Dihedral Angles of Trialanine in D_2O Determined by Combining FTIR and Polarized Visible Raman Spectroscopy

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Abstract: We have measured the polarized visible Raman and FTIR spectra of trialanine and triglycine in D_2O at acid, neutral, and alkaline pD. From the Raman spectra we obtained the isotropic and the anisotropic scattering. A self-consistent spectral analysis of the region between 1550 and 1800 cm^{-1} was carried out to obtain the intensities, frequencies, and halfwidths of the respective amide I bands. A model was developed by means of which the intensity ratios of the amide I bands in all spectra and the respective frequency differences were utilized to determine the orientational angle θ between the peptide groups and the strength of excitonic coupling between the corresponding amide I modes. By exploiting results from a recent ab initio study on triglycine (Torii, H; Tasumi, M. *J. Raman Spectrosc.* **1998**, *29*, 81), we used these parameters to determine the dihedral angles ϕ and ψ between the peptide groups. Our results show that trialanine adopts a 3_1-helical structure in D_2O for all of its three protonation states. The structure is insensitive to the carboxylate protonation and changes only slightly with N-terminal protonation. Triglycine is structurally more heterogeneous in the zwitterionic and the cationic state. Our spectral analysis suggests that 3_1-helices coexist with right-handed α-helical and/or with β-turn conformations. The N-terminal protonation stabilizes the 3_1-structure. Our study provides compelling evidence that tripeptides adopt stable conformations in aqueous solution and that they are suitable model systems to investigate the initiation of secondary structure formation.

Introduction

A thorough understanding of protein folding is still one of the major topics of contemporary biochemistry and structural biology. Three of the open questions refer to the early phase of the folding process, which leads to the formation of secondary structure motifs, namely: (1) how many amino acids are necessary to allow for the formation of a stable conformation in aqueous solution, (2) what are the optimum compositions with respect to the most prominent types of secondary structures, and (3) what are the structures of segments constituting the initial step of secondary structure formation and how do they depend on the amino acid composition.[1]

Numerous studies have been undertaken to identify the propensity of the 20 naturally proteinogenic amino acids for one of the natural abundant secondary structures. This was then used as a basis for secondary structure prediction. Chou and Fasman[2] undertook a statistical survey of the crystal structures of 15 proteins to obtain conformational parameters for all amino acid residues, which were used as a measure of their respective propensities. For α-helices the upper 6 of the propensity hierarchy are $E^- > A > L > H^+ > M > Q$, for the β-sheet they obtained $M > V > I > C > Y > F$, and for turn structures $N > D > G > K > S > P$. Slightly different orders of the helix propensity were obtained by O'Neil and DeGrado (A and K as the dominant helix formers)[3] and Lyu et al.,[4] who, by means of guest−host experiments, obtained the order $A > L > M > E > I > V > S$. All these studies agree in pointing to A as a strong helix former. This is supported by the fact that short (16−20 member) alanine-based peptides were found to form stable α-helices in water.[5] Studies on these peptides also reveal that charged side chains can stabilize helical conformations, in particular, if they are positioned close to the terminal groups.[6] Positively charged side chains have to be built in at the C-terminal while negatively charged groups are most appropriate for the N-terminal to provide proper capping.

All these propensity studies are in principle based on the assumption that the stability of a given structure is determined by local interactions. The current literature indicates that this is at least partially the case. Zimmerman and Scheraga[7] compared the calculated probability for bend formations of 47 different amino acid sequences in *N*-acetyl-*N*′-methylamide dipeptides with the occurrence of this structure in real proteins and found good correlations for 26 of these peptides. They also showed that the majority of the remaining peptides had at least one charged residue. This led them to conclude that the solvent−side chain interaction affects their propensity. The notion of local interactions was confirmed by the guest/host experiments of Lyu et al.[4] These findings suggest that it would be very useful to study the secondary structure of short peptide fragments in solution under different conditions (temperature, pH). The

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identification of such structures would also facilitate the identification of initiation sites of protein folding.

