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Calculated Raman Optical Activity Signatures of Tryptophan Side Chains

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Vibrational Raman optical activity (ROA) spectroscopy[1] measures the difference in Raman scattering intensity of chiral molecules in right- and left-polarized incident light, and allows one to determine the structure and absolute configuration of biomolecules *in aqueous solution*.[2] While most bands in the ROA spectra of proteins are assigned to the peptide backbone and can provide information on secondary structure elements (see, e.g., ref. [3] for experimental work and refs. [4, 5] for theoretical studies), some bands are related to the conformation of specific side chains. By comparing the ROA backscattering spectra of different viral coat proteins, Blanch et al. suggested[6] that a ROA band at $\approx$1550 cm^{-1}, which is assigned to the W3 vibration of the indole ring in tryptophan, can be used to determine the absolute stereochemistry of the tryptophan side chain. While it had been shown earlier in an analysis of the Raman spectra of different crystalline tryptophan derivatives[7] that the wavenumber of this W3 vibration correlates with the magnitude of the $\chi^{2,1}$ torsion angle (see Figure 1 for the definition of this angle), they inferred that its sign can be deduced

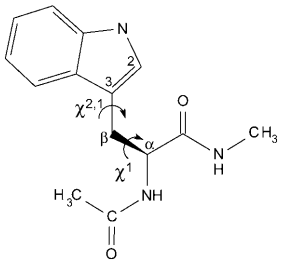

Figure 1. Structure of *N*-acetyl-(*S*)-tryptophan-*N*′-methylamide and definition of the torsion angles $\chi^{2,1}$ and χ^1. $\chi^{2,1}$ is defined by the atoms C_2–C_3–C_β–C_α, the torsion angle χ^1 is defined by the atoms C_3–C_β–C_α–N. Also see ref. [19].

from the sign of the corresponding ROA band. From the positive W3 ROA signal of hen lysozyme in solution and the observation that its crystal structure shows a positive sign of $\chi^{2,1}$ for four of the six tryptophan residues, they concluded that a positive W3 ROA signal corresponds to a positive sign of $\chi^{2,1}$. Additional support for this assignment is given by an observed increase of the magnitude of the W3 ROA signal for a complex of hen lysozyme with the trimer of *N*-acetylglucosamide in which, according to its crystal structure, five out of six tryptophans show a positive sign of $\chi^{2,1}$.

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However, there are a number of uncertainties in this assignment which could not be resolved by experiment. The sign and the magnitude of the W3 ROA signal might depend not only on $\chi^{2,1}$ (Blanch et al. assumed a $\sin\chi^{2,1}$ dependence of the magnitude of the ROA intensity), but could also be influenced by other structural parameters, such as the χ^1 torsion angle. This would complicate the comparison of the W3 ROA signal observed in hen lysozyme to the crystal structure, which contains six tryptophan residues in different conformations. Furthermore, it is not certain that the conformation of the tryptophan side chains in solution is the same as in the crystal.

To address these issues, we have investigated the influence of the torsion angles $\chi^{2,1}$ and χ^1 on the intensity of the W3 ROA signal in tryptophan by performing density functional theory (DFT) calculations. These calculations allow us to study the effect of the different structural changes on the ROA spectrum in a controlled manner and further make it possible to analyze the origin of the ROA signals of interest. As model system, we chose *N*-acetyl-(*S*)-tryptophan-*N*′-methylamide (see Figure 1), which is the simplest model that, in addition to the tryptophan side chain, also contains the main structural features of the peptide backbone. This structural model allows for an extrapolation of the results to extended peptide chains and proteins because it is reasonable to assume that the normal mode responsible for the W3 signal is localized on the tryptophan side chain and does not couple with normal modes on other parts of the protein. We further expect that the ROA intensity is not significantly influenced by contributions of other chiral residues that are not included in the model to the total wavefunction.

We optimized the structures of six different conformers of *N*-acetyl-(*S*)-tryptophan-*N*′-methylamide, which differ in the torsion angles $\chi^{2,1}$ (for which two different orientations, corresponding to a positive and a negative torsion angle, are possible) and χ^1 (for which three different orientations are possible). The values of these angles and the relative energies of these conformers are summarized in Table 1. All conformers fall within an energy range of only 13 kJ mol^{-1}. Therefore, in larger proteins the conformation of the backbone and the protein environment may change the energy ordering of the conformers, and all orientations of the torsion angles can be realized.

