^{13}C Isotope Labeling of Hydrophobic Peptides. Origin of the Anomalous Intensity Distribution in the Infrared Amide I Spectral Region of β-Sheet Structures

Joseph W. Brauner,* Christine Dugan, and Richard Mendelsohn*

Contribution from the Department of Chemistry, Newark College, Rutgers University, 73 Warren Street, Newark, New Jersey 07102

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Abstract: A series of isotopically substituted derivatives of the hydrophobic peptide $K_2(LA)_6$ including $K_2LA^*(LA)_5$, $K_2L^*A^*(LA)_5$, and K_2 $LA^*LA^*(LA)_4$ (where the asterisk represents a residue with ^{13}C substitution in the peptide bond C═O) has been synthesized. The peptides adopt antiparallel β-sheet conformations as revealed by solution CD and IR measurements. The amide I region of the IR spectrum is substantially altered by the isotopic labeling. Peaks of anomalously large intensity are observed on the low frequency (∼1610 cm^{-1}) side of the major conformation marker at 1625−1630 cm^{-1} present in the unlabeled isotopomer. The spectral changes cannot be described by the appearance of a pure mode based on the substitution of an oscillator of increased mass within the sequence. A semiempirical model incorporating transition dipole coupling and through-bond interactions within the context of the Wilson GF matrix method produces excellent agreement between calculated and observed amide I spectra with a single set of four parameters (through H-bond interaction force constant, through valence bond interaction force constant, transition dipole magnitude, and physical size) for four amide I β-sheet contours. In addition, the model reproduces the amide I contour for an isotopically labeled derivative of the α-helical peptide $K_2(LA)_{10}$. The excellent agreement between calculated and experimental spectra suggests that the model accounts for the most important interactions between peptide groups with β-sheet or α-helical structures.

Introduction

Isotopic labeling of peptides provides a convenient means for determining local elements of secondary structure within a primary sequence using vibrational spectroscopic techniques. The technique has immense potential in several areas of biological chemistry, including elucidation of protein folding pathways and studies of peptide−protein interactions including antigen−antibody and enzyme−substrate interaction. Tadesse et al.[1] demonstrated the feasibility of the method via synthesis of isotopically labeled derivatives of a water-soluble, monomeric peptide rich in alanine, glutamine, and glycine. FT-IR spectroscopy was used to infer the existence of particular conformations at particular sites in the molecule. Halverson et al.[2] extended the approach for β-sheet regions through ^{13}C isotopic labeling of the peptide bond C═O in selected residues in a nine-residue peptide (H_2NLMVGGVVIACOOH) representing the carboxyl terminus of the amyloid-forming protein. The potential of this approach for studying protein−protein interactions was recognized by Haris et al.[3] and applications to bacteriorhodopsin[4,5] and calmodulin[6] have been reported. Ludlam et al.[7] labeled two separate leucine sites in phospholambam, a 52 amino acid membrane protein that regulates Ca-ATPase activity in SR of cardiac muscle cells. Polarized attenuated total reflectance studies of these peptides in a lipid environment showed the labeled bonds to be located within an α-helical secondary structure with an axial tilt of ∼30° from the membrane normal.

The IR spectrum of the amide I (energetically mostly C═O stretch) region between 1610 and 1700 cm^{-1} undergoes marked changes in response to secondary structure alterations and is widely used in studies of protein conformation. For derivatives isotopically labeled with ^{13}C in a single peptide bond, the IR data acquired by Ludlam et al.[7] showed that the amide I contour of a helical sequence is essentially that expected from the inclusion of an oscillator of increased mass within the molecule. That is, ^{13}C substitution should produce a downward shift of 37 cm^{-1} for an isolated C═O stretching frequency, reasonably close to the observed shift of 44 cm^{-1}. In contrast, Halverson et al.[2] noted that ^{13}C substitution at a single site within an (intermolecular) antiparallel β-sheet produced altered spectral patterns in the amide I region which were very different from those expected and could not be explained from simple shifts in the mass of a harmonic oscillator. No detailed explanation of the observed complex patterns was attempted although the authors suggested that the transition dipole coupling between the oscillators was altered upon isotopic substitution.

To prepare the way for a complete understanding of the effects of isotopic substitution on peptide IR spectra in the

(1) Tadesse, L.; Nazarbaghi, R.; Walters, L. *J. Am. Chem. Soc*. **1991**, *113*, 7036−7037.

(2) Halverson, K.; Sucholeiki, I.; Ashburn, T. T.; Lansbury, R. T. *J. Am Chem. Soc*. **1991**, *113*, 6701−6703.

(3) Haris, P. I.; Robillard, G. T.; van Dijk, A. A.; Chapman, D. *Biochemistry* **1992**, *31*, 6279−6284.

(4) Ludlam, C. F. C.; Sonar, S.; Lee, C.-P.; Coleman, M.; Herzfeld, J.; Rajbhandary, U. L.; Rothschild, K. J. *Biochemistry* **1995**, *34*, 2−6.

(5) Sonar, S.; Lee, C.-P.; Coleman, M.; Patel, N.; Liu, X. M.; Marti, T.; Khorana, H. G.; Rajbhandari, U. L.; Rothschild, K. J. *Nature Struct. Biol.* **199**4, *1*, 512−517.

(6) Zhang, M. J.; Fabian, H.; Mantsch, H. H.; Vogel, H. J. *Biochemistry* **1994**, *33*, 10833−10888.

(7) Ludlam, C. F. C.; Arkin, I. T.; Liu, X. M.; Rothman, M. S.; Rath, P.; Aimoto, S.; Smith, S. O.; Engleman, D. M.; Rothschild, K. J. *Biophys. J*. **1996**, *70*, 1728−1736.

