Published in final edited form as:

J Phys Chem B. 2009 August 13; 113(32): 11252–11259. doi:10.1021/jp809857y.

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, PA 15260

Abstract

We utilized UV-resonance Raman (UVRR) measurements and density functional theory (DFT) calculations to relate that the AmII'p frequency to the ψ -angle. The AmII'p frequency shifts by $\sim 25 \text{ cm}^{-1}$ 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 angles 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 polypyrroline peptide. The temperature-induced 7 cm^{-1} downshift in the AmII'p frequency of the polypyrroline peptide results from a $\sim 45^\circ$ rotation of the ψ dihedral angle from $\psi = 145^\circ$ (ideal PPII conformation) to $\psi = 100^\circ$ (collapsed PPII conformation).

Keywords

AmII' of Proline; UV-Resonance Raman Spectroscopy; volume phase transition; *cis-trans* isomerization; DFT calculations

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_α (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 inter-peptide 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 regions¹. 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 T₁, the pro *cis-trans* isomerization rate constant is estimated to be $1 \times 10 \text{ s}^{-1}$.¹⁹ In contrast, a typical protein such as cytochrome *b*₅₆₂ has a refolding rate of $2 \times 10 \text{ s}^{-1}$.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 ¹³C-NMR spectroscopy,²³⁻²⁶ no such clear-cut quantitative markers yet exist in IR²⁷ or Raman

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

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 Hudson³⁰ 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 Harada³⁴ 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 ~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's³⁴ 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's³⁴ interpretation of the AmII'p frequency shift.⁴⁰ In their UV-Raman study, utilizing pre-resonance 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) show no significant dependence on hydrogen bonding.⁴⁰

In a recent theoretical study of NMA and NMA-water complexes (and their deuterated isotopomers) we recently 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 C=O's 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 pro rich 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 (UVR) 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 $\sim 8 \text{ cm}^{-1}$. We find that the AmII'p band position is very sensitive to non-planarity of the pro peptide bond. The peptide bond non-planarity 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

The UV resonance Raman (UVR) 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 3rd harmonic of a Nd:YAG laser (Coherent, Infinity) in H₂ 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 D₂O were acquired from Sigma-Aldrich. The chemicals were used as received. 1 mg/ml of peptide concentration in 0.2 M sodium perchlorate solution were used for UVR measurements.

Computational details

All calculations were performed using the Gaussian'03⁴⁴ calculation package at the DFT⁴⁵⁻⁴⁷ level of theory employing the B3LYP⁴⁸⁻⁵⁰ 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". 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 ($\phi = -90^\circ$, $\psi = 145^\circ$) were calculated in vacuum ($\epsilon = 1.00$) and in water ($\epsilon = 78.39$), acetonitrile ($\epsilon = 36.64$), and heptane ($\epsilon = 1.92$) 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 $\psi = -90^\circ, -70^\circ, -60^\circ, -50^\circ, -45^\circ, 60^\circ, 90^\circ, 120^\circ, 140^\circ, 145^\circ, 150^\circ$ were calculated in water. In heptane (PCM) only $\psi = -90^\circ, -60^\circ, -45^\circ, 60^\circ, 120^\circ, 145^\circ, 160^\circ$ values were calculated. In gas phase calculations ala-pro conformers were calculated for $\phi = -80^\circ$ and $\psi = -90^\circ, -60^\circ, -45^\circ, 60^\circ, 120^\circ, 145^\circ$.
4. A series of zwitterionic ala-pro conformers with $\psi = 145^\circ$ and $\phi = -60^\circ, -90^\circ, -100^\circ$ and -120° were calculated for ala-pro in water and in the gas phase. In heptane and

acetonitrile, we calculated conformers with $\phi = -60^\circ$, -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 ($\phi = -80^\circ$, $\psi = 100^\circ$) and canonical PPII polyproline ($\phi = -80^\circ$, $\psi = 145^\circ$) were calculated by utilizing the *protein* utility in Tinker⁵³ and visualized using VMD software.⁵⁴ End-to-end distance and radii of the two polymers were estimated using CACheTM (Fujitsu). 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.

