Uncoupled Peptide Bond Vibrations in α-Helical and Polyproline II Conformations of Polyalanine Peptides

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We examined the 204-nm UV resonance Raman (UVR) spectra of the polyproline II (PPII) and α-helical states of a 21-residue mainly alanine peptide (AP) in different H_2O/D_2O mixtures. Our hypothesis is that if the amide backbone vibrations are coupled, then partial deuteration of the amide N will perturb the amide frequencies and Raman cross sections since the coupling will be interrupted; the spectra of the partially deuterated derivatives will not simply be the sum of the fully protonated and deuterated peptides. We find that the UVR spectra of the AmIII and AmII′ bands of both the PPII conformation and the α-helical conformation (and also the PPII AmI, AmI′, and AmII bands) can be exactly modeled as the linear sum of the fully N−H protonated and N−D deuterated peptides. Negligible coupling occurs for these vibrations between adjacent peptide bonds. Thus, we conclude that these peptide bond Raman bands can be considered as being independently Raman scattered by the individual peptide bonds. This dramatically simplifies the use of these vibrational bands in IR and Raman studies of peptide and protein structure. In contrast, the AmI and AmI′ bands of the α-helical conformation cannot be well modeled as a linear sum of the fully N−H protonated and N−D deuterated derivatives. These bands show evidence of coupling between adjacent peptide bond vibrations. Care must be taken in utilizing the AmI and AmI′ bands for monitoring α-helical conformations since these bands are likely to change as the α-helical length changes and the backbone conformation is perturbed.

Introduction

Vibrational spectroscopy[1−25] is a very powerful tool for studying peptide and protein structure. The utility of vibrational spectroscopic methods derive from their inherently high resolution. In addition, these methods are uniquely powerful since they can be used kinetically to examine short time (nanosecond, picosecond, and femtosecond) dynamics.[26−37] UV resonance Raman spectroscopy (UVRS)[4−7,27,28] is an especially powerful approach, since it can be used to selectively excite within the amide backbone $\pi \rightarrow \pi^*$ transition[38] in order to selectively examine numerous amide vibrations.[39−42] In addition, it shows little interference from the vibrations of the ubiquitous solvent water,[27,43] in contrast to IR absorption-based methods.[44]

We recently demonstrated that UVRS is the most powerful method to determine secondary structure of proteins and peptides in dilute solutions.[6,27,45] The UVRS method utilizes the unique spectral signature of each of the protein secondary structure motifs.[6] The intrinsic assumption is that the basis spectra of each motif can be linearly added to fit the observed spectra and that the linear weighting factors give the relative abundancies of each motif.

The use of this approximation could be problematic. For example, it is clear that the Raman cross section of the α-helix conformation is relatively small, due to the dependence of the Raman cross sections on the molar absorptivity of the peptide bond electronic transitions.[39] It is well-known that the amide backbone $\pi \rightarrow \pi^*$ transitions suffer hypochromism due to excitonic interactions.[38,39,46−49] This causes hypochromism in the resonance Raman cross sections. Thus, the Raman spectra of an amide bond with α-helical Φ and Ψ angles, but not in an α-helix, will display a spectrum identical to that of a peptide bond within a long α-helix at similar hydrogen bonding conditions.[7] However, the long α-helix peptide bond Raman cross sections will be smaller due to hypochromism.[50] The α-helical Raman intensities increase as the helix length shortens, exactly the opposite behavior seen for CD α-helical molar ellipticities. In fact, the CD molar ellipticity vanishes for short helices.[45,51,52]

The intrinsic assumption utilized in many vibrational spectroscopic studies is that the measured spectra derive from the linear summation of spectra contributed by the individual peptide bonds. This assumption ignores the possibility of coupling of the vibrational motion of adjacent peptides. This assumption is used even though there are numerous reports that indicate coupling of the amide I (AmI) bands of peptides.[36,53−59]

The nature of amide vibrations is very complex[50,60−65] and not yet fully understood. The vibrations of a peptide group involve atomic motions such as CO stretching (CO s), CN stretching (CN s), C_α−C stretching (C_α−C s), NH bending (NH b), and C_α−H bending (C_α−H b). The individual vibrational modes of adjacent peptide bonds could strongly couple to each other to form complex collective vibrations. This coupling could occur as a result of through-bond interactions between adjacent amide groups,[54,58,59] through hydrogen-bond interactions,[58] and/or through the interactions between the oscillating electronic charge densities of adjacent peptide groups. The latter interactions can be expressed either in terms of a relatively simple transient dipole coupling model (TDC),[55−57,59] or in terms of a somewhat more complex transient charge model (TCM).[13,54,55] TDC is expected to have the greatest impact on vibrations with

