Dihedral Angles of Tripeptides in Solution Directly Determined by Polarized Raman and FTIR Spectroscopy

Reinhard Schweitzer-Stenner
Department of Chemistry, University of Puerto Rico, Rio Piedras Campus, San Juan, Puerto Rico 00931-3346

ABSTRACT The amide I mode of the peptide linkage is highly delocalized in peptides and protein segments due to through-bond and through-space vibrationally coupling between adjacent peptide groups. Woutersen and Hamm (2000. *J. Phys. Chem. B.* 104:11316–11320) used coherent femtosecond infrared (IR) spectroscopy to determine the excitonic coupling energy and the orientational angle between the transition dipole moments of the interacting amide I modes of cationic tri-alanine in D_2O. Recently, the same parameters were determined for all protonation states of tri-alanine by analyzing the amide I bands in the respective IR and isotropic Raman spectra (Schweitzer-Stenner et al., 2001. *J. Am. Chem. Soc.* 119:1720–1726.). In both studies, the dihedral angles ϕ and ψ were then obtained by utilizing the orientational dependence of the coupling energy obtained from ab initio calculations on tri-glycine in vacuo (Torri and Tasumi, 1998. *J. Raman Spectrosc.* 29:81–86) to obtain an extended 3_1 helix-like structure for the tripeptide. In the present paper, a novel algorithm for the analysis of excitonic coupling between amide I modes is presented, which is based on the approach by Schweitzer-Stenner et al. but avoids the problematic use of results from ab initio calculations. Instead, the dihedral angles are directly determined from infrared and visible polarized Raman spectra. First, the interaction energy and the corresponding degree of wave-function mixing were obtained from the amide I profile in the isotropic Raman spectrum. Second, the depolarization ratios and the amide I profiles in the anisotropic Raman and IR-absorption spectra were used to determine the orientational angle between the peptide planes and the transition dipole moments, respectively. Finally, these two geometric parameters were utilized to determine the dihedral angles ϕ and ψ between the interacting peptide groups. Stable extended conformations with dihedral angles in the β-sheet region were obtained for all protonation states of tri-alanine, namely $\phi_+ = -126°$, $\psi_+ = 178°$; $\phi_\pm = -110°$, $\psi_\pm = 155°$; and $\phi_- = -127°$, $\psi_- = 165°$ for the cationic, zwitterionic, and anionic state, respectively. These values reflect an extended β-helix structure. Tri-glycine was found to be much more heterogeneous in that different extended conformers coexist in the cationic and zwitterionic state, which yield a noncoincidence between isotropic and anisotropic Raman scattering. Our study introduces vibrational spectroscopy as a suitable tool for the structure analysis of peptides in solution and tripeptides as suitable model systems for investigating the role of local interactions in determining the propensity of peptide segments for distinct secondary structure motifs.

INTRODUCTION

The exploration of protein and peptide structures provides the backbone for today's research in molecular biology and biochemistry. The most accurate methods in this regard are x-ray crystallography and, for smaller molecules also, multidimensional nuclear magnetic resonance (NMR) spectroscopy, by which atomic resolution can be achieved. The disadvantage of the former is that it provides a static picture of the investigated molecule. NMR can be applied to peptides and small proteins in solution. Its capability to detect conformational changes is limited to processes, which are slower than a millisecond. As a consequence, only average structures are obtained for molecules with faster structural variations between multiple conformers (Wüthrich and Grathwohl, 1974). Doubtless complementary methods for the structure analysis of peptides and proteins in solution are warranted, which are also applicable to probe fast structural variations on a micro- or nanosecond time scale.

Vibrational spectroscopies such as fourier transform infrared (FTIR) and Raman spectroscopy are techniques suitable for monitoring structural changes even in the range of picoseconds, but they are generally considered as low-resolution techniques, which are used to quantify the contribution of distinct secondary structure motifs to an overall protein structure (Griebenow et al., 1999; Bandekar, 1992, Chi et al., 1998). More site-specific investigations are possible by isotopically labeling distinct peptide groups (Arkin et al., 1997; Dong et al., 2001). Additionally, one can probe contributions of side chains, which are clearly discernable in even complex spectra, e.g., the IR band of the antisymmetric carboxylate-stretching vibrations (Hu et al., 1998) or Raman lines of aromatic residues that are resonance enhanced with UV excitation (Chi and Asher, 1998). Vibrational Circular Dichroism (Yoder et al., 1997) and Raman Optical Activity spectra (Barron et al., 2000) can be used to discriminate between α and 3_{10} helices. Generally, however, all these techniques cannot compete with NMR with respect to structure resolution.

The dihedral angles ϕ and ψ (Fig. 1) are the key determinants of the backbone structure of peptides and proteins. Only recently, vibrational spectroscopies have been suc-

Submitted November 28, 2001 and accepted for publication January 23, 2002.

Address reprint requests to Reinhard Schweitzer-Stenner, Dept. of Chemistry, Univ. of Puerto Rico, Rio Piedras Campus, P.O. Box 23346, San Juan, Puerto Rico 00931-3346. Tel.:1-787-764-0000, Ext. 2417; Fax: 1-787-756-8242; E-mail: rstenner_upr_chemistry@gmx.net.

0006-3495/02/07/523/10 $2.00

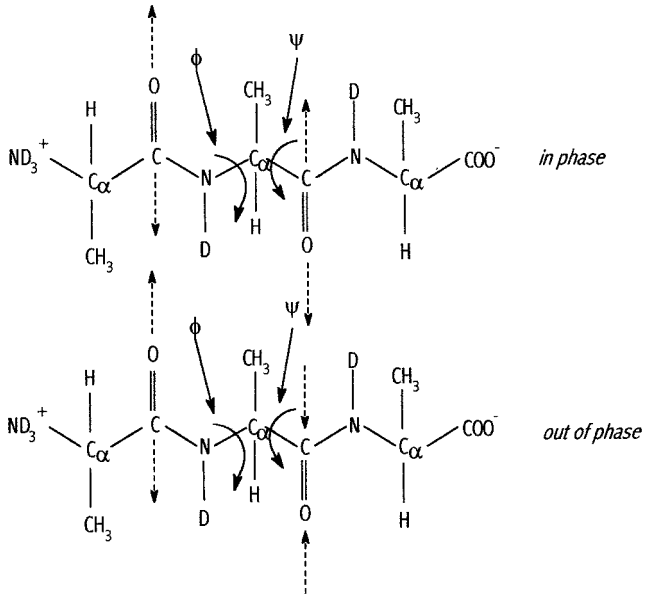

FIGURE 1 Structure of zwitterionic tri-alanine and representation of the in-phase (*upper figure*) and the out-of-phase combination of the amide I modes. For the sake of simplicity, the latter are represented by the CO stretching vibrations that provided the predominant contribution to the mode's eigenvector.

cessfully used to determine these angles for tripeptides in D_2O. These studies use the excitonic coupling between adjacent amide I modes in a polypeptide chain, which depends on the distance and the orientational angle between the respective transition dipole moments (Krimm and Bandekar, 1986). Woutersen and Hamm (2000, 2001) performed two-dimensional IR spectroscopy experiments to determine the excitonic coupling energy and the dihedral angle between the amide I transition dipole moments of the two peptide groups in cationic tri-alanine. The dihedral angles were then estimated by comparison with the orientational dependence of the coupling energy obtained from ab initio calculations on tri-glycine in vacuo (Torri and Tasumi, 1992). An alternative technically less demanding approach from our laboratory utilized the visible isotropic Raman scattering and IR-absorption to determine the excitonic coupling energy and the relative transition dipole moment orientation for all protonation states of tri-alanine (Schweitzer-Stenner et al., 2001). The dihedral angles were again determined by comparison with earlier ab initio calculations on tri-glycine (Torii and Tasumi, 1998). Both studies yielded very similar results, indicating that this tripeptide adopts a very stable extended structure in the β-sheet region of the Ramachandran plot, which can be characterized as 3_1 helix or polyglycine II structure. The exact structure was found to slightly change upon N-terminal deprotonation. For triglycine, we obtained significant conformational heterogeneity, which makes the interpretation of the spectra more complicated (Schweitzer-Stenner et al., 2001).

