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Published on Web 11/07/2002

Tripeptides Adopt Stable Structures in Water. A Combined Polarized Visible Raman, FTIR, and VCD Spectroscopy Study

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Received June 20, 2002

Abstract: We have measured the band profile of amide I in the infrared, isotropic, and anisotropic Raman spectra of L-alanyl-D-alanyl-L-alanine, acetyl-L-alanyl-L-alanine, L-vanyl-L-vanyl-L-valine, L-seryl-L-seryl-L-serine, and L-lysyl-L-lysyl-L-lysine at acid, neutral, and alkaline pD. The respective intensity ratios of the two amide I bands depend on the excitonic coupling between the amide I modes of the peptide group. These intensity ratios were obtained from a self-consistent spectral decomposition and then were used to determine the dihedral angles between the two peptide groups by means of a recently developed algorithm (Schweitzer-Stenner, R. Biophys. J. **2002**, *83*, 523−532). The validity of the obtained structures were checked by measuring and analyzing the vibrational circular dichroism of the two amide I bands. Thus, we found two solutions for all protonation states of trialanine. Assuming a single conformer, one obtains a very extended β-helix-like structure. Alternatively, the data can be explained by the coexistence of a 3_1-(PII) and a β-sheet-like structure. Acetyl-L-alanyl-L-alanine exhibits a structure which is very similar to that obtained for trialanine. The tripeptide with the central D-alanine adopts an extended structure with a negative ψ and a positive ϕ angle. Trivaline and triserine adopt single β_2-like structures such as that identified in the energy landscape of the alanine dipeptide. Trilysine appears different from the other investigated homopeptides in that it adopts a left-handed helix which at acid pD is in part stabilized by hydrogen bonding between the protonated carboxylate (donor) and the N-terminal peptide carbonyl. Our result provides compelling evidence for the capability of short peptides to adopt stable structures in an aqueous solution, which at least to some extent reflect the intrinsic structural propensity of the respective amino acids in proteins. Furthermore, this paper convincingly demonstrates that the combination of different vibrational spectroscopies provides a powerful tool for the determination of the secondary structure of peptides in solution.

Introduction

For a long time, it was generally believed that peptide fragments exhibit a variety of coexisting conformations in vacuo as well as in solution.[1−7] One of the few exceptions was the C-peptide of ribonuclease A.[8] The situation changed somewhat 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,[2,4] but two-dimensional NMR has modified this view by providing evidence that the conformational space of even tripeptides such as trialanine is more restricted than originally thought so that structures of limited stability can be formed.[10]

A detailed knowledge of the preferred conformations of small peptide fragments is of multiple biological relevance. First, it will aid in broadening the experimental basis for determining the intrinsic propensity of amino acids for the most prominent secondary structures which thus far is mostly determined by statistical analyses of proteins.[11,12] Second, it will provide a sound basis for computational work dedicated to explore the contributions of local peptide−peptide and the amino acid−solvent interactions to the respective conformational preference.[13] Third, it will be useful for identifying the initiation sites of the secondary structure formation during the folding process.[10]

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10.1021/ja027381w CCC: $22.00

Interesting information about the stability of small tri- and tetrapeptides emerged from numerous computational studies. Only a few particularly relevant results for our project can be mentioned. Molecular dynamics calculations on Ac−$(A)_{3-}$-NHMe and Ac−$(V)_{3-}$NHMe in water were performed by Brooks and associates.[14,15] For the alanine peptide, they found that an (extended) β-sheet structure is only at slightly lower energies than the helical conformation, indicating a much larger helix formation probability than predicted by the Zimm−Bragg theory.[16] 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 a blocked trialanine peptide analogue (called alanine dipeptide, ADP, in the following) revealed that a C_7 structure ($\phi = -82°$, $\psi = 59°$) is the most stable one in a reaction field mimicking the influence of the solvent.[17] The right-handed α-helix was obtained at 6.7 kJ/mol higher energies. However, a recent DFT study on ADP hydrogen bonded to water in a reaction field suggests that an aqueous solution stabilizes the P_{II} structure with (ϕ,ψ) = (−93.5°, 127.6°) as well as an α_R-like structure (ϕ, ψ) = (−82°, −44°) , while the C_7 (γ-turn) structure (ϕ, ψ) = (−81.9°, 72.3°) is favored in vacuo.[18] Very recently, Mu and Stock performed for the first time an MD-simulation on unblocked cationic trialanine in water and obtained coexisting PII (ϕ, ψ) = (−67°, 132°), β (ϕ, ψ) = (−122°, 130°), and α_R-conformers (ϕ, ψ) = (−76°, −44°).[19] These studies strongly indicated that tripeptides are capable of adopting rather stable structures in water, which are closely related to the prominent secondary structure types. This notion is corroborated by results of recent NMR/ CD studies on oligopeptides with a seven alanine[20] and a seven lysine motif,[21] respectively, which both reveal a PII structure as the most populated conformer.

Several spectroscopic studies particularly on model peptides have been undertaken but they remained inconclusive with respect to the determination of dihedral angles.[22−24] The situation, however, has changed over the last two years. First, Woutersen and Hamm[25] obtained a PII (3_1 helix) like structure for cationic trialanine in water by exploring the excitonic states of amide I by coherent multidimensional IR-spectroscopy. Subsequently, our research group obtained similar structures for all protonation states of L-alanyl-L-alanyl-alanine (AAA) by combining isotropic Raman scattering, IR-absorption, and earlier results from ab initio studies.[26] Very recently, we obtained a modified picture for this tripeptide from a combined analysis of amide I by IR absorption and isotropic and anisotropic Raman scattering, that is, somewhat more extended PII-like structures which bears some similarity with a β-helix.[27] However, the differences to the earlier results[29] are quantitative rather than qualitative. Gnanakaran and Hochstrasser[28] combined the same method with detailed computational studies to obtain a mixture of PII- and α_R-like conformers for ADP, in excellent accordance with the DFT study from Han et al.[18] The C-terminal ψ-angle of dialanine in water was recently obtained by exploiting the structural sensitivity of amide III.[29,30]Altogether, these studies provide compelling evidence for stable structures of small peptides in water. Moreover, they demonstrate that vibrational spectroscopy can be used to quantitatively determine their secondary structure.

In the present paper, we use our previously developed algorithm[27] to determine the structure of a series of homo-tripeptides in water from the amide I band profile in their Raman and FTIR-spectra. For some of the investigated tripeptides, we additionally performed and analyzed the vibrational circular dichroism (VCD) spectra to check the results of the structure analysis. First, we investigated the modified trialanine peptides acetyl-L-alanyl-L-alanine (AcAA) and L-alanyl-D-alanyl-L-alanine (AA^DA) and compared them with AAA to determine the influence of the C-terminal group and of D-alanine substitution on the structure of trialanine. Second, we explored the structure of L-vanyl-L-vanyl-L-valine (VVV), L-seryl-L-seryl-L-serine (SSS), and L-lysyl-L-lysyl-L-lysine (KKK) as representatives of tripeptides with aliphatic, polar, and charged amino acid residues. To make use of the structural sensitivity of amide III,[29] we investigated some of these peptides (AAA, AA^DA, VVV, KKK) in H_2O.

To avoid confusion, we emphasize that our designation of peptides is determined by the number of amino acids, that is, a tripeptide contains three amino acids and two peptide groups.

Theoretical Background

Excitonic Coupling of Amide I Modes. The theory used to obtain the dihedral angles of tripeptides from the amide I bands in their visible Raman and IR-spectra has been described in detail elsewhere.[27] Only the basic principles are briefly summarized in the following.

We assume a two-oscillator model to describe the mixing between the two amide I modes of tripeptides by transition dipole and through bond coupling.[31] The corresponding excitonic states are written as:

$$|\chi_-\rangle = \cos\nu|\chi_1\rangle - \sin\nu|\chi_2\rangle$$
$$|\chi_+\rangle = \sin\nu|\chi_1\rangle + \cos\nu|\chi_2\rangle \qquad (1)$$

The parameter ν describes the degree of mixing between the unperturbed states $|\chi_1\rangle$ and $|\chi_2\rangle$, which is maximal for $\nu = 45°$. This requires the unperturbed modes to be accidentally degenerated. $|\chi_+\rangle$ and $|\chi_-\rangle$ are the excitonic states of the in-phase (ip) and out-of-phase (oop) combination of the interacting modes.

The mixing parameter ν can be determined from the intensity ratio $R_{iso} = I^-_{iso}/I^+_{iso}$ of the two amide I bands in the spectrum of isotropic Raman scattering (I^-_{iso} and I^+_{iso} are the isotropic

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intensities of $|\chi_-\rangle$ and $|\chi_+\rangle$). In the next step, we use the mixing parameter and the intensity ratio $R_{IR} = I^-_{IR}/I^+_{IR}$ in the FTIR spectrum to obtain the angle $\tilde{\theta}$ between the transition dipole moments of amide I. Third, we made use of the fact that intensity ratio R_{aniso} of the amide I in anisotropic Raman spectrum depends on the mixing parameter and on the orientation angle θ between the peptide normals. We calculate R_{aniso} as a function of θ and compared the result with the experimental value. Thus, one generally obtains two values θ_1 and θ_2. In the final step, we calculate θ and $\tilde{\theta}$ as functions of the dihedral angles ϕ and ψ. This normally yields two pairs of values, which reproduce the obtained orientational angles; one pair corresponding to $\tilde{\theta}$ and θ_1, the other one to $\tilde{\theta}$ and θ_2. Two or three of the four solutions can generally be excluded because they correspond to forbidden regions of the Ramachandran space. All the mathematical details are reported in ref 27.

