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Local structure of β -hairpin isotopomers by FTIR, 2D IR and ab initio theory

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Abstract

The 12-residue tryptophan zipper β -hairpin (SWTWENGKWTWK) and two ^{13}C -isotopomers were examined in the amide-I region using FTIR and femtosecond two-dimensional infrared (2D IR) spectroscopies. Spectroscopic features of the labeled transitions with ^{13}C -substituted amide unit present in the terminal or turn region of the hairpin, including their frequency shifts and distributions, line broadenings, orientations and anharmonicities of diagonal peaks, allow the peptide local structure and local environment to be examined. The results suggest a larger structure fluctuation in the terminal region than in the turn region as a result of the side chain effect and solvent-peptide interaction. The results also suggest that the uncoupled amide-I modes are not degenerate and that this is likely to be a common situation for solvated polypeptides. In addition, the amide-I states in the terminal and turn regions were found to be delocalized over several neighboring amide units. Cross peaks between the various labeled and unlabeled structural regions were clearly observed in the 2D IR correlation spectra, allowing them to be characterized for monitoring structural changes. These results illustrate the sensitivity of 2D IR to the local environment of solvated peptides. The simulated 1D and 2D IR spectra of the hairpin, obtained by using the vibrational exciton model incorporating coupling constants from quantum chemical computations and semi-empirical calculations, were found to reproduce the essential features of the experimental results.

Introduction

The β -hairpin is a key element of secondary structure and therefore it is of great interest to obtain chemical bond scale descriptions of its conformations and their dynamics. It is thought to be the smallest representation of β -sheets having cross strand interactions. It is composed of a single polypeptide chain in the antiparallel conformation with a β -type turn near the center of the sequence. The stability and folding dynamics of several water-soluble model hairpins have been studied.^{1–9} Among which, the so-called tryptophan zippers are unique.⁶ The NMR structure of one of them, Trpzip2, shows a twisted β -hairpin containing a hydrophobic cluster of Trp residues in the terminal region. This robust, relatively well-defined structure is an excellent model with which to characterize the vibrational states of peptides in aqueous solution, to investigate the relations of the vibrational spectra with peptide conformations and to evaluate the distributions of structures. In particular the amide-I mode vibrations in these structures while likely to be delocalized across the complete length scale of the peptide, probe local environments that range from very hydrophobic, in the region of the Trp's, to solvent exposed. Furthermore, some of the structures have relatively sharp melting curves which make them very suitable for studies of fast folding and unfolding kinetics.^{6,8,10–12}

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The delocalization of vibrational excitations in peptides is a topic that has been frequently discussed and considerable experimental^{13–18} and theoretical^{19–23} work has been aimed at determining the coupling coefficients that lead to the delocalization. The amide-I mode of peptides has a large IR transition dipole and so electrostatic coupling is expected to lead to vibrational excitation exchange amongst amide units in peptides where the local amide modes form a nearly degenerate set of oscillators. The experimental spectra of small peptides^{18,24,25} and secondary structures such as helices^{17,26,27} and sheets^{28,29} are consistent with their vibrational states being delocalized. The coupling constants have also been estimated by fitting linear IR spectra to exciton-like models.^{13,30} In the theoretical models a zero order set of states is modified by through bond and electrostatic coupling of the modes. However it has proved difficult to establish the frequency distributions of the zero order transitions that form the basis for the exciton calculation for various systems of interest. These frequencies must depend on the local secondary structure details, the type of amino acids adjoining the amide mode and on the coupling of the modes to the surrounding solvent and to the side chains of other amino acids. The heterogeneity of all these effects tends to localize the vibrational excitations. Although one can build effects of disorder into theoretical descriptions^{22,31,32} it may be expected that each peptide will have its own unique characteristics. There is therefore scope for detailed study of specific systems having well defined structures. Traditionally the study of the spectra of isotopic impurities has been the approach to finding the underlying structure of exciton bands.^{33,34}

Isotope labeling of amide groups has proved to be an effective strategy for characterizing local structural features of polypeptides by means of FTIR,^{9,35} Raman³⁶ and most recently 2D IR.^{15,17,18,26,27,37,38} The scope of both coherent nonlinear and conventional vibrational spectroscopy and circular dichroism^{39–42} have been greatly enlarged by isotope editing techniques applied to lipids, membranes, peptides and proteins⁴³ enzyme reaction intermediates⁴⁴ and even shifting the IR spectra of whole proteins.⁴⁵ Isotope substitution has always been a successful strategy of IR spectroscopy and the applications to biological systems are a natural development⁴⁶ whose use is growing.^{17,47–55} The amide-I mode is largely a carbonyl stretch and so its frequency is significantly modified by isotopic replacement of: ^{12}C by ^{13}C (shift 38 cm^{-1}); ^{16}O by ^{18}O (shift 35 cm^{-1}); and both ^{12}C and ^{16}O by ^{13}C and ^{18}O (shift 60 cm^{-1}). Any one of these substitutions can be employed to spectrally select a particular residue. Of these isotopes only ^{13}C occurs significantly enough in natural abundance (1%) to interfere with spectral selectivity hence the recent use of $^{13}\text{C}=^{18}\text{O}$.^{17,26,27,35,37} Isotopic selectivity also proved useful in nonequilibrium folding experiments on peptides.⁵⁶ The trpzip2 β -hairpin offers an interesting paradigm to which the isotopic labeling strategies can be applied because of its twisted conformation. In addition, the four-Trp hydrophobic cluster in the terminal region may have a different role than the residues in the turn region in controlling the folding and stability of the hairpin.¹¹

The 2D IR method used in this work and its applications have been reviewed frequently.^{57–62} When combined with isotopic selectivity 2D IR presents many advantages for the study of peptides. While the observed frequencies and transition intensities obtained from FTIR experiments can be fitted to structural models that imply coupling and specific angular relations between amide units, the 2D IR experiment provides the opportunity to identify these parameters directly and hence provide a less assumptive description of the peptide structures and their distributions. Because 2D IR spectra separate the effects of molecular structure distributions from other relaxation processes they are line narrowed compared with FTIR spectra. Therefore, they can access local structural parameters that are not available from other methods. Significant questions remain regarding the detailed description of the structure distributions and equilibrium dynamics of the hairpin in aqueous solution. For example, a recent 2D IR experiment on trpzip2⁶³ has concluded that stable hydrogen bond interactions persist between the two β -strands at temperatures up to 82°C ,

which would normally be considered as denaturing conditions. In the present work we examine the local structures and structural distributions of the β -hairpin using linear and 2D IR techniques in association with $^{13}\text{C}=^{16}\text{O}$ isotope selection of particular amide groups of the hairpin.

