

Conformational analysis of tetrapeptides by exploiting the excitonic coupling between amide I modes

Qing Huang¹ and Reinhard Schweitzer-Stenner^{2*}

¹ Department of Chemistry, University of Puerto Rico, Río Piedras Campus, P.O. Box 23346, San Juan, Puerto Rico 00931, USA

² Department of Chemistry, Drexel University, 4141 Chestnut Street, Philadelphia, Pennsylvania 19104, USA

Received 23 December 2003; Accepted 13 March 2004

The amide I band of the IR and to a lesser extent also of the corresponding visible Raman spectra of peptides and proteins are frequently used to determine their secondary structure composition. Thus far, however, this analytical approach is generally a low-resolution technique, particularly because it mostly discriminates only between α -helical, β -sheet (parallel and antiparallel) and so-called random coil conformations. This study shows that for tetrapeptides the combined use of the IR and Raman amide I band profiles allows one to discriminate currently known secondary structure motifs. To exploit the spectral information to its fullest extent, we developed an algorithm which calculates the amide I intensity profiles of IR, isotropic and anisotropic Raman scattering and also the depolarization ratios of the Raman bands as a function of the dihedral angles of the two central amino acid residues. The approach is based on a quantum mechanical treatment of the vibrational coupling between the amide I modes of the three peptide groups in the framework of a coupled oscillator model. We calculated the band profiles of a representative set of secondary structures, i.e. a right-handed α -helix, a 3_{10} -helix, β -sheets and a polyproline II (PPII)-type 3_1 -helix and β -turns. Our results unambiguously show that all these secondary structure motifs can be identified by comparing experimentally observed IR and Raman amide I bands with their respective calculated intensity distributions. Copyright © 2004 John Wiley & Sons, Ltd.

KEYWORDS: peptide structure analysis; tetrapeptides; excitonic coupling; amide I; Raman and IR spectroscopy

INTRODUCTION

The wavenumber position and profile of the amide I band in the IR and Raman spectra of peptides and proteins is often used to determine their secondary structure composition.^{1–4} The corresponding normal mode of the peptide linkage is predominately a CO stretching vibration with some admixture from NH in-plane bending, CN stretching and C α (C)H in-plane bending.^{1,5} Amide I modes of adjacent peptide groups can interact by through-bond and through-space coupling.^{1,6,7} This gives rise to delocalized, excitonic vibrational states. The respective excitonic coupling strength depends on the distance between the interacting transition dipole moments and on the dihedral angles ϕ and ψ ,¹ which are the key determinants of the backbone structure of peptides and proteins (Fig. 1). Torii and Tasumi⁶ calculated the excitonic coupling strength for the interaction between

adjacent amide I modes of triglycine *in vacuo* as a function of the central dihedral angles. They found that irrespective of the secondary structure, through-space and through-bond coupling contribute nearly equally to the total coupling strength. The authors also calculated the coupling strength for second nearest amide I modes in a regular peptide and found it to be mainly determined by a through-space transition dipole coupling (TDC) mechanism.

The relation between coupling strength and dihedral angles obtained by Torii and Tasumi⁶ can be utilized for determining the secondary structure of peptides. Woutersen and Hamm,^{8,9} for instance, carried out two-dimensional femtosecond IR measurements to obtain directly the excitonic coupling strength for the two interacting amide I' modes of cationic trialanine in D₂O and the orientational angle between their transition dipole moments. Subsequently, they used the orientational dependence of these parameters to determine the dihedral angles of the central residue, i.e. $(\phi, \psi) \approx (-60^\circ, 140^\circ)$. This is close to the values for a canonical polyproline II (PPII) conformation. Schweitzer-Stenner *et al.*¹⁰ obtained very similar values for this peptide by exploiting the isotropic Raman scattering of amide I' to

*Correspondence to: Reinhard Schweitzer-Stenner, Department of Chemistry, Drexel University, 4141 Chestnut Street, Philadelphia, Pennsylvania 19104, USA. E-mail: rschweitzer-stenner@drexel.edu
tele: 215 895 2268, fax: 215 895 1265
Contract/grant sponsor: NIH; Contract/grant number: P20 RR16439-01.

