α-Helix and Associated Loop Signatures in Vibrational Raman Optical Activity Spectra of Proteins

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Abstract: A comparison of the aqueous solution vibrational Raman optical activity (ROA) spectra of poly-L-lysine in random coil and α-helix conformations with those of bovine serum albumin and insulin, which are both rich in α-helix, is reported. Possible ROA signatures of α-helix include a broad positive band in the range ~900–1000 cm^{-1} and a couplet, negative at low wavenumber and positive at high, centred at ~1103 cm^{-1}, both features originating in backbone C_α–C and C_α–N stretch modes; a negative–positive couplet centred at ~1275 cm^{-1} originating in backbone amide III $C_\alpha H$ and NH deformations; and a positive band in the amide I region peaking at ~1665 cm^{-1}. Bovine serum albumin, but not insulin, shows an intense positive ROA band at ~1339 cm^{-1} which might originate in some of the many rigid loops present in this protein: a similar ROA band occurs in α-helical, but not in random coil, poly-L-lysine at ~1335 cm^{-1}, which suggests that the α-helical sections are connected by similar loop structures. Our results indicate that ROA is a more incisive probe of biopolymer conformation than conventional vibrational spectroscopy because only those few vibrational coordinates within a complicated normal mode which sample the skeletal chirality directly contribute to the corresponding ROA band intensity.

Introduction

Raman optical activity (ROA), which refers to a small difference in the intensity of Raman scattering from chiral molecules in right and left circularly polarized incident light,[1,2] measures vibrational optical activity and thus, like the complementary technique of vibrational circular dichroism,[2,3] can provide much new stereochemical information. Recently, new instrumental developments[4] have enabled ROA spectra to be measured routinely on a large range of biological molecules is aqueous solution and provide a completely new perspective on solution structure.[5,6] In particular, proteins show rich ROA spectra that contain information about both secondary backbone and side group conformation.[6-8] Although it is unlikely that ROA will provide complete structures like X-ray crystallography and two-dimensional NMR, its simple application to aqueous solution samples with no restrictions on the size of the biopolymer (unlike two-dimensional NMR) makes it attractive for studying many current problems in protein chemistry.

Preliminary results suggest that protein ROA spectra contain signals characteristic of loops and turns in addition to those from basic secondary conformation elements such as α-helix and β-sheet. These signals reflect the ability of ROA to probe short-range structural relationships and could be particularly valuable since loops and turns are central to protein folding and function but are not easily probed in aqueous solution by other physical techniques. Here we present the ROA spectra of poly-L-lysine in random coil and α-helix conformations and point out some interesting correlations with the ROA spectral features of two proteins with a high α-helix content which suggest that ROA provides clear signatures of α-helix structures and certain associated loops. A parallel study of β-sheet and associated turn ROA signatures is reported elsewhere.[9]

Experimental Details

The instrument used for the ROA measurements is based on backscattering, which is essential for aqueous soltion samples such as proteins, and employs a single-grating spectrograph fitted with a backthinned CCD detector and a holographic notch filter to block the Rayleigh line.[4]

The sample of poly--lysine was purchased from Sigma (as the hydrobromide, mol wt 15000–30000), and bovine serum albumin (crystallized, salt-free, lyophilized) and insulin (bovine pancreas) were purchased from Fluka. All the samples were used without further purification and dissolved in H_2O at a concentration of ~150 mg/mL. The required peptide pH values were obtained with use of HCl or NaOH. Insulin runs were conducted at pH = 2 in HCl to increase the solubility. The solutions were filtered through 0.22-μm Millipore filters into quartz microfluorescence cells, 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 ~15 h for the peptides and ~10 h for the proteins.

Results and Discussion

Poly-L-lysine is particularly favored by spectroscopists because α-helix, β-sheet, and random coil conformations can be easily induced in aqueous solution by changing conditions such as pH and temperature.[3,10-13] We obtained good ROA spectra of poly-L-lysine under the conditions required for α-helix (pH = 11, T

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= 20 °C) and random coil (pH = 3, T = 20 °C). It can be seen from Figure 1 that they are quiet different, despite the fact that that corresponding parent Raman spectra are rather similar with only a few small differences. Conventional Raman spectra of poly-L-lysine in random coil and α-helix conformations have been reported previously:[11,12] our random coil spectrum is very similar to the corresponding spectra from these earlier studies, but our α-helix spectrum does not look quite the same (it seems to have more random coil character). However, the possibility that our poly-L-lysine sample at pH = 11 is not a well-defined structure serves to emphasise that ROA is a more incisive probe of solution conformation than conventional vibrational spectroscopy because, as discussed below, the ROA spectrum appears to carry new information about the nature of the conformational elements present.

