Dependence of the AmII′p Proline Raman Band on Peptide Conformation

Zeeshan Ahmed, Nataliya S. Myshakina, and Sanford A. Asher*

Department of Chemistry, University of Pittsburgh, Pennsylvania 15260

Received: November 8, 2008; Revised Manuscript Received: May 15, 2009

We utilized UV resonance Raman (UVRR) measurements and density functional theory (DFT) calculations to relate the AmII′p frequency to the ψ angle. The AmII′p frequency shifts by $\sim$25 cm^{-1} as the ψ angle is varied over allowed angles of the Pro peptide bond. The AmII′p frequency does not show any significant dependence on the φ dihedral angle. The conformation sensitivity of the AmII′p frequency derives from conformation-induced changes in the planarity of the Pro peptide bond; ψ angle changes push the amide nitrogen out of the peptide bond plane. We use this AmII′p frequency dependence on the ψ angle to track temperature-induced conformation changes in a polyproline peptide. The temperature-induced 7 cm^{-1} downshift in the AmII′p frequency of the polyproline peptide results from an $\sim$45° rotation of the ψ dihedral angle from ψ = 145° (ideal PPII conformation) to ψ = 100° (collapsed PPII conformation).

Introduction

The unique pyrrolidine ring side chain of the proline amino acid loops back onto itself to form a tertiary amide that imposes significant restrictions on the N−C$_\alpha$ (φ) bond rotation.[1−5] The reduced conformational freedom of Pro residues enforces local order in proteins and peptides which is often utilized in the nucleation and control of secondary structure motifs.[1−10] Lacking an amide hydrogen, Pro residues cannot engage in more than one interpeptide hydrogen bond.[8,9,11−14] Consequently, Pro residues are typically found at the start of α-helices, the edges of β-sheets, and, most frequently, loops, unordered, and turn regions.[1] When located in the middle of stable helices, such as in trans-membrane proteins, Pro residues induce a kink along the α-helical axis.[15,16]

The Pro peptide bond's *cis*−*trans* isomerization can influence protein conformation during folding, as it often controls the rate limiting step.[17,18] For example, in refolding of ribonuclease T$_1$, the Pro *cis*−*trans* isomerization rate constant is estimated to be 1×10^3 s^{-1}.[19] In contrast, a typical protein such as cytochrome b_{562} has a refolding rate of 2×10^5 s^{-1}.[20−22]

Given the important impact of Pro peptide bond isomerization on folding kinetics, it is important to identify spectroscopic markers that can differentiate between *cis* and *trans* isomers of the Pro peptide bond. While it is possible to differentiate isomeric states of Pro by ^{13}C NMR spectroscopy,[23−26] no such clear-cut quantitative markers yet exist in IR[27] or Raman spectroscopy.[28,29] Recent Raman studies have investigated the AmII′ band of Pro (AmII′p) as a possible marker for Pro isomerization.[29−34]

The AmII′p vibration is similar to the AmII′ vibration of deuterated amide bonds in that it involves significant C−N stretching without any N−H(D)$_b$ bending component.[35−38] The Raman AmII′p frequency and intensity has been experimentally observed to depend upon protein conformation.[31−33] In addition, the band frequency appears to depend on the identity of the neighboring (i − 1) residue.[34] These studies also led to the suggestion that the AmII′p frequency is sensitive to the isomeric state of the Pro peptide bond. However, significant disagreements exist in the literature over the quantitative interpretation of the AmII′p band frequency dependence.[31−34]

Caswell and Spiro reported that in polyproline the AmII′ band downshifts from 1465 to 1435 cm^{-1} upon conversion of polyproline from the PPII (*trans*) to the PPI (*cis*) conformation.[31] However, Harhay and Hudson[30] reported that, at 200 nm excitation, simple X-Pro dipeptides did not show any changes in the AmII′p band frequencies when their *cis* content was increased via pH increases. These authors also attributed the observed decrease in the AmII′p band intensity to a pH-induced bathochromic shift of the UV absorption.[30]

An alternative interpretation of the AmII′p spectral frequency dependence was suggested by Takeuchi and Harada[34] who proposed that the shift in the band position observed during denaturation of proteins could be due to changes in the hydrogen bonding of the Pro peptide bond. The authors reported that in aprotic solvents such as acetonitrile, the AmII′p downshifts by $\sim$25 cm^{-1} as compared to aqueous solution, suggesting that solvent−amide hydrogen bonding is primarily responsible for the observed changes in band position.[34]

Takeuchi and Harada's[34] hydrogen bonding mechanism, however, fails to reconcile the frequency differences observed in small, solvent accessible, X-Pro dipeptides where the band positions are known to differ by as much as 10 cm^{-1} depending upon the identity of the neighboring residue (i − 1). Jordon et al.[39] suggested that the side chain modes of the i − 1 residue likely couple with the C−N$_s$ vibration of the Pro peptide bond.

A more recent study by Triggs and Valentini, however, directly contradicts Takeuchi and Harada's[34] interpretation of the AmII′p frequency shift.[40] In their UV-Raman study, utilizing preresonance enhancement, Triggs and Valentini systematically examined the impact of solvation and hydrogen bonding by using model peptide bonds of ε-caprolactam, *N*,*N*-dimethylacetamide (DMA), and *N*-methylacetamide (NMA) in the liquid, aqueous, and gaseous phases.[40] Their results demonstrate that the AmI (C═O$_s$) frequency is sensitive to hydrogen bonding. However, the frequency of the AmII′-like vibrations of DMA (a tertiary amide) and ε-caprolactam (a *cis* amide) shows no significant dependence on hydrogen bonding.[40]

In a recent theoretical study of NMA and NMA−water complexes (and their deuteratred isotopomers), we recently

* To whom correspondence should be addressed. Phone: 412 624 8570. Fax: 412 624 0588. E-mail: asher@pitt.edu.

10.1021/jp809857y CCC: $40.75
Published on Web 07/23/2009

demonstrated that the AmII and AmII′-like vibrations of NMA and *d*-NMA lack significant dependence on C═O hydrogen bonding because the C—N_s motion makes a relatively small contribution to the AmII (~25%) and AmII′-like vibrations (~8%).[41] In contrast, the hydrogen-bond-dependent AmI vibration is >75% C═O_s. The hydrogen bond dependence of the AmII (C—N_s and NH_b) vibration of NMA derives from its N—H_b component which makes up to 50% of the AmII normal mode composition.[41]

The apparent lack of an AmII′p hydrogen bonding frequency dependence obscures our understanding of AmII′p frequency shifts. In particular, it frustrates our understanding of conformation/hydration changes in Pro rich peptides such as the elastin peptides. These biologically important peptides undergo a large volume change in response to specific stimuli such as temperature or ionic strength.[42]

Here, we systematically examine the conformation, isomerization, and hydrogen bond dependence of the AmII′p frequency of various Pro derivatives using a combination of UV resonance Raman (UVRR) measurements and density functional theory (DFT) calculations. Our results indicate that the AmII′p band position is insensitive to changes in amide—water hydrogen bond strength.

The frequency of the *cis* and *trans* conformers differs by ~8 cm^{-1}. We find that the AmII′p band position is very sensitive to nonplanarity of the Pro peptide bond. The peptide bond nonplanarity is modulated by conformation changes that alter the ψ angle such that the amide nitrogen is pushed out of the peptide bond plane. This result allows us to correlate the AmII′p Raman band frequency to the local conformation of the Pro peptide bond.