The shortcoming of the above studies is that they rely on ab initio calculations for a peptide in vacuo. The latter does not take into account the local dielectricity of the aqueous solvent, which generally causes a reduction of the transition dipole coupling (Krimm and Bandekar, 1986). In the present study, we report an algorithm that avoids this uncertainty in that it allows the direct determination of the dihedral angles of tripeptides solely from the amide I band profile in their polarized Raman and FTIR spectra. We argue that the reported formalism will provide a suitable basis for a more extended theory by which vibrational spectra of higher-order peptide can be analyzed for structure determination. Thus, the method will become applicable to biotechnologically relevant peptide fragments (e.g., for drug design) (Dyson et al., 1988; Schmidt and Langner, 1997; Nikiforovich et al., 2000) and model systems used for investigating the initial steps of protein folding (Marqusee et al., 1987). The structure analysis of tripeptides in solution is relevant for exploring the initial structures and local interactions that govern the initiation of secondary structure formation (Zimmermann and Scheraga, 1977; Lyu et al., 1990; Tobias and Brooks, 1991). Tripeptides themselves are biomedically relevant as protease inhibitors (Kiso et al., 1999), taste receptors (Goodman et al., 1997) and for enzyme regulation (Torreggiani et al., 2000).

THEORY

Excitonic coupling between amide I modes

Amide I modes of adjacent peptide groups interact due to through-bond and nonbonding interactions (Krimm and Bandekar, 1986; Torii and Tasumi, 1992; Hamm et al., 1998). The latter results from coupling between these modes' strong transition dipole moments and depends on their strength and relative orientation (Krimm and Bandekar, 1986). Figure 1 schematically displays the structure of tri-alanine together with an eigenvector representation of in-phase and out-of-phase coupled amide I modes. For the sake of simplicity, amide I is solely represented as carbonyl stretching vibration. The total interaction between two amide I modes is accounted for by a Hamiltonian $\hat{H}'$ so that the Schrödinger equation reads as

$$\hat{H}|n\rangle = (\hat{H}_0 + \hat{H}')|n\rangle, \tag{1}$$

where $\hat{H}_0$ is the Hamiltonian for the two unperturbed amide I modes described by the vibrational wavefunctions χ_1 (Q_1) and χ_2 (Q_2) (Q_1 and Q_2 denote nuclear coordinates). In the matrix representation, the total Hamiltonian is written as (Woutersen and Hamm, 2000; 2001)

$$H = \begin{pmatrix} \tilde{\nu}_1 & \Delta \\ \Delta & \tilde{\nu}_2 \end{pmatrix}, \tag{2}$$

where $\tilde{\nu}_1$ and $\tilde{\nu}_2$ denote the wavenumbers of the uncoupled amide I modes, and Δ is the coupling energy expressed in

units of cm^{-1}. $\hat{H}$ can be diagonalized by standard procedures to yield the following expression for the eigenfunctions,

$$|\chi_-\rangle = \cos\nu|\chi_1\rangle - \sin\nu|\chi_2\rangle, \quad (3)$$
$$|\chi_+\rangle = \sin\nu|\chi_1\rangle + \cos\nu|\chi_2\rangle,$$

and eigenenergies

$$\tilde{\nu}_\pm = \frac{\tilde{\nu}_1 + \tilde{\nu}_2}{2} \pm \sqrt{\frac{\Delta_0^2}{4} + \Delta^2}, \quad (4)$$

where Δ_0 is the energy between the unperturbed amide I modes. The parameter ν describes the degree of mixing between χ_1 and χ_2. χ_+ and χ_- are the vibrational wavefunctions of the in-phase and out-of-phase combination of the interacting modes as visualized in Fig. 1. The excitonic coupling energy Δ is related to the mixing parameter ν by the equation

$$\Delta = \frac{\Delta_{exp}}{2 \cdot \sqrt{1 + 4/\tan\nu}}, \quad (5)$$

where Δ_{exp} is the experimentally observed wavenumber difference between the two amide I bands.

Different parameters are obtainable by using IR- and Raman spectroscopy to explore the excitonic states of amide I. The isotropic part of the Raman scattering can be used to determine ν and thus also Δ. The respective anisotropic scattering additionally depends on the orientational angle θ between the peptide groups. IR-absorption is a function of the orientational angle $\tilde{\theta}$ between the transition dipole moments (cf. Fig. 2). Together, θ and $\tilde{\theta}$ can be used to determine the dihedral angles ϕ and ψ. Details of the mathematical tools are developed in the following.

Raman tensor of the coupled oscillators

To formulate the Raman tensor of the coupled oscillators, we first have to define our coordinate systems. Figure 2 exhibits the chosen reference structure of tri-alanine with $\phi = 180°$ and $\psi = 0°$. System $S_1(x_1, y_1, z_1)$ has its x_1 axis along the direction of the NC_α bond, which is also the rotational axis with respect to the dihedral angle ϕ. $S_2(x_2, y_2, z_2)$ is defined correspondingly for peptide 2. The orientational angle θ is the direction cosinus between the respective z-axes. Note, that θ is different from the angle $\tilde{\theta}$ between the transition dipole vectors. Because neither NC_α nor $C_\alpha N$ are exactly in the plane of the N- and C-terminal peptide group, respectively, the two peptide groups are not exactly coplanar in the reference structure. The very small angle between their z-axis ($\sim 1.1°$) was neglected in our analysis, i.e., we assumed that $\theta = 0°$.

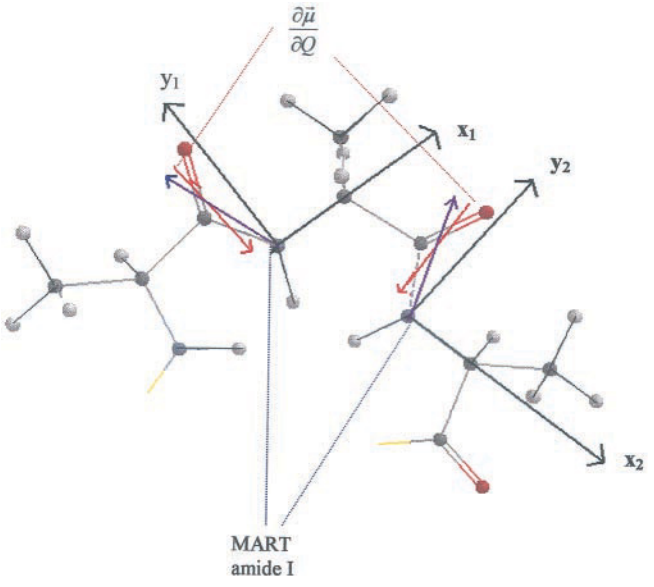

FIGURE 2 Reference structure of tri-alanine ($\phi = 180°$, $\psi = 0°$) and representation of the coordinate system chosen to describe the transition dipole moment (*red arrow*) and the Raman tensor of the noninteracting peptide groups. The pink arrows represent the MART of amide I as obtained by Pajcini et al. (1996). The structure was obtained by using the Spartan program from Wavefunction, Inc. Note that the terminal groups are truncated.