One comment is relevant in this context. In view of the well-established fact that through bond coupling contributes to amide I mixing, one may argue that it is inappropriate to use the coupled oscillator model with a single coupling constant. The breakdown of the coupled oscillator model even for tripeptides has been proposed by Keiderling and associates on the basis of VCD-experiments.[32,33] We think that our approach is justified because IR absorption and Raman scattering mostly reflect the contributions of CO (IR, Raman) and CN (Raman) stretch to the eigenvectors.[34,35] We performed DFT based normal mode calculations for various trialanine structures in vacuo and obtained comparable mixing of these two coordinates for $|\chi_+\rangle$ and $|\chi_-\rangle$ (Schweitzer-Stenner, unpublished). In the following, we introduce a somewhat modified coupled oscillator model for VCD, by which we were able to explain our data.

VCD-Signal of Amide I. In absence of any intrinsic chirality (no VCD signal of the unperturbed amide I), excitonic coupling creates a VCD couplet. For a coupled oscillator, one obtains for the rotational strength of $|\chi_+\rangle$ and $|\chi_-\rangle$:[36]

$$R^{\pm} = \mp \frac{1}{2}\cdot\sin 2\nu\cdot\pi\tilde{\nu}_0\vec{T}_{12}\cdot(\Delta\vec{\mu}_1 \times \Delta\vec{\mu}_2) \qquad (2)$$

where $\tilde{\nu}_0$ is the average wavenumber of the two amide I bands, $\vec{T}_{12}$ is the distance vector between the two oscillators, and $\Delta\vec{\mu}_{1,2}$ are their transition dipole moments. Chirality is brought about by the different orientation of the two transition dipoles; it disappears for $\tilde{\theta} = 0°$. Generally, eq 2 yields a negative signal for $|\chi_+\rangle$ and a positive one of equal intensity for $|\chi_-\rangle$ in the case of extended structures, while it is just the other way round if the structure is helical.[33]

As shown below, the amide I of the C-terminal group exhibits some rotational strength depending on the protonation state. To take this into account, we modified eq 2 to obtain

$$R^- = \Delta\vec{\mu}_1\Delta\vec{m}_1\cos^2(\nu) - {}^1/_2\cdot\Delta\vec{\mu}_2\Delta\vec{m}_1\sin(2\nu) + {}^1/_2\cdot\sin(2\nu)\cdot\pi\tilde{\nu}_0\vec{T}_{12}\cdot(\Delta\mu_1 \times \vec{\mu}_2)$$

$$R^+ = \Delta\vec{\mu}_1\Delta\vec{m}_1\sin^2(\nu) + {}^1/_2\cdot\Delta\vec{\mu}_2\Delta\vec{m}_1\sin(2\nu) - {}^1/_2\cdot\sin(2\nu)\cdot\pi\tilde{\nu}_0\vec{T}_{12}\cdot(\Delta\vec{\mu} \times \Delta\vec{\mu}_2) \quad (3)$$

where $\Delta\vec{m}_1$is the intrinsic magnetic transition dipole moment associated with the C-terminal amide I. For $\Delta\vec{m}_1 \neq 0$, one obtains an asymmetric VCD-couplet.

The band shape of the amide I couplet is written as

$$\Delta\epsilon = \frac{\tilde{\nu}_0}{2.3\cdot10^{-39}}\left[\frac{R^-}{\sigma_-\sqrt{2\pi}}\exp\left(\frac{-(\tilde{\nu}-\tilde{\nu}_-)^2}{2\cdot{\sigma_-}^2}\right) + \frac{R^+}{\sigma_+\sqrt{2\pi}}\exp\left(\frac{-(\tilde{\nu}-\tilde{\nu}_+)^2}{2\cdot{\sigma_+}^2}\right)\right] \quad (4)$$

We thus approximated the individual bands by Gaussian centered at $\tilde{\nu}_-$ (out-of-phase) and $\tilde{\nu}_+$ (in phase) thus neglecting the small Lorentzian contribution to the amide I band shape.[26] σ_- and σ_+ are the half-half-widths of the Gaussian bands. The conversion factor in the denominator accounts for rotational strengths expressed in units of [esu^2·cm^2].[37]

Error Analysis. A detailed estimation of the error intervals for the ϕ and ψ values obtained from the IR and Raman data has been given in ref 27. We followed the same procedure to obtain the statistical errors for the dihedral angles of the tripeptides investigated in the present study (Table 1). Because of the highly nonlinear propagation of errors, asymmetric error intervals are obtained.

Material and Methods

Materials. **L**-Alanyl-**L**-alanine-**L**-alanyl (AAA), acetyl-**L**-alanyl-**L**-alanine (AcAA), **L**-alanyl-**D**-alanyl-**L**-alanine (AA^{D}A), vanylvanylvaline (VVV), lysyllysyllysine (KKK), and serylserylserine (SSS) were purchased from Bachem Bioscience Inc. (>98% purity). AAA, AcAA, AA^{D}A, and KKK were used without further purification. $NaClO_4$ were obtained from Sigma-Aldrich Chemical company (St. Louis, MO). All chemicals were of analytical grade. The peptides were dissolved in D_2O and H_2O at a concentration between 0.2 and 0.5 M. The pD and pH of the solutions were adjusted by adding small aliquots of DCl or NaOD and HCl or NaOH, respectively, to obtain the cationic, zwitterionic, and anionic states of the peptides. The pD values were determined by utilizing the method of Glasoe and Long[38] to correct the values obtained from pH electrode measurements. For the Raman experiments, the solvent contained 0.25−0.1 M $NaClO_4$ whose 934 cm^{-1} Raman band was used as an internal standard.[39] Additionally, we recorded the polarized Raman spectra of AAA, AA^{D}A, VVV, and KKK in H_2O at acid, neutral, and alkaline pH.

Methods. Raman Spectroscopy. 457.9 and 488-nm excitations (300 mW, 1 W) were obtained from an argon ion laser (Lexel). Laser filters were used to eliminate plasma lines. The polarized exciting laser beam was focused onto the sample with a lens 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 collimator and the entrance slit of the spectrometer were employed to

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Table 1. Spectral and Structural Parameters of the Investigated Tripeptides

	AAA[a] (+)[b]	AAA[a] (+,-)[b]	AAA[a] (–)[b]	AA^{D}A (+)[b]	AA^{D}A (+-)[b]	AA^{D}A (–)[b]	AcA A (–)[c]	VV V (+)[b]	VVV (+-)[b]	VVV (–)[b]	SSS (–)[b]	KK K (4+)[d]	KK K (-,3+)[d]
$\tilde{\nu}_1$[cm^{-1}]	1652	1646/ 1648[e]	1638	1649	1642	1632	1630	1646	1645	1633	1638	1647	1642
$\tilde{\nu}_2$[cm^{-1}]	1676	1673/ 1675[e]	1649	1673	1671	1649	1649	1667	1667	1645	1657	1674	1670
Γ_{G_1}[cm^{-1}][f]	21.3	25.5/ 23.4[e]	29.6	21.3	27.0	23.4	25.5	20.5	20.5	25.0	25.0	20.5	26.2
Γ_{G_2}[cm^{-1}][f]	18.9	17.2	30.5	18.9	20.4	22.6	24.8	17.9	17.9	25.6	27.0	24.5	30.0
R_{iso}	0.39	0.48	0.41	0.45	0.47	0.57	0.7	0.51	0.52	0.67	0.37	0.19	0.27
R_{aniso}	1.16	1.14	1.1	1.2	1.11	1.03	1.09	0.96	0.98	0.99	0.95	0.69	0.57
R_{IR}	1.52	1.52	1.69	1.42	1.7	1.27	1.2	1.47	1.5	1.35	1.67	0.73	1.12
ρ_-	0.24	0.22	0.28	0.24	0.23	0.24	0.19	0.22	0.23	0.17	0.22	0.2	0.14
ρ_+	0.12	0.11	0.14	0.12	0.11	0.16	0.14	0.14	0.15	0.13	0.11	0.07	0.08
Δ[cm^{-1}]	5.2	4.9	2.3	4.6	5.2	2.3	1.7	3.4	3.5	1.2	4.4	9.2	8.0
$\tilde{\theta}$ [deg]	119	126	128	118	125	116	121	127	125	129	123	76	95
θ [deg]	124	124	131	118	130	135	122	137	140	135	138	137	145
ϕ [deg]	−123	−120	−127	115	130	130	−125	−170	−170	−165	−135/−175	15	37
	+7/− 3	+7/−3	+8/− 4	+7/−3	+7/−3	+8/−4	+8/−4	−5/+ 10	−5/+1 0	±10	±15	+10/ −5	+10/ −5
ψ [deg]	173	164	165	−175	−175	−180	173	140	135	135	170/135	40	20
	+7/− 2	+7/−2	+5/− 10	+7/−2	+7/−2	+5/−1 0	+5/−1 0	±5	±5	±10	+5/− 10	+5/− 10	+5/− 10

[a] Taken from ref 28. [b] (+): cationic, (+−): zwitterionic, (−):anionic. [c] (−) cationic. [d] (4+): carboxylate and three residues protonated, (−3+): carboxylate deprotonated, three residues protonated. [e] Different wavenumbers were obtained from the isotropic and anisotropic Raman spectrum. [f] Gaussian half-width of the Voigtian profile.

