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Couplings between Peptide Linkages across a 3_{10}-Helical Hydrogen Bond Revealed by Two-Dimensional Infrared Spectroscopy

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Peptide linkages connected through different types of hydrogen bonding networks are fundamental to various protein secondary structures. For example, the 3_{10}-helix exhibits intramolecular C═O···H−N hydrogen bond pattern between the *i*th and $(i + 3)$th residues, whereas the α-helix is between the *i*th and $(i + 4)$th.[1] Vibrational couplings between peptide linkages are sensitive to the underlying structure, a feature extensively explored in IR experiments of amide-I modes to determine secondary structures.[2,3] Here we demonstrate that vibrational coupling between hydrogen-bonded peptide linkages in a 3_{10}-helix can be directly probed by a combination of two-dimensional infrared (2D IR) spectroscopy and isotope editing of amide-I and amide-II modes. The isotope-dependent cross-peaks provide valuable information on local structure, such as the formation of a single helix turn, important for studying protein folding processes.

Recently, we have demonstrated that 2D IR of amide-I modes is a powerful technique that can distinguish the subtle conformational differences between 3_{10}- and α-helices as well as probe the onset of 3_{10}-helical secondary structure.[4] The doublet amide-I cross-peak pattern is characteristic of the 3_{10}-helix structure, but contains no site-specific information. To elucidate the 3_{10}-helix formation at the residue level, we need to devise a strategy to directly probe the coupled vibrational modes that are connected through a 3_{10}-helical C═O···H−N hydrogen bond and involve significant motions of these atoms. Isotope substitution with $^{13}C═^{16}O$ and $^{13}C═^{18}O$ proves to be useful for isolating local amide-I modes and probing local structure and dynamics.[5] Isotope labeling the hydrogen bonding partner with N-D is the most intuitive choice, but cannot be achieved in a site specific manner. The amide-II mode, consisting mainly of the N−H bending and C−N stretching,[2] is chosen as our reporter. ^{15}N labeling decreases the amide-II frequency of *N*-methylacetamide (NMA) in nitrogen matrix by 13−15 cm^{-1},[6] a sizable shift compared to typical line widths (~30 cm^{-1}). Most 2D IR studies of peptides have focused on the amide-I mode.[3−5] Only a recent few studies explored the couplings between the amide-I and amide-II modes in NMA and dipeptides,[7,8] but cross-peaks have yet to be observed for modes connected through an intramolecular hydrogen bond in peptides.

We performed linear and 2D IR experiments on three hexapeptides in $CDCl_3$ (Figure 1a): Z-Aib-L-Leu-$(Aib)_2$-Gly-Aib-O*t*Bu (**1**, Z, benzyloxycarbonyl; Aib, α-aminoisobutyric acid; O*t*Bu, *tert*-butoxy), monolabeled peptide with $^{13}C═^{18}O$ at Leu (**1***), and bis-labeled peptide with $^{13}C═^{18}O$ at Leu and ^{15}N at Gly (**1****). The 2D IR cross-peak pattern of **1** in $CDCl_3$ exhibits a clear doublet in the amide-I region (Figure S2), indicating that the peptide forms a 3_{10}-helix. This structure is not unexpected because many peptides with a high fraction of Aib are known to adopt the 3_{10}-helical conformation.[9]

Figure 1b shows the linear IR spectra of hexapeptides in the region covering the $^{13}C═^{18}O$ amide-I mode and the amide-II modes. Two

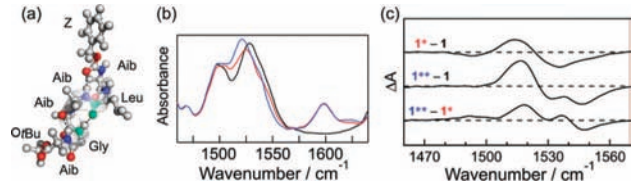

Figure 1. (a) Molecular structure of **1** taking an ideal 3_{10}-helical conformation with the dihedral angles $(\phi, \psi) = (-57°, -30°)$.[1] The atoms labeled with ^{13}C, ^{18}O, and ^{15}N for **1*** and **1**** are colored green. (b) Normalized FTIR spectra of **1** (black), **1*** (red), and **1**** (blue) in $CDCl_3$. (c) Difference spectra in the amide-II region.

