Tryptophan Absolute Stereochemistry in Viral Coat Proteins from Raman Optical Activity

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Raman optical activity (ROA), which measures vibrational optical activity spectra by means of a small difference in the intensity of Raman scattering from chiral molecules in right- and left-circularly polarized incident light, is finding many applications in studies of solution structure and function of biomolecules.[1] Although many protein ROA bands originate in vibrations of the peptide backbone, which contains the most rigid, chiral parts of the structure, there are a few distinct ROA bands from side chains which also contain useful information. A tryptophan ROA band in the 1550 cm^{-1} region, assigned to a W3 type vibration of the indole ring,[2] is especially prominent and, as reported here, appears to reflect the absolute stereochemistry of the tryptophan side chain conformation.

In a Raman study of tryptophan derivatives in the crystalline state, Miura et al.[2] found that the wavenumber of W3 correlates with the magnitude of the torsion angle $\chi^{2,1}$ defined by the three bonds $C_2{=}C_3{-}C_\beta{-}C_\alpha$ illustrated in Figure 1. This correlation was shown to apply also to tryptophan side chains in proteins in aqueous solution, including the coat proteins of viruses,[3] and has been exploited in time-resolved ultraviolet resonance Raman studies of hemoglobin.[4] The sign is not available from the conventional Raman spectrum but, as we show here, it is provided by the ROA spectrum since this is sensitive to absolute stereochemistry. This additional information could be valuable in many applications. For example, since different models of the coat protein structures of filamentous bacterial viruses can accommodate $\chi^{2,1}$ angles related by a 180° flip, which for values $\sim\pm90°$ produces angles with similar magnitudes but opposite signs,[5,6] an experimental determination of the sign of $\chi^{2,1}$ would remove this ambiguity.

ROA spectra of several filamentous bacterial viruses were reported recently.[7] In those strains containing a single tryptophan in the major coat proteins, namely M13 and IKe, a sharp *positive* ROA band appears at $\sim$1554 cm^{-1} similar to one observed in most native globular proteins containing tryptophans. For the strain Pf1, which contains no tryptophans, no ROA band occurs in this region. The Raman and ROA spectra of these three strains recorded in the earlier study are displayed in Figure 2 (that of IKe was not shown in the earlier report). We have since measured the ROA spectrum of another filamentous bacterial virus, PH75, which has unusual thermophilic properties.[8] Its Raman and ROA spectra are presented as the bottom pair in Figure 2. Although

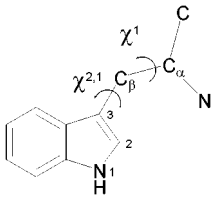

Figure 1. Definition of the tryptophan torsion angles χ^1 and $\chi^{2,1}$. The zero of $\chi^{2,1}$ corresponds to the eclipsed conformation of $C_2{-}C_3{-}C_\beta{-}C_\alpha$. A torsion angle is considered positive or negative according as when the system is viewed along the central bond in the direction $C_3 \rightarrow C_\beta$, the bond to the front atom C_2 requires a rotation to the right or left, respectively, in order that it may eclipse the bond to the rear atom C_α.[14]

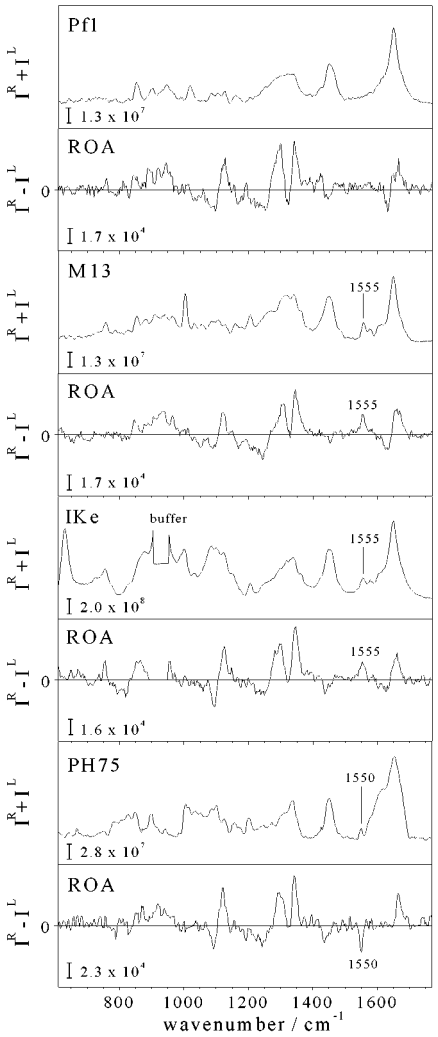

Figure 2. The backscattered Raman and ROA spectra of filamentous bacteriophages Pf1 (top pair), M13 (second pair), IKe (third pair), and PH75 (bottom pair) in 150 mM NaCl, 15 mM Tris-HCl (pH 7.6) measured at room temperature ($\sim$20 °C) on an instrument described previously.[15] The concentrations were $\sim$10 mg/mL. Instrumental conditions: laser wavelength 514.5 nm; laser power $\sim$700 mW at the sample; spectral resolution $\sim$10 cm^{-1}; recording time $\sim$48 h.