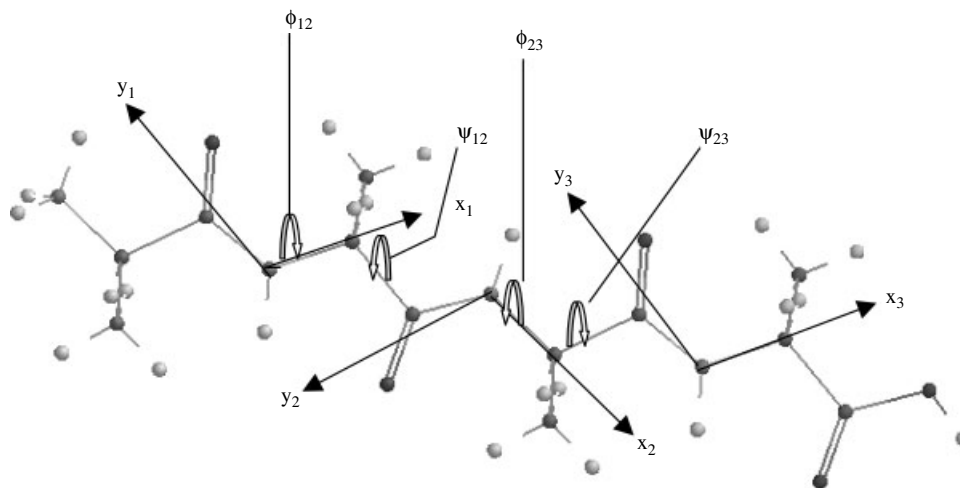


Figure 1. Planar structure of tetraalanine ($\phi = 180^\circ$, $\psi = 180^\circ$). The coordinate systems $S_1(x_1, y_1, z_1)$, $S_2(x_2, y_2, z_2)$ and $S_3(x_3, y_3, z_3)$ were used to express the Raman tensors of the individual, uncoupled amide I modes and their transition dipole moments (the z-components have been omitted for the sake of clarity; they all point out of the drawing plane). The structure was obtained by using the program TITAN from Wavefunction.

obtain the coupling strength and the corresponding IR band to derive the angle between the transition dipole moments. Thus, they obtained similar values to Woutersen and Hamm⁸ and, as a consequence, also a similar conformation for the central residue by utilizing the results of Torii and Tasumi.⁶ Moreover, they found that this conformation is only weakly affected by the protonation of the terminal residues. An extended approach for the structure analysis of tripeptides was reported in a subsequent paper, which was based entirely on spectroscopic data in that anisotropic Raman scattering of amide I was used to determine additionally the orientational angle between the peptides' normals.¹¹ The two orientational angles derived from IR and anisotropic Raman spectra were eventually employed to determine the corresponding dihedral angles. The result of this analysis was checked by measuring and analyzing the vibrational and electronic circular dichroism spectra of the peptide investigated.^{12,13} The structures of a variety of tripeptides in water have been determined by this protocol.^{12,14–16}

In this study, we extended the above algorithm to explore the relationship between the secondary structure of tetrapeptides and the IR and Raman band profiles of amide I. We calculated Raman and IR intensities for a set of typical secondary structure motifs, i.e. right α -helix, 3_{10} -helix, β -sheets, PPII helix and β -turns I and II, by utilizing the coupling strength values reported by Torii and Tasumi.⁶ Our results clearly showed that all these conformations can be distinguished by determining the fractional intensities of the individual bands in the respective IR, isotropic and anisotropic amide I profile and the respective Raman depolarization ratios. We thus demonstrated that vibrational spectroscopy is a much more sensitive tool for secondary structure analysis than generally assumed. The results of this work can be used to identify the structure of tetrapeptides just

by comparing experimental band profiles with the reported theoretical distributions. Hence it should be particularly possible to check whether tetrapeptides can adopt turn structures as predicted by molecular dynamics calculations.¹⁷ A more quantitative structure analysis of tetraalanine is reported in a separate publication.¹⁸

THEORY

Figure 1 schematically displays the structure of a tetrapeptide (tetraalanine). In what follows, the excitonic states of amide I are evaluated in terms of the indicated central dihedral angles (ϕ_{12} , ψ_{12}) and (ϕ_{23} , ψ_{23}).¹⁸ We assumed that the distance between the interacting amide vibrations is large enough to treat them as separated, coupled oscillators rather than part of a very delocalized normal mode.¹⁹ This notion was recently corroborated by *ab initio* calculations on tri- and higher order peptides.⁷ The excitonic state of the three coupled amide I oscillators can be obtained by solving the Schrödinger equation:

$$\hat{H}|\chi\rangle = (\hat{H}_0 + \hat{H}')|\chi\rangle \quad (1)$$

where \hat{H}_0 is the Hamiltonian for the uncoupled amide I modes and \hat{H}' accounts for excitonic coupling. $|\chi\rangle = |\chi_1, \chi_2, \chi_3\rangle$ is the state vector for the vibrational states of the uncoupled oscillators. The Hamiltonian for the excitonic states in tetrapeptides is expressed in the matrix representation as