amide-II bands are observed at 1498 and 1528 cm^{-1}. Roughly speaking, the higher (lower) frequency band can be attributed to the vibrational exciton band of the hydrogen-bonded (free) amide-II modes. The amide-II mode is known to exhibit a blue shift when the N−H group is hydrogen bonded.[10] If the hexapeptide forms a full 3_{10}-helix, four out of the five amide N−H groups will participate in intramolecular hydrogen bonding except for the first one at the N-terminus (Figure 1a). The relative intensity of the two bands in Figure 1b is consistent with this picture when compared to the trend observed in FTIR measurements of Z-$(Aib)_n$-O*t*Bu (n = 1, 2, 3, 5, 8, and 10) in $CDCl_3$, where the intensity of the higher frequency band increases with n (Figure S3). The spectrum of Z-Aib-O*t*Bu (Figure S1) shows that the capping groups provide only small contributions to the spectral window of interest.

The $^{13}C═^{18}O$ labeling on the Leu residue of **1*** results in an amide-I peak at 1598 cm^{-1}, completely separated from the much stronger $^{12}C═^{16}O$ amide-I bands (Figures 1b and S1). The isotopes also affect the amide-II modes. As shown in the difference spectrum (Figure 1c, **1***−**1**), the intensity at 1536 cm^{-1} decreases and at 1514 cm^{-1} increases. Such a red shift of the higher frequency band indicates that the N−H group of the second peptide linkage is hydrogen-bonded with the urethane C═O group, as expected for a full 3_{10}-helix (Figure 1a).

The bis-labeled peptide **1**** has almost the same amide-I bands as **1***, so ^{15}N labeling primarily affects the amide-II modes. The higher frequency band of **1**** is red-shifted by ~4 cm^{-1} from that of **1***. The difference spectrum between **1**** and **1*** reveals clearly the additional effect of ^{15}N labeling, with major spectral changes occurring in the high frequency band. This indicates that the N−H group of the fourth peptide linkage is involved in hydrogen bonding. For a 3_{10}-helix, its bonding partner is the C═O group of the second peptide linkage.

The isotope effects on amide-II bands in Figure 1c are quite complex because the isotope shifts are small. Our DFT calculations on NMA in the gas phase at the B3LYP/6-311++G(d,p) level[11] predict 7 and 13 cm^{-1} red shifts for $^{13}C═^{18}O$ and ^{15}N substitution, respectively. The labeled amide-II local mode is still coupled to the network of unlabeled amide-II local modes. The labels on hydrogen-bonded linkages slightly affect the low frequency exciton band (see the minor intensity changes

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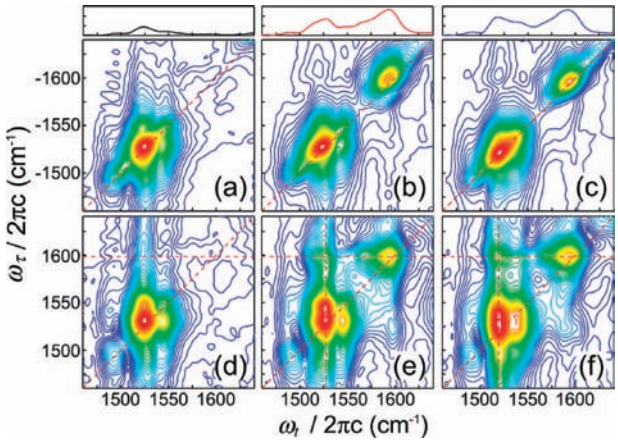

Figure 2. Normalized absolute magnitude 2D IR rephasing (R, a−c) and nonrephasing (NR, d−f) spectra of **1**, **1***, and **1**** (from left to right) in $CDCl_3$. Horizontal slices of the NR spectra along the dashed line at $\omega_\tau = 1598$ cm^{-1} are shown in the top panels.

at 1492 cm^{-1} in Figure 1c), indicating that the free and hydrogen-bonded amide-II local modes are coupled.

