Peptide Structures

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Solution Structures of β Peptides from Raman Optical Activity**

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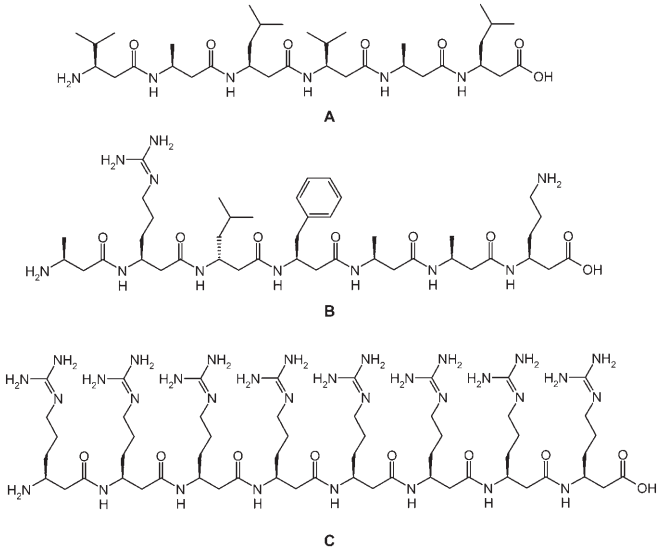
Dedicated to Professor Andreas Pfaltz on the occasion of his 60th birthday

There is much current interest in the synthesis and characterization of compounds, which form chiral helical and other secondary structures as key building blocks of supramolecular and functional nanosystems.[1–3] Some of the work in this field is inspired by—and attempts to mimic—the homochirality and function of biological macromolecules, exemplified by the recent discovery of a β-dodecapeptide folding into a helix that aggregates to an octameric complex, just like a true protein forming a quaternary structure.[4] β-Peptides consisting of homologated proteinogenic α-amino acids contain an additional CH_2 group in each and every residue. They can adopt stable helices in methanol or water and can interact with cellular proteins and membranes.[5] However, as with their natural counterparts, determination of the solution structures of the associated oligomers and polymers, unfolded or folded, remains a key problem. 2D NMR spectroscopy is the method of choice, so far, but is not always applicable and can be cumbersome when several interconverting conformers are present. The NMR method provides structures averaged over ca. 10^{-6} s, a very long time scale as compared to that of rotations, vibrations, and electronic excitations. Studies of β-peptides using circular dichroism suggested that this conventional chiroptical UV/Vis technique is unable to provide reliable information about their conformational preferences.[6] Here we report a promising study of this problem using the chiroptical technique of vibrational Raman optical activity (ROA).[7,8]

We studied the three β-peptides **A**, **B**, and **C**. Their backscattered Raman and ROA spectra in methanol solution are displayed in Figure 1 a–c and that of **C** in water as Figure 1 d. From the average Δ values, it is apparent that **A** generates much stronger ROA signals than **B** and **C**. From a 2D NMR investigation, it is known that the β-peptide **A** adopts the conformation of a left-handed (*M*)-3_{14}-helix in methanol solution.[5c,9] The much weaker ROA spectra of **B** and **C** compared with that of **A** suggest that their conformations are much less well-ordered than that of **A**. Compound **B** was in fact designed to suppress secondary-structure formation[10] by incorporating one β-amino acid residue ($\beta hLeu^3$) of opposite absolute configuration to the others. The ROA spectra of **C** in methanol and water are quite similar, which indicates that whatever residual structures are present are similar in the two solvents; however, the much weaker ROA of **C** in water suggests that their amounts may be rather less than in methanol.[11]



Quantum-chemical simulations of the ROA spectra of oligomers and polymers now being possible,[12–14] we calculated the Raman and ROA spectra of **A** in the (*M*)-3_{14}-helical conformation. Figure 2 shows a comparison of these simulated spectra with the experimental spectra. The overall agreement is good, bearing in mind the approximations introduced to make the simulation tractable at our present level of capability. Some of the differences of detail can be attributed to the side-chains being fixed in one conformation. Conformational freedom of side-chains within a limited range, together with backbone flexibility, needs to be incorporated in order to simulate the observed Raman and ROA bandshapes more realistically[13,15] (for example, some of the strong sharp simulated bands below around 600 cm^{-1}, which originate in delocalized backbone and side-chain

