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UV Resonance Raman Elucidation of the Terminal and Internal Peptide Bond Conformations of Crystalline and Solution Oligoglycines

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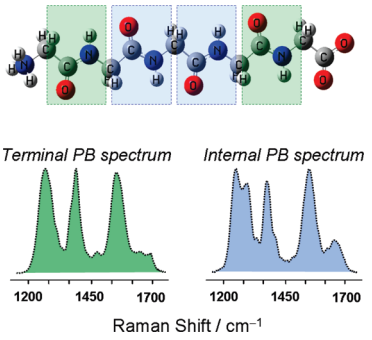
ABSTRACT Spectroscopic investigations of macromolecules generally attempt to interpret the measured spectra in terms of the summed contributions of the different molecular fragments. This is the basis of the local-mode approximation in vibrational spectroscopy. In the case of resonance Raman spectroscopy, independent contributions of molecular fragments require both a local-mode-like behavior and the uncoupled electronic transitions. Here, we show that the deep-UV resonance Raman spectra of aqueous solution-phase oligoglycines show independent peptide bond molecular fragment contributions, indicating that peptide bond electronic transitions and vibrational modes are uncoupled. We utilize this result to separately determine the conformational distributions of the internal and penultimate peptide bonds of oligoglycines. Our data indicate that in aqueous solution, the oligoglycine terminal residues populate conformations similar to those found in crystals (3_1-helices and β-strands) but with a broader distribution, while the internal peptide bond conformations are centered around the 3_1-helix Ramachandran angles.

SECTION Kinetics, Spectroscopy



Spectroscopic methodologies are of a great importance in chemical analysis and for the determination of molecular structure. Interpretations of spectra generally use reductionist strategies whereby the spectrum of a macromolecule is first interpreted as the sum of the contributions of the individual molecular fragments. This first-order analysis is then reanalyzed to take into account higher-order phenomena such as the coupling of molecular dynamics between molecular fragments and the environment. For example, in UV resonance Raman (UVRR) spectra of polypeptides, the vibrations could be localized within the individual peptide bond (PB), such that the PBs independently contribute. This would lead to spectra that are easily analyzed, where each PB vibration is not context-dependent. Alternatively, spectra of polypeptides may result from coupled motion of the backbone atoms of adjacent PBs.[1] This would lead to vibrational modes which depend upon their adjacent PB secondary structures.

Our previous study of a mainly ala-peptide in H_2O/D_2O mixtures indicated that the spectra of the partially deuterated chains could be roughly modeled (except for the amide I band) as a statistically weighted sum of deuterated and protonated independent segments.[2,3] This could only occur if the amide vibrations were essentially localized within the individual PB.

Here, we show that the deep-UV resonance Raman spectra of aqueous solution-phase oligoglycines show independent peptide bond molecular fragment contributions; the peptide bonds independently resonance Raman scatter. The peptide bond electronic transitions and vibrational modes are uncoupled. We utilize this result to separately determine the conformational distributions of the internal and penultimate peptide bonds of oligoglycines.

Vibrational and Electronic Coupling. Figure 1 compares the 204 nm UVRR difference spectrum between gly_6 and gly_5 to that of gly_3. If there were negligible coupling between the PB vibrations, the gly_3 spectrum should closely approximate the two terminal PB spectra of oligopeptides, while the $gly_6 - gly_5$ spectrum should approximate a single internal PB vibration.

Summation of the $gly_6 - gly_5$ difference spectra and the gly_3 spectra essentially perfectly models the UVRRS of gly_5 and gly_6 (Figure 1). The differences are below shot noise levels. This result indicates the lack of coupling of peptide bond vibrations between adjacent PB. The ability to accurately model the spectral intensities indicates that couplings between these vibrations and the resonant electronic transitions are localized within each PB.

Internal PB Conformation Preferences. The $gly_6 - gly_5$ spectrum differs significantly from that of gly_3, with a clear doublet spanning the CH_2 twist/Am III spectral region, while gly_3 shows a single complex band shape. The CH_2 wagging, as well as the Am II and Am I bands of $gly_6 - gly_5$, also differs from that of gly_3, which may imply different structural preferences of the internal and terminal residues.