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 nitrogen cooled charge-coupled device (CCD) with 256 × 1024 pixels in the chip. The spectral resolution was 3.8 cm^{-1} at 457 nm and 3.2 cm^{-1} at 488-nm excitation. 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.[39]

IR Spectroscopy. FTIR spectra were measured with a Nicolet Magna-IR System 560 optical bench as described elsewhere.[40] 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 6-μm thick Mylar spacers were used. The 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 using Nicolet OMNIC 3.1 software.

VCD Spectroscopy. VCD spectra were measured with a Chiralir FT-VCD spectrometer from Bomem/BioTools. This spectrometer is equipped with a HgCdTe detector having a cutoff at 8 cm^{-1} and a ZeSe photoelastic modulator (PEM) to create left and right circularly polarized radiation. VCD and IR spectra were measured in D_2O with resolution of 4 cm^{-1} using a CaF_2 cell with a path length of 56 microns. The VCD spectra were collected in blocks for a total collection time of approximately 12 h depending on the peptide sample investigated. The PEM was optimized for maximum quarter-wave response at 1400 cm^{-1}. Other experimental conditions are provided in the figure captions referring to the VCD spectra.

Spectral Analysis. All spectra were analyzed using the program MULTIFIT.[41] They were normalized to the internal standard, that is, 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. These and the corresponding IR spectrum were self-consistently analyzed in that they were fitted with a set of identical frequencies, half-widths, and band profiles. The isotropic and anisotropic Raman intensities and the depolarization ratios ρ were calculated as

$$I_{iso} = I_x - \frac{4}{3} I_y$$
$$I_{ansio} = I_y$$
$$\rho = \frac{I_x}{I_y} \qquad (5)$$

In principle, I_{aniso} should be written as $2{,}33 \cdot I_y$. As in earlier papers,[26,27] we prefer to identify it with I_y in the depicted figures so that the polarization properties of different lines can be better inferred.

Results and Discussion

Notation. To provide the consistent basis for the structure analysis of the investigated tripeptides, we introduce the following notations: PII, if $|\psi| > |\phi|$ and $180° < \psi < 110°$, $130° < \phi < 50°$, so that a left-handed helix is formed; β, if $|\psi| < |\phi|$ and $150° < \psi, \phi < 100°$, α_R, if $-60° < \psi < -0°$, $-50° < \phi < -150°$, α_L, if $10° < \psi < 80°$, $-20° < \phi < -50°$; βII-turn, if $130° < \psi < 90°$, $-70° < \phi < -40°$ (overlaps with PII) or $0 < \psi < 80°$, $0° < \phi < 40°$. Other structures are not considered in this study. For all peptides investigated, the respective amide I band at lower wavenumbers could be assigned to the in-phase combination of the two coupled amide I modes and is therefore labeled as AI$_-$. The corresponding band at higher wavenumbers represent the in-phase combination and is designated as AI$_+$. For convenience, we use the notation amide I rather than amide I′ in the paper.

Alanine Containing Peptides. We have recently used our approach to investigate the structure of trialanine in D_2O for all three protonation states and identified stable, slightly left-handed helical PII-like structures,[27] which are somewhat more extended than the PII(3_1) structure proposed on the basis of two-dimensional IR experiments[25] and an earlier Raman/IR study.[26] The corresponding dihedral angles are listed in Table 1.

It should be noted that the coupling energies for the AAA species listed in Table 1 are somewhat higher than those reported in refs 26 and 27. This discrepancy results from an error in eq

(40) Gribenow, K.; Diaz Laureano, Y.; Santos, A. M.; Montañez Clemente, I.; Rodriguez, L.; Vidal, M.; Barletta, G. *J. Am. Chem. Soc.* **1999**, *121*, 8157.
(41) Jentzen, W.; Unger, E.; Karvounis, G.; Shelnutt, J. A.; Dreybrodt, W.; Schweitzer-Stenner, R. *J. Phys. Chem.* **1996**, *100*, 14184.

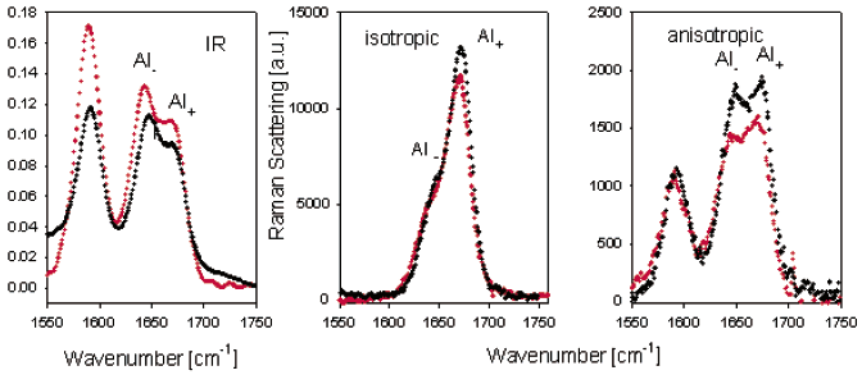

Figure 1. FTIR and isotropic and anisotropic Raman spectra of zwitterionic AAA (black) and AA^DA in D_2O (red) between 1550 and 1750 cm^{-1}. The AAA spectra were taken from ref 25. The corresponding polarized Raman spectra of AA^DA were measured with 457-nm excitation (laser power: 200 mW, concentration: 0.2 M)

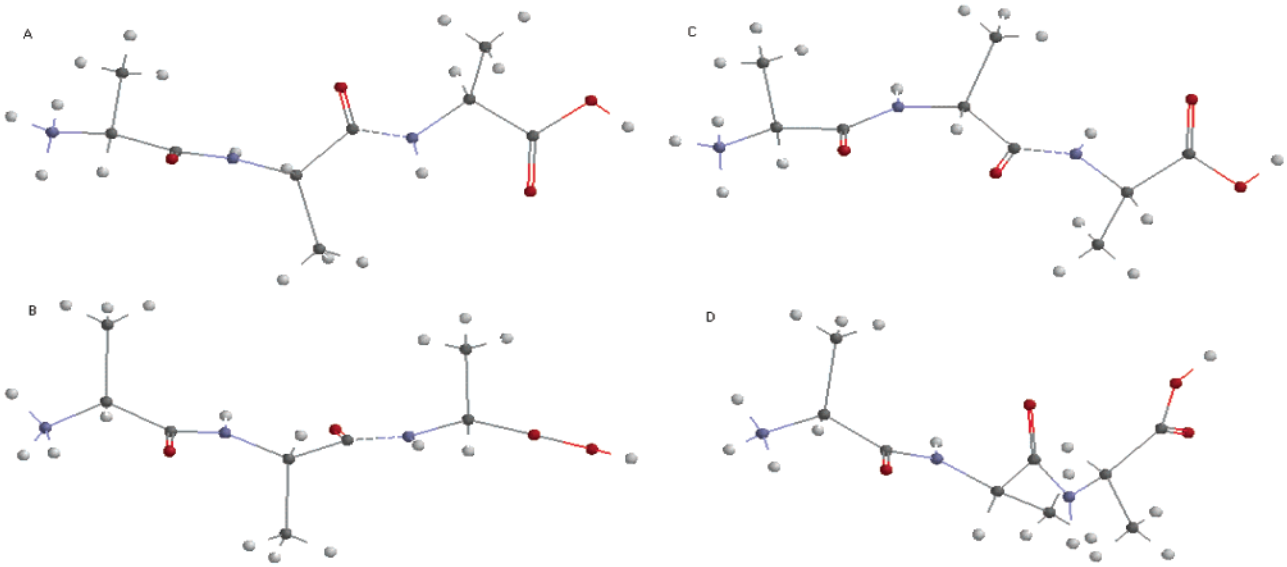

Figure 2. Upper panel: Structure of cationic AAA (A) and AA^DA (C) as obtained from the analysis of the amide I band profiles in IR absorption and Raman scattering. Lower panel: Representation of the two coexisting structures (extended (B) and PII (D)) of cationic AAA inferred from an alternative analysis of the spectroscopic data.

