

Results and Discussion

A. The Optimized Geometry of PPMIA

The optimized torsion angles for PPMIA are listed in Table 1.

The notation for torsion angles of PPMIA is illustrated in Figures 2 and 3. Three kinds of torsion angles are compared with each other: the first column corresponds to the result of calculation with a dielectric constant $\varepsilon = 1.0$, the second to those of the hydrated compound with a distance-dependent dielectric constant described previously, and the third to X–Ray crystallographic results in ref. 2. For *tans* PPMIA, experimental torsional angles are not available from X–Ray crystallography. One sees that torsion angles of hydrated PPMIA are closer to those of X–Ray crystallographic results than those of nonhydrated PPMIA.

B. Hydration of *cis* and *trans* PPMIA

To investigate the stability of *cis* and trans PPMIA, it is necessary to calculate the internal conformational entropy of *cis* and *trans* PPMIA themselves as well as the conformational entropy of *cis* and *trans* PPMIA, and water molecules bound to them. The conformation of *cis* and *trans* was initially minimized using the data of X–Ray crystallographic study in Table 1 without considering the hydration. However, the hydrated *cis* and *trans* PPMIA are optimized using the same initial geometry with the distance dependent dielectric constant. Since the water molecules in the first hydration shell are mainly responsible for the hydration structure of solute,[22,23] the directly bound water molecules to *cis* and *trans* PPMIA are considered in this calculation. Whereas
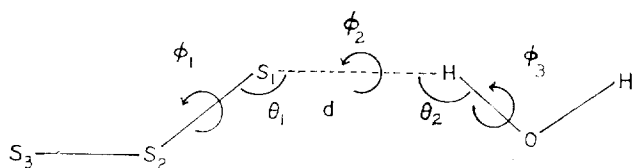
Figure 1. Geometrical parameters for water molecule.

TABLE 1: Backbone Torsion Angles of PPMIA

Torsion Angles	cis			trans	
	Free	Hydrated	Exp	Free	Hydrated
ω_1	−193	−189	−178	−200	−199
ϕ_1	−63	−65	−62	−66	−67
ψ_1	141	142	135	148	147
ω_2	0	−2	−13	−161	−166
ϕ_2	−113	−112	−119	145	145
ψ_2	52	51	60	148	141
ω_3	−177	−176	−176	180	180

four water molecules were optimized around *cis* and *trans* PPMIA, respectively. In *cis* PPMIA, two water molecules are bound to oxygen of L–alanyl and proyl, respectively. In *trans* PPMIA, one water molecule is bound to oxygen of pivaloyl, proyl, L–alanyl and nitrogen of N–isopropyl, respectively.

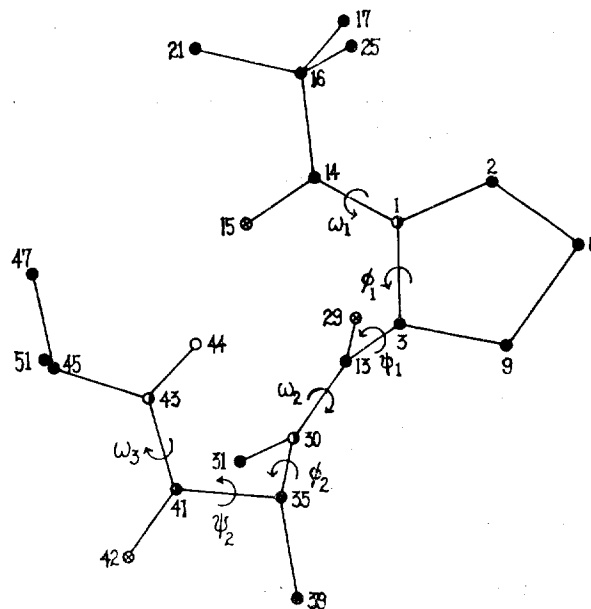The optimum and binding energies of water molecules are

Figure 2. Model compound of *cis*–PPMIA and definition of torsion angles. For clarity, hydrogen atoms are not shown. Each atom is designated as follows: carbon ●, oxygen ⊗, nitrogen ○.

