β-Sheet and associated turn signatures in vibrational Raman optical activity spectra of proteins

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Abstract

We have measured the aqueous solution vibrational Raman optical activity (ROA) spectra of concanavalin A, α-chymotrypsin, and β-lactoglobulin, all of which are rich in β-sheet, together with that of the model β-turn peptide L-pro-L-leu-gly-NH_2. Possible ROA signatures of antiparallel β-sheet include a strong sharp positive band at $\sim 1,313$ cm^{-1} associated with backbone amide III C_αH and NH deformations, and an amide I couplet, negative at low wavenumber and positive at high, centered at $\sim 1,658$ cm^{-1}. Negative ROA bands in the range $\sim 1,340$–1,380 cm^{-1}, which might originate in glycine CH_2 deformations, appear to be characteristic of β-turns. Our results provide further evidence that ROA is a more incisive probe of protein conformation than conventional vibrational spectroscopy, infrared, or Raman, because only those few vibrational coordinates within a given normal mode that sample the skeletal chirality directly contribute to the corresponding ROA band intensity.

Keywords: protein secondary and supersecondary structure; Raman optical activity; vibrational optical activity of β-sheet proteins

Raman optical activity (ROA) refers to a small difference in the intensity of vibrational Raman scattering from chiral molecules in right and left circularly polarized incident light (Barron, 1982; Barron & Hecht, 1993). It provides complete vibrational optical activity spectra and so, like the complementary technique of vibrational circular dichroism (Keiderling & Pancoska, 1993), can provide much new stereochemical information. Thanks to new instrumental developments (Hecht et al., 1992), ROA spectra can now be measured routinely on a large range of biological molecules in aqueous solution and provide a completely new perspective on solution structure (Barron & Hecht, 1993). Proteins show particularly rich ROA spectra that contain information about both secondary backbone and sidegroup conformation (Barron et al., 1992a, 1992b; Wen, 1992): ROA builds upon, and adds a new dimension to, conventional Raman spectroscopy, which is already well established as a valuable probe of protein structure (e.g., Williams, 1986). Although it is unlikely that ROA will provide complete structures like X-ray crystallography and 2-dimensional NMR, its simple application to aqueous solution samples with no restrictions on the size of the biopolymer (unlike 2-dimensional NMR) makes it ideal for studying many timely problems in protein science.

The ability of ROA to probe short-range structural relationships seems to be reflected in signals characteristic of loops and turns in addition to basic secondary conformation elements such as α-helix and β-sheet: this could be particularly valuable because these motifs are central to protein folding and function but are not easily probed by other physical techniques. In this short paper, we discuss the ROA spectra of several proteins with a high β-sheet content together with that of a model type II β-turn peptide and point out some interesting correlations which suggest that ROA provides clear signatures of β-sheet structures and certain associated turns.

Results

β-sheet proteins

We measured the backscattered aqueous solution Raman and ROA spectra of concanavalin A, α-chymotrypsin, and β-lactoglobulin, all of which contain significant amounts of antiparallel β-sheet. The ROA spectra of the last two proteins are shown in Figure 1. Experimental difficulties associated with aggregation of the protein under prolonged exposure to the laser beam prevented us from obtaining a complete ROA spectrum of concanavalin A of good enough quality to present here; nonetheless certain important features developed sufficiently strongly to be included in the discussion. We shall also include the ROA spectrum of ribonuclease A, which has been reported previously (Barron et al., 1992a, 1992b), in the discussion since this protein is also rich in β-sheet.