The corresponding Raman tensor of unperturbed amide I_1 mode has the general form,

$$\hat{\alpha}_1(S_1) = \begin{pmatrix} a & c & 0 \\ c & b & 0 \\ 0 & 0 & 0 \end{pmatrix}. \quad (6)$$

We assume that the corresponding Raman tensor $\hat{\alpha}_2(S_2)$ has the same elements as $\alpha_1(S_1)$, which means that the Raman scattering cross sections of amide I_1 and I_2 are identical. To derive the Raman tensor of a coupled amide I vibration, we rotate S_2 into S_1 to obtain the Raman tensor $\hat{\alpha}_2'(S_1)$. To this end, we first rotate S_2 by θ into the x_1, y_1 plane of S_1 and subsequently by the azimuthal angle η around its z-axis to orient it parallel to S_1. The translation shift between the two systems is irrelevant. These operations are accounted for by

$$\hat{\alpha}_2'(S_1) = R_2^T(\eta)\,[R_1^T(\theta)\hat{\alpha}_2(S_2)R_1(\theta)]R_2(\eta), \quad (7a)$$

where

$$R_2(\theta) = \begin{pmatrix} \cos\theta & 0 & -\sin\theta \\ 0 & 1 & 0 \\ \sin\theta & 0 & \cos\theta \end{pmatrix} \quad (7b)$$

and

$$R_1(\eta) = \begin{pmatrix} \cos\eta & -\sin\eta & 0 \\ \sin\eta & \cos\eta & 0 \\ 0 & 0 & 1 \end{pmatrix}. \quad (7c)$$

$R_1^T(\eta)$ and $R_2^T(\theta)$ are the transposed of R_1 and R_2, respectively. It should be noted that η depends weakly on θ. This

has been considered in the analysis of tripeptide spectra described below. We transfer $\hat{\alpha}_2(S_2)$ into S_1 by using Eqs. 7a and 7c to obtain

$$\hat{\alpha}'_2(S_1) = \begin{pmatrix} a'\cos^2\theta & c'\cos\theta & -\frac{a'}{2}\sin 2\theta \\ c'\cos\theta & b' & -c'\sin\theta \\ -\frac{a'}{2}\sin 2\theta & -c'\sin\theta & a'\sin^2\theta \end{pmatrix}, \tag{8a}$$

where

$$\begin{aligned} a' &= a\cos^2\eta + b\sin^2\eta + c\sin 2\eta, \\ b' &= a\sin^2\eta + b\cos^2\eta - c\sin 2\eta, \\ c' &= (b-a)\sin 2\eta + c(\cos^2\eta - \sin^2\eta). \end{aligned} \tag{8b}$$

The Raman tensors $\hat{\alpha}^+$ and $\hat{\alpha}^-$ of the in-phase and out-of-phase combination of amide I_1 and I_2 are written as

$$\begin{aligned} \alpha^- &= \cos\nu\cdot\hat{\alpha}_1(S_1) - \sin\nu\cdot\hat{\alpha}'_2(S_1), \\ \alpha^+ &= \sin\nu\cdot\hat{\alpha}_1(S_1) + \cos\nu\cdot\hat{\alpha}'_2(S_1). \end{aligned} \tag{9}$$

By inserting Eqs. 6, 8a, and 8b into Eq. 9 in the manuscript, one obtains

$$\begin{aligned} \alpha^-_{xx} &= a\cdot\cos\nu - (a\cdot\cos^2\eta + b\cdot\sin^2\eta \\ &\quad + c\cdot\sin 2\eta)\cdot\cos^2\theta\sin\nu \\ a^-_{yy} &= b\cdot\cos\nu - (a\cdot\sin^2\eta + b\cdot\cos^2\eta \\ &\quad - c\cdot\sin 2\eta)\cdot\sin\nu \\ \alpha^-_{zz} &= -(a\cdot\cos^2\eta + b\cdot\sin^2\eta + c\cdot\sin 2\eta)\cdot\sin^2\theta\cdot\sin\nu \\ \alpha^-_{xy} &= \alpha^-_{yx} = c\cdot\cos\nu + \left[\frac{(a-b)}{2}\sin 2\eta \right. \\ &\quad \left. - c\cdot\cos 2\eta\right]\cos\theta\cdot\sin\nu \end{aligned} \tag{10a}$$

$$\begin{aligned} \alpha^-_{xz} &= \alpha^-_{zx} \\ &= \tfrac{1}{2}(a\cdot\cos^2\eta + b\cdot\sin^2\eta \\ &\quad + c\cdot\sin 2\eta)\cdot\sin 2\theta\cdot\sin\nu \\ \alpha^-_{yz} &= \alpha^-_{zy} = \left[c\cdot\cos 2\eta - \frac{(a-b)}{2}\right]\sin\theta\cdot\sin\nu \end{aligned}$$

for the out-of-phase combination and

$$\begin{aligned} \alpha^+_{xx} &= a\cdot\sin\nu + (a\cdot\cos^2\eta + b\cdot\sin^2\eta \\ &\quad + c\cdot\sin 2\eta)\cdot\cos^2\theta\cdot\cos\nu \\ a^+_{yy} &= b\cdot\sin\nu + (a\cdot\sin^2\eta + b\cdot\cos^2\eta \\ &\quad - c\cdot\sin 2\eta)\cdot\cos\nu \\ \alpha^+_{zz} &= (a\cdot\cos^2\eta + b\cdot\sin^2\eta + c\cdot\sin 2\eta)\cdot\sin^2\theta\cdot\cos\nu \\ \alpha^+_{xy} &= \alpha^+_{yx} = c\cdot\sin\nu - \left[\frac{(a-b)}{2}\sin 2\eta \right. \\ &\quad \left. - c\cdot\cos 2\eta\right]\cos\theta\cdot\cos\nu \end{aligned} \tag{10b}$$

$$\begin{aligned} \alpha^+_{xz} &= \alpha^+_{zx} = -\tfrac{1}{2}(a\cdot\cos^2\eta + b\cdot\sin^2\eta \\ &\quad + c\cdot\sin 2\eta)\cdot\sin 2\theta\cdot\cos\nu \\ \alpha^+_{yz} &= \alpha^+_{zy} = -\left[c\cdot\cos 2\eta - \frac{(a-b)}{2}\right]\sin\theta\cdot\cos\nu \end{aligned}$$

for the in-phase combination. It should be emphasized in this context that Eq. 9 is different from the corresponding equation reported by Schweitzer-Stenner et al. (2001) due to the choice of different coordinate systems. As shown below, this is irrelevant for the isotropic scattering but affects the formalism for anisotropic scattering. The present formalism is more accurate because the chosen coordinate system reflects the rotational properties of the tripeptides system.

Isotropic scattering intensities of coupled amide I modes

The Raman tensor of amide I gains contributions from isotropic and anisotropic scattering. Experimentally, the corresponding intensities are obtained from polarized Raman spectra by (Sieler and Schweitzer-Stenner, 1997)

$$I_{aniso} = I_y \qquad I_{iso} = I_x - \tfrac{4}{3} I_y, \tag{11}$$

where x and y label the polarization of the scattered light parallel and perpendicular to the polarization of exciting laser beam, respectively. The isotropic scattering for the two delocalized amide modes is proportional to the tensor invariant,

$$\beta^2_{s\pm} = \tfrac{1}{9}(\mathrm{Tr}\,\alpha^{\pm})^2. \tag{12}$$

By using Eqs. 10a and 10b, one derives the following simple expression for the respective isotropic scattering:

$$\sin 2\nu = \frac{1 - R_{iso}}{1 + R_{iso}}, \tag{13}$$

by which we can use the experimentally determined value for $R_{iso} = I^-_{iso}/I^+_{iso}$ to determine the mixing parameter, and,

by Eq. 5, also the coupling energy. This equation was reported earlier (Schweitzer-Stenner et al. 2001).

Anisotropic intensities of coupled amide I modes

We proceed by recalling that the anisotropic intensity is proportional to the tensor invariant (Long, 1977),

$$\gamma^2_{\text{aniso}} = \tfrac{1}{2}[(\alpha_{xx} - \alpha_{yy})^2 + (\alpha_{yy} - \alpha_{zz})^2 + (\alpha_{zz} - \alpha_{xx})^2] + \tfrac{3}{4}[(\alpha_{xy} + \alpha_{yx})^2 + (\alpha_{yz} + \alpha_{zy})^2 + (\alpha_{xz} + \alpha_{zx})^2]. \quad (14)$$

By using the tensor elements in Eqs. 10a and 10b, one can calculate the intensity ratio for anisotropic scattering $R_{\text{aniso}} = \gamma^2_{\text{aniso},-}/\gamma^2_{\text{aniso},+}$ where − and + label the out-of-phase and in-phase combination of amide I. Of course, one can derive the explicit equation for R_{aniso}, but this is rather intricate and does not allow a direct calculation of θ. Instead, we calculated R_{aniso} for different orientational angles and compared the result with the respective experimental value.

