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Water Molecule Adsorption on Protonated Dipeptides

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Abstract: Equilibrium constants for the adsorption of the first water molecule on six protonated dipeptides (Gly−Gly+H$^+$, Gly−Ala+H$^+$, Ala−Gly+H$^+$, Ala−Ala+H$^+$, Pro−Gly+H$^+$, and Gly−Trp+H$^+$) have been measured as a function of temperature, and $\Delta H°$ and $\Delta S°$ determined. Density functional theory calculations were performed for both the unsolvated peptides and the peptide water complexes at the B3LYP/6-311++G** level. MP2/6-311++G** calculations were also carried out for Gly/Ala peptides. The calculations suggest that adsorption of a water molecule by these simple dipeptides is a complex process, both the unsolvated peptide and the peptide−water complexes have multiple conformations with similar free energies. Average $\Delta H°$ and $\Delta S°$ values derived from the calculations are in reasonable agreement with the experimental results. According to the calculations, the dominant water adsorption process involves a significant conformational change to accommodate a bridging water molecule. $\Delta H°$ is diminished for Pro−Gly+H$^+$ mainly because the water interacts with a secondary amine, whereas for Gly−Trp+H$^+$, $\Delta H°$ is significantly decreased by the loss of cation-π interactions upon water adsorption. For unsolvated peptides the proton affinities of the N-terminus and the backbone carbonyl groups are known to be similar. Addition of a single water molecule causes a significant stabilization of the N-terminus protonation site.

Introduction

It has been known for some time that water interactions play an important structural role in proteins and there have been many studies of these interactions using a variety of methods.[1−3] In particular, small peptides have received a lot of attention because of their experimental tractability and relative simplicity. Recent advances in experimental techniques have afforded some exciting new views,[4−11] including detailed information about the interaction of individual water molecules with amino acids and small peptides isolated in the gas phase.[12−14] Small peptides are also particularly well suited for theoretical treatment and many studies have been conducted using Monte Carlo,[15,16] molecular dynamics,[17−19] and quantum chemistry calculations.[20−25] Dipeptides, tripeptides, and larger peptides,[26−32] have been investigated by both theoretical and experimental means.

In an earlier publication, we presented the results of a study of the addition of the first water molecule onto relatively large unsolvated polypeptides (15−20 residues) where it was found that water adsorption was extremely sensitive to the secondary structure.[33] In this publication, we describe a study of water addition to unsolvated dipeptides where our goal is to obtain a

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more detailed understanding of the interactions of water with peptides in general. We have performed equilibrium measurements for the addition of the first water molecule to six protonated dipeptides (Gly−Gly+H^+, Gly−Ala+H^+, Ala−Gly+H^+, Ala−Ala+H^+, Pro−Gly+H^+ and Gly−Trp+H^+). Density functional theory calculations were performed at the B3LYP/6-311++G** level for the low energy conformations of both the unsolvated peptide and the peptide−water complex. MP2/6-311++G** calculations were then performed for the Gly/Ala peptides. Knowledge about the conformation is important in understanding a variety of mass spectrometric measurements for small peptides, including H/D exchange[34] and gas-phase basicity measurements,[35] in addition to ion-equilibrium measurements.[8,36] In the present case, the results indicate that there are multiple conformations with similar free energies involved in the addition of the first water molecule.

Experimental Methods

All experimental data were obtained using a temperature-variable injected-ion drift tube apparatus that has been described in detail elsewhere.[37] Briefly, desolvated ions are produced by an electrospray source with a heated capillary. The ions then enter a 30.5 cm long drift tube which consists of four sections that can be cooled with liquid nitrogen. The temperature of each section is regulated to better than ±0.5 K with microprocessor-based temperature controllers. The drift tube has a series of guard rings that establish a uniform electric field along its length and contains helium buffer gas at a pressure of around 4 Torr. After traveling down the drift tube under the influence of a weak electric field, some of the ions exit through a small aperture. These ions are focused into a quadrupole mass spectrometer and after being mass analyzed, they are detected by an off-axis collision dynode and dual microchannel plates.

Equilibrium measurements are performed by admitting a known partial pressure of water vapor into the drift tube and recording the intensity of the reactants and products in the mass spectrum. A leak valve was used to regulate water pressure and the measured pressure was corrected for the buffer gas flow. The corrected water vapor pressure was typically around 1−7 mTorr in the measurements reported here. We have considered in detail the possible sources of errors in our equilibrium measurements in a previous publication.[33] Most measurements were performed with an E/p (drift field/total pressure) of ∼2.3 V/cm Torr which meets the low-field criteria[38] in similar studies.[8,39] The effective temperature increase in the drift tube due to the drift field is below 1 K.[33,40] Lowering the drift voltage from 280 V to 180 V gave identical results. This also shows that the measurements are independent of time, as they should be if equilibrium is established. Drift time distributions were obtained by admitting a short pulse of ions into the drift tube and recording the arrival time distribution at the detector. The drift time distributions of unsolvated species was identical to that of the water adduct, as is expected for two species in equilibrium.

Equilibrium constants were calculated from the following

$$K = \frac{I_{p+w}}{I_p \cdot P_w} \quad (1)$$

where I_p and I_{p+w} are intensities in the mass spectrum of peptide and peptide−water complex, respectively, and P_w is the partial pressure of water vapor in the drift tube in atmospheres. As described previously,[33] the standard Gibbs free energy change for addition of a water molecule to Gly−Gly+H^+ was measured for calibration purposes. Our value of −35.1 kJ mol^{-1} at 293 K is in good agreement with −36.8 kJ mol^{-1} obtained by Klassen et al.[8] These authors noted that their value may be slightly elevated by condensation of water onto the peptides at the exit aperture. Our value may be slightly diminished by collision induced dissociation of the peptide−water complex outside of the drift tube. Gly−Gly+H^+ has a strong affinity for water and so even a few collision events can influence the equilibrium constant.

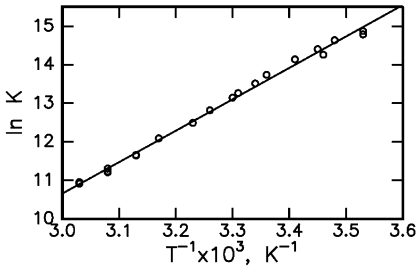

Figure 1. van't Hoff plot of ln K against $1/T$ for the adsorption of a water molecule onto the Ala−Gly+H^+ peptide. The slope of the line is $-\Delta H°/R$ and the intercept is $\Delta S°/R$. The points are experimental data and the line is a linear regression.