The calculated ROA spectra of the six conformers are depicted in Figure 2. In all spectra, the W3 ROA band at $\approx$1540 cm^{-1} can be clearly identified as the one with the highest absolute ROA intensity. For the conformers (**1**+) to (**3**+), this band has a positive sign, while it is negative for the conformers (**1**−) to (**3**−). In the other parts of the spectrum, no clear similarities of the spectra of the different conformers can be identified. The wavenumbers and the ROA intensity of the W3 mode of all conformers are collected in Table 1.

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Table 1. Torsional angles $\chi^{2,1}$ and χ^{1} of different conformers of *N*-acetyl-(*S*)-tryptophan-*N*′-methylamide, relative energies of the different conformers E_{rel} (in kJ mol^{-1}) with respect to the minimum energy conformer (**2+**), as well as wavenumbers ν (in cm^{-1}) and ROA intensity I_R–I_L (in Å^4 a.m.u.$^{-1}$) of the W3 vibration.

	$\chi^{2,1}$	χ^{1}	E_{rel}	ν	I_R–I_L
(**1+**)	+113.7	−47.1	8.0	1538.8	0.2434
(**2+**)	+79.4	+45.6	0.0	1530.6	0.2916
(**3+**)	+91.6	−156.4	12.9	1535.6	0.1804
(**1−**)	−81.6	−48.8	7.0	1539.0	−0.1168
(**2−**)	−102.2	+30.1	7.8	1530.7	−0.2806
(**3−**)	−86.1	−164.5	5.7	1538.2	−0.3034

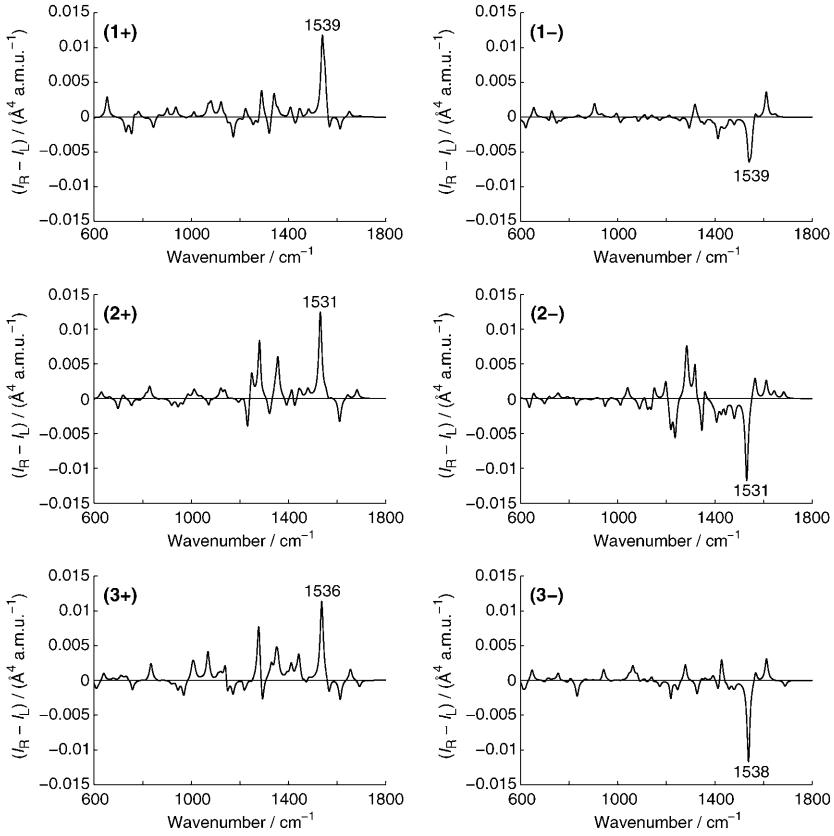

Figure 2. Calculated backscattering ROA spectra (excitation wavelength $\lambda = 514.5$ nm) of different conformers of *N*-acetyl-(*S*)-tryptophan-*N*′-methylamide.