6 of ref 26 and eq 5 of ref 27. Herein, the term $4/\tan 2\nu$ has to be substituted by $1/\tan^2(2\nu)$, which leads to the final expression $\Delta = (\Delta_{exp}/2) \sin 2\nu$. The thus-obtained δ-value for cationic AAA is now very close to that reported by Woutersen and Hamm (6 cm^{-1})).[25]

Figure 1 compares the IR, isotropic, and anisotropic Raman spectra of zwitterionic AAA and zwitterionic AA^DA. The corresponding spectra are very similar in that AI_- dominates the IR spectra, while AI_+ is more intense in the isotropic Raman spectra. The anisotropic spectra exhibit amide I bands of comparable intensity, but the spectral analysis reveals that AI_1 is always slightly more intense. All these spectra are characteristic for an extended structure. Slight differences between the respective intensity distributions of the two peptides are clearly detectable. We subjected these data and spectra recorded for the remaining protonation states of AA^DA to a self-consistent decomposition as described under Material and Methods. This yielded the spectral parameters and the intensity ratios of the amide I bands listed in Table 1. The latter were then used to obtain the dihedral angles by means of the formalism and the protocol described by Schweitzer-Stenner.[27] This generally yields two and sometimes even four solutions assignable to different quadrants in the Ramachandran plot. For AAA and AA^DA, only one of them can be regarded as sterically allowed and as shown below, can be disregarded on the basis of the respective VCD signal. Thus, one obtains unequivocal results which are also listed in Table 1. For AA^DA, our analysis revealed structures of the dihedral angles which differ from the respective pairs of AAA mostly by their opposite signs. Thus, AA^DA exhibits an extended structure with some right-handed helicity. Figure 2 compares the obtained structures of cationic AAA and AA^DA.

We also analyzed the spectrum of the AcAA, which is nearly identical with what we obtained for anionic AAA (data not shown). The spectral and structural parameters are listed in Table 1. Our result indicates that the N-terminal group does not have any significant influence on the dihedral angles between the two interacting peptide groups.

In our analysis, we did not consider coupling between amide I and the C-terminal modes (i.e., CO stretch (s) in the cationic and COO^- antisymmetric stretch (as) in zwitterionic and anionic states). This is based on two experimental facts. First, Woutersen and Hamm did not identify any substantial coupling between the C-terminal amide I and CO s in the cationic state.[25] Second,

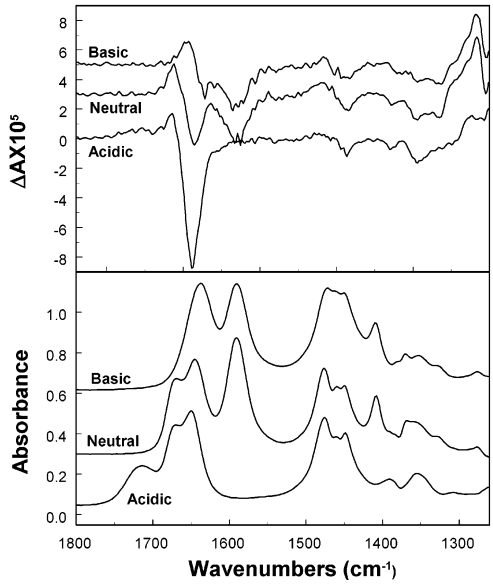

Figure 3. VCD and FTIR spectra of cationic, zwitterionic, and anionic AAA in D_2O (sample concentration: 0.3 M) recorded between 1250 and 1800 cm^{-1}.

coupling between the COO^- as and the C-terminal amide I in the other two protonation states would admix isotropic scattering into the totally depolarized Raman band of the carboxylate vibration. This is not observed in our spectra.

We used the VCD signal of amide I to carry out an independent check of the structures obtained for all protonation states of trialanine. The corresponding spectra recorded between 1250 and 1800 cm^{-1} are shown in Figure 3. In all cases, amide I shows a negative couplet. Additionally, we obtain a small positive signal for the CO s at 1710 cm^{-1} in the spectrum of the cationic species and large and broad negative signals at 1590 cm^{-1} in the spectra of the zwitterionic and anionic species, which result from the COO^- as. Other signals arising from CH_3 deformation (1400−1450 cm^{-1}) and combinations of CH bending modes (1300−1400 cm^{-1}) are comparatively weak.

The amide I couplet of the cationic state is clearly asymmetric in that the negative signal at amide I_- is stronger than the positive signal at amide I_+. The asymmetry is significantly reduced by the carboxylate deprotonation and further by the N-terminal deprotonation so that the couplet of the anionic species becomes nearly symmetric. This indicates that a pure coupling oscillator model (i.e., $\Delta\vec{m}_1 = 0$) is valid only for the anionic state. As shown by Woutersen and Hamm,[42] amide I_- can be described as the amide I mode of the C-terminal peptide which some (out-of-phase) admixture of the corresponding N-terminal vibration. Apparently, it has intrinsic rotational strength, which is strongest when the carboxylate group is protonated. This interpretation is supported by the amide I VCD signals in the spectra of the protonation states of dialanine (data not shown).

We have employed the above outlined theoretical approach to model the obtained VCD signals of amide I (Figure 4) as follows. The transition dipole moment (2.0 esu cm) for a single amide I mode was determined from the IR absorption spectra as described by Nafie et al.[37] The vector products of the transition dipole moments were calculated using the dihedral

(42) Woutersen, S.; Hamm, P. *J. Chem. Phys.* **2001**, 114, 2727.

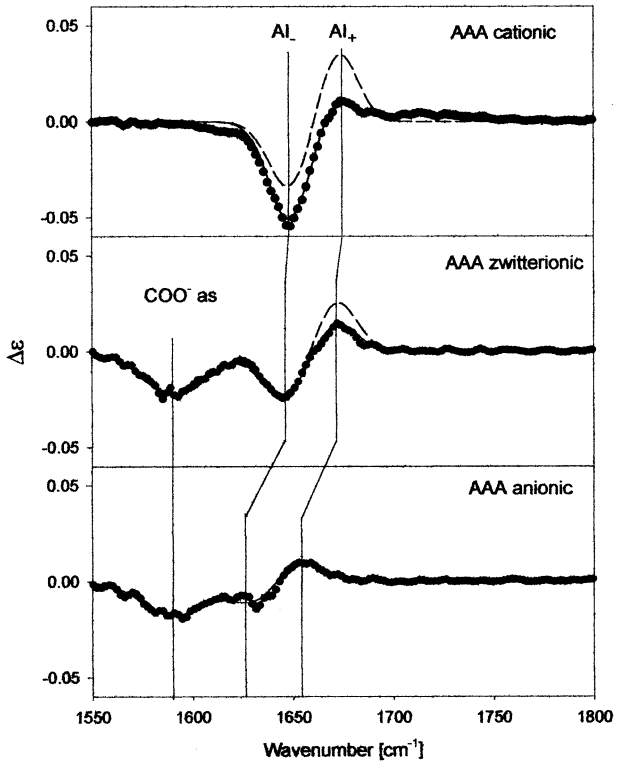

Figure 4. VCD of amide I for cationic, zwitterionic, and anionic AAA in D_2O. The solid and dashed lines result from calculations described in the text.

angles listed in Table 1. To this end, we used the geometrical factors in eq 23 in ref 27. The distance vector $\vec{T}_{12}$ points from the N-terminal to the C-terminal peptide carbon. The Gaussian half-widths were obtained for the spectral analysis of the Raman and IR spectra. The bands at 1590 and 1711 cm^{-1} were accounted for by scaled Gaussians. By inserting all these data into eq 3, we were able to satisfactorily reproduce the amide I couplet of anionic AAA (Figure 4, lower panel), but we overestimated the positive and underestimated the negative signal of the cationic state (dashed line in Figure 4, upper panel). For the zwitterionic peptide, only the positive signal was overestimated (dashed line in Figure 4, middle panel). To eliminate this difference, we assumed an intrinsic magnetic transition moment $\Delta\vec{m}_1$ for amide I_1. Since this parameter appears in two different scalar products with $\Delta\vec{\mu}_1$ and $\Delta\vec{\mu}_2$ in eq 3, we had to make an assumption about its relative orientation. For the sake of simplicity, we assumed that it has a negligible *z*-component perpendicular to the peptide plane. We obtained good fits to the couplets of the cationic peptide by choosing an angle of 87° between $\Delta\vec{m}_1$ and $\Delta\vec{\mu}_1$, indicating that the intrinsic chirality probed by amide I is very small. The respective magnetic moment was $2.3 \cdot 10^{-23}$ esu cm. The zwitterionic signal could only be reproduced by assuming a somewhat less extended structure $(\phi, \psi) = (-110°, 150°)$ and an intrinsic magnetic moment of $0.7 \cdot 10^{-23}$ esu cm, respectively. Alternatively, one can invoke the possibility that the zwitterionic state is heterogeneous, that is, that two or even more conformers coexist. This would be consistent with the already reported noncoincidence between isotropic and anisotropic scattering.[26] This point will be discussed in more detail below. Altogether, our successful reproduction of the VCD signals confirm the trialanine structure obtained from our Raman and IR data.