$$H = \begin{pmatrix} \nu_1 & \Delta_{12} & \Delta_{13} \\ \Delta_{21} & \nu_2 & \Delta_{23} \\ \Delta_{31} & \Delta_{23} & \nu_3 \end{pmatrix} \quad (2)$$

where ν_i denote the wavenumbers of the uncoupled amide I modes, and $\Delta_{ij} = \Delta_{ji}$ ($i, j = 1, 2, 3$) are the coupling energies

expressed in units of cm^{-1} . The eigenstates of H can be obtained by diagonalizing Eqn. (2). In general terms, they are written as

$$|\tilde{\chi}_i\rangle = a_{i1}|\chi_1\rangle + a_{i2}|\chi_2\rangle + a_{i3}|\chi_3\rangle \quad (3)$$

where a_{ij} ($i, j = 1, 2, 3$) are mixing parameters with $\sum_i a_{ij}^2 = \sum_j a_{ij}^2 = 1$. Assuming that the three amide I bands of the uncoupled modes have identical Raman cross-sections and IR oscillator strengths, the mixing coefficients were used to calculate the respective intensities of the coupled modes. For the IR intensities of the coupled modes one derives

$$I_i^{\text{IR}} \sim |a_{i1}\tilde{\mu}_1 + a_{i2}\tilde{\mu}_2 + a_{i3}\tilde{\mu}_3|^2 \quad (4)$$

where $\tilde{\mu}_j$ ($j = 1, 2, 3$) is the vibrational electronic transition dipole moment of the j th amide I mode.

The Raman tensor of the excitonic states reads

$$\begin{aligned} \hat{\alpha}'_1 &= a_{11}\hat{\alpha}_1 + a_{12}\hat{\alpha}_2 + a_{13}\hat{\alpha}_3 \\ \hat{\alpha}'_2 &= a_{21}\hat{\alpha}_1 + a_{22}\hat{\alpha}_2 + a_{23}\hat{\alpha}_3 \\ \hat{\alpha}'_3 &= a_{31}\hat{\alpha}_1 + a_{32}\hat{\alpha}_2 + a_{33}\hat{\alpha}_3 \end{aligned} \quad (5)$$

where $\hat{\alpha}_1$, $\hat{\alpha}_2$ and $\hat{\alpha}_3$ are the amide I Raman tensors of the three peptide groups. In order to calculate $\hat{\alpha}'$, all $\hat{\alpha}_i$ have to be expressed with respect to the same coordinate system. Thus, the Raman tensors of the excitonic states become dependent on the mutual orientation of the peptide groups. As in our earlier studies, we selected the C-terminal peptide group as our reference system with the respective nitrogen atom as origin of the coordinate system. The x -axis of the coordinate system coincides with the NC_α bond, which is also the rotational axis for the dihedral angle ϕ (Fig. 1). The y -axis is (nearly) coplanar with the peptide group and z is the out-of-plane coordinate. The small deviation from co-planarity (1.1°) results from the fact that the NC_α bond is not exactly coplanar with the peptide plane.²⁰ We assigned $\hat{\alpha}_1$, $\hat{\alpha}_2$ and $\hat{\alpha}_3$ to the N-terminal, central and C-terminal peptide, respectively, and rotated first $\hat{\alpha}_1$ from the coordinate systems S_1 (N-terminal) into S_3 (C-terminal) by the following matrix operations:

$$\begin{aligned} \hat{\alpha}_1(S_2) &= R^T(\omega_{12})(R^T(\psi_{12})(R^T(\xi_{12})(R^T(\phi'_{12})) \\ &\quad \times \hat{\alpha}_1(S_1)R(\phi'_{12}))R(\xi_{12}))R(\psi_{12})R(\omega_{12}) \\ \hat{\alpha}_1(S_3) &= R^T(\omega_{23})(R^T(\psi_{23})(R^T(\xi_{23})(R^T(\phi'_{23})) \\ &\quad \times \hat{\alpha}_1(S_2)R(\phi'_{23}))R(\xi_{23}))R(\psi_{23})R(\omega_{23}) \end{aligned} \quad (6)$$

which can be understood as follows. First, S_1 has to be rotated by an angle $\phi'_{12} = \phi_{12} - \pi$. Subsequently, a rotation by ξ_{12} in the xy -plane is necessary so that the y -coordinate coincides with the C_αC bond, which is the rotational axis for ψ_{12} . ξ_{12} is the angle formed by the y_1 -axis and the C_αC bond. Next, the system is rotated by the dihedral angle

ψ_{12} . The fourth step involves the rotation by an angle ω_{12} , which is formed by the C_αC bond and the y_2 -axis. This rotation causes the x -axis to coincide with the NC_α bond. Subsequently, a similar sequence of rotations around the angles ϕ_{23} , ξ_{23} , ψ_{23} and ω_{23} has to be carried out to obtain $\hat{\alpha}_1$ in the coordinate system S_3 . The same transformation has to be carried out to obtain $\hat{\alpha}_2$ in S_3 . ω and ξ can be obtained from textbooks on peptide structure with 96° and 20° , respectively.²⁰