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 (Fig. 1). Our calculations show that *trans* $\rightarrow$ *cis* isomerization results in a slight elongation of the C-N bond length (~ 0.004 Å) and a nearly equal contraction of 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 $n \rightarrow \pi^*$ 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 occurs when the $\text{O}_{i-1} \dots \text{C}_i$ distance is ≤ 3.2 Å and the $\text{O}_{i-1} \dots \text{C}_i = \text{O}_i$ 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 re-distribution 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.

The impact of hydrogen bonding

As discussed above, the work of Triggs and Valentini⁴⁰ contradicts Takeuchi and Harada's³⁴ 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 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 ($-0.07\text{ cm}^{-1}/^\circ\text{C}$).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 ($\sim 4\text{ cm}^{-1}$ 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 Valentini⁴⁰ 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 Fig 2, the frequency of the AmII'p of ala-pro barely downshifts from 1488 cm⁻¹ to 1487 cm⁻¹ as the solution temperature increases from 4 to 65 °C. The band intensity however, shows a ~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 (~25%) and the AmII' like vibrations (~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 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 ~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 ~25 cm⁻¹ downshift in the AmII'p frequency observed by Takeuchi and Harada³⁴ in acetonitrile cannot mainly result from *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's³⁴ 25 cm⁻¹ downshift of AmII'p frequency could derive from conformational alterations about the ϕ, 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 those observed in aprotic solvents with similar dielectric constants.^{62,63}

Our temperature dependent UVRR experiment (Fig 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's³⁴ experiment replaces water with acetonitrile as the solvent media, incurring large changes in the 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 AmII'p frequency of ala-pro by using DFT calculations in PCM water ($\epsilon = 78.39$), acetonitrile ($\epsilon = 36.64$), heptane ($\epsilon = 1.92$) and vacuum ($\epsilon = 1.00$). Our results indicate that the 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 Harada³⁴ 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 ($\epsilon = 78.39$), 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 (Fig 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 Harada³⁴ 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 φ = -80° (Fig 3). To simplify the discussion, we divide all calculated conformers into two groups: helical conformers (ψ < 0°) and extended conformers (ψ > 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° (Fig. 3). The AmII'p frequency shift in both the helical and extended conformation linearly correlates with changes in C-N bond length (Fig. 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 ω¹ 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 (Fig. 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 sp³ hybridized, while the planar nitrogen corresponds to sp² hybridization. Rehybridization directly impacts the C-N bond length. Structures with more sp³ hybridization have longer C-N bond lengths than the shorter C-N bond lengths of sp²-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 non-planarity of the peptide bond. The C-N bond length change deriving from increased non-planarity of the peptide bond correlates with changes in the AmII'p band frequency (Fig. 4).

Our results are in agreement with recent statistical analyses of protein conformation and its correlation with the ω-angle. Previously, Macarthur and Thornton's⁶⁹ 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 AmIII₃ vibration (C-N_s with in-phase NH_b) sinusoidally depends on the ψ-dihedral angle. Recently, Mirkin and Krimm's⁷⁷ DFT calculations indicate that 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 amongst the various amide vibrations is due to differences in normal mode composition, e. g. the AmIII₃ 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 indicate that in addition to amide nitrogen pyramidalization, ψ -angle changes impact the coupling of C α -H_b to N-H_b which significantly impact the AmIII₃ frequency.⁷⁶ The AmII'p vibration lacks the amide NH.

We probe the impact of 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 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 (Fig 3 and 4). However, the frequency shifts are larger in low dielectric environments like heptane (Fig 3). This effect derives from stabilization of the non-planar peptide bond in low dielectric environments. Consequently, the deviations from peptide bond planarity are larger in low dielectric environments.