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large dipole moment changes. As expected, there are examples of coupling between the AmI modes of peptide groups due to their large dipole moment changes.[36,53−59]

In a previous investigation[66] we attempted to examine the coupling between adjacent peptide bonds by investigating the UVR spectra of two linked amides in mixed H_2O/D_2O solutions. Deuteration of the amide N (N−D) dramatically perturbs the amide vibrational modes.[60] The experiment probed whether deuteration of one amide perturbed the vibrational modes of the linked amide. In the case studied, we clearly demonstrated independent AmIII, AmII, and AmII′ vibrations in this small peptide. Unfortunately, we were unable to come to a definitive conclusion for the AmI bands because of their weak intensities.[66]

In the work here, we examine the same issue in a 21-residue mainly ala peptide AP which is ∼50% α-helix at 0 °C[27,28] and melts to a polyproline II (PPII) conformation at higher temperatures.[67] We already examined the UVR spectra and the normal modes of this peptide in both its α-helical and PPII states in detail.[50] We demonstrated that, as in the amide functional group, N-deuteration of a peptide bond dramatically changes the normal mode composition of the peptide bond vibrations due to the decoupling of CN s and NH b motion.[60,68] This gives rise to completely differentiable spectra of the N−H and N−D peptide bonds, which permits us to monitor spectral changes due to the deuteration of adjacent peptide bonds. We searched for evidence of perturbation of the spectra due to adjacent peptide bond deuteration. As shown below, we see no evidence for coupling of the amide vibrations, except for the lone case of the AmI/AmI′ vibrations of the α-helix conformation of AP. This indicates that, in most cases, vibrational spectra can be simply interpreted as the linear sum of the spectra of the individual peptide bonds. This result increases the utility of vibrational spectroscopic methods for studying protein structure and function.

Experimental Section

Sample Preparation. The 21-residue ala-based peptide $AAAAA(AAARA)_3A$ (AP) was prepared (HPLC pure) at the Pittsburgh Peptide Facility by using the solid-state peptide synthesis method. The AP H_2O/D_2O mixtures contained 1 mg/mL AP and 0.2 M sodium perchlorate. All Raman spectra were normalized to the intensity of the ClO_4^- internal standard Raman band (932 cm^{-1}).

To ensure the random deuteration of the peptide groups along the AP chain in its α-helical state, all the AP solutions in H_2O/D_2O mixtures were held at 62 °C for 5 min prior to lowering the temperature to form the α-helix conformations. At 62 °C, AP exists predominantly in a highly water-exposed PPII conformation, which ensures fast and random H/D exchange of the amide nitrogens. All UVRS measurements were cycled through the following temperature sequence: 0, 50, 62, 0 °C.

L-Arginine was purchased from Sigma Chemical (St. Louis, MO) and used as received. All the arg solutions in H_2O/D_2O mixtures contained 5 mg/mL concentrations of arg, and 0.5 M concentrations of sodium perchlorate.

Instrumentation. The UV resonance Raman instrumentation has been described in detail elsewhere.[27,28] A Coherent Infinity Nd:YAG laser produced 355-nm (3rd harmonic) 3-ns pulses at 100 Hz. This beam was Raman shifted to 204 nm (5th anti-Stokes) by using a 1-m tube filled with hydrogen (60 psi). A Pellin Broca prism was used to select the 204-nm excitation beam. The Raman scattered light was imaged into a subtractive double spectrometer,[69] and the UV light was detected by a Roper Scientific nitrogen-cooled CCD camera. All samples were measured in a thermostated free surface flow stream.

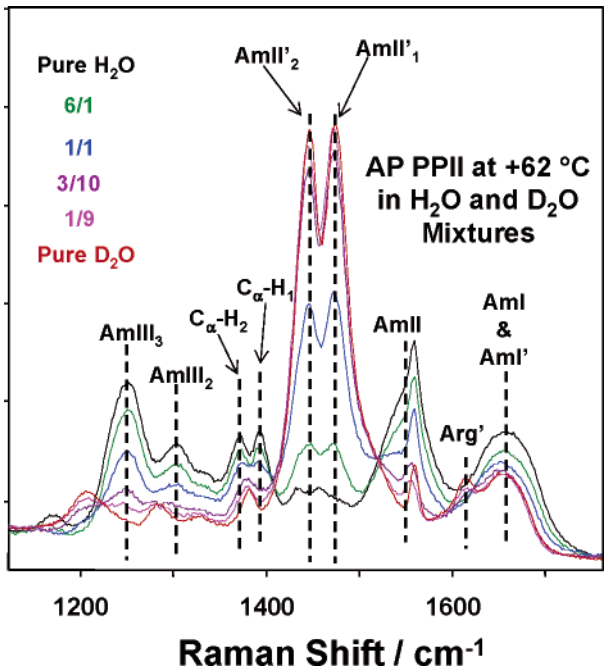

Figure 1. The 204-nm UVRR spectra of the AP PPII-state in different H_2O/D_2O mixtures at 62 °C. Neither H_2O nor D_2O Raman spectra were subtracted. The O_2 stretching contribution (sharp band at ∼1555 cm^{-1}, overlapping the AmII band) was not removed.