According to the secondary structure analysis method of Levitt and Greer (1977), concanavalin A and α-chymotrypsin
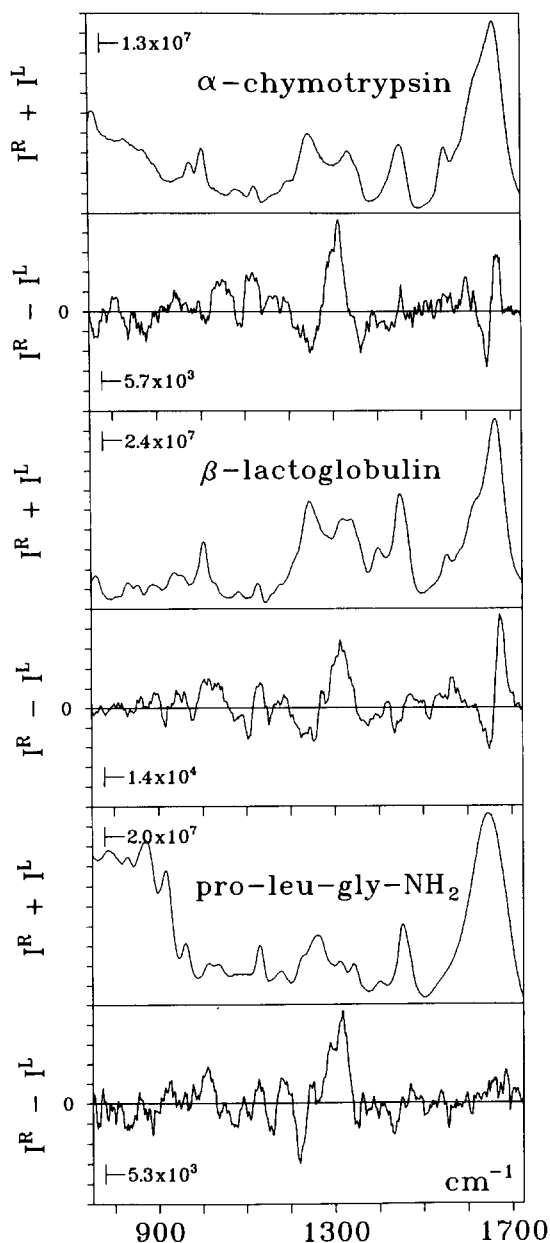
Fig. 1. The backscattered Raman and ROA spectra of α-chymotrypsin, β-lactoglobulin and L-pro-L-leu-gly-NH₂ in aqueous solution.

are classified as all β, containing 64% β-sheet with 2% α-helix, and 51% β-sheet with 10% α-helix, respectively. Although ribonuclease A is classified as (α + β), there is nonetheless much more β-sheet (46%) than α-helix (23%). The β-sheet in concanavalin A takes the form of a β-barrel made up from 2 antiparallel pleated sheets, one a twisted sheet of 7 strands and the other a bowed sheet of 6 strands (Reeke et al., 1975): this protein therefore provides a good model for ROA signatures of antiparallel β-sheet and associated turns and loops with negligible interference from α-helix bands. In α-chymotrypsin the β-sheet is contained in 2 domains each centered on a 6-stranded antiparallel β-barrel (Branden & Tooze, 1991). The structure of ribonuclease A is similar to that of ribonuclease S, which is known

to have antiparallel β-sheet in the form of a highly twisted sheet rather than a barrel (Mathews & van Holde, 1990). The molecule of β-lactoglobulin contains approximately 50% β-sheet and 15% α-helix (Casal et al., 1988): the core is an 8-stranded antiparallel β-barrel made from 2 4-stranded slabs; in addition a 9th strand, together with a stretch of α-helix and one of the constituent strands of the β-barrel, defines a β-α-β motif (Papiz et al., 1986).

An interesting spectral region for protein ROA is the extended amide III where peptide backbone modes involving $C_\alpha H$ and NH deformations occur (Barron & Hecht, 1993; Ford et al., 1993). A dominant extended amide III feature of α-chymotrypsin is a strong, sharp positive ROA band at ~1,313 cm⁻¹. A similar feature appears in the ROA spectra of concanavalin A and β-lactoglobulin and can also be discerned in ribonuclease A; but because a peak is not observed at this wavenumber in highly α-helical proteins such as bovine serum albumin and insulin (Wen et al., 1994), we tentatively assign it to antiparallel β-sheet. All 4 proteins also show a negative ROA band in the range ~1,340–1,380 cm⁻¹, but again it is not seen in proteins rich in α-helix: from comparisons with a model β-turn peptide (vide infra) we suggest that it is a β-turn signature.