Our results differ from the earlier VCD study of Diem and co-workers,[22] who reported that only the amide I of the zwitterionic AAA displays an amide I couplet. From this they concluded that the Coulomb interaction between the terminal groups is necessary to stabilize a well-defined structure. Particularly, our VCD experiments argue to the contrary in that they reveal that stable structures exist for all protonation states, in accordance with conclusions drawn from Raman optical activity experiments by Ford et al.[23] Bour and Keiderling obtained the amide I signal of ADP from ab initio calculations.[32] For a PII structure, they obtained a signal which qualitatively and quantitatively compares well with our experimental data.

In view of the most recent theoretical studies on trialanine and its blocked analogue, however, the question arises whether we can exclude the coexistence of different conformers from our experimental results. For zwitterionic AAA, the noncoincidence between isotropic and anisotropic Raman scattering points into this direction. An even stronger hint comes from recent NMR results on cationic AAA reported in a study by Mu et al.,[43] which are consistent with a ϕ value of approximately $-60°$. This is close to the values reported in our earlier study[26] and by Woutersen and Hamm[25] but not with the ϕ-value reported in the present and our most recent study.[27] Several checks of our spectroscopic data revealed that this ϕ-value cannot be brought into accordance with our R_{aniso} value. However, a possible solution of this contradiction can indeed be offered by assuming the coexistence of two different conformers. To check this possibility for cationic AAA, we proceeded as follows. First, we assumed that one of the conformers is close to the PII_{WH} structure reported by Woutersen and Hamm,[25] namely, (ϕ_{PII}, ψ_{PII}) = ($-60°$, 150°). We then utilized the algorithm of Schweitzer-Stenner[27] to calculate R_{iso}, R_{aniso}, and R_{IR} values for different mixing values ν for this particular conformer. Second, we allowed PII_{WH} to coexist with another structure and calculated the R_{iso}, R_{aniso}, and R_{IR} values for the amide I bands of this binary mixture by using the equations

$$R_j = \left(\frac{1 + \lambda - \left(\frac{1}{R_{j,1} + 1} + \frac{\lambda}{R_{j,2} + 1} \right)}{\left(\frac{1}{R_{j,1} + 1} + \frac{\lambda}{R_{j,2} + 1} \right)} \right) \qquad (6)$$

where the index j represents iso, aniso, and IR, and λ is the concentration of conformer 2 divided by the concentration of conformer 1. Since the noncoincidence between isotropic and anisotropic Raman scattering is small or nondetectable for AAA, we assumed further that both conformers have nearly the same amide I frequencies and half-widths. We checked various (ϕ, ψ)-pairs in allowed regions of the Ramachandran plot as possible solutions for the second conformer. Eventually, we obtained only one solution which was consistent with the experimental data, namely, a very extended structure with (ϕ, ψ) = ($-165°$, 150°) with a coupling energy of 3.4 cm^{-1}. For the PII_{WH} structure, we obtained a coupling energy of 6.5 cm^{-1}. The mixing ratio was nearly 50:50. We employed this result to recalculate the VCD signal of cationic AAA and obtained excellent agreement with the experimental data. The reason is that the PII structure provides a much smaller and the extended structure a much larger VCD couplet than the observed signal so that a 50:50 mixing of both just reproduces the experimental spectrum. Finally, we performed a comparison with the NMR data discussed by Mu et al. (these authors used unreported data by Dorai and Griesinger), that is, a value of 5.17 Hz for the ^3J coupling between the hydrogen of the central $C_\alpha H$ bond and the amide proton of the N-terminal peptide group.[43] This coupling depends on the dihedral angle χ between these two hydrogens and thus on ϕ. By using the analytical relationship between ^3J and χ reported by Karplus,[44] one obtains ^3J values of 5.0 and 6.1 Hz for PII_{WH} and the extended structure, respectively, so that a 50:50 mixture would produce a signal at 5.55 Hz. Only slight variations in the confidence intervals of our dihedral angles allow a perfect reproduction for the experimental ^3J value. We are therefore led to the conclusion that there are indeed two coexisting conformers of AAA. The dihedral angles obtained by assuming a single conformer have to be understood as representing an average structure of the tripeptide.

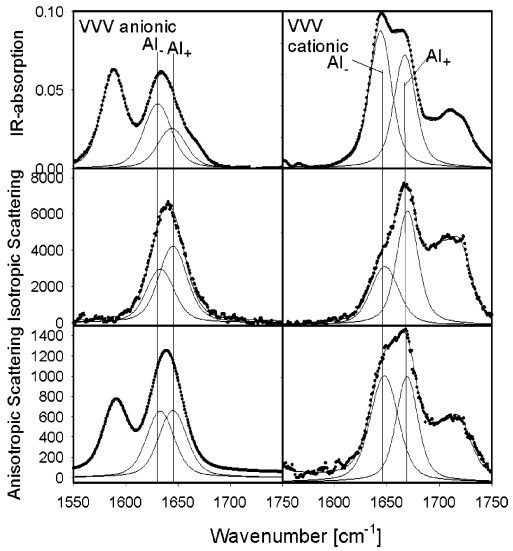

Figure 5. FTIR and isotropic and anisotropic Raman spectra of cationic, zwitterionic, and anionic VVV in H_2O or D_2O. The Raman spectra were measured with 457-nm excitation (laser power: 200 mW; sample concentration: 0.2 M in H_2O, 0.3 M in D_2O). The solid lines and the band profiles result from the spectral fitting described in the text.

Any attempt to reproduce the experimental intensity ratios by assuming the coexistence of an extended and a helical structure rendered unsuccessful. We therefore exclude any substantial fraction of helical conformers to exist in the investigated samples.

The similarity of the orientational angles of all protonation states of AAA and of AcAA strongly suggests that also these peptides exhibit the above coexistence of PII and β-sheet-like structure. The same holds in principle for AA^DA but of cause with conformers belonging to the lower left field of the Ramachandran plot.

Trivaline. Figure 5 depicts the IR absorption and the isotropic and anisotropic Raman scattering observed for cationic and anionic VVV. The zwitterionic amide I profile is very similar to that of the cationic species (data not shown). All these spectra are very similar to those observed for AAA, but amide I_- exhibits a larger relative intensity for VVV, which is indicative

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(44) Karplus, M. J. *J. Chem. Phys.* **1959**, *30*, 11.

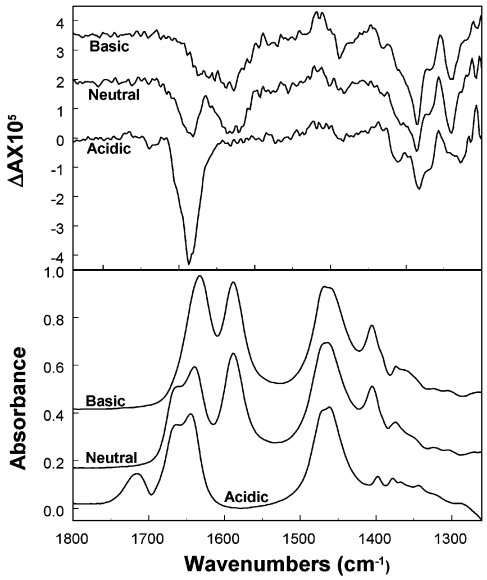

Figure 6. VCD and FTIR spectra of cationic, zwitterionic, and anionic VVV in D_2O recorded between 1250 and 1800 cm^{-1} (sample concentration: 0.3 M).

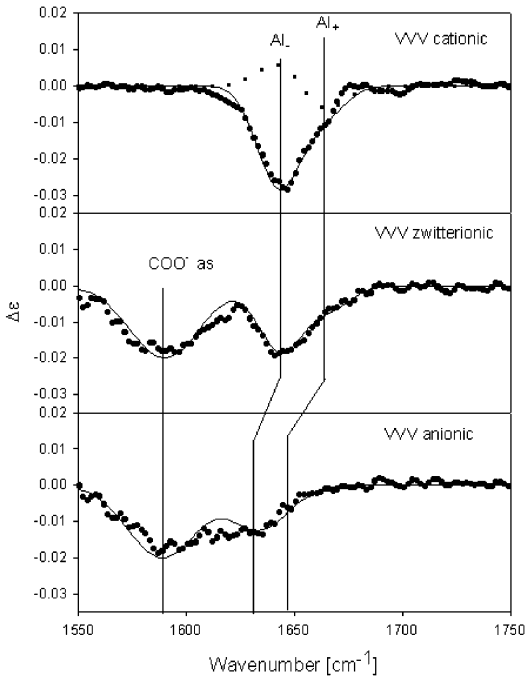

Figure 7. VCD of amide I for cationic, zwitterionic, and anionic VVV in D_2O. The solid and dashed lines result from calculations described in the text.