In this paper we present the FTIR and 2D IR spectra of the trpzip2 and its two ^{13}C -isotopomers in the amide-I region. We examine the degree of delocalization of the amide-I modes in different segments of the β -hairpin, and seek to explore the usefulness of this delocalization and its spectral signatures as a probe of the peptide local structure through a single ^{13}C -replacement in two different spatial regions. The perturbation of both the one- and two-quantum band structure by a single ^{13}C -substituted amide-I state has been analyzed by employing the vibrational exciton model. The coupling and zero order transition frequencies have been evaluated by DFT computations and semi-empirical calculations. We suggest assignments of the FTIR spectrum by computing the distribution of the amide-I local mode transition dipole strength of the hairpin. We compare the observed and estimated ^{13}C -enhancements in the two ^{13}C -isotopomers. The 1D and 2D IR spectra of the trpzip2 were simulated satisfactorily using the exciton model. Both diagonal and off-diagonal peaks appeared in the 2D IR spectra and their relationships to the peptide structure are discussed for the three β -hairpin isotopomers.

Experimental

The trpzip2 consists of 13 amide units (Figure 1): units 1 to 12 are those associated with the peptide backbone from the N- to C-terminus and amide 13 is on the side chain of Asn6. The sequence is SWTWENGKWTWK. The unlabeled peptide (UL) and two isotopomers were synthesized by AnaSpec (San Jose, CA) using a standard solid-phase Fmoc-protocol. The isotopomers have ^{13}C substitution in the amide carbonyl of Trp2 (the isotopomer L2) and Gly7 (the isotopomer L7). Deuterated samples were obtained by lyophilization using 0.1M DCl. Residual trifluoroacetic acid from peptide synthesis was also removed by the lyophilization. Peptides were dissolved in D_2O at a concentration of *ca.* 10-mM (pD = 4–5). Linear IR (FTIR) and 2D IR measurements were carried out using a CaF_2 IR sample cell with 56- μm spacer at 278 K. The 2D IR spectra in the amide-I region were collected using the heterodyned spectral interferometry in the 6- μm region as described recently.¹⁸ Briefly, 2D IR experiments were carried out using a 1 kHz repetition rate Ti-Sapphire laser generating *ca.* 74 fs pulses (FWHM), having *ca.* 200 cm^{-1} spectral width. Three 300-nJ pulses (k_1 , k_2 and k_3 , with delay times τ and T respectively), were focused on sample and the phase matched signal in the $-k_1 + k_2 + k_3$ direction was collinearly overlapped with a local oscillator pulse separated from k_3 by 1ps. The signal (along time axis t) plus local oscillator is dispersed by a 50 lines/mm grating and then projected onto a 64-element MCT array IR detector at the focal plane. Although the pulses are 74 fs in width the frequency bandwidth does not provide a flat spectrum across the complete amide-I region of the ^{13}C -substituted peptide. Therefore two measurements were carried out, one with the central frequency of IR laser pulses tuned either at 1592 cm^{-1} and the other at 1680 cm^{-1} . The center element of the array detector set at *ca.* 1640 cm^{-1} and 1650 cm^{-1} for experiments with these two pulses. The time domain signal $S(\tau, T=0, t)$ is obtained from a discrete inverse Fourier transform of the raw data in the frequency domain.¹⁸ A double Fourier transform along the coherence time τ and the detection time t axes generates the rephasing spectrum $S_R(-\omega_\tau, \omega_t)$ and the nonrephasing spectrum $S_{NR}(\omega_\tau, \omega_t)$. A 2D IR correlation spectrum $S(\omega_\tau, \omega_t)$, also called absorptive spectrum, is obtained by adding equally weighted spectra $S_R(-\omega_\tau, \omega_t)$ and $S_{NR}(\omega_\tau, \omega_t)$. All the 2D IR correlation spectra reported here correspond to the $\langle zzzz \rangle$ tensor element of the third-order response.

Results and Discussion

FTIR spectra

The linear IR spectra shown in Figure 2a are normalized by area in the frequency region from 1565.0 to 1750.0 cm^{-1} . The UL peptide shows two distinct peaks at 1635.3 cm^{-1} and 1674.0 cm^{-1} . The most intense peak is on the lower frequency side, a known characteristic of the β -hairpin conformation.³ For L2 the maximum frequency of this most intense band is shifted to 1639.5 cm^{-1} with a decreased peak intensity. A new diffuse band appears at lower frequency stretching from 1575.0 to 1610.0 cm^{-1} and centered at *ca.* 1600.0 cm^{-1} . For L7, the frequency of the strong band is almost unchanged (1636.3 cm^{-1}) compared with that of UL, but a new peak appears with a maximum at 1590.0 cm^{-1} which is presumed to be due to the amide having the $^{13}\text{C}=^{16}\text{O}$ group. The ^{13}C -labeled peak in L7 is *ca.* 10 cm^{-1} lower in frequency than that in L2. If it is assumed that the zero order isotopic shift of the amide-I excitation is similar for each mode, this result indicates that zero order mode frequencies in this peptide are not degenerate as might be assumed in a very simple exciton modeling of the amide-I vibrational excitations.^{14,22} In addition, in each case there is a very weak high frequency transition observed between 1700 and 1725 cm^{-1} .

The FTIR difference spectra (L2–UL) and (L7–UL) are given in Figure 2b. The positive peaks represent new absorption caused by ^{13}C substitution. The difference spectra provide a qualitative picture of the delocalization of the isotopically substituted amide unit. In each case, three pairs of positive/negative peaks are shown, indicating that the amide units at the isotopically selected regions are involved in delocalized states of the β -hairpin. In addition, the major positive component associated with mainly the $^{13}\text{C}=^{16}\text{O}$ transition at the lowest frequency end is slightly broader in width in the case of L2 than in L7, suggesting different structural heterogeneity or relaxation dynamics for the amide-I modes in these two regions.