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conformation-sensitive amide I spectral region, the current work seeks a quantitative elucidation of the spectra of isotopically labeled peptides. We have selected a relatively simple hydrophobic peptide, $K_2(LA)_6$, designed to form stable monolayers at the air/water interface, but also known to form an intermolecular antiparallel β-sheet in MeOH solution. The amide I region of the IR spectrum of $K_2(LA)_6$ and a series of synthetic ^{13}C isotopically labeled derivatives of this molecule is well described with a semiempirical model for coupling between vibrational modes that includes transition dipole coupling and electronic interactions through bonds. This model also reproduces the spectra of a primarily α-helical molecule, i.e., normal and (singly) isotopically labeled $K_2(LA)_{10}$ in a lipid medium. This agreement suggests that the model accounts for the most important interactions shaping the amide I envelope in the IR spectra of isotopically labeled $^{13}C{=}O$ peptides, and that it may be relevant for eventual biochemical applications.

Materials and Methods

Materials. Reagents for peptide synthesis were obtained from Perseptive Biosystems, Inc. (Framingham, MA). Isotopically labeled amino acids were purchased from Cambridge Isotope Laboratories, Inc. (Andover, MA). Solvents used for purification were HPLC grade while those used during the synthesis were reagent grade and were purchased from Aldrich Chemical Co. (Milwaukee, WI) and Fisher Scientific Co. (Pittsburgh, PA). 1,2-Dipalmitoylphosphatidylcholine, which was required to induce complete helical structure in $K_2(LA)_{10}$, was obtained from Avanti Polar Lipids Inc. (Alalbaster, AL).

Peptide Synthesis and Purification. The peptides $K_2(LA)_x$ ($x = 6$ and 10) and their isotopic derivatives labeled with ^{13}C at selected sites were synthesized on a Millipore 9050Plus peptide synthesizer using solid-phase FMOC chemistry. The amino acids were coupled as pentafluorophenyl esters in the presence of BtOH. PAL-PEG PS support resin was used to obtain neutral C-terminal carboxyamides. Peptides were deprotected and cleaved with TFA/phenol/triisopropylsilane/water (88:5:2:5) to produce crude protein. The purity of the protein was monitored by reverse-phase HPLC and electrospray mass spectroscopy. A C-18 column was used to purify the K_2LA_6. To eliminate interference in the amide I region by the carboxyl band (1678 cm^{-1}) of TFA, the peptides were treated with 50 mM methanolic HCl to replace bound TFA counterions with chloride ions.

FT-IR Spectroscopy and Data Analysis. Methanolic solutions of $K_2(LA)_6$ were prepared at concentrations ranging from 1 to 2.5 mg/mL. K_2LA_{10} solutions in 2-chloroethanol were prepared at concentrations ranging from 1 to 15 mg/mL. A fixed path length (200 μm) CaF_2 cell was used for FTIR transmission measurements. Spectra were obtained with a Mattson RS-1 (Madison WI) spectrometer equipped with an MCT detector. Four shuttled blocks of 125 interferograms each were co-added at 4 cm^{-1} resolution and Fourier transformed with two levels of zero filling to provide spectra encoded at ~1 cm^{-1} intervals. To obtain spectra of the peptide amide I region, methanol and 2-chloroethanol spectra were subtracted as required. Data were analyzed with Grams/32 software (Galactic Industries, Salem NH) and then imported into SigmaPlot 5.0 software (SSPS, Inc.) for direct comparison with calculated spectra. The latter were generated in MathCad8 software (MathSoft, Inc.) and imported into SigmaPlot 5.0 software.

Circular Dichroism Spectroscopy. CD spectra were collected with an AVIV Model 60 DS Spectropolarimeter with a 0.1 cm path length cell. Samples were prepared in methanol solutions and measured at ambient temperature.

Theory

The Wilson FG matrix method, also known as normal coordinate analysis, is used to determine molecular normal modes of vibration and corresponding frequencies. It has had considerable success in elucidating vibrational spectra of a great number of molecules of increasing complexity, even approaching molecules as large as polymers. The approach requires as input the molecular geometry, atomic masses, and estimated force constants for assumed harmonic atomic motions such as bond stretches, bends, and twists. However, the approach is unable to account for the broad amide I contour between 1600 and 1700 cm^{-1} in the IR spectra of peptides and proteins. The contour, which arises from vibrational coupling among the localized amide I motions of each peptide group, must be explained with inclusion of factors generally considered to lie outside the realm of traditional normal coordinate calculations. The current study approaches the problem by employing a higher level treatment originated by Miyazawa[8] which begins by representing each peptide group as a harmonic oscillator vibrating at some common unperturbed frequency.

There is some disagreement as to the relative importance of various coupling mechanisms. Miyazawa,[8] in his pioneering analysis of the amide I contour of an α-helix, only allowed for interactions through valence bonds between adjacent oscillators and through H-bonds between oscillators i and $i + 3$, each peptide group being represented by an oscillator. Torii and Tasumi[9,10] considered only transition dipole coupling (TDC) to model the amide I bands of globular proteins. Krimm and coworkers[11] added TDC to normal coordinate analysis of polypeptides to account for the observed splitting of the amide I frequency. In the current study the inclusion of all three types of interaction was found to bring about excellent agreement between the calculated and experimental spectra of isotopically labeled β-sheets and α-helices.

More specifically, the amide I motion of each peptide group is represented as a harmonic oscillator having a mass, a force constant, and a time varying dipole moment. These are then coupled by the three mechanisms mentioned above. In general, one oscillator may exert a force on another by virtue of its position, velocity, or acceleration. It is assumed here that the oscillators only couple by the first condition which is equivalent to connecting them by massless, frictionless springs. This affects only the potential energy of the system and is accounted for by including interaction force constants in an **F** matrix as indicated below.