All dipeptides were obtained from Sigma Aldrich and used without further purification. The best signals were found with solutions of 1 mg of dipeptide in 10 mL of methanol with 5 drops of formic acid. A recent study of dipeptides in aqueous solution by Scherer et al. has shown that the cis/trans ratio for Gly−Gly is 0.003.[41] The cis isomers of Ala−Gly and Gly−Ala have also been observed.[42] It is not known whether electrospraying and injection into the drift tube will lead to different isomer populations than present in aqueous solution. Drift time distributions measured for the dipeptide ions at both 20 °C and at −150 °C could be fit assuming that only a single conformation was present.[43] However, this does not rule out the presence of two or more isomers with nearly identical cross sections. These results are also consistent with multiple conformations that are rapidly interconverting on the experimental time scale. This would lead to a single narrow peak at a position characterized by the average cross section. Calculations presented below favor this interpretation.

Experimental Results

Equilibrium constants were obtained over a range of temperatures and a van't Hoff plot was constructed for each peptide. A typical example is shown in Figure 1 for the Ala−Gly+H^+ peptide. The results show good reproducibility and the van't Hoff plots show excellent linearity ($R^2 > 0.99$). The slope of the van't Hoff plot is $-\Delta H°/R$ and the intercept is $\Delta S°/R$. The enthalpy and entropy change for the addition of the first water molecule to Ala−Gly+H^+ obtained from Figure 1 is $\Delta H° = -66.7$ kJ mol^{-1} and $\Delta S° = -111$ J K^{-1} mol^{-1}. All of the experimental results obtained in this way are summarized in Table 1. The experimental uncertainties are calculated from the standard deviations of the slopes and intercepts of the van't Hoff plots of n points multiplied by the 95% confidence interval coefficients from the student's t-distribution for n-2 degrees of freedom.[36] Previous experience for similar equilibrium-based measurements suggests that the experi-

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Table 1. Measured Enthalpy and Entropy Changes for Addition of the First Water Molecule to the Dipeptides and the Calculation Result[a]

peptide	$\Delta H°$ expt. kJ mol^{-1}	calculated $\Delta H°_{AVG}$	$\Delta S°$ expt. J K^{-1} mol^{-1}	calculated $\Delta S°_{AVG}$
Gly−Gly+H$^+$	−68.0 ± 2.5	−71.4	−109 ± 8	−134
Ala−Gly+H$^+$	−66.7 ± 1.7	−76.8	−111 ± 5	−142
Gly−Ala+H$^+$	−70.7 ± 2.6	−74.3	−124 ± 8	−139
Ala−Ala+H$^+$	−61.5 ± 1.8	−66.9	−105 ± 6	−124
Pro−Gly+H$^+$	−53.2 ± 1.0	−53.9[b]	−94 ± 4	−108[b]
Gly−Trp+H$^+$	−47.7 ± 1.9	−56.5[b]	−79 ± 7	−101[b]

[a] The uncertainty estimates are derived from the standard deviations of slopes and intercepts of the van't Hoff plots of n points multiplied by the 95% confidence interval coefficients from the student's t-distribution for n-2 degrees of freedom.[36] Calculation results are population-averaged enthalpies (in kJ mol^{-1}) and entropies (in J K^{-1} mol^{-1}) derived from the calculations (see text). The calculated results for Pro−Gly+H$^+$ and Gly−Trp+H$^+$ are derived from B3LYP/6-311++G** calculations, whereas for the other peptides the results are derived from MP2/6-311++G** calculations. [b] Calculated at the B3LYP/6-311++G** level.

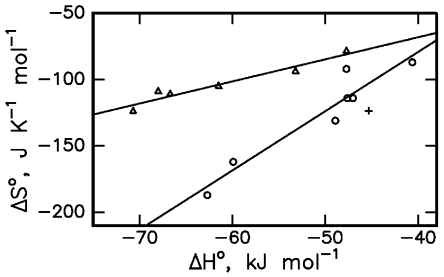

Figure 2. Plot of the entropy change against the enthalpy change for addition of the first water molecule onto unsolvated peptides. The triangles are the results for the peptides studied here. The circles are the results for the 15−20 residue peptides reported in ref 33. The lines are least-squares fits to the two data sets. The cross shows $\Delta H°$ and $\Delta S°$ for the adsorption of a vapor phase water molecule onto liquid water.

mental error is usually within 4 kJ mol^{-1} for $\Delta H°$ and within 8 J K^{-1} mol^{-1} for $\Delta S°$. Comparison to $\Delta H°$ and $\Delta S°$ for the addition of a water molecule to related molecules (Table 3 in ref 33) show that the values in Table 1 are comparable to those of molecules of similar size.

The experimental results largely fall into two groups: the four glycine/alanine peptides (Gly−Gly+H$^+$, Gly−Ala+H$^+$, Ala−Gly+H$^+$, and Ala−Ala+H$^+$) and the two other peptides (Pro−Gly+H$^+$ and Gly−Trp+H$^+$). In the first group, the enthalpy changes for Gly−Ala+H$^+$, Ala−Gly+H$^+$, and Gly−Gly+H$^+$ are within experimental uncertainty. These three dipeptides were found to adsorb water over approximately the same temperature range (283 K to 343 K). The enthalpy change for the Ala−Ala+H$^+$ peptide is slightly smaller than for the rest of the alanine/glycine group. Both $\Delta H°$ and $\Delta S°$ are appreciably smaller for both Pro−Gly+H$^+$ and Gly−Trp+H$^+$ than for the Gly/Ala group. A lower temperature is also required in order to observe the hydrated ions of these peptides (248 to 283 K). Figure 2 shows a plot of the measured entropy change against the measured enthalpy change for addition of the first water molecule onto unsolvated peptides. The triangles are the results for the peptides studied here. The circles are the results for the 15−20 residue peptides reported in ref 33. The lines are least-squares fits to the two data sets. For the dipeptides studied here (triangles) the plot is close to linear ($R^2 = 0.93$).