To perform this evaluation of our data, however, additional knowledge about the tensor elements a, b, and c is required. To this end, we made use of the experimental depolarization ratios of the two amide I bands in the Raman spectrum, which are related to isotropic and anisotropic scattering by the relation,

$$\rho_{\pm} = \frac{3\gamma^2_{\text{aniso},\pm}}{45\beta^2_{\text{iso},\pm} + 4\gamma^2_{\text{aniso},\pm}}. \quad (15)$$

Thus, one generally obtains a set of different values for θ and the relative Raman tensor elements $b^* = b/a$ and $c^* = c/a$, which are all consistent with the experimentally observed R_{aniso} and $\rho_{\pm}$ values. To impose further restrictions, we use the recently obtained information about the principal axis of the Raman tensor (PART) of amide I.

PART of uncoupled amide I modes

The exact determination of the Raman tensor elements generally requires measurements on single crystals. For di-glycine, this has been done by Pajcini et al. (1996) for visible (488 nm) and ultraviolet (244nm) excitation. With respect to amide I, they obtained that, for visible excitation, the major axis of the Raman tensor (MART) in the diagonal frame is inclined 33.3° from carbonyl bound, as shown in Fig. 2 (*blue arrows*). Because the amide I mode has negligible side chain contribution (Krimm and Bandekar, 1986), this result is representative for peptide groups in the absence of any interpeptide coupling.

We exploited the result of Pajcini et al. (1996) by rotating the Raman tensor from S_1 so that the x_1-axis coincides with the MART,

$$\begin{pmatrix} \alpha_{xx}^{\text{PART}} & 0 & 0 \\ 0 & \alpha_{yy}^{\text{PART}} & 0 \\ 0 & 0 & 0 \end{pmatrix} = R^{\text{T}}(\beta) \begin{pmatrix} a & c & 0 \\ c & b & 0 \\ 0 & 0 & 0 \end{pmatrix} R(\beta). \quad (16)$$

$R(\beta)$ is given by

$$R(\beta) = \begin{pmatrix} \cos\beta & -\sin\beta & 0 \\ \sin\beta & \cos\beta & 0 \\ 0 & 0 & 1 \end{pmatrix}. \quad (17)$$

$R^{\text{T}}(\beta)$ is the respective transposed matrix. The rotational angle β can be derived from textbook peptide structures (Schulz and Schirmer, 1978) to 96°. For this value, one obtains the relationship

$$b^* = -9.3 \cdot c^* + 1. \quad (18)$$

Only b^* and c^* values fulfilling Eq. 18 can be used in the Raman tensor (Eqs. 10a and 10b) to calculate R_{aniso} as a functional of θ between the peptide groups. Thus, the orientational angle can be obtained by comparison with the experimental R_{aniso} value.

Infrared intensities of coupled amide I modes

The infrared intensities of the delocalized states depend on the angle $\tilde{\theta}$ between the transition dipole moments of the amide I_1 and I_2,

$$I^-_{\text{IR}} \propto \left(\cos\nu \cdot \frac{\partial\vec{\mu}}{\partial Q_1} - \sin\nu \cdot \frac{\partial\vec{\mu}}{\partial Q_2}\right)^2, \quad (19)$$

$$I^+_{\text{IR}} \propto \left(\sin\nu \cdot \frac{\partial\vec{\mu}}{\partial Q_1} + \cos\nu \frac{\partial\vec{\mu}}{\partial Q_2}\right)^2.$$

Woutersen and Hamm (2001) have shown that $\partial\vec{\mu}/\partial Q_1 = \partial\vec{\mu}/\partial Q_2 \equiv \vec{\mu}'$. Hence, we obtain the following vector representation for infrared intensities:

$$I^-_{\text{IR}} \propto |\vec{\mu}'|^2 \cdot \begin{pmatrix} \cos\nu - \sin\nu \cdot \cos\tilde{\theta} \\ \sin\nu \cdot \sin\tilde{\theta} \end{pmatrix}^2, \quad (20)$$

$$I^+_{\text{IR}} \propto |\vec{\mu}'|^2 \cdot \begin{pmatrix} \sin\nu + \cos\nu \cdot \cos\tilde{\theta} \\ \cos\nu \cdot \sin\tilde{\theta} \end{pmatrix}^2.$$

This leads to the following expression for the relationship between $\tilde{\theta}$ and the intensity ratio $R_{\text{IR}} = I^-_{\text{IR}}/I^+_{\text{IR}}$,

$$\tilde{\theta} = \arccos\left(\frac{1 - R_{\text{IR}}}{\sin 2\nu \cdot (1 + R_{\text{IR}})}\right), \quad (21)$$

which were used also by Schweitzer-Stenner et al. (2001) to determine $\tilde{\theta}$ from the experimentally obtained IR intensity

ratio. The mixing parameter ν can be obtained from the experimental value for R_{iso} as described above.

Determination of the dihedral angles

In this subsection, we show that the latter depend differently on the dihedral angles. We consider the unit vector $\vec{n}_1 = (x_{n1}, y_{n1}, z_{n1})$ and $\vec{n}_2 = (x_{n2}, y_{n2}, z_{n2})$ in the directions of the transition dipole moments of amide I_1 in S_1 and S_2. The angle $\tilde{\theta}$ can be determined by

$$\tilde{\theta} = \arccos(\vec{n}_1 \vec{n}_2). \tag{22}$$

We express the components of $\vec{n}_1$ and $\vec{n}_2$ in terms of the respective polar angle,

$$x_{n_1} = x_{n_2} = \cos\vartheta \qquad y_{n_1} = y_{n_2} = \sin\vartheta \qquad z_{n_1} = z_{n_2} = 0, \tag{23}$$

where we assumed that the angle ϑ between the transition dipole moment and the x-axis is identical in both coordinate systems. To calculate the scalar product in Eq. 22, we use S_2 as reference system and transfer S_1 into S_2. This requires the evaluation of the product,

$$\vec{n}_1^* = R(\omega)R(\psi)R(\xi)R(\phi') \cdot \vec{n}_1. \tag{24}$$

Figure 2 can be used to understand Eq. 24. First, S_1 has to be rotated by the dihedral angle ϕ' around x_1. This angle is related to the dihedral angle ϕ by $\phi' = \phi - \pi$. Subsequently, a rotation by ξ in the xy-plane is necessary so that the y-ordinate coincides with the $C_\alpha C$ bond, which is the rotational axis for ψ. ξ is the angle formed by the y_1-axis and the $C_\alpha C$ in Fig. 2. Next, the system is rotated by the dihedral angle ψ. The fourth step involves the rotation by an angle ω, which is formed by the $C_\alpha C$ bond and the y_2-axis. This causes the abscissa to coincide with the NC_α bond. Note that, for a coplanar arrangement of the peptide groups as depicted in Fig. 2, $\eta = \omega - \xi$.

Carrying out the operations in Eq. 24 and inserting the result and Eq. 23 into Eq. 22 eventually yields

$$\tilde{\theta} = \arccos\left\{ \begin{array}{l} (\cos\omega \cdot \cos\xi \cdot \cos\psi - \sin\omega \cdot \sin\xi)x^2 \\ + \left[\begin{array}{r} \sin\psi \cdot \sin\phi' \cdot \cos\omega \\ -\cos\phi' \cdot (\cos\psi \cdot \sin\xi \cdot \cos\omega + \sin\omega \cdot \cos\xi) \\ +\sin\omega \cdot \cos\xi \cdot \cos\psi + \cos\omega \cdot \sin\xi \end{array} \right] xy \\ - \left[\begin{array}{r} \cos\phi'(\cos\psi \cdot \sin\xi \cdot \sin\omega - \cos\omega \cdot \cos\xi) \\ -\sin\psi \cdot \sin\phi' \cdot \sin\omega \end{array} \right] y^2 \end{array} \right\}, \tag{25}$$

which reveals a pretty complex dependence of $\tilde{\theta}$ on the dihedral angles ϕ' and ψ. ω and ξ can be obtained from textbooks on peptide structure to 96° and −20°. We checked Eq. 25 by reproducing the functional dependence of $\tilde{\theta}$ on ϕ and ψ reported by Woutersen and Hamm (2001).