Ribonuclease A shows a strong negative ROA band peaking at ~1,245 cm⁻¹, which we have previously suggested might be a signature of a certain type of loop with local order (Wen et al., 1994). A similar ROA band, but broader and weaker, is shown here by concanavalin A and α-chymotrypsin. On the other hand, the ROA spectrum of β-lactoglobulin in this region is strikingly different from that of any other protein studied to date; namely a sharp couplet, negative at low wavenumber and positive at high centered at ~1,264 cm⁻¹: because this is the first ROA spectrum of a protein containing a β-α-β motif to have been measured, we tentatively suggest that it might originate in this supersecondary element. This idea could be tested by measuring the ROA spectrum of plasma retinol-binding protein, which has an antiparallel β-barrel structure remarkably similar to that in β-lactoglobulin but without the β-α-β motif (Papiz et al., 1986).

Both α-chymotrypsin and concanavalin A show an approximately conservative couplet in the amide I region, where modes originating mostly in the amide carbonyl stretch occur, that is negative at low wavenumber and positive at high with a crossover at ~1,658 cm⁻¹. Since the α-helix appears to give a single positive ROA band in the amide I region (Wen et al., 1994), we suggest that this couplet is a signature of antiparallel β-sheet. It is interesting that β-lactoglobulin shows a larger amide I ROA couplet than α-chymotrypsin and concanavalin A with more intensity in the higher-wavenumber positive component: this might be connected with the fact that the antiparallel β-strands within the β-barrel in β-lactoglobulin are in a simple up-and-down arrangement, whereas in α-chymotrypsin and concanavalin A they are arranged in a Greek key motif with connections across the ends of the barrel (Branden & Tooze, 1991). Ribonuclease A also shows a similar amide I ROA couplet to that in β-lactoglobulin, which again might be associated with its motif structure. In addition, the α-helix content of both β-lactoglobulin and ribonuclease A might also boost the positive higher-wavenumber component. It should be mentioned that bovine serum albumin, which contains only α-helix and loop structures, also shows an amide I ROA couplet with a small negative low-wavenumber component (Wen et al., 1994): however, the crossover occurs at

~1,647 cm^{-1}, significantly lower than in the β-sheet proteins, so the negative component might originate in other structural elements that are present in this protein.

There could be a simple explanation for the different α-helix and β-sheet ROA signatures in the amide I region, first suggested to us by P. Pancoska, which depends on the fact that ROA intensity, like vibrational circular dichroism (Bour & Keiderling, 1993), appears to be dominated by short-range mechanisms with little contribution from delocalized exciton-type mechanisms centered on extended helix and sheet modes of vibration. In the α-helix, all the C=O groups are in the same hydrogen-bonded environment with the same local chirality so, assuming there is no significant contribution from a delocalized exciton-type mechanism, a single ROA band is expected. On the other hand, there are at least 2 different environments for the C=O groups in β-sheet: if the group is in an edge strand, it can point in toward an adjacent β-strand or out into the environment. Thus more ROA structure in the amide I region is anticipated from β-sheet than from α-helix. This interpretation also suggests that association of protein molecules into dimers, tetramers, etc. via β-sheet linkages, as is known to occur in concanavalin A (Arrondo et al., 1988) and β-lactoglobulin (Casal et al., 1988), must be taken into account when interpreting amide I β-sheet signatures and indeed could be used to monitor the formation of such species. However, at this early stage in protein ROA studies, exciton-type mechanisms cannot be ruled out as an alternative explanation for the characteristic bands of extended secondary structures in the amide I region.

ROA signals can also be discerned in the lower-wavenumber spectral region ~800–1,200 cm^{-1} of our β-sheet proteins, where backbone C–C and C–N skeletal stretch coordinates contribute significantly to the normal modes. In particular, a broad positive ROA band in the range ~1,000–1,060 cm^{-1} could well originate in β-sheet as α-helix proteins show little ROA intensity in this range (Wen et al., 1994).