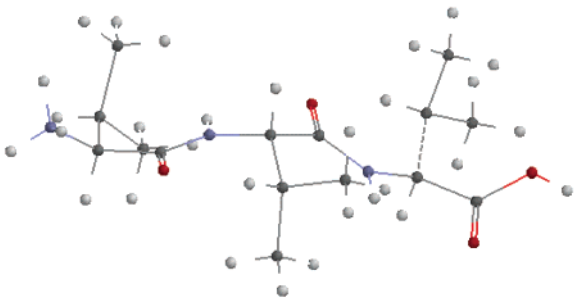

Figure 8. Structure of cationic VVV as obtained from the analysis of the amide I band profiles in IR absorption and Raman scattering.

of weaker excitonic coupling. This is confirmed by our spectral analysis, the results of which are listed in Table 1. The obtained coupling parameters ($\Delta = 3.5$ and 3.4 cm^{-1}) of the cationic and zwitterionic states are smaller than the corresponding values of AAA ($\Delta = 5.2$ and 4.9 cm^{-1}). For all three protonation states, the IR as well as the isotropic and anisotropic Raman bands could be fitted with the same spectral parameters. This absence of any noncoincidence strongly suggests the dominance of a single conformer. The dihedral angles were determined on the basis of the amide I bands' intensity and depolarization ratios. Thus, we obtained two (sterically) possible solutions for all three protonation states, respectively, for which $(\phi_1, \psi_1) \approx (-\phi_2, -\psi_2)$. Structural differences between the three protonation states are in the limit of experimental uncertainty.

The VCD spectra of VVV shown in Figure 6 are selective with respect to the above two solutions. The analysis of amide I is depicted in Figure 7. The signals of both amide bands are negative with a dominant contribution from amide I_-. Amide I_+ solely appears as a shoulder at higher wavelengths. Bour and Keiderling[32] computed very similar signals for β-sheet structures by ab initio methods. We reproduced all these VCD signals by superimposing the coupled oscillator signal for the geometries with $|\phi| > |\psi|$ with a contribution arising from an intrinsic magnetic moment of the C-terminal amide I. The second pair of dihedral angles obtained from the Raman and IR data ($|\phi| < |\psi|$) were inconsistent with the obtained VCD signal.

For all fits, we used the angle between $\Delta\vec{\mu}_1$ and $\Delta\vec{m}_1$ as obtained for AAA. Thus, only the magnetic moment was used as free parameter. Our analysis reveals that the coupled oscillator couplet is weak for the considered tripeptide structure (dotted lines in Figure 7) and opposite to that of AAA, thus causing the small negative signal at amide I_+. The finally obtained ϕ and ψ values of the three protonation states are listed in Table 1. They suggest that VVV is more extended than AAA, its structure is very much comparable with that of the β_2' conformer emerging from DFT calculations on ADP.[18] The obtained structure of cationic VVV is shown in Figure 8.

Another line of evidence for the thus obtained structure deserves to be mentioned. It has recently been demonstrated first by Asher et al.[29] and subsequently by Schweitzer-Stenner et al.[30] that the frequency of the most intense amide III band of dipeptides can be used as a measure of the dihedral angle ψ. Figure 9 shows the isotropic Raman spectrum of VVV in H_2O measured at acid pH. The amide III of VVV is at 1251 cm^{-1}, whereas it is observed at 1261 cm^{-1} for AAA. Ab initio calculations on a MP2 level by Asher et al.[29] predict a 13 cm^{-1} downshift of amide III for the obtained difference between the ψ angles of AAA and VVV. In our recent paper,[30] we presented an empirical equation calibrated by using the amide III frequency of AAA. For the ψ-angle of VVV, it predicts an amide III wavenumber of 1251 cm^{-1}, in perfect agreement with the experimental value. The spectrum in Figure 9 also depicts the amide S band at 1400 cm^{-1}, which only exists for extended structures.

We checked for the possibility to reproduce the experimental intensity ratios of VVV by a binary mixture of two conformers. To this end, we made use of PII, α_R, C_7, and C_5 structures reported by Han et al.[18] We did not find any evidence for structural heterogeneity. This leads us to conclude that VVV favors a single conformer in water.

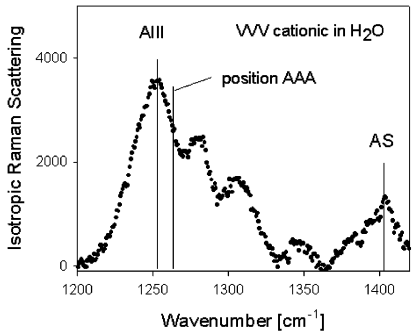

Figure 9. Isotropic Raman spectrum of cationic VVV in H_2O between 1200 and 1450 cm^{-1}. The amide III band is marked and compared with the respective position in the spectrum of aqueous cationic AAA. The spectrum was measured with 457-nm excitation (laser power: 200 mW, concentration: 0.2 M)

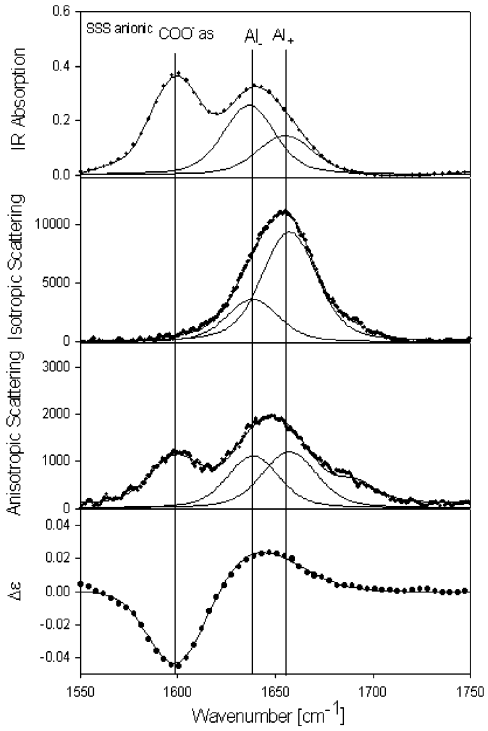

Figure 10. FTIR, isotropic Raman, anisotropic Raman, and VCD spectrum of the amide I region of anionic SSS in D_2O. The Raman spectra were measured with 457-nm excitation (laser power: 150 mW, sample concentration: 0.25 M). The VCD spectrum was recorded with a concentration of 0.125 M. The solid lines and the band profiles result from the spectral fitting described in the text.

Triserine. The investigation of this tripeptide was hampered by the substantial fluorescence due to sample impurities. Only the measurements on the anionic species yielded a good signal-to-noise ratio. The corresponding IR and Raman spectra are shown in Figure 10. As usual for the anionic state, amide I appears as one band with the anisotropic band at lower wavenumbers, indicating that the profile contains two overlapping bands with different depolarization ratios. In principle, these band profiles are difficult to analyze because multiple solutions exist for a good fit, which cannot be discriminated by statistical arguments. Fortunately, however, only the spectral parameters within a very restricted interval yield orientational angles $\tilde{\theta}$ and θ, which can be related to the same dihedral pair of dihedral angles. A further check of the analysis is provided by the capability to reproduce the depolarization ratios of the two amide

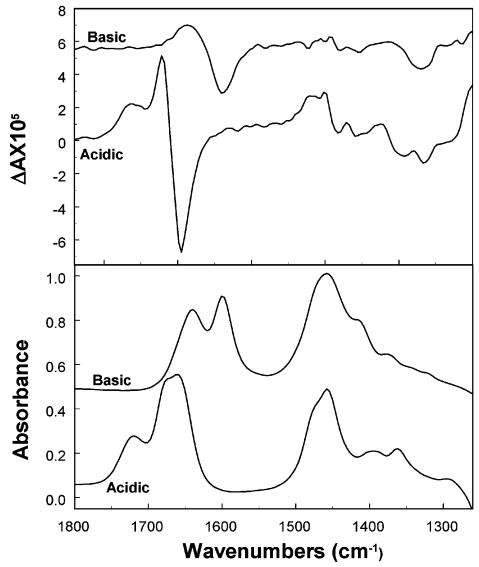

Figure 11. VCD and FTIR spectra of cationic and anionic VVV in D_2O recorded between 1250 and 1800 cm^{-1} (sample concentration: 0.25 M).

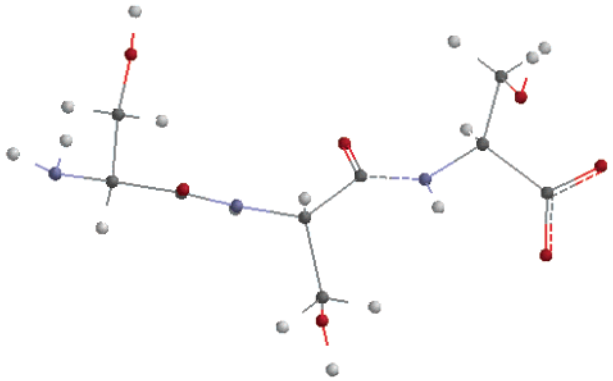

Figure 12. Structure of anionic SSS as obtained from the analysis of the amide I band profiles in IR absorption and Raman scattering.

I bands (Table 1). Thus, we managed to obtain two reliable values for the dihedral angles, that is, $(\phi_1, \psi_1) = (-135°, 178°)$ and $(\phi_2, \psi_2) = (-175°, 135°)$.