Figure 3. Model compound of *trans*–PPMIA and definition of torsion angles. For clarity, hydrogen atoms are not shown. Each atom is designated as follows: carbon ●, oxygen ⊗, nitrogen ○.
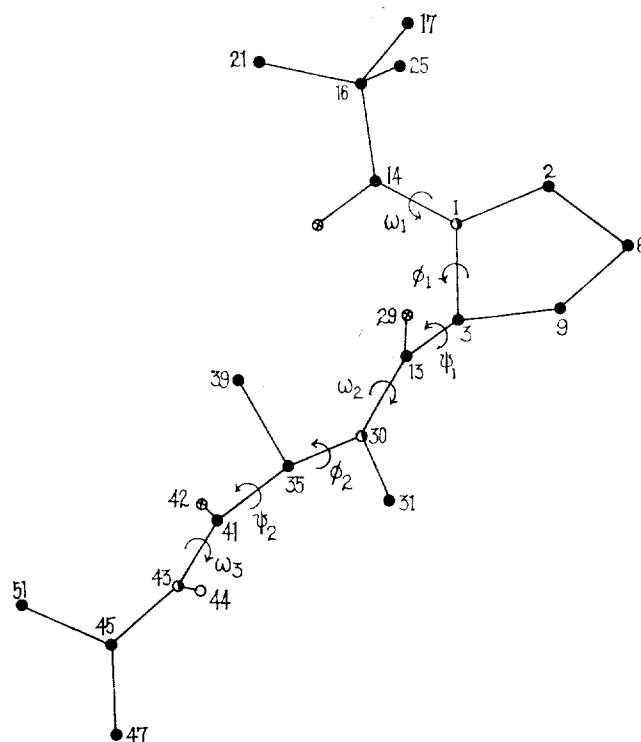
summarized in Table 2 and the hydration scheme is shown in Figures 4 and 5. The interaction energies of water molecules bound to oxygen are on the average -14.8 kcal/mole, and the interaction to nitrogen is -7.97 kcal/mole.

Configurjational entropies of water molecules bound to PPMIA are on the average 16.0 e.u, which is shown in Table 2.

C. The Stability of *cis* and *trans* PPMIA due to the Hydration

The interaction energies for the minimized PPMIA in the free and hydrated states are summarized in Table 3.

The internal conformational energies of *cis* and *trans* PPMIA in the free state are -9.65 kcal/mole, and -2.09 kcal/mole compared to -7.29 kcal and -0.96 kcal/mole in the hydrated state respectively. This difference between those energies in the free and hydrate state, is due to the loss of internal conformational energies through the hydration. And the difference between configuration energies of *cis* and *trans* PPMIA is caused by the molecular geometric structures due to a ring opening of hydrogen bond.

TABLE 2: Optimum Geometry, Interaction Energy and Configurational Entropy of Water Molecules Bound to N-Pivaloyl-prolyl-N-methyl-N'-isopropy-L-alaninamide [a]

Water molecule		Reference Atoms			Optimum geometry								
		S_1	S_2	S_3	d	θ_1	θ_2	ϕ_1	ϕ_2	ϕ_3	ΔE	S_W	ΔF
cis	W_1	29	13	30	1.74	184	171	235	4	174	-12.75	14.46	17.06
	W_2	42	41	43	1.74	147	180	13	6	330	-13.34	18.77	-18.94
	W_3	29	13	30	1.78	94	172	284	262	206	-16.97	12.25	-19.90
	W_4	42	41	43	1.95	94	185	111	214	335	-9.82	16.64	-14.78
trans	W_1	15	14	1	1.71	127	170	125	38	158	-18.41	18.91	-24.05
	W_2	29	13	30	1.68	242	171	299	279	177	-14.72	16.63	-19.68
	W_3	42	41	43	1.77	231	177	331	148	230	-17.61	15.99	-22.38
	W_4	44	43	41	2.43	170	27	228	187	148	-7.97	14.31	-12.24

[a] Each number of reference atoms is shown in Figures 2 and 3.

TABLE 3: Intramolecular Interaction Energy of N-pivaloyl-L-prolyl-N-methyl-N'-isopropyl-L-alaninamide