2D IR spectra

The main characteristics of 2D IR spectra of peptides have been discussed in a number of recent publications.^{18,22,25,26,64} The diagonal peaks are elongated along the diagonal by an amount that depends on the width of the distribution of frequencies. Each linear absorption peak has a corresponding diagonal contribution in 2D IR. The positive peaks sitting close to diagonal are due to the $v=0 \rightarrow v=1$ transitions of the vibrators. The negative peaks at slightly lower ω_i values than the positive diagonal peaks correspond to the $v=1 \rightarrow v=2$ transitions of the vibrators. We refer to the shift as the diagonal anharmonicity although the modes being observed are often not localized to individual amide units. The 2D IR correlation spectra of UL, L2 and L7 are shown in Figure 3. The results obtained by fixing the IR laser central frequencies at 1592 cm^{-1} and 1680 cm^{-1} are given in the left column (a, b, c) and right column (d, e, and f) respectively. In this section, a general description of 2D IR characteristics is given first, followed by a discussion of the difference between the results in the left and right columns of Figure 3. We then focus on the influence of ^{13}C -substitutions on the diagonal and off-diagonal peaks. In Figure 3, the cross peaks manifest coupling between modes at ω_i and ω_j and are found in the region of $(\omega_\tau = \omega_i, \omega_t = \omega_j)$ and $(\omega_\tau = \omega_j, \omega_t = \omega_i)$. Each cross peak has one positive and one negative component (with a common ω_τ value), which are marked with “+” and “−” respectively in the figure when they can be identified easily. The separation between each pair of the negative and positive off-diagonal peak is the so-called off-diagonal anharmonicity. Each diagonal peak also has one positive and one negative component with a common ω_τ value. In the following text, we use only the ω_τ value to describe a diagonal peak, and use the (ω_τ, ω_i) values of the positive component when we refer to a cross peak.

The 2D IR spectra of the three isotopomers collected with two different laser center frequencies show different spectral features (Figure 3). For UL, the major spectral difference between the left and right column is the peak intensity of the high frequency diagonal peaks (1674 cm^{-1}) with respect to the low frequency diagonal peaks (1635 cm^{-1}). The cross peaks above and below diagonal are also stronger in Figure 3d than in Figure 3a. The coordinates of these two cross peaks are (1674 cm^{-1} , 1635 cm^{-1}) and (1635 cm^{-1} , 1674 cm^{-1}). For L2, similar features are evident in addition to new diagonal peak (1600 cm^{-1}) due to the ^{13}C -label that is independent of the laser pulse center frequency. The off-diagonal peak linking the ^{13}C -shifted transition and the un-shifted strong transition located at (1600 cm^{-1} , 1640 cm^{-1}), is slightly stronger in Figure 3b than in Figure 3e. For L7, the situation is quite similar to that of L2, except that the ^{13}C -shifted diagonal transition is significantly lower in frequency (1590 cm^{-1}). This result is evident from both Figures 3c and 3f though the peak intensities are much weaker in the latter. In this case, the cross peaks located at (1636 cm^{-1} , 1590 cm^{-1}) and (1590 cm^{-1} , 1636 cm^{-1}) are more prominent than their counterparts in L2. In addition to these changes a diagonal peak at very high frequency ($\omega_t \geq 1700\text{ cm}^{-1}$) is more clearly shown in the spectra of each of the isotopomers when 1680 cm^{-1} is the center frequency: it corresponds to the high frequency weak transition in the linear IR spectra (Figure 2a). Note that both the $v=0 \rightarrow v=1$ and $v=1 \rightarrow v=2$ transitions are revealed for this high frequency transition in the three isotopomers. The cross peaks between this weak transition and the lower frequency strong transitions are also evident in the spectra, for example, at the location of (1674 cm^{-1} , 1710 cm^{-1}), which is labeled by a “+” in Figure 3d. Further, the peak intensities in the region with ω_t less than 1600 cm^{-1} are generally weaker in the three spectra using the 1680 cm^{-1} pulse. The intensity-affected regions contain the tails of the $v=1 \rightarrow v=2$ transitions of the strong diagonal peaks, and that of the ^{13}C -shifted transitions. These results show that tuning the laser center frequency enables spectral features associated with very weak transitions that are not clearly visible in linear spectra to be revealed. The pulse of 74 fs used in the present experiments would need to be reduced to ca. 35 fs to enable a single center frequency experiment to show up these details.

The 2D IR spectrum of UL shown in Figure 3d is generally in agreement with the spectrum reported recently by Tokmakoff and coworkers.⁶³ Although their sample conditions were not identical to ours, the essential 2D IR spectral characteristics are similar in regard to the relative intensity of the two diagonal signals and the observation of off-diagonal signals located below and above diagonal. These pairs of signals give rise to the so-called “Z”-shaped 2D IR characteristics of the β -hairpin.⁶³ Clearly distinguishable 2D spectral characteristics as a result of a single ^{13}C -substitution are the changed shape and position of the diagonal peaks and the appearance of new cross peaks that vary from one isotopomer to another. Introduction of ^{13}C -labeling to the amide unit tends to separate the amide-I diagonal peaks of the labeled amides from the remainder, allowing the local structural distribution in the labeled region to be examined separately. The 2D IR spectrum of UL peptide shows mainly two pairs of elliptically shaped diagonal peaks at 1636 cm^{-1} and 1674 cm^{-1} (Figures 3a, 3d). This elongation along the diagonal signifies a persistent inhomogeneous broadening. The introduction of ^{13}C -labeling on site-2 isolates a diagonal peak of the selected region which has significant inhomogeneous broadening (Figures 3b, 3e). In addition, the peak frequency of the main transition is slightly increased by ca. 3 cm^{-1} with respect to the unlabeled case. For L7, the ^{13}C -labeled amide-I transition appears at a much lower frequency position and has a clear separation from the ^{12}C transitions (Figures 3c, 3f).