Computational Methods

Molecular dynamics (MD) simulations were first performed to obtain starting structures for more sophisticated calculations on the charged dipeptides and their water adducts. The MD simulations were performed with the MACSIMUS suite of programs[44] using either the CHARMM21 or the CHARMM22 parameter set[45,46] and the TIP3P model for water.[47]

The starting conformation for the MD runs was the fully extended structure with backbone dihedral angles $\phi = 180°$ and $\psi = 180°$ (see below for an explanation of these angles). At least twenty 240-ps runs were performed at 300 K for each of the dipeptides and their water adducts. The average potential energy was determined from the final 35 ps of each simulation. Ramachandran plots of the MD simulations indicate that they explore the available conformational space reasonably well. Gly−Gly+H$^+$ was chosen as a test case since this peptide has the greatest conformational flexibility. For this peptide an additional thirty conformations were randomly selected from ten 960-ps 400-K simulations. All fifty structures were then optimized at the HF/6-31G* level using Jaguar v.4.1.[48] Calculations on the alanine dipeptide and other peptides have shown that, whereas larger basis sets and electron correlation affect the depth of a well on the potential surface, smaller basis sets such as HF/6-31G* can provide reasonable agreement with the relative energies of different conformers,[49,23] though changes in the relative energies have been reported.[24] For the Gly−Gly+H$^+$ dipeptide, 18 out of the 30 structures derived from the 400 K simulations optimized to a structure identical to the lowest energy structure derived from the 300 K simulations, even though the initial conformations were often significantly different. A similar result was obtained for the dipeptide−water complex. These results confirm that the MD simulations provide an effective tool for generating initial conformations for optimization using more sophisticated methods.

For all peptides, the lowest energy structure and other representative, low-lying conformations derived from the MD simulations were initially optimized at the HF/6-31G* level. For Gly−Gly+H$^+$, Pro−Gly+H$^+$, and Gly−Trp+H$^+$ the number of trial structures was 78, 15, and 51, respectively; for the other Gly/Ala peptides at least four trial structures were used. The lowest energy structure and a few other structures were then re-optimized with B3LYP/6-311++G** using Gaussian 98.[50] The outputs of DFT calculations for Gly−Gly+H$^+$, Gly−Ala+H$^+$, Ala−Gly+H$^+$, and Ala−Ala+H$^+$ were then used as starting structures for MP2 optimization and frequency calculations with Gaussian 98. Pro−Gly+H$^+$ and Gly−Trp+H$^+$ were only calculated only up to the B3LYP/6-311++G** level due to time constraints.

Harmonic frequencies and zero-point energies were computed for all structures. All calculated frequencies were verified to be real. For MP2 calculations, the frequency was scaled by 0.94 and ZPE by 0.98.[51] The DFT results were used unscaled. The calculation of $\Delta H°$ was performed using the following equation

$$\Delta H° = \Delta E_{\text{bind}} + \Delta E_{\text{thermal}} + \Delta(PV) + \Delta\text{ZPE} - \text{CP} \qquad (2)$$

where ΔE_{bind} is the difference in the calculated binding energy for the peptide + water and the peptide−water complex, $\Delta E_{\text{thermal}}$ is the

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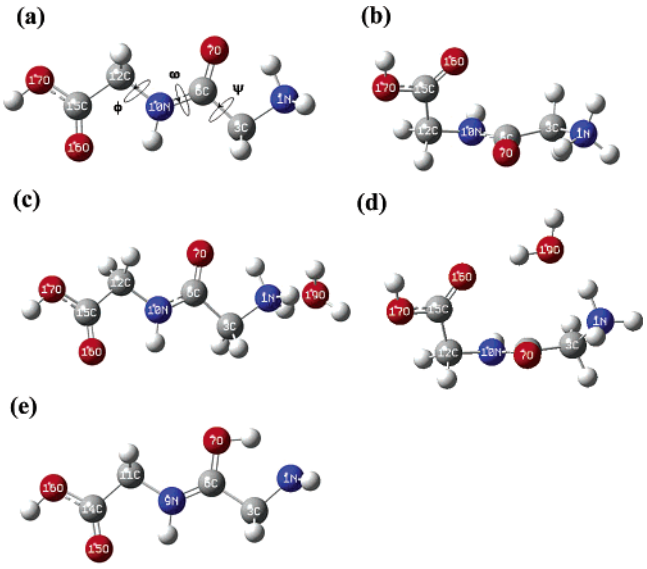

Figure 3. MP2/6-311++G** optimized structures of Gly−Gly+H^+ and Gly −Gly+H_2O+H^+. (a) and (b) are Gly−Gly+H^+ in the extended and $\mathbf{L}^+$ conformations (see text), respectively. (c) and (d) are Gly−Gly+H_2O+H^+ in the extended and $\mathbf{U}^+$ conformation (see text). (e) is the optimized structure of Gly−Gly+H^+ protonated at the backbone carbonyl group. Only non-hydrogen atoms are labeled.

difference in the thermal energy, $\Delta(PV)$ is the change in PV, ΔZPE is the difference in the calculated zero point energies, and CP is the counterpoise correction. The counterpoise correction calculated with DFT for the extended Gly−Gly+H_2O+H^+ water adduct (see below) is 4.02 kJ mol^{-1}, and for the water bridging Gly−Gly+H_2O+H^+ $\mathbf{U}^+$ structure the counterpoise correction is 5.17 kJ mol^{-1}. These calculated values were used for the Gly/Ala peptides. For the peptide−water complexes of Pro−Gly+H^+ and Gly−Trp+H^+ in the extended (or **I**) conformation an estimated value of 4.3 kJ mol^{-1} was used for the counterpoise correction, and for the **U** conformation a value of 5.0 kJ mol^{-1} was used. The counterpoise corrections calculated at the MP2 level for the extended Gly−Gly+H_2O+H^+ water adduct and the water bridging Gly−Gly+H_2O+H^+ $\mathbf{U}^+$ structure are 8.8 kJ mol^{-1} and 12.8 kJ mol^{-1}, respectively. These values were used for the other Gly/Ala dipeptides calculated at the MP2 level. $\Delta S°$ is simply the entropy of the peptide−water complex at STP minus the sum of the entropies of water and the peptide ion by themselves. The entropies were calculated using standard statistical mechanical methods.[52] Unless otherwise noted, the protonation site is the N-terminus.