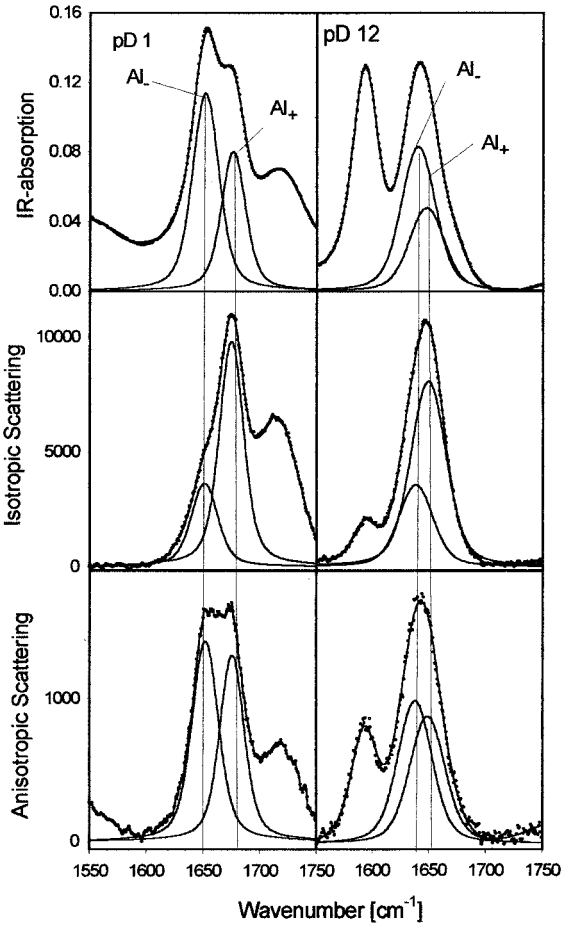

FIGURE 3 FTIR, isotropic Raman and anisotropic Raman spectrum of tri-alanine between 1500 and 1800 cm^{-1} measured at the indicated pD values, which represent the cationic (1.5) and anionic state (12). The line profiles result from the spectral decomposition, which is described in detail in Schweitzer-Stenner et al. (2001).

The angle θ between the peptide groups obtained from anisotropic scattering is given by

$$\theta = \arccos(z_{n_1} z_{n_2}) = \cos\phi \cdot \cos\psi - \sin\psi \cdot \sin\xi \cdot \sin\phi'. \tag{26}$$

STRUCTURE ANALYSIS OF TRIPEPTIDES IN D_2O

We now use the formalism outlined above to reanalyze the amide I profiles of tri-alanine and tri-glycine in D_2O reported earlier (Schweitzer-Stenner et al., 2001). D_2O has been chosen as solvent to avoid vibrational mixing with water-bending vibrations (Sieler and Schweitzer-Stenner, 1997). For illustration, the spectra of the cationic and anionic state are shown in Fig. 3. R_{IR} R_{iso}, and R_{aniso} of the two lines AI_- and AI_+ are apparently different for both protonation states and indicate excitonic coupling. The two lines are assigned to the out-of-phase (−) and in-phase (+) combination of the amide I modes.

TABLE 1 Spectroscopic and structural parameters obtained from the analysis of the FTIR, isotropic, and anisotropic Raman spectra of tri-alanine at the pD indicated

	pD 1	pD 6	pD 12
$\tilde{\nu}_1$ [cm^{-1}]	1652	1646*/1648†	1638
$\tilde{\nu}_2$ [cm^{-1}]	1676	1673*/1675†	1649
Γ_{G1} [cm^{-1}]	21.3	22.5*/23.4†	29.6
Γ_{G2} [cm^{-1}]	18.9	17.2	30.5
R_{iso}	0.39	0.48	0.41
R_{aniso}	1.16	1.14	1.1
R_{IR}	1.52	1.52	1.69
Δ [cm^{-1}]	4.0	3.8	1.8
θ [°]	119 (116)‡	126 (131)‡	128
θ [°]	124 (130)‡	124 (117)‡	131
ϕ [°]	−123 (−126)‡	−120 (−110)‡	−127
ψ [°]	173 (178)‡	164 (155)‡	165

*Isotropic scattering
†Anisotropic scattering
‡Calculated with an extended model assuming inequivalent intensities.

AI$_-$ is more depolarized ($\rho_- = 0.25$ and 0.22 at pD 1.5 and 6, respectively) than AI$_+$ ($\rho_- = 0.11$ at both pDs). The most relevant spectral parameters are listed in Table 1 (taken from Schweitzer-Stenner et al., 2001). In what follows, we use the spectra of the cationic state to demonstrate the different steps of our analysis in detail.

We use Eqs. 13 and 21 to obtain the respective mixing parameter ν and the angle $\tilde{\theta}$ between the transition dipole moments. To obtain the orientational angle θ we proceed as follows. First, we use Eqs. 9, 14, and 15 to determine the relative tensor elements c^* and b^*, for which one obtains the experimentally observed intensity ratio R_{aniso} and depolarization ratios ρ_- and ρ_+ of the two amide I bands. Thus, one obtains multiple pairs c^*, b^*. Each pair of c^*, b^* corresponds to two orientational angles θ. The relationships between c^*, b^* and θ are graphically illustrated in Fig. 4.

In the next step, we make use of the MART of amide I, which Pajcini et al. (1996) recently obtained for di-glycine. We use Eq. 18 to select a single pair from the above-mentioned set of multiple c^*, b^* values, i.e., $c_t^* = 0.055$ and $b_t^* = 0.49$. A Raman tensor with these elements in S_1 is diagonal if the x_1-axis is rotated to coincide with the MART of amide I. We then identify $\theta_1(b_t^*, c_t^*) = 128°$ and $\theta_2(b_t^*, c_t^*) = 52°$ (graphical illustration in Fig. 4). To estimate its uncertainty, we assume that the MART angle of amide I is determined with an accuracy of $\pm 5°$. From this we estimate an error interval of $-1.0/+0.5$ for $\theta(b_t^*, c_t^*)$. In the following, we solely use $\theta_1(b_t^*, c_t^*)$, because $\theta_2(b_t^*, c_t^*)$ was found to have no structural meaning because it cannot be related to any pair of dihedral angles.

Next, we use Eqs. 25 and 26 to calculate the angle $\tilde{\theta}$ between the transition dipole moments and the orientational angle θ for different ϕ and ψ values. The transition dipole moment forms an angle of 20° with the carbonyl bond and is pointed toward the amide proton (Fig. 2) (Lee and Krimm, 1998). This corresponds to an angle of $\vartheta = 95°$ in