The tensors calculated by means of Eqns (5) and (6) can be used to calculate the isotropic and anisotropic scattering for the i th excitonic states:

$$\begin{aligned} \beta_{si}'^2 &= \frac{1}{9}(\text{Tr}\hat{\alpha}'_i)^2 \\ \gamma_{\text{aniso},i}'^2 &= \frac{1}{2}[(\alpha'_{xx,i} - \alpha'_{yy,i})^2 + (\alpha'_{yy,i} - \alpha'_{zz,i})^2 \\ &\quad + (\alpha'_{zz,i} - \alpha'_{xx,i})^2] \\ &\quad + \frac{3}{4}[(\alpha'_{xy,i} + \alpha'_{yx,i})^2 + (\alpha'_{yz,i} + \alpha'_{zy,i})^2 \\ &\quad + (\alpha'_{zx,i} + \alpha'_{xz,i})^2] \end{aligned} \quad (7)$$

Equation (7) can be used to calculate the intensity ratios of the amide I bands in the isotropic and anisotropic Raman spectrum of tetrapeptides:

$$R_{\text{iso},i'} = \beta_{s,i}'^2 / \beta_{s,i'}^2 \quad (8a)$$

$$R_{\text{aniso},i'} = \gamma_{\text{aniso},i'}^2 / \gamma_{\text{aniso},i'}^2 \quad (8b)$$

as function of the mixing parameters a_{ij} and the dihedral angles $\phi_{i'}$ and $\psi_{i'}$. The depolarization ratios can also be calculated as

$$\rho_i = \frac{3\gamma_i^2}{4\gamma_i^2 + 45\beta_i^2}$$

or

$$\rho_i^{-1} = \frac{4}{3} + 15 \frac{I_i^{\text{iso}}}{I_i^{\text{aniso}}}$$

RESULTS AND DISCUSSION

Concepts

The goal of this study was to determine the intensity distributions of amide I' for a tetrapeptide in various conformations resembling a set of classical secondary structure motifs. Table 1 lists the respective dihedral angles which we used for our calculations.

To calculate the amide I bands in IR and Raman spectra, we first obtained the coupling energies for the conformations in Table 1 by utilizing the results from *ab initio* calculations in triglycine reported by Torii and Tasumi.⁶ Ham and Cho²¹ have recently published somewhat different values for the alanine dipeptide. Since our experimental results for PPII and extended β -strand conformations^{10,15,18} are

Table 1. Dihedral angles of secondary structures²⁹ for which amide I IR and Raman intensity distributions were calculated (a)

	ϕ°	ψ°
Right-handed α -helix (RH)	-57	-47
Parallel β -sheet strand (PBS)	-119	113
Anti-parallel β -sheet strand (APBS)	-139	135
Right-handed 3_{10} -helix (3-10-H)	-49	-26
Left-handed PPII (PPII)	-79	150

(b)

β -Turn	$(\phi, \psi)_1$	$(\phi, \psi)_2$
Turn type I (BT1)	(-60, -30)	(-90, 0)
Turn type II (BT2)	(-60, 120)	(90, 0)

closer to those of Torrii and Tasumi, we decided to use their values throughout this study. Figure 2 depicts the contour plot, which illustrates the relationship between nearest neighbor coupling energy and the dihedral angles. In tetrapeptides, the strength of the coupling between amide I modes of second nearest peptide group, i.e. Δ_{13} in Eqn (7), must additionally be taken into consideration. It is predominantly governed by TDC. Table 2 lists the Δ_{12} and Δ_{13} values of the conformations for which we calculated the spectral distributions in the following. The coupling strength between the second nearest peptide group is apparently much smaller than that among the nearest group. The largest values were obtained for a 3_{10} -helix. Generally, the coupling between second nearest peptide groups can be even neglected for PPII and α -helices, but should be taken into account in β -sheet and 3_{10} -helix structures.