The first structure is very extended and comparable to C^5_{ext}[18] but still shows some PII character. It produces a negligibly small coupled oscillator couplet in the VCD spectrum. The experimental signal can only be explained by assuming $\Delta\vec{m}_1 \neq 0$. The fit depicted by the solid line in Figure 10 was obtained by assuming $\Delta\vec{m}_1 = -1.0 \cdot 10^{-23}$ esu cm and an orientational angle being substantially different from that used for the other tripeptides investigated. With the second solution, however, we obtained a nearly perfect fit to the VCD signal, suggesting that it is closer to reality. Figure 11 compares the entire VCD and IR spectra of anionic and cationic SSS. The amide I VCD of the cationic species displays a strong and nearly symmetric coupling signal which indicates a PII structure. Apparently, the structure of SSS is much more pD-dependent than that of AAA and VVV. For illustration, Figure 12 displays one of the two structures of the anionic state.

Trilysine. Figure 13 shows the IR and Raman spectra of trilysine as measured at acid and neutral pD. We denote these samples as KKKA and KKKN in the following. The spectra of KKKN appear qualitatively similar to those obtained for AAA and VVV, though the anisotropic Raman spectrum of KKKN

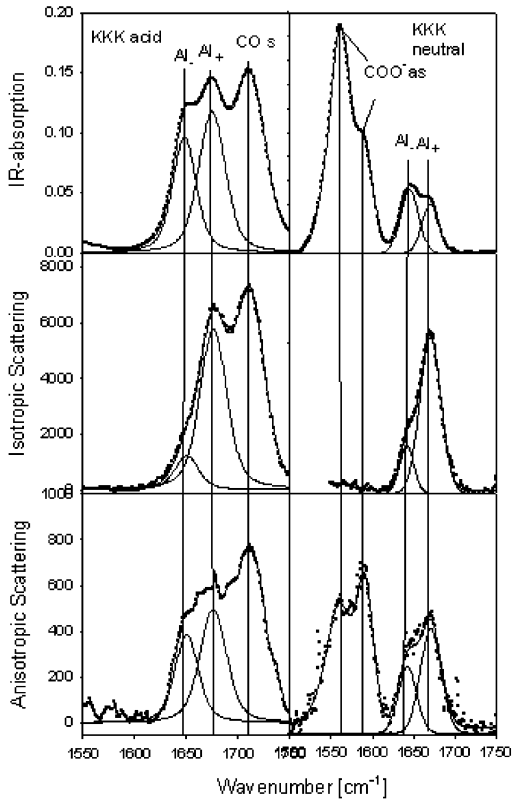

Figure 13. FTIR and isotropic and anisotropic Raman spectra of acid (pD 1) and neutral (pD 7) KKK. The Raman spectra were measured with 457-nm excitation (laser power: 200 mW, sample concentration: 0.125 M). The solid lines and the band profiles result from the spectral fitting described in the text.

is indicative to a lower R_{aniso}. For KKKA, we observed a significant change in that the most intense IR band now appears at a higher frequency. This is not caused by a sign change of the excitonic coupling energy, since the isotropic spectrum still depicts a more intense amide I_+ band. Generally, the spectra of KKKA are consistent with a turn or helical structure of the peptide.

We also performed Raman measurements at alkaline pH but particularly the anisotropic spectrum exhibits a bad signal-to-noise ratio in the amide I region. The analysis is further complicated by a strong overlap with the Raman band of the antisymmetric carboxylate stretch (data not shown).

VCD and IR spectra were taken at pD 1 and 2 and are depicted in Figure 14. All bands associated with C-terminal vibrations as well as the amide I band shape exhibit a strong pD dependence. The VCD signal of amide exhibits a clear couplet similar to what was obtained for AAA and cationic SSS. Magnitude and shape of the signal change with pD variation.

The analysis of the amide I band is now complicated by the presence of multiple protonation states of the peptide. However, as discussed in more detail below, only the fully protonated species exists at pD 1. The analysis of amide I excitonic coupling yields two solutions which are both acceptable, that is, a right-handed turn or even helical structure with (ϕ_R, ψ_R) = (−20°, −30°) and a left-handed helix (or β II turn) (ϕ_{L1}, ψ_{L1}) = (20°, 40°) or (ϕ_{L2}, ψ_{L2}) = (40°, 20°).

Fortunately, we can reduce the number of possible solutions by utilizing the VCD spectra shown in Figures 14 and 15. If the structure were right-handed helical, we should have observed

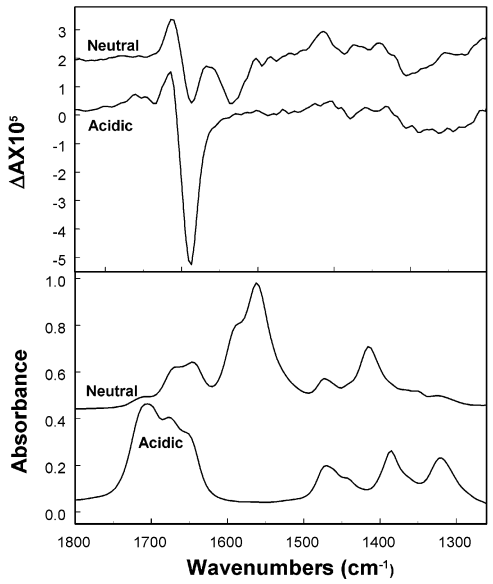

Figure 14. VCD and FTIR spectra of KKK in D_2O measured between 1250 and 1800 cm^{-1} at the indicated pD (sample concentration: 0.15 M).

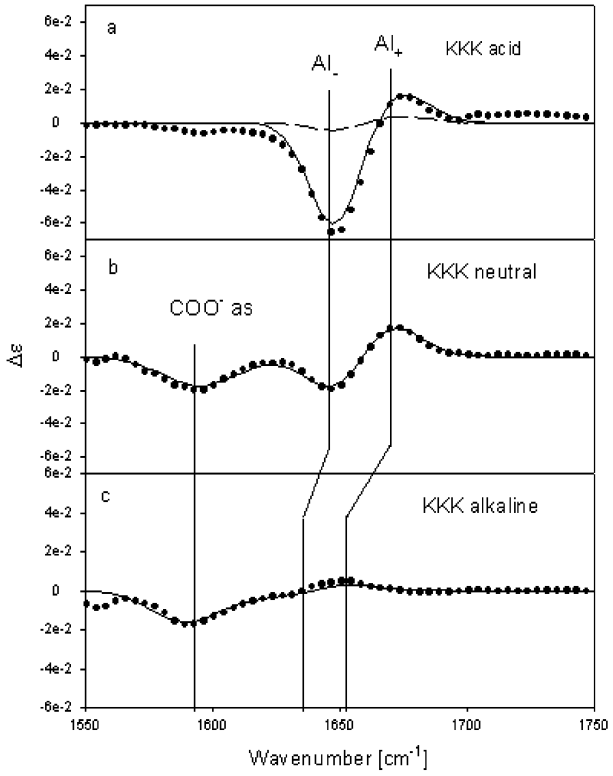

Figure 15. VCD of amide I for acid (pD 1) and neutral KKK (pD 7). The solid and dashed lines result from calculations described in the text.

a couplet with a positive signal at amide I_- and a negative one at amide I_+. On the contrary, the measured VCD spectrum is very similar to the asymmetric signal, with a strong negative amide I_- contribution, which we observed for cationic AAA. We used the above (ϕ_{L1}, ψ_{L1}) and (ϕ_{L2}, ψ_{L2}) values to calculate the spectrum. In addition, we assumed again a magnetic moment for amide I with the same orientation as that used for AAA and VVV. Thus, we obtained a nearly perfect agreement with the experimental signal for (ϕ_{L1}, ψ_{L1}) but not for (ϕ_{L2}, ψ_{L2}). Thus, only (ϕ_{L1}, ψ_{L1}) is listed in Table 1. The composition of the couplet is qualitatively different from that obtained for cationic

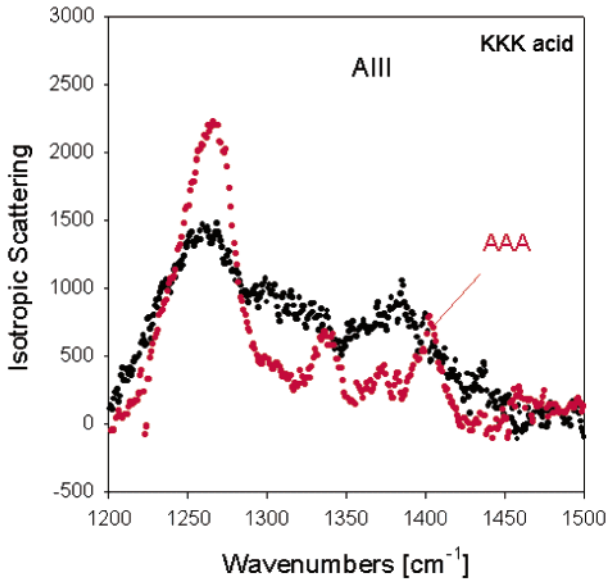

Figure 16. Isotropic Raman spectrum of KKK in H_2O between 1200 and 1500 cm^{-1} measured at pH 1 with 457-nm excitation (laser power: 150 mW, concentration: 0.125 M). The corresponding spectrum of AAA (red) is shown for comparison.

