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Empirical Relationships between Isotope-Edited IR Spectra and Helix Geometry in Model Peptides

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Abstract: Infrared spectroscopy (IR) is commonly used to study secondary structure of both peptides and proteins. The amide I band is very sensitive to peptide secondary structure, and the conformation of a peptide can be probed at the residue level by introducing site-specific isotope-labels into the peptide backbone. The replacement of a carbonyl ^{12}C with a ^{13}C results in a $\sim$40 cm^{-1} shift in the amide I′ band. The amide I bands of specifically labeled helices should vary systematically as a function of the number and relative spacing of the labeled residues; thus one should be able to describe the conformation of a polypeptide in substantial detail by probing the changes in IR spectra as a function of the number and positioning of isotope labels. In this study, we report IR spectra of a series of differently labeled helical peptides. A series of 25mer peptides were synthesized based on the repeat sequence $(AAAAK)_n$. We have varied the number and spacing of the labels on each peptide and studied the changes in the ^{12}C and ^{13}C amide I′ band due to label position. Our results indicate that changing the number of labels changes the frequency and intensity of both the ^{12}C and the ^{13}C amide mode. We also found that varying the spacing between labels causes these amide peaks to shift. Isotope labeling, combined with IR spectroscopy and theoretical predictions, may generate a description of peptide backbone conformations at the residue level.

Introduction

Infrared spectroscopy (IR) is widely used as a method for determining the secondary structure of proteins and short polypeptides and monitoring conformational changes upon protein unfolding, ligand binding, or other functional activity. Most studies have focused on the amide I band, which occurs between 1700 and 1600 cm^{-1} and has been assigned to a mode consisting primarily of stretching of the carbonyl backbone. The properties of this band depend on the secondary structure of the polypeptide; α-helices, β-sheets, and random coil can all be distinguished based on differences in the frequency, splitting, bandwidth, and intensity of their amide I bands.[1] This conformational dependence can be attributed to differences in intrapeptide hydrogen bonding among the different secondary structures and transition dipole coupling between amide I modes of adjacent peptide moieties.[2] Theoretical and computational approaches to relating the amide I bands of polypeptides to protein structure have been explored in detail, including normal-mode analysis,[3] coupled-oscillator models,[4] and ab initio calculations.[5]

Typically, IR spectroscopy is a low-resolution technique for studying proteins, analogous to ultraviolet circular dichroism: the overall secondary structure content can be determined by analysis and deconvolution of the amide I band, but the structural elements cannot be assigned to specific amino acid residues in the polypeptide sequence. However, residue-level information can be extracted by introducing site-specific isotope labels into a polypeptide. For example, when ^{12}C of a backbone carbonyl is replaced with a ^{13}C, the amide I band of this residue is shifted by about 40 cm^{-1}, typically bringing it outside the ^{12}C band. This isotope-shifted band serves as a local probe of the conformation of specific residues or groups of residues. In recent studies, this approach of "isotope-edited" FTIR has been used to determine the conformation of specific residues within proteins,[6] probe protein−protein interactions,[7] and study the equilibrium and kinetics of the helix-coil transition in model peptides.[8−11] These experiments fill an important gap in biophysical studies of protein conformation. High-resolution

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techniques, such as multidimensional NMR spectroscopy, can provide very specific information about the conformation of residues within peptides and proteins, but this technique is limited in application due to large amounts of sample required and time-resolution of experiments. UVCD is very sensitive (requiring small amounts of dilute solutions), and can be used to follow secondary structure changes as a function of time or as a rapid-screening approach, but without residue-level information. Isotope-edited FTIR holds the potential of providing both local probes of secondary structure and the capability of obtaining time-resolved information.

While previous studies demonstrate the potential of the isotope-editing approach to elucidate residue-level information on protein structure and dynamics, the full quantitative information contained within the spectra has yet to be fully exploited; in these studies, spectral differences are largely interpreted in crude terms ("helical or not-helical) instead of specific quantitative parameters (backbone torsion angles, distances/dimensions of helices, etc.). However, we should be able to construct a more precise description of backbone conformation from the data available from isotope-edited IR experiments, since the coupling between amide I modes of labeled residues should vary systematically with the distances and angles between their dipoles. In other words, pushed a bit further, one should be able to elucidate and quantitate subtle differences in backbone conformation (perhaps even differences which are difficult to resolve by NMR) by IR spectroscopy.