For a long period of time it was generally believed that peptide fragments exhibit a diversity of coexisting conformations in solution. One of the few exceptions was the C-peptide of ribonuclease A.[8] The situation changed when it was discovered that synthetic peptides are able to cause an immune response which produces antibodies recognizing the corresponding sequence in the folded protein.[9] Earlier NMR experiments on very short linear peptides in water typically indicated a random distribution of conformations,[10] but two-dimensional NMR has modified this view by suggesting that the conformational space of even tripeptides (e.g., trialanine) is more restricted than originally thought so that structures of limited stability can be formed.[1]

Tripeptides are important model substances for exploring the initial steps of secondary structure formation in protein folding and for identifying the impact of local nearest neighbor interactions between residues on the propensity of peptide segments. Trialanine is of particular importance due to the high helical propensity of its amino acid residues.[2] Interesting insight on the stability of small tri- and tetrapeptides emerged from numerous computational studies. Molecular dynamics calculations on Ac-$(A)_3$-NHMe and Ac-$(V)_3$-NHMe in water were performed by Brooks and associates.[11] For the alanine peptide, they found that an (extended) β-sheet structure at only slightly lower energies than the helical conformation, indicating a much larger helix-forming probability than predicted by the Zimm−Bragg theory.[12] For the valine peptide, the authors obtained a significant stabilization of the β-sheet structure. For both peptides they obtained turn structures as folding intermediates. Ab initio studies on blocked trialanine peptide analogues revealed that a C_7 structure ($\phi = -83°$, $\psi = 63°$) is the most stable one in a reaction field mimicking the influence of the solvent.[13] The right-handed α-helix was obtained at 6.7 kJ/mol higher energies. However, the recent study on *N*-acetyl-L-alanine *N*′-methylacetamide (AAMA) in water suggests that an aqueous solution stabilizes a polyglycine 3_1-helix (P_{II}) like structure with $\phi = -92.7°$ and $\psi = 128.6°$ and a right-handed α-helix with $\phi = -80.3°$ and $\psi = -47.61°$, while the C_7 (γ-turn) structure ($\phi = -81.9°$ and $\psi = 72.3°$) is favored in vacuo.[14] These and other studies strongly indicated that tripeptides are much more capable of adopting rather stable structures in water than indicated even by the above NMR experiments.[1] Moreover, it seems that these structures are closely related to the prominent secondary structure types.

Compared with computational investigations the number of experimental studies particularly by spectroscopic techniques is still rather limited. Results from FTIR, vibrational dichromism, and Raman optical activity measurements[15,16] on tri- and tetraalanine suggested that these peptides exhibit well-defined conformations rather than sampling large parts of the Ramachandran plot, but no information could be drawn with respect to their secondary structures. Only recently, Woutersen and Hamm[17,18] performed femtosecond two-dimensional IR spectroscopy experiments to obtain $\phi = -60°$ and $\psi = 140°$ for the fully protonated state of trialanine in D_2O from the excitonic coupling of amide I′. Their result is close to the $3_1(P_{II})$ structure predicted by ab initio calculations on blocked trialanine analogues.[14]

In the present study we employed an alternative and novel spectroscopic method for determining the dihedral angles of tripeptides. It combines conventional polarized visible Raman with FTIR spectroscopy. For the sake of comparison with the work of Woutersen and Hamm[17,18] we applied our method to trialanine in D_2O but extended the focus by investigating all protonation states of this peptide. We also investigated triglycine, which is the simplest tripeptide and serves as a reference system, since it is expected to show a maximum of conformational flexibility. Our results enabled us to determine the secondary structures of the peptides investigated and their dependence on the protonation of the terminal groups.

Theoretical Background

In the following we confine ourselves to a brief description of the theoretical background and the underlying physical principles and assumptions of the method by which we obtain the excitonic coupling energy and the angle between the two peptide groups from FTIR and polarized visible Raman spectra of tripeptides. More details on the somewhat tedious mathematics used to obtain the reported equations will be given in a forthcoming paper.[19]

As Woutersen and Hamm[17,18] we assume that the two amide I modes of the peptide groups interact via transition dipole and through bond coupling.[20] If we neglect interactions with other normal modes, the Schrödinger equation reads as:

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

where $\hat{H}_0$ is the Hamiltonian for the two unperturbed amide I modes, which are describable by the vibrational wave functions $\chi_1(Q_1)$ and $\chi_2(Q_2)$ (Q_1 and Q_2 denote nuclear coordinates). $\hat{H}'$ accounts for the coupling between the two modes. In the matrix representation the total Hamiltonian is written as:[17,18]