The most important ROA features of random coil poly-L-lysine appear in the amide I and extended amide III regions. Thus the strong positive band peaking at ~1677 cm^{-1} originates mostly in the amide I C=O stretch while the broad negative band between ~1180 and 1261 cm^{-1} and the strong positive band peaking at ~1318 cm^{-1} originate in extended amide III modes involving $C_\alpha H$ and in-plane NH deformations.[14] We also measured the ROA spectrum of random coil poly-L-glutamic acid (not shown)[6] which displays similar features to random coil poly-L-lysine in the amide I and extended amide III regions, but little ROA intensity elsewhere.

Poly-L-lysine at pH = 11 shows a remarkably similar ROA spectrum to that of the highly α-helical protein bovine serum albumin (BSA) also shown in Figure 1 (human serum albumin has a 67% α-helix content[15]). We shall focus initially on the following common ROA features: a broad positive band between ~915 and 993 cm^{-1} in poly-L-lysine but slightly lower in BSA (~900–970 cm^{-1}), a couplet centred at ~1103 cm^{-1} negative at low wavenumber and positive at high, and a similar couplet centered at ~1275 cm^{-1} but with most intensity in the positive high-wavenumber band which peaks at ~1300 cm^{-1}. Since they occur in the ROA spectra of other proteins such as insulin (61%[16])-with significant α-helix content (also shown in Figure 1) but not in proteins with little α-helix,[6-9] we attribute these ROA features to α-helix modes, the first two involving backbone C_α–C and C_α–N stretch coordinates and the third extended amide III modes. In fact a Raman band between 909 and 945 cm^{-1} originating in skeletal motions has been identified previously as a conventional Raman marker for α-helical structure, but this band could not be used to uniquely identify the α-helix since random coil polypeptides also show a similar band in this region (see the top two spectra in Figure 1).[17] However, ROA distinguishes the two types of structure here since random coil poly-L-lysine shows a negative ROA band in this region.

The fourth common ROA feature shown by poly-L-lysine at pH = 11 and by BSA, a large positive band at ~1335 cm^{-1} in the former and at ~1339 cm^{-1} in the latter, is not shown by insulin and does not appear in β-sheet proteins,[9] which reinforces our earlier suggestion[6-8] that it is associated with the many double rigid helix–loop–helix motifs possessed by BSA on account of the large number of adjacent disulfide bridges.[15,18] This also has the intriguing implication that, in our sample of poly-L-lysine, the α-helical sections are connected by "BSA-type" loop structures, which is consistent with the indication from the conventional