A clear exposition of the coupled oscillator problem has been given by Symon.[12] To find the frequencies of the normal modes of vibration for the coupled system and the contribution of each peptide group to each mode, the secular determinant ($|\mathbf{GF} - \lambda E| = 0$, see Wilson Decius and Cross[13]) must be set up and the secular equation solved. Comparison of this matrix formalism with the algebraic version for a simple coupled system presented by Symon[12] where only potential energy interactions are present shows that the **G** matrix is diagonal with the ith diagonal element being $1/m_i$ where m_i is the mass of the ith oscillator. The **F** matrix has both diagonal and off-diagonal elements. The ijth off-diagonal element contains the sum of all the interaction terms that apply between the ith and the jth oscillators. The computation of these interaction force constants is described below. Following Symon,[12] the ith diagonal element of the **F** matrix consists of k_i, the force constant of the ith oscillator, plus the sum of all the k_{ij}'s where $j \neq i$, i.e., the off-

(8) Miyazawa, T. *J. Chem. Phys.* **1960**, *32*, 1647−1652.
(9) Torii, H.; Tasumi, M. *J. Chem. Phys.* **1992**, *96*, 3379−3387.
(10) Torii, H.; Tasumi, M. In *Infrared Spectroscopy of Biomolecules*; Mantsch, H. H., Chapman, D., Eds.; Wiley-Liss; New York, 1996; Chapter 1.
(11) Krimm, S.; Bandekar, J. *Adv. Protein Chem.* **1986**, *38*, 181−364. Krimm, S.; Abe, Y. *Proc. Natl. Acad. Sci. U.S.A.* **1972**, *69*, 2788−2792. Moore, W. H.; Krimm, S. *Proc. Natl. Acad. Sci. U.S.A.* **1975**, *72*, 4933−4935.
(12) Symon, K. R. *Mechanics*; Addison-Wesley: Reading; 1960; p 188.
(13) Wilson, E. B., Jr.; Decius, J. C.; Cross, P. C. *Molecular Vibrations*; McGraw-Hill Book Co., Inc; New York; 1955.

diagonal interaction force constants in the *i*th row. The first effect of coupling is thus to change the frequency of the independent motion of a given oscillator.

Transition Dipole Coupling

It is shown here how through space electrical interaction between a collection of oscillating dipoles can be represented in terms of force constants in an **F** matrix. Even though the dipoles are time harmonic they can be treated as quasi-static if the wavelength of their radiation is much greater than both their physical size and the range of their effects.[14] The potential energy of a collection of static electric dipoles, *V*, is the sum of all the pairwise interaction energies W_{ij} where

$$W_{ij} = \mathbf{p}_j \cdot \nabla \Phi_i$$

and $\mathbf{p}_j$ is electric dipole moment of the *j*th oscillator and $\nabla\Phi_i$ is the gradient of the electric potential of dipole *i* at the location of dipole *j*. Dipole *i* of moment **p** can be located between $+a/2$ and $-a/2$ along a local *z* axis and its potential expanded in the axial multipole expansion[14] as

$$\Phi(r,\theta) = pP_1(\cos\theta)/r^2 + pa^2P_3(\cos\theta)/4r^4 + ...$$

where P_i is the *i*th Legendre polynomial, and *r* and θ are spherical polar coordinates. The point dipole approximation that is generally used results from retaining only the first term. This gives

$$W_{ij} = \mathbf{p}_i \cdot \mathbf{p}_j / {r_{ij}}^3 - 3(\mathbf{p}_i \cdot \mathbf{r}_{ij})(\mathbf{p}_j \cdot \mathbf{r}_{ij})/{r_{ij}}^5$$

This is a good approximation when $a/r \ll 1$. But for nearest or next nearest neighbor peptide groups in a polypeptide this condition may not be met. Therefore the first two terms in the expansion are retained as a refinement of the point dipole approximation. For axial symmetry in spherical polar coordinates the gradient operator is:

$$\nabla = \mathbf{i}_r\, \partial/\partial r + \mathbf{i}_\theta (1/r)\, \partial/\partial\theta$$

Then,

$$W_{ij} = \mathbf{p}_i \cdot [\mathbf{i}_r(-2p_j\cos(\theta)/{r_{ij}}^3 - p_j a^2(5\cos^3(\theta) - 3\cos(\theta))/2r^5) + \mathbf{i}_\theta(-p_j\sin(\theta)/r^3 + p_j a^2(-15\cos^2(\theta)\sin(\theta) + 3\sin(\theta))/8r^5)]$$

The force constant for this interaction $F_{ij} = (\partial^2 V/\partial q_i\, \partial q_j)_0 = (\partial^2 W_{ij}/\partial q_i\, \partial q_j)_0$, where q_i is the normal coordinate for the mode of interest of the *i*th oscillator and the derivative is evaluated at the equilibrium values of the normal coordinates. On substituting the expression for W_{ij}

$$F_{ij} = (\partial p_i/\partial q_i)(\partial p_j/\partial q_j)_0\, \mathbf{u}_i \cdot [\mathbf{i}_r(-2\cos(\theta)/r^3 - a^2(5\cos^3(\theta) - 3\cos(\theta))/2r^5) + \mathbf{i}_\theta(-\sin(\theta)/r^3 + a^2(-15\cos^2(\theta)\sin(\theta) + 3\sin(\theta))/8r^5)]$$

where $\mathbf{u}_i$ is a unit vector along $(\partial p_i/\partial q_i)_0$. The derivative of the electric dipole moment of a polar group with respect to the normal coordinate for a given mode is its transition dipole moment for that mode. Since the force constants which determine in part the splitting pattern of the coupled oscillators depend on the transition moments, this effect is called transition dipole coupling (TDC).