For our calculations we had to guess the wavenumbers of the uncoupled modes. The analysis of vibrational spectra of cationic and zwitterionic di- and tripeptides in D₂O revealed amide I wavenumbers around 1675 cm⁻¹ for the N-terminal peptide group (ν_1 in our notation).^{10,22} For the sake of simplicity and in close agreement with experimental results,¹⁸ we assumed an identical splitting Δ_0 between the uncoupled amide I modes, so that the wavenumbers of ν_2 and ν_3 are 1675 cm⁻¹ - Δ_0 and 1675 cm⁻¹ - 2 Δ_0 , respectively. Finally, we neglected the possibility that like amide III²³ the intrinsic frequency of amide I might be dependent on the dihedral angles owing to the admixture of C _{α} H in-plane bending, as suggested by theoretical calculations.¹⁹

In order to formulate the Raman tensor of the uncoupled amide I mode, we made use of the work of Pajcini *et al.*²⁴ for diglycine combined with some recent results obtained for trialanine.¹²

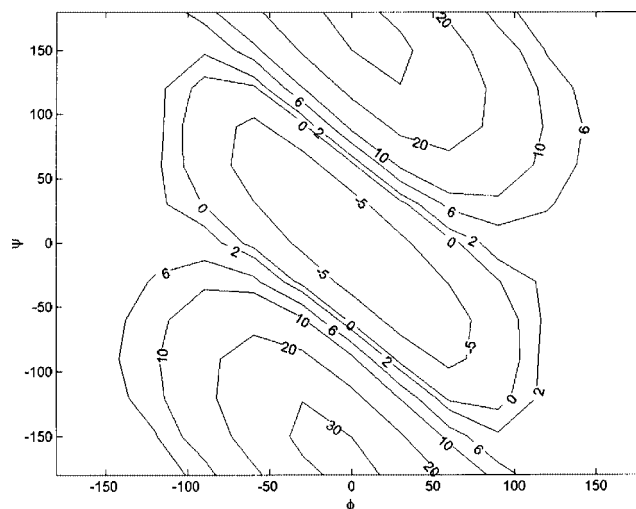


Figure 2. Contour plot illustrating the strength of the nearest neighbor excitonic coupling between amide I modes as a function of the dihedral angles ϕ and ψ of the residue between the interacting peptide groups (similar but not identical plots have been published earlier^{8,9,14}).

Table 2. Energies of excitonic coupling between amide I vibrations of nearest and second nearest peptide groups (courtesy of Drs H. Torrii and M. Tasumi; cf. Ref. 6)

	Δ_{12} cm ⁻¹	Δ_{23} cm ⁻¹	Δ_{13} cm ⁻¹
Right-handed α -helix	12.1	12.1	-1.5
Parallel β -strand	2.6	2.6	1.2
Anti-parallel β -strand	3.9	3.9	0.9
Right-handed 3_{10} -helix	3.1	3.1	-3.0
Left-handed PPII	4.5	4.5	-0.8
BT1	7.0	4.0	-0.1
BT2	-1.5	4.0	-0.1

As shown earlier, the Raman tensor of amide I' can be written with sufficient accuracy as¹⁵

$$\hat{\alpha}_1(S_1) = \begin{pmatrix} a & d & 0 \\ d & b & 0 \\ 0 & 0 & c \end{pmatrix} \quad (9)$$

Equation (9) is based on the assumption that the Raman cross-section stems mostly from vibronic coupling to electronic $\pi \rightarrow \pi$ transitions in the xy -plane of the peptide group.⁵ A weak z -component can be brought about either by coupling to the charge-transfer transitions $n_{\text{COO}} \rightarrow \pi_{\text{peptide}}$ and $\pi_{\text{COO}} \rightarrow \pi_{\text{peptide}}$ or to an electronic transition of the adjacent amino acid residue.^{22,24} The parameters in Eqn (9) are not independent. The tensor element d can be obtained as a function of a and b by rotating S_1 into the principal axis system, in which the Raman tensor is diagonal. For diglycine, Pajcini *et al.*²⁴ found that the major axis of the principal axis system forms an angle of 33.3° with the

peptide's carbonyl bond for visible excitation. The transformation between S_1 and the principal axis system is given by¹¹

$$\begin{pmatrix} \cos \beta & \sin \beta & 0 \\ -\sin \beta & \cos \beta & 0 \\ 0 & 0 & 1 \end{pmatrix} \begin{pmatrix} a & d & 0 \\ d & b & 0 \\ 0 & 0 & c \end{pmatrix} \times \begin{pmatrix} \cos \beta & -\sin \beta & 0 \\ \sin \beta & \cos \beta & 0 \\ 0 & 0 & 1 \end{pmatrix} = \begin{pmatrix} r_1 & 0 & 0 \\ 0 & r_2 & 0 \\ 0 & 0 & r_3 \end{pmatrix} \quad (10)$$

where β is the angle between the x -axis in the principal axis system and $x_1(S_1)$. From the fixed geometry of the peptide linkage we obtained $\beta = 96^\circ$. Hence one obtains from Eqn (10)

$$\begin{aligned} a &= r_1 \cos^2 \beta + r_2 \sin^2 \beta \approx 0.01r_1 + 0.99r_2 \\ b &= r_1 \sin^2 \beta + r_2 \cos^2 \beta \approx 0.99r_1 + 0.01r_2 \\ c &= r_3 \\ d &= 0.5 \tan(2\beta)(a - b) = 0.1(a - b) \end{aligned} \quad (11)$$

