Solvent Effects on the Secondary Structures of Proteins

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We examined the effect of solvation on the conformational preferences (e.g., α-helix versus β-sheet) of tripeptides using ab initio quantum mechanics (Hartree−Fock 6-31G**) with solvation in the Poisson−Boltzmann continuum solvent approximation. We find that aqueous solvent preferentially stabilizes the α-helix conformation over β-sheet conformations by 3.5 kcal/mol for Ala, 2.4 kcal/mol for Gly, and 2.0 kcal/mol for Pro. We determined the torsional potential surfaces of the tripeptides, Gly-Ala-Gly, Gly-Gly-Gly, and Gly-Pro-Gly using both aqueous solvent and nonpolar solvent conditions. These results were used to determine force-field torsional parameters for the protein main chains.

1. Introduction

One of the most important problems in both theoretical and experimental biochemistry is to understand how the final folded structure of a protein is determined by its primary sequence of amino acids.[1−4]

Starting with Chou and Fasman, statistical information from the protein data bank is commonly used to predict the most-likely secondary structure of a protein given its primary sequence.[5,6] Although useful, such predictions are not sufficiently accurate. Because good model systems having stable α-helices in water have been developed[7,8] there has been significant experimental progress in determining the preferences for α-helix conformations. However no model systems lead to stable β-sheet conformations in water, with the result that there is little direct experimental evidence for the preferences of β-sheets.[9,10]

Many theoretical studies have been directed at understanding the preferences in secondary structures of a protein.[11,12] However, most such ab initio studies (usually on dipeptide model systems) have been carried out in a vacuum, ignoring the solvent effects which must play an important role in determining the amino acid conformations during the protein folding.[13−16] Consequently, we included solvation effects in calculating the conformations.

We have also been concerned about the accuracy of the force-field (FF) parameters for the main chains of proteins. Clearly, those forces can be important in understanding how the final folded structure of a protein is determined by its primary sequence of amino acids.[1−4]

We report here ab initio quantum mechanical calculations (Hartree−Fock, 6-31G** basis) for the conformational energies of the Gly-Ala-Gly, Gly-Gly-Gly, and Gly-Pro-Gly tripeptides in water. This is expected to mimic the solvation effects in hydrophilic environments (surface regions).

The results show that solvation preferentially stabilizes the α-helix conformations over β-sheet conformations by 2 to 3.5 kcal/mol.

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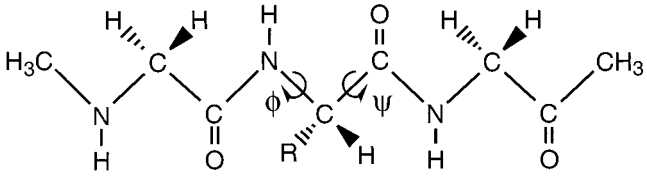

Figure 1. The tri-amino acid model system for ab initio calculations. Both glycines have the extended conformation shown for all the conformations of the center amino acid. The conformational angles (χ_i) of the amino acid side chain was optimized for each ϕ and ψ. Shown is $\phi = 180°$ and $\psi = 180°$.

2. Calculations

2.1 Calculational Details. All quantum chemical calculations were at the Hartree−Fock (HF) level using the 6-31G** basis for all atoms. All calculations used the Jaguar quantum chemistry program.[17,18] Solvation was included by solving the Poisson−Boltzmann equations with a realistic molecular surface (van der Waals radius plus solvent radius about each atom) using the Jaguar solvation model (PBF).[19] We assumed $\epsilon = 80$ and $R_0 = 1.4$ Å based on using water as the solvent to mimic hydrophilic environments. The solvent effects were calculated self-consistently. At each iteration the wavefunction is calculated in the field of the solvent and then the charges (based on the electrostatic potential from the HF wavefunction) are used to calculate a new reaction field.[19] This process is repeated until convergence.