Through-Bond Interactions

Ab initio formalism does not exist at present for calculating force constants representing the interactions between peptide groups separated by an intervening α-carbon atom or between H-bonded groups for peptide systems as large as those considered here. Therefore these values were adjusted to a certain fraction of the force constant of an oscillator so as to give the best fit between the calculated and experimental amide I contours. We note, however, that Bour and Keiderling[15] have presented ab initio quantum mechanical calculations of dipole and through-bond interactions to explicitly calculate the vibrational circular dichroism of a dipeptide. They too observe the necessity of including both types of interaction, even for this relatively small system.

Spectral Simulations

The peptides in this study were known from solution CD and IR data to be either α-helices or antiparallel *β*-sheets. To locate the relative positions of the transition dipoles, so that the coupling between them can be treated quantitatively, the Cartesian coordinates of the N, C′, and O atoms in each peptide group were calculated from data given in Fraser and MacRae[16] for a standard α-helix or the *β*-form of poly(L-alanine). For *β*-sheets, intermolecular antiparallel forms were modeled. A unit vector representing the transition dipole was placed at the C′ atom of each group extending out along the C′−O bond. The vector was then rotated 20° off the C═O bond toward the C_α atom in the NC═O plane, as other analyses[9,11] have assumed. This sets up the spatial relationship between the transition dipoles. The magnitude of the transition dipole moment was set empirically at 300 $cm^{3/2}/s$ (3.86 D/(A $amu^{1/2}$)). The corresponding magnitude for a ^{13}C group was set to 319 $cm^{3/2}/s$ (4.10 D/(A $amu^{1/2}$)). These values are close to that of 3.70 D/(A $amu^{1/2}$) found appropriate by Torii.[9] In computing the TDC contribution to an interaction force constant, the dielectric constant of the intervening matter was taken as $r^{1/2}$. The physical size of the transition dipole was taken as 2.2 Å, the distance between the N and O atoms in the peptide group.

The force constant of an unsubstituted oscillator divided by its mass was set to $\lambda_0 = 4\pi^2c^2\nu_0^2$ where the unperturbed frequency ν_0 was empirically adjusted to 1678 cm^{-1} for a *β* structure and 1690 cm^{-1} for an α-helix. The same force constant was used for both substituted and unsubstituted oscillators. The mass ratio of an unsubstituted to a substituted oscillator was set equal to 0.951. For antiparallel *β* forms the best value for the through-hydrogen bond force constant was −0.022 times the force constant of an oscillator, while the best value of the through-valence bond force constant was found to be +0.013 times the force constant. The corresponding factors for the α form were −0.01 and +0.005, respectively.

The *n* by *n* (*n* = total number of peptide groups in both strands of the simulated *β*-sheet or in the α-helix) **G** and **F** matrixes were set up in Mathcad 8; the eigenvalues, $\lambda_i = 4\pi^2c^2\nu_i^2$, and eigenvector matrix, **L**, of the **GF** matrix were then computed. The eigenvalues give the frequencies of the normal modes in the collective system of oscillators. The elements in the *i*th row of the $\mathbf{L}^{-1}$ matrix give the weighting factor for the

(14) Eyges, L. *The Classical Magnetic Field*; Dover Publications: New York, 1972.

(15) Bour, P.; Keiderling, T. A. *J. Am. Chem. Soc.* **1993**, *115*, 9602−9607.

(16) Fraser, R. D. B.; MacRae, T. P. *Conformation of Fibrous Proteins*; Academic Press: New York, 1973.

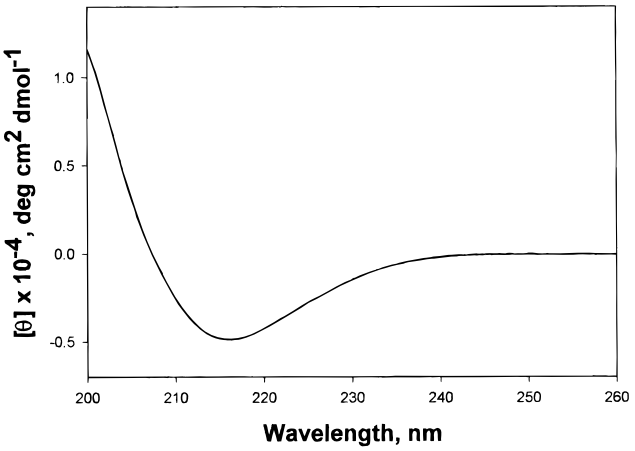

Figure 1. CD spectrum of a 1 mg/mL solution of $K_2LA^*(LA)_5$ in methanol. The asterisk after the single letter code indicates the position of the ^{13}C group. Spectra have been smoothed with a spline function.