The stabilization of the non-planar 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 sp² character of the C-N bond in the charged state results in a more planar peptide bond.⁴¹ Thus, in polar solvents the non-planar 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 $\psi > 120^\circ$. This deviation in the AmII'p frequency derives from the terminal NH₃⁺ 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 of the peptide bond. Consequently, as the sp² 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 the ϕ -angle from -60° to -120° with $\psi = 145^\circ$. Within this range of ϕ , the AmII'p frequency varies by $\sim 2 \text{ cm}^{-1}$ (Fig 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 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° (Fig 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 $\phi = -60^\circ$ 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 $\phi = -60^\circ$ ala-pro conformer in water as compared to ala-pro in heptane/gas phase.

Temperature Dependent Spectra of Polyproline

As shown in Fig 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 100% conversion from the *trans* to *cis* conformation or a conformation change along the ψ -dihedral angle.

As shown in Fig 7 (insert) the CD spectra of polyproline at both 5 and 50°C show a small positive peak at 225 nm and a global minima at $\sim 205\text{ nm}$ indicating a predominantly *trans* (PPII) conformation⁷⁹⁻⁸³ 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 $198\text{--}200\text{ nm}$, a strong positive band at $\sim 214\text{ nm}$ and a weak negative band at $\sim 231\text{ nm}$.⁸³ These features are clearly lacking in the polyproline spectra at either temperature (Fig 7, insert).

Our CD results demonstrate that the temperature induced downshift in the Raman AmII'p frequency of polyproline (Fig 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* $\rightarrow$ *cis* isomerization is expected to upshift the AmI' band by $\sim 13\text{ cm}^{-1}$.

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 Fig 3, starting from an ideal PPII conformation ($\phi = -80^\circ$, $\psi = 145^\circ$) the observed 7 cm^{-1} shift results from a 45° rotation of the ψ angle from $\psi = 145^\circ$ to $\psi = 100^\circ$, thus resulting in a distorted PPII conformation (Fig 8). Previously, Swenson and Formanek⁸⁴ 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 non-planarity can be modulated by ψ -angle changes that push the amide nitrogen out of the peptide bond plane. The non-planar 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 $\sim 25\text{ cm}^{-1}$ downshift in the AmII'p frequency of pro-pro dipeptide between water and acetonitrile observed by Takeuchi and Harada³⁴ likely derives from a $\sim 85^\circ$ rotation of the ψ -dihedral angle from $\psi \sim 60^\circ$ to $\psi \sim 145^\circ$. We correlate the 7 cm^{-1} downshift in the AmII'p frequency of polyproline to a temperature-induced distortion of the native PPII structure ($\psi = 145^\circ$). At high temperatures the polyproline peptide adopts a compact PPII structure with $\psi = 100^\circ$.

Acknowledgments

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

References

1. Reiersen H, Rees AR. Trends in Biochemical Sciences 2001;26:679. [PubMed: 11701327]
2. Madison V. Biopolymers 1977;16:2671.
3. Venkatachalam CM, Price BJ, Krimm S. Biopolymers 1975;14:1121. [PubMed: 1164539]
4. Johnston N, Krimm S. Biopolymers 1971;10:2597. [PubMed: 5126529]
5. Dorman DE, Torchia DA, Bovey FA. Macromolecules 1973;6:80. [PubMed: 4778412]
6. Tamburro AM, Guantieri V, Pandolfo L, Scopa A. Biopolymers 1990;29:855. [PubMed: 2383648]
7. Chiu CH, Bersohn R. Biopolymers 1977;16:277. [PubMed: 831862]
8. McLachlan AD. Biopolymers 1977;16:1271. [PubMed: 880354]
9. Nemethy G, Scheraga Harold A. Biopolymers 1984;32:2781. [PubMed: 6525401]
10. Harper ET, Rose GD. Biochemistry 1993;32:7605. [PubMed: 8347570]
11. Tamaki M, Akabori S, Muramatsu I. Biopolymers 1996;39:129.
12. Garrett, RHG.; Charles, M. Biochemistry. Vol. Second. Saunders College Publishing; Philadelphia, Pa: 1999.
13. Glaser, R. Biophysics. Springer; New York: 2000.
14. Piela L, Nemethy G, Scheraga Harold A. Biopolymers 1987;26:1587. [PubMed: 3663874]
15. Sankaramakrishnan R, Vishveshwara S. Biopolymers 1990;30:287. [PubMed: 2279068]
16. Deber CM, Glibowicka M, Woolley GA. Biopolymers 1990;29:149. [PubMed: 2328283]
17. Seshadri S, Oberg KA, Fink AL. Biochemistry 1994;33:1351. [PubMed: 8312253]
18. Houry WA, Scheraga Harold A. Biochemistry 1996;35:11719. [PubMed: 8794753]
19. Mayr LM, Odefey C, Schutkowski M, Schmid FX. Biochemistry 1996;35:5550. [PubMed: 8611546]
20. Pascher T, Chesick JP, Winkler JR, Gray HB. Science 1996;27:1558. [PubMed: 8599112]