Computational Results

Gly−Gly+H^+. Figure 3 shows MP2/6-311++G** optimized structures for Gly−Gly+H^+ and Gly−Gly+H_2O+H^+. An extended structure with two C_5 rings (C_5 because five atoms close an intramolecular hydrogen bond) was found to be the lowest energy structure for all the unsolvated Gly/Ala dipeptides ions at the MP2/6-311++G** level. The Gly−Gly+H^+ version of this structure is shown in Figure 3(a). The backbone dihedral angles of the dipeptides can be used to classify the structures. The backbone dihedral angles are the C-terminal ϕ (C_{i-1}−N_i−$C_{\alpha,i}$−C_i), the N-terminal ψ (N_{i-1}−$C_{\alpha,i-1}$−C_{i-1}−N_i), and peptide bond ω ($C_{\alpha,i-1}$−C_{i-1}−N_i−C_i), where i is the residue number and C_α is a backbone carbon with the side-chain. These angles are shown in Figure 3a. For the extended structure in Figure 3a the dihedral angles are $\phi = 180°$ and $\psi = 180°$. This structure has previously been described by Cassady and co-workers for protonated Gly−Gly+H^+ as well as for Ala−Gly+H^+, Gly−Ala+H^+ and Ala−Ala+H^+.[53−55] Paizs et al. have also reported a similar lowest energy structure for Gly−Gly+H^+.[56] Variants of these extended C_5 structures have also been found for neutral peptides..[25]

Table 2. Relative Enthalpies (as defined by eq 2) and the Relative Free Energies of Different Conformations from the DFT (B3LYP/6-311++G**) and MP2 (MP2/6-311++G**) Calculations[a]

		B3LYP/6-311++G**		MP2/6-311++G**	
peptide	structure	relative enthalpy, (kJ mol^{-1})	relative free energy (kJ mol^{-1})	relative enthalpy (kJ mol^{-1})	relative free energy (kJ mol^{-1})
Gly−Gly+H^+	$\mathbf{L}^+$	6.5	5.6	0.3	4.4
Gly−Gly+H_2O+H^+	$\mathbf{U}^+$	−7.9	5.2	−16.7	−1.2
Ala−Gly+H^+	$\mathbf{L}^-$	6.5	5.4	−0.9	2.0
Ala−Gly+H_2O+H^+	$\mathbf{U}^+$	−11.5	1.0	−15.5	−5.3
Gly−Ala+H^+	$\mathbf{L}^-$	5.5	3.9	1.5	−0.2
Gly−Ala+H_2O+H^+	$\mathbf{U}^-$	−8.8	2.8	−18.9	−3.9
Ala−Ala+H^+	$\mathbf{L}^-$	5.9	4.5	−0.1	1.0
Ala−Ala+H_2O+H^+	$\mathbf{U}^-$	−6.6	6.7	−13.4	−0.6
Pro−Gly+H^+	$\mathbf{L}^-$	3.4	2.9		
Pro−Gly+H_2O+H^+	$\mathbf{U}^+$	−3.3	5.9		
Gly−Trp+H_2O+H^+	$\mathbf{U}^-$	−6.2	0.8		

[a] The tabulated values are the energies of the non-extended isomer for both the hydrated and unhydrated peptide minus the corresponding energies of the extended isomer. A negative number favors the non-extended isomer.

The other low energy conformation that appears to be important for the Gly−Gly+H^+ peptide, shown in Figure 3b, has the C-terminal group rotated to $\phi = 66.5°$ (ϕ is the 6C−10N−12C−15C dihedral angle). We refer to this structure as $\mathbf{L}^+$ because the atoms of the backbone approximate an L-shape and because the C-terminal ϕ angle is positive. The electronic energy difference between the extended and $\mathbf{L}^+$ conformations of Gly−Gly+H^+ at the MP2/6-311++G** level is 2.0 kJ mol^{-1} favoring the extended structure (5.7 kJ mol^{-1} at the B3LYP/6-311++G** level). Thus, the two structures are energetically almost indistinguishable at the highest level of theory. The difference between the extended and $\mathbf{L}^+$ form is simply the rotation of the C-terminus around the ϕ bond. This requires the breaking of the C_5 ring which is formed by a weak C═O to N−H hydrogen bond at the C-terminus. An $\mathbf{L}^-$ analogue with negative ϕ angle also exists and it is isoenergetic with the $\mathbf{L}^+$ form. These **L** structures are equivalent to the second lowest energy structures A2 and A2e reported by Paizs et al.[55] The potential energy surfaces of small peptides are known to have many local minima and thus many stable conformations are possible.[25,53,57,58] Free energy (at 298 K) favors the extended structure by a significant but small 4.4 kJ mol^{-1}. This suggests that both structures will contribute under experimental conditions. At the B3LYP/6-311++G** level the free energy favors the L^+ structure by 5.6 kJ mol^{-1}. The relative enthalpies (as defined by eq 2) and the relative free energies of the different conformations from the DFT and MP2 calculations are summarized in Table 2.

Finally, an alternative protonation site was considered at the MP2 level of theory. Instead of protonation at the N-terminus, the charge was placed on the central amide carbonyl group. The optimized structure, shown in Figure 3e, is closely related to the extended conformation described above. Protonation at the amide carbonyl group is less favorable than protonation at the N-terminus by 6.7 kJ mol^{-1} at the MP2/6-311++G** level. In a recent study of protonated triglycine using density functional theory, Rodriquez et al. found that protonation

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at the N-terminus amine was energetically favored over protonation at the carbonyl group nearest the N-terminus.[28] However, when the free energies were considered it was found that protonation at the carbonyl group was preferred at room temperature. This reversal apparently does not occur for protonated diglycine: when the free energies are considered protonation at the carbonyl group is less favorable than protonation at the N-terminus by 8.8 kJ mol^{-1} at room temperature. This difference is large enough that the N-terminus should be the dominant protonation site at room temperature.