The 2D IR cross peaks appear to be different in the unlabeled and labeled peptides. In the case of the UL peptide they appear in the spectral region both above and below the diagonal, although their intensities are much weaker than the diagonal signals. For the L2 and L7 peptides, in addition to the cross peaks linking the two main transitions, cross peaks are also

observed between the strong transition and the ^{13}C -shifted transition. This observation indicates that the lowest frequency transition associated with ^{13}C isotopic labeling is not localized on the labeled amide, *but involves nearby amides*. There is a collection of the cross peaks hidden under the intense diagonal peaks in the case of the UL peptide (Figure 3a). These cross peaks are split into two parts as a result of ^{13}C -labeling (Figure 3b and c): the cross peaks that originate from the interaction between labeled transitions and the unlabeled transitions; and the hidden cross peaks that originate from the interaction between the remaining unlabeled transitions. Such a splitting is more dramatic in the case of L7 because of the larger ^{13}C -isotopic shift, so the shifted cross peaks are clearly seen in the region of (1636 cm^{-1} , 1590 cm^{-1}) and (1590 cm^{-1} , 1636 cm^{-1}) as shown in Figure 3c and f. This splitting is of great importance because it allows two types of cross peaks to be characterized independently. A similar splitting of the cross peaks should also occur in the region of the high frequency weak transition, however, the splitting is limited and the associated cross peaks are too weak to be seen in the 2D IR spectra. The strength of the cross peaks in 2D IR is determined by several factors, the coupling between amide units, the angle between their transition dipole moments, and the correlation coefficient between the frequency distributions of the coupled transitions.⁶⁴

Spectral Simulation Methods

Spectral simulation was based on the vibrational exciton Hamiltonian of polypeptide amide-I transitions that was recently reported.²² The model has been used to interpret the linear IR and 2D IR results of the α -helices,^{17,26} β -sheets,^{29,47} and dipeptides.¹⁸ The validity of this approach has been examined recently by comparing the model derived off-diagonal anharmonicity with that from ab initio DFT computations for dipeptides and tripeptides in various conformations.⁶⁵ Briefly the amide-I states in a polypeptide were treated as an independent set of coupled vibrators. One- and two-exciton Hamiltonian matrices were constructed in the basis of uncoupled local modes. An empirical value for the zero order diagonal anharmonicity was assumed for each mode (16 cm^{-1}), and the bilinear coupling was assumed. Matrix diagonalization yields both the one- and two-quantum transition energies. The resultant diagonal and off-diagonal anharmonicities are functions of the coupling and the zero order anharmonicity.¹⁶ The Bloch dynamics was assumed: $\gamma_{01} = 7.0\text{ cm}^{-1}$ and $\gamma_{12} = 7.7\text{ cm}^{-1}$ are the homogeneous linewidths for the $v=0\rightarrow v=1$ and $v=1\rightarrow v=2$ transitions. A standard deviation $\sigma = 8.5\text{ cm}^{-1}$ was used for the Gaussian inhomogeneous broadening obtained by averaging over 5000 diagonally disordered and correlated samplings. The 1D spectra and 2D IR correlation spectra of the amide-I transitions were calculated in the frequency domain as previously described.²² To compare the simulations with experimental results, the correlation coefficient between the fluctuations of all the $v=0\rightarrow v=1$ transitions was assumed to be 0.9 in the site state basis. The simulated 2D spectra presented here assume delta laser pulses so the results do not incorporate the central frequency and spectral width of the IR laser pulses used in the 2D IR experiment. Furthermore, both spectral diffusion and noise are omitted from the simulation, so an exact reproduction of the experiments is not expected.

Vibrational Coupling

Ab initio density functional theory (DFT) calculations have been used to evaluate vibrational couplings in the β -hairpin by using Gaussian03 program.⁶⁶ A model type-I' β -turn for trpzip2, Ac-Asn-Gly-NMe with two pair of dihedral angles ($\phi_1 = +60^\circ$, $\psi_1 = +30^\circ$; $\phi_2 = +90^\circ$, $\psi_2 = 0^\circ$)¹⁹ was used to evaluate the couplings in the turn region by DFT calculations at the level of B3LYP/6-31G*. It was found that $\beta_{5,6} = +2.5$, $\beta_{5,7} = -3.6$, $\beta_{5,13} = +1.1$, $\beta_{6,7} = +0.7$, $\beta_{6,13} = -5.9$ and $\beta_{7,13} = +0.5\text{ cm}^{-1}$, where the indices were defined in Figure 1. The nearest neighbor couplings along chain and cross chain were evaluated from DFT calculation of glycine tripeptide dimer in an ideal antiparallel β -sheet configuration ($\phi =$

-139° , $\psi = +135^\circ$) at the same level of theory. The averaged $\beta_{i,i+1}$ was $+6.5 \text{ cm}^{-1}$ and averaged coupling of two hydrogen-bonded amide-I modes was found to be -5.5 cm^{-1} . These coupling constants were used in the simulations of the 1D and 2D IR spectra. Remaining couplings were obtained using the interaction of the transition charge with charge fluxes.^{22,24}

Site state and exciton state

We carried out the parametric method 3 (PM3) semi-empirical calculations on Trpzip2 in vacuum using Gaussian98.⁶⁷ Starting from the NMR structure in the protein data bank⁶⁸ (PDB ID 1LE3,⁶ model 1), the geometry of Trpzip2 was fully optimized and the normal mode calculations were performed. Local mode frequencies were obtained by the wave function de-mixing of the harmonic normal modes.⁶⁵ It was found that the PM3 predicted local mode frequencies have a mean frequency of 1901 cm^{-1} but are not degenerate. The normal mode and local mode frequencies are shown in Figure 4 (upper curves, circles and up-triangles respectively). It is seen that the amide-I units that participate in forming intramolecular hydrogen bonds (sites-1, 3, 5, 8 and 10) have relatively lower frequency in their local modes than those with their C=O groups facing outside (sites-2, 4, 6, 7, 9, 11 and 13). Site-12 has its C=O weakly hydrogen bonded and therefore has high frequency. Site-13 amide unit is located in the side chain of Asn residue, having the highest frequency. The largest site-to-site frequency difference was found to be nearly 25 cm^{-1} (site-13 vs. 10). In our simulation, we first use a simple model in which the zero order frequencies are fully degenerate. However, we find that in order to reproduce the observed linear IR spectra of the three isotopomers by using the couplings described above, the zero order frequencies have to be non-degenerate. A few assumptions were made. First, the mean zero order frequency was set to be 1658.5 cm^{-1} . Second, the local mode transition energy for site-7 was set to be ca. 1645 cm^{-1} , which is ca. 16 cm^{-1} lower than that of site-2. We find this is crucial to reproduce the ^{13}C -shifted transitions in L2 vs. L7. Third, since the local hairpin structure has two-fold symmetry, the frequency at site-5 was also lowered to the same level as site-7. The resulting set of zero order frequencies is plotted in Figure 4 (lower curve). As we show below, such a simple picture of the zero order states produces the essential feature of the linear IR and 2D IR results. However, we believe that the zero order amide-I mode frequencies needed for spectral simulations of solvated polypeptides are generally non-degenerate. We tried many other variations in zero order frequencies and small shifts of other transitions could improve the agreement with experiment. However we did not find a unique set of shifts, thus the simplest model that exhibited the main experimental features was used. A better description of the zero order frequencies would require additional 1D and 2D IR measurements. In addition, the zero order frequencies may have a distribution when there are multiple structures. Further, to reproduce the observed linear IR and 2D IR of L2 and L7 isotopomers, the zero order isotope shift for the ^{13}C -labeled amide-I mode was assumed to be 47 cm^{-1} . The DFT anharmonic frequency calculation of *N*-methylacetamide in vacuum at the level of B3LYP/6-31+G** predicts 44 cm^{-1} by using Gaussian03.

