Vibrational Raman Optical Activity of Peptides and Proteins

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Vibrational Raman optical activity spectra in the range 1100—1500 cm^{-1} of aqueous solutions of L-alanyl-L-alanine, D-alanyl-D-alanine, lysozyme, and α-chymotrypsin show features originating in coupled C_α–H and N–H deformations of the peptide backbone and appear to be sensitive to the details of the secondary conformation.

Vibrational Raman optical activity (ROA) measurements on chiral molecules can provide a wealth of stereochemical information because a vibrational spectrum contains bands associated with every part of the molecular framework.[1—3] ROA is expected to be particularly valuable in studies of biologically significant molecules. However, owing to its greater sensitivity, the complementary technique of vibrational circular dichroism (VCD) has so far set the pace in this area.[4] However, a recent dramatic advance in ROA instrumentation based on the use of a backscattering geometry[5,6] (in place of the usual 90° scattering arrangement) together with a cooled charge coupled device (CCD) detector[7] has now provided sufficient sensitivity to render biologically significant molecules in aqueous solution accessible to ROA studies. This communication reports the first ROA spectra of peptides and proteins.

The peptide and protein samples were studied as near-saturated solutions in water contained in quartz microfluorescence cells, and ROA measurements were made using a focused 500 mW argon-ion laser beam at 488.0 nm and a spectral resolution (FWHH) of ~8 cm^{-1}.

Figure 1 shows the backscattered Raman and ROA spectra of L-alanyl-L-alanine and D-alanyl-D-alanine solutions acquired in 4 h. It can be seen that there is excellent reflection symmetry between the enantiomeric spectra. Interpretation of these spectra is aided considerably by the assignment work of Diem *et al.* using conventional Raman measurements[8,9] together with VCD,[10] and by our recent experimental and *ab initio* theoretical ROA work on alanine.[11] This report concentrates on a few ROA features that appear to be characteristic of the peptide conformation. The large couplet in the Raman bands at 1340 and 1372 cm^{-1} is also shown by

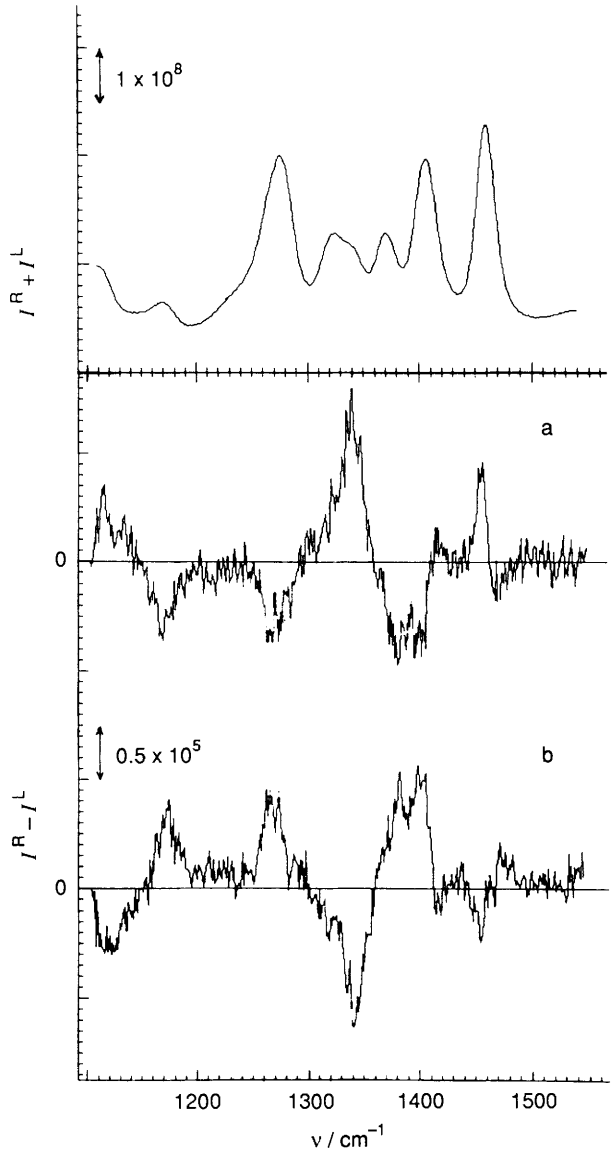

Figure 1. The backscattered Raman ($I^R + I^L$) and ROA ($I^R - I^L$) spectra of (a) L-alanyl-L-alanine and (b) D-alanyl-D-alanine in water. The intensity scales (in electron counts) are arbitrary.

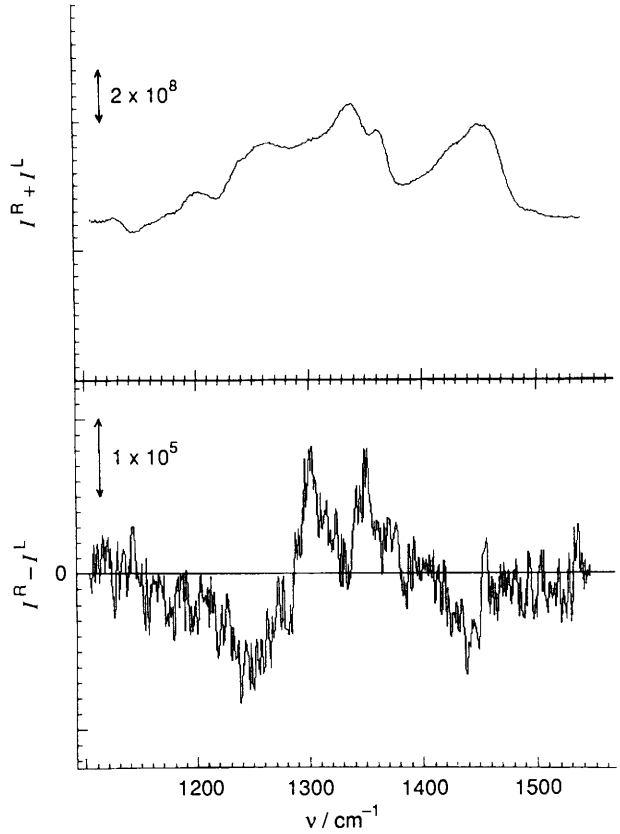

Figure 2. The backscattered Raman and ROA spectra of lysozyme in water.