MP2/6-311++G** optimized structures for the Gly−Gly+H$^+$ water adducts are also shown in Figure 3. Figure 3c is the extended structure with water bound to the protonated amine. The other structure shown in Figure 3d is close to the lowest energy conformation found in the MD simulations. This structure is designated **U**$^+$ because the structure is U-shaped with a bridging water between the protonated N-terminus and the C-terminal carbonyl group. The + designates positive ϕ and ψ angles (ϕ = 68.30° and ψ = 150.10°) (ϕ is the dihedral angle of 6C−10N−12C−15C and ψ is 1N−3C−6C−10N). Another form with negative ϕ and ψ angles (**U**$^-$) was also found to exist, with an energy essentially identical to that of **U**$^+$ at the HF 6-31G* level. The electronic energy of the **U**$^+$ complex is lower than for the extended complex by a large 19.4 kJ mol^{-1} at the MP2/6-311++G** level (10.9 kJ mol^{-1} with DFT) presumably because of the multiple interactions with the bridging water molecule. However, the difference in the free energies of these two structures is small, only 1.2 kJ mol^{-1} in favor of the **U**$^+$ form at the MP2/6-311++G** level. Thus, while the **U**$^+$/ **U**$^-$ structure is the lowest energy structure for Gly−Gly+H_2O+H$^+$ by a significant amount, it is likely that the extended form is also present under the experimental conditions. The **U**$^+$/ **U**$^-$ conformations are obviously related to the **L**$^+$/**L**$^-$ structures of the unsolvated Gly−Gly+H$^+$ peptide. A structure with the water bridging from the protonated N-terminus amine to the central amide carbonyl group does not have a low energy for geometric reasons: the hydrogen bonding partners are too close together to form good hydrogen bonds with both sites.

We also considered addition of water to the diglycine peptide protonated at the backbone carbonyl group. The energy gap between the two protonation sites is relatively small. Thus, it is possible that addition of a water molecule may preferentially stabilize one of the protonation sites over the other, perhaps even switching their order. The lowest energy structure found for the water complex of the diglycine peptide protonated at the backbone carbonyl group is an extended conformation with the water bound to the protonation site and the N-terminus pointing away. This structure has an electronic energy that is 18.3 kJ mol^{-1} higher (free energy 20.6 kJ mol^{-1} higher) than the lowest energy structure found for the peptide protonated at the N-terminus at the MP2/6-311++G** level. Without the water, the energy difference between the protonation sites is 6.7 kJ mol^{-1} (8.8 kJ mol^{-1} for free energy), so addition of a water molecule causes a significant stabilization of the protonation site at the N-terminus. When protonated at the amide carbonyl group, the peptide-water complex cannot form a more stable structure analogous to the **U**$^+$/ **U**$^-$ conformations (with a water molecule bridging between the protonated carbonyl group and the N-terminus amine or C-terminus carboxyl group) due to geometric restrictions: the hydrogen bonding partners are too close together for water to form good hydrogen bonds with both sites. We optimized structures of this type (taken from the MD simulations) at the HF/6-31G* level, but they had high energies compared to the **U**$^+$/ **U**$^-$ structures for Gly−Gly+H_2O+H$^+$ protonated at the N-terminus. Thus, there is a significant demerit to hydrating a charged carbonyl group. In solution, the backbone carbonyl groups are much less basic than the N-terminus and the N-terminus is the preferred protonation site, so addition of water to the unsolvated peptides is expected to favor protonation at the N-terminus.

Ala−Gly+H$^+$, Gly−Ala+H$^+$, and Ala−Ala+H$^+$. MP2/6-311++G** optimized structures for the nonextended forms of Ala−Gly+H$^+$, Gly−Ala+H$^+$, and Ala−Ala+H$^+$ are shown in Figure 4. As

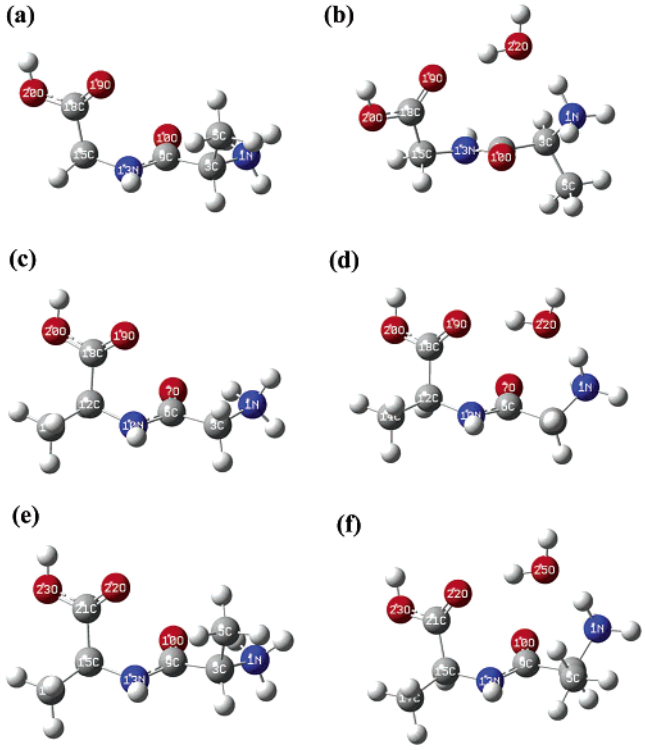

Figure 4. (a) MP2/6-311++G** optimized structure of Ala−Gly+H$^+$ in the **L**$^-$ conformation. (b) Ala−Gly+H_2O+H$^+$ in the **U**$^+$ conformation. (c) Gly−Ala+H$^+$ in the extended conformation. (d) Gly−Ala+H_2O+H$^+$ in the **U**$^-$ conformation. (e) Ala−Ala+H$^+$ in the extended conformation. (f) Ala−Ala+H_2O+H$^+$ in the **U**$^-$ conformation. Only non-hydrogen atoms are labeled.

with Gly−Gly+H$^+$, there are extended conformations that are only slightly less stable. The **L**$^-$ forms shown in Figure 4 for Ala−Gly+H$^+$, Gly−Ala+H$^+$, and Ala−Ala+H$^+$ have electronic energies that are 2.9, 0.1, and 0.8 kJ mol^{-1} lower than the extended conformations, respectively. The free energy differences are 2.0, −0.2, and 1.0 kJ mol^{-1}, respectively, so for Gly−Ala+H$^+$ the free energy favors the **L** structure (though by a negligible amount). The same general trends observed for Gly−Gly+H_2O+H$^+$ are also found for the other Gly/Ala peptide−water complexes. For Ala−Gly+H_2O+H$^+$, the **U**$^+$ form shown in Figure 4b has an electronic energy that is lower than for the extended complex by 20.9 kJ mol^{-1}. The lowest energy Gly−Ala+H_2O+H$^+$ peptide-water complex is the **U**$^-$ form shown in Figure 4d, which is 17.6 kJ mol^{-1} lower than the extended conformation. The Ala−Gly+H_2O+H$^+$ **U**$^+$ conformer was found to be 5.5 kJ mol^{-1} higher in energy than the **U**$^-$ structure at the B3LYP/6-311++G** level presumably because of unfavorable steric interactions involving the methyl side chain. The **U**$^-$ peptide−water complex, Ala−Ala+H_2O+H$^+$, shown in Figure 4f is more stable than the extended conformation by 15.1 kJ mol^{-1} (and 3.4 kJ mol^{-1} more stable than the **U**$^+$ structure at the B3LYP/6-311++G** level). In terms of free energy, the **U**$^+$/**U**$^-$ peptide−water complexes are favored over the extended structures by 0−5 kJ mol^{-1}.