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

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}. H can be diagonalized by standard procedures to yield the following expressions for the new eigenstates:

$$|\chi_-\rangle = \cos\nu|\chi_1\rangle - \sin\nu|\chi_2\rangle$$
$$|\chi_+\rangle = \sin\nu|\chi_1\rangle + \cos\nu|\chi_2\rangle \tag{3}$$

and eigenenergies:

$$\tilde{\nu}_\pm = \frac{\tilde{\nu}_1 + \tilde{\nu}_2}{2} \pm \sqrt{\frac{\Delta_0^2}{4} + \Delta^2} \tag{4}$$

where Δ_0 is the energy difference between the unperturbed amide I modes. The parameter ν describes the degree of mixing between χ_1 and χ_2, which is maximal for $\nu = 45°$. This requires the unperturbed modes to be accidentally degenerated. χ_+ and

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χ_- are the vibrational wave functions of the in-phase (ip) and out-of-phase (oop) combination of the interacting modes.

The mixing parameter ν was determined as follows. As described in the Materials and Methods section, we measured the Raman scattering polarized parallel (I_x) and perpendicular (I_y) to the polarization of the exciting laser beam. From this one obtains the isotropic ($I_{iso} = I_x - {}^4/_3 I_y$) and anisotropic scattering ($I_{aniso} = I_y$). *Per definitionem* the intensity ratio of the isotropic Raman bands of χ_- and χ_+ is independent of the orientation between the two peptide groups. Provided that the isotropic scattering is identical for the uncoupled modes a straightforward calculation yields:

$$\sin 2\nu = \frac{1 - R_{iso}}{1 + R_{iso}} \tag{5a}$$

with

$$R_{iso} = \frac{I^-_{iso}}{I^+_{iso}} \tag{5b}$$

where I^-_{iso} and I^+_{iso} are the isotropic intensities of χ_- and χ_+. The coupling energy Δ is related to the mixing parameter ν by:

$$\Delta = \frac{\Delta_{exp}}{2\sqrt{1 + 4/\tan 2\nu}} \tag{6}$$

where Δ_{exp} is the difference between the experimental band positions. The mathematical background of these equations will be published elsewhere.[19] As one reads from eq 5 the isotropic intensity of the oop-vibration disappears for maximal mixing ($\nu = \pi/4$) as expected.

In contrast to the isotropic scattering, the infrared intensities of the delocalized states depend on the orientational angle θ between the transition dipole moments of the amide **I** modes. This results from the fact that:

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

$$I^+_{IR} \propto \left(\sin\nu \frac{\partial\vec{\mu}}{\partial Q_1} + \cos\nu \frac{\partial\vec{\mu}}{\partial Q_2}\right)^2 \tag{7}$$

where $\partial\vec{\mu}/\partial Q_1$ and $\partial\vec{\mu}/\partial Q_2$ are the transition dipole moments of the uncoupled amide I modes. Since the intrinsic IR intensities of the uncoupled modes are identical,[18] elementary vector algebra yields:[19]

$$\cos\theta = \frac{1 - R_{IR}}{\sin 2\nu(1 + R_{IR})} \tag{8}$$

where $R_{IR} = I^-_{IR}/I^+_{IR}$. With the knowledge of the mixing parameter from the isotropic Raman scattering we used eq 8 to determine the angle between the transition dipole moments.

The anisotropic Raman scattering also depends on θ. To estimate the corresponding contribution to the Raman tensor of χ_+ and χ_- we rotated the respective coordinate system of peptide 2 into that of peptide 1 by an orthogonal transformation. Hence, the Raman tensor of oop and ip is calculated by:

$$\alpha^- = \cos\nu\cdot\alpha_1 - \sin\nu\cdot R(\theta)^T\,\alpha_2 R(\theta)$$

$$\alpha^+ = \sin\nu\cdot\alpha_1 + \cos\nu\cdot R(\theta)^T\,\alpha_2 R(\theta) \tag{9}$$

where $R(\theta)$ is the rotational matrix and R^T is its transposed. If we assume that the anisotropic Raman scattering is identical for the uncoupled modes and that their Raman tensor can be approximated by:[19]

$$\alpha = \begin{pmatrix} a & 0 & 0 \\ 0 & a & 0 \\ 0 & 0 & 0 \end{pmatrix} \tag{10}$$

then a tedious but straightforward calculation yields:

$$\cos 2\theta = \frac{4(1 - R_{aniso}) - (1 + R_{aniso})\sin 2\nu}{3(1 + R_{aniso})\sin 2\nu} \tag{11}$$

where $R_{aniso} = I^-_{aniso}/I^+_{aniso}$. This equation can be used to check the determination of θ from the IR spectrum. In eqs 9−11 we neglect the fact that even for the fully extended structure the transition dipole moments are not exactly parallel.