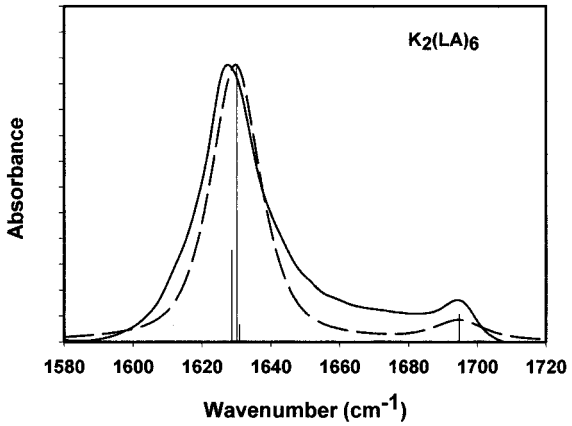

Figure 2. A comparison of experimental and simulated spectra of the amide I region (1580−1720 cm^{-1}) for $K_2(LA)_6$. The intensities of the components calculated from the model are represented by vertical lines at the corresponding frequencies. The dashed-line spectrum is generated by representing each component as a 30/70 Gaussian/Lorentzian function with a half width (w) of 8 cm^{-1}. The solid line is the experimental spectrum.

vector contribution of each local transition dipole to the net transition dipole for the ith mode. This is based on the equation $\mathbf{L}^{-1}\mathbf{R} = \mathbf{Q}$ (ref 13) where **R** is a column vector of internal coordinates and **Q** is a column vector of normal coordinates. The square of the length of the net transition dipole for each mode was taken as the intensity for that mode.

Results

(1) Conformation of Synthetic Peptides. The CD spectrum of $K_2(LA\text{-}^{13}C)(LA)_5$ in MeOH solution is shown in Figure 1 and the IR spectrum of unlabeled $K_2(LA)_6$ is shown in Figure 2. The IR spectra were taken from 5-fold more concentrated solutions. Both spectra are consistent with the presence of a single secondary structure, namely the antiparallel β-sheet form. IR spectra are particularly diagnostic for this secondary structure and show the characteristic components of the split amide I mode at 1628 (strong) and 1694 (weak) cm^{-1}. The IR spectra in the amide I region of $K_2(LA)_{10}$ in a lipidic environment reveal (Figure 7) a single intense feature at 1657 cm^{-1} characteristic of an α-helix. Spectra of this molecule in 2-chloroethanol (data not shown) indicate populations of additional conformers.

(2) β-Sheet Peptides: Experimental and Simulated Spectra for Normal and Isotopically Substituted $K_2(LA)_6$. The

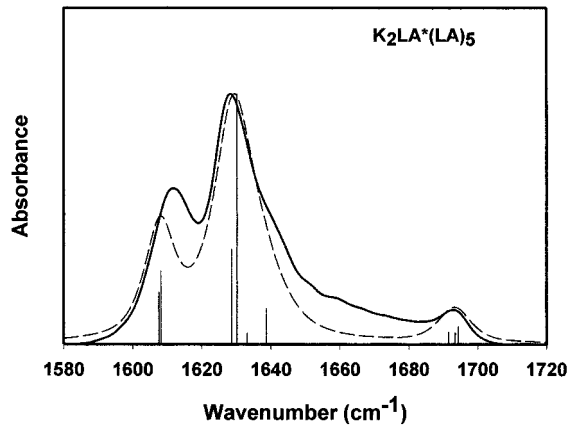

Figure 3. A comparison of experimental and simulated spectra of the amide I region (1580−1720 cm^{-1}) for $K_2LA^*(LA)_5$. The intensities of the components calculated from the model are represented by vertical lines at the corresponding frequencies. The dashed-line spectrum is generated by representing each component as a 30/70 Gaussian/Lorentzian function with w = (5, 5, 5, 8, 8, 8, 5, 5, 5) cm^{-1}, respectively. The solid line is the experimental spectrum.

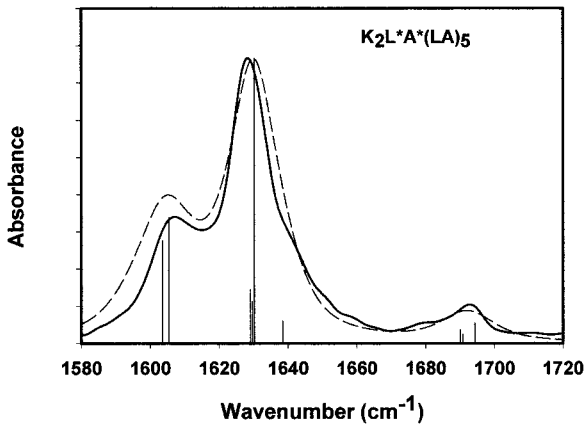

Figure 4. A comparison of experimental and simulated spectra of the amide I region (1580−1720 cm^{-1}) for $K_2L^*A^*(LA)_5$ in methanol. The intensities of the components calculated from the model are represented by vertical lines at the corresponding frequencies. The dashed-line spectrum is generated by representing each component as a 30/70 Gaussian/Lorentzian function with w = (10, 10, 8, 8, 8, 8, 8, 8, 8) cm^{-1}, respectively. The solid line is the experimental spectrum.

experimental and simulated IR spectra in the amide I spectral region for $K_2(LA)_6$ (peptide I), $K_2(LA\text{-}^{13}C)(LA)_5$ (peptide II), $K_2(L\text{-}^{13}C,A\text{-}^{13}C)(LA)_5$ (peptide III), and $K_2(LA\text{-}^{13}C)(LA\text{-}^{13}C)(LA)_4$ (peptide IV), where all the ^{13}C substitutions are in the C═O bond, are shown in Figures 2−5, respectively. ^{13}C substitution induces remarkable changes in the spectra. Peptide II, the singly substituted derivative, displays a fairly intense peak near 1611 cm^{-1} with an intensity about 17% of the whole contour. This is substantially greater than that expected from an isolated oscillator, which constitutes about 7% (1/14th) of the population. The disubstituted derivative with two adjacent labels, peptide III, shows a spectrum quite similar to peptide I (single label), with a slightly broadened feature at 1606 cm^{-1}. For both peptides II and III, the peaks characteristic of the unsubstituted antiparallel β-sheet, at 1693 and 1629 cm^{-1}, are present. The most dramatic spectral alteration is noted for peptide IV, the disubstituted derivative with ^{13}C substituents separated by a single ^{12}C residue. Anomalously large intensity is seen in a low-frequency mode at 1609 cm^{-1} that occupies about 30% of the amide I contour. Thus, shifting the position of the second label by a single residue has almost doubled the