Pro−Gly+H$^+$. Calculations were performed only up to the B3LYP/6-311++G** level for this peptide. The lowest energy structure found for unsolvated Pro−Gly+H$^+$ is the extended structure shown in Figure 5a. It has two C_5 rings closed by weak intramolecular hydrogen bonding. The **L**$^-$ structure shown in Figure 5b is 2.7 kJ mol^{-1} less stable than the extended conformation at this level of theory. This difference is 2.9 kJ mol^{-1} when free energies are considered. We also considered an alternative protonation site for this peptide. Protonation at the CO group adjacent to the N-terminus was disfavored by 12.5 kJ mol^{-1} compared to protonation at the N-terminus at the B3LYP/6-311++G** level. For the Pro−Gly+H_2O+H$^+$ complex the extended structure in

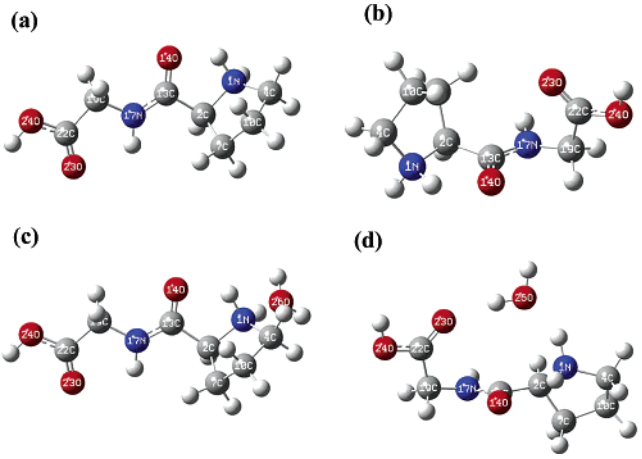

Figure 5. B3LYP/6-311++G** optimized structure of Pro−Gly+H^+ in the (a) extended conformation and (b) $\mathbf{L}^-$ conformation. Pro−Gly+H_2O+H^+ in the (c) extended conformation and (d) $\mathbf{U}^+$ conformation. Only non-hydrogen atoms are labeled.

Figure 5c lies 5.8 kJ mol^{-1} above the $\mathbf{U}^+$ conformation shown in Figure 5d. Interestingly, the extended conformation is favored over the $\mathbf{U}^+$ conformation in terms of free energy by 5.9 kJ mol^{-1}. It is important to note that DFT calculations for the Ala/Gly peptides favor the extended structure by free energies of 0−6 kJ mol^{-1} (see Table 2). This is reversed at the higher MP2 level of theory, where the $\mathbf{U}^+/\mathbf{U}^-$ peptide−water complexes are favored over the extended ones by free energies of 0−5 kJ mol^{-1}. Thus, the $\mathbf{U}^+$ structure for Pro−Gly+H_2O+H^+ is expected to become more favorable at the higher level of theory.

Gly−Trp+H^+. With the Gly−Trp+H^+ peptide there is the possibility of interactions between the cation and the delocalized π ring. These interactions have been an object of considerable interest.[59] The CHARMM force field does not include parameters to specifically describe this interaction. Thus, there is the possibility that the CHARMM force field may be biased against structures where cation-π interactions are important. Therefore, we performed a more extensive conformational search for this peptide. An additional 26 structures were randomly selected from ten 400-K 960-ps MD runs. All of these were optimized at the HF/6-31G* level. Again, the same lowest energy structure was found many times. This structure was essentially identical to the lowest energy structure obtained from the 300 K MD simulations performed for this peptide. The lowest energy structure after B3LYP/6-311++G** optimization is shown in Figure 6a. The charge at the N-terminus is interacting with the indole ring (this structure is denoted **I** to indicate the presence of this cation−π interaction). In a recent publication it was suggested that the exact manner in which the charge interacts with the ring is quite flexible; meaning that many configurations can coexist with little energy barrier between them.[60] Structure **I** can be viewed as a variant of the extended structures discussed above.

A procedure identical to that described above for the unsolvated Gly−Trp+H^+ peptide was also employed for the water complex. Here, however, optimization of the 26 structures randomly chosen from the 400 K simulations led to a diverse range of different conformations only one of which (the lowest energy one) corresponded to the lowest energy structure found in the 300 K simulations. The lowest energy B3LYP/6-311++G** optimized structure is shown in Figure 6b. It is analogous to the other $\mathbf{U}^-$ structures discussed above. Other $\mathbf{U}^-$ structures were found, but they were less favorable by at least 6 kJ mol^{-1} at the HF/6-31G* level. The critical factor appears to be the sign of the side-chain dihedral angle χ_1 (the dihedral angle described by 10N−12C−14C−17C in Figure 6) and $\chi_2^{3,1}$ (dihedral angle described by 12C−14C−17C−18C in Figure 6). For the $\mathbf{U}^-$ structure in Figure 6b, these angles are positive ($\chi_1 = 54.34°$, $\chi_2^{3,1} = 94.19°$), whereas the less favored structures have negative torsion angles of the same magnitude. The extra stability arises from the proximity of the indole ring to both the backbone N−H and the N-terminal charge when both dihedral angles are positive. Another structure that is 11.4 kJ mol^{-1} less stable at HF/6-31G* level than the lowest energy $\mathbf{U}^-$ structure is shown in Figure 6c. This structure is simply the **I** structure in Figure 6a with the water dangling off of the N-terminal charge. At the B3LYP/6-311++G** level the electronic energy of the $\mathbf{U}^-$ form is 7.5 kJ mol^{-1} lower than for the hydrated **I** form. In terms of free energy, the **I** form is more favorable by an insignificant 0.8 kJ mol^{-1}. How the free energy would change with a higher level of theory is difficult to predict.