Experimental Section

The UV resonance Raman (UVRR) spectrometer has been described in detail elsewhere.[43] Briefly, 204 nm UV light was obtained by generating the fifth anti-Stokes Raman harmonic of the third harmonic of a Nd:YAG laser (Coherent, Infinity) in H_2 gas. The sample was circulated in a free surface, temperature controlled stream. A 165° backscattering geometry was used for sampling. The collected light was dispersed by a subtractive double monochromator onto a back thinned CCD camera (Princeton Instruments-Spec 10 System).[43]

Ac-Pro and X-Pro dipeptides (X = Trp, Ala, Gly, Val, Leu, Ser, and Phe) were acquired from Bachem, while polyproline (m.w. = 5800), sodium perchlorate, and D_2O were acquired from Sigma-Aldrich. The chemicals were used as received. A 1 mg/mL peptide concentration in 0.2 M sodium perchlorate solution was used for UVRR measurements.

Computational Details. All calculations were performed using the Gaussian 03[44] calculation package at the DFT[45−47] level of theory employing the B3LYP[48−50] combinational functional and 6-311+G* basis set. Calculated frequencies were scaled by a 0.98 scaling factor.[51,52] The polarizable continuum model (PCM) as implemented in Gaussian 03 was utilized to account for solvent effects. We optimized the geometry and calculated the harmonic vibrational frequencies of the following species:

(1) The *cis* and *trans* isomers of Ac-Pro-Me, where the *cis* and *trans* isomers were defined by the ω torsional angle C′—N—C—C″. During geometry optimization, this torsional angle was fixed at 180° for the *trans* conformer and 0° for the *cis* conformer.

(2) The frequencies of the optimized *trans* zwitterionic Ala-Pro molecule ($\varphi = -90°$, $\psi = 145°$) were calculated in a vacuum ($\varepsilon = 1.00$) and in water ($\varepsilon = 78.39$), acetonitrile ($\varepsilon = 36.64$), and heptane ($\varepsilon = 1.92$) to probe the impact of the dielectric constant on the AmII′p vibrational frequency. Similarly, we calculated the frequency of *trans* zwitterionic Ala-Pro in water (PCM) hydrogen bonded to an explicit water molecule at the C═O site. The angle between the water molecule and the C═O group was fixed at 180° during optimization.

(3) A series of zwitterionic Ala-Pro conformers with the φ dihedral angle fixed at −80° and $\psi = -90$, −70, −60, −50, −45, 60, 90, 120, 140, 145, and 150° were calculated in water. In heptane (PCM), only $\psi = -90$, −60, −45, 60, 120, 145, and 160° values were calculated. In gas phase calculations, Ala-Pro conformers were calculated for $\varphi = -80°$ and $\psi = -90$, −60, −45, 60, 120, and 145°.

(4) A series of zwitterionic Ala-Pro conformers with $\psi = 145°$ and $\varphi = -60$, −90, −100, and −120° were calculated for Ala-Pro in water and in the gas phase. In heptane and acetonitrile, we calculated conformers with $\varphi = -60$, −90, and −120°. To prevent any impact from possible charge transfer/electrostatic interactions between the C═O and the N-termini, we froze the NH_3^+ rotation during the geometry optimization.

The structures of a 10-mer collapsed polyproline ($\varphi = -80°$, $\psi = 100°$) and canonical PPII polyproline ($\varphi = -80°$, $\psi = 145°$) were calculated by utilizing the *protein* utility in Tinker[53] and visualized using VMD software.[54] End-to-end distance and radii of the two polymers were estimated using CAChe (Fujitsu). The solvent accessible surface area of both polymers was calculated using the Spacefill utility of Tinker, utilizing a 1.4 Å radius probe to calculate the accessible and excluded volumes.

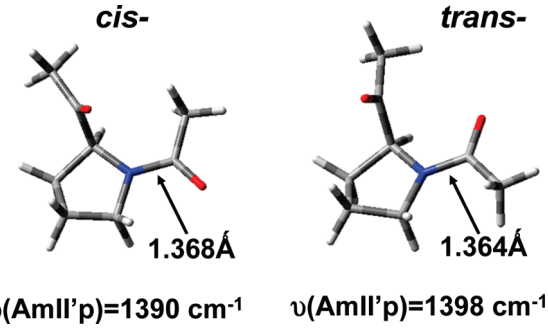

Figure 1. Calculated structures of *cis* and *trans* isomers of Ac-Pro-Me. The C—N bond length elongates (0.004 Å) in the *cis* conformation, resulting in an 8 cm^{-1} downshift of the AmII′p vibration. The C═O bond length contracts by 0.003 Å which results in a 13 cm^{-1} upshift of the AmI′ vibration.

Results and Discussion

Impact of *cis*—*trans* Isomerization. We examined the impact of *cis*—*trans* isomerization on the AmII′p frequency by calculating the vibrational spectra of the *cis* and *trans* conformers of methylated Ac-Pro (Figure 1). Our calculations show that *trans* → *cis* isomerization results in a slight elongation of the C—N bond length (~0.004 Å) and a nearly equal contraction of the C═O bond length (0.003 Å). The elongation of the C—N bond length results in an 8 cm^{-1} downshift of the *cis*-AmII′p vibration, while the *cis*-AmI′ vibration upshifts by 13 cm^{-1}.

Changes in the calculated peptide bond geometry likely derive from differences in electron distribution between the *cis* and *trans* conformers. According to Hinderaker and Raines,[55] the PPII conformation of proline peptides is stabilized by n → π^*

TABLE 1: Calculated Geometric Parameters of *cis* and *trans* Conformers of Ac-Pro-Me

	trans-proline	*cis*-proline
d(C—N), Å	1.364	1.368
d(C═O), Å	1.227	1.223
$d(O_{i-1}\cdots C_i)$, Å	3.045	4.393
$\angle(O_{i-1}\cdots C_i{=}O_i)$, deg	102.51	78.27
$d(C_{i-1}{=}O_{i-1})$, Å	1.211	1.209

interactions which result in delocalization of a nonbonding pair of electrons from the amide oxygen's n orbital to the neighboring amide oxygen π^* orbital.[55] The authors suggest that significant n $\rightarrow \pi^*$ interactions occur when the $O_{i-1}\cdots C_i$ distance is $\leq$3.2 Å and the $O_{i-1}\cdots C_i{=}O_i$ angle falls between 99 and 119°.[55] As shown in Table 1, only the calculated *trans* proline geometry satisfies these criteria.

The n $\rightarrow \pi^*$ interaction results in redistribution of electronic density away from the oxygen's lone pair orbital.[55] Therefore, in the *trans* PPII conformer, the C═O double bond character decreases, while the C—N bond order increases. Lacking this n $\rightarrow \pi^*$ charge transfer, *cis*-proline has a lower C—N bond order, which gives rise to the calculated 8 cm^{-1} downshift of the AmII′p vibration upon *trans* $\rightarrow$ *cis* isomerization.