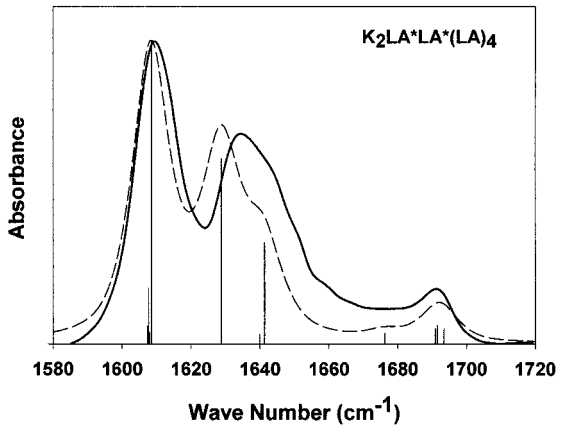

Figure 5. A comparison of experimental and simulated spectra of the amide I region (1580−1720 cm^{-1}) for $K_2LA^*LA^*(LA)_4$ The intensities of the components calculated from the model are represented by vertical lines at the corresponding frequencies. The dashed-line spectrum is generated by representing each component as a function with $w = (6, 6, 6, 6, 7, 6, 6, 5, 6)$ cm^{-1}, respectively. The solid line is the experimental spectrum.

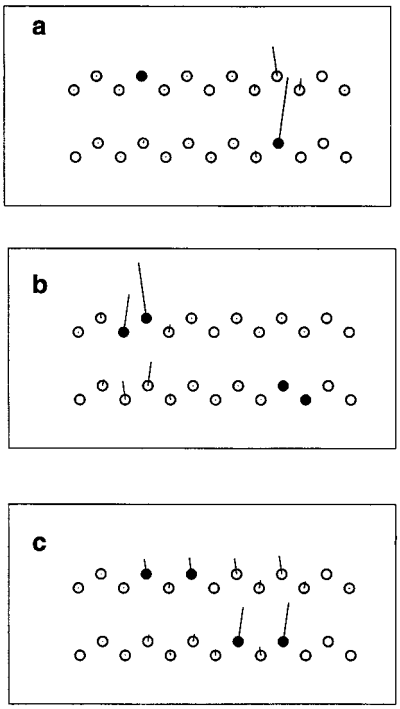

Figure 6. Graphical depiction of the relative activity of the transition dipoles in the antiparallel β-sheet for the ~1611 cm^{-1} band: (a) mode responsible for 60% of the intensity for $K_2LA^*(LA)_5$; (b) mode responsible for 55% of the intensity for $K_2L^*A^*(LA)_5$; and (c) mode responsible for 78% of the intensity for $K_2LA^*LA^*(LA)_4$. Isotopically substituted peptide groups are denoted by a filled circle.

intensity of the new spectral feature (compare Figures 4 and 5). In addition, the primary β-sheet marker band, now at 1634 cm^{-1}, exhibits shoulders on the high-frequency side.

The semiempirical model described above closely reproduces the main features of the amide I region of the spectra of peptides I−IV. A single set of parameters (noted above) was used for the collection of the β-sheet peptides. The calculated spectra are presented in two ways in each of Figures 2−5. First the calculated peaks and their relative intensities are depicted as vertical lines with heights proportional to their relative intensities at the calculated frequencies. Next, the lines are broadened by a 30% Gaussian−70% Lorentzian function with the widths

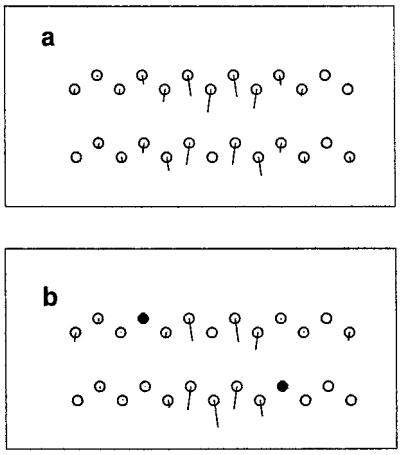

Figure 7. Graphical depiction of the relative activity of the transition dipoles in the antiparallel β-sheet that contribute to the ~1630 cm^{-1} band: (a) mode responsible for 71% of the intensity for $K_2(LA)_6$ and (b) mode responsible for 75% of the intensity for $K_2LA^*(LA)_5$. Isotopically substituted peptide groups are denoted by a filled circle.

(indicated in the figure captions) selected to best match the overall contour in the experimental spectra. The agreement between the observed and simulated spectra is quite good. The qualitative features of the unsubstituted and ^{13}C-substituted peptides are accounted for and the relative intensities of the observed amide I features are closely maintained.

(3) Transition Dipole Activity in Normal and Isotopically Substituted β-Sheets. The origin of the low-frequency peak (1606−1611 cm^{-1}) for the substituted peptides II−IV is clarified by examining the contribution of each peptide group to the modes in this region as shown in Figure 6a−c. The relative contributions are indicated by the length of the line emanating from circles which represent the oscillators which in turn represent the peptide groups in this highly schematic version of the molecule. Isotopically substituted groups are marked by filled circles. Only the most intense modes are shown.