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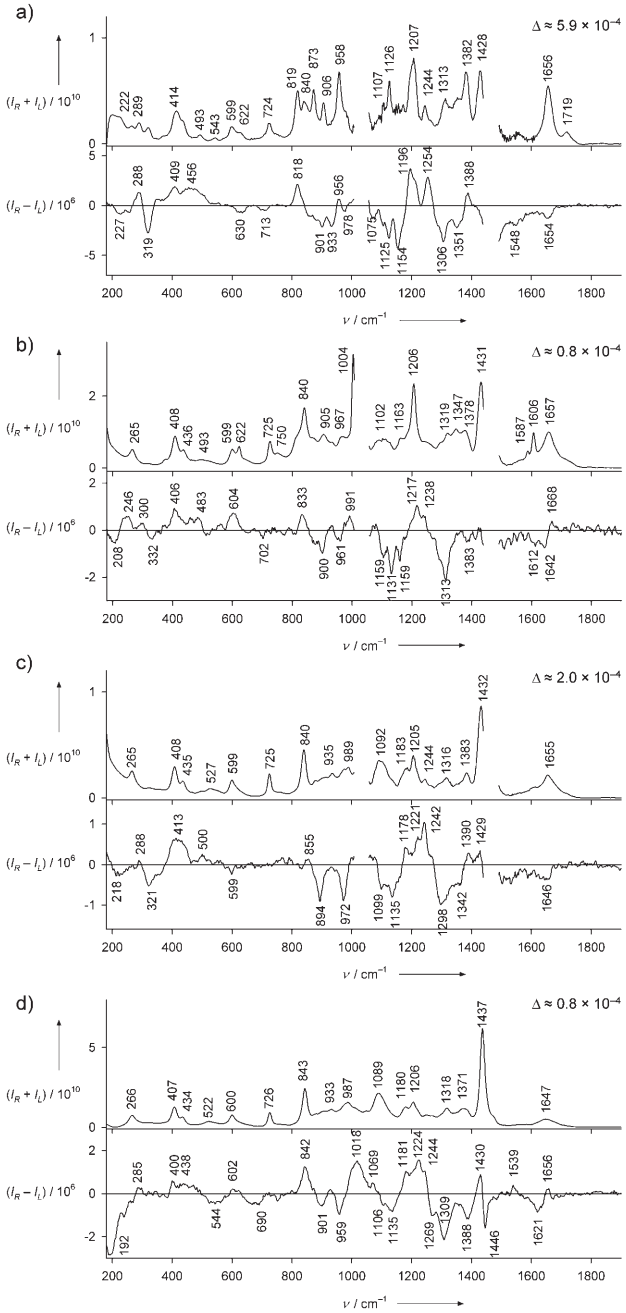

Figure 1. Back-scattered SCP Raman ($I_R + I_L$) and ROA ($I_R - I_L$) spectra of a) **A** in MeOH, b) **B** in MeOH, c) **C** in MeOH, and d) **C** in H_2O. The solvent spectra have been subtracted from the parent Raman spectra (the gaps correspond to intense solvent bands under which reliable ROA data cannot be acquired). The specified Δ values are the averages over the ranges 300–1007 and 1057–1440 cm^{-1}, at spectral points at 1 cm^{-1} intervals, of the ratios of the ROA to the Raman band intensities at each point. Although only approximate (due to uncertainties associated with solvent subtraction, especially for weak Raman bands), they provide a useful indication of the relative intensities of the four ROA spectra.

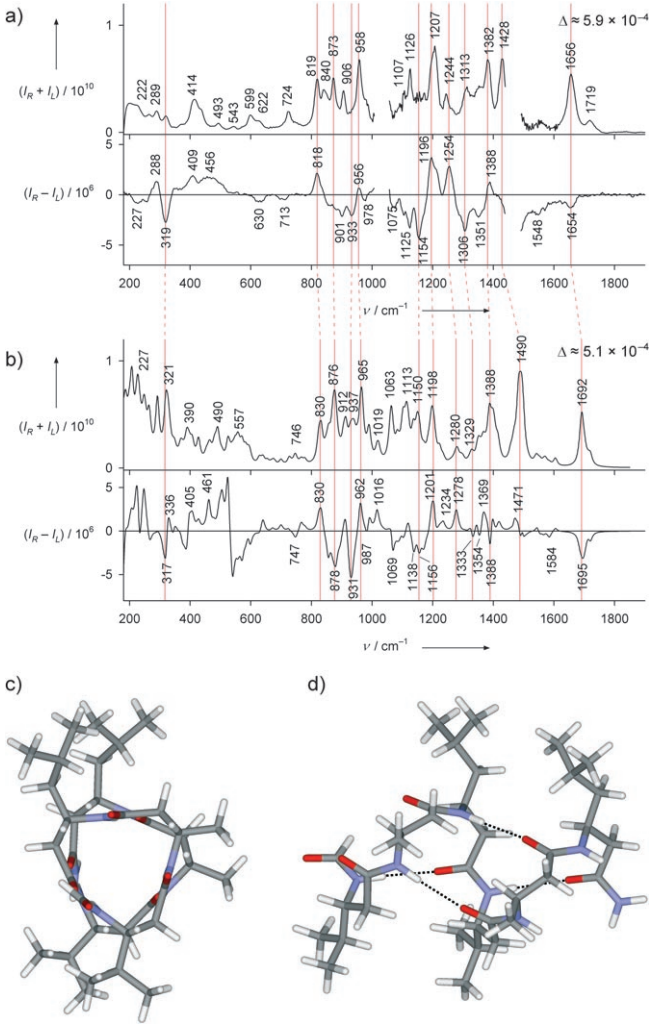

Figure 2. a,b) Comparison of the experimental (a) and simulated (b) Raman and ROA spectra of **A**. c,d) Orthogonal views of the (*M*)-3_{14}-helical conformation used for the calculation; blue N, red O, gray C, white H.