From the calculated ROA spectra, we can draw the following conclusions. 1) A positive $\chi^{2,1}$ torsion angle corresponds to a positive W3 ROA signal, whereas a negative $\chi^{2,1}$ angle corresponds to a negative W3 ROA signal, irrespective of the torsion angle χ^{1} or other structural parameters. Thus, our calculations confirm the experimental assignment. 2) We do not find any correlation between the magnitude of the W3 ROA signal and the magnitude of $\chi^{2,1}$. In particular, we cannot confirm the $\sin\chi^{2,1}$-dependence suggested earlier.[6] 3) For the backbone ROA bands of the model compounds considered here, in particular the amide I, II, and III regions, large differences between the spectra of the different conformers are observed, even though the conformation of the backbone is identical. This relates to the earlier theoretical results for different diastereomers of trialanine that showed a large influence of the side chains on the amide ROA bands.[5]

Further insight into the origin of the W3 ROA signal can be gained by analyzing the associated normal mode, which is shown in Figure 3. The normal mode is a combination of an in-plane vibration of the indole ring, a deformation of the $C_\beta H_2$ group, and an in-plane N–H deformation of an amide group in the backbone. For conformers with different signs of $\chi^{2,1}$, the orientations of the indole ring with respect to the $C_\beta H_2$ group differ and, therefore, the relative phases of the indole and the $C_\beta H_2$ vibrations differ. Furthermore, a different backbone N–H deformation is involved for different conformers, and the amplitude of this backbone N–H deformation differs.

However, these differences in the normal modes are not responsible for the different sign of the ROA signal. This can be seen by analyzing the contributions of these different groups (i.e. the indole ring, the $C_\beta H_2$ group and the backbone) to the ROA signal according to the local decomposition analysis proposed by Hug.[8] The group coupling matrices shown in Figure 3 clearly demonstrate that the main contribution to the ROA signal is generated by those distortions in the normal mode that originate from the (achiral) indole ring. The change in the sign is also governed by the contribution of the indole ring. At the indole ring, the normal modes of the different conformers are almost identical. Therefore, the change in the sign of the ROA signal must be caused by a change in the derivatives of the electric-dipole–electric-dipole polarizability tensor α and the electric-dipole–magnetic-dipole polarizability tensor G' with respect to nuclear displacements of the atoms in the indole ring. This change in the property tensor derivatives is due to the different local chirality at the C_β atom in conformers with opposite sign of $\chi^{2,1}$, which affects the total wavefunction from which the property is calculated. This different local chirality is probed by the nuclear displacements of the atoms in the non-chiral indole ring, while the vibration of the $C_\beta H_2$ group (or its coupling with the vibration of the indole ring) only give a small contribution to the ROA intensity. The observed sign change is in accordance with a planar symmetry rule that can be established using a simple "one-electron theory" of ROA.[9]

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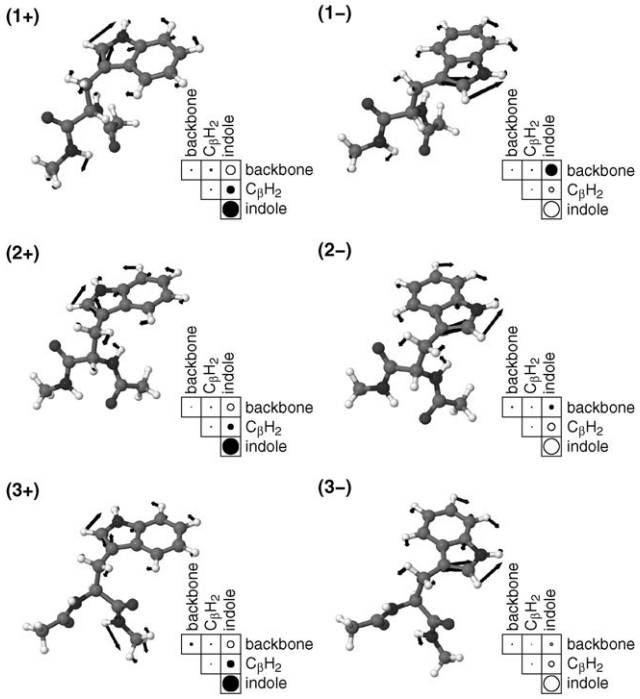

Figure 3. W3 normal mode of different conformers of *N*-acetyl-(*S*)-tryptophan-*N*′-methylamide and associated group coupling matrices of the ROA invariant $\beta(G')^2$ for this normal mode.