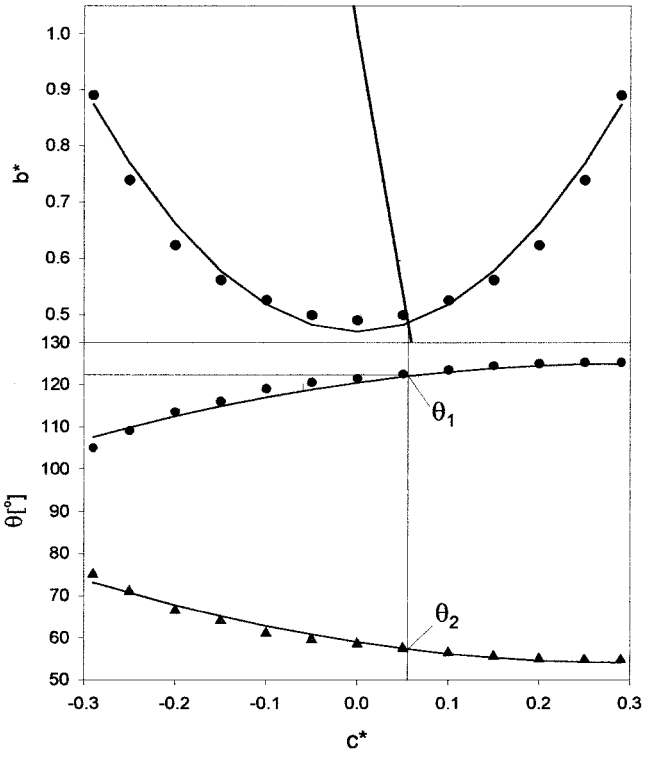

FIGURE 4 *Upper panel*: Graphical representation of the relationship between the elements b^* and c^* of the amide I Raman tensor, for which the experimentally observed depolarization ratios ρ_- and ρ_+ and the intensity ratio R_{aniso} of the cationic tri-alanine could be reproduced. The straight line represents the solutions of Eq. 18. The intersection of both representations yield the values b_t^* and c_t^*, of the Raman tensor for the coordinate systems S_1 and S_2 shown in Fig. 2 and described in the text. *Lower panel*: Graphical representation of the relationship between values of the orientational angle θ and of the tensor element c^*, which are consistent with the experimentally observed depolarization ratios ρ_- and ρ_+ and the intensity ratio R_{aniso} of the cationic tri-alanine. Two values are obtained for each c^*. The solid perpendicular line-map the coordinates b_t^*, c_t^* in the upper panel onto the $\theta = f(c^*)$ representation in the lower panel.

S_1. We then determine the pairs of ϕ and ψ values for which the experimentally determined angle $\tilde{\theta}(R_{IR})$ and the above obtained $\theta(b_t^*, c_t^*)$ could be simultaneously reproduced. Thus, we derive two solutions for the dihedral angles of tri-alanine, namely $\phi_1 = -123° \pm 2°$, $\psi_1 = 173° \pm 2°$ and $\phi_2 = 45° \pm 2°$, $\psi_2 = -55° \pm 3°$. We can disregard (ϕ_2, ψ_2) because it is not in an allowed region of the Ramachandran plot. The obtained conformation, (ϕ_1, ψ_1) is somewhat more extended than the 3_1-helix structures recently reported by Woutersen and Hamm (2000, 2001), $(\phi, \psi) = (-60°, 140°)$ and by our research group (Schweitzer-Stenner et al., 2001), $(\phi_2, \psi_2) = (-70°, 145°)$. All these coordinate pairs belong to the extended region in the interval $-180° \leq \phi \leq 45°$ and $110° \leq \psi \leq 180°$ (Fig. 4) (Tanaka and Scheraga, 1977). Our structure also deviates from the β-sheet conformations of polyglycine I ($(\phi, \psi) = (-138.4°, 135.7°)$, Fig. 5) and of poly(L-alanine) ($(\phi, \psi) = (-138.4°, 135.7°)$, Fig. 5)

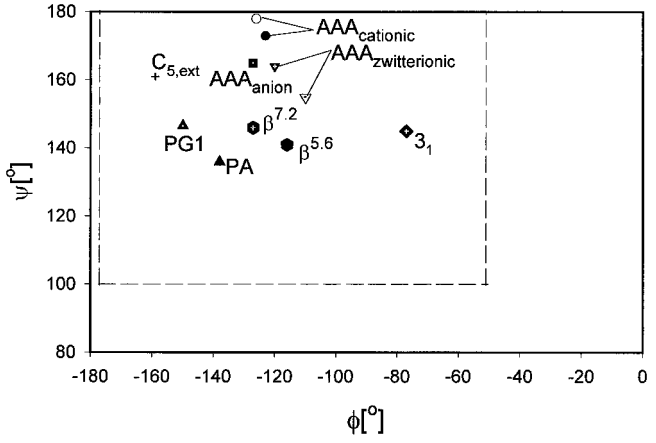

FIGURE 5 Ramachandran representation of the dihedral angles obtained from the analysis of the protonation states of tri-alanine. The box drawn with dashed lines mark the region for extended structures due to the criteria described by Tanaka and Scheraga (1977). The assignments of all data points are indicated. Two coordinates are plotted for the cationic and anionic states of tri-alanine. The *filled symbols* represent the results obtained with the assumption that the two amide I modes show the same intrinsic intensities in the absence of coupling, whereas the corresponding *open symbols* depict the coordinate values obtained by considering slightly different intensities obtained from the Raman spectra of the three protonation states of di-alanine in D_2O. The coordinates for some known structures are indicated for comparison.

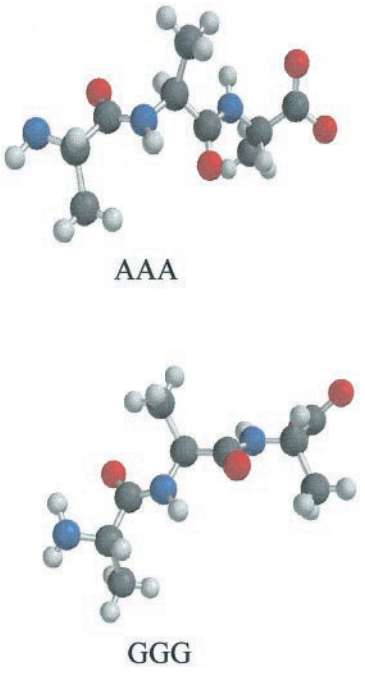

FIGURE 6 Structure of anionic tri-alanine and tri-glycine as determined from the corresponding IR_ and Raman band profiles of amide I. The drawing was produced using the program Spartan of Wavefunction, Inc.

(Arnott et al., 1967) and is less extended than the $C_5^{ext}{}_5(-157°, 165°)$ structure obtained for blocked tri-alanine analogs (Han et al., 1998). With respect to the ϕ-coordinate, it is, however, comparable with the so-called $\beta^{6.5}$ $(-116°, 141°)$ and $\beta^{7.2}$ helices $(-127°, 146°)$, which are formed by mixture of L- and D-amino acids with 6.5 residues per turn (Fig. 5). The most prominent representative of this type of peptides is the transmembrane ion channel gramidicin A (Krimm and Bandekar, 1986).

The above error interval of the ϕ and ψ reflects only uncertainties of the mathematical procedure. Additional errors, however, arise from uncertainties of the spectral analysis. We carried out a detailed error analysis of our spectral parameters and estimated their influence on the dihedral angles. Thus, we obtained $\phi_1 = -123° + 7°/-3°$ and $\psi_1 = 173° + 7°/-2°$. Additionally, we checked how our results are affected if one allows slightly different intensities for the two unperturbed amide bands in the Raman spectrum. To this end, we first determined the intensities of amide I in the 457-nm Raman spectra of the three protonation states of di-alanine in D_2O and found that $I_{cation}(AI) = 1.2 \cdot I_{zwitt}(AI) = 1.3 \cdot I_{anion}(AI)$. (F. Eker, R. Schweitzer-Stenner, unpublished). We then used these intensity ratios to obtain $(\phi, \psi) = (-126°, 178°)$ for the cationic state (Table 1). The coordinate is also represented in Fig. 5. This structure can still be described as extended and β-helix-like.

We also reinvestigated the spectra of the remaining protonation states of tri-alanine by using the spectroscopic data reported by Schweitzer-Stenner et al. (2001). The results are listed in Table 1. Qualitatively, all protonation states are very similar in that they can be characterized as extended β-helix-like structures (Fig. 5). As an example, Fig. 6 depicts the obtained structure of anionic tri-alanine.