AAA. The pure coupled oscillator contribution is very weak (dashed line in Figure 15a) but interferes constructively with the respective contributions arising from the intrinsic magnetic moment $\Delta\vec{m}_1$=2.2·10^{-23} esu cm, which is negative for amide I −, but positive for amide I$_+$. For the extended structure of AAA, the respective signals are both negative.

We have also analyzed IR, Raman, and VCD spectra measured at pD 7. As shown below, at this pD most of the peptides are zwitterionic with respect to the terminal groups with still completely protonated lysine residues. The spectral analysis was somewhat difficult because of the low signal-to-noise ratio of the anisotropic spectrum and the overlap of the amide I region with the band of the antisymmetric carboxylate in the IR spectrum. Even though R_{IR} is now slightly larger than 1, the analysis still yields a left- and right-handed helical structure. The VCD signal (Figure 15b) suggests again a left-handed helical (or β II) structure, and we used the obtained dihedral angles (ψ_{L1}, ϕ_{L1}) = (30°, 50°) and (ψ_{L2}, ϕ_{L2}) = (30°, 50°) for our calculation. The data reproduction is nearly perfect for (ψ_{L1}, ϕ_{L1}) but insufficient for (ψ_{L2}, ϕ_{L2}). The couplet is symmetric, that is, $\Delta\vec{m}_1 = 0$.

Unfortunately, we could not analyze the spectra of the fully deprotonated species because of the low anisotropic amide signal. We tried to reproduce the VCD spectrum (Figure 15c) with the structural parameters obtained for the KKKN. The agreement with the amide I VCD signal is not perfect but still satisfactory. As usual the couplet appears much weaker in the spectrum of the alkaline species because of larger band overlap and half-widths.

We have also measured the polarized Raman spectra of KKKA amide III region in H_2O, but the results are somewhat inconclusive. The amide III band appears weak and broadened with peaks at 1266 and 1300 cm^{-1} (Figure 16). The problem is that lysine-based peptides are not very suitable for using the amide III as structural marker mode because of its heavy mixing with an in-phase combination of the residue's CH_2 bending vibrations.[29,30] This reduces the Raman intensity and the amide III frequency. It is remarkable, however, that the amide S mode does not appear around 1400 cm^{-1} in the spectrum of KKKA. This clearly indicates that the peptide does not adopt an extended or β-sheet structure.[45]

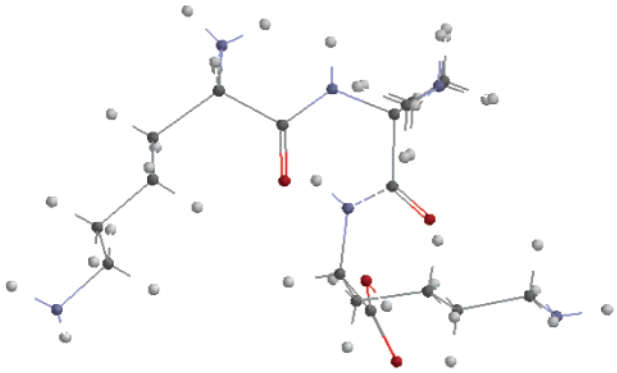

Figure 17. Structure of KKK at acid PD as obtained from the analysis of the amide I band profiles in IR absorption and Raman scattering.

We also checked whether the intensity ratios of KKK can be rationalized by a mixture of two conformers. Various simulations of intensity ratios revealed that this is very unlikely. This is due to the fact that the obtained R_{aniso} is close to the lowest possible theoretical value so that it cannot represent an averaging of conformers with very different R_{aniso} values. Very extended structures can also cause low R_{aniso} values, but they can be excluded on the basis of the observed R_{IR}. Thus, our results indicate that more sterically demanding residues lock tripeptides into a single conformer, at least in water. Only GGG[26] and to a minor extent AAA exhibit conformational heterogeneity.

Hence, we conclude that KKK adopts a left-handed turn or helical structure in water. Figure 17 shows the structure obtained at acid pD.

Conformational Propensity. The structure of peptides and proteins are determined by their primary sequence. This knowledge has initiated efforts to predict the protein structure from known amino acid sequences. The basic idea behind different concepts was that propensities for the most prominent secondary structure motifs can be assigned to amino acids. Chou and Fasman,[11] for instance, calculated so-called conformational parameters which are the relative frequencies of residues in helix and β-sheet conformation. For the amino acids used in the present study, they observed the hierarchies A > K$^+$ > V > S for α-helices, V ≫ A > S > K$^+$ for β-sheets, S ≫ K$^+$ > A > V for β-turns, and S > K > A > V for so-called coil structures. The strong β-sheet propensities of V and S coincide with our finding that VVV and SSS adopt structures, which can be described as extended β-sheet conformations. K$^+$ appears second for helices as well as for turns; this relative propensity has at least some correspondence to the left-handed helix obtained from our results. The well-established high α_R helical propensity of A cannot be inferred from our data. Instead, A gives rise to slightly left-handed helical structures which are structurally similar but less extended than the classical 3_1 or polyproline II structure. This result is of interest because Tiffany and Krimm[46] had hypothesized that coil structures are not random but exhibit a local, 3_1-like local order which depends on local interactions

(45) Mix, G.; Schweitzer-Stenner, R.; Asher, S. A. *J. Am. Chem. Soc.* **2000**, *132*, 9028.
(46) Tiffany, M. L.; Krimm, S. *Biopolymers* **1968**, *6*, 1379.

and thus on the steric and physical properties of the respective amino acids. Our results indicate that in the absence of other scaffolding forces (i.e., hydrogen bonding) A and S have a high coil propensity.

A very early investigation by Wu and Kabat[47] deserves attention in this context. These authors have determined repeated values for tripeptides in eleven known proteins. The AAA motif was most found in the α_R-domain, but PII structures with (ϕ, ψ) = (−77°, 143°) have also been observed. Interestingly, the closely related VAA motif was found with (ϕ, ψ) = (−137°, 168°), which is very close to one of the AAA structures obtained in the present study. The VVA motif which might be comparable with VVV shows a clear β-sheet structure with (ϕ, ψ) = (−123°, 120°, 125°, −129°), which parallels at least qualitatively the tendency represented by the VVV-tripeptide. Lysine containing peptides appear very frequently as right-handed helical. Our results confirm the helical propensity of lysine even though the left-handed helicity obtained for KKK is not biologically representative. Altogether, the study of Wu and Kabat indicates that structures similar to those obtained in the present study exist in proteins.

The biological relevance of our results are strongly underscored by recently reported results from various spectroscopic investigations on peptides and proteins. Dukor and Keiderling identified the structure of the poly(L-glutamic acid) at neutral pH as PII.[48] Park et al.[49] used CD spectroscopy to obtain an equilibrium between random coil and PII structure for a monomeric alanine based peptide with 17 residues. Rucker and Creamer[21] and Shi et al.[20] employed CD and NMR spectroscopy to investigate oligopeptides with seven lysine and alanine residues in solution and obtained that these peptides are predominantly PII in water. These results support an early hypothesis of Tiffany and Krimm who proposed that the so-called random coil structure of proteins can in reality be described as an ensemble of different local PII-like structures.[50] All these results combined with our findings suggest that local interactions (residue−peptide and residue−solvent) determine the structure of the unfolded state of proteins. Additionally, the PII structure is also of relevance in native structures of proteins.[51,52] A particularly interesting observation concerning the biological relevance of PII has recently been reported by Blanch et al.[53] They found that the amyloidogenic prefibrillar intermediate of human lysozyme exhibits contributions from a PII motif which substitutes the hydrated helix of the native state.

Currently, our results do not allow us to specify the forces which give rise to the obtained tripeptide structures. Some experiments on XAA peptides strongly suggest that the N-terminal peptides have a limited influence on the secondary structure. We are in the process of investigating series of XAA, AXA, and XAA peptides to check our current hypothesis that the central amino acid is the main structural determinant. If this is true, the results from these investigations will also allow us to determine the intrinsic structural propensity of amino acids in different solvents. The results will provide a good basis for an extended survey of protein structures to correlate the structural propensity of tripeptides with the structure of respective fragments in proteins.

Acknowledgment. Financial support for R.S.S. was provided by NSF (PR EPSCOR) Grant No. OSR-9452893, from the NIH-COBRE II grant for the *Center for Research in Protein Structure, Function, and Dynamics* and from the Fondos Institucionales para la Investigación of the University of Puerto Rico (20-02-2-78-514). We thank Dr. Brad Weiner for allowing us to build up a temporary setup for Raman experiments in his laboratory. R.S.S. thanks Timothy A. Keiderling for very useful discussions concerning the excitonic coupling mechanism for amide I and Peter Hamm and Gerhard Stock for very intensive discussions concerning the comparison of spectroscopic and computational studies on cationic trialanine. Both colleagues have provided us reprints and important results (^{3}J-coupling) prior to publication. The Syracuse authors wish to acknowledge support from the National Institutes of Health grant GM for financial support.

JA027381W

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