Results and Discussion

AP is >50% α-helical at 0 °C and melts as the temperature increases to 30 °C.[27] The unfolded state of AP peptide was initially assumed to be a random coil state.[27,28,70] However, we recently demonstrated that the AP α-helix melts to a predominantly polyproline II (PPII) conformation.[67] The kinetic studies[27,28,70] reveal fast (∼200 ns) α-helix melting to the PPII-state without any significantly populated intermediates.

AP PPII Bands in H_2O. The 204-nm excitation occurs within the $\pi \rightarrow \pi^*$ electronic transitions of the amide backbone,[38] which enhances the backbone amide bond vibrational modes.[39−42] UVR spectra of the AP PPII-state in water at 62 °C (Figure 1) show an AmI band at 1660 cm^{-1} (mainly CO stretching), the AmII band at 1558 cm^{-1} (mainly CN s and NH b), and the C_α−H b doublet (with a contribution from the CH_3-umbrella mode) at 1370 and 1394 cm^{-1}. The C_α−H b bands are resonancely enhanced only in PPII-like and β-sheet-like conformations and do not appear in the α-helix conformation.[6,7,50,67,71] Our recent study of the AmIII region[50] has demonstrated the complex origin of these bands which involve CN s and NH b coupled to C−C_α s and C_α−H b motions.[7,50] The 1245 cm^{-1} ($AmIII_3$) band,[50] which is assigned to the "classic" AmIII band, shows dominating contributions from NH bending and C−N stretching. The 1303 cm^{-1} ($AmIII_2$) band derives mainly from C−C_α stretching mixed with C_αH bending, with possibly a small contribution from CN stretching.[50] The weak $AmIII_1$ band at 1336 cm^{-1} was suggested[50] to result from a minor β-strand conformation of AP. In this case, the band would contain contributions from mainly C_α−C s and N−C s.

AP PPII bands in pure D_2O. The 204 nm UVRR spectrum of PPII AP in pure D_2O at 62 °C (Figure 1) shows a dominating Am II′ doublet at 1444 and 1476 cm^{-1} due to the exchange of the peptide bonds to form the N−D derivatives. In the N−D derivative the ND b is decoupled from the CN s. This doublet

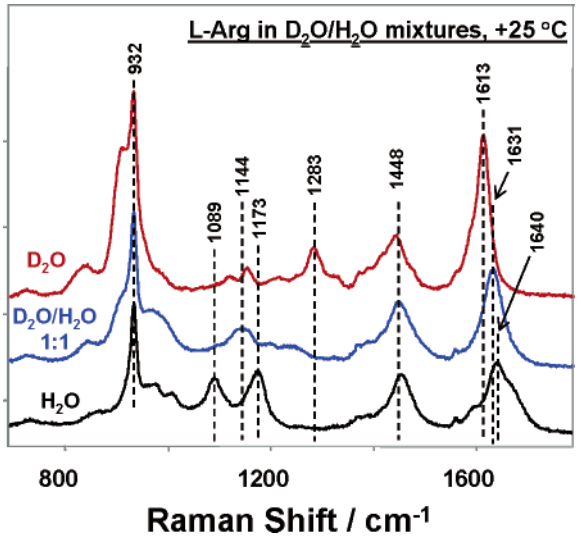

Figure 2. The 204-nm UVRR spectra of L-arg (5 mg/mL) in pure D_2O, in pure H_2O, and in a H_2O/D_2O mixture (1:1) at 25 °C. The ~1640 cm^{-1} band in H_2O shifts to ~1613 cm^{-1} in D_2O.