The energy level diagram of the simulated one- and two-quantum states for UL, L2, and L7 is shown in Figure 5 where the zero order energies (both one and two-quantum states without couplings) are plotted on the left and the exciton states of three isotopomers on the right. There are 13 one-quantum states and 91 two-quantum states.¹⁴ The introduction of the coupling and the diagonal anharmonicity leads to a broadening in the two-exciton band. Assuming a similar $^{13}\text{C}=^{16}\text{O}$ frequency shift of the amide-I local excitation for each site state, ^{13}C substitution of amide at site-2 or site-7 perturbs the one- and two-exciton band structures quite differently because of the mode localization. Broader bands are predicted in the case of L7. The lowest one-exciton states in both L2 and L7 are mostly due to the

localized ^{13}C -substituted amides, as shown in the wave function analysis (see below). The overtones for these two localized one-exciton states, which are the lowest two-exciton states in each of the ^{13}C isotopomers, are also separated substantially from the corresponding two-exciton bulk states.

1D IR simulation

The simulated 1D IR spectra and difference spectra of three isotopomers are shown in Figure 6. The simulation reproduces all the basic features of the measured FTIR spectra: the overall spectral bandwidth, the relative peak intensity, peak positions of the main transitions and the shifted transitions due to ^{13}C -substitution. An idea of the delocalization of the 2- and 7-position amide-I mode can be glanced from the differences in their linear spectra from that of the unlabeled peptide. Two simulated difference spectra L2-UL and L7-UL (Figure 6b) resemble those given in Figure 2b, with positive signals associated with ^{13}C -substitution spanning almost the entire amide-I region in each case. This suggests that the exciton model roughly reproduces the one-exciton states and the delocalization of the amide-I modes of both site-2 and site-7. The mode delocalization can be further demonstrated by

examining the excitonic state wave function $\psi_k = \sum_n^N c_{nk} \varphi_n$, where c_{nk} is the coefficient of n th site state wave function φ_n to the k th exciton state ψ_k . Figure 7 illustrates the relative amide-I mode amplitudes (c_{nk}) of the site states in the low-frequency band due to ^{13}C -substitution at site-2 or site-7. Clearly, in the case of L2 (Figure 7a), this low-frequency transition contains mostly site-2, and minor contributions from site-1, 3, and 9–11. The contribution from the remaining sites is predicted to be insignificant. In addition, the result shows that in this transition, the inter-strand amide-I motions are in phase (having the same sign in c_{nk}) for the hydrogen-bonded pairs (site-1 and 11; site-2 and 10; and site-3 and 9), because only then can a strong IR transition result from parallel C=O groups. For the L7-isotopomer (Figure 7b), it is seen that the low-frequency transition contains mostly the amide-I motion from site-7. Site-4, 5 and 8 contribute to the mode moderately, whereas the remainder contributes insignificantly.

IR band assignments

To clarify the linear IR spectra of the UL peptide, the projections of a specific site state transition dipoles onto the excitonic states, k , were evaluated to display the spectral distribution $S^{(n)}(\omega)$ of the local modes, as:

$$S^{(n)}(\omega) = \left\langle \sum_k^N |V_{kn} \vec{\mu}_{0n}|^2 \frac{\gamma_{k0}/\pi}{(\omega - \omega_{k0})^2 + \gamma_{k0}^2} \right\rangle, \quad (1)$$

where V_{kn} is the eigenvector, μ_{0n} is the transition dipole of the n th local state, γ_{k0} is a the homogeneous line width of the exciton transition, and the angle brackets imply averaging over a distribution of local state frequencies that appear in the eigenvectors. Calculations using Equation (1) were carried out in three groups: $n = 1, 2, 3$ and 4 (the N-terminus strand); $n = 5, 6, 7$ and 13 (the β -turn); and $n = 8, 9, 10, 11$ and 12 (the C-terminus strand) respectively. The result is shown in Figure 8. Here the cross-terms, between the strands and turn which may contain negative contributions were neglected in the calculation. It is found that the maximum peak position of $S(\omega)$ for the β -turn is at the higher frequency end ($>1675 \text{ cm}^{-1}$) of the linear spectrum whereas those of the two strands are at the lower frequency end ($<1645 \text{ cm}^{-1}$). These results are in general agreement with previous band assignment of β -hairpins.^{3,69} The results shown in Figure 8 can be easily understood by examining the magnitude of the couplings. Using our transition charge coupling scheme, we found that most of the inter-mode couplings are smaller than 0.1 cm^{-1} (absolute value) when two

amide units are separated by more than two residues and not hydrogen-bonded nor next to a hydrogen-bond pair. This results in a picture in which the terminal and the turn regions can be viewed approximately from the low frequency and high frequency components respectively in its 1D spectrum of the UL peptide.

¹³C-Enhancement

Although only one of 13 residues is substituted by ¹³C the relative intensity contributed by the isotopically shifted line in the spectrum is considerably different from 1/13. This effect arises from the coupling between the sites and provides strong evidence for existence of such interactions. The numerical simulations of the linear IR spectra predict enhancement factors of 1.9 and 1.3 for the L2 and L7 isotopomer respectively. The enhancement is calculated with respect to the transition dipole squared of an uncoupled single site. The experimental values are ca. 1.6 and 1.2 so the trend of the predicted values is in agreement

with the experiment. The computed delocalized states, $\psi_k = \sum_n^N c_{nk} \varphi_n$, allow computation of the transition dipole strength in the k th state contributed from site n as the fraction c_{nk}^2 of the local mode transition dipole strength. The contribution to the k th state from a cross term between site- m and n is the fraction $2c_{mk} c_{nk} \cos \theta_{mn}$ of the local mode transition dipole strength, where θ_{mn} is the angle between m th and n th local transition dipoles. For example, the isotopically shifted transition ($k=1$) in the simulated linear IR spectrum in the L2

isotopomer (site $n=2$ is labeled) has $c_{2,1}^2=0.94$. It is predicted that the cross term contributions between site-2 and each of the sites 1, 3, 9, 10, and 11 are 0.19, 0.20, 0.10, 0.19, and 0.10, respectively. The sum of these contributions to the $k=1$ transition intensity is 1.7, which accounts for 90% of the total enhancement. Therefore the linear and 2D IR spectra of the ¹³C-shifted transition must depend on the coupling between just a few sites

around the labeled region. For the L7 isotopomer, $c_{7,1}^2=0.98$. Local mode transition dipole strength contributions to this isotopically shifted band come from cross terms of $n=7$ with site-4 (0.08), 5 (0.09) and 8 (0.13). The sum of these four contributions to the $k=1$ transition is ca. 1.3, which is essentially the whole intensification effect.