21. Hagen SJ, Hofrichter L, Szabo A, Eaton WA. *Proc Nat Acad Sci USA* 1996;93:11615. [PubMed: 8876184]
22. Kubelka J, Hofrichter J, Eaton WA. *Current Opinion in Structural Biology* 2004;14:76. [PubMed: 15102453]
23. Lyubovitsky JG, Gray HB, Winkler JR. *J Am Chem Soc* 2002;124:5481. [PubMed: 11996590]
24. Reimer U, Scherer G, Drewello M, Kruber S, Schutkowski M, Fischer G. *Journal of Molecular Biology* 1998;279:449. [PubMed: 9642049]
25. Grathwohl C, Wuthrich K. *Biopolymers* 1976;15:2043. [PubMed: 963242]
26. Grathwohl C, Wuthrich K. *Biopolymers* 1976;15:2025. [PubMed: 963241]
27. Swenson CA. *Biopolymers* 1971;10:2591. [PubMed: 5126528]
28. Rippon WB, Koeing JL, Walton AG. *J Am Chem Soc* 1970;92:7455. [PubMed: 5487551]
29. Harhay GP, Hudson B. *J Phys Chem* 1993;97:8158.
30. Harhay GP, Hudson BS. *J Phys Chem* 1991;95:3511.
31. Caswell DS, Spiro TG. *Journal of the American Chemical Society* 1987;109:2796.
32. Mayne L, Hudson B. *J Phys Chem* 1987;91:4438.
33. Mayne L, Hudson B. *Methods Enzymol* 1986;130:331. [PubMed: 3773739]
34. Takeuchi H, Harada I. *Journal of Raman Spectroscopy* 1990;21:509.
35. Song S, Asher SA, Krimm S. *J Am Chem Soc* 1991;113:1155.
36. Qian W, Mirkin NG, Krimm S. *Chemical Physics Letters* 1999;315:125.
37. Mirkin NG, Krimm S. *Journal of Molecular Structure* 1996;377:219.
38. Cheam TC, Krimm S. *Spectrochimica Acta Part A: Molecular Spectroscopy* 1984;40:481.
39. Jordon T, Mukerji I, Yang W, Spiro TG. *J Biol Chem* 1996;379:51.
40. Triggs NE, Valentini JJ. *J Phys Chem* 1992;96:6922.
41. Myshakina NS, Ahmed Z, Asher SA. *J Phys Chem B*. 2008in press
42. Urry DW. *Journal of Physical Chemistry B* 1997;101:11007.
43. Bykov SB, Lednev IK, Ianoul A, Mikhonin AV, Asher SA. *Appl Spectrosc* 2005;59:1541. [PubMed: 16390595]
44. Frisch, MJ.; T, GW.; Schlegel, HB.; Scuseria, GE.; Robb, MA.; Cheeseman, JR.; Montgomery, JA., Jr; Vreven, T.; Kudin, KN.; Burant, JC.; Millam, JM.; Iyengar, SS.; Tomasi, J.; Barone, V.; Mennucci, B.; Cossi, M.; Scalmani, G.; Rega, N.; Petersson, GA.; 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, JE.; Hratchian, HP.; Cross, JB.; Bakken, V.; Adamo, C.; Jaramillo, J.; Gomperts, R.; Stratmann, RE.; Yazyev, O.; Austin, AJ.; Cammi, R.; Pomelli, C.; Ochterski, JW.; Ayala, PY.; Morokuma, K.; Voth, GA.; Salvador, P.; Dannenberg, JJ.; Zakrzewski, VG.; Dapprich, S.; Daniels, AD.; Strain, MC.; Farkas, O.; Malick, DK.; Rabuck, AD.; Raghavachari, K.; Foresman, JB.; Ortiz, JV.; Cui, Q.; Baboul, AG.; Clifford, S.; Cioslowski, J.; Stefanov, BB.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Martin, RL.; Fox, DJ.; Keith, T.; Al-Laham, MA.; Peng, CY.; Nanayakkara, A.; Challacombe, M.; Gill, PMW.; Johnson, B.; Chen, W.; Wong, MW.; Gonzalez, C.; Pople, JA. *Gaussian 03*. Vol. Revision C.01. Gaussian, Inc.; Wallingford CT: 2004.
45. Kohn W, Sham LJ. *Physical Review* 1965;137:1697.
46. Parr, RG.; Yang, W. *Density-functional theory of atoms and molecules*. Oxford Univ Press; Oxford: 1989.
47. Hohenberg P, Kohn W. *Phys Rev* 1964;136:B864.
48. Becke AD. *Journal of Chemical Physics* 1993;98:5648.
49. Lee C, Yang W, Parr RG. *Physical Review B: Condensed Matter and Materials Physics* 1988;37:785.
50. Miehlich B, Savin A, Stoll H, Preuss H. *Chemical Physics Letters* 1989;157:200.
51. Irikura KK, Johnson RD III, Kacker RN. *Journal of Physical Chemistry A* 2005;109:8430.
52. Halls MD, Velkovski J, Schlegel HB. *Theoretical Chemistry Accounts* 2001;105:413.
53. Ponder, JW. TINKER, Software Tools for Molecular Design. <http://dasher.wustl.edu/tinker>
54. Humphrey W, Dalke A, Schulten K. *J Molec Graphics* 1996;14:33.
55. Hinderaker MP, Raines RT. *Prot Sci* 2003;12:1188.