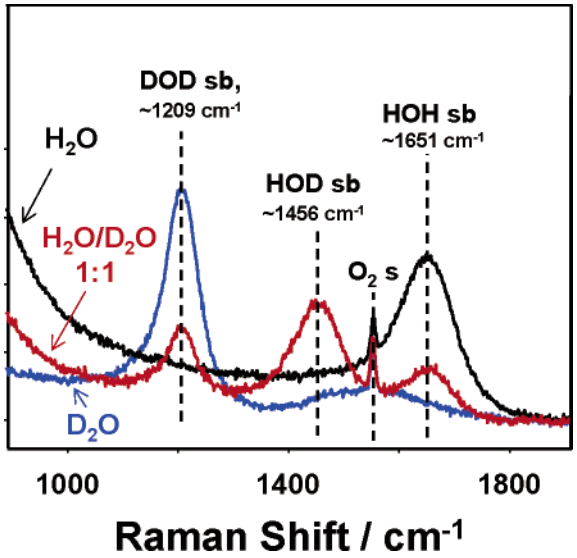

Figure 3. The 204-nm UVRS of pure H_2O, pure D_2O, and 1:1 H_2O/D_2O mixture. Spectra clearly show that the 1:1 mixture consists of 25% H_2O, 50% HOD, and 25% D_2O. Note: the contribution from atmospheric oxygen (O_2 s) has not been removed.

derives from the resonance coupling of an almost pure CN s vibration with a CH_3 asymmetric bending vibration.[28,50,60,72] This coupling disappears[50] upon substitution of natural abundance ala by perdeuterated 2,3,3,3-D_4 ala to form the deuterated derivative AdP.

AP in D_2O also shows a doublet in the AmI′ region, with bands at 1614 and 1660 cm^{-1} (Figure 1). We recently demonstrated that the 1660 cm^{-1} band is the AmI′ vibration,[50] while the ~1614 cm^{-1} band results from arg side-chain vibration(s) (Figure 2), as discussed in detail below. The protonated ala side chains in D_2O give rise to a small band at ~1380 cm^{-1} from the CH_3 umbrella mode.[50,60] Thus, N−D deuteration of AP in D_2O results in the loss of the $AmIII_3$, $AmIII_2$, the weak $AmIII_1$, and the AmII bands, which are replaced by the dominating AmII′ doublet. Small frequency shifts occur between the AmI and AmI′ bands. Thus, we can separately monitor the spectral features of protonated and deuterated peptide groups since most strong bands do not overlap.

AP PPII Bands in H_2O/D_2O Mixtures. We can examine the UVRS of PPII AP in mixed H_2O/D_2O solutions to determine whether there is coupling of the amide vibrations between peptide bonds. The hypothesis states that if coupling occurs between protonated segments, random deuteration will break this coupling and cause changes in the coupled normal modes and shifts in the vibrational frequencies. This will result in an inability to model the spectra of randomly deuterated AP simply as the weighted sums of AP in pure H_2O and D_2O. Obviously, if coupling is absent, then we can treat the Raman scattering from PPII conformations as independent scattering from individual peptide bonds. Our earlier study of two linked amides concluded that there was no coupling between adjacent peptide groups in that small peptide(s).[66]

The rate of H/D exchange at the amide nitrogen depends mainly on the peptide/ protein conformation as well as on the pH/pD value.[73−75] Exposed amino acid residues at neutral pH values exchange rapidly, while the rates for buried amino acid residues in the native state may be reduced by factors as large as 10^8 relative to the same residues in unfolded state.[76]

Since the PPII state of AP is highly exposed to H_2O and/or D_2O, and/or H_2O/ D_2O, the H/D exchange at the amide nitrogen will be complete at neutral pH/pD values within minutes, as we confirmed by our spectral measurements. Thus, we have complete statistical exchange of H and D, and the numbers of both N-protonated and N-deuterated amide groups is simply proportional to the relative concentrations of H_2O and D_2O.

Figure 1 shows AP PPII spectra taken at 62 °C in various mixtures. Though water, D_2O, and HOD contributions are not subtracted from these UVR spectra, their relative contributions for AP concentration of 1 mg/mL are low. The broad H_2O OH bending band at ~1651 cm^{-1} contributes only to the AmI region, while the broad D_2O OD bending band at ~1209 cm^{-1} does not overlap any band in the 1240−1670 cm^{-1} region of interest. However, the HOD bending band, in D_2O/H_2O mixtures at ~1456 cm^{-1}, may overlap the AmII′ band (Figure 3).

In contrast, the arg side-chain bands complicate the AmI spectral region (Figure 2). For example, the ~1640 cm^{-1} arg band in water downshifts to ~1614 cm^{-1} in D_2O. There is also an arg band at ~1450 cm^{-1} which overlaps the AmII′ doublet. However, the AmII and AmIII regions are free from any overlap from arg, H_2O, HOD, and D_2O vibrations.