2.2 The Gly-X-Gly Conformation Surface. To establish the effect of environment on the conformation of amino acids, we first carried out quantum mechanical calculations on the model system (Gly-Ala-Gly) (Figure 1) for all ϕ and ψ torsional angles of the center alanine. The two glycines are used to provide a proper environment for the polypeptide and have extended forms ($\phi = 180°$ and $\psi = 180°$) for all conformations of the alanine. The quantum mechanical calculations were carried out for every 60° of the ϕ and ψ torsional angles (36 points) of the center alanine plus three additional conformations corresponding to α-helix ($\phi = -57°$ and $\psi = -47°$) and β-sheet ($\phi = -119°$ and $\psi = 113°$ for parallel β-sheet, and $\phi = -139°$ and $\psi = 135°$ for antiparallel β-sheet) conformations (total 39 points). The geometry of each conformation was fully optimized by

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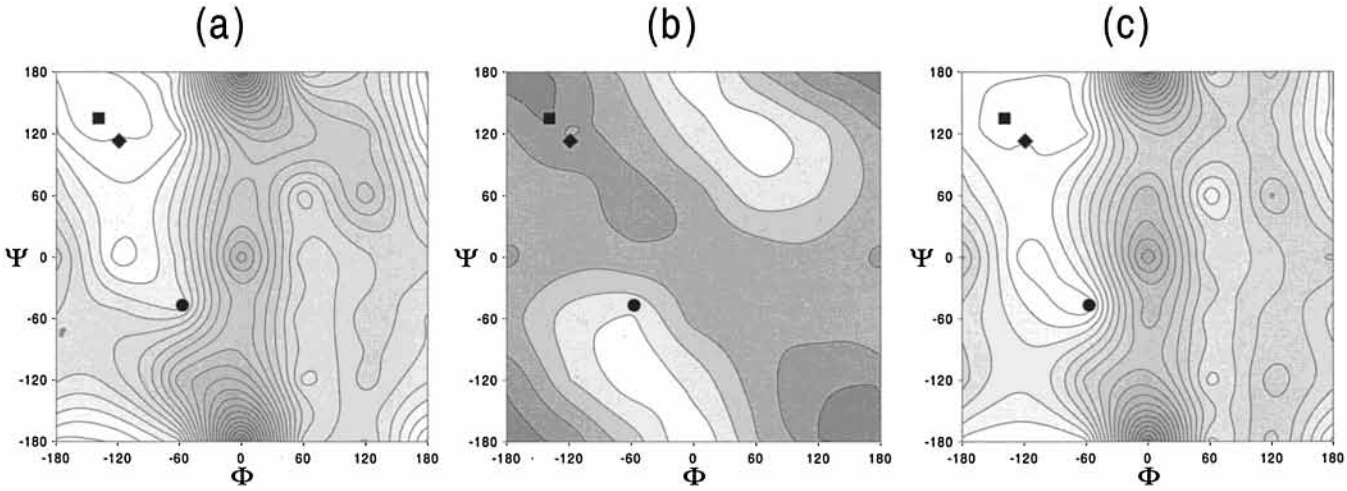

Figure 2. Conformation energies for Gly-Ala-Gly. Each map is based on the energies for 36 pairs of torsional angles ($\Delta\phi = 60°$, $\Delta\psi = 60°$) plus three additional energies corresponding to the α-helix ($\phi = -57°$, $\psi = -47°$) indicated by solid circle, the parallel β-sheet ($\phi = -119°$ and $\psi = 113°$) indicated by solid diamond, and the antiparallel β-sheet ($\phi = -139°$ and $\psi = 135°$) conformations. The bright region indicates stable conformations and the dark region indicates unstable conformations. The maps show clearly that solvent effects tend to stabilize the α-helical conformation compared to the β-sheet conformation. The contour spacing is 1.0 kcal/mol. (a) Vacuum HF results, (b) solvation energy for H_2O, (c) total energy in H_2O.