It should be noted that our model can easily be extended to account for excitonic coupling in higher order peptides, in particular because Torii and Tasumi[20] have shown that only nearest neighbor and second neighbor interactions have to be taken into consideration. This will be described in detail in a future publication.[19]

Materials and Methods

Materials. Alanylalaninealanine (AAA) and glycylglycineglycine (GGG) were purchased from Bachem Bioscience Inc. (>98% purity) and used without further purification. D_2O and $NaClO_4$ were obtained from Sigma-Aldrich Chemical company (St. Louis, MO). All chemicals were of analytical grade. The peptides were dissolved in D_2O at a concentration of 0.3 M. The pD of the solutions was adjusted to 1, 6, and 12 by adding DCl or NaOD to obtain the cationic, zwitterionic, and anionic state of the peptide. The pD values were determined by utilizing the method of Glasoe and Long[21] to correct the values obtained from pH electrode measurements. For the Raman experiments the solvent contained 0.25 M $NaClO_4$ whose 934 cm^{-1} Raman band was used as an internal standard.[22]

Methods. (a) Raman Spectroscopy. 457.9-nm excitation (300 mW) was obtained from an argon ion laser (Lexel). A laser filter was used to eliminate plasma lines. The polarized exciting laser beam was focused onto the sample with a lense of 100 mm focus length. The Raman scattered light was collected in a 135° backscattering geometry. The scattered radiation was imaged onto the entrance slit (width adjusted to 100 μM) of a triple-grating spectrometer (T64000, Jobin Yvon Inc.). A polarization analyzer followed by a scrambler between the collimator and the entrance slit of the spectrometer were employed to measure the Raman intensity polarized parallel (I_x) and perpendicular (I_y) to the scattering plane. The scattering light was dispersed by the spectrometer and then detected by a liquid-cooled charge-coupled device (CCD) with 256 × 1024 pixels in the chip. The spectral resolution was about 3.2 cm^{-1}. The frequency calibration of the recorded Raman spectra was checked by means of the 934 cm^{-1} band of the internal standard, the frequency of which had been determined earlier with high accuracy.[22]

(b) IR Spectroscopy. FTIR spectra were measured with a Nicolet Magna-IR System 560 optical bench as described.[23] A total of 256 scans at 2 cm^{-1} resolution using Happ-Ganzel apodization were averaged to obtain each spectrum. For all experiments, a Spectra Tech liquid cell equipped with CaF_2 windows and 15-μm thick spacers were used. The

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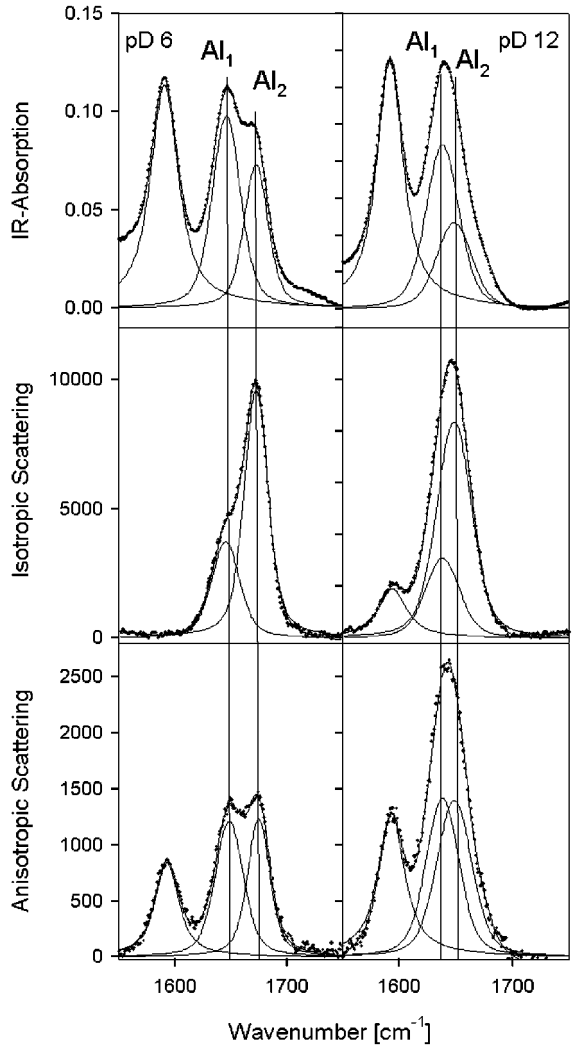

