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# Vibrational Raman Optical Activity Characterization of Poly(L-proline) II Helix in Alanine Oligopeptides

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Although originally defined for the conformation adopted by polymers of L-proline, the poly(L-proline) II (PPII) helix can be supported by amino acid sequences other than those based on L-proline and has been recognized as a common structural motif within loops in the X-ray crystal structures of many proteins.[1,2] It consists of a left-handed helix with three-fold rotational symmetry ($n = -3$) in which the $\phi,\psi$ angles of the constituent residues are restricted to values around $-78°$, $+146°$, corresponding to a region of the Ramachandran surface to one side of the $\beta$-region. The extended nature of the PPII helix precludes intrachain hydrogen bonds, the structure being stabilized instead by main-chain hydrogen bonding with water molecules and with side chains,[3] and possibly also by an $n \rightarrow \pi^*$ interaction between the $O_{i-1}$ peptide carbonyl oxygen and mainly the $C_i$ peptide carbon.[4] PPII currently attracts much interest as a major conformational element of disordered polypeptides and unfolded proteins in aqueous solution.[5] It can be distinguished from random coil in polypeptides using ultraviolet circular dichroism (UVCD),[5,6] vibrational circular dichroism (VCD),[7] and FTIR and Raman,[8] but in the presence of other conformational elements such as in proteins, it is difficult to identify it with these techniques. However, it appears to be readily identified, even in proteins, using Raman optical activity (ROA), one version of which provides vibrational optical activity spectra by measuring a small difference in the intensity of Raman scattering from chiral molecules in right- and left-circularly polarized incident light.[9−12]

ROA has been valuable for studying PPII in unfolded and partially folded proteins,[13] especially its potential role in promoting fibril formation in amyloidogenic protein systems.[14,15] The assignment of PPII ROA bands in this earlier work has been a little insecure since it was based on dominant features observed in the ROA spectra of disordered poly(L-lysine) and poly(L-glutamic acid), relying mainly on the UVCD and VCD evidence that these polypeptide states contain large amounts of PPII.[5−7] However, a recent study using a combination of NMR and UVCD has demonstrated that a seven-residue alanine peptide adopts predominantly the PPII conformation in aqueous solution.[16] Independently, a recent Raman, FTIR, and VCD study has revealed that cationic tetra-alanine ($Ala_4$) also adopts predominantly the PPII conformation in aqueous solution.[8] We have therefore measured the ROA spectra of these two alanine peptides, along with those of cationic $Ala_2$, $Ala_3$, $Ala_5$, and $Ala_6$, to confirm the earlier ROA band assignments for PPII structure and to monitor PPII formation as a function of chain length.

The backscattered Raman and ROA spectra of cationic $Ala_2$ to $Ala_5$ are displayed in Figure 1, and of Acetyl-OOAAAAAAAOO-Amide (OAO), where O represents ornithine, which is a slightly modified version of the one shown to adopt a predominantly PPII conformation,[16] in Figure 2, all in aqueous solution. These were measured on an incident circular polarization instrument described previously.[17] The corresponding spectra of disordered poly(L-glutamic acid) (PLG) are also shown in Figure 2 for comparison. Due to low solubility, the signal-to-noise ratio of the ROA spectrum of $Ala_6$ was poor, and it is not shown; but the main features are similar to those of the $Ala_5$ spectrum. The ROA spectrum of disordered PLG, which we have previously taken as characteristic of PPII structure, is dominated by a strong positive band at ~1319 $cm^{-1}$. This is within the extended amide III region where normal vibrational modes made up of various combinations of the in-plane N−H deformation, the C−N stretch, and $C_\alpha$−H deformations occur.[18] However, a detailed analysis of the actual normal mode composition is outside the scope of this article. This positive ~1319

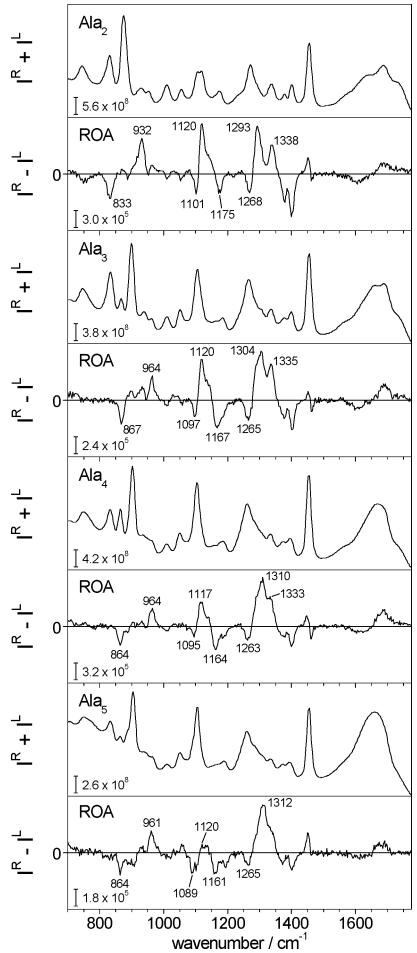


***Figure 1.*** Backscattered Raman ($I^R + I^L$) and ROA ($I^R - I^L$) spectra of aqueous solutions of cationic $Ala_2$ to $Ala_5$ (pH 1.0). Conditions: concentrations ~40−80 mg/mL; laser wavelength 514.5 nm; laser power ~700 mW at the sample; spectral resolution ~10 $cm^{-1}$.

