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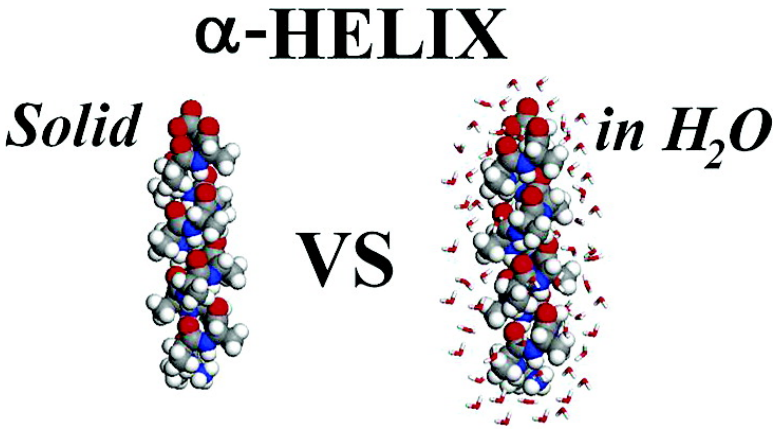

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UV Raman Examination of α-Helical Peptide Water Hydrogen Bonding

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Both intramolecular protein−protein interactions and protein−solvent interactions determine native protein secondary and tertiary structures. For example, intramolecular protein hydrogen bonding (H-bonding) stabilizes α-helical and β-sheet conformations,[1−3] while protein−water interactions stabilize peptide and protein PPII conformations.[4−7] However, even though intramolecular H-bonding is expected to dominate the amide carbonyl and N−H groups H-bonding of α-helical peptides, an exterior sheath of water should hydrogen bond to exposed intramolecularly bonded N−H···O═C linkages.[1,8−10] Such interactions may also be important in the stabilization of the α-helix conformation.[11−13] These H-bonding interactions are expected to depend on the extent that the peptide side chains shield the peptide backbone from water.

In this work, we directly examine water H-bonding to the backbone of α-helical peptides by comparing 204 nm UV Raman (UVR) spectra of an α-helical peptide between aqueous solution and the dehydrated solid state. We examine here the extent of H-bonding to α-helices and the consequence of this H-bonding to the amide vibrational spectra. One of our goals is to develop a method to monitor this H-bonding.

It has been demonstrated that the UVR amide band spectra are sensitive to both the peptide secondary structure composition[14−16] and the peptide−water interactions.[15,17] Excitation within the ∼200 nm peptide bond $\pi \rightarrow \pi^*$ transitions yields UVR spectra dominated by the peptide bond vibrations.[16]

In the present work, we examine the peptide−water H-bonding interactions of a 21 residue, mainly Ala peptide (AP), which exists at low temperatures in an equilibrium between the α-helical and PPII conformations.[15,17,18] We previously characterized the temperature dependence of the UVR spectrum of the pure AP α-helix in water between −5 and 30 °C[18] and described in great detail the numerical procedure used to remove the PPII contribution from the observed spectra.[15,18,19]

Briefly, we determined the aqueous AP structural composition between 0 and 80 °C and found that it was essentially >95% PPII above 45 °C. We monitored the temperature dependence of the PPII spectrum between 45 and 80 °C and assumed a similar temperature dependence of the PPII spectra at lower temperatures. It should be noted that we earlier had assumed that these PPII spectra were due to a random coil conformation.[15]

Because we knew the temperature dependence of the frequencies, intensities, and bandwidths of the PPII spectra at all temperatures, we could subtract the amount necessary to end up with a pure α-helix spectra.[15,18,19] The amount to be subtracted was easily determined from the intensity of the UVR C_α−H bending bands which serve as sensitive markers of the PPII spectral contribution.[16,17,20,21] These bands do not appear in the α-helix spectra.