Referring to the values $r_1 = 1$, $r_2 = 0.3$ and $r_3 = -0.02$ obtained from Pajcini *et al.*,²⁴ we obtained relative values $a = 0.31$, $b = 1.0$, $c = -0.02$ and $d = -0.07$. These values were used in our simulation. (It should be noted that the peptide plane is assigned to xz rather than to xy as in the paper of Pajcini *et al.*²⁴)

Calculation of intensity distributions

Plate 1 depicts a histogram representation of the amide I intensity distributions calculated for the above secondary structure conformations. For the calculations we assumed that $\Delta_0 = 15 \text{ cm}^{-1}$, so that unperturbed modes $\nu_1 = 1675 \text{ cm}^{-1}$, $\nu_2 = 1660 \text{ cm}^{-1}$ and $\nu_3 = 1645 \text{ cm}^{-1}$. This is close to what was observed experimentally for tetraalanine.¹⁸ Plate 1(a) displays panels exhibiting the distributions of IR, Raman isotropic and anisotropic scattering amide I intensities. Plate 1(b) shows the depolarization ratios (DPRs) of the three amide I bands for the conformations investigated. Apparently, different secondary structures give rise to significantly different intensity and depolarization ratio distributions. Some conformations can be identified solely by just using one of the distributions. The RH conformation, for instance, distinguishes itself from other conformations by exhibiting the largest fractional intensity for band 1 and the weakest one for band 3 in the IR spectra. With respect to isotropic scattering, the RH conformation also distinguishes itself from the other conformation because it has a strong amide band 3 and a weak band 1, which reflect the large positive nearest neighbor coupling strength for this conformation. With respect to the DRP distribution, RH is peculiar in that it exhibits the most different DPRs among all the distribution calculated. Another example is given by BT2. From its DPR distribution alone, it can

be easily identified since it has the smallest DPR for the band 2.

In many cases, however, the identification of a conformation requires the comparison of all intensity distributions, because different conformations can give rise to similar spectral features for up to three calculated distributions. It is difficult, for instance, to distinguish between the respective isotropic and anisotropic band profiles to identify the conformations of PBS and APBS, because they have similar intensity distributions. This generally requires spectra with good signal-to-noise ratios and a reliable spectral analysis, as performed for several tripeptides.^{10,12,14} 3_{10}H and BT1 cannot be discriminated from IR and isotropic scattering because they share very similar intensity distribution patterns with comparable intensities for the three bands. The PBS, APBS, 3_{10}H , PPII and BT1 conformations can hardly be discriminated by solely using their DPR distribution.

We now discuss how some most prominent secondary structures can be inferred from the depicted distributions. The RH conformation can be unambiguously identified by its IR, Raman isotropic scattering and DRP distribution in that band 1 dominates in the spectral profiles while it exhibits a relatively small depolarization ratio. Furthermore, band 2 has the weakest fractional intensity in anisotropic scattering. 3_{10}H distinguishes itself from the other conformers by exhibiting very similar, homogeneous distributions in all spectra. BT1 is similar to RH with respect to its IR and isotropic scattering profile, but can be distinguished from this conformation by means of its anisotropic Raman scattering profile from which the distribution for BT1 is contrastingly much different with band 3 the strongest. BT2 can easily be identified by means of its DPR distribution, which is the only one for which band 2 shows the smallest depolarization ratio. Also, by comparison of their respective isotropic and anisotropic scattering, BT1 can easily be discriminated from BT2 from isotropic scattering, because the former depicts a dominant band 1, whereas band 2 is most intense for the latter. Also, they have remarkably different distributions in anisotropic scattering. PBS, APBS and PPII show very similar spectral and depolarization ratio distributions. This is understandable, because they have very close geometries and coupling strengths as listed in Tables 1 and 2. Therefore, a decision about the conformation requires a more quantitative analysis of the intensity ratios, as described in a recent paper.¹⁸

The wavenumbers of the amide I bands used for the above calculations are somewhat arbitrary. However, simulations for other Δ_0 values revealed an invariance of the intensity distributions. Our calculations can therefore be considered as representative. For the sake of clarity we did not consider the fact that different secondary structures yield different magnitudes of excitonic splitting, which from an experimental viewpoint provide another piece of information for the structure analysis.