alanine,[11] where it is known that the lower and higher frequency component bands originate in a methine C_α–H deformation and the symmetric methyl deformation, respectively. However, interaction with the symmetric methyl deformation is probably not the main source of ROA intensity in the 1340 cm^{-1} C_α–H band because a positive feature of similar intensity appears at the same frequency in the ROA spectrum of the L-L-L-alanyl tripeptide ROA spectrum (not shown) with a much reduced negative feature in the methyl symmetric deformation; also a large positive feature is seen at slightly lower frequency in the ROA spectrum of L-polyglutamic acid (not shown) which contains no methyl groups. The next band, at 1325 cm^{-1}, which shows ROA of the same sign as that at 1340 cm^{-1} (but weaker) is probably related to one in alanine at slightly lower frequency with similar ROA and which is known to originate in another C_α–H deformation.[11] The band at ~1279 cm^{-1} showing ROA of opposite sign to that in the 1325 and 1340 cm^{-1} bands is conventionally assigned to the 'amide III' vibration, which is supposed to consist mainly of the N–H deformation.[12] However, Diem *et al.*[8—10] have shown that in fact this band, together with those at 1325 and 1340 cm^{-1}, involves much more mixing between the C_α–H and the N–H deformations than previously supposed, which explains the well known geometric sensitivity of the amide III band.

Figures 2 and 3 show the backscattered Raman and ROA spectra of lysozyme and α-chymotrypsin solutions, the first being run for 16 h and the second for 13 h. The dominant feature common to both ROA spectra is a broad couplet, negative on the lower frequency side and positive at higher frequency, centred at ~ 1275 cm^{-1}, which is within the conventional amide III region. However, a major difference between the two protein ROA spectra appears to the high frequency side of this broad couplet, where α-chymotrypsin shows a second couplet centred at ~1320 cm^{-1} that is positive at lower frequency and negative at higher frequency. The lysozyme ROA drops to zero rapidly towards 1320 cm^{-1} and then becomes positive again suggesting a similar couplet to that in α-chymotrypsin but with opposite sign. Extrapolating from the peptide results discussed above, we tentatively propose that these major protein ROA features originate in coupled C_α–H and N–H deformations from the peptide backbone and so represent a superposition of ROA bands from the various types of secondary backbone conformations, being particularly sensitive to the range of angles φ around the different C_α–N bonds present in the particular protein. It is intriguing that the region from ~1300 to 1380 cm^{-1} where the ROA spectra of the two proteins differ significantly is the region where 'amide III' modes characteristic of reverse turns in polypeptide chains are expected to be observed.[13]

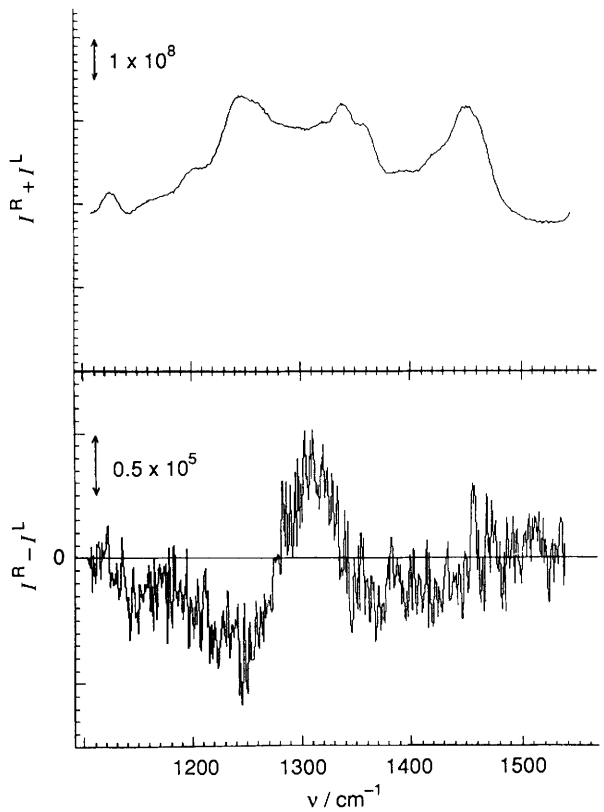

Figure 3. The backscattered Raman and ROA spectra of α-chymotrypsin in water.

The importance of the preliminary results presented here is that they demonstrate that it is now possible to obtain ROA spectra of biological molecules in aqueous media and that the spectra appear to be sensitive to important conformational features. For the same reasons that have led conventional Raman spectroscopy to find many applications in biochemistry (water is a good solvent for Raman studies, the complete vibrational spectrum is accessible, resonance enhancement enables sites of biological function to be probed directly, *etc.*[14]), ROA is now expected to become a powerful new method in biochemical spectroscopy.

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