Figure 1. FTIR (upper panel), isotropic, and anisotropic Raman spectra ($\lambda_{exc} = 457$ nm) of trialanine measured at the indicated pD. The solid lines and the band profiles arise from the fitting procedure described in the text.

peptide sample was put between CaF_2 windows. Each peptide sample was measured at least four times. Spectra were corrected for the solvent background in an interactive manner with use of Nicolet OMNIC 3.1 software.

(c) Spectral Analysis. All spectra were analyzed by using the program MULTIFIT.[24] They were normalized to the internal standard, i.e., the ClO_4^- band at 934 cm^{-1}. To eliminate solvent contributions we measured the solvent reference spectra for both polarizations, which were then subtracted from the corresponding peptide spectra. The intensities of the normalized polarized Raman bands were derived from their band areas and used to calculate the depolarization ratio. The isotropic and anisotropic Raman spectra were obtained from the polarized spectra as described in the paper. These and the corresponding IR spectra were self-consistently analyzed in that they were fitted with a set of identical frequencies, halfwidths, and band profiles. The intensities of the polarized bands were derived from their band areas.

Results

Secondary Structure of Trialanine. The IR and Raman spectra of the zwitterionic and the alkaline state of trialanine in D_2O and the results of the spectral analysis are shown in Figure 1. Protonation of the carboxylate group causes only small changes of the spectral parameters (data not shown). The two amide I bands are clearly discernible in the spectra of the zwitterionic and cationic species. Their intensity ratio is very different in the isotropic and anisotropic Raman and IR spectrum due to excitonic coupling between the amide modes of the two peptide groups. We first used eq 5a to determine the mixing parameter ν and subsequently the orientational angle θ by means of eqs 8 and 11. The agreement between the thus obtained values is remarkable (122° from the IR and 123° from the anisotropic Raman spectrum). This corroborates the validity of the spectral analysis and the underlying assumptions. The situation is more difficult for the sample at alkaline pD. The deprotonation of the N-terminal group causes the two bands to nearly coalesce into a single band at lower frequencies. A broad variety of spectral parameters can be used to obtain fits of comparable quality. It turned out, however, that only a few of these fits provided consistent and meaningful solutions of eqs 5a and 8. This set can be reduced further to a very small range of spectral parameters by requiring consistency between the solutions of eqs 8 and 11. Thus, very reliable parameters were obtained despite the difficulties with the spectral analysis.

Table 1. Spectroscopic and Structural Parameters Obtained from the Analysis of the FTIR, Isotropic, and Anisotropic Raman Spectra of Trialanine and Triglycine at the PD Indicated

(A) Trialanine

	pD 1	pD 6	pD 12
$\tilde{\nu}_1$ [cm^{-1}]	1652	1649[a]	1638
$\tilde{\nu}_2$ [cm^{-1}]	1676	1675[a]	1649
Γ_{G1} [cm^{-1}]	21.3	23.4	29.6
Γ_{G2} [cm^{-1}]	18.9	17.2	30.5
Δ [cm^{-1}]	4.0	4.2	1.8
θ [deg]	119	122	128
ϕ [deg]	−66	−70	−95
ψ [deg]	140	145	150

(B) Triglycine

	pD 6	pD 12
$\tilde{\nu}_1^A$ [cm^{-1}]	1638	1640
$\tilde{\nu}_2^A$ [cm^{-1}]	1683	1662
$\tilde{\nu}_1^B$ [cm^{-1}]	1653	
$\tilde{\nu}_2^B$ [cm^{-1}]	1673	
Γ_{G1} [cm^{-1}]	13.0	24.3
Γ_{G2} [cm^{-1}]	19.2	27.2
Δ^A [cm^{-1}]	9.2	3.3
θ^A [deg]	30[a]	115[b]
Δ^B [cm^{-1}]	3.2	
θ^B [cm^{-1}]	115[b]	
ϕ^A [deg]	−15/110	−75
ψ^A [deg]	−57/65	150

[a] Obtained from the IR spectrum. [b] Average of the values obtained from the IR and anisotropic Raman spectra.