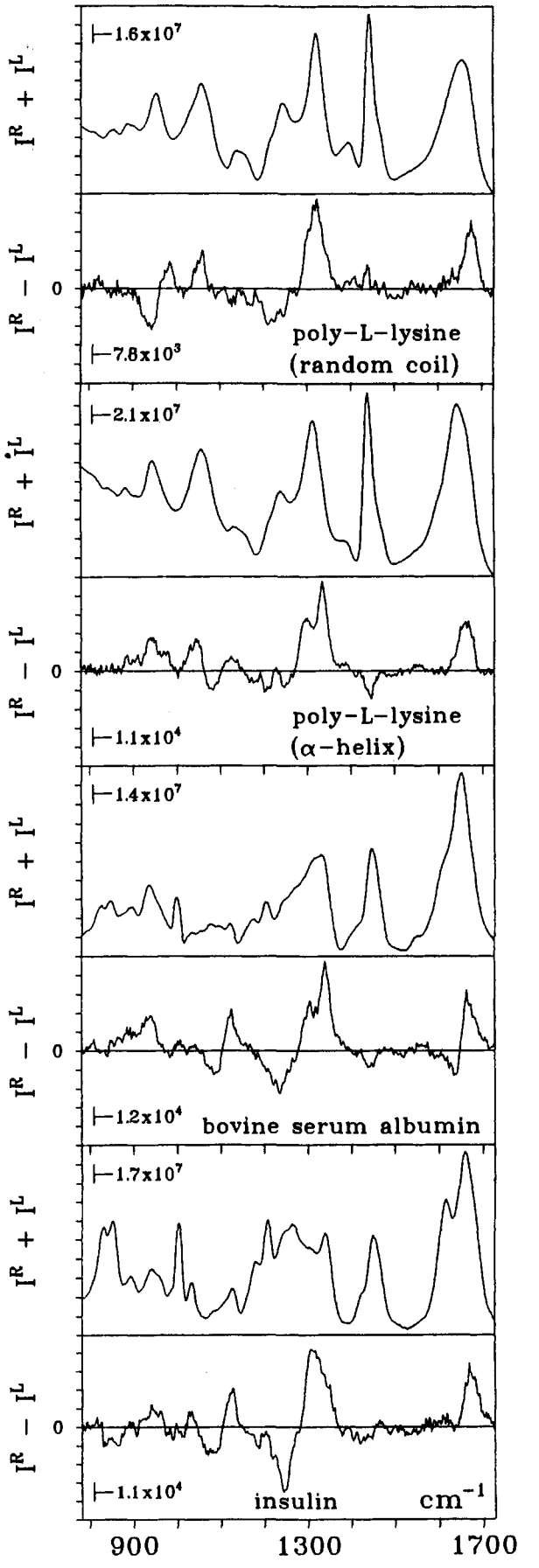

Figure 1. The backscattered Raman ($I^R + I^L$) and ROA ($I^R - I^L$) spectra of poly-L-lysine in random coil and α-helix conformations and of bovine serum albumin and insulin, all in H_2O.

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Raman spectrum that our sample was not completely α-helical. To our knowledge no other physical technique has detected evidence for this type of aqueous solution loop conformation in poly-L-lysine.

On the other hand, insulin shows a strong sharp negative ROA band peaking at ~1245 cm^{-1}. ROA therefore appears to reveal here two types of protein loop structure, each with a different but well-defined (from the sharpness of the corresponding ~1245- and 1339-cm^{-1} ROA bands) type of local order. BSA also probably contains some of the "insulin-type" loop structure since it shows a negative ROA peak at ~1237 cm^{-1}, but weaker and broader than in insulin. Another example of the strong negative ~1245-cm^{-1} ROA band is shown by ribonuclease A^{6-8} which contains a large amount of structure of "irregular geometry"[19] which we therefore suggest is similar to the "insulin-type" loop structure (at least in our aqueous solution samples).

Our earlier ROA results[6-8] now reveal that both types of locally ordered loop occur in lysozyme and α-lactalbumin, but in different proportions in accordance with the different functions of these two proteins: thus the ROA spectra of both proteins reveal the positive ~1339-cm^{-1} band and the negative ~1245-cm^{-1} band; but the former is much stronger in α-lactalbumin and the latter much stronger in lysozyme, from which we deduce the more of the "BSA-type" loop is found in α-lactalbumin and more of the "insulin-type" loop in lysozyme. Since helix–loop–helix motifs can function as calcium binding sites,[20] it is possible that a "BSA-type" loop is involved in calcium binding in α-lactalbumin.

The α-helix also appears to give rise to a single positive ROA band in the amide I region peaking at ~1665 cm^{-1}. This contrasts with the couplets, negative at low and positive at high wavenumber and centered at ~1658 cm^{-1}, that appear to be characteristic of antiparallel β-sheet.[9] It can be seen from Figure 1 that BSA also shows an amide I ROA couplet: however, the crossover occurs at ~1647 cm^{-1}, significantly lower than in the β-sheet proteins, so the small negative component in the low-wavenumber side might originate in other structural elements that are present. Insulin also shows a hint of a similar negative ROA band on the low-wavenumber side of the strong positive amide I band. However, the protein amide I band is usually intense and quite strongly polarized which can lead to distortions in the associated ROA, so further studies are necessary before these particular signatures can be applied with confidence.

Concluding Remarks

The results presented here indicate that α-helix and associated loop signatures might be readily identifiable in protein ROA spectra. However, on account of the high concentrations and certain pH values employed in order to obtain good ROA spectra, the peptide and protein reference states were not necessarily well-defined (for example, insulin is known to associate in aqueous solution at high concentration[21]), 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 protein structure analysis.

Our results suggest that, rather than relying on correlations with ROA spectra of peptides in known model conformations, an alternative "bootstrap" approach to the analysis of protein ROA spectra might be useful. 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 method is sometimes used 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,[22] and it is the basis of protein VCD spectral analysis.[3]

The normal vibrational modes of proteins can be very complex, with contributions from many skeletal and side group local vibrational coordinates all of which can affect the intensity and frequency. We have therefore not dwelt much on vibrational assignments, particularly in the lower wavenumber region. However, the results presented here demonstrate that ROA can cut through the resulting complexity of the conventional vibrational spectra: only those few local vibrational coordinates within a complicated normal mode which sample the skeletal chirality most directly make significant contributions to the associated ROA intensity, thereby generating characteristic ROA band patterns which are usually much simpler than the parent Raman band patterns.

The preliminary identifications of α-helix and locally-ordered loop protein ROA signatures reported here, together with those of β-sheet and associated turns reported elsewhere,[9] suggest that ROA will soon be able to provide useful information on protein secondary and supersecondary (motif) structure in aqueous solution. Such studies are currently hampered by the high sample concentrations and long spectral aquisition times that are necessary to achieve an acceptable signal-to-noise ratio, but hopefully these restrictions will be removed in the near future with anticipated improvements in ROA instrumentation.

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