Impact of Hydrogen Bonding. As discussed above, the work of Triggs and Valentini[40] contradicts Takeuchi and Harada's[34] suggestion that the AmII′p frequency is sensitive to the hydrogen bonding state of the Pro peptide bond. The frequencies of the AmII′-like vibrations of tertiary (DMA) and cis (ε-caprolactum) amides do not a show significant sensitivity to hydrogen bonding.[40]

Here, we re-examine the impact of water—peptide hydrogen bonds by examining the temperature dependence of the Raman spectra of Ac-Pro, Ala-Pro, Gly-Pro, Phe-Pro, Ser-Pro, and Val-Pro dipeptides.[56−59] The AmII′ vibration of N-deuterated NMA (*d*-NMA) in D_2O shows a significant temperature dependence (−0.07 cm^{-1}/°C).[56] If the observed frequency shift of the AmII′ band of N-deuterated NMA (*d*-NMA) primarily derives from hydrogen bonding changes at the carbonyl, then the AmII′p should show a similar temperature dependence ($\sim$4 cm^{-1} shift over a 60 °C interval). However, if the temperature dependence of the AmII′ band of *d*-NMA derives from its small N—D_b component (5%),[40,60,61] as suggested by Triggs and Valentini,[40] then the AmII′p, which altogether lacks the N—H bond, will not be significantly impacted by changes in carbonyl hydrogen bonding.

As shown in Figure 2, the frequency of the AmII′p of Ala-Pro barely downshifts from 1488 to 1487 cm^{-1} as the solution temperature increases from 4 to 65 °C. The band intensity, however, shows an $\sim$22% decrease. We observe similarly insignificant temperature-induced frequency shifts in other Pro dipeptides (Table 2). These results clearly indicate that changes in hydrogen bond strength do not significantly impact the AmII′p frequency.

Our recent theoretical study of NMA—water complexes demonstrated that C═O—water hydrogen bonding impacts the peptide bond geometry, resulting in the elongation of the C═O bond and contraction of the C—N bond.[41] The AmII and AmII′-like vibrations of NMA and *d*-NMA, however, lack significant dependence on C═O hydrogen bonding because C—N_s motion makes a relatively small contribution to the AmII ($\sim$25%) and AmII′-like vibrations ($\sim$8%).[41] In contrast, the C═O hydrogen-bond-sensitive AmI vibration is 75% C═O_s.

A lack of significant hydrogen bond strength dependence of the AmII′p frequency in small, water accessible Pro dipeptides

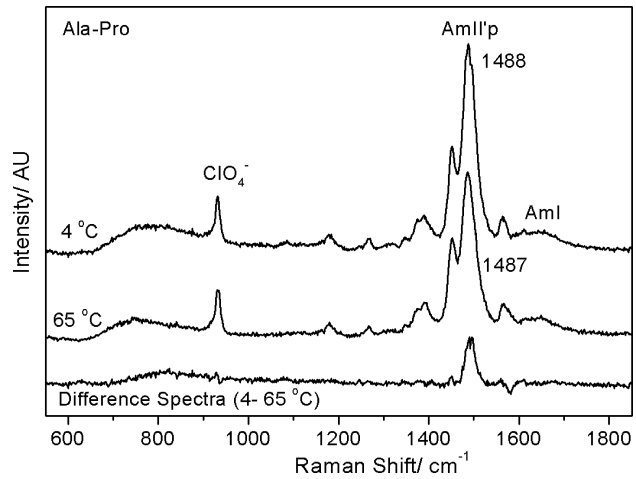

Figure 2. The 204 nm excited AmII′p band of Ala-Pro (1 mg/mL) showing an insignificant change in frequency ($\Delta\nu$ = 1 cm^{-1}) as the temperature is increased from 4 to 65 °C. The band intensity, however, shows a 22% decrease with increasing temperature.

TABLE 2: Temperature Dependence of AmII′p Frequency

peptide	AmII′p frequency at 4 °C (cm^{-1})	AmII′p frequency at 65 °C (cm^{-1})	$\Delta\nu$/°C (cm^{-1}/°C)
Ala-Pro	1488	1487	−0.017
Gly-Pro	1486	1485	−0.017
Ser-Pro	1482	1480	−0.03
Val-Pro	1476	1475	−0.017

suggests that the normal mode composition of the AmII′p vibration contains relatively little C—N_s. Indeed, our theoretical calculations of Ala-Pro with PCM water indicate that the AmII′p normal mode composition contains only $\sim$26−28% C—N_s motion (Table 3). A relatively small C—N_s contribution minimizes the impact of hydrogen-bond-induced peptide bond geometry changes.

The $\sim$25 cm^{-1} downshift in the AmII′p frequency observed by Takeuchi and Harada[34] in acetonitrile cannot mainly result form *cis*—*trans* isomerization of the proline peptide bond. As discussed above, we calculate that the *cis* conformer downshifts 8 cm^{-1} from that of the trans conformer. Takeuchi and Harada's[34] 25 cm^{-1} downshift of AmII′p frequency could derive from conformational alterations about the φ and ψ angles. Alternatively, the AmII′p frequency downshift may derive from differences in the solvent dielectric constant.[62] For the AmI vibration, previous studies indicate that the hydration-induced frequency downshift requires both the solvent dielectric constant increase (bulk water) and explicit hydrogen bonding of the peptide bond.[62−64] The frequency downshifts of the AmI vibration in NMA observed in protic solvents (explicit hydrogen bonding) are far larger than those observed in aprotic solvents with similar dielectric constants.[62,63]

Our temperature-dependent UVRR experiment (Figure 2) directly probes the impact of hydrogen bond strength on the AmII′p frequency. Dielectric constant changes are relatively minor. In contrast, Takeuchi and Harada's[34] experiment replaces water with acetonitrile as the solvent media, incurring large changes in both the dielectric constant and the hydrogen bonding state of the peptide bond. Such large changes in the environment may impact the peptide bond geometry and/or the normal mode composition of the AmII′p vibration.

We evaluated the impact of the dielectric constant changes on the AmII′p frequency of Ala-Pro by using DFT calculations in PCM water (ε = 78.39), acetonitrile (ε = 36.64), heptane (ε = 1.92), and a vacuum (ε = 1.00). Our results indicate that the

TABLE 3: Calculated Normal Mode Composition of Ala-Pro ($\psi = 145°$)

	gas phase		water	
conformation	υ(AmII′p)	PED(>5%)	υ(AmII′p)	PED(>5%)
$\varphi = -60°$	1442	CH_3 sym def (25) −C−N s (21) −C═O s (10) C−C s (8) CH_3 asym def′ (6) −C═O inp b (6)	1470	C−N s (28) $-CH_3$ asym def′ (23) −C−C s (8) C═O s (7) C═O inp b (6)
$\varphi = -90°$	1457	C═O s (20) C−N s (19) $-CH_3$ asym def′ (8) −C−C s (8) $-CH_3$ sym def (7) C═O inp b (6)	1471	CH_3 asym def′ (26) −C−N s (26) C−C s (7) −C═O s (7) −C═O inp b (6)

TABLE 4: Ala-Pro ($\varphi = -90°$, $\psi = 145°$) AmII′p Frequency Dependence on Solvent Dielectric Effect and Hydrogen Bonding

solvent media	dielectric constant	AmII′p frequency/cm^{-1}	AmI′ frequency/cm^{-1}
water	78.39	1471	1661
water + H_2O[a]	78.39	1470	1648
acetonitrile	36.64	1472	1664
heptane	1.92	1466	1703
vacuum	1.00	1457	1715

[a] Ala-Pro hydrogen bonded to an explicit water molecule, immersed in PCM water.