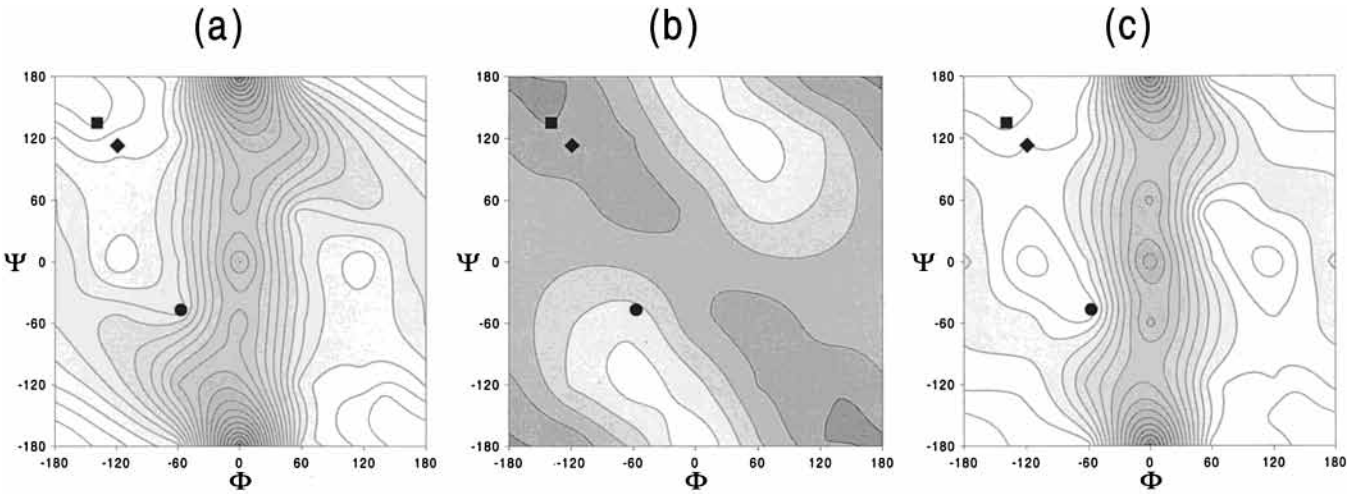

Figure 3. Conformation energies for Gly-Gly-Gly. Each map is based on the energies for 36 pairs of torsional angles ($\Delta\phi = 60°$, $\Delta\psi = 60°$) plus three additional energies corresponding to the α-helix ($\phi = -57°$, $\psi = -47°$) indicated by solid circle, the parallel β-sheet ($\phi = -119°$ and $\psi = 113°$) indicated by solid diamond, and the antiparallel β-sheet ($\phi = -139°$ and $\psi = 135°$) conformations. The bright region indicates stable conformations and the dark region indicates unstable conformations. The maps show clearly that solvent effects tend to stabilize the α-helical conformation compared to the β-sheet conformation. The contour spacing is 1.0 kcal/mol. (a) Vacuum HF results, (b) solvation energy for H_2O, (c) total energy in H_2O.

quantum mechanical calculations (Hartree−Fock, 6-31G** basis) in vacuum. This leads to the contour maps in Figure 2 for the potential energy and solvation energy of alanine.

We also carried out the same calculations for the Gly-Gly-Gly (Figure 3) and Gly-Pro-Gly (Figure 4) cases.

3. Results

Without solvation, our results are similar to those of previous calculations.[13,20] Table 1 shows the apparent local minima and Table 2 shows the relative energy differences of the α-helix, parallel β-sheet, and antiparallel β-sheet conformations to the global minimum in each case with ϕ and ψ angles used in the current calculations.

The absolute minima of alanine at ($\phi = -138$, $\psi = 138$) and proline at ($\phi = -72$, ψ) = 120) correspond to a β-sheet while the absolute minimum of glycine at ($\phi = 180$, $\psi = 180$) is the extended conformation. For alanine, the potential surface has a channel pointing toward the α-helix region with local minima at ($\phi = -120$, $\psi = 0$) about 1.343 kcal/mol higher. The actual α-helix conformations of alanine, glycine, and proline are not minima ($E = 3.448$ kcal/mol, 4.603 kcal/mol, and 2.492 kcal/mol, respectively). In addition, there are relative minima for alanine at ($\phi = -120$, $\psi = 0$) with $E = +1.343$, at ($\phi = 60$, $\psi = 60$) with +5.234 and at ($\phi = 60$, $\psi = -120$) with +4.558 kcal/mol. Glycine has two relative minima at ($\phi = 120$, $\psi = 0$) and ($\phi = -120$, $\psi = 0$), but proline has none.

In water the solvation energies for alanine (22.508 to 27.875 kcal/mol) (Figure 2b), glycine (24.113 to 29.212 kcal/mol) (Figure 3b), and proline (21.306 to 27.336 kcal/mol) (Figure 4b) are large. Though the solvation energies of proline show the strongest preferences only at ($\phi = 0$, $\psi = 180$), those of alanine and glycine show the strongest preferences at ($\phi = 60$, $\psi = 120$), ($\phi = -60$, $\psi = -120$), and ($\phi = 0$, $\psi = 180$). However, the solvation energies with water are strongly biased toward the α-helix conformation for all the three cases. For alanine, this biased solvation effect leads to a minimum at (ϕ