The values for Δ and θ are listed in Table 1. It should be noted that we were also able to determine the sign of these parameters. Δ is positive, because the lower intensity of the low-frequency band in the isotropic Raman spectrum suggests that it has to be assigned to the oop combination of the two amide I modes.[20] θ can be determined unambiguously from eq 8 (not from eq 11, which always has two solutions). Both parameters depend on the dihedral angle between the peptide groups, which we obtained by adopting the procedure of Woutersen and Hamm.[17,18] These authors have utilized results from recent ab initio calculations on a triglycine peptide in vacuo by Torii and Tasumi,[20] who calculated the force constants reflecting amide I excitonic coupling as a function of ϕ and ψ. Woutersen and Hamm superimposed their contour plots with that reflecting the relationship between the dihedral angles and

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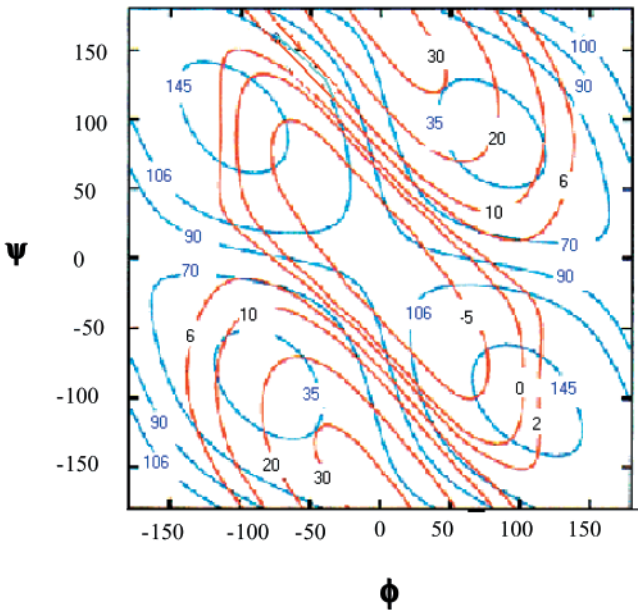

Figure 2. Contour plots of the coupling constant Δ and the orientational angle θ as a function of the dihedral angles ϕ and ψ (modified after Woutersen and Hamm[17,18]).

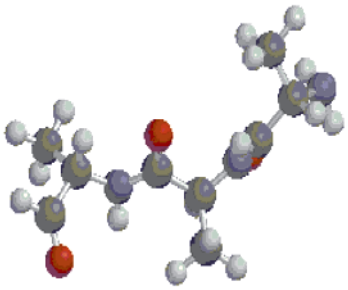

Figure 3. Turn structure of trialanine inferred from the IR and Raman spectra.

the orientational angle θ. A modified copy of their plot is shown in Figure 2. For the zwitterionic as well as for the cationic state the values obtained for Δ and θ correspond either to the upper left or lower right of the Ramachandran plot. Since the latter does not correspond to any biological structure we disregard this possibility. Thus, we end up with a very small area of possible ϕ and ψ values for the protonated and zwitterionic states, the center values of which are −66° and 140°, respectively (Table 1).

Our results are in reasonable agreement with those of Woutersen and Hamm[17,18] and suggests that the fully protonated and zwitterionic peptide exhibits a 3_1 helix structure (Figure 3) comparable with polyglycine II (P_{II}). In the Ramachandran plot this conformation lies very close to the region sampled by the $(\phi,\psi)_2$ angles of type II β-turns. The protonation of the N-terminus causes a structural change, which reduces the exciton coupling and slightly increases the orientational angle between the peptide bonds. Our values for Δ and θ are consistent with two structures. One of them is somewhat more extended than the zwitterionic state, but still 3_1 helical, and exhibits dihedral angles of $\phi = -95°$ and $\psi = 150°$. The second possible structure has $\phi = -100°$ and $\psi = 55°$. Though it is in proximity to the C_7 structure obtained for AAMA in vacuo,[14] we disregard this solution for the following reasons. First, all ab initio calculations suggest that this structure is not stabilized in water.[13,14] Second, Raman optical activity data by Ford et al.[16]