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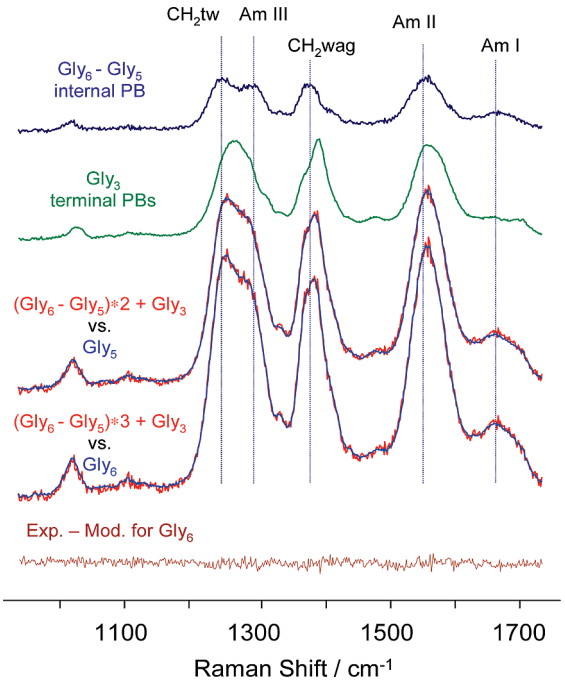

Figure 1. The 204 nm UVRR spectra of oligoglycines in aqueous solutions containing 0.5 M $LiClO_4$, at neutral pH. The $gly_6 - gly_5$ difference spectrum approximates the spectrum of an internal peptide bond, while that of gly_3 approximates that of the two terminal peptide bonds. This is demonstrated by the fact that the summation of these spectra accurately models gly_5 and gly_6 spectra, as demonstrated by the lack of features in the bottom gly_6 difference spectrum between the experimental and modeled spectra. All of the UVRRS were scaled relative to the internal standard perchlorate 932 cm^{-1} band (not shown).

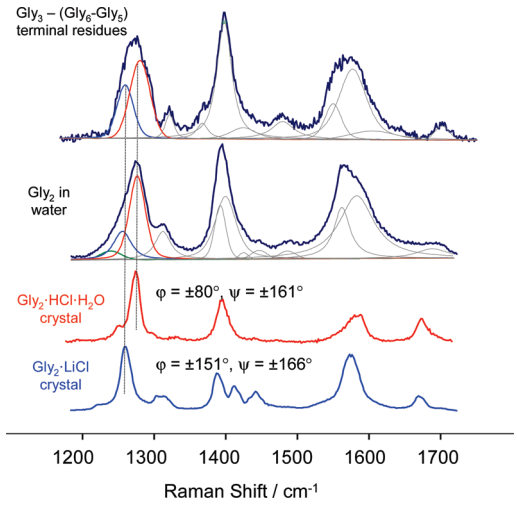

Figure 2. UVRR difference spectrum $gly_3 - (gly_6 - gly_5)$ approximates the spectrum of the two terminal residues of oligoglycines in solution. Also shown is the spectra of gly_2 in solution and two gly_2 crystal samples of known structure, $Gly_2 \cdot HCl \cdot H_2O$ ($\varphi = \pm 80°$, $\psi = \pm 161°$) and $Gly_2 \cdot LiCl$ ($\varphi = \pm 154°$, $\psi = \pm 168°$).[5,6] The complex amide III band shapes of the solution samples can be well modeled with the amide III bands of these crystal gly_2 derivatives.

In a separate study reported elsewhere,[4] we compared the internal residue oligoglycine UVRR spectra in solution to that of solid polyglycines of known conformations. These studies indicate that the internal PBs of oligoglycines occur as a broad ensemble of conformations centered around the 3_1-extended helix conformation.

Penultimate PB Conformation Preferences. The UVRR spectrum of gly_3 closely approximates the spectrum of the terminal PB of longer oligoglycines. Gly_3 shows a complex amide III band shape, suggesting that multiple conformational states of the peptide are populated in solution. Subtraction of the spectrum of an internal PB ($gly_6 - gly_5$) from the spectrum of gly_3 produces a spectrum which is very similar to that of gly_2 in water (Figure 2), which should approximate the spectrum of the average of the two penultimate oligoglycine PBs.

We characterized the conformations of the $gly_3 - (gly_6 - gly_5)$ PB and gly_2 in solution by comparing their complex amide III bands to those of the amide III bands of crystalline gly_2 derivatives of known structures.

The solution UVRR spectra of $gly_3 - (gly_6 - gly_5)$ and gly_2 show amide III spectral features similar to those in the spectra of gly_2 crystals with known φ and ψ angles (Figure 2). These frequencies and bandwidths are listed in Table 1.

Crystalline $Gly_2 \cdot HCl \cdot H_2O$ have $\varphi = \pm 80°$ and $\psi = \pm 161°$ dihedral angles that are close to those of a 3_1-extended helix.[5] Crystals of $Gly_2 \cdot LiCl$ have $\varphi = \pm 154°$ and $\psi = \pm 168°$, which are close to that of a β-strand.[6] The amide III band of the β-strand gly_2 is 19 cm^{-1} downshifted compared to that of the 3_1-extended helix, indicating significant conformational sensitivity (Table 1). The amide III frequencies also depend upon the hydrogen bonding to the PB N−H since N−H bending significantly contributes to the amide III vibration. However both crystal structures have N−H hydrogen bonded to the Cl^-, which implies similar HB strengths.