Even the "crude" information about the conformation of local residues is clouded by limitations in our understanding of these spectra. In studies of model helical peptides, frequencies and intensities of both the ^{12}C and ^{13}C amide I bands vary as a function of location within the helix reflecting subtle differences in coupling between ^{12}C and ^{13}C amide I modes, due to both differences in local backbone conformation and the relative placement of the labels within the helix.[8] These variations may overshadow spectral differences due to inhomogeniety in local environment, clouding interpretations of the isotope-editing experiment in terms of residue-level differences in structure. Moreover, in most studies involving sequences taken from naturally occurring proteins or peptides, placement of labels is dictated primarily by the availability of specifically labeled amino acids and the amenability of particular sequences to labeling. The number of labels used and their relative positioning often varies a great deal from experiment to experiment, or even within an experiment; for example, one region of a protein may be labeled at a string of adjacent residues, whereas in another region the labels are separated form each other by one or more residues. This variability complicates the quantitative interpretation and comparison of the isotope-shifted spectra, since it remains unclear whether frequency differences observed are due to the conformations of the labeled positions or the arrangement and number of labeled residues.

Unfortunately, there has been no systematic experimental or computational study of how coupling between ^{13}C residues, ^{12}C residues, and ^{13}C−^{12}C coupling vary within a helix as a function of the number and relative placement of ^{13}C labels. With a thorough set of correlations between amide I band characteristics and the geometry and arrangement of labels, it should be possible to obtain detailed structural information (distances, torsional angles, etc.) about the polypeptides by quantitative analysis of spectra of labeled peptides. We are seeking to broaden the applicability and clarify the information content of isotope-edited IR spectroscopy via experimental and computational studies of model peptides labeled systematically with ^{13}C in backbone carbonyls.

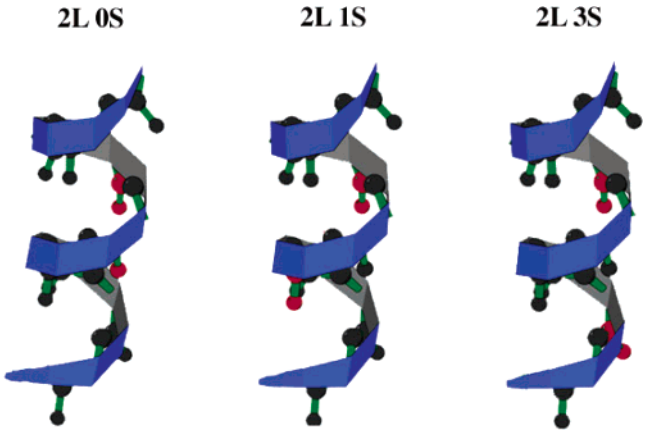

Figure 1. Ribbon diagrams of model helices illustrating spacing of labeled carbonyls. "Labeled" backbone carbonyls are indicated in red. In the 2L3S peptide, the labels are separated by a full helical turn, but are aligned on the same face of the helix. This relative geometry is responsible for the coupling behavior observed in the FTIR spectra.

In this paper, we report IR spectra of a series of ^{13}C labeled model helical peptides with twenty-five residues, based on the repeat $(AAAAK)_n$. These peptides form stable α-helices in aqueous solution, and many experimental studies have demonstrated that the central residues in these peptides are predominantly helical at low temperatures.[12] We have used these helical peptides as a scaffold for arranging ^{13}C-labeled backbone carbonyls in different configurations, by varying the number of adjacent labeled groups and by varying the spacing between the labeled groups (for illustration, see Figure 1). The amide I′ spectra in these peptides vary a great deal; both the ^{12}C and ^{13}C amide I bands can change by up to 10 cm^{-1} by merely rearranging label placement with the helix. Correlations between these spectral properties and the relative geometry of the labeled residues may be used as a guide in future studies for elucidating specific quantitative parameters (distances, torsional angles, etc.) about local regions of a helix backbone.

Materials and Methods

Peptide Synthesis. Ten, twenty-five residue peptides based on the $(AAAAK)_n$ repeat were synthesized, each differing the number and position of ^{13}C labeled residues (Table 1). All peptides were synthesized on a Pioneer automated peptide synthesizer (Applied Biosystems, Foster City, CA) using Fmoc chemistry. ^{13}C labeled peptides were synthesized by addition of N−Fmoc-1−^{13}C−alanine at the designated position in sequence. Peptides were purified using reverse-phase HPLC.