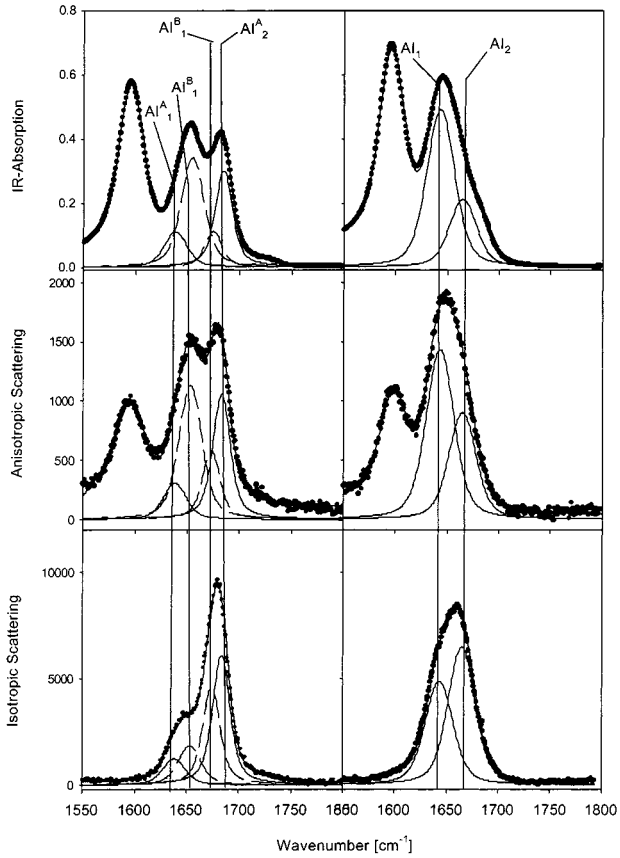

Figure 4. FTIR (upper panel), isotropic, and anisotropic Raman spectra ($\lambda_{exc} = 457$ nm) of triglycine measured at the indicated pD. The solid lines and the band profiles arise from the fitting procedure described in the text.

are not indicative of major structural changes due to the N-terminal protonation. Third, the dihedral angels of the latter are close to those obtained from ab initio calculations of AAMA, which is certainly a good model substance for the deprotonated state of trialanine.[14]

While the isotropic and the anisotropic spectra of the protonated and the deprotonated species could be fitted with the same spectral parameters, the anisotropic amide I′ bands of the zwitterion are downshifted by 1−2 cm^{-1} with respect to their band positions in the isotropic spectrum. The small discrepancy likely reflects some conformational heterogeneity, i.e., peptides with another structure coexist with the 3_1 species. The former give rise to a more isotropic low-frequency band which causes the entire isotropic amide I spectrum to exhibit slightly higher peak frequencies. Since this shift is small the concentration of this additional conformer must be much lower than that of the 3_1 helix and it is therefore impossible to extract its spectral parameters. From the fact that such a discrepancy between isotropic and anisotropic spectra does not exist for the anionic and cationic states, we can conclude that the obtained structures are by far the most stable ones in aqueous solution.

Secondary Structure of Triglycine. To study the influence of conformational flexibility on the amide I spectra we also investigated the three protonation states of triglycine. Computational evidence suggests that this peptide occupies more conformational space than trialanine due to the absence of sterically demanding residues.[25] Figure 4 shows the IR and Raman spectra of the zwitterionic and the anionic state of

(25) Schulz, G. E.; Schirmer, R. H. *Principle of Protein Structure*; Springer: Berlin, Germany, 1979.