2D IR simulation

The simulated 2D IR correlation spectra of UL, L2 and L7 isotopomers are shown in Figure 9. The simulated 2D IR spectra have all the essential spectral features that are shown in experimental results collected with the laser center frequencies at 1592 cm⁻¹ and 1680 cm⁻¹ (Figure 3). For the UL peptide (Figure 9a), two diagonal peaks ($\omega_\tau \approx 1635$ and 1674 cm⁻¹) each containing a $v=0 \rightarrow v=1$ transition and an anharmonically shifted $v=1 \rightarrow v=2$ transition are evident and two cross peaks appear above and below the diagonal, each containing a positive and a negative component, marked with “+” and “-” respectively. For both the L2 and L7 peptides, three diagonal peaks are seen at $\omega_\tau \approx 1600$, 1640, and 1674 cm⁻¹ (L2) and $\omega_\tau \approx 1590$, 1635, and 1674 cm⁻¹ (L7), in which the lowest frequency transitions appear as a result of ¹³C-labeling (Figure 9b and c). The simulated peak separation between the ¹³C-shifted and un-shifted transitions is larger in L7 than in L2, reproducing the essential experimental observations (Figure 3bc). The main characteristics of the cross peaks between the ¹³C-shifted and the main exciton transitions are predicted for both L2 and L7. The peak positions and the diagonally elongated and anti-diagonally narrowed features of these diagonal signals (Figure 3abc) are well reproduced by the simulation. These results show that the 2D IR spectra of the β -hairpin are very sensitive to the spatial location of the ¹³C substitution and that the essential features of the 2D IR spectra of the β -hairpin including a ¹³C-perturbation can be understood on the basis of a vibrational exciton model incorporating coupling constants from ab initio DFT computations and semi-empirical

calculations. In the simulation the vibrational frequency fluctuations of different sites was assumed to be 90% correlated. The profile of the tilted diagonal peaks of both the $\nu=0\rightarrow\nu=1$ and $\nu=1\rightarrow\nu=2$ transitions and the tilting of the off-diagonal peaks in each isotopomers observed in 2D IR experimental results (see Figure 3abc) can be approximately reproduced by the simulation. It was found that completely uncorrelated diagonal disordered samplings do not fully reproduce these spectral features. These results seem to suggest that the frequency distributions of the amide-I anharmonic oscillators at different sites of the β -hairpin are significantly correlated. Since it is clear that the solvent induced fluctuations of different regions of the hairpin are unlikely to be highly correlated we conclude that the frequency deviations defining the overall shapes of the spectra are mainly originating from structure distributions that are relatively static on the few ps time scale of the experiments, justifying the use of a Bloch model in the simulations.

The local structural signature of the hairpin is reflected by the shift of the strong diagonal peak induced by ^{13}C isotope labeling. This is because the ^{13}C -shifted transition is delocalized into a few amide units around the labeling site, and also because the diagonal peak elongation signifies the static inhomogeneous structure distributions. We find that the ^{13}C -shifted diagonal peaks in the L2 and L7 isotopomers shown in Figure 3(b and c) are quite different in regard to their peak orientation in the 2D IR spectrum plane, their shapes and their frequency separations that reveal different local structures in the two spatial regions of the hairpin. The orientation of the diagonal peaks can be characterized by the “slope”,⁷⁰ which is found along the zero contour line that separates the $\nu=0\rightarrow\nu=1$ and $\nu=1\rightarrow\nu=2$ transitions at the ω_{τ} of their peak position.²⁷ When the inhomogeneous broadening is dominant, the slope approaches to unity; The slope is positive infinity when there is only homogeneous broadening. The slope was found to be ca. 1.1 for L2 and ca. 1.9 for L7 (Figure 10), indicating more inhomogeneous broadening contribution in the case of L2. Further, the slope is associated with the shape of the diagonal peaks. We may use the “aspect ratio” to characterize the shape of either the $\nu=0\rightarrow\nu=1$ or $\nu=1\rightarrow\nu=2$ transition. Here the aspect ratio, R , is defined as the ratio of the half width at half maximum along the major to that along the minor axis of an elliptically shaped 2D IR peak. Pure homogeneously broadened and spectrally isolated transitions have $R=1$. We measure the major axis from the peak center in the direction of decreasing ω_{τ} , and the minor axis from the center toward increasing ω_{τ} in order to minimize the influence of nearby 2D peaks in Figure 10. The major axis is not parallel to the diagonal direction when the slope is not unity. It is found that $R = 2.3$ in L2 and $R = 1.9$ in L7 for the $\nu=0\rightarrow\nu=1$ transitions (Figure 10), suggesting there is more inhomogeneous broadening for L2. Even though L2 is not such an isolated transition as L7 this qualitative change in inhomogeneity is marked. The diagonal anharmonicities of the ^{13}C -shifted transitions in the L2 and L7 isotopomers also have been examined. In the case of L2, the diagonal anharmonicity was estimated to be ca. 13.8 cm^{-1} by curve fitting the real part of the 2D IR slice at $\omega_{\tau}=1600.0\text{ cm}^{-1}$ with Gaussian functions for both the $\nu=0\rightarrow\nu=1$ and $\nu=1\rightarrow\nu=2$ transitions (Figure 11a). For L7, the anharmonicity was estimated to be ca. 14.6 cm^{-1} , by similarly fitting the 2D IR slice at $\omega_{\tau} = 1590.0\text{ cm}^{-1}$ (Figure 11b). Note that the apparent peak separation of the ^{13}C -shifted $\nu=0\rightarrow\nu=1$ and $\nu=1\rightarrow\nu=2$ transitions is estimated to be ca. 22 cm^{-1} in the case of L7, much larger than the actual anharmonicity. In addition, the diagonal anharmonicity of the ^{13}C -shifted transition in the cases of L2 and L7 are predicted by the exciton modeling to drop from the 16 cm^{-1} zero order value to 14.0 cm^{-1} and 14.5 cm^{-1} (Figure 11c and d). The magnitude of the anharmonicity is associated with the coupling and thus the torsional angles of peptide backbone. Our recent study of model di- and tripeptides⁶⁵ suggests that as the intermode coupling increases, the diagonal anharmonicity decreases. The ^{13}C -shifted transition has a smaller diagonal anharmonicity in the case of L2, suggesting that stronger couplings are involved in the terminal region; whereas the anharmonicity is larger in the turn region, suggesting weaker couplings. DFT computations and transition charge coupling calculations