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Table 1. Sequences of Peptides Used in This Study[a]

name'	sequence
unlabeled	Ac−AAAAKAAAAKAAAAKAAAAKAAAAY−NH_2
1L	Ac−AAAAKAAAAKAAAAKAAAAKAAAAY−NH_2
2L	Ac−AAAAKAAAAK$\underline{A}\underline{A}$AAKAAAAKAAAAY−$NH_2$
3L	Ac−AAAAKAAAAK$\underline{A}\underline{A}\underline{A}$AKAAAAKAAAAY−$NH_2$
4L	Ac−AAAAKAAAAK$\underline{A}\underline{A}\underline{A}\underline{A}$KAAAAKAAAAY−$NH_2$
N4L	Ac−$\underline{A}\underline{A}\underline{A}\underline{A}$KAAAAK$\underline{A}\underline{A}\underline{A}\underline{A}$KAAAAKAAAAY−$NH_2$
2LT	Ac−$\underline{A}\underline{A}\underline{A}\underline{A}$KAAAAKAAA$\underline{A}$KAAAAKAAAAY−$NH_2$
2L1S	Ac−AAAAKAAAAK$\underline{A}\underline{A}\underline{A}$KAAAAKAAAAY−$NH_2$
2L2S	Ac−AAAAKAAAAK$\underline{A}\underline{A}\underline{A}\underline{A}$KAAAAKAAAAY−$NH_2$
2L3S	Ac−AAAAKAAAAK$\underline{A}$KAAA$\underline{A}$KAAAAKAAAAY−NH_2
2L4S	Ac−AAAAKAA$\underline{A}$KAA$\underline{A}$AKAAAAKAAAAY−NH_2
2L5S	Ac−AAAAKAA$\underline{A}$AKAA$\underline{A}\underline{A}$KAAAAKAAAAY−$NH_2$
2L6S	Ac−AAAAKAA$\underline{A}\underline{A}$KAAAA$\underline{K}\underline{A}$AAAKAAAAY−$NH_2$
3L1S	Ac−AAAAKAAA$\underline{A}$KAAAAK$\underline{A}$AAAKAAAAY−NH_2
4L1S	Ac−AAAAKA$\underline{A}\underline{A}\underline{A}K\underline{A}\underline{A}\underline{A}$AKAAAAKAAAAY−$NH_2$

[a] Underlined residues indicate location of ^{13}C-labeled carbonyls.

The masses of purified peptides were determined by electrospray mass spectrometry, and all were found to be in agreement with the expected values.

FTIR Sample Preparation. To remove residual trifluoroacetic acid (TFA) from the peptides as well as exchange amide N−H to N−D, about 2 mg of peptide were dissolved in 1−2 mL of 0.1% phosphoric acid/D_2O solution.[13] This mixture was frozen and lyophilized to dryness. Lyophilized samples were then dissolved in 100 μL D_2O. The concentration of the peptide was measured via the ultraviolet absorbance of the tyrosine residue (at 274 nm, $\epsilon = 1450$ M^{-1} cm^{-1}).

FTIR Spectra. Samples were loaded into a water-jacketed, heatable solution cell with CaF_2 windows (Wilmad) and a 50 μm Teflon spacer. The water jacket of the cell was connected to a refrigerated circulating water bath (Neslab) used to control the sample temperature. Spectra were measured on a Bruker Equinox 55/S FTIR spectrometer, with the sample compartment purged continuously with dry N_2. Samples were equilibrated at the desired temperature for 10 minutes before measurement of spectra. Typically, 512 scans were collected and averaged at each temperature at a resolution of 4 cm^{-1}. Spectra of D_2O were also measured at each temperature and subtracted from the peptide spectra. All data analysis was performed using GRAMS/32 (Galactic Software).

Results

IR Spectra of Unlabeled Peptide. The conformation of the unlabeled 25-mer peptide (Table 1) has been reported previously; at 2 °C, the peptide is helical, and undergoes a transition to random coil as the temperature increases.[9] IR spectra of the amide I′ band of the unlabeled peptide at varying temperatures are shown in Figure 2. At low temperature (∼2 °C), the amide I′ band is sharp with maximum at 1631 cm^{-1}; as the temperature increases, the band decreases in intensity, broadens, and shifts to higher frequency, until at 65 °C the band reaches 1645 cm^{-1}. These spectra are typical of a model helical peptide in aqueous solution.[14]