In summary, we have shown that the ROA intensity of the W3 band at $\approx$1540 cm^{-1} of tryptophan reflects the absolute conformation with respect to the $\chi^{2,1}$ torsion angle of the tryptophan side chain. We have confirmed the earlier experimental assignment that a positive W3 ROA signal corresponds to a positive $\chi^{2,1}$ torsion angle, while a negative signal corresponds to a negative $\chi^{2,1}$, that is, the W3 ROA band reflects the different local chirality at the C_β atom. According to our local decomposition analysis, this sign change can be attributed to the parts of the W3 normal mode that are localized at the indole ring, and that are very similar in all investigated conformers. This shows that the different local chirality of an adjacent group can cause a different sign of the ROA intensity, even though the normal mode is unchanged. For establishing ROA sign rules it will, therefore, not be sufficient to consider the composition of the normal modes alone, but it will be as important to also take into account the local chirality of groups not directly involved in the normal mode generating a specific ROA signal.

Computational Methods

Structure optimizations were performed with the Turbomole program package.[10] The BP86 exchange–correlation functional[11] and Ahlrichs' valence triple-zeta basis with one set of polarization functions (TZVP) and the corresponding auxiliary basis sets were employed.[12] The model structures were treated as isolated species without consideration of solvent effects. The program SNF[13] was used to calculate the normal modes and vibrational frequencies, as well as the derivatives of the property tensors required for the calculation of the ROA intensities (i.e., of the electric-dipole–electric-dipole polarizability α, the electric-dipole–magnetic-dipole polarizability G', and the electric-dipole–electric-quadrupole polarizability A) by numerical differentiation. The analytic energy gradients (needed for the semi-numerical calculation of the harmonic force field) were calculated with Turbomole for distorted structures.[13] The required property tensors were calculated with time-dependent DFT by a modified version of Turbomole's ESCF program[14,15] using the same exchange–correlation functional and basis set as for the structure optimization. It has been shown previously that the ROA intensities of organic molecules are not sensitive to the choice of the exchange–correlation functional.[16] To ensure gauge-invariance, the velocity representation of the electric-dipole operator was employed for the $\beta(G')^2$ invariant.[15] The experimental excitation wave length of 514.5 nm was used. The calculated ROA backscattering intensities contain both the contributions of the $\beta(G')^2$ and of the $\beta(A)^2$ invariant. For the local decomposition analysis, only the $\beta(G')^2$ invariant, which dominates the ROA backscattering intensity of the W3 mode, is employed.[17] ROA spectra were plotted using Gnuplot with a Lorentzian band shape and a half-width of 15 cm^{-1}. Pictures of molecular structures and normal modes were prepared with JMOL.[18]

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[1] a) L. D. Barron, *Molecular Light Scattering and Optical Activity,* 2nd ed., Cambridge University Press, Cambridge, **2004**; b) L. D. Barron, M. P. Bogaard, A. D. Buckingham, *J. Am. Chem. Soc.* **1973**, *95*, 603–605; c) W. Hug, S. Kint, G. F. Bailey, J. R. Scherer, *J. Am. Chem. Soc.* **1975**, *97*, 5589–5590.

[2] a) L. D. Barron, E. W. Blanch, L. Hecht in *Advances in Protein Chemistry, Vol. 62* (Ed.: G. D. Rose), Elsevier, San Diego, **2002**, pp. 51–90; b) L. D. Barron, L. Hecht, I. H. McColl, E. W. Blanch, *Mol. Phys.* **2004**, *102*, 731–744; c) F. Zhu, N. W. Isaacs, L. Hecht, G. E. Tranter, L. D. Barron, *Chirality* **2006**, *18*, 103–115.