suggest the dominant coupling terms in the terminal region are $\beta_{21} = \beta_{23} = +6.5$, $\beta_{2,9} = +4.5$, $\beta_{2,10} = -5.5$, and $\beta_{2,11} = +2.3 \text{ cm}^{-1}$; and those in the turn region are $\beta_{47} = +3.9$, $\beta_{57} = -3.6$, and $\beta_{87} = +6.5 \text{ cm}^{-1}$. Because the couplings are intimately linked to the conformation, the (ϕ, ψ) angle difference in two different spatial regions of a peptide can be revealed by the diagonal anharmonicities.

The observed 1D and 2D IR signals of the isotopically labeled β -hairpin provide chemical bond level insight into the peptide equilibrium conformational dynamics. Since the wave function analysis suggests that site-2 is predominant in the ^{13}C -shifted transition of L2 and site7 is predominant in that of L7, one can examine the local environments in these two regions that influence the frequency distributions of the ^{13}C -shifted transitions. The amide C=O bonds of Trp2 and Gly7 face towards solvent according to an NMR study,⁶ so that the solvent electrostatic potential at the peptide perturbs the C=O bond length and causes the amide-I mode to have a frequency distribution. However, the two C=O groups have quite different local environments. The C=O of Trp2 is located in a hydrophobic region with several relatively large side chain groups in its neighborhood (including Trp4, 9, and 11), the nearby water molecules can adopt a range of structures so the influence of the solvent potential on the C=O bond is to cause more inhomogeneous broadening. This causes the amide-I mode to have a relatively broader frequency distribution. On the contrary, the C=O of Gly7 is located in a hydrophilic region so that the influence of the solvent potential on the amide frequency distribution is expected to be weaker. In addition, because of the more hydrophilic environment of L7 its local mode frequency is shifted to lower frequency as observed. We suggest that the solvent effect is the dominant structural origin of the observed difference in the ^{13}C -labeled transition frequency distributions in the two isotopomers.

The coupling between different spatial regions of the β -hairpin and the dynamics of the structural changes for these regions is sensed by the 2D IR cross peaks. The experiment and simulation clearly show the cross peaks between the two main transitions of the UL peptide as well as those cross peaks between unlabeled transitions and the high frequency weak transition for both the L2 and L7 isotopomers. All the simulated cross peaks appear both below and above the diagonal, with their features in agreement with the experimental results. However, the cross peaks are more clearly shown in the simulated spectra than in the measurements, partly because the simulation is free from noise. The introduction of 2% noise into the simulation tends to localize the 2D IR peaks making them more like the experiments. The high frequency transition, near 1670 cm^{-1} in UL, results from an in-phase interaction along chain and an out-of-phase interaction across chain.^{47,71,72} The lowest frequency strong transition of UL is due to an in-phase interaction across chain and an out-of-phase interaction along chain. Therefore the cross peaks in the 2D IR spectra involve coupling between sites delocalized throughout the entire hairpin. This is one of the reasons why these predicted cross peaks have similar intensities (relative to the diagonal peaks) in the three isotopomers. Significant effects on these cross peaks may require changes in the inter-chain amide geometry (hydrogen bond distances).⁶³ We believe they may require changes in the intra-chain conformation (dihedral angles), or in both inter- and intra-chain conformations, because these cross peaks characterize the global secondary structure of the β -hairpin.

The cross peaks between the ^{13}C -band and the low frequency strong transition are more local structural indicators. They originate from the interaction between two groups of transitions, the relatively local labeled transitions and the remaining unlabeled transitions. The patterns of the cross peaks shown in Figures 3b and 3c (experiments), and Figures 9b and 9c (simulations) depend on the total coupling strength between the two groups of transitions in each of the two isotopomers. The relative peak intensity of the diagonal of the ^{13}C -shifted transition and the cross peak was examined for L2 and L7. A spectral slice

cutting through the ω_t -axis at specific ω_t value can be obtained. The results are shown in Figures 11a and 11b for L2 ($\omega_t = 1600 \text{ cm}^{-1}$) and L7 ($\omega_t = 1590 \text{ cm}^{-1}$) respectively. It is found that the ratio of the peak-to-peak intensity of the off-diagonal to the diagonal signals is ca. 0.22 in both L2 and L7, indicating strong couplings between the ^{13}C -labeled and unlabeled transitions in both cases. If the coupling is weak, the off-diagonal and diagonal peak ratio can be as small as 0.04, as seen in the alanine dipeptide in D_2O .¹⁸

The intensity of cross peak with respect to the diagonal peak in the region below the diagonal has been examined. The slices obtained from the simulated 2D IR spectra are given in Figures 11c and 11d. Comparing the relative peak-to-peak intensities of the diagonal and the off-diagonal signals, the simulation agrees with experiment reasonably for the cases of L7 (Figures 11d vs. 11b). This suggests that the overall couplings among the amide-I modes in the turn and other regions of the hairpin that were used in our model calculations are representative of the actual coupling. For the L2 isotopomer, the simulated cross peaks are somewhat stronger than found in the experiment (Figures 11c vs. 11a). This implies that the couplings associated with this cross peak are probably overestimated in the model. This disagreement can be reconciled by postulating a distribution of structures in which the turn region is relatively rigid and type-I' β -turn like, whereas the terminal is relatively flexible and deviates significantly from an ideal β -hairpin ($\phi = -139^\circ$, $\psi = +135^\circ$). This idea is supported by the multiple NMR structures,⁶ from which the mean value of dihedral angles in the terminal region varies from -80° to -136° for ϕ and $+70^\circ$ to $+145^\circ$ for ψ . Our measurements and simulations show that the 2D IR cross peaks associated with a single ^{13}C -substituted amide are sensitive to how the labeled region interacts with the remaining peptide structure, and thus the results have provided information on peptide local conformations. Our results also confirm that amide-I inter-mode couplings that are indeed characteristics of particular conformations.