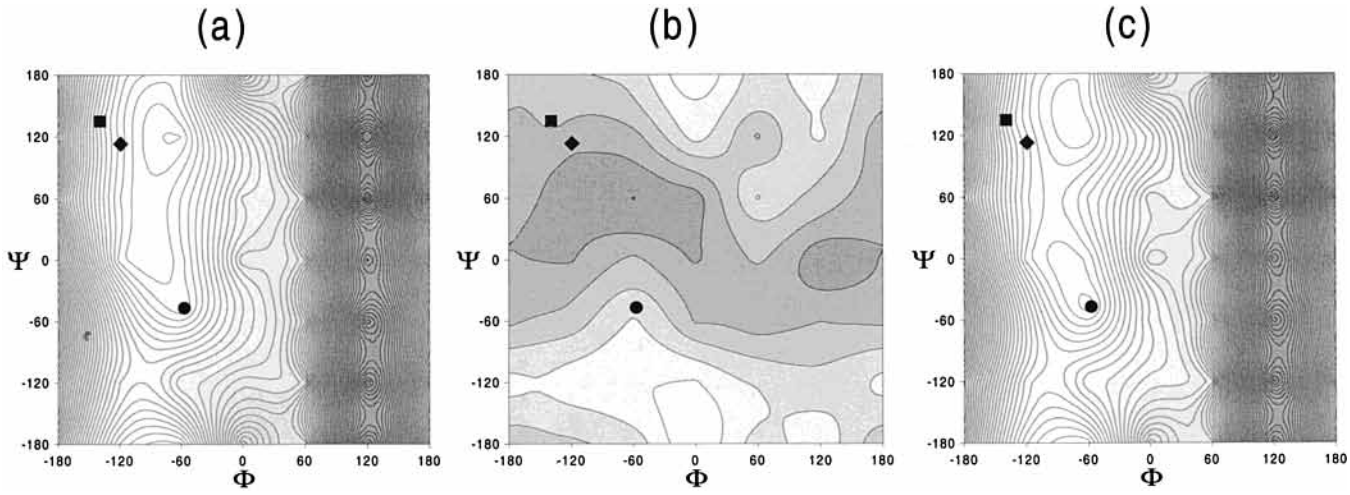

Figure 4. Conformation energies for Gly-Pro-Gly. Each map is based on the energies for 36 pairs of torsional angles ($\delta\phi = 60°$, $\delta\psi = 60°$) plus three additional energies corresponding to the α-helix ($\phi = -57°$, $\psi = -47°$) indicated by solid circle, the parallel β-sheet ($\phi = -119°$ and $\psi = 113°$) indicated by solid diamond, and the antiparallel β-sheet ($\phi = -139°$ and $\psi = 135°$) conformations. The bright region indicates stable conformations, and the dark region indicates unstable conformations. The maps show clearly that solvent effects tend to stabilize the α-helical conformation compared to the β-sheet conformation. The contour spacing is 1.0 kcal/mol. (a) Vacuum HF results, (b) solvation energy for H_2O, (c) total energy in H_2O.

TABLE 1: The Energy Minima and the Energy Differences of the Minima to the Global Minimum Are Shown with the Conformational ϕ and ψ Angles[a]

residue					β-sheet	α-helix	α-helix
alanine	(ϕ, Ψ)	(−120, 0)		(60, −120)	(−138, 138)		(60, 60)
	ΔE_{vac}	1,343		4.558	0.000		5.234
	(ϕ, Ψ)	(0, −180)	(60, 120)	(−60, −120)			
	ΔE_{sol}	0.039	0.000	0.238			
	(ϕ, Ψ)	(−120, 0)		(60, −120)	(−120, 120)	(−60, −48)	(60, 60)
	ΔE_{wat}	0.338		3.592	0.000	0.281	2.138
glycine	(ϕ, Ψ)	(±180, −180)	(±120, 0)				
	ΔE_{vac}	0.000	2.280				
	(ϕ, Ψ)	(0, −180)	(−60, −120)	(60, 120)			
	ΔE_{sol}	0.000	0.408	0.408			
	(ϕ, Ψ)	(±180, ±180)	(±120, 0)				
	ΔE_{wat}	0.000	0.322				
proline	(ϕ, Ψ)				(−72, 120)		
	ΔE_{vac}				0.000		
	(ϕ, Ψ)	(0, 180)					
	ΔE_{sol}	0.000					
	(ϕ, Ψ)				(−72, 126)	(−60, −48)	
ΔE_{wat}					0.000	0.263	

[a] ΔE_{vac}: relative total energy in vacuum. ΔE_{sol}: relative solvation energy in water. ΔE_{wat}: relative total energy in water.