The Figure 1 spectra show that the AmIII and AmII band intensities are proportional to the relative concentration of H in the H_2O/D_2O mixtures, while the AmII′ band intensity is proportional to a relative concentration of D. There are no obvious changes in spectral band shapes as the relative H_2O and D_2O concentrations change. No new bands appear.

Figure 4 compares the experimentally measured AP PPII spectrum in a 1:1 H_2O/D_2O mixture at 62 °C to the equally weighted sum of the AP PPII spectra in pure water and AP PPII in pure D_2O at 62 °C. In Figure 4, we digitally removed the spectral contributions from H_2O, HOD, D_2O, as well as from arg side chains. Figure 4 clearly shows no evidence of spectral differences between the mixed and pure H_2O/D_2O spectra; the entire 1:1 H_2O/D_2O spectrum is modeled well by linearly summing AP spectra in pure H_2O and D_2O.

This observation is not particularly surprising for the AmII, II′, and III vibrations since they have modest dipole moments and would not couple well through a transition dipole coupling mechanism.[55−57,59] However, the lack of coupling for the AmI vibration may be surprising in view of the Hochstrasser group's recent 2-D IR spectral observation of AmI coupling in an α-helical peptide.[53,54] There is no real conflict here since our

TABLE 1: Population of Protonation/Deuteration States of Three Adjacent Peptide Bonds in Different D_2O/Water Mixtures

H_2O/D_2O ratio	Population Fraction of Pure Protonation or Deuteration States			Population Fraction of Mixed Protonati on and Deuterat ion States						
	HHH	DDD	Total	HHD	DHH	DHD	DDH	HDD	HDH	Total
6:1	63	0.29	63.3	10.5	10.5	1.7	1.7	1.7	10.5	36.7
1:1	12.5	12.5	**25**	12.5	12.5	12.5	12.5	12.5	12.5	75
3:10	1.2	45.5	46.7	4.1	4.1	13.7	13.7	13.7	4.1	53.3
1:9	0.1	72.9	**73**	0.9	0.9	8.1	8.1	8.1	0.9	27

PPII structure is quite different; the carbonyls are not coparallel and the PPII structure does not have intrapeptide hydrogen bonds. Further, because of their relative orientation and because the through-space distance between carbonyls is longer in the PPII structure compared to that in the α-helix, we expect a decreased transition dipole coupling in the PPII conformation compared to that in the α-helix conformation.[55,56]

Deuteration dramatically changes the amide normal modes,[28,50,60] where the UVR AmIII and AmII bands disappear and are replaced by an AmII′ band which contains a large amount of CN s. In a 1:1 mixed H_2O/D_2O solution, 75% of the N−H (N−D) derivatives possess an adjacent N−D (N−H) peptide bond; in the 6:1 mixture, only 36.7% of the N−H (N−D) have at least one adjacent N−D (N−H) (Table 1). *Thus, we can clearly conclude that coupling between the identical vibrations of adjacent peptide groups (namely, AmI, II, III, II′, and I′) does not occur in the AP PPII conformation and each peptide bond vibrates independently from its neighbors.* Alternatively, we would conclude that coupling results in insignificant spectral changes in either intensity or frequency.

Calculation of AP α-Helical UVR Spectra in Different H_2O/D_2O Mixtures. It is not possible to prepare pure α-helical conformation samples of AP. Even low-temperature samples are a mixture of α-helical and PPII AP conformations. We calculated the pure AP α-helical spectra by numerically removing the PPII spectral contribution. This is straightforward, since we previously characterized the AP PPII spectrum in pure water and pure D_2O[27,28,50,67] and determined the temperature dependence of the PPII amide bands between −5.5 °C and 80 °C.