L-Pro-L-leu-L-gly-NH$_2$

The tripeptide L-pro-L-leu-L-gly-NH$_2$ is known to take up a type II β-turn conformation in the crystal (from X-ray diffraction: Reed & Johnson, 1973) and in dimethylsulfoxide solution (from NMR: Higashijima et al., 1978). As shown in Figure 2, in this conformation a rigid 10-membered turn structure is stabilized by an intramolecular hydrogen bond between the carbonyl of the proline residue and a proton of the terminal amide group. Conventional Raman studies of this molecule in both the solid and solution state have been reported (Hseu & Chang, 1980; Fox et al., 1981) together with a detailed normal mode analysis of the crystalline structure (Naik & Krimm, 1984). Unfortunately the aqueous solution conformation of this tripeptide is not known with certainty: on the basis of Raman studies, it has been suggested that a rotational isomer is formed, which is different from that in the crystal and dimethylsulfoxide on account of hydrogen bonding between the carbonyl groups and surrounding water molecules (Hseu & Chang, 1980). Nonetheless, as shown below there are some highly suggestive correlations between its aqueous solution ROA spectrum and those of certain proteins.

The backscattered Raman and ROA spectra of L-pro-L-leu-gly-NH$_2$ in H$_2$O are shown at the bottom of Figure 1. It is striking how several of the ROA features in the range ~1,200–1,400 cm^{-1} also appear in the ROA spectra of the β-sheet proteins. These

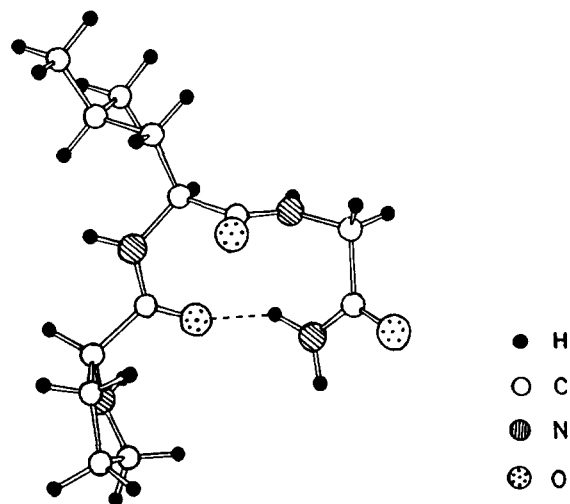**Fig. 2.** The crystal conformation of L-pro-L-leu-gly-NH$_2$ (adapted from Naik & Krimm, 1984).

● H
○ C
◕ N
◉ O

ROA features in the tripeptide include a sharp negative band at ~1,348 cm^{-1} and a strong sharp positive ROA band at ~1,317 cm^{-1}, both of which appear in similar positions in all 3 protein spectra; and a large negative band at ~1,222 cm^{-1} that also appears in β-lactoglobulin but is not so apparent in the other 2 proteins. Naik and Krimm (1984) assign significant contributions from glycine and leucine CH$_2$ wag and twist coordinates, and proline CH$_2$ wag coordinates (at the higher wavenumber end of this range), together with in-plane NH deformations, to the normal modes of crystalline pro-leu-gly-NH$_2$ in this region, and it is reasonable to expect similar contributions in aqueous solution. We therefore suggest that CH$_2$ deformations coupled with in-plane NH deformations will provide signatures of β-turn structures in protein ROA spectra and are probably responsible for the negative ROA band in all 3 proteins in the range ~1,340–1,380 cm^{-1}, and perhaps for the negative ROA band shown by β-lactoglobulin at ~1,224 cm^{-1}. Whether or not the ~1,317-cm^{-1} positive ROA band in the peptide has anything to do with the similar-looking ~1,313-cm^{-1} protein ROA band remains an open question; but our current opinion is that the protein band originates more in extended amide III β-sheet vibrations than in the turns.

Little ROA intensity is shown by L-pro-L-leu-gly-NH$_2$ in the amide I region. Although this suggests that the amide I ROA couplet shown by the proteins discussed above does indeed originate mostly in antiparallel β-sheet, rather than in β-turns, much more experimental data on model β-turn peptides is needed to properly characterize β-turn amide I ROA signatures.