AmII′p frequency downshifts by 5 cm^{-1} whereas the AmI′ frequency upshifts by 42 cm^{-1} as the dielectric constant decreases from 78.39 (water) to 1.92 (heptane, Table 4). The calculated 9 cm^{-1} difference in the AmII′p frequency between the gas phase and heptane derives from the PCM perturbation to the AmII′p mode composition. The relative change in the AmII′p frequency between water and acetonitrile is negligible, which suggests that the 25 cm^{-1} downshift in the AmII′p frequency observed by Takeuchi and Harada[34] does not derive from differences in the solvent dielectric constant.

We examined the impact of local hydrogen bonding on the AmII′p frequency by calculating the frequency of the AmII′p vibration of Ala-Pro in PCM water ($\varepsilon = 78.39$) versus Ala-Pro in PCM water but hydrogen bonded to an explicit water molecule. The presence of an explicit water molecule has a negligible impact on the AmII′p frequency, indicating that the AmII′p vibration does not show any significant dependence on water−C═O hydrogen bonding (Table 4). This result is in agreement with our UVRR results (Figure 2) which indicate the frequency of AmII′p is insensitive to C═O hydrogen bonding.

Our results, thus, indicate that hydrogen bond strength and solvent dielectric effect have a negligible impact on the AmII′p frequency. We therefore conclude that the 25 cm^{-1} downshift in the AmII′p frequency observed by Takeuchi and Harada[34] likely derives from (φ, ψ) conformational changes in the Pro peptide bond.

***ψ* Angle Dependence.** We explore the impact of ψ angle rotation on the AmII′p frequency by calculating vibrational frequencies for a series of zwitterionic Ala-Pro conformers spanning the allowed ψ angles at a fixed $\varphi = -80°$ (Figure 3). To simplify the discussion, we divide all calculated conformers into two groups: helical conformers ($\psi \leq 0°$) and extended conformers ($\psi > 0°$).

Analysis of the zwitterionic Ala-Pro reveals that the calculated AmII′p frequencies of extended conformers upshift by ~25 cm^{-1} when the ψ angle is varied from 60 to 150°. In contrast, the AmII′p frequency of *helical* conformers shows a weak ψ angle dependence. The AmII′p frequency downshifts by 4 cm^{-1} as the ψ angle is varied from −90 to −45° (Figure 3). The AmII′p frequency shift in both the helical and extended conformation linearly correlates with changes in C−N bond length (Figure 4).

Our calculations reveal that the C−N bond length changes derive from changes in planarity of the peptide bond which can be monitored by the torsional angle Θ.[65,66]

$$\Theta = -\omega + \omega^1 + \pi$$

where the ω^1 torsional angle in the Pro peptide bond is defined by atoms C_α, C, N, and C*, where C* is the carbon atom of the pyrrolidine ring (Figure 5). The magnitude of Θ correlates with the extent of peptide bond nitrogen pyramidalization. Large Θ values indicate more extensive pyramidalization due to rehybridization of the amide nitrogen.[65−74]

The pyramidal nitrogen is sp^3 hybridized, while the planar nitrogen corresponds to sp^2 hybridization. Rehybridization directly impacts the C−N bond length. Structures with more sp^3 hybridization have longer C−N bond lengths than the shorter C−N bond lengths of sp^2-like structures.[65−74] Figure 4d displays the dependence of Θ upon ψ rotation. As the ψ angle is varied, Θ changes, indicating that the ψ conformation changes directly impact the nonplanarity of the peptide bond. The C−N bond length change deriving from increased nonplanarity of the peptide bond correlates with changes in the AmII′p band frequency (Figure 4).

Our results are in agreement with recent statistical analyses of protein conformation and its correlation with the ω angle. Previously, Macarthur and Thornton's[69] statistical analysis of 85 high resolution X-ray structures of proteins from the protein databank (PDB) indicated a systematic dependence of the ω angle on the (φ, ψ) angles. Recently, Esposito et al.'s[75] statistical analysis of 163 high resolution protein X-ray structures from the PDB suggested that the ω angle values are strongly correlated with the ψ dihedral angle. In contrast, the ω angle values shows an insignificant dependence on the φ dihedral angle.[75]

It should be noted that *ab initio* calculations of Asher et al.[76] indicate that the frequency of the $AmIII_3$ vibration ($C-N_s$ with in-phase NH_b) sinusoidally depends on the ψ dihedral angle. Recently, Mirkin and Krimm's[77] DFT calculations indicated that

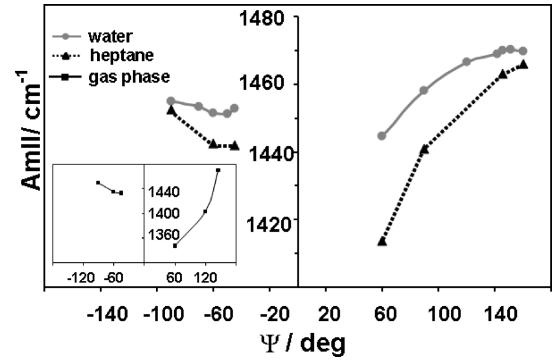

Figure 3. Calculated conformational dependence of the AmII′p frequency of Ala-Pro on the ψ dihedral angle in water, heptane, and gas phase (inset).

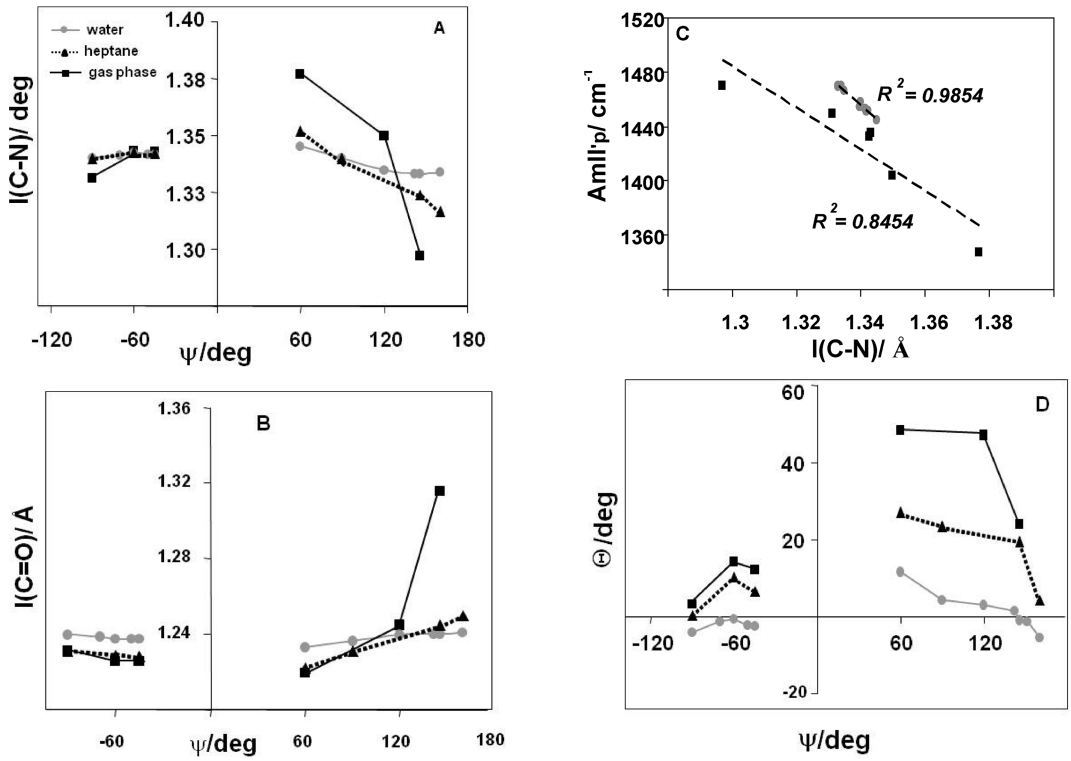

Figure 4. Calculated ψ dependence of the (A) C−N bond length and (B) C═O bond length of Ala-Pro in water, heptane, and gas phase. (C) Calculated dependence of Ala-Pro AmII′p frequency and C−N bond length in water (gray circles) and gas phase (black squares). (D) Calculated ψ dependence of the peptide bond planarity angle (Θ) in water, heptane, and gas phase.