Amide bands of both the pure α-helix[8,11,18] and PPII[15,17,22] conformations undergo characteristic changes with temperature. These changes cannot be attributed to conformational transitions since they show up in AP kinetic spectra at the shortest delay times,[15,22] in a manner very similar to that in dilute solutions of *N*-methylacetamide[18] and in a manner expected from theoretical predictions.[23]

In the work here, we prepared an α-helical solid AP sample by quickly freezing a solution of AP initially at −5 °C (where the α-helix content[15] is ∼63%, while the PPII content is ∼37%) in liquid nitrogen. The frozen sample was then dehydrated by lyophilization at low temperature, after which it was warmed to room temperature under vacuum. We expect that this process results in a solid AP sample which contains significant amounts of α-helix as well as other conformations, which probably include β-sheet, β-strand, and disordered conformations, with some non-hydrogen-bonded amide bonds. It is known that polyalanine can occur in α-helix[24] and β-sheet[2] conformations in the solid phase.

We also prepared a solid AP sample which we expect contains similar *non-α-helical* conformations. This was prepared by slow 40 °C evaporation of an AP solution. At 40 °C, low concentration AP occurs mainly in a PPII conformation.[15,17,22] PPII is expected to convert to β-sheet or β-strand-like conformations upon dehydration since as peptide−water H-bonds are lost, the H-bonding switches to intermolecular hydrogen bonds.

We expect a similar process will occur when frozen PPII conformations lose water. Further, the resulting conformations will still be limited to the allowed regions of the Ramachadran Ψ and Φ angles within the broad β-sheet region. Since peptide bond UVR spectra are mainly dependent on their Ψ Ramachadran angle,[17,20] the non-α-helical intermolecularly hydrogen-bonded conformations in the lyophilized and evaporated solid samples should show roughly similar UVR spectra. We also implicitly assume that the lyophilized sample α-helices do not unfold during freezing and the consequent dehydration.

Figure 1 compares the spectra of the solid AP samples prepared by these two methods to the 70 °C solution β-sheet spectrum of a 1:1 molar mixture of polyglutamic acid (PGA) and polylysine (PLL), and to solution spectra of AP α-helix at various temperatures. The PGA−PLL spectrum shown is a pure β-sheet spectrum.[25]

The high-temperature-evaporated solid AP spectrum is roughly similar to that of β-sheet. For example, the AmI bands occur at ∼1670 cm^{-1} (the increased AmI band intensity in AP and the ∼1690 cm^{-1} shoulder result from Arg side chain contributions which are absent in the PGA−PLL sample). Similar AmII frequencies occur in the evaporated AP and β-sheet spectra, although the band shapes show differences. A higher AmIII frequency occurs in the evaporated AP spectrum than in the PGA−PLL β-sheet spectrum. These differences probably result from the existence of additional slightly different conformations in the evaporated AP sample.

We calculated the solid α-helix spectrum from the −5 °C liquid nitrogen frozen lyophilized AP sample by subtracting 37% of the evaporated AP spectrum (the rest remained in α-helix). This yielded a spectrum similar in shape to those of the α-helix solution spectra shown at the bottom of Figure 1. These α-helix solution spectra

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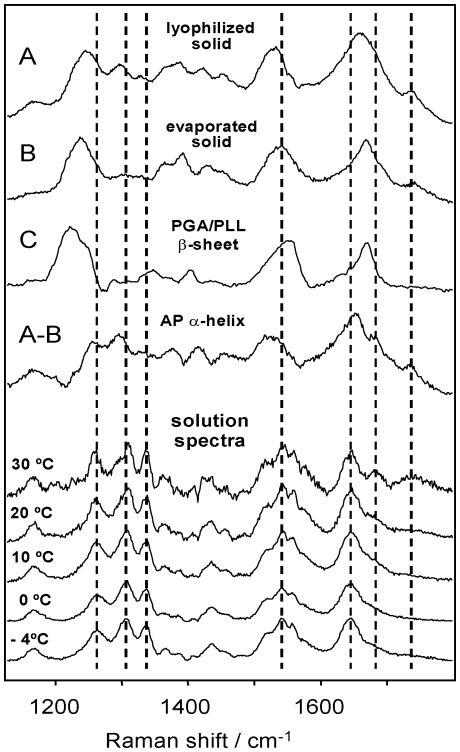