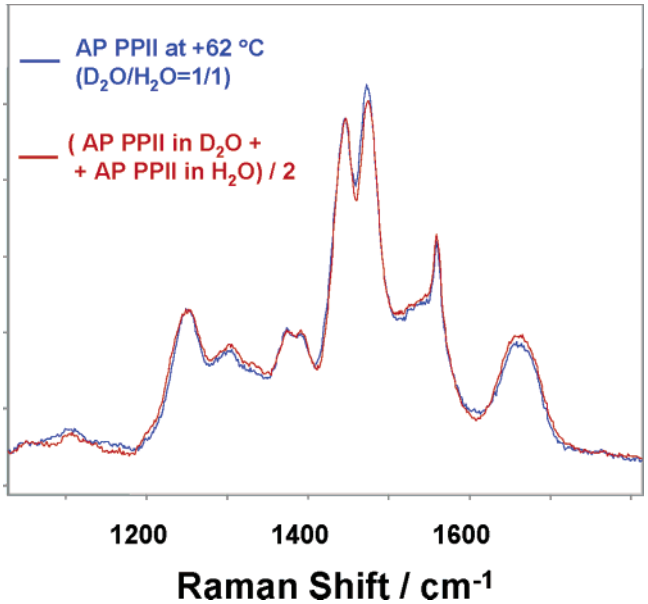

Figure 4. The 204-nm UVRS of the AP PPII state in a D_2O/water mixture (1:1) at 62 °C can be modeled well by the sum of spectra of AP PPII in pure water and AP PPII in pure D_2O. Note that the contributions of the arg side chain in D_2O, in H_2O, and in H_2O/D_2O were digitally removed, as were the D_2O, H_2O, and HOD spectral contributions.

Thus, we calculate the AP α-helix spectra at 0 °C from the following relationship:

$$S_\alpha(0\ ^\circ\mathrm{C}) = [S_{obs}(0\ ^\circ\mathrm{C}) - (1 - f_\alpha)^* S_{II}(0\ ^\circ\mathrm{C})]/f_\alpha$$

where $S_\alpha(0\ ^\circ\mathrm{C})$ is the calculated AP α-helix spectrum, $S_{obs}(0\ ^\circ\mathrm{C})$ is the observed AP spectrum, and $S_{II}(0\ ^\circ\mathrm{C})$ is the calculated AP PPII spectrum, all at $T = 0\ ^\circ\mathrm{C}$ in a specific H_2O/D_2O mixture. $f_\alpha = 0.55$ is the α-helix fraction of AP at 0 °C as determined by Lednev et al.[27] Figure 5 shows an example of the decomposition of the $T = 0\ ^\circ\mathrm{C}$ AP spectrum in H_2O/D_2O (1:1) into its α-helix and PPII components, while Figure 6 compares the calculated α-helix spectra of six different H_2O/D_2O mixtures ranging from pure water to pure D_2O.

AP α-Helix Bands in Pure Water. We recently examined the assignment of the UVR bands of α-helical AP in water in detail.[50] The AmI band occurs at ∼1646 cm^{-1}, while the AmII band occurs at 1547 cm^{-1} (Figure 6). The AmI and AmII bands of the α-helix are 13 and 11 cm^{-1} downshifted compared to those of the PPII conformation.[50] In addition, the AP α-helix, N−H amide bond AmIII region shows a characteristic triplet of bands between 1250 and 1350 cm^{-1}, which are denoted as $AmIII_3$ (∼1261 cm^{-1}), $AmIII_2$ (∼1306 cm^{-1}), and $AmIII_1$ (∼1337 cm^{-1}).[50] The normal mode composition of these bands is complicated; the vibrations were shown to involve different combinations of NH ib, CN s, NC s, C_α−H b, and C_α−C s.[50]

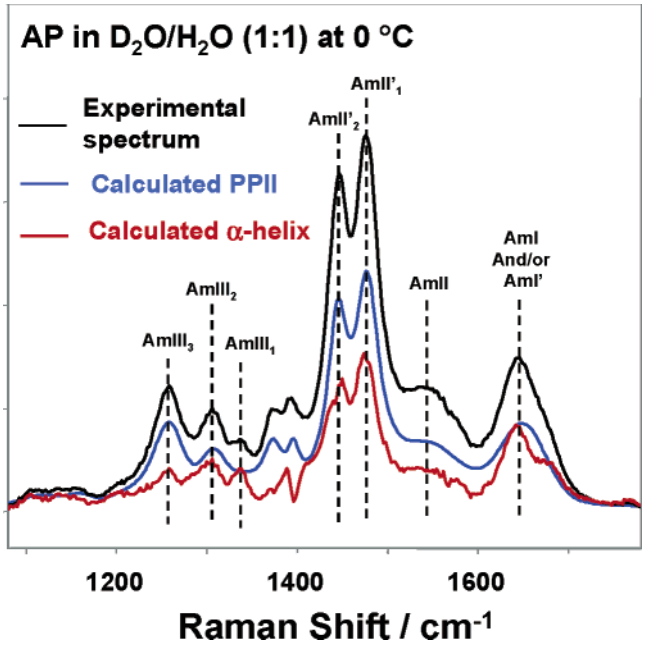

Figure 5. Example of decomposition of 204-nm UVRR spectra of AP into its PPII and α-helical components in a H_2O/D_2O mixture (1:1) at 0 °C. The PPII spectrum at 0 °C was calculated from the experimental AP PPII spectrum at 50 °C utilizing the measured temperature dependence of its UVRR bands. The α-helix spectrum at 0 °C was calculated by subtracting the calculated 0 °C PPII UVRS from the 0 °C experimental UVRS. Contributions from arg, water, DOD, and D_2O were not subtracted.