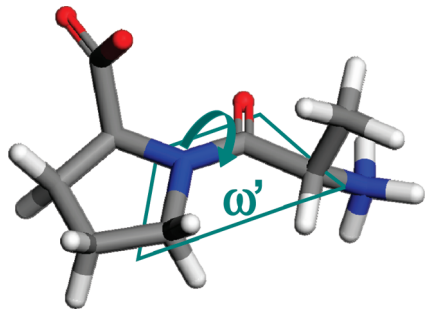

Figure 5. The torsional angle ω' of Ala-Pro is defined as a rotation around the C−N bond in the dihedral plane defined by the C−C(O)−N−C* atoms.

the N−H_s (amide A) frequency is also conformation sensitive. These authors attribute the conformation sensitivity of the N−H_s vibration to conformation-induced pyramidalization of the amide nitrogen.[77]

The conformational sensitivity of the various amide vibrations all appear to derive from the pyramidalization of the amide nitrogen, which directly impacts the amide bond geometry, resulting in significant changes in the amide vibrational frequencies. The general trend relating vibrational frequencies to (φ, ψ) conformation changes, however, shows differences between the different amide vibrations. A lack of uniform conformation sensitivity among the various amide vibrations is due to differences in normal mode composition; e.g., the $AmIII_3$ frequency sinusoidally depends on the ψ dihedral angle, while the AmII′p frequency does not show such a simple ψ dependence. Normal mode composition analysis of non-Pro, non-Gly peptide bonds indicates that, in addition to amide nitrogen pyramidization, ψ angle changes impact the coupling of C_α−H_b to N−H_b which significantly impact the $AmIII_3$ frequency.[76] The AmII′p vibration lacks the amide NH.

We probe the impact of the solvent dielectric effect on the calculated ψ angle dependence of the AmII′p frequency by computing the AmII′p frequency of various Ala-Pro conformers in a vacuum, water, heptane, and acetonitrile utilizing the PCM model. Our results indicate that changes in the dielectric constant of the surrounding media do not significantly impact the general trend relating the ψ angle to the AmII′p frequency (Figures 3 and 4). However, the frequency shifts are larger in low dielectric environments like heptane (Figure 3). This effect derives from stabilization of the nonplanar peptide bond in low dielectric environments. Consequently, the deviations from peptide bond planarity are larger in low dielectric environments.

The stabilization of the nonplanar peptide bond in low dielectric environments can be understood from the solvent's impact on the peptide bond's resonance structure. In polar solvents, the high dielectric environment stabilizes the charged form of the peptide bond [$^-$O(C)N$^+$H].[41,78] In this charged state, the carbonyl bond is elongated, whereas the C−N bond contracts as its double bond character increases. The increased sp^2 character of the C−N bond in the charged state results in a more planar peptide bond.[41] Thus, in polar solvents, the nonplanar peptide bond is energetically unfavorable.

In the gas phase, the general trend relating the ψ angle changes to the AmII′p frequency, however, appears to deviate at ψ >120°. This deviation in the AmII′p frequency derives from the terminal NH_3^+ group's attempt to donate a proton to the peptide bond C═O. Enol formation is unfavorable in aqueous solutions.

Our investigations of the conformation and solvent dependence of the AmII′p frequency indicate that the ψ angle and environment dependence of various amide vibrations derive from pyramidalization of the amide nitrogen. Deviations from planarity, whether induced via ψ angle conformation changes or changes in solvent dielectric constant, impact the planarity

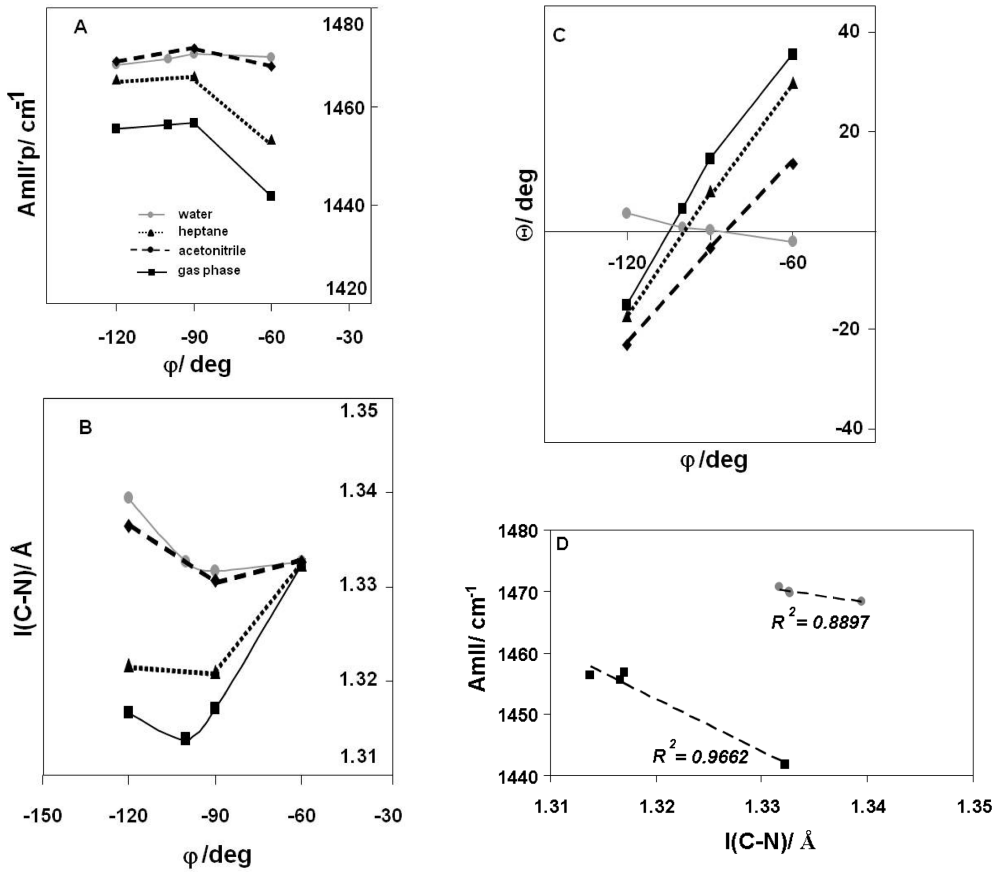

Figure 6. Calculated φ dependence of Ala-Pro (A) AmII′p frequency, (B) C−N bond length, and (C) Θ planarity angle in water, heptane, acetonitrile, and gas phase. (D) The calculated Ala-Pro AmII′p frequencies and C−N bond lengths in water (gray circles) and gas phase (black squares) are linearly correlated.

of the peptide bond. Consequently, as the sp^2 character of the amide nitrogen decreases, the C−N bond elongates, whereas the C═O bond contracts. Consequently, those amide vibrations containing significant contributions from the nitrogen stretching (AmII, AmII′, AmII′p, AmIII, and N−H_s vibrations) show a frequency downshift, while the C═O_s (AmI) show frequency upshifts.