Figure 1. The 204 nm UVR spectra of (A) solid sample obtained by freezing a −5 °C AP solution and lyophilizing (∼63% α-helix); (B) solid sample obtained by evaporating 40 °C AP solution; and (C) calculated spectrum of pure β-sheet PGA−PLL stoichiometric mixture in solution at 70 °C. (A−B) solid AP α-helix spectrum calculated by subtracting spectrum B (subtraction factor 0.37) from spectrum A. Lower spectra are pure α-helix AP solution spectra at the indicated temperatures. See text for details.

were calculated by subtracting[15,18] pure PPII spectra from the measured spectra until the C_α−H b band disappeared.

The 204 nm UVR AP solution phase α-helical spectra are distinguished by a triplet of AmIII bands [labeled $AmIII_1$ (1337 cm^{-1}), $AmIII_2$ (1306 cm^{-1}), and $AmIII_3$ (1261 cm^{-1}) at −4 °C][18] and the absence of a ∼1400 cm^{-1} C_α−H band. The AmII band occurs at 1542 cm^{-1}, while the AmI band occurs at 1647 cm^{-1}. Peptides in which the amide bonds are hydrogen bonded to water show downshifts for the AmII and AmIII bands and upshifts for the AmI band when the temperature increases due to weakening of water H-bonding.[8,11,18] In contrast, few changes occur for the solution phase α-helix conformation, where most of the H-bonding is satisfied by intramolecular interactions. Only the $AmIII_3$ band shows a significant frequency decrease with temperature.

The calculated 204 nm UVR spectrum of solid α-helical AP is similarly dominated by a triplet of AmIII bands, which are all downshifted from that in solution. Compared to the −4 °C solution spectrum, the solid α-helix $AmIII_1$ band downshifts 3 cm^{-1}, while the $AmIII_2$ band downshifts 11 cm^{-1} and the $AmIII_3$ band downshifts 5 cm^{-1}. Thus, removal of water H-bonding decreases the AmIII triplet frequencies much more than does the modest temperature-induced weakening of the water hydrogen bond strengths.

Both the AmII and AmIII bands, which have contributions from C−N stretching and N−H bending, upshift as H-bonding to the amide bond increases. This upshift occurs for two reasons: a water molecule hydrogen bonded to the amide hydrogen increases the N−H bending force constant, and H-bonding stabilizes a resonance structure which increases the double bond character of the C═N bond.[11,17] Thus, these vibrational frequencies increase.

The AmII band at 1524 cm^{-1} shows the largest frequency decrease (20 cm^{-1}) upon the loss of water H-bonding. This behavior is similar to that previously observed for the temperature dependence of the PPII conformation.[15,17] The AmII vibration is the most sensitive to hydration according to the theoretical *N*-methylacetamide calculations of Torii et al.[23]

In contrast, the ∼1652 cm^{-1} AmI band of solid α-helical AP is ∼7 cm^{-1} upshifted compared to that of α-helical AP in water, which is consistent with a loss of peptide−water H-bonding. Namely, this AmI band upshift occurs due to the increased contribution of carbonyl double-bonded resonance structure.[11,17] In addition, another band appears in solid α-helix AP at ∼1734 cm^{-1}, which also emerges in α-helix AP in water at the highest temperature of 30 °C. This 1734 cm^{-1} band probably derives from a non-hydrogen-bonded amide C═O groups.

This study allows us to conclude that a H-bonding water sheath occurs around a polyalanine α-helical peptide. These peptide−water hydrogen bonds increase the AmII and AmIII band frequencies and decrease the AmI frequency, as expected. We can use this correlation to monitor amide−water H-bonding of α-helices in proteins. As shown elsewhere,[8,18,26] one can isotopically edit particular peptide bonds to determine amide band frequencies for particular bonds to determine H-bonding at particular sites.

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