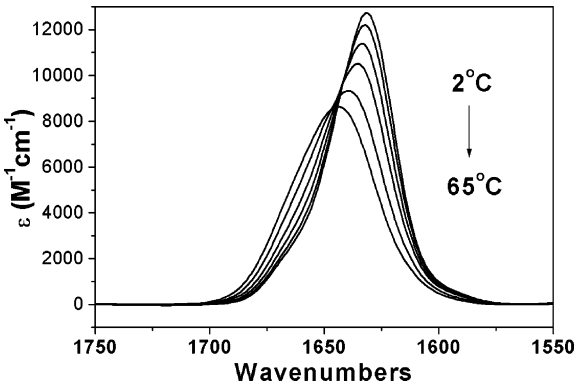

Figure 2. IR spectra of unlabeled peptide. Spectra were measured at 2, 10, 20, 30, 45, and 65 °C; changes in spectra with increasing temperature are indicated by the arrows.

IR Spectra of Peptides with Adjacent Labels. Peptides 1L, 2L, 3L, and 4L contain 1, 2, 3, and 4 adjacent ^{13}C labeled residues in the center of the helix, respectively; IR spectra for these peptides are shown in Figure 3; trends in frequency and intensity as a function of number of labels are plotted in Figure 4. Introduction of ^{13}C labels at the backbone carbonyl results in a new, isotope-shifted amide I′ band, ∼40 cm^{-1} shifted from the ^{12}C band. Frequencies of the ^{12}C and ^{13}C amide I′ bands for these peptides are given in Table 2. The ^{12}C amide I band of 1L at 2 °C is very similar to that of the unlabeled peptide in frequency, having maximum at ∼1631 cm^{-1}. Addition of a second label causes a 4 cm^{-1} shift in the 2 °C ^{12}C amide I′ band to ∼1635 cm^{-1}, and this band continues to shift to higher frequency as the number of labels increases. Interestingly, when four adjacent ^{13}C labels are added to the N-terminus of the peptide instead of the center, the frequency of the ^{12}C amide I′ band is unaffected.[8c] At 65 °C, when the peptide is in the random coil conformation, the frequency of the ^{12}C band does not vary as a function of the number of labels (Table 2).

The ^{13}C amide I′ band of 1L appears at 1596 cm^{-1} at 2 °C, and this band becomes an unresolved tail to the ^{12}C band as the temperature is increased to 65 °C. When the number of labels is increased to two in 2L, the ^{13}C amide I′ band at 2 °C is at ∼1600 cm^{-1}, shifted 4 cm^{-1} from the band of the 1L peptide. The ^{13}C band position does not change in the 3L and 4L peptides (Table 2).

The intensity of the ^{13}C and ^{12}C bands also vary as a function of number of labeled residues. As the number of labels increases from 1 to 2, the ^{13}C intensity experiences a large increase in intensity, with a corresponding decrease in ^{12}C intensity. Addition of subsequent labels results in smaller intensity changes (Figure 4b).

IR Spectra of Peptides with Two Labels Spaced at Different Intervals. Spectra of the peptides 2L2T through 2L6S, which contain two ^{13}C labels separated by 0 through 6 unlabeled residues respectively, are shown in Figure 5; trends in the amide I band frequencies as a function of residue spacing are shown in Figure 6. The 2L2T peptide has a very similar spectrum to the 2L peptide. As the peptide residues are spaced further apart, the frequency of the ^{13}C amide I′ band decreases (to 1590 cm^{-1} in 2L2S). The frequency increases with the 2L3S peptide (1596 cm^{-1}), then decreases again in 2L4S and 2L5S (1592 cm^{-1}), then increases in 2L6S (1594 cm^{-1}). In all cases, the frequencies of both the ^{12}C and ^{13}C amide I′ bands are similar at the 65 °C,

(13) All FTIR spectra were measured in D_2O solution. Under these conditions, the backbone amide protons of the peptides exchanged for deuterium. The amide I band of deuterated peptides is shifted to lower frequency and is referred to as the amide I′ band.