$= -120$, $\psi = 120$) corresponding to the β-sheet and a second minimum at ($\phi = -60$, $\psi = -48$) corresponding to the α-helix, now only 0.281 kcal/mol higher.

Table 2 shows that solvation dramatically changes the relative energy between the α-helix and parallel β-sheet conformations. Thus, ΔE_{vac}(α-helix) − ΔE_{vac}(p-β-sheet), changes

- from +2.210 kcal/mol in vacuum to −0.986 kcal/mol in water for alanine ($\delta = -3.2$ kcal/mol),
- from +1.533 kcal/mol in vacuum to −0.948 kcal/mol in water for glycine ($\delta = 2.5$ kcal/mol), and
- from −1.369 kcal/mol in vacuum to −4.033 kcal/mol in water for proline ($\delta = 2.7$ kcal/mol).

The net aqueous stabilizations of α-helix in kcal/mol are Ala (3.2), Gly (2.1), and Pro (2.7). Similar stabilization is observed between the α-helix and antiparallel β-sheet conformation, where ΔE_{vac}(α-helix) − ΔE_{vac}(a-β-sheet) changes

- from +3.268 kcal/mol in vacuum to −0.501 kcal/mol in water for alanine,
- from +3.836 kcal/mol in vacuum to 0.509 kcal/mol in water for glycine, and
- from −4.674 kcal/mol in vacuum to −6.148 kcal/mol in water for proline.

TABLE 2: The Relative Energy (kcal/mol) of the α-Helix and β-Sheet Conformations to the Global Minimum in Vacuum and Water[a]

residue	conformation	ΔE_{vac} (kcal/mol)	ΔE_{sol} (kcal/mol)	ΔE_{wat} (kcal/mol)
alanine	α-helix	3.448	1.227	0.282
	p-β-sheet	1.238	4.483	1.268
	a-β-sheet	0.180	5.057	0.783
glycine	α-helix	4.603	1.664	1.168
	p-β-sheet	3.070	4.145	2.116
	a-β-sheet	0.767	4.991	0.659
proline	α-helix	2.492	2.198	0.281
	p-β-sheet	3.861	4.861	4.314
	a-β-sheet	7.166	3.672	6.429

[a] All energies are from ab initio calculations (HF, 6-31G** basis) on Gly-X-Gly with a Poisson−Boltzman description of the solvent. [b] α-Helix: right-handed α-helix, where (ψ, Ψ) = (−57, −47); p-β-sheet: parallel β-sheet, where (ψ, Ψ) = (−119, 113); a-β-sheet: antiparallel β-sheet, where (ψ, Ψ) = (−139, 135); ΔE_{vac}: relative total energy in vacuum; ΔE_{sol}: relative solvation energy in water; ΔE_{wat}: relative total energy in water.