The excitation pattern for the substituted peptides II−IV demonstrates that the low-frequency bands are the result of combined ^{13}C group and ^{12}C group motions. In peptide II and peptide III, the ^{13}C group excitation migrates to the opposite ^{12}C group via the intervening H-bond and propagates to neighboring ^{12}C groups, albeit at a reduced level, via connecting valence bonds. The ^{13}C modes pick up substantial intensity from sympathetically excited ^{12}C groups thus explaining the "anomalous" intensity of the ^{13}C band. The transition dipole activity pattern of peptide IV (Figure 6c) shows that spacing the ^{13}C groups permits the through-bond interactions to involve even more ^{12}C groups so that its resultant intensity dominates the spectrum.

On comparing the patterns in the unsubstituted and singly substituted β-sheets it is clear that substitution truncates the coupled region reducing the intensity of the 1630 cm^{-1} band ascribed to the ^{12}C oscillators (Figure 7).

(4) α-Helical Peptides: Experimental and Simulated Spectra for Normal and Isotopically Substituted $K_2(LA)_{10}$. Experimental and simulated IR spectra in the amide I spectral region for α-helical $K_2(LA)_{10}$ (peptide V) and $K_2(LA)_4(LA\text{-}^{13}C)(LA)_5$ (peptide VI) are shown in Figures 8 and 9. The unlabeled helix spectrum (Figure 8) is dominated by the anticipated α-helical peak at 1656 cm^{-1}. In addition to the 1656 cm^{-1} band, the singly substituted α-helix spectrum (Figure 9) introduces a weak feature at 1618 cm^{-1}. The intensity of this band is ~7% of the integrated fractional intensity of the contour.

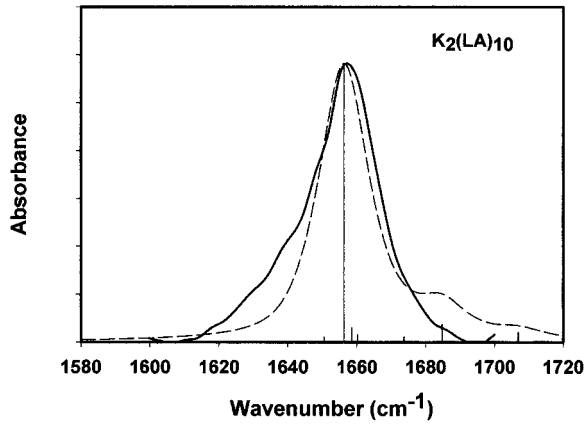

Figure 8. A comparison of experimental (in vesicles with 1,2-dipalmitoylphosphatidylcholine) and simulated spectra of the amide I region (1580−1720 cm^{-1}) for $K_2(LA)_{10}$. The intensities of the components calculated from the model are represented by vertical lines at the corresponding frequencies. The dashed-line spectrum is generated by representing each component as a 30/70 Gaussian/Lorentzian function with a halfwidth of 8 cm^{-1}. The solid line is the experimental spectrum.

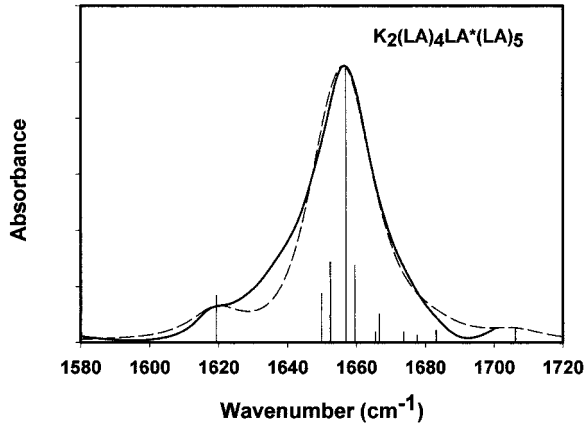

Figure 9. A comparison of experimental (in vesicles with 1,2-dipalmitoylphosphatidylcholine) and simulated spectra of the amide I region (1580−1720 cm^{-1}) for $K_2(LA)_4LA^*(LA)_5$. The intensities of the components calculated from the model are represented by vertical lines at the corresponding frequencies. The dashed-line spectrum is generated by representing each component as a 30/70 Gaussian/Lorentzian function with a halfwidth of 8 cm^{-1}. The solid line is the experimental spectrum.

In the α-helix, the ^{13}C substitution produces a downward shift of 36 cm^{-1}, which is close to the calculated isolated C═O stretching shift of 37 cm^{-1} . The intensity is approximately that predicted if the ^{13}C group were an isolated oscillator.

(5) Transition Dipole Activity in the α-Helix Peptides. The contributions of the peptide groups to the mode at 1618 cm^{-1} (Figure 10) show that the substituted group is the prime contributor to this frequency with minor contributions from the ^{12}C groups H bonded to it.

Discussion

The current model is the logical extension of the work of Miyazawa[8] and Krimm[11] and their respective co-workers. The current model incorporates several features not previously utilized. First, the finite size of the transition dipoles is allowed for instead of assuming the point dipole approximation. Second, through-bond interactions as well as transition-dipole coupling have been included in the analysis. Third, the diagonal force constants in the **F** matrix are modified by adding in the interaction force constants in the same row as indicated by the analysis of Symon.[12] This was not done in previous treatments.