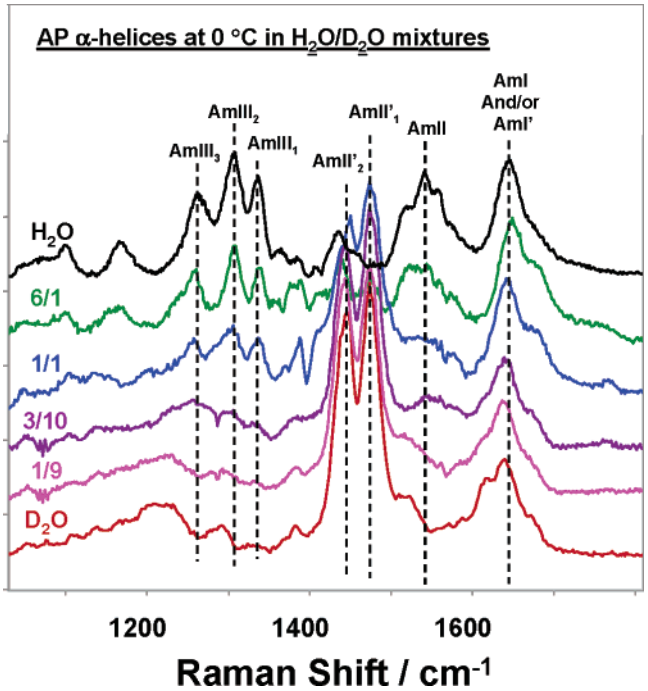

Figure 6. UVRS spectra of AP α-helices calculated at 0 °C in different H_2O/D_2O mixtures. Contributions from arg, water, DOD, and D_2O are not subtracted.

The shape of the AmIII band envelope dramatically changes between the PPII and α-helix conformations (see Figure 12 of Mikhonin et al.[50]). It was also shown that the $AmIII_3$ band frequency is very sensitive to the Ψ dihedral angle, which specifies the backbone secondary structure,[7,67] due to the Ψ dependence of the coupling between NH b and C_α−H b vibrations. NH b was shown to be highly mixed with C_α−H b at Ψ ∼ 120° (*β*-sheet-like or PPII-like), but almost free from this mixing at Ψ = −60° (α-helical Ψ-angle). The AmIII band envelope suffers a large relative intensity decrease upon α-helix formation (see Figure 12 of Mikhonin et al.[50]) due to the hypochromism due to α-helix excitonic interactions.[38,39,46−49]

AP α-Helical Bands in Pure D_2O. N-deuteration also dramatically affects the AP α-helix UVR (Figure 6). The decoupling of ND b from CN s and C_α−H b again leads to a dominating enhancement of the AmII′ band, where CN s motion is resonantly coupled with one of the components of the CH_3 bending mode.[28,50,60,72] Though the AmII′ band of the AP α-helix is only slightly downshifted compared to the AmII′ band of the AP PPII, it shows a ∼2-fold intensity loss[50] due to the α-helix excitonic interactions.[38,39,46−49]

The AmI′ band, which occurs in α-helical AP at 1639 cm^{-1}, shows no significant change in relative intensity compared to that of AmI′ band of AP PPII. However, the frequency of the AmI′ band downshifts ∼20 cm^{-1} upon α-helix formation. The AmIII′ bands, which are almost pure N−D b, are weak and occur between 900 and 1000 cm^{-1} (not shown).

Coupling of AP α-Helical Amide Bond Vibrations. We can determine whether coupling occurs between amide vibrations in adjacent α-helical bonds in AP by testing whether the UVR spectra of mixed H_2O/D_2O mixtures can be modeled as the sum of α-helical spectra of AP in pure H_2O and D_2O. We can easily and independently monitor the N−H and N−D AmIII and AmII′ bands since they do not significantly overlap with themselves nor with water or D_2O. Even though the ∼1450 cm^{-1} arg side chain, as well as the ∼1456 cm^{-1} HOD bands overlap the AmII′ band, they are too weak to significantly interfere (Figures 2 and 3).