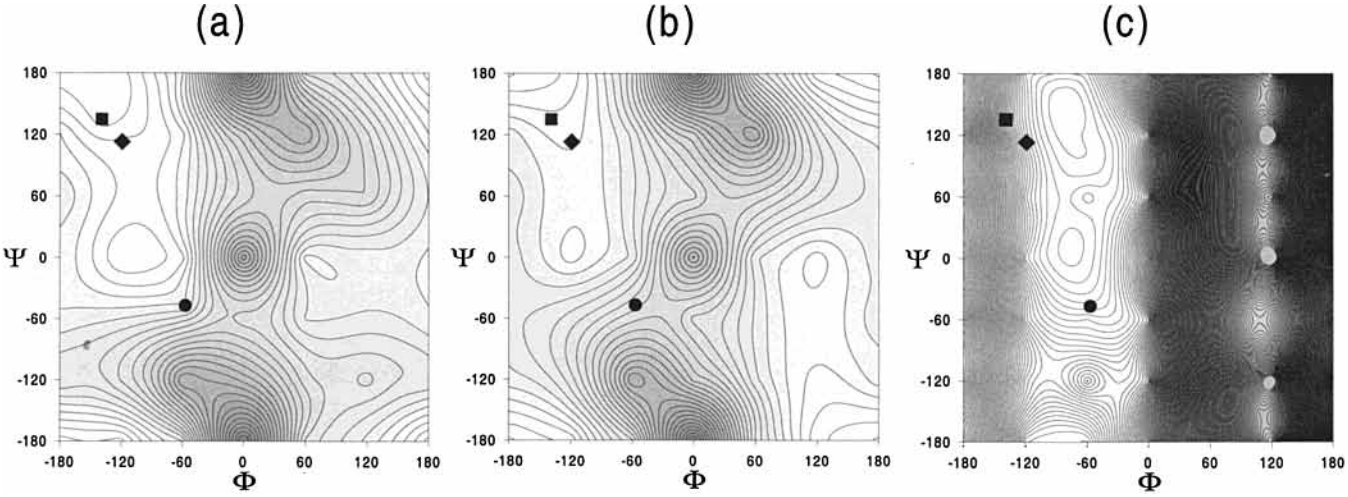

Figure 5. Force-field conformation energies for (a) Gly-Ala-Gly, (b) Gly-Gly-Gly, and (c) Gly-Pro-Gly in vacuum. Each map can be compared to the corresponding HF results in Figures 2a, 3a, and 4a, respectively. The force field used in the fit was based on the exponential-6 form of DREIDII[37] nonbonds, UFF[38] valence terms (with all but the ω torsion zeroed out), and charges were derived from a HF calculation on the extended form of each tripeptide. Gly-Gly-Gly was used to fit the ϕ and ψ torsions. The two torsions involving the C_β atom were added to fit the Gly-Ala-Gly map. One additional torsion, listed in Table 3, was added to properly reproduce the Gly-Pro-Gly map. To improve the torsional fit for the lower energy conformations, each tripeptide conformation was weighted with a Boltzmann factor.

TABLE 3: The Force-Field Torsional Cosine Expansion Terms Used in Fit to the Quantum Mechanical Data[a,b,c]

	A (kcal/mol)	*B* (kcal/mol)	*C* (kcal/mol)
all amino acids			
$C-N-C_\alpha-C$ (ϕ)	1.00	−1.00	−1.25
$N-C-C_\alpha-N$ (ψ)	1.50	−2.25	0.00
needed for non-glycine			
$C_\beta-C_\alpha-N-C$	−1.70	−1.70	−0.20
$C_\beta-C_\alpha-C-N$	1.20	0.60	0.40
proline-specific			
$C_\delta-N-C_\alpha-C$	0.00	0.00	1.50

[a] The torsion function is a simple cosine sum: torsional energy = $A\cos(\theta) + B\cos(2\theta) + C\cos(3\theta)$; prior to the torsional fit, all torsions but the ω torsion were zeroed; the ω torsion $C_\alpha-N-C-C_\alpha$ was not fit, but was left with a barrier of 10 kcal/mol and a periodicity of 2. [b] C_δ is the side-chain δ-carbon of proline adjacent to the main chain nitrogen. [c] See Figure 5 for a more complete description of the force-field fitting procedure.

The net solvent stabilizations of α-helix in kcal/mol are Ala (3.8), Gly (3.3), and Pro (2.5).

4. Discussion

Starting with Chou and Fasman, statistical information from the protein data bank is commonly used to predict the most-likely secondary structure of a protein given its primary sequence.[5,6] Although useful, such predictions are not sufficiently accurate. Because many good model systems having stable α-helices in water have been developed[7,8] there has been significant experimental progress in determining the preferences for α-helix conformations. However developing model systems leading to stable β-sheet conformations in water has been with the result that there is little direct experimental evidence for the preferences of β-sheets.[9,10]

Various results of theoretical calculations[21−23] on model systems for amino acids in vacuum have shown that the right-handed α-helix conformation is not stable (with a few exceptions[24]) while the β-sheet conformation is quite stable. This is not consistent with experiment, and hence it has been proposed that the right-handed α-helix must be stabilized by specific nonbonded interactions.[13] It has been suggested that the α-helical conformation is destabilized compared to the β-sheet conformation by the dipole moment interaction between the side chain and the backbone.[25]