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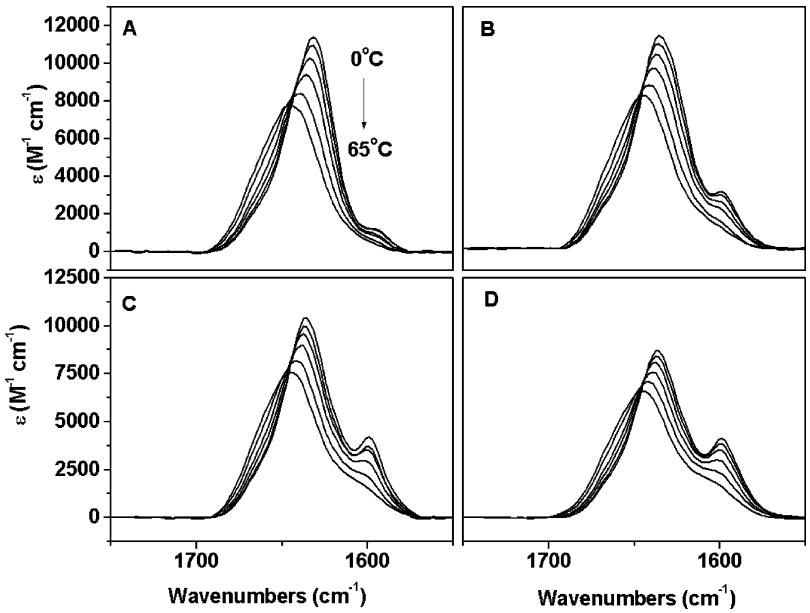

Figure 3. IR spectra of peptides with increasing number of labels (a) 1 L; (b) 2 L; (c) 3 L; (d) 4 L. Spectra were measured at 2, 10, 20, 30, 45, and 65 °C. Intensities were calculated by dividing the measured absorbance by the path length (0.05 cm) and the concentration, as determined by the absorption of tyrosine at 274 nm. Errors in the intensities are approximately ±10%.

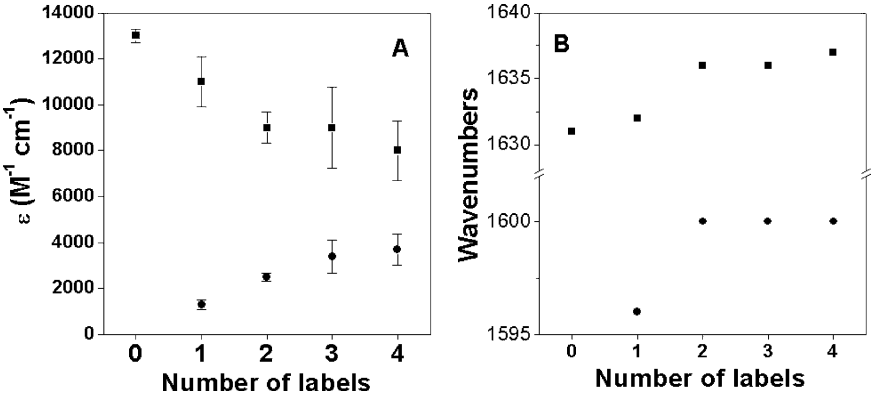

Figure 4. Trends in ^{12}C (squares) and ^{13}C (circles) amide I′ frequencies (a) and intensities (b) as a function of number of labeled residues. Error bars determined from standard deviations of at least four measurements (on different samples). Frequency error bars are smaller than symbols ($\sim \pm 0.2$ cm^{-1}). Frequencies and peak heights taken from spectra mesured at 2 °C.

Table 2. Amide I′ Band Frequencies of Adjacent Labeled Peptides at 2 °C and 65 °C[a]

	^{12}C amide I′ (cm^{-1})		^{13}C amide I′ (cm^{-1})
peptide	2 °C	65 °C	2 °C
AKA	1631	1644	N/A
1L	1632	1645	1596
2L	1636	1645	1600
3L	1636	1645	1600
4L	1637	1645	1600
N4L[b]	1633	1645	1598

[a] Frequencies are the average of at least four measurements; errors are all (0.2 cm^{-1}). [b] Data taken from ref 9.

where the peptide is mainly random coil. The frequencies of the bands are listed in Table 3.

IR Spectra of Peptides with Multiple Labels Spaced by One Residue. Spectra of the peptides 2L1S, 3L1S, and 4L1S, which contain two, three, and four ^{13}C labels separated by one unlabeled residue, are shown in Figure 7. The frequencies of both the ^{12}C and ^{13}C labeled bands do not change significantly as the number of labels is increased, but the intensity of the ^{13}C band increases with an increase in number of labels.