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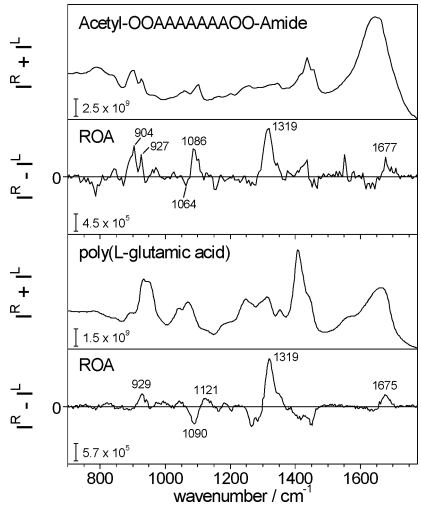


***Figure 2.*** Backscattered Raman and ROA spectra of aqueous solutions of Acetyl-OOAAAAAAAOO−Amide (pH 4.6) (prepared using Fmoc chemistry on a Rainin solid state synthesizer) and disordered poly(L-glutamic acid) (pH 12.6). Conditions as for Figure 1.

$cm^{-1}$ ROA band, together with weak positive amide I ROA intensity at ∼1675 $cm^{-1}$, have been taken previously as the main ROA signatures of PPII structure.[13−15] It is gratifying that the ROA spectrum of OAO is very similar to that of disordered PLG in the extended amide III and amide I regions, which supports our previous PPII assignments. This comparison also demonstrates that the ROA in these regions is largely independent of the nature of the side chains and thus does reliably reflect the backbone conformation.

By analyzing the amide I′ band profile in Raman, FTIR, and VCD spectra of cationic $Ala_4$ in $D_2O$ solution in terms of excitonic coupling between the amide I′ modes of the constituent peptide groups, the Ramachandran ($\phi$,$\psi$) angles of the two central amino acid residues were determined to be (−70°,155°) and (−80°,145°),[8] which are close to the angles for PPII helix. $Ala_4$ appears to exhibit a higher propensity for PPII than $Ala_3$ for which an ∼50:50 mixture of PPII and extended $\beta$-strand was deduced by a similar method.[19] The series of alanine peptide ROA spectra in Figure 1 reinforces these conclusions in a transparent way, because they clearly evolve toward that of the PPII-rich peptide OAO with increasing length: two positive ROA bands at ∼1291 and 1341 $cm^{-1}$ in $Ala_2$ gradually coalesce through $Ala_3$ and $Ala_4$ into a strong positive ROA band at ∼1312 $cm^{-1}$ in $Ala_5$ that looks similar to those at ∼1319 $cm^{-1}$ in OAO and disordered PLG. Furthermore, most of the other ROA bands, which arise from motions of the $-NH_3^+$ and $-CO_2H$ end groups together with the $-CH_3$ side chains and which are very prominent in $Ala_2$, having been discussed previously for this molecule,[20] are increasingly suppressed as the extended amide III pattern strengthens, thereby reinforcing its assignment to peptide backbone modes.

This study has confirmed the previous assignment of positive ROA at ∼1319 $cm^{-1}$, together with weak positive ROA at ∼1675 $cm^{-1}$, to PPII. However, positive ROA as low as ∼1310 $cm^{-1}$ should now also be considered as sometimes diagnostic of PPII, perhaps in short segments. The precise position of this band appears to be sensitive to pH since in $Ala_4$ it has previously been reported to peak at ∼1312, ∼1315, and 1325 $cm^{-1}$ at low, neutral, and high pH, respectively,[20] suggesting that small changes in the PPII conformational parameters occur with changes in pH. Positive protein ROA bands in the range ∼1315−1325 $cm^{-1}$ have already been taken as diagnostic of PPII.[13]

A theoretical study of the energy landscapes and folding kinetics of $Ala_{12}$ found that solvation has a dramatic effect.[21] In a vacuum, the narrow basin corresponding to the α-helical structure is more stable than the broad basin associated with coil and $\beta$-structures, but in water the relative energies of the two basins are reversed. The present work, together with the earlier reports,[8,16] provides experimental support for this conclusion, but with the refinement that it is the PPII region of the coil/$\beta$-structure basin that is the most stable in aqueous solution, at least for $Ala_4$−$Ala_7$ the structures of which are nothing like a random coil.

Our observations also support the earlier finding that segments of at least four alanine residues are required to fully stabilize the PPII conformation in aqueous solution.[8] Interestingly, molecular dynamics simulations suggest that a peptide segment of four alanine residues can support the formation of a strongly hydrated groove around the peptide backbone.[22] It has also been pointed out that the $n \rightarrow \pi^*$ interaction between the $O_{i-1}$ peptide carbonyl oxygen and the $C_i$ peptide carbon, which is especially favorable in the PPII conformation, can be cooperative.[4] However, another theory based on a minimization of chain-packing density predicts noncooperative PPII-like structures for alanine peptides in aqueous solution.[23]

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