In contrast, our calculations for water show that this unfavorable dipole moment of the α-helix induces a stronger solvent effect in water, leading to an α-helical conformation nearly as stable as the β-sheet conformation in water (the solvation energy is directly related to the dipole moment of the solute). This strong solvent effect in water for the α-helical conformation agrees with earlier thermodynamic studies which included the solvent effects on an alanine dipeptide.[13] Our calculations show that for proline the α-helix conformation is more stable (4 to 6 kcal/mol) than the β-sheet conformation and for alanine the α-helical conformation is slightly more stable than the β-sheet conformation (0.501 and 0.986 kcal/mol). For glycine the α-helical conformation is more stable than parallel β-sheet conformation (0.948 kcal/mol) but less stable than the antiparallel β-sheet conformation (0.509 kcal/mol). This becomes clear for the calculations on the 10 nonpolar amino acids for the α-helix and β-sheet conformations. For all 10 relatively hydrophobic amino acids the β-sheet is more stable than the α-helix conformation in vacuum (hydrophobic environment), but in water the stability of α-helix conformation becomes very close to that of β-sheet conformation due to the strong solvent stabilization of the α-helix conformation (unpublished data).

These results support the observation that β-sheets usually occur only inside folded proteins. This is because the interior of proteins is usually hydrophobic favoring the β-sheet conformation.

These results are also supported by experiments which show (i) a transition of polylysine from the α-helix to β-sheet conformations by the addition of anesthetics, and (ii) a transition of polylysine from β-sheet to α-helix occurs by applying pressure.[26] The anesthetics induce a partial dehydration of the polypeptide side chains, creating a more hydrophobic environment favorable for β-sheet conformation for the polypeptide.[27] In contrast the applied pressure seems to push waters near the side chains and makes the environment more hydrophilic.[28,29]

These results support the observation that hydrophobic residues have high preferences for the β-sheet secondary

structure and polar residues have low preferences for the β-sheet secondary structure.[5,6] This is because hydrophobic residues are more likely to be inside the protein (hydrophobic environment) than are hydrophilic residues while hydrophilic residues have relatively high probabilities to be placed on the exterior of proteins compared to the hydrophobic residues. These results also explain why there are many α-helix models stable in water, making it easy to study the properties of α-helices, while very few β-sheet models are stable in water, making it difficult to study β-sheets.[13,24] These conclusions are supported by the recent results that the presence of a hydrophobic core is essential for the formation of a β-sheet.[9,10]

Goodwin and co-workers observed that an alcoholic cosolvent increased the stability of helix conformations of peptides.[32] They proposed that the helical conformation is more solvated by water than the random coil and is the most perturbed by the addition of cosolvent. Indeed our results show that α-helices are more hydrated than β-sheets which explain the effect of alcohol cosolvent.[32−35]

Recent experimental results show that the peptides from the prion protein induce conformational transitions due to addition of acetonitrile and/or salts.[34] The added denaturants make the microenvironment around the peptides more hydrophobic, causing a conformational change in the peptides from α-helix to β-sheet. Our results are consistent with the observation, thus providing a possible insight into explaining the Creutzfeldt−Jakob disease, the most common human prion disease.[36]

Our results do show that for the case of alanine and glycine the α-helical conformation is comparable compared to the β-sheet conformation in water. For the case of proline the α-helical conformation is much more stable than the β-sheet conformations both in water and even in vacuum. These results seem to contradict the observation that a proline residue tends to destroy the α-helix conformations. Proline residues destabilize the α-helix because of the pyrrolidine ring attached to the imide nitrogen. Its presence matters only when the *succeeding* residue is a proline. The steric interactions of a residue are independent of the nature of the *predecessor* because only the carbonyl group (C═O) of the preceding residue is involved.[30] This is supported by the observations that proline residues are one of the best residues to initiate α-helix.[31]

Our results show that the α-helix conformation is stabilized by solvation with water, providing insight into understanding the role of interactions between solvents and proteins in guiding protein folding.

These results should be useful for deriving a force field for use in molecular dynamics.

5. Force Field Parameters

We determined torsional FF parameters to fit the QM results in both vacuum and solvent for all three cases. The resulting torsional parameters are listed in Table 3. The final conformation energies for each case in vacuum are shown in Figure 5.

6. Summary

We find that solvents have a significant effect on the conformation of polypeptides in solvation. We believe that these effects play an important role in protein folding. We report torsional parameters to use in chemical MD calculations.

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Supporting Information Available: QM energies and solvation energies for all 39 conformations of all cases listed in Table 2. Tables S-1, S-2, and S-3 are for G-A-G, G-G-G, and G-P-C, respectively, and Table S-4 shows the special points for all cases. This material is available free of charge via the Internet at http://pubs.acs.org.

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