Discussion

General Qualitative Observations. Consistent with previous experiments, the frequency and intensity of the ^{13}C amide I′ bands of these peptides vary with conformation. As the temperature increases, and the peptide changes conformation from helix to random coil, the ^{13}C amide I′ band shifts to higher frequency and decreases in intensity, becoming a poorly resolved tail on the main ^{12}C band. This can be clearly observed even for a singly labeled peptide (1L); thus even a single ^{13}C labeled residue can be used as a local probe of peptide conformation.

While these spectra confirm that the isotope-edited IR method can be used to probe local peptide conformation, the qualitative trends in spectral features as a function of the number and positioning of labeled residues provides some useful insights and guides for the design of future isotope-editing experiments. One of the more striking observations is the significant variation in IR spectra as a function of relative positioning of labels, independent of local conformation and structural issues. For example, the frequency difference between the ^{13}C amide I′ bands of the 2L2T and 2L2S peptides is about 10 cm^{-1}, which

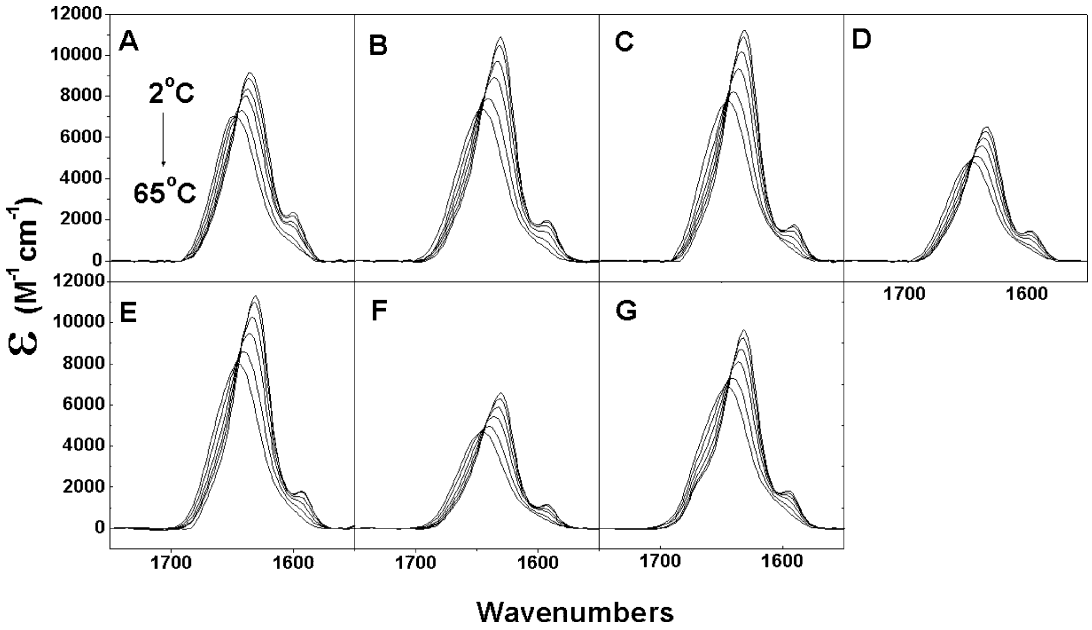

Figure 5. IR spectra of peptides with two labels spaced at different intervals: (a) 2L2T; (b) 2L1S; (c) 2L2S; (d) 2L3S; (e) 2L4S; (f) 2L5S; and (g) 2L6S. Spectra were measured at 2, 10, 20, 30, 45, and 65 °C. For each peptide, the errors in intentisities are ±10%.

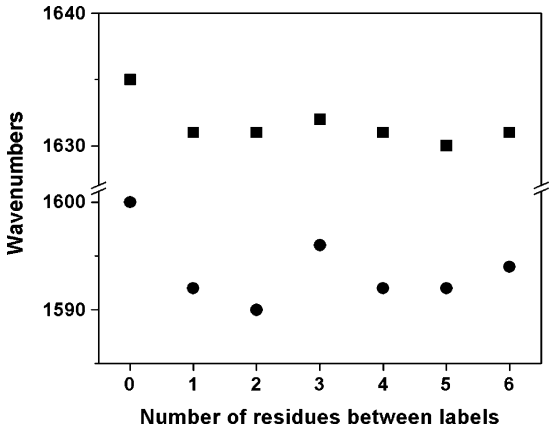

Figure 6. Trends in ^{12}C (squares) and ^{13}C (circles) amide I′ frequencies as a function of spacing between two labeled residues. Frequencies taken from spectra measured at 2 °C.