vibrations, should be significantly broadened and suppressed in this way). An important observation is that the handedness of the helix is given immediately from the simulation even without perfect agreement in all the ROA spectral details. This may be due partly to the fact that, like in natural peptides and proteins,[7] the largest ROA signals are generated by the backbone, which is the most rigid twisted part of the structure. More sophisticated (but much more computationally-expensive) calculations employing more refined basis sets[16] and incorporating several side-chain and backbone conformations should provide even better agreement.

These preliminary results reveal that typical β-peptides are excellent samples for ROA measurements, generating rich strong spectra containing valuable new information. A first simple analysis can provide information about whether a well-defined secondary structure dominates, or whether the structure is significantly disordered, with quantum-chemical spectral simulations having the potential to provide complete solution structures, including right- or left-handedness of corresponding helices. As more ROA data accumulate, it should be possible to identify distinct ROA band patterns characteristic of the repertoire of structural types adopted by various β-peptide sequences, and perhaps eventually to apply

multivariate analysis methods to identify distinct folds of protein analogs, as is done for their natural counterparts.[17] With more experimental ROA data it will also become possible to provide a test of the results of extensive molecular dynamics simulations of β-peptidic conformations.[18]

Experimental Section

The syntheses of peptides **A**, **B**, and **C** as well as their full characterizations have been described previously.[5c,9,10]

The Raman and ROA spectra of **A**, **B**, and **C** were measured in back-scattering using the previously described ChiralRAMAN instrument (BioTools, Inc.),[7b] which employs the scattered circular polarization (SCP) measurement strategy. The ROA spectra are presented as $(I_R - I_L)$ and the parent Raman spectra as $(I_R + I_L)$, where I_R and I_L are the Raman-scattered intensities with right- and left-circular polarization, respectively. The samples were studied at concentrations ca. 16 mg mL^{-1} for **A** and ca. 50 mg mL^{-1} for **B** and **C** at ambient temperature. Experimental conditions: laser wavelength 532 nm; laser power at the sample 350 mW; spectral resolution 10 cm^{-1}; acquisition times 40 h for **A** and 18 h for **B**, 7 h for **C** in methanol, and 35 h for **C** in water.

The quantum-chemical simulations of the Raman and ROA spectra of **A** were performed on formyl-(βhVal-βhAla-βhLeu)$_2$-amide. The initial geometrical parameters were taken from the average of the (*M*)-3$_{14}$-helical solution structures of a β-hepta-, a β-octa-[19a] and a β-icosapeptide[19b] determined from 2D NMR measurements (hitherto unpublished PDB coordinates) with backbone torsion angles ϕ(C-*N*-C$^\alpha$-C$^\beta$) = −140°, ψ(N-C$^\alpha$-C$^\beta$-C) = +60°, ζ(C$^\alpha$-C$^\beta$-C-N) = −140°, and ω(C$^\beta$-C-*N*-C$^\alpha$) = 180°. It was not computationaly tractable to simulate molecular properties of the whole structure directly, hence force field and polarizability tensors of the whole structure were obtained from two formyl-(βhAla)$_5$-amide fragments adopting the backbone configuration and four side-chain amino acid residue fragments based on βhVal and βhLeu by transfer in Cartesian coordinates.[20] All fragments were subjected to a restricted normal-mode optimization[21] with modes fixed in the range −100 to +200 cm^{-1} and they were only slightly distorted during optimization. The backbone torsion angles of the optimized structure were $\phi = 137 \pm 1°$, $\psi = +59 \pm 1°$, $\zeta = -140 \pm 2°$, and $\omega = 180 \pm 1°$, with side-chain torsion angles of βhVal δ(N-C$^\alpha$-C-H) = 63° and of βhLeu δ(N-C$^\alpha$-C-C) = −62°, ε(C$^\alpha$-C-C-H) = 7° (several structures with different side-chain conformations were simulated, but this one gives best agreement with experiment). The optimizations and the force field computations were performed at the B3LYP/COSMO[22]/6-311G** level, while the polarizability and optical activity tensor computations were performed at the HF/vacuum/rDP[16] level for backbone and the HF/vacuum/rDPS[16] level for side-chain fragments. The Raman and ROA spectra were then generated within the harmonic approximation using the usual procedure.[23]

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