Discussion

These observations indicate that β-sheet and β-turn signatures might be readily identifiable in protein ROA spectra. However, due to the high concentrations employed in order to obtain good ROA spectra, the peptide and protein reference states were not necessarily well defined (for example, concanavalin A and β-lactoglobulin are known to associate under the conditions used here), so the structural origins of some of the ROA signals discussed in this paper should not yet be taken as definitive. Many

studies of other model peptides and proteins in both H_2O and D_2O are necessary before the correlations pointed out here can be applied with confidence to the analysis of protein structure in solution.

Using a conventional approach to the spectroscopic analysis of protein secondary structure, it would be necessary to perform ROA measurements on aqueous solutions of polypeptides in model β-sheet conformations and on a number of small peptides with well-characterized model β-turns before protein β-sheet and associated turn signals can be fully characterized. Unfortunately, experimental difficulties have hampered such studies: model β-sheet polypeptides tend to assume a glue-like consistency with large random birefringence, which prevents ROA measurements; and model β-turn peptides tend to have too low a solubility. Our only success so far has been with the pro-leu-gly-NH_2 peptide discussed here.

On the other hand, the results presented here and elsewhere suggest that an alternative "bootstrap" approach to the analysis of protein ROA spectra might be fruitful. Because only those few local vibrational coordinates within a normal mode that sample the skeletal chirality directly make significant contributions to the ROA intensity, ROA is able to cut through the complexity of conventional vibrational spectra to provide an incisive probe of backbone conformation by means of characteristic ROA band patterns, which are usually much simpler than the parent Raman band patterns. Thus, provided well-refined X-ray crystal structures are available, the proteins themselves might provide examples of model conformational features for ROA characterization; and this in turn could be used to identify new solution conformational features of peptides. A similar philosophy is sometimes applied to analyze the conventional electronic ultraviolet circular dichroism spectrum of a protein with unknown structure in terms of the spectra of proteins that have a known secondary structure (Johnson, 1990) and is the basis of protein vibrational circular dichroism amide I band analysis (Keiderling & Pancoska, 1993).

The preliminary identification of β-sheet and associated turn protein ROA signatures reported here, together with those of α-helix and associated loops reported elsewhere (Wen et al., 1994), suggest that ROA will soon be able to provide useful information on protein secondary and supersecondary (motif) structure in aqueous solution. Anticipated developments in ROA instrumentation in the near future should greatly facilitate such studies.

Materials and methods

The instrument used for the ROA measurements has been described previously (Hecht et al., 1992). It is based on backscattering, which is essential for aqueous solution samples such as proteins, and employs a single-grating spectrograph fitted with a backthinned CCD camera and a holographic notch filter to block the Rayleigh line.

All the samples were purchased from Sigma and used without further purification. L-pro-L-leu-gly-NH_2 was prepared as a near-saturated solution in H_2O (~0.4 M). The protein concentrations were ~150 mg/mL, with concanavalin A and α-chymotrypsin in acetate buffer at pH 5.1, and β-lactoglobulin in H_2O. If dissolved in H_2O, concanavalin A produces a highly scattering milky solution unsuitable for ROA studies, presum-

ably due to aggregation of the tetramers that are known to exist at pH 7.0 (Arrondo et al., 1988); however, below pH 6.0 the tetramers dissociate into dimers and a clear solution can be obtained. The solutions were filtered through 0.22-μm Millipore filters into quartz microfluorescence cells and centrifuged, followed by prolonged exposure to the laser beam to reduce fluorescence from traces of impurities.

The ROA spectra are presented in the form of a circular intensity difference (in ADC counts) $I^R - I^L$, where I^R and I^L are the Raman-scattered intensities in right- and left-circularly polarized incident light. The conventional Raman intensities are presented as a corresponding circular intensity sum $I^R + I^L$. The experimental conditions were as follows: laser wavelength, 514.5 nm; laser power at the sample, 600 mW; spectral band width, 10 cm^{-1}; recording time ~37 h for the tripeptide, ~17 h for α-chymotrypsin, and ~25 h for β-lactoglobulin.

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