Table 3. Amide I′ Band Frequencies of Peptides with Variably Spaced Labels at 2 °C and 65 °C

	^{12}C amide I′ (cm^{-1})		^{13}C amide I′ (cm^{-1})
peptide	2 °C	65 °C	2 °C
unlabeled	1631	1644	N/A
2L2T	1635	1646	1600
2L1S	1631	1645	1592
2L2S	1631	1645	1590
2L3S	1632	1645	1596
2L4S	1631	1644	1592
2L5S	1631	1645	1592
2L6S	1631	1644	1594

is about as large as an amide I′ band shift which arises from a conformational change.[8,9] Thus, the results of an isotope-edited IR experiment must be interpreted with caution; the number and position of labels must be taken into account when making qualitative comparisons of spectra of proteins labeled at different positions. Moreover, since the "spaced apart" labeling patterns increase the splitting between the ^{12}C and ^{13}C amide I′ bands, the ^{13}C bands in these peptides are better resolved and more easily characterized than the spectra of peptides with adjacent labels.

As one might expect, the relative intensity of the ^{13}C amide I′ band depends on the number of labeled residues present. However, the largest increase in intensity comes upon going from one to two labeled residues; as the number of labels is increased beyond two, the intensity increases with a smaller slope (Figure 4b).[15] Thus, not only does increasing the number of labels reduce the residue-level resolution of the experiment, it also has diminishing returns in terms of improvement in relative ^{13}C band intensity. Combining these two effects, the 4L1S peptide gives the best resolved, largest intensity amide I′ band of the series. For probing α-helices, the ideal isotope-editing experiment might involve two or three labels arranged at a spacing of one or two residues apart.

Effects of ^{13}C Labels on ^{12}C Amide I′ Band. Introduction of one ^{13}C-labeled residue into the center of the helix does not significantly perturb the frequency of the ^{12}C amide I′ band (1632 cm^{-1} vs 1631 cm^{-1}), but the introduction of two or more labels results in a significant increase in the ^{12}C amide I′ (Figure 4a). This is consistent with previous studies of isotope-labeled helices in which peptides labeled in the center of a helix had a ^{12}C amide I′ band shifted to higher frequency than peptides labeled at the ends.[9,10] A block of labeled residues may disrupt coupling between ^{12}C amide I′ modes along the helix; in other words, the presence of the labels creates two unlabeled helices, both much shorter than the original. These two ^{12}C helices may couple only weakly through the distance spanned by the ^{13}C labeled residues. Since shorter helical segments give rise to higher frequency amide I′ bands, this results in a shifted, shortened, and broadened ^{12}C amide I′ in peptides 2L through 4L. This model is consistent with the observed shift of the ^{12}C amide I′ band in a peptide labeled exclusively at N-terminus;

(15) This trend in ^{13}C amide I′ band intensity as a function of number of labels suggests that there are some interactions between the ^{13}C and ^{12}C amide I bands, and that the simple dipole coupling mechanism involving fully isolated ^{12}C and ^{13}C modes is inadequate to fully explain the observed spectral trends.

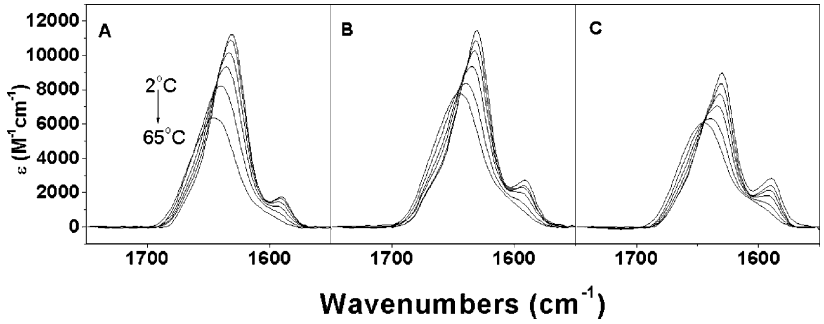

Figure 7. IR spectra of peptides with varying number of labels spaced alternately in sequence: (a) 2L1S; (b) 3L1S; (c) 4L1S. Spectra were measured at 2, 10, 20, 30, 45, and 65 °C.

in this case (N4L; Table 2), the ^{12}C residues still form a single helical segment, and the resulting ^{13}C amide I′ band frequency is not shifted significantly from that of the unlabeled peptide. Since the ^{12}C amide I′ band in 1L is very similar to that of the unlabeled peptide, the presence of a single label is not sufficient to significantly disrupt the coupling between ^{12}C amide I′ of the helix.