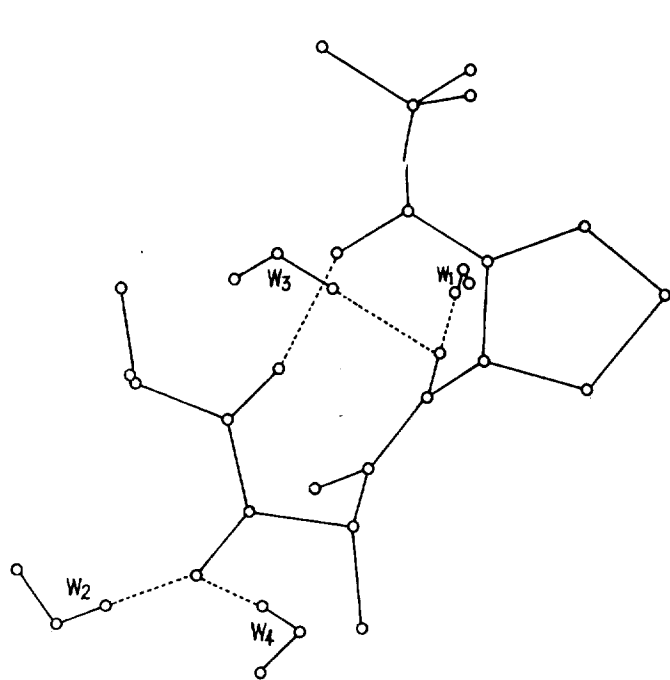|	*cis*			*trans*		
	Free	Hydrated	ΔE_{intra}	Free	Hydrated	ΔE_{intra}
E_{el}	-4.97	-2.83	2.14	-16.83	-15.57	1.26
E_{pol}	-5.86	-5.88	-0.02	-6.16	-6.37	-0.21
E_{nb}	15.18	15.50	0.32	14.50	15.65	1.15
E_{tor}	5.56	4.48	-0.68	6.40	5.33	-1.07
E_{hb}	-19.56	-19.59	-0.03	—	—	—
E_{tot}	-9.65	-7.92	1.73	-2.09	-0.96	1.13

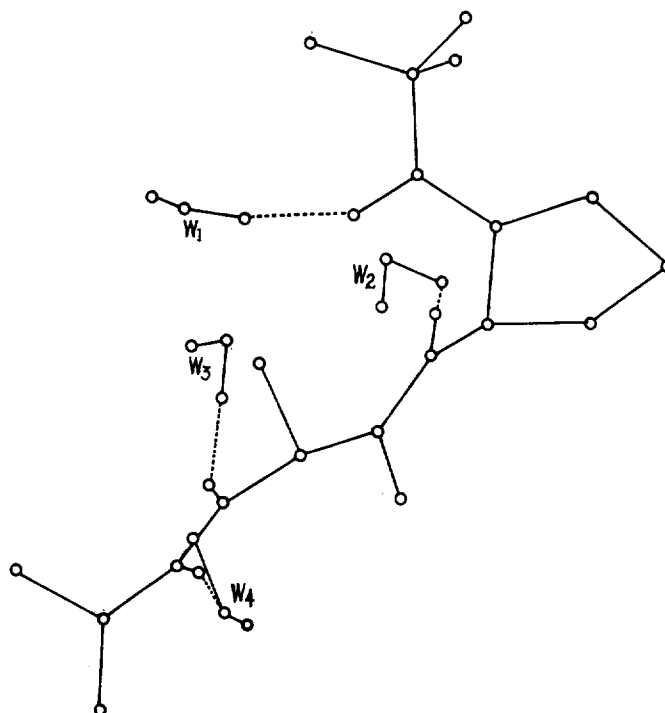**Figure 4.** Structure of hydrated *cis*-PPMIA.

Figure 5. Structure of hydrated *trans*-PPMIA.

TABLE 4: Intermolecular Interaction Energy of N–pivaloyl–L–prolyl–N′–isopropyl–L–alaninamide [a]

	cis			trans		
	$E_{s,w}$	$E_{w,w}$	ΔE_{inter}	$E_{s,w}$	$E_{w,w}$	ΔE_{inter}
E_{el}	48.53	−1.26	46.97	57.45	3.96	61.41
E_{pol}	−3.50	0.46	−3.04	−2.31	1.76	−0.55
E_{nb}	−2.96	0.43	−2.53	−3.00	−0.14	−3.14
E_{hb}	−95.40	0.01	−95.39	−99.79	−0.05	−99.84
E_{tot}	−53.63	−0.36	−53.99	−47.65	5.54	−42.12

[a] $E_{s,w}$ and $E_{w,w}$ are the interaction energies for the substrate–bound water molecules and the bound water molecules, respectively.

TABLE 5: Interaction Energy Changes due to the Hydration

	cis			trans		
	ΔE_{intra}	ΔE_{inter}	ΔE_{tot}	ΔE_{intra}	ΔE_{inter}	ΔE_{tot}
ΔE_{el}	2.14	46.97	49.11	1.26	61.41	62.47
ΔE_{pol}	−0.02	−3.04	−3.06	−0.21	−0.55	−0.76
ΔE_{nb}	0.32	−2.53	−2.21	1.15	−3.14	−1.99
ΔE_{tor}	−0.68	—	−0.68	−1.07	—	−1.07
ΔE_{hb}	−0.03	−95.39	−95.42	—	−99.84	−99.84
ΔE_{tot}	1.73	−53.99	52.26	1.13	−42.12	−40.99

The intermolecular interaction energies of the hydrated PPMIA are shown in Table 4. The total energies of them are −53.63 kcal/mole and −47.65 kcal/mole, respectively, in which the interaction energies between PPMIA and water molecules bound to them are the major factors. However, PPMIA becomes stable due to the hydrogen bond between PPMIA and water molecules bound to it.