Recently, Gnanakaran and Hochstrasser (2001) performed a combined molecular dynamics and multidimensional IR study on blocked dipeptide analog of tri-alanine in H_2O and CCl_4. The results for the molecular dynamics yielded two allowed region for the peptide, one in the β-sheet region $(-130 < \phi < -80, 140 < \psi < 180)$ and the helical region $(-130 < \phi < -65, -180 < \psi < 30)$. The structures obtained in this study fit perfectly into β-sheet region. From their experimental data, the authors derived an α_R-like conformer, which is apparently neither in agreement with the results of the present study nor with those reported by Schweitzer-Stenner et al. (2001) and Woutersen and Hamm (2000, 2001). In fact, an α-helix can be ruled out from our data because it would give rise to very similar IR and Raman spectra, in which the same band is dominating (Torii and Tasumi, 1998; Lee and Krimm, 1998). These discrepancies might be due to the neglect of through-bond coupling in the study of Gnanakaran and Hochstrasser (2001), which accounts for half of nearest neighbor excitonic coupling strength (Torii and Tasumi, 1998).

The question arises whether the dihedral angles obtained represent an average structure of a conformationally heterogeneous sample. Heterogeneity of tripeptides is suggested by results from NMR studies (Wright et al., 1988), whereas spec-

troscopic (Woutersen and Hamm, 2000; Ford et al., 1994) and computational studies suggest rather stable structures in water (Han et al., 1998). Our data strongly support the absence of significant heterogeneity for tri-alanine for the following reasons. First, the Gaussian contributions to the Voigtian band profiles of amide I_- and amide I_+ (21.3° and 18.9°) are even smaller than that observed for di-alanine in D_2O (24.4°) (F. Eker, R. Schweitzer-Stenner, unpublished). All these values are larger than the width of the Gaussiam frequency distributions Gnanakaran and Hochstrasser (2001) obtained from molecular dynamics studies on blocked dipeptide analog of tri-alanine in water. Apparently, excitonic coupling does not cause any additional spectral broadening. Second, the coexistence of structures with significantly different dihedral angles (e.g., extended and helical conformers) would differently affect the isotropic and anisotropic scattering profile, so that both could not be fitted with the same spectral parameters. This is not the case for the cationic and anionic state. The zwitterionic tri-alanine exhibits indeed a very small noncoincidence between isotropic and anisotropic frequencies ($\sim$1 cm^{-1}). This discrepancy indicates to a small contamination by other conformers.

Our results rule out a structure that Lee et al. (1989) derived from the vibrational circular dichroism signals of zwitterionic tri-alanine, namely (ϕ, ψ) = (120°, −25°). The authors argued that this structure is stabilized by electrostatic interaction between the terminal charges, so that it is absent in the cationic and anionic state. First, this structure is very unlikely because it is in the forbidden region of the Ramachandran plot. Second, the corresponding excitonic coupling energy of 8 cm^{-1} (Woutersen and Hamm, 2000) is too high. Third, our data and earlier Roman optical activity data by Ford et al. (1994) exclude the possibility of major structural changes due to the protonation of the terminal groups. The difference between the amide I profiles of the anionic and zwitterionic/cationic states result from a significant downshift of the (unperturbed) amide I frequency of the N-terminal peptide due to the deprotonation of the ammonium group (Sieler et al., 1999). Therefore, we rule out any significant influence of the terminal charges on the structure of tri-alanine.

In our earlier study (Schweitzer-Stenner et al., 2001) we have also analyzed the IR and Raman spectra of tri-glycine. This was rendered more difficult, because at least two conformers coexist in the zwitterionic and cationic state, which give rise to a substantial noncoincidence between isotropic and anisotropic scattering ($\sim$6 cm^{-1}). The minimal model to which all band profiles could be fitted is composed of four bands of different intensities and depolarization ratios. The result was interpreted as reflecting the coexistence between extended, helical, and turn structures, but the coexistence of multiple conformers cannot be ruled out. Thus, the Raman spectra of tri-glycine demonstrate that heterogeneity is indeed detectable by our methods.

In the anionic state, the noncoincidence between the isotropic and anisotropic scattering is eliminated, but the two bands exhibit somewhat larger Gaussian contributions (27.9° and 26.2°) than the amide I band of anionic di-glycine in D_2O (21°) (Sieler et al., 1999), indicating some conformational flexibility in a limited part of the Ramachandran space. From the frequencies and intensity ratios reported earlier (Schweitzer-Stenner et al., 2001) we calculated the dihedral angles (ϕ, ψ) = (−123°, −176°). This structure is in the so-called ε-region of the Ramachandran plot, which is generally sampled by glycine-containing peptides. For illustration, it is displayed in Fig. 6.

SUMMARY AND OUTLOOK

Altogether, the present study shows for the first time that the combination of FTIR and polarized Raman spectroscopy provides sufficient information to obtain the dihedral angles of tripeptides without utilizing any additional information from computational studies. This opens a new pathway in using vibrational spectroscopy for the structure analysis of peptides in solution.

The question arises whether the theoretical concept can be extended to analyze oligopeptides with more than two peptide groups. We have no doubt that this is possible. Possible concepts and formalism depend on the adopted structure. It is comparatively clear for helices and β-sheet structures because Raman and IR intensities are dictated by well-known group theoretical rules (Krimm and Bandekar, 1986). Deviations from these rules (e.g., an IR-active mode of a β-sheet structure displays also some intensity in the Raman spectrum) can be analyzed in terms of structural distortions. The dihedral angles (or their distribution) can, in principle, be obtained from the analysis of frequency values and depolarization ratios. Turn structures, which exhibit very different dihedral angles of their peptide linkages, give rise to multiple and resolvable bands (Hamm et al., 1999). Their analysis requires an extension of the excitonic coupling matrix. Hamm et al. (1999) showed that the Schrödinger equation can be solved by using time-independent perturbation theory. The mathematical formalism for analyzing IR and Raman spectra will become pretty complex, but the above algorithm can be used as a corner stone.

The most relevant biological result of our study is that, in contrast to conventional wisdom derived from NMR-experiments (Dyson et al., 1988), tripeptides can adopt a well-defined structure in solution, which is not stabilized by electrostatic interactions between the terminal groups. This suggests that, in accordance with earlier suggestions by Zimmermann and Scheraga (1977), local interactions are strong enough to give rise to structure propensities of small peptide fragments. This is certainly of relevance for understanding the general secondary structure propensity of amino acid residues, or more appropriate, of pairs of amino acid residues. In our laboratory, we have started a series of investigations on various tripeptides to further characterize the physical basis of these interactions.

The author acknowledges financial support by a National Science Foundation Experimental Program to Stimulate Competitive Research (EPSCOR) grant (Puerto Rico EPSCOR grant OSR-9452893) and the receipt of some very useful information from Peter Hamm.

REFERENCES

Arkin I. T., K. R. MacKenzie, and A. T. Brünger. 1997. Site-directed dichroism as a method for obtaining rotational and orientational constraints for oriented polymers. *J. Am. Chem. Soc.* 119:8973–8980.

Arnott, S., and S. D. Dover. 1967. Refinement of bond angles of an alpha-helix. *J. Mol. Biol.* 30:209–212.

Barron L. D., L. Hecht, E. W. Blanch, and A. F. Bell. 2000. Solution structure and dynamics of biomolecules from Raman optical activity. *Prog. Biophys. Mol. Biol.* 73:1–49.

Bandekar, J. 1992. Amide modes and protein conformation. *Biochim. Biophys. Acta.* 1120:123–143.

Chi, Z., and S. A. Asher 1998. UV resonance Raman determination of protein acid denaturation: selective unfolding of helical segments of horse myoglobin. *Biochemistry* 37:2865–2872.

Chi, Z., X. G. Chen, J. S. W. Holtz, and S. A. Asher. 1998. UV resonance Raman-selective amide vibrational enhancement: quantitative methodology for determining protein secondary structure. *Biochemistry.* 37:2855–2864.

Dong, J., Z.-L. Whan, Y.-C. Chu, S. N. Nakagawa, P. G. Katsoyannis, M. A. Weiss, and P. M. Carey. 2001. Isotope-edited Raman spectroscopy of proteins: a general strategy to probe individual peptide bonds with applications to insulin. *J. Am. Chem. Soc.* 123:7919–7920.