***φ* Angle Dependence.** We calculated the φ dependence of the AmII′p frequency for zwitterionic Ala-Pro conformers in water, spanning φ angles from −60 to −120° with $\psi = 145°$. Within this range of φ, the AmII′p frequency varies by ∼2 cm^{-1} (Figure 6), indicating the AmII′p frequency does not show any significant dependence on the φ dihedral angles. We calculate similar results for Ala-Pro conformers in acetonitrile. This result is not surprising. As discussed above, Esposito et al.'s[75] statistical analysis of 163 high resolution protein X-ray structures from the PDB indicates the variations in the ω angle do not show a significant correlation with the φ dihedral angle.

In low dielectric constant media like heptane and a vacuum, the calculated AmII′p frequency shows a small dependence on the φ dihedral angle. In particular, the AmII′p frequency dramatically decreases as the φ dihedral angle decreases from −90 to −60° (Figure 6). However, at high dielectric constant as in water or acetonitrile, there is no change in the AmII′p frequency over this range of φ angles. This can be explained by the normal mode composition analysis (Table 3). In the gas phase, the normal mode composition of the AmII′p vibration of the $\varphi = -60°$ conformer contains significant amounts (25%) of methyl symmetric deformation. At higher dielectric constant, the AmII′p normal mode composition changes because methyl symmetric deformation is replaced by methyl asymmetric deformation. This normal mode composition change results in an increase in the AmII′p frequency of the $\varphi = -60°$ Ala-Pro conformer in water as compared to Ala-Pro in the heptane/gas phase.

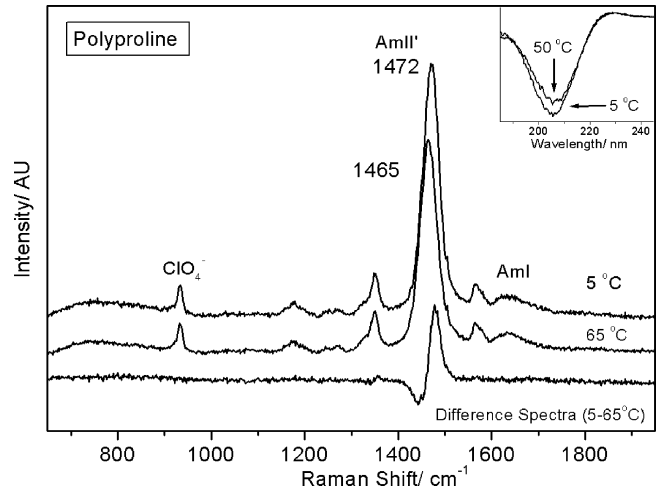

Figure 7. Polyproline shows a 7 cm^{-1} downshift in the AmII′p band frequency as the temperature increases from 5 to 65 °C. The CD spectra (inset) of polyproline at 5 and 50 °C show characteristic features of the PPII conformation, indicating a lack of significant *trans*-to-*cis* isomerization with increasing temperature.

Temperature-Dependent Spectra of Polyproline. As shown in Figure 7, the AmII′p band of polyproline downshifts from 1472 to 1465 cm^{-1} as the solution temperature is increased from 5 to 65 °C. The 7 cm^{-1} downshift derives from either a nearly

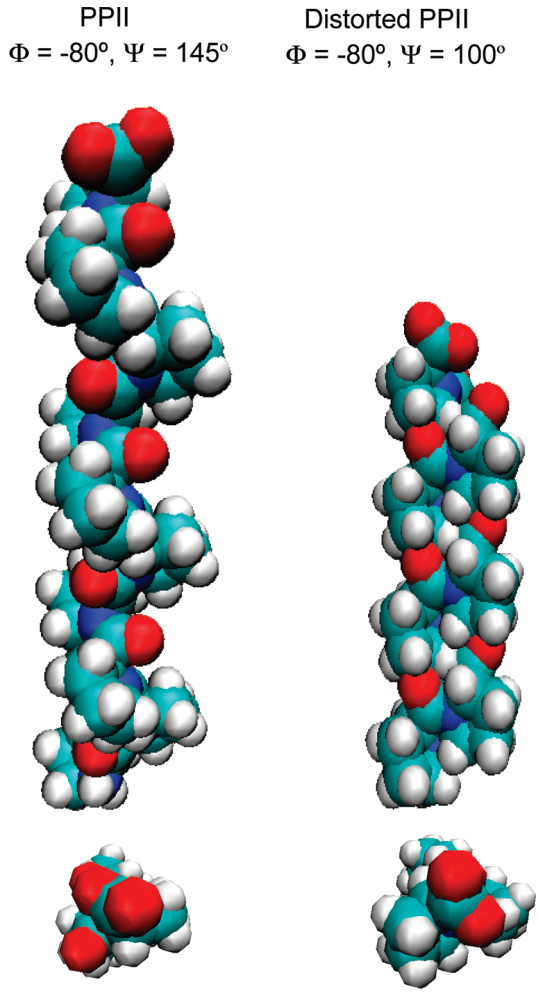

Figure 8. Structure of ideal PPII Pro peptide (left) and the proposed structure of collapsed polyproline (right).

100% conversion from the *trans* to *cis* conformation or a conformation change along the ψ dihedral angle.

As shown in Figure 7 (inset), the CD spectra of polyproline at both 5 and 50 °C show a small positive peak at 225 nm and a global minima at ~205 nm, indicating a predominantly *trans* (PPII) conformation[79−83] at both temperatures. We do not observe any spectral features corresponding to the *cis* (PPI) conformation, which is known to show a medium intensity negative band at 198−200 nm, a strong positive band at ~214 nm, and a weak negative band at ~231 nm.[83] These features are clearly lacking in the polyproline spectra at either temperature (Figure 7, inset).

Our CD results demonstrate that the temperature-induced downshift in the Raman AmII′p frequency of polyproline (Figure 7) does not derive from isomerization of the Pro peptide bond. Furthermore, the AmI′ of polyproline does not show any significant change in band position with increasing temperature. As discussed above, a *trans* → *cis* isomerization is expected to upshift the AmI′ band by ~13 cm^{-1}.

Our theoretical results, discussed above, indicate that the observed temperature-induced downshift in the AmII′p frequency of polyproline is due to a small conformation change that distorts the native PPII conformation. As shown in Figure 3, starting from an ideal PPII conformation ($\varphi = -80°$, $\psi = 145°$), the observed 7 cm^{-1} shift results from a 45° rotation of the ψ angle from $\psi = 145°$ to $\psi = 100°$, thus resulting in a distorted PPII conformation (Figure 8). Previously, Swenson and Formanek[84] had suggested that the temperature-induced upshift in the AmI′ frequency of polyproline may derive from slight changes in the ψ angle. These authors attributed the observed changes in polyproline to a temperature-induced disruption of Pro−water interactions.[84]

Conclusions

Utilizing UVRR experiments and DFT calculations, we systematically examined the dependence of the AmII′p frequency on hydrogen bonding, *cis*−*trans* isomerization, and conformation changes. Our UVRR results show that the AmII′p band does not show any significant change in frequency with increasing temperature. These results indicate that the frequency of the AmII′p is not sensitive to changes in carbonyl−water hydrogen bonding.[40] Our theoretical calculations indicate the AmII′p frequency shows an 8 cm^{-1} downshift upon *trans*-to-*cis* isomerization of the peptide bond. This frequency dependence arises due to a slight elongation of the C−N bond in the *cis* conformer.