[3] a) I. H. McColl, E. W. Blanch, A. C. Gill, A. G. O. Rhie, M. A. Ritchie, L. Hecht, K. Nielsen, L. D. Barron, *J. Am. Chem. Soc.* **2003**, *125*, 10019–10026; b) I. H. McColl, E. W. Blanch, L. Hecht, L. D. Barron, *J. Am. Chem. Soc.* **2004**, *126*, 8181–8188; c) I. H. McColl, E. W. Blanch, L. Hecht, N. R. Kallenbach, L. D. Barron, *J. Am. Chem. Soc.* **2004**, *126*, 5076–5077.

[4] a) C. Herrmann, K. Ruud, M. Reiher, *ChemPhysChem* **2006**, *7*, 2189–2196; b) J. Kapitán, V. Baumruk, P. Bouř, *J. Am. Chem. Soc.* **2006**, *128*, 2438–2443; c) J. Kapitán, F. Zhu, L. Hecht, J. Gardiner, D. Seebach, L. D. Barron, *Angew. Chem.* **2008**, *120*, 6492–6494; *Angwe. Chem. Int. Ed.* **2008**, *47*, 6392–6394.

[5] C. Herrmann, K. Ruud, M. Reiher, *Chem. Phys.* **2008**, *343*, 200–209.

[6] E. W. Blanch, L. Hecht, L. A. Day, D. M. Pederson, L. D. Barron, *J. Am. Chem. Soc.* **2001**, *123*, 4863–4864.

[7] a) T. Miura, H. Takeuchi, I. Harada, *J. Raman Spectrosc.* **1989**, *20*, 667–671; b) H. Takeuchi, *Biopolymers* **2003**, *72*, 305–317.

[8] W. Hug, *Chem. Phys.* **2001**, *264*, 53–69.

[9] L. D. Barron, *J. Chem. Soc. A* **1971**, 2899–2904.

[10] a) R. Ahlrichs, et al., TURBOMOLE, URL: http://www.turbomole.com; b) R. Ahlrichs, M. Bär, M. Häser, H. Horn, C. Kölmel, *Chem. Phys. Lett.* **1989**, *162*, 165–169.

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[11] a) A. D. Becke, *Phys. Rev. A* **1988**, *38*, 3098–3100; b) J. P. Perdew, *Phys. Rev. B* **1986**, *33*, 8822–8824.
[12] a) A. Schäfer, C. Huber, R. Ahlrichs, *J. Chem. Phys.* **1994**, *100*, 5829–5835; b) TURBOMOLE basis set library, URL: ftp://ftp.chemie.uni-karlsruhe.de/pub/basen; c) TURBOMOLE auxiliary basis sets, URL: ftp://ftp.chemie.uni-karlsruhe.de/pub/jbasen.
[13] a) J. Neugebauer, M. Reiher, C. Kind, B. A. Hess, *J. Comput. Chem.* **2002**, *23*, 895–910; b) J. Neugebauer, C. Herrmann, S. Luber, M. Reiher, SNF 4.0—A program for the quantum chemical calculation of vibrational spectra, URL: http://www.theochem.ethz.ch/software/snf.
[14] a) R. Bauernschmitt, R. Ahlrichs, *Chem. Phys. Lett.* **1996**, *256*, 454–464; b) R. Bauernschmitt, M. Häser, O. Treutler, R. Ahlrichs, *Chem. Phys. Lett.* **1997**, *264*, 573–578; c) F. Furche, R. Ahlrichs, *J. Chem. Phys.* **2002**, *117*, 7433–7447; d) S. Grimme, F. Furche, R. Ahlrichs, *Chem. Phys. Lett.* **2002**, *361*, 321–328.
[15] S. Luber, M. Reiher, *Chem. Phys.* **2008**, *346*, 212–223.
[16] M. Reiher, V. Liégeois, K. Ruud, *J. Phys. Chem. A* **2005**, *109*, 7567–7574.
[17] S. Luber, C. Herrmann, M. Reiher, *J. Phys. Chem. B* **2008**, *112*, 2218–2232.
[18] JMOL—An open-source molecule viewer, URL: http://jmol.sourceforge.net.
[19] IUPAC–IUB Commission on Biochemical Nomenclature, *J. Biol. Chem.* **1970**, *245*, 6489–6497.

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