Dyson, H. J., R. A. Lerner, and P. E. Wright. 1988. The physical basis for induction of protein-reactive antipeptide antibodies. *Ann. Rev. Biophys. Biophys. Chem.* 17:305–324.

Ford, S. J., Z. Q., Wen, L. Hecht, and L. D. Barron. 1994. Vibrational Raman optical activity of alanyl peptide oligomers: a new perspective on aqueous solution conformation. *Biopolymers.* 34:303–314.

Gnanakaran, S. and R. M. Hochstrasser. 2001. Conformational preferences and vibrational frequency distributions of short peptides in relation to multidimensional infrared spectroscopy. *J. Am. Chem. Soc.* 123: 12886–12898.

Goodman, M., Q. Zhu, D. R. Kent, Y. Amino, R. Iacovino, E. Benedetti, and A. Santini. 1997. Conformational characterization of the 1-aminocyclobutane-1-carboxylic acid residue in model peptides *J. Pept. Sci.* 3:321–241.

Griebenow, K., A. M. Santos, and K. G. Carrasquillo. 1999. Secondary structure of proteins in the amorphous dehydrated state probed by FTIR spectroscopy. Dehydration-induced structural changes and their prevention. *Internet J.* Vib. Spectr. [www.ijvs.com] 3:1–20.

Hamm, P., M. Lim, W. F. DeGrado, and R. M. Hochstrasser. 1999. The two-dimensional IR nonlinear spectroscopy of a cyclic penta-peptide in relation to its three-dimensional structure. *Proc. Natl. Acad. Sci. U.S.A* 96:2036–2041.

Hamm, P., M. Lim, and R. M. Hochstrasser. 1998. Structure of the amide I band of peptides measured by femtosecond nonlinear-infrared spectroscopy. *J. Phys. Chem. B.* 102:6123–6138.

Han, W.-G., K. J. Jalkanen., M. Elstner, and S. Suhai. 1998. Theoretical study of aqueous N-Acetyl-L-alanine *N′*-methylamide: structures and Raman, VCD, and ROA spectra. *J. Phys. Chem. B.* 101:2587– 2602.

Hu, X., L. A. Dick, and T. G. Spiro. 1998. Fourier transform infrared evidence against Asp beta 99 protonation in hemoglobin: nature of the Tyr alpha 42-Asp beta 99 quaternary H-bond. *Biochemistry.* 37: 9445–9448.

Kiso, Y., H. Matsumunotot, S. Mizumoto, T. Kimura, Y. Fujiwara, and K. Akaji. 1999. Small dipeptide-based HIV protease inhibitors containing the hydroxymethylcarbonyl isostere as an ideal transition-state mimic. *Biopolymers.* 51:59–68.

Krimm, S, and J. Bandekar. 1986. Vibrational spectroscopy of peptides and proteins. *Adv. Prot. Chem.* 38:181–360.

Lee, O., G. M. Roberts, and M. Diem. 1989. IR Vibrational CD in Alanyl Tripeptide: Indication of a Stable Solution Conformer. *Biopolymers* 28:1759–1770.

Lee, S.-H., and S. Krimm. 1998. General treatment of vibrations of helical molecules and application to transition dipole coupling in amide I and amide II modes of α-helical poly(L-alanine). *Chem. Phys.* 230:277–295.

Long, D. A. (1977). *Raman Spectroscopy.* McGraw-Hill, Chichester, Great Britain

Lyu, P. C., M. L. Liff, L. A. Marky, and N. R. Kalenbach. 1990. Side chain contributions to the stability of alpha-helical structure in peptides. *Science.* 258:669–673.

Marqusee, S., and R. L. Baldwin. 1987. Helix stabilization by Glu$^-\cdots$Lys$^+$ salt bridges in short peptides of de novo design. *Proc. Natl. Acad. Sci. U.S.A.* 84:8898–8902.

Nikiforovich, G. V., K. E. Köver, W.-J. Zhang, and G. Marshall. 2000. Cyclopeptides as flexible conformational templates. *J. Am. Chem. Soc.* 122:3262–3273.

Pajcini, V., X. G. Chen, R. W. Bormett, S. J. Geib, P. Li., S. A. Asher, and E. G. Lidiak. 1996. Glycylglycine $\pi\rightarrow\pi^*$ and charge transfer transition moment orientations: near-resonance Raman single-crystal measurements. *J. Am. Chem. Soc.* 118:9716–9726.

Schmidt, U., and J. Langner. 1997. Cyclotetrapeptides and cyclopentapeptides: occurrence and synthesis. *J. Pept. Res.* 49:67–73.

Schulz, G. E., and R. H. Schirmer. 1978. Principles of Protein Structure. Springer Verlag, Berlin.

Schweitzer-Stenner, R., F. Eker, Q. Huang, and K. Griebenow. 2001. Dihedral angles of trialanine in D_2O determined by combining FTIR and polarized visible Raman spectroscopy. *J. Am. Chem. Soc.* 123:9628–9633.

Sieler, G., and R. Schweitzer-Stenner. 1997. The amide I mode of peptides in aqueous solution involves vibrational coupling between the peptide group and water molecules of the hydration shell. *J. Am. Chem. Soc.* 119: 1720–1726.

Sieler, G., R. Schweitzer-Stenner, J. S. W. Holtz, V. Pajcini, and S. A. Asher. 1999. Different conformers and protonation states of dipeptides probed by polarized Raman, UV-resonance Raman, and FT-IR spectroscopy. *J. Phys. Chem. B.* 103: 372–384.

Tanaka, S., and H. Scheraga. 1977. Statistical mechanical treatment of protein conformation. 5. A multistate model for specific-sequence copolymers of amino acids. *Macromolecules.* 10:9–20.

Tobias, D. J., and C. L. Brooks. 1991. Thermodynamics and mechanism of α helix initiation in alanine and valine peptides. *Biochemistry.* 30: 6059–6070.

Torii, H., and M. Tasumi, M. 1992. Application of the three-dimensional doorway-state theory for analyses of the amide I infrared bands of globular proteins. *J. Chem. Phys.* 97: 92–102.

Torii, H., and M. Tasumi 1998. *Ab initio* molecular orbital study of the amide I vibrational interactions between the peptide groups in di- and tripeptides and considerations on the conformation of the extended helix. *J. Raman Spectrosc.* 29:81–86.

Torreggiani, A., M. Tampa, and G. Fini. 2000. Raman and IR spectroscopic investigation of zinc(II)-carnosine complexes *Biopolymers* 57:149–159.

Woutersen, S., and P. Hamm. 2000. Structure determination of trialanine in water using polarization sensitive two-dimensional vibrational spectroscopy. *J. Phys. Chem. B. 104*:11316–11320.

Woutersen, S., and P. Hamm. 2001. Isotope-edited two-dimensional vibrational spectroscopy of trialanine in aqueous solution. *J. Chem. Phys.* 114: 2727–2737.

Wright, P. E., H. J. Dyson, and R. A. Lerner. 1988. Conformation of peptide fragments of proteins in aqueous solution: implications for initiation of protein folding. *Biochemistry.* 27:7167–7175.

Wüthrich, K., and C. Grathwohl. 1974. A novel approach for studies of the molecular conformations in flexible polypeptides. *FEBS Lett.* 43:337–340.

Yoder, G., A. Polese, R. A. G. D. Silva, F. Formaggio, M. Crisma, Q. B. Broxterman, J. Kamphuis, C. Toniolo, and T. A. Keiderling. 1997. Conformational characterization of thermally blocked L-(αMe)Val homopeptides using vibrational and electronic circular dichroism. 3_{10}-Helical stabilization by peptide–peptide interaction. *J. Am. Chem. Soc.* 119:10278–10285.

Zimmermann, S. S., and H. A. Scheraga. 1977. Local interactions in bends of proteins. *Proc. Natl. Acad. Sci. U.S.A.* 74:4126–4129.