Correlations between Frequency Changes and the Helix Structure. The variations in ^{13}C and ^{12}C amide I′ frequencies correlate qualitatively with the labels position in the peptide structure. For example, in Figure 5, the correlation between frequency and spacing between labels has the general shape of a damped sinusoidal curve with "peaks" occurring at 2L3S and 2L6S peptides. Structurally, these two spacings correspond to having the labels separated by one turn and two turns, respectively (Figure 1).

Quantitatively, the trends observed in these spectra can be described in terms of dipole coupling between the two labeled ^{13}C amide I modes, as modeled by a simple dipolar interaction energy matrix. Following the coupled-oscillator approach of Torii and Tasumi,[16] the interactions of the amide I modes of two ^{13}C residues *i* and *j*, assuming no coupling with the surrounding ^{12}C residues, can be determined by a 2 × 2 interaction matrix, where the diagonal terms are the intrinsic frequencies of the individual modes and the off-diagonal terms give the interaction energies (V_{ij}). These terms are proportional to the dot product of the dipoles (μ_i and μ_j) and inversely proportional to the cube of distance between the dipoles (R_{ij}^3)

$$V_{ij} \propto \frac{\mu_i \cdot \mu_j - 3(\mu_j \cdot n_{jk})(\mu_k \cdot n_{jk})}{R_{ij}^{\,3}}$$

where n_{jk} is the unit vector along the line between diopoles μ_j and μ_k. In other words, the coupling energies should vary with the angle and distance between the dipoles of the amide I modes (which lie fairly close to the C═O axis). Using coupling parameters for an alpha helix given by Torii and Tasumi, we have calculated band shifts based on this very simple (and crude) dipole coupling model and compared them to the experimental results (Figure 8). The basic trend of the data for the first for peptides in the series is reproduced well by this simple model. Deviations occur at long separations (for example, 2L5S and 2L6S peptides); however, the modest success at predicting the coupling energies using this very simple approximation holds promise for the success of more sophisticated calculations.[17] The combination of isotope-edited IR spectroscopy with spectral simulation should enable the derivation of quantitative descriptions of the helix backbone geometry.

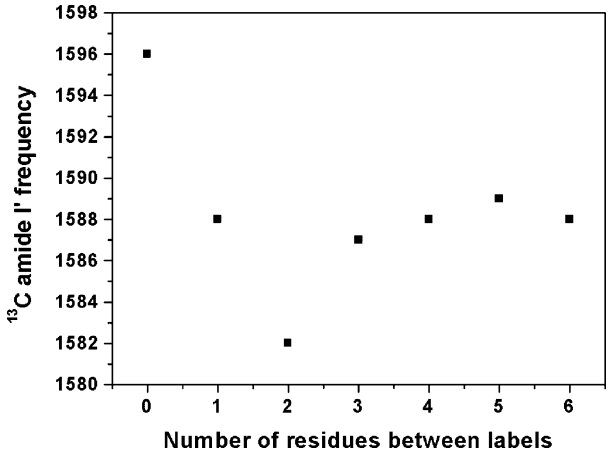

Figure 8. Predicted frequencies of ^{13}C amide I band in double labeled helices as a function of the number of residues between the labels.

Table 4. Amide I′ Band Frequencies of Peptides with Multiple Alternate Labeling at 2 °C and 65 °C

	^{12}C amide I′	^{13}C amide I′
peptide	2 °C	2 °C
unlabeled	1631	N/A
2L1S	1631	1592
3L1S	1631	1590
4L1S	1632	1596

Conclusions

The ^{12}C and ^{13}C amide I′ bands in specifically labeled helices are rich with information about the relative geometries of the labeled carbonyls. On one hand, this means that isotope-edited IR experiments should be designed and interpreted with caution, since large changes in the ^{13}C amide I′ band (10 cm^{-1} or greater) may be a function of label placement (separated versus adjacent) rather than differences in backbone conformation. However, these experiments also demonstrate that these band shifts can be interpreted quantitatively in terms of the relative geometry of the labeled carbonyls, opening the way to detailed quantitative descriptions of backbone geometry. The combination of specific

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isotope labeling, IR spectroscopy (equilibrium or time-resolved), and high-level spectral simulations should be a powerful tool for deriving protein secondary structure.

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