Figure 2 presents 2D IR rephasing (R) and nonrephasing (NR) spectra. Overall, we observed better resolved diagonal peaks and more distinct cross-peaks in the NR spectra than in the R spectra. The NR sequence has higher resolving power due to destructive interference effects.[8] It is also very useful for line-narrowing cross-peaks between anticorrelated vibrators,[12] such as hydrogen-bonded amide-I and amide-II modes. In the R spectra of **1*** and **1****, the $^{13}C{=}^{18}O$ amide-I mode at the second peptide linkage is observed at $(\omega_t, \omega_\tau) \approx (1593, -1598)$ cm^{-1} and no peak appears here in the spectrum of **1**. For the amide-II region, the strongest diagonal peak at (1525, −1528) cm^{-1} in the R spectrum of **1** corresponds to the high frequency band in the linear spectrum. Upon isotope labeling, the amide-II band maximum maintains at about the same position for **1***, but shifts to (1520, −1521) cm^{-1} for **1****. The free amide-II band appears in the 2D R spectrum of **1** as a diagonally elongated shoulder on the red side of the main peak, but it is clearly resolved as a separate peak in the NR spectrum at (1493, 1497) cm^{-1}. The complex couplings among the amide-II modes manifest as many cross-peaks in the NR spectra.

The most interesting feature in the 2D spectra is the cross-peaks between the $^{13}C{=}^{18}O$ amide-I mode and the amide-II modes. They are weak in the R spectrum, but much stronger in the NR spectrum. For **1***, the cross-peaks are located at (1527, 1598) and (1596, 1524) cm^{-1}. These cross-peaks reveal the presence of couplings between the labeled amide-I mode and some modes within the high frequency amide-II band. The couplings can involve anharmonic interactions between amide-I/II modes within the second peptide linkage and/or between the second amide-I and amide-II on other linkages. Whether the fourth linkage contributes to the latter interactions can be revealed by observing the changes of these cross-peaks upon ^{15}N labeling.

The top panels in Figure 2 show the slices through 2D NR spectra at $\omega_\tau = 1598$ cm^{-1}. The cross-peak maximum of **1**** is at 1518 cm^{-1}, significantly red-shifted from that of **1*** by 9 cm^{-1}. This is a clear evidence that the amide-II local mode on the fourth peptide linkage is coupled to the amide-I local mode on the second peptide unit. ^{15}N labeling changes the fourth amide-II local mode frequency and results in corresponding shifts in the cross-peak frequencies. Such coupling information is not obtainable from linear IR. The cross-peak line shapes observed here are quite complex because there are many amide-II exciton states that the $^{13}C{=}^{18}O$ labeled amide-I mode can couple to. Although detailed analysis is still underway, it may be worthwhile to estimate how this coupling depends on the existence of 3_{10}-helix based on the transition charge coupling model.[13] Using partial charges and charge derivatives along the amide-I and -II modes of NMA from a DFT calculation, we estimated the coupling between the two modes separated by two peptide units to be −12 cm^{-1} in the ideal 3_{10}-helix [$(\phi, \psi) = (-57°, -30°)$][1] and −0.1 cm^{-1} in semiextended structure [$(-78°, 146°)$]. Such a difference in the coupling strength suggests that these cross-peaks would be useful for detecting helix formation. In a recent experiment the couplings between labeled amide-I modes separated by 1−3 residues have been measured in an alanine rich α-helix.[5a] Measuring the amide-I/II couplings with labels at several different sites will enable further refinement of the peptide local structure. Also it would be interesting to theoretically investigate the amide-I/II coupling mechanism, including through-(hydrogen)-bond and through-space interactions. Although our new observation for the coupled amide-I and amide-II modes was made in a 3_{10}-helix, the technique is generally applicable to systems involving couplings between C═O stretching and N−H bending modes.

In summary, the combination of multiple isotope labels and 2D IR enabled us to observe the cross-peaks between the amide-I and amide-II modes connected through a C═O···H−N intramolecular hydrogen bond in a 3_{10}-helical peptide. Although the ^{15}N isotope effect on the amide-II band is not as strong as ^{13}C and ^{18}O on the amide-I band, the 2D IR cross-peaks exhibit a clearly discernible ^{15}N shift, twice that of linear IR. Thus, the ^{15}N label is potentially useful for probing local conformations by 2D IR spectroscopy. The strategy demonstrated here is promising for directly detecting the transient formation of a single helix turn, which would be difficult to obtain from 2D IR of unlabeled amide-I modes.

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Supporting Information Available: Complete ref 11. Hexapeptide synthesis; measurement procedure; double-crossed polarization 2D IR spectrum of **1**; and linear spectra of hexapeptides and Z-$(Aib)_n$-O*t*Bu. This material is available free of charge via the Internet at http://pubs.acs.org.

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