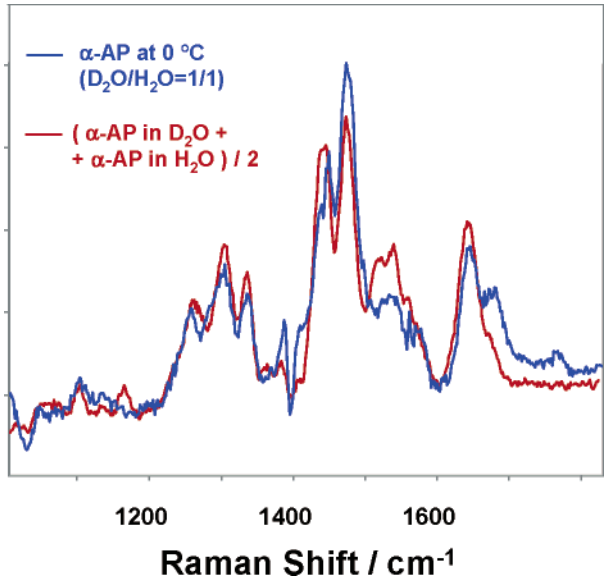

Figure 7. The 204-nm calculated UVRR spectra of AP α-helix in a 1:1 D_2O/water mixture at 0 °C. The AmIII and AmII′ regions are well modeled by the sum of the AP α-helix in pure H_2O and AP α-helix in pure D_2O. We attempted to digitally remove overlapping bands from arg in D_2O, H_2O, and H_2O/D_2O solutions, as well as D_2O, H_2O, and HOD contributions. The AmII band fitting is unreliable because of the overlapping contributions of these bands to the AmII region and the contribution from atmospheric oxygen. The spectral differences in the AmI/AmI′ region clearly indicate vibrational coupling between adjacent peptides in this α-helix conformation.

Figure 6 shows that the intensity in the AmIII and AmII regions in the N−H α-helix UVR spectra smoothly decreases as the concentration of D_2O increases. In contrast, the AmI region shows discontinuous changes such as the appearance of a high-frequency AmI shoulder at ∼1675 cm^{-1}. The low-frequency AmI′ shoulder at ∼1614 cm^{-1} simply results from an arg side chain band downshifted in D_2O to ∼1613 cm^{-1} from its ∼1640 cm^{-1} value in H_2O (Figure 2).

Figure 7 clearly shows that the AmIII and AmII′ regions of α-helical AP can be satisfactorily modeled as the sum of spectra of α-helical AP in pure H_2O and D_2O. The situation is difficult to resolve for the AmII N−H α-helix region, since it is plagued by overlapping bands from atmospheric oxygen, arg, and HOD, which make the results unreliable.

In contrast, it is clear that the AmI/AmI′ region cannot be modeled as the weighted sum of individual α-helices in pure water and pure D_2O. *We, thus, find evidence of adjacent peptide bond coupling in the AmI/AmI′ region of α-helical AP.* The high-frequency shoulder at ∼1675 cm^{-1} derives from a band contributed by the N−H peptide bonds (Figure 6), which is not, however, present in pure H_2O (Figure 3). It is a collective vibrational frequency which is not observed in the absence of partial deuteration.

Our observation of coupling of the AmI/AmI′ bands of α-helical AP is consistent with the Hochstrasser group's[53,54] recent demonstration of coupling between the AmI′ bands of α-helical peptides. These studies were accomplished by ultrafast, transient 2-D IR studies of $^{13}C = {}^{18}O$ isotopically edited peptides.

The lack of coupling of the amide vibrations in the PPII conformation as well as the lack of coupling of the AmIII and AmII′ (and probably AmII) bands in the α-helix conformation is important, since it makes these bands very straightforward indicators of the conformation of peptides and proteins. The UVR spectra can be assumed to result from the independent

Raman scattering of different peptide bonds. Obfuscation of spectral interpretations of conformational changes will not result from alterations in the characteristic basis spectra as the peptide or protein adopts different conformations. This promises to make vibrational spectroscopy of even higher utility for protein structural studies. This is especially important because it increases the utility of kinetic vibrational spectroscopy to elucidate the initial steps in protein folding.[26−33]

Conclusions

UV Raman studies of α-helical and PPII conformations of AP in mixed H_2O and D_2O samples show that the AmIII (PPII and α-helix), AmII (PPII), and AmII′ (PPII and α-helix) bands can be modeled as being independently Raman scattered by the individual peptide bonds. Negligible coupling occurs for these vibrations between the adjacent peptide bonds. This dramatically simplifies the use of vibrational spectral modeling to elucidate peptide and protein structure. The AmI and AmI′ vibrations of PPII also show no evidence of coupling between adjacent peptide bonds. In contrast, the AmI and AmI′ bands of the α-helical conformation clearly indicate the occurrence of interamide coupling. The interamide coupling in the α-helix is probably the result of the proximity of the amide C═O groups to one another, the fact that they are oriented parallel to one another, and because the C═O groups of different amides are linked through interamide hydrogen bonding.

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