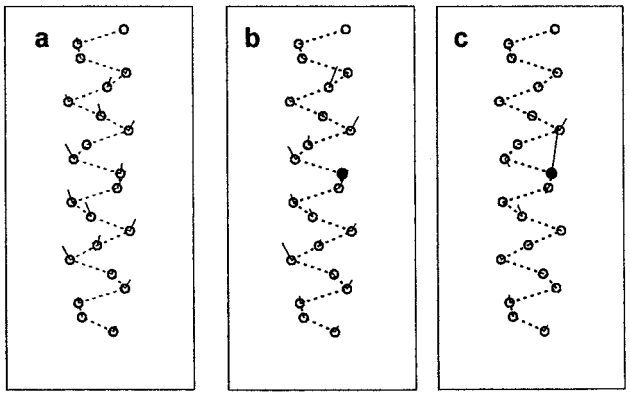

Figure 10. Graphical depiction of the relative transition dipole activity in an α-helix that contributes to the 1656 and 1617 cm^{-1} bands: (a) mode responsible for 100% of the intensity of the 1656 band in $K_2(LA)_{10}$; (b) mode responsible for 80% of the intensity of the 1656 band in $K_2(LA)_4LA^*(LA)_5$; and (c) mode responsible for 100% of the intensity of the 1617 band in the $K_2(LA)_4LA^*(LA)_5$. The dashed lines connect adjacent carbon atoms in each case.

The use of isotopic labeling severely constrains the range of parameter space that provides a reasonable fit to the data. The use of four β-sheet peptides, including three different patterns of ^{13}C peptide-bond substitution, provides a severe test for the formalism. The agreement between the simulated and experimental spectra in Figures 3−6 is excellent. Particularly noteworthy is the replication of the spectral changes observed for the two disubstituted derivatives, peptide III (consecutive substitution) and peptide IV (substitution of two ^{13}C groups, separated by a ^{12}C group), which have very different spectra (compare Figures 4 and 5). Changes in any of the parameters by more than a few percent result in much poorer overall agreement for the entire set of spectra.

The introduction of both transition dipole coupling and through-bond (valence and H bond) interactions requires some justification. Miyazawa's[8] original formalism utilized symmetry analysis and through-bond interactions to explain the observed splittings for the amide I β-sheet (both parallel and antiparallel) structure. The intrachain and interchain interaction constants are then determined from the observed IR and Raman data. Although the predictive value of this approach is limited, the utility of the amide I region for secondary structure analysis, especially for the identification of β-sheets, was clearly demonstrated. Krimm and co-workers[11] ambitiously extended the above approach through complete normal coordinate analysis of polypeptides of regular secondary structure. To fully explain several features of modes arising from peptide bonds, they introduced transition dipole (through space) coupling, assuming the point dipole approximation, to add to the observed normal coordinate analysis. In a recent extension of this approach, Torii and Tasumi[9,10] attempted to calculate the amide I contours for a full set of globular proteins. They stated that "valence bond and H-bond interactions play only a minor role in coupling the amide I motions of different peptide groups", and used a TDC-based mechanism to obtain fair agreement between experimental and simulated contours. On the other hand, they found it necessary to raise the diagonal force constants (DFCs, in their notation) by 1.6% for some peptide groups that have the C═O bonds pointing toward the inside of β-sheets and for those belonging to some α-helices. They state that the "physical significance of such variation in DFCs is not clear", but better

agreement with experiment evidently results by such procedures. In the current work, we choose to assume the presence of direct through-bond interactions as a plausible mechanism for vibrational coupling between peptide groups. Thus, to obtain reasonable agreement between the calculated and experimental spectra, we do not have to arbitrarily alter the diagonal force constants.

It is of some interest to compare the values of the through hydrogen bond and through valence bond interaction constants found empirically by Miyazawa and Blout[17] for α-helices and β-sheets to the values used in this study. There is correspondence in that both studies found the through H-bond term to be negative while the through valence bond term is positive, and in addition the former has about twice the magnitude of the latter. Also the magnitudes of these terms are a small fraction of the unperturbed frequency or force constant, respectively. Later studies[16] found a range of values and relative magnitudes for these interaction terms for the β-sheet but the sign difference persists. This study finds the through H-bond interaction to be weaker in the α-helix compared to the β-sheet which may be accounted for in part by an N···O distance about 0.034 Å longer in the former. Also the H-bonding pattern is different in the two cases.

The primary motivation for the current study is to provide a more quantitative analysis of the amide I spectra of ^{13}C isotopically labeled spectra for eventual biochemical applications. Lansbury and co-workers[2] showed that the substitution of a single peptide bond in a β-sheet sequence with ^{13}C produces a low-frequency component in the spectrum whose intensity depends on the position of the substituent. They assigned that component (which ranged in frequency between 1604 and 1611 cm^{-1}) to ^{13}C amide I. The current work indicates that such an assignment is only partially correct and that the mode in question involves contributions from the labeled moiety and neighboring unlabeled groups. The anomalously high intensity of the mode is a manifestation of unexpected coupling. Further, the relationship suggested in ref 2 between spectral changes and the formation of cross-fibrillar structures must be considered unproven.

The biological utility of the current calculations will be manifest in systems where the induction of β structure (either in proteins or in peptides bound to proteins) is anticipated. Using isotopic labels, it will be possible to determine specific sites where such structures form. The current work suggests that the optimal experimental strategy would be to synthesize peptides with a labeling pattern such as in K_2(LA-^{13}C) (LA-^{13}C)$(LA)_4$ (peptide IV), with a ^{12}C residue between two labeled groups. This would be expected to produce the largest perturbation in the spectra.

In contrast to β-sheets, α-helices show the intensity patterns for their isotopically substituted analogues expected on the basis of an isolated oscillator. The presence of a new band for a singly substituted derivative therefore produces a weak spectral feature, which would likely be buried under other peaks in experimental situations where labeled peptides are bound to proteins.

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(17) Miyazawa, T.; Blout, E. R. *J. Am. Chem. Soc.* **1961**, *83*, 712−719.