triglycine in D_2O and the results of their spectral decomposition. The spectra of the cationic and the zwitterionic state are again very similar. For both, an attempt to fit all corresponding spectra with the same set of parameters failed, because the frequency positions of two discernible amide I bands are significantly different in the isotropic and anisotropic spectrum. In accordance with expectations this indicates conformational heterogeneity, i.e., more than one conformer contributes significantly to the spectrum. The spectral analysis for the neutral triglycine is not straightforward since we have no a priori knowledge about how many conformers coexist. In the following we consider two extreme cases, i.e., (1) the coexistence of multiple conformers with various dihedral angles mostly in the β-sheet and helical region of the Ramachandran plot and (2) only two clearly discernible conformers exist which represent different secondary structure motifs. Model 1 can be disregarded because Figure 2 shows that even relatively small changes of the dihedral angles give rise to significant alterations of Δ which would cause a much larger broadening of the Raman and IR spectra than obtained. If model 2) were to apply, all spectra must be composed of four bands. To reduce the ambiguity of a fit with such an extended model we assumed identical Lorentzian and Gaussian halfwidths for the two bands arising from the oop and ip combinations of the isolated peptide modes, respectively. Even with this restriction, we did not obtain a unique fit of the three spectra. Fortunately, however, the range of possible frequencies could again be substantially narrowed by demanding that the obtained intensity ratios and frequencies yield a consistent and meaningful solution of eqs 5, 8, and 11. We could not totally eliminate discrepancies between the θ-values obtain from the IR and anisotropic scattering spectra, but they are close enough to allow a consistent identification of the secondary structure.[26] The obtained values for Δ and θ are listed in Table 1B. For conformer A they are consistent with two possible structures, i.e., a right-handed α-helix with a somewhat large ϕ angle ($\phi = -115°$, $\psi = -57°$) and with the $(\phi,\psi)_3$ angles of type II β-turns ($\phi = 110°$, $\psi = 65°$). Computational studies suggest that both conformations are indeed possible for glycine-based peptides.[25] For conformer B we found $\phi = -64°$ and $\psi = 135°$, which is again a 3_1 helix.

The corresponding spectra of the anionic species measured at alkaline pD could be fitted with identical spectral parameters. This indicates that a single conformation is predominant, which resembles that of the anionic trialanine but with a somewhat smaller ϕ-value.

The analysis of the triglycine spectra is certainly on a less strong footing than that of the trialanine spectra due to the occurrence of conformational heterogeneity. The result of our simple two-conformer model, however, makes sense, because the obtained structures lie well within the allowed part of the Ramachandran plot.

Discussion

This study introduces conventional polarized Raman and IR spectroscopy as comparatively cheap and easy to handle tools for a rather precise determination of the secondary structure of small peptides. The obtained results show that the two simplest tripeptides triglycine and trialanine adopt well-defined structures in water, in contrast to what has been suggested from results of NMR experiments.[1] Trialanine exhibits a polyglycine II like 3_1-helix that is not very different from conformations found in the first half of type II β turn structures. This structure is maintained for all three protonation states though with some minor differences between the zwitterionic and the fully protonated states. Interestingly, the ϕ-angle is already very close to the optimum value of right-handed α-helices so that a transition to this structure involves solely changes in ψ. Triglycine was found to be somewhat heterogeneous in its cationic and zwitterionic state. An analysis with a minimum model yielded the coexistence of 3_1-helices with right-handed α-helices and/or with conformations resembling the second half of type II β-turn structures. This heterogeneity is eliminated at alkaline pD in that the N-terminal deprotonation stabilizes a 3_1 helix.

Several classical and quantum mechanical computational studies have been carried out on blocked tripeptides to obtain the most likely conformations in vacuo and in aqueous solution. Tobias and Brooks[11] performed molecular dynamics simulation for Ac-AlaAla-NHMe and Ac-ValVal-NHMe. They identified three different minima corresponding to an extended β-sheet conformation, a right-handed α-helix, and a reverse turn structure, respectively. For both peptides, the extended conformation is predicted to be lowest in energy, but the energy difference between extended and helical structure was found to be small for Ac-AlaAla-NHMe. The reverse turn was identified as an intermediate in the "folding" pathway between β-sheet and α-helix. Head-Gordon and co-workers[13] performed ab initio calculations on blocked triglycine and trialanine analogues in vacuo and in a water representing reaction field. They found that the latter stabilizes right- as well as left-handed helical structures and obtained the global minimum for a C_7 structure. More recently, Han et al. conducted a very detailed ab initio study on *N*-acetyl-L-alanine *N*′-methylamide in water.[14] This molecule can be considered as a suitable model for trialanine. By comparing computational results with experimentally obtained vibrational spectra (IR, Raman, Raman optical activity) the authors found that a polyglycine II like structure (termed P_{II} in their paper) is stabilized in water. This is in excellent agreement with our results on trialanine. Altogether the above computational studies and our results corroborate each other in suggesting that tripeptides adopt well-defined structures in water rather than sampling large parts of the Ramachandran space. This indicates that they can be used as model substances to study (1) the contribution of local interactions for the initiation of secondary structure formation in folding processes and (2) the propensity of amino acid residues to form secondary structures in different solvents.

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(26) A preliminary calculation for which we dropped the assumption that the *xx* and *yy* component of the Raman tensor are identical substantially narrowed the gap between the θ-values obtained from the IR and the anisotropic spectrum.