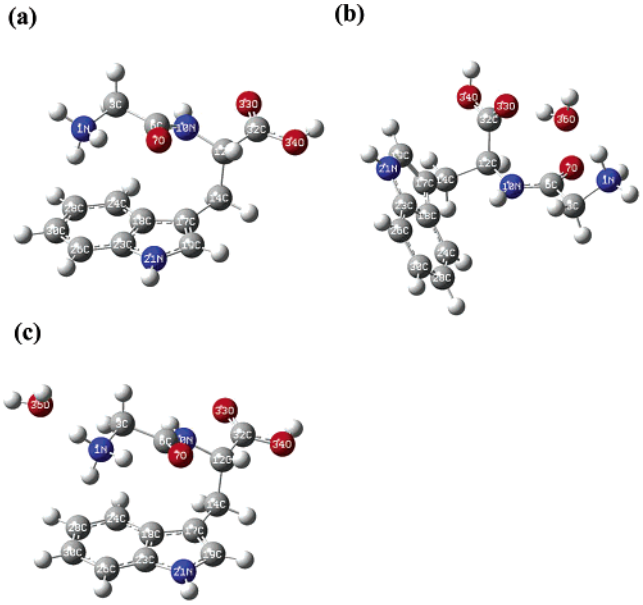

Figure 6. B3LYP/6-311++G** optimized structure of (a) Gly−Trp+H^+ in the **I** conformation. Gly−Trp+H_2O+H^+ in the (b) $\mathbf{U}^-$ conformation and in the (c) **I** conformation. Only non-hydrogen atoms are labeled.

Discussion

The calculations suggest that the equilibria studied experimentally do not involve single reactant and product conformations. For the unsolvated peptides studied here (except Gly−Trp+H^+) there are extended and bent forms ($\mathbf{L}^+/\mathbf{L}^-$) with similar energies and similar free energies. For the peptide−water complexes the $\mathbf{U}^+/\mathbf{U}^-$ conformations with a bridging water molecule are 15−20 kJ mol^{-1} more stable in electronic energy than the extended conformations for the Gly/Ala peptides because of the stabilization offered by the extra hydrogen bond between the C-terminal carbonyl group and water. However, the free energy difference shrinks to 0−5 kJ mol^{-1} because of the large entropic demerit inherent in the **U** type structures, which lack the low-frequency "wriggling" mode available to the extended counterparts. For Pro−Gly+H_2O+H^+ (where calculations were only done up to the B3LYP/6-311++G** level) the ordering of the extended and **U**-type structures is reversed (the extended is lower) when free energies are considered, but as mentioned earlier, this is most likely an artifact from the DFT calculations. For Gly−Trp+H_2O+H^+, the $\mathbf{U}^-$ structure is competitive with a conformations stabilized by cation-π interactions (the **I**-form). The MD simulations suggest that the different conformations freely interconvert and so they are expected to be in equilibrium with each other on

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the experimental time scale. Therefore, the different conformations cannot be separated by ion mobility methods, at least at the temperatures employed in the experiments.

Because multiple conformations are involved, it is not very useful to compare the enthalpy changes and entropy changes calculated for particular conformations to the experimental values. To derive average enthalpy and entropy changes for comparison with the measured values we established a thermodynamic cycle involving water addition to the main species and interconversion between the different conformations

$$E \rightleftarrows L$$

$$L + H_2O \rightleftarrows U{\cdot}H_2O$$

$$E + H_2O \rightleftarrows E{\cdot}H_2O$$

$$U{\cdot}H_2O \rightleftarrows E{\cdot}H_2O$$

The calculated enthalpy and entropies for these species were used to determine equilibrium constants for the individual steps. We then calculated overall equilibrium constants for the addition of a water molecule to the unsolvated peptides over the temperature range accessed experimentally, and used these values to derive average enthalpy and entropy changes for comparison with the experimental values. The results of this analysis are shown in Table 1. The obvious shortcoming to this procedure is that it assumes that only a few structures contribute while the potential energy surfaces of these small dipeptides are relatively flat. On the other hand, it is not realistic to do high level calculations for a large number of conformations and so the approach adopted here is a compromise. The average enthalpy and entropy changes derived from the calculations using this analysis lie between the extremes obtained by considering the individual steps.

It is evident from Table 1 that the calculated average enthalpy and entropy changes are in good overall agreement with the experimental results. For the Gly/Ala peptides the calculated $\Delta H°$ values are systematically larger than the measured quantities. The average deviation is 5.6 kJ mol^{-1}, but the deviation for Ala−Gly+H$^+$ stands out as being significantly larger than the others. The calculated $\Delta S°$ values for the Gly/Ala peptides are all systematically larger than the measured quantities. The average deviation is 22 J K^{-1} mol^{-1}. For the unsolvated Gly/Ala peptides the extended conformations have slightly lower free energies in the calculations (by 0−2 kJ mol^{-1}), whereas the **U**$^+$/**U**$^-$ peptide water complexes are favored (by 0−5 kJ mol^{-1}). Thus the equilibrium is primarily between the extended forms of the unsolvated peptides and the **U** forms of the peptide water complex, which involves a significant conformational change. The origin of the discrepancy between the measured and calculated $\Delta S°$ is almost certainly the vibrational component of the entropy. It is most likely due to an underestimate of the entropy for the low-frequency water−peptide modes in the water complex due to the effects of anharmonicity. Although it is also possible that there is a systematic error in the calculated vibrational frequencies for these low frequency modes. Regardless of the source, the discrepancy in the entropies will also lead to a systematic underestimate of the free energies of the complexes (i.e., the free energies of the complexes should be slightly more negative than determined from the calculations).

Table 2 summarizes the difference between DFT and MP2 results. In both the unhydrated and hydrated forms, DFT is biased toward the extended structure by at least 6 kJ mol^{-1} in both enthalpy and free energy (and in the electronic energy by a comparable magnitude). The discrepancy between the DFT and MP2 is presumably due to the absence of dispersion interactions in the DFT calculations. Dispersion interactions are expected to favor the more compact conformations over the extended ones, which is consistent with the discrepancies observed here. Regardless of the origin of the differences between the MP2 and DFT results, caution is clearly necessary in relying solely on DFT to determine the energetic details of even small and simple peptides.