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(1) Barron, L. D.; Hecht, L.; Blanch, E. W.; Bell, A. F. *Prog. Biophys. Mol. Biol.* **2000**, *73*, 1−49.
(2) Miura, T.; Takeuchi, H.; Harada, I. *J. Raman Spectrosc.* **1989**, *20*, 667−671.
(3) Miura, T.; Thomas, G. J., Jr. In *Subcellular Biochemistry, Vol. 24. Proteins: Structure, Function and Engineering*; Biswas, B. B., Roy, S., Eds.; Plenum Press: New York, 1995; pp 55−99.
(4) Rodgers, K. R.; Su, C.; Subramaniam, S.; Spiro, T. G. *J. Am. Chem. Soc.* **1992**, *114*, 3697−3709.
(5) Takeuchi, H.; Matsuno, M.; Overman, S. A.; Thomas, G. J., Jr. *J. Am. Chem. Soc.* **1996**, *118*, 3498−3507.
(6) Tsuboi, M.; Overman, S. A.; Thomas, G. J., Jr. *Biochemistry* **1996**, *35*, 10403−10410.
(7) Blanch, E. W.; Bell, A. F.; Hecht, L.; Day, L. A.; Barron, L. D. *J. Mol. Biol.* **1999**, *290*, 1−7.
(8) Pederson, D. M.; Welsh, L. C.; Marvin, D. A.; Sampson, M.; Perham, R. N.; Yu, M.; Slater, M. R. *J. Mol. Biol.* In press.

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generally similar to the other three ROA spectra, the main features of which reflect the extended helix fold containing roughly equal amounts of hydrated and hydrophobic α-helix, the ∼1550 cm^{-1} tryptophan W3 ROA band of PH75 is *negative*. This suggests that the absolute stereochemistry of the indole ring relative to the plane defined by $C_3-C_\beta-C_\alpha$ in PH75 is quasienantiomeric to that in M13 and IKe.

The parent W3 Raman band wavenumbers are ∼1555 cm^{-1} for M13 and IKe which correspond, according to ref 2, to a value for the torsion angle magnitude $|\chi^{2,1}|$ of |105°|, and ∼1550 cm^{-1} for PH75 corresponding to |93°|. The uncertainties in these angles may be of the order ±10°.[5] Tryptophan side chains in a sample of 19 highly refined high-resolution protein X-ray structures were reported to cluster within six distinct rotamers with $\chi^{2,1}$ taking values in the range −87° to +113°.[9] The values deduced for M13, IKe, and PH75 fall within this range. Since large W3 ROA is generated by tryptophan conformations with $|\chi^{2,1}|$ in the vicinity of |90°|, the ROA intensity may have a $\sin\chi^{2,1}$ dependence, which would be consistent with a mechanism involving coupled C−H deformations,[10] possibly the large C_2-H deformation of W3 with $C_\beta-H$ deformations.

Since hen lysozyme shows a strong sharp *positive* W3 ROA band associated with a parent Raman band at ∼1553 cm^{-1},[1] the absolute sign of $\chi^{2,1}$ may be deduced by checking $\chi^{2,1}$ for the six tryptophans in this protein. For PDB structure 1lse the values are +102°, −79°, +106°, −90°, +80°, and +107° for tryptophans 28, 62, 63, 108, 111, and 123, respectively. A $\sin\chi^{2,1}$ dependence for the W3 ROA intensity would produce significant cancellation among four of the six tryptophan ROA signals. The observed positive W3 ROA signal would then result from the excess of tryptophans with positive $\chi^{2,1}$ angles. Further evidence for the validity of this approach is our observation of an ∼40% increase in the intensity of the positive W3 ROA band of hen lysozyme on binding to the trimer of *N*-acetylglucosamine (data not shown), which accords with the X-ray crystal structure of this complex (1lzb) in which the sign of $\chi^{2,1}$ for tryptophan 62 is reversed (the corresponding angles are +102°, +95°, +110°, −85°, +83°, and +103°). We therefore associate a positive W3 ROA signal with a positive value of $\chi^{2,1}$ and hence deduce that $\chi^{2,1}$ is +105° for M13 and IKe and −93° for PH75.

In partially denatured molten globule states of equine lysozyme[11] and human lysozyme,[12] the positive W3 ROA bands present in the native states are absent. This was suggested to arise because the tryptophans become less constrained, resulting in conformational heterogeneity with cancellation from oppositely signed ROA contributions. The present results validate this idea. The W3 ROA band may therefore also be used as a probe of conformational heterogeneity among a set of tryptophans in disordered protein sequences. It is similar in this respect to the near-ultraviolet electronic circular dichroism (UVCD) bands from aromatic side chains which also disappear when tertiary structure is lost on partial denaturation.[13] These two techniques provide complementary perspectives because ROA probes the intrinsic skeletal chirality of the tryptophan side chain whereas UVCD probes the chirality in the general environment of the chromophore.

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(9) Ponder, J. W.; Richards, F. M. *J. Mol. Biol.* **1987**, *193*, 775−791.
(10) Barron, L. D. *Molecular Light Scattering and Optical Activity*; Cambridge University Press: Cambridge, 1982
(11) Blanch, E. W.; Morozova-Roche, L. A.; Hecht, L.; Noppe, W.; Barron, L. D. *Biopolymers (Biospectroscopy)* **2000**, *57*, 235−248.
(12) Blanch, E. W.; Morozova-Roche, L. A.; Cochran, D. A. E.; Doig, A. J.; Hecht, L.; Barron, L. D. *J. Mol. Biol.* **2000**, *301*, 553−563.
(13) Woody, R. W.; Dunker, A. K. In *Circular Dichroism and the Conformational Analysis of Biomolecules*; Fasman, G. D., Ed.; Plenum Press: New York, 1996; pp 109−157.
(14) IUPAC-IUB Commission on Biochemical Nomenclature. In *J. Biol. Chem.* **1970**, *245*, 6489−6497.
(15) Hecht, L.; Barron, L. D.; Blanch, E. W.; Bell, A. F.; Day, L. A. *J. Raman Spectrosc.* **1999**, *30*, 815−825.