56. Mikhonin AV, Ahmed Z, Ianoul A, Asher SA. *Journal of Physical Chemistry B* 2004;108:19020.
57. Lednev IK, Karnoup AS, Sparrow MC, Asher SA. *J Am Chem Soc* 1999;121:8074.
58. Manas ES, Getahun Z, Wright WW, DeGrado WF, Vanderkooi JM. *J Am Chem Soc* 2000;122:9883.
59. Neidigh JW, Fesinmeyer RM, Andersen NH. *Nature Structural Biology* 2002;9:425.
60. Chen XG, Asher SA, Schweitzer-Stenner R, Mirkin NG, Krimm S. *Journal of the American Chemical Society* 1995;117:2884.
61. Torii H, Tasumi M. *Journal of Raman Spectroscopy* 1998;29:81.
62. Torii H, Tatsumi T, Tasumi M. *J Raman Spectrosc* 1998;29:537.
63. Eaton G, Symons CR, Rastogi PP. *J Chem Soc Faraday Trans1* 1989;85:3257.
64. Ham S, Kim JH, Lee H, Cho M. *Journal of Chemical Physics* 2003;118:3491.
65. Ramachandran GN, Lakshminarayanan AV, Kolaskar AS. *Biochimica et Biophysica Acta, Protein Structure and Molecular Enzymology* 1973;303:8.
66. Ramek M, Yu CH, Sakon J, Schafer LJ. *Phys Chem A* 2000;104:9636.
67. Selvarengan P, Kolandaivel P. *Bioorganic Chemistry* 2005;33:253. [PubMed: 16023485]
68. Ramachandran GN. *Biopolymers* 1968;6:1494. [PubMed: 5685106]
69. MacArthur MW, Thornton JM. *J Mol Biol* 1996;264:1180. [PubMed: 9000639]
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. [PubMed: 14653754]
71. Krimm S, Mirkin NG. *J Phys Chem A* 2004;108:5438.
72. Lopez-Garriga JJ, Hanton S, Babcock GT, Harrison JF. *J Am Chem Soc* 1986;108:7251.
73. Lopez X, Mujika JI, Blackburn GM, Karplus M. *J Phys Chem A* 2003;107:2304.
74. Alkorta I, Cativiela C, Elguero J, Gil AM, Jimenez AI. *New J Chem* 2005;29:1450.
75. Esposito L, De Simone A, Zagari A, Vitagliano L. *J Mol Struct* 2005;347:483.
76. Asher SA, Ianoul A, Mix G, Boyden MN, Karnoup A, Diem M, Schweitzer-Stenner R. *Journal of the American Chemical Society* 2001;123:11775. [PubMed: 11716734]
77. Mirkin NG, Krimm S. *Journal of Physical Chemistry A* 2004;108:5438.
78. Milner-White EJ. *Protein Sci* 1997;6:2477. [PubMed: 9385654]
79. Creamer TP. *Proteins: Structure, Function, and Genetics* 1998;33:218.
80. Tiffany ML, Krimm S. *Biopolymers* 1968;6:1379. [PubMed: 5669472]
81. Tiffany ML, Krimm S. *Biopolymers* 1969;8:347.
82. Tiffany ML, Krimm S. *Biopolymers* 1972;11:2309. [PubMed: 4634868]
83. Kakinoki S, Hirano Y, Oka M. *Polymer Bulletin* 2005;53:109.
84. Swenson CA, Formanek R. *The Journal of Physical Chemistry* 1967;71:4073. [PubMed: 6074049]
85. Mezei M, Fleming PJ, Srinivasan R, Rose GD. *Proteins: Structure, Function, and Bioinformatics* 2004;55:502.