For Pro−Gly+H$^+$ and Gly−Trp+H$^+$, calculations were only done up to the B3LYP/6-311++G** level. By comparing the B3LYP/6-311++G** results to those obtained from the MP2/6-311++G** calculations for the Gly/Ala peptides we anticipate that the B3LYP/6-311++G** calculations will underestimate the overall $\Delta H°$ by 0−4 kJ mol^{-1} and overestimate the overall $\Delta S°$ by 0−10 J K^{-1} mol^{-1} compared to the MP2/6-311++G** results. The calculations correctly reproduce the significant decrease in the overall $\Delta H°$ and $\Delta S°$ values for Pro−Gly+H$^+$ compared to the Gly/Ala peptides (see Table 1). This decrease can be attributed largely to the fact that the water is interacting with a secondary amine in Pro−Gly+H$^+$ as opposed to a primary amine in the Gly/Ala peptides. This is supported by the observation that $\Delta H°$ and $\Delta S°$ for hydration of Pro−Gly+H$^+$ are similar to those for protonated pyrrolidine (proline without the carboxylic acid group) for which $\Delta H° = -57.3$ kJ mol^{-1} and $\Delta S° = -88.3$ J K^{-1} mol^{-1}.[61] Another important factor is the destabilization of the **U**-type conformation of Pro−Gly+H_2O+H$^+$ peptide−water complex. The **U**-type Pro−Gly+H_2O+H$^+$ is more strained than the Gly/Ala analogues. The distance between the two termini is larger, and the hydrogen bonds to the water molecule are longer than in the Gly/Ala peptides. For example, in the Gly/Ala **U**-type structures the typical distance between the oxygen atom in water and the charged amine hydrogen is 1.68 Å. This distance is 1.79 Å in Pro−Gly+H_2O+H$^+$. The distance between the C-terminal carbonyl oxygen and the nearest hydrogen in water is typically 1.83 Å in the Gly/Ala **U**-type structures. This distance increases to 1.90 Å for Pro−Gly+H_2O+H$^+$. These conformational differences are presumably caused by the proline ring. They lead to a decrease in the energy difference between the extended and **U**-type conformations (the **U**-type conformation is destabilized) compared to the energy differences between these conformations for the Gly/Ala peptides.

The lowest energy conformation for unsolvated Gly−Trp+H$^+$ is the **I**-form which is stabilized by cation-π interactions. The **I**-form of the Gly−Trp+H_2O+H$^+$ peptide−water complex is 7.5 kJ mol^{-1} less stable than the **U**-form at the B3LYP/6-311++G** level, but the advantage in terms of free energy dwindles to an insignificant 0.8 kJ mol^{-1}. This ordering may reverse, in favor of the U form, at the MP2/6-311++G** level. It is evident from the results shown in Table 1 that the $\Delta H°$ for addition of a water molecule to Gly−Trp+H$^+$ is significantly smaller than for the other dipeptides studied here. The dominant water adsorption process on this peptide involves a geometry

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change form the **I**-form and the **U**-form, at least at the B3LYP/6-311++G** level. A cation-π interaction stabilizes the unsolvated peptide, but this interaction is disrupted in the lowest free energy peptide−water complex, and so $\Delta H°$ for addition of a water molecule is expected to be diminished by the strength of this interaction when compared to the other peptides. The deviation between the measured and calculated $\Delta H°$ and $\Delta S°$ is significant for Gly−Trp+H^+ (see Table 1). The calculations overestimate the enthalpy change by more than 8 kJ mol^{-1}.

Figure 2 shows a plot of $\Delta S°$ vs $\Delta H°$ for the results reported here (triangles) and results obtained previously on larger alanine-based polypeptides[33] (circles). There are obvious correlations between $\Delta S°$ and $\Delta H°$. In general, a small (less negative) ΔH° indicates weaker interactions between the water molecule and the peptide (though this generalization ignores the effects of structural changes such as those discussed above for Gly−Trp+H^+). Weaker interactions should result in lower frequency vibrations between the water and the peptide in the complex, which in turn causes a larger vibrational entropy for the complex and a smaller (less negative) $\Delta S°$ for the reaction. It is evident from Figure 2 that the results for the dipeptides and the larger peptides are not correlated. The line for the larger peptides has a significantly steeper slope. The large negative entropy changes for addition of a strongly bound water molecule to the larger peptides result because the water molecule stiffens up the peptide, compensating for the additional vibrational entropy that results from the new petide−water vibrational modes. This stiffening does not occur for the dipeptides.

Conclusions

The addition of water to the dipeptides studied here is a complex process involving multiple conformations with similar free energies. For Gly/Ala peptides the equilibrium probed experimentally is primarily between the extended forms of the unsolvated peptides and the **U**-forms of the peptide water complex, and involves a significant conformational change. Comparison of DFT and MP2 results shows that for both the unhydrated and hydrated forms, DFT is biased toward the extended structure. Caution is clearly necessary in relying solely on DFT to determine the energetic details of these systems.

For the Pro−Gly+H_2O+H^+ peptide-water complex, the overall decrease in enthalpy and entropy of water adsorption is mainly due to the location of the protonation site at a secondary amine. A contributing factor is that the **U**-type conformation is destabilized by strain (because of the proline ring) but the equilibrium is still expected to involve conformational change. The lowest energy conformation for unsolvated Gly−Trp+H^+ is stabilized through cation-π interactions. However, these interactions are disrupted in the lowest energy Gly−Trp+H_2O+H^+ peptide−water complex which has a **U**-type conformation. Loss of the cation-π interactions leads to a substantial decrease in the enthalpy change for addition of a water molecule. Overall, there is reasonably good agreement between the calculated average enthalpy and entropy changes and the measured quantities. The largest discrepancy was observed for the Gly−Trp+H^+ peptide. Finally, we note that adsorbing a single water molecule on the Gly−Gly dipeptide significant increases the proton affinity of the N-terminus amine relative to the backbone carbonyl groups.

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Supporting Information Available: Electronic energies, enthalpies, entropies, bond angles, and bond lengths for all the conformations optimized using B3LYP/6-311++G** and MP2/6-311++G**. This material is available free of charge via the Internet at http://pubs.acs.org.

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