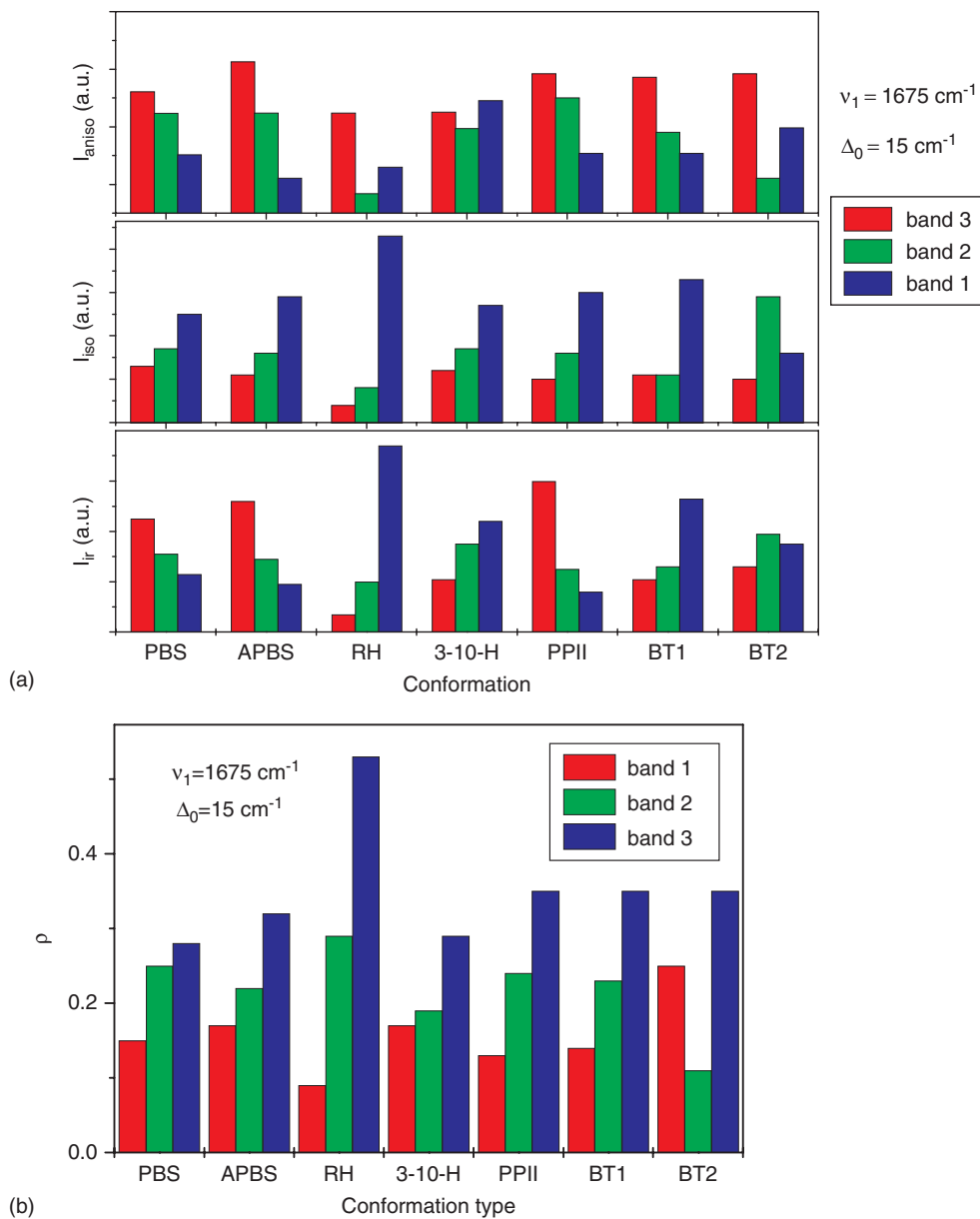


Plate 1. Histogram plots of (a) amide I intensity distributions of FTIR, Raman isotropic and anisotropic spectra and of (b) the Raman depolarization ratios of the amide I bands for the indicated secondary structures of a tetrapeptide. Wavenumber settings: $\nu_1 = 1675 \text{ cm}^{-1}$, $\nu_2 = 1660 \text{ cm}^{-1}$ and $\nu_3 = 1645 \text{ cm}^{-1}$ for the local modes.