Conclusion

In this paper, a synthetic peptide⁶ that has a unique conformation (antiparallel chains linked by a type-I' β -turn) in the aqueous solution has been examined by vibrational spectroscopies. The peptide has been designed to have a hydrophobic cluster of Trp residues in the chain region. Selective ^{13}C -isotopic substitution of a peptide was shown to provide an effective spectroscopic probe of the local structure. FTIR and 2D IR correlation spectral characteristics of the 12-residue tryptophan zipper that forms a twisted β -hairpin show that single ^{13}C -substitution perturbs both one- and two-exciton states, causing shifts of the IR transitions. The diagonal peak profiles are altered and new cross peaks appear between the ^{13}C -shifted and un-shifted strong transitions. These cross peaks would be otherwise buried inside the tail of the intense low frequency diagonal signal as in the case of the UL peptide. Therefore the ^{13}C -substitution allows the frequency distribution of the delocalized mode in the isotopically selected region of the peptide to be characterized by decreasing the interference with other delocalized modes.

The 2D IR signal is very sensitive to local structure fluctuations of the solvated peptides. The principal signatures are the mean frequency, the frequency distributions of the ^{13}C -shifted transitions, the shape, orientation and line broadening of the diagonal and off-diagonal peaks, and the diagonal and off-diagonal anharmonicities. The profile of the diagonal peak reveals the frequency distribution of the labeled and nearby amide units that are coupled to it. Peptide-solvent interactions and peptide side chain heterogeneity are believed to be two primary factors that influence the amide-I local mode frequency distributions and frequency shifts.

The zero order peptide amide-I mode transitions in the trpzip2 β -hairpin are not degenerate. This is confirmed by both 1D and 2D IR experiments. A set of non-degenerate zero order local mode frequencies of the hairpin have been assumed and three sets of 1D and 2D IR spectra (UL, L2 and L7) have been simulated using the vibrational exciton model leading to general agreement between experiment and computation. Characteristics of the vibrational mode delocalization and the global and local peptide conformations are shown in the simulated 1D and 2D IR spectra. The 2D IR spectral simulation of three isotopomers predicts that the 2D IR spectra of the β -hairpin are highly sensitive to the positions of the ^{13}C -substitution.

Although the peptide system we studied here is unique in having 2D IR cross peaks between two strong transitions that can be used to characterize the equilibrium global conformation dynamics, the approach is generally applicable to other polypeptide and protein conformations. The introduction of a single ^{13}C -substitution at a desired spatial region of a polypeptide allows characterization of both the local and global structures. Another example where 1D and 2D IR studies have shown that the observed ^{13}C -enhancement can report on the spatial extent of the excitations, and the profile of 2D IR peaks can report the structural distributions, is the multi-stranded β -sheet formed by the AcWL₅ peptide in lipids.^{29,47} Mode delocalization permits the use of a single ^{13}C -isotopic substitution as the local structural probe when the couplings in the neighborhood of the substitution are non-negligible. In the hairpin, both the amide-I states in the turn region and in the terminal region were found to be delocalized. Thus the 1D and 2D IR signal in the isotopically labeled spectral region reveals a coherent state of the labeled sites plus a few neighboring sites. Such a coherent state depends on the coupling constants among the amide units involved in the exciton state hence has the local structure character of more than just the labeled residue. A single ^{13}C -isotopic substitution would reveal the structural distribution at one residue only if the amide-I modes were completely uncoupled or strongly localized by solvent or structure fluctuations.

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Figure 1.
A simplified molecular structure of the 12-residue trpzip2 with 13 amide units ($\text{O}=\text{C}-\text{N}-\text{H}$).
The peptide forms a twisted anti-parallel β -hairpin conformation.

Figure 2.

(a) FTIR spectra of three trpzip2 isotopomers in D₂O: the unlabeled (UL), L2 (¹³C=¹⁶O labeled on Trp2) and L7 (¹³C=¹⁶O labeled on Gly7). (b) Difference spectra: L2-UL and L7-UL. Arrows indicate positive going peaks.

Figure 3.

Real part of the 2D IR correlation spectra of trpzip2 isotopomers: (a and d) UL, (b and e) L2, and (c and f) L7. The cross peaks are marked with “+” and “-” for positive and negative components. (a, b, and c) were collected with IR laser central frequency at 1592 cm^{-1} and (d, e, and f) were collected with IR laser central frequency at 1680 cm^{-1} .

Figure 4.

The zero order transition frequencies obtained from the PM3 computation (upper curve, up-triangles) and assumed in the model calculation (lower curve, down-triangles). The normal mode frequencies are also given (circles, top axis).

Figure 5.

A level diagram of the simulated one- and two-quantum states for UL, L2, and L7. The zero order energy levels are given on the left.

Figure 6. Plot of relative amplitudes of the local amide-I mode in the low-frequency band due to ^{13}C -substitution in (a) L2 and (b) L7 isotopomers.

Figure 7.

(a) Simulated linear IR spectra of three trzip2 isotopomers: UL, L2 and L7. (b) Simulated difference spectra: L2-UL and L7-UL.

Figure 8. Suggested band assignment of the 1D IR spectrum of the unlabeled hairpin based on the exciton model.

Figure 9. Simulated real part of the 2D IR correlation spectra of trpzip2 isotopomers: (a) UL, (b) L2, and (c) L7. The cross peaks are marked with “+” and “-” for positive and negative components.

Figure 10.

Slope and aspect ratio of the ^{13}C -labeled 2D IR correlation spectra: (a) L2, (b) L7. Spectra were taken from Figures 3b and 3c with more different number of contour lines. The zero-contour slope (dashed line) between the $v=0 \rightarrow v=1$ and $v=1 \rightarrow v=2$ transitions is shown in each case. The dashed arrows indicate the major and minor axes of the elliptical peak of the $v=0 \rightarrow v=1$ transition.

Figure 11. kSlices of the real part of the 2D IR correlation spectra at two different ω_τ values for L2 (a, c) and L7 (b, d). (a, b) experimental results collected with IR laser central frequency at 1592 cm⁻¹. (c, d) simulated results.