The amide III region of the gly_3-(gly_6-gly_5) difference spectrum, as well as that of the gly_2 spectrum have a complex shape indicating significant conformational inhomogeneity of the terminal residues.

We can estimate the conformational distribution of the solution oligoglycine terminal residues by modeling the amide III region of the $gly_3 - (gly_6 - gly_5)$ difference spectrum with the well-defined amide III bands of crystal gly_2 of known structures. Assuming that the integrated intensities of the amide III bands are proportional to the populations of corresponding conformations in solution, we estimate that in solution, ~60% of terminal residues are in a 3_1-extended helix-like conformation while ~40% are in extended β-strand-like conformation. Modeling of the gly_2 in solution gives similar results (~65% in a 3_1-extended helix-like conformation and ~35% in an extended β-strand-like conformation).

As discussed above, the internal residues of oligoglycines in solution mainly populate a 3_1-extended helix-like conformation. The higher preferences of the terminal residues for a β-strand conformation may result from the favorable interaction between terminal $-NH_3^+$ and the adjacent PB carbonyl, as well as between the terminal $-COO^-$ and the adjacent PB N−H, which are maximized for the completely extended conformation.

The amide III bands of the crystalline samples show full widths at half height of ~15−18 cm^{-1}, which is likely to be their homogeneous bandwidth. The corresponding

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Table 1. Frequencies (ν) and Bandwidths (w) of UVRR Amide Bands in the $gly_3 - (gly_6 - gly_5)$ Difference Spectrum, gly_2 in Solution and gly_2 Crystals

	$gly_3 - (gly_6 - gly_5)$ difference spectrum ν (w) /cm^{-1}	gly_2 in solution ν (w) /cm^{-1}	$Gly_2 \cdot HCl \cdot H_2O$ crystals ν (w) /cm^{-1}	$Gly_2 \cdot LiCl$ crystals ν (w) /cm^{-1}
Am III	1261(27), 1281(33)	1245(34),1261(29), 1281(26)	1286(15)	1267(18)
CH_2 wag	1400(25)	1395(18), 1402(34)	1404(17)	1393(16)
Am II	1553(27), 1580(46)	1562(27), 1584(57)	1580(38), 1593(20)	1575(29)
Am I	1705(24)	1686(51)	1678(16)	1670(17)

solution amide III bands show almost two-fold broader bandwidths of ~26−34 cm^{-1}, which indicate that the terminal residues of oligoglycines in solution populate a broad ensemble of extended conformations with some preference for the 3_1-helix.

In conclusion, the UV resonance Raman spectra of oligoglycines in aqueous solution can be accurately modeled as a linear sum of the spectra of their internal and terminal peptide bonds, which indicates the absence of vibrational and electronic coupling between adjacent PBs in polypeptides in solution. This result significantly simplifies polypeptide UVRR spectral analysis. The UVRR spectra of the oligoglycine internal PBs significantly differ from those of the terminal PBs, which implies different structural preferences. Our data indicates that in aqueous solution, oligoglycine terminal residues populate a broad range of extended conformations between 3_1-helices and β-strands while the internal PBs conformation are centered around the Ramachandran angles of the 3_1-helix.

Experimental Methods

Materials. Gly_2, gly_3, gly_5, and gly_6 were purchased from Bachem and used as received. Gly_2 hydrochloride (Sigma Inc.) was recrystallized from water to obtain $Gly_2 \cdot HCl \cdot H_2O$ crystals. Lithium chloride (T.J. Baker Inc.) was used for cocrystallization with gly_2 to grow $Gly_2 \cdot LiCl$ crystals. Lithium perchlorate (Fisher Scientific Inc.) was used to increase the solubility of gly_5 and gly_6 in water and as an internal Raman intensity standard.

Raman Measurements. For solution samples, we used 204 nm Raman excitation near the maximum absorbance of the peptide bond $\pi \rightarrow \pi^*$ transition. The third harmonic of the Nd:YAG laser operating at 100 Hz was anti-Stokes Raman shifted in hydrogen to 204 nm (fifth anti-Stokes) . The peptides were studied in a flow stream to avoid any contribution from photochemical degradation processes. Scattered light was dispersed by a double spectrometer and was detected by a Princeton Instruments Spec-10:400B CCD camera (Roper Scientific). A detailed description of the instrumentation is given elsewhere.[7] For solid samples, we used preresonance 229 nm excitation to minimize sample photodegradation. Raman measurements were performed using an intracavity doubled Ar ion Laser (Coherent Inc.) A custom-made, rotating metal cell was used for the solid powder samples to avoid light-induced sample degradation. The crystal powder was pressed into a circular groove in the rotating metal cylinder. Typical accumulation times were less than 1 min.

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