CONCLUSIONS

Taken together, the combined use of all spectroscopies enables one to discriminate, to a significant extent, among all conformations investigated. Short peptides are suitable model systems for investigating the role of local and residue–residue interactions in determining the propensity of peptide segments for distinct secondary structure motifs and for investigating the initial steps of protein folding.^{25–28} In this study we have focused on tetrapeptides and showed that different secondary structures give rise to distinguishable spectral distributions with respect to the amide I band profile in IR, isotropic and anisotropic Raman spectra. The depolarization ratios of the Raman bands provide an additional tool for structure identification. This study not only provides the results which can be used by spectroscopists to identify the structure of tetrapeptides in solution, but also a new approach which sheds light on how to take advantage of Raman and FTIR spectra to identify secondary structural contents in longer peptides and proteins. It makes use of intensity distributions rather than heavily overlapped wavenumbers to discriminate different peptide motifs. We shall employ this approach to analyze oligo- and polypeptides in solution, and thus establish the powerful application of conventional vibrational spectroscopic methods in the study of biologically relevant biomolecules.

Acknowledgments

Financial support was provided from the NIH-COBRE II grant for the Center for Research in Protein Structure, Function and Dynamics (P20 RR16439-01). We thank Dr Torii for generously providing us the coupling strength values used in this paper and Dr Hamm for his advice concerning the production of the contour plot in Fig. 2.

REFERENCES

- Krimm S, Bandekar J. *Adv. Protein Chem.* 1986; **38**: 181.
- Mantsch HH, Casal HL, Jones RN. *Advances in spectroscopy. In Spectroscopy of Biological Systems*, Clark RJH, Hester RE (eds). Wiley: New York, 1986; 1.
- Jackson M, Haris PI, Chapman D. *J. Mol. Struct.* 1989; **214**: 329.
- Torii H, Tasumi M. In *Infrared Spectroscopy of Biomolecules*, Mantsch HH, Chapman D (eds). Wiley: New York, 1996; 1.
- Schweitzer-Stenner R. *J. Raman Spectrosc.* 2002; **32**: 711.
- Torii H, Tasumi M. *J. Raman Spectrosc.* 1998; **29**: 81.
- Choi J-H, Ham S, Cho M. *J. Phys. Chem. B* 2003; **107**: 9132.
- Woutersen S, Hamm P. *J. Phys. Chem. B* 2000; **104**: 11 316.
- Woutersen S, Hamm P. *J. Chem. Phys.* 2001; **114**: 2727.
- Schweitzer-Stenner R, Eker F, Huang Q, Griebenow K. *J. Am. Chem. Soc.* 2001; **123**: 9628.
- Schweitzer-Stenner R. *Biophys. J.* 2002; **83**: 523.
- Eker F, Cao X, Nafie L, Schweitzer-Stenner R. *J. Raman Spectrosc.* 2002; **124**: 14 330.
- Eker F, Griebenow K, Schweitzer-Stenner R. *J. Am. Chem. Soc.* 2003; **125**: 8178.
- Eker F, Cao X, Nafie L, Huang Q, Schweitzer-Stenner R. *J. Phys. Chem. B* 2003; **107**: 358.
- Schweitzer-Stenner R, Eker F, Perez A, Griebenow K, Cao X, Nafie L. *Biopolymers (Pept. Sci.)* 2003; **71**: 558.
- Eker F, Griebenow K, Cao X, Nafie L, Schweitzer-Stenner R. *Biochemistry* 2004; **43**: 613.
- Blatt HD, Smith PE, Pettit BM. *J. Phys. Chem B* 1997; **101**: 7682.
- Schweitzer-Stenner R, Eker F, Griebenow K, Cao X, Nafie L. *J. Am. Chem. Soc.* 2004; **18**: 126, 2768.
- Bour P, Keiderling TA. *J. Am. Chem. Soc.* 1993; **115**: 9602.
- Schulz GE, Schirmer RH. *Principles of Protein Structure*. Springer: Heidelberg, 1978; 18.
- Ham, S, Cho, M. *J. Chem. Phys.* 2003; **118**: 6915.
- Sieler G, Schweitzer-Stenner R, Holtz JSW, Pajcini V, Asher SA. *J. Phys. Chem. B* 1999; **103**: 372.
- Asher SA, Ianoul A, Mix G, Boyden MN, Karnoup A, Diem M, Schweitzer-Stenner R. *J. Am. Chem. Soc.* 2001; **123**: 11 775.
- Pajcini V, Chen XG, Bormett RW, Geib SJ, Li P, Asher SA, Lidiak EG. *J. Am. Chem. Soc.* 1996; **118**: 9716.
- Marqusee S, Baldwin RL. *Proc. Natl. Acad. Sci. USA.* 1987; **84**: 8898.
- Zimmermann SS, Scheraga HA. *Proc. Natl. Acad. Sci. USA* 1977; **74**: 4126.
- Lyu PC, Liff ML, Marky LA, Kalenbach NR. *Science* 1990; **258**: 669.
- Tobias DJ, Brooks CL. *Biochemistry* 1991; **30**: 6059.
- Voet D, Voet JG. *Biochemistry* (2nd edn). Wiley: New York, 1995.