The configurational entropy changes of PPMIA through the hydration is evaluated and cis PPMIA is −1.13 e.u compared to −2.99 e.u in trans PPMIA.

The interaction energies for PPMIA due to the hydration are summarized in Table 5. The total entropy changes and the total free energy changes through the hydration between cis and trans are illustrated in Figure 6.

These values are −5.87 e.u, −50.51 kcal/mole, −40.01 e.u, −39.79 kcal/mole, 7.49 e.u, 5.34 kcal/mole, 9.35 e.u, and 16.04 kcal/mole, respectively. It is clear that since the entropy changes are caused by the conformational entropy changes of PPMIA, we can not neglect the contribution of conformational entropy of conformer to the total free energy changes of PPMIA. The decrease of the conformational entropies of PPMIA is caused by the reduction of flexibility of PPMIA by means of hydration. The conformational changes for the free and hydration state of PPMIA are shown in Figures 4 and 5.

Conclusions

The hydration schemes of cis and trans PPMIA are obtained from the conformational analysis of cis and trans PPMIA by using an empirical potential function and are in good agreement with the results of X–Ray crystallography study. From these results, it is found that the hydration effect is essential to the stability of PPMIA. In cis PPMIA, this bend is stabilized by strong intramolecular interaction between the terminal CO and NH bonds.
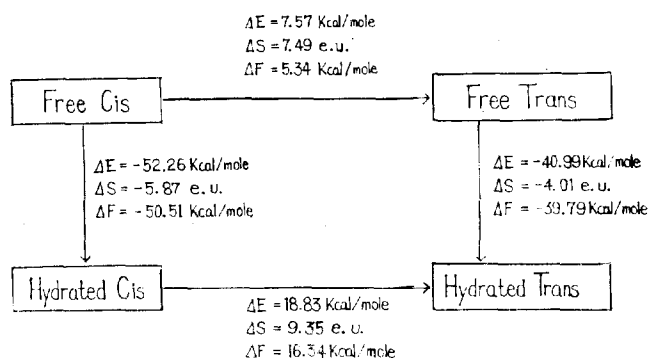

Figure 6. The overall free energy changes among cis–PPMIA and trans–PPMIA in the free and hydrated state.

The minor trans conformer adopts an opening conformation. The change of configurational entropy decreases due to the hydration and a ring opening. The result has shown that the environment effect including the hydration and a ring opening is important in determining the conformation of PPMIA.

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S_H2 Reaction on Silicon-Carbon Bond in the Photoreactions of 2,3-Benzo-1,1-diphenyl(or dimethyl)-1-sila-2-cyclobutene with Carbonyl Compounds

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The photoreaction of 2, 3–benzo–1, 1–diphenyl (or dimethyl)–1–sila–2–cyclobutene (**9** or **10**) with an aldehyde or ketone results in 1:1 cycloadduct of [4+2] type. In the reactions of 2, 3–benzo–1, 1–dimethyl–1–sila–2–cyclobutene (**10**) with acetone and butanone, another 1:1 adducts (**13**) were also formed, respectively. The following facts indicate that the formation of adduct involves an attack of a triplet carbonyl compound on the silicon of the benzosilacyclobutene, an S_H2 process. (1) Even when the reaction of **9** with acetophenone was carried out under conditions such that more than 99 % of incident light was absorbed only by acetophenone using the filter solution of aq. cupric sulfate, the same adduct was still formed. (2) When the reaction of **9** with acetone was carried out under oxygen atmosphere, only trace amount of adduct was formed.

Introduction

In recent years, much attention has been paid to the chemistry of multiple bonds involving silicon.[1] In the course of study on the chemistry of *o*–quinonoid compounds,[2] we became more interested in the properties of *o*–silaquinone methide (**2**) which is unknown yet.

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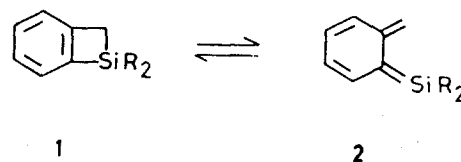
Benzocyclobutene and its heteroatom–substituted analogs (**3**) undergo thermal and photochemical ring opening to give