Our results indicate the AmII′p frequency is most sensitive to the planarity of the Pro peptide bond as measured by its Θ dihedral angle. The peptide bond nonplanarity can be modulated by ψ angle changes that push the amide nitrogen out of the peptide bond plane. The nonplanar amide bond has a larger sp^3 character at the amide nitrogen and hence shows a larger C−N bond length as compared to the planar amide bond. The change in C−N bond length directly correlates with changes in the AmII′p frequency.

Our calculations indicate that, in the allowed region of the Ramachandran space, the AmII′p frequency shows the largest variation in the extended state (PPII/β-strand) region, whereas the AmII′p frequency shows only a weak conformational dependence when it occurs within the α-helical region. Conformational changes causing alterations of the φ dihedral angle do not significantly impact the AmII′p frequency.

These results allow us to correlate changes in AmII′p frequency with conformation changes at the Pro peptide bond. We calculate that the ~25 cm^{-1} downshift in the AmII′p frequency of the Pro-Pro dipeptide between water and acetonitrile observed by Takeuchi and Harada[34] likely derives from an ~85° rotation of the ψ dihedral angle from $\psi \sim 60°$ to ψ ~145°. We correlate the 7 cm^{-1} downshift in the AmII′p frequency of polyproline to a temperature-induced distortion of the native PPII structure ($\psi = 145°$). At high temperatures, the polyproline peptide adopts a compact PPII structure with $\psi = 100°$.

Acknowledgment. The authors would like to thank Dr. Sasmita Das for helpful discussions and NIH grant RO1 EB002053 for financial support.

References and Notes

(1) Reiersen, H.; Rees, A. R. *Trends Biochem. Sci.* **2001**, *26*, 679.
(2) Madison, V. *Biopolymers* **1977**, *16*, 2671.
(3) Venkatachalam, C. M.; Price, B. J.; Krimm, S. *Biopolymers* **1975**, *14*, 1121.
(4) Johnston, N.; Krimm, S. *Biopolymers* **1971**, *10*, 2597.
(5) Dorman, D. E.; Torchia, D. A.; Bovey, F. A. *Macromolecules* **1973**, *6*, 80.
(6) Tamburro, A. M.; Guantieri, V.; Pandolfo, L.; Scopa, A. *Biopolymers* **1990**, *29*, 855.
(7) Chiu, C. H.; Bersohn, R. *Biopolymers* **1977**, *16*, 277.
(8) Mclachlan, A. D. *Biopolymers* **1977**, *16*, 1271.
(9) Nemethy, G.; Scheraga Harold, A. *Biopolymers* **1984**, *32*, 2781.
(10) Harper, E. T.; Rose, G. D. *Biochemistry* **1993**, *32*, 7605.
(11) Tamaki, M.; Akabori, S.; Muramatsu, I. *Biopolymers* **1996**, *39*, 129.