Figure 1.

The 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.

Figure 2.

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

Figure 3.
Calculated conformational dependence of AmII'p frequency of ala-pro on Ψ -dihedral angle in water, heptane and gas phase (insert).

Figure 4.

Calculated ψ -dependence of the (A) C-N bond length (B) and C=O bond length of ala-pro in water, heptane and gas phase. C) Calculated dependence of ala-pro AmI'_{p} frequency and C-N bond length in water (grey 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 C-N bond in the dihedral plane defined by the C-C(O)-N-C* atoms.

Figure 6.

Calculated ϕ -dependence of ala-pro (A) $A_{mlI'p}$ frequency (B) C-N bond length (C) and the Θ planarity angle in water, heptane, acetonitrile and gas phase. (D) The calculated ala-pro $A_{mlI'p}$ frequencies and C-N bond lengths in water (grey circles) and gas phase (black squares) are linearly correlated.

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

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

Table 1Calculated geometric parameters of *cis*- and *trans*-conformer of Ac-Pro-Me

	<i>Trans</i> -Proline	<i>Cis</i> -Proline
d(C-N), Å	1.364	1.368
d(C=O), Å	1.227	1.223
d(O _{i-1} ...C _i), Å	3.045	4.393
∠ (O _{i-1} ...C _i =O _i), °	102.51	78.27
d(C _{i-1} =O _{i-1}), Å	1.211	1.209

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)	$\Delta\nu/^\circ\text{C}$ (cm ⁻¹ /°C)
Ala-Pro	1488	1487	-.017
Gly-Pro	1486	1485	-.017
Ser-Pro	1482	1480	-0.03
Val-Pro	1476	1475	-0.017

Table 3Calculated normal mode composition of ala-pro ($\psi = 145^\circ$).

Conformation	Gas Phase		Water	
	$\nu(\text{AmII}'\text{p})$	PED(>5%)	$\nu(\text{AmII}'\text{p})$	PED(>5%)
$\varphi = -60^\circ$	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)
$\varphi = -90^\circ$	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 4Ala-Pro ($\phi = -90^\circ$, $\psi = 145^\circ$) 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 ₂ O ^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.