(12) Garrett, R. H. G.; Charles, M.; *Biochemistry*, 2nd ed.; Saunders College Publishing: Philadelphia, PA, 1999.
(13) Glaser, R. *Biophysics*; Springer: New York, 2000.
(14) Piela, L.; Nemethy, G.; Scheraga, H. A. *Biopolymers* **1987**, *26*, 1587.
(15) Sankararamakrishnan, R.; Vishveshwara, S. *Biopolymers* **1990**, *30*, 287.
(16) Deber, C. M.; Glibowicka, M.; Woolley, G. A. *Biopolymers* **1990**, *29*, 149.
(17) Seshadri, S.; Oberg, K. A.; Fink, A. L. *Biochemistry* **1994**, *33*, 1351.
(18) Houry, W. A.; Scheraga Harold, A. *Biochemistry* **1996**, *35*, 11719.
(19) Mayr, L. M.; Odefey, C.; Schutkowski, M.; Schmid, F. X. *Biochemistry* **1996**, *35*, 5550.
(20) Pascher, T.; Chesick, J. P.; Winkler, J. R.; Gray, H. B. *Science* **1996**, *27*, 1558.
(21) Hagen, S. J.; Hofrichter, L.; Szabo, A.; Eaton, W. A. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 11615.
(22) Kubelka, J.; Hofrichter, J.; Eaton, W. A. *Curr. Opin. Struct. Biol.* **2004**, *14*, 76.
(23) Lyubovitsky, J. G.; Gray, H. B.; Winkler, J. R. *J. Am. Chem. Soc.* **2002**, *124*, 5481.
(24) Reimer, U.; Scherer, G.; Drewello, M.; Kruber, S.; Schutkowski, M.; Fischer, G. *J. Mol. Biol.* **1998**, *279*, 449.
(25) Grathwohl, C.; Wuthrich, K. *Biopolymers* **1976**, *15*, 2043.
(26) Grathwohl, C.; Wuthrich, K. *Biopolymers* **1976**, *15*, 2025.
(27) Swenson, C. A. *Biopolymers* **1971**, *10*, 2591.
(28) Rippon, W. B.; Koeing, J. L.; Walton, A. G. *J. Am. Chem. Soc.* **1970**, *92*, 7455.
(29) Harhay, G. P.; Hudson, B. *J. Phys. Chem.* **1993**, *97*, 8158.
(30) Harhay, G. P.; Hudson, B. S. *J. Phys. Chem.* **1991**, *95*, 3511.
(31) Caswell, D. S.; Spiro, T. G. *J. Am. Chem. Soc.* **1987**, *109*, 2796.
(32) Mayne, L.; Hudson, B. *J. Phys. Chem.* **1987**, *91*, 4438.
(33) Mayne, L.; Hudson, B. *Methods Enzymol.* **1986**, *130*, 331.
(34) Takeuchi, H.; Harada, I. *J. Raman Spectrosc.* **1990**, *21*, 509.
(35) Song, S.; Asher, S. A.; Krimm, S. *J. Am. Chem. Soc.* **1991**, *113*, 1155.
(36) Qian, W.; Mirkin, N, G.; Krimm, S. *Chem. Phys. Lett.* **1999**, *315*, 125.
(37) Mirkin, N. G.; Krimm, S. *J. Mol. Struct.* **1996**, *377*, 219.
(38) Cheam, T. C.; Krimm, S. *Spectrochim. Acta, Part A* **1984**, *40*, 481.
(39) Jordon, T.; Mukerji, I.; Yang, W.; Spiro, T. G. *J. Biol. Chem.* **1996**, *379*, 51.
(40) Triggs, N. E.; Valentini, J. J. *J. Phys. Chem.* **1992**, *96*, 6922.
(41) Myshakina, N. S.; Ahmed, Z.; Asher, S. A. *J. Phys. Chem. B* **2008**, *112*, 11873.
(42) Urry, D. W. *J. Phys. Chem. B* **1997**, *101*, 11007.
(43) Bykov, S. B.; Lednev, I. K.; Ianoul, A.; Mikhonin, A. V.; Asher, S. A. *Appl. Spectrosc.* **2005**, *59*, 1541.
(44) Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Montgomery, J. A., Jr.; Vreven, T.; Kudin, K. N.; Burant, J. C.; Millam, J. M.; Iyengar, S. S.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, G. A.; Nakatsuji, H.; Hada, M.; Ehara, M.; Toyota, K.; Fukuda, R.; Hasegawa, J.; Ishida, M.; Nakajima, T.; Honda, Y.; Kitao, O.; Nakai, H.; Klene, M.; Li, X.; Knox, J. E.; Hratchian, H. P.; Cross, J. B.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, R. E.; Yazyev, O.; Austin, A. J.; Cammi, R.; Pomelli, C.; Ochterski, J. W.; Ayala, P. Y.; Morokuma, K.; Voth, G. A.; Salvador, P.; Dannenberg, J. J.; Zakrzewski, V. G.; Dapprich, S.; Daniels, A. D.; Strain, M. C.; Farkas, O.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Ortiz, J. V.; Cui, Q.; Baboul, A. G.; Clifford, S.; Cioslowski, J.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Challacombe, M.; Gill, P. M. W.; Johnson, B.; Chen, W.; Wong, M. W.; Gonzalez, C.; Pople, J. A. *Gaussian 03*, revision C.01; Gaussian, Inc.: Wallingford, CT, 2004.
(45) Kohn, W.; Sham, L. J. *Phys. Rev.* **1965**, *137*, 1697.
(46) Parr, R. G.; Yang, W. *Density-functional theory of atoms and molecules*; Oxford Univ. Press: Oxford, U.K., 1989.
(47) Hohenberg, P.; Kohn, W. *Phys. Rev.* **1964**, *136*, B864.
(48) Becke, A. D. *J. Chem. Phys.* **1993**, *98*, 5648.
(49) Lee, C.; Yang, W.; Parr, R. G. *Phys. Rev. B: Condens. Matter Mater. Phys.* **1988**, *37*, 785.
(50) Miehlich, B.; Savin, A.; Stoll, H.; Preuss, H. *Chem. Phys. Lett.* **1989**, *157*, 200.
(51) Irikura, K. K.; Johnson, R. D., III; Kacker, R. N. *J. Phys. Chem. A* **2005**, *109*, 8430.
(52) Halls, M. D.; Velkovski, J.; Schlegel, H. B. *Theor. Chem. Acc.* **2001**, *105*, 413.
(53) Ponder, J. W. TINKER, Software Tools for Molecular Design. In http://dasher.wustl.edu/tinker.
(54) Humphrey, W.; Dalke, A.; Schulten, K. *J. Mol. Graphics* **1996**, *14*, 33.
(55) Hinderaker, M. P.; Raines, R. T. *Protein Sci.* **2003**, *12*, 1188.
(56) Mikhonin, A. V.; Ahmed, Z.; Ianoul, A.; Asher, S. A. *J. Phys. Chem. B* **2004**, *108*, 19020.
(57) Lednev, I. K.; Karnoup, A. S.; Sparrow, M. C.; Asher, S. A. *J. Am. Chem. Soc.* **1999**, *121*, 8074.
(58) Manas, E. S.; Getahun, Z.; Wright, W. W.; DeGrado, W. F.; Vanderkooi, J. M. *J. Am. Chem. Soc.* **2000**, *122*, 9883.
(59) Neidigh, J. W.; Fesinmeyer, R. M.; Andersen, N. H. *Nat. Struct. Biol.* **2002**, *9*, 425.
(60) Chen, X. G.; Asher, S. A.; Schweitzer-Stenner, R.; Mirkin, N. G.; Krimm, S. *J. Am. Chem. Soc.* **1995**, *117*, 2884.
(61) Torii, H.; Tasumi, M. *J. Raman Spectrosc.* **1998**, *29*, 81.
(62) Torii, H.; Tatsumi, T.; Tasumi, M. *J. Raman Spectrosc.* **1998**, *29*, 537.
(63) Eaton, G.; Symons, C. R.; Rastogi, P. P. *J. Chem. Soc., Faraday Trans. 1* **1989**, *85*, 3257.
(64) Ham, S.; Kim, J.-H.; Lee, H.; Cho, M. *J. Chem. Phys.* **2003**, *118*, 3491.
(65) Ramachandran, G. N.; Lakshminarayanan, A. V.; Kolaskar, A. S. *Biochim. Biophys. Acta* **1973**, *303*, 8.
(66) Ramek, M.; Yu, C.-H.; Sakon, J.; Schafer, L. *J. Phys. Chem. A.* **2000**, *104*, 9636.
(67) Selvarengan, P.; Kolandaivel, P. *Bioorg. Chem.* **2005**, *33*, 253.
(68) Ramachandran, G. N. *Biopolymers* **1968**, *6*, 1494.
(69) MacArthur, M. W.; Thornton, J. M. *J. Mol. Biol.* **1996**, *264*, 1180.
(70) Otani, Y.; Nagae, O.; Naruse, Y.; Inagaki, S.; Ohno, M.; Yamaguchi, K.; Yamada, G.; Uchiyama, M.; Ohwada, T. *J. Am. Chem. Soc.* **2003**, *125*, 15191.
(71) Krimm, S.; Mirkin, N. G. *J. Phys. Chem. A.* **2004**, *108*, 5438.
(72) Lopez-Garriga, J. J.; Hanton, S.; Babcock, G. T.; Harrison, J. F. *J. Am. Chem. Soc.* **1986**, *108*, 7251.
(73) Lopez, X.; Mujika, J. I.; Blackburn, G. M.; Karplus, M. *J. Phys. Chem. A* **2003**, *107*, 2304.
(74) Alkorta, I.; Cativiela, C.; Elguero, J.; Gil, A. M.; Jimenez, A. I. *New J. Chem.* **2005**, *29*, 1450.
(75) Esposito, L.; De Simone, A.; Zagari, A.; Vitagliano, L. *J. Mol. Struct.* **2005**, *347*, 483.
(76) Asher, S. A.; Ianoul, A.; Mix, G.; Boyden, M. N.; Karnoup, A.; Diem, M.; Schweitzer-Stenner, R. *J. Am. Chem. Soc.* **2001**, *123*, 11775.
(77) Mirkin, N. G.; Krimm, S. *J. Phys. Chem. A* **2004**, *108*, 5438.
(78) Milner-White, E. J. *Protein Sci.* **1997**, *6*, 2477.
(79) Creamer, T. P. *Proteins: Struct., Funct., Genet.* **1998**, *33*, 218.
(80) Tiffany, M. L.; Krimm, S. *Biopolymers* **1968**, *6*, 1379.
(81) Tiffany, M. L.; Krimm, S. *Biopolymers* **1969**, *8*, 347.
(82) Tiffany, M. L.; Krimm, S. *Biopolymers* **1972**, *11*, 2309.
(83) Kakinoki, S.; Hirano, Y.; Oka, M. *Polym. Bull.* **2005**, *53*, 109.
(84) Swenson, C. A.; Formanek, R. *J. Phys. Chem.* **1967**, *71*, 4073.
(85) Mezei, M.; Fleming, P. J.; Srinivasan, R.; Rose, G. D. *Proteins: Struct., Funct., Bioinf.* **2004**, *55*, 502.

JP809857Y