APPLICATION OF LASER RAMAN SPECTROSCOPY TO THE STRUCTURAL ANALYSIS OF POLYPEPTIDES IN DILUTE AQUEOUS SOLUTION

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1. Introduction

To obtain information on the structure-function relationships of proteins, both soluble and within membrane systems, it is necessary to study these species in their native aqueous environments. Of the physical techniques presently available, laser Raman spectroscopy appears to offer unusual opportunities to realize this goal.

The information supplied by laser Raman spectroscopy is similar to that yielded by infrared spectroscopy and complementary to it. When a transparent medium is irradiated with monochromatic photons of frequency ν_0, most of these pass through unchanged, but a small proportion is scattered by solute and solvent molecules. The bulk of the scattered photons have the same frequency as the incident light, but a small number have frequencies, $\nu_0-\nu_1$, $\nu_0-\nu_2$, etc., different from that of the incident light. A series of Raman scattering bands result whose frequency shifts, ν_1, ν_2, etc. are characteristics of the covalent bonds within the molecules producing the scattering [1, 2]. The frequency shifts of covalent bonds in organic substances lie in the range of 150–4000 cm^{-1} and are, in general, close to the infrared absorption frequency of these bonds. Both Raman and infrared spectroscopy yield information about intramolecular vibrational and rotational frequencies, but the symmetry selection rules which specify whether a particular mode will appear in the spectrum are different for the two techniques [3, 4]. Thus, Raman spectroscopy of dilute aqueous solutions is possible in frequency regions inacessible to infrared measurements, because the OH-bending vibration which causes intense infrared absorption produces only weak Raman scattering.

2. Measurement of Raman shifts of polypeptides in dilute aqueous solution

Fig. 1. is a schema of our apparatus. The light source is the 4879.8 Å line of an argon ion laser with 1.0 W maximum power. Power to the sample is stabilized to ± 3% per scan of 60–125 Å and is regulated by adjustment of laser output and use of neutral-density filters. The beam diameter within the sample cell is 0.05–0.10 mm. Light scattered at 90° is focussed by an f 1.2 lens upon the slit of a Spex 1400 double monochromator with slit width 150–200 μm (resolution 3.5–4.5 cm^{-1}). The volume of the beam sampled by the monochromator is about 0.1 μl. The system thus permits analysis of extremely small amounts of material, although in our experiments we use a total sample volume of 2–3 ml, contained in rectangular cuvettes of synthetic fused silica. The detection system employs an EMI 9558 photomultiplier (S20 cathode) cooled by the boil-off of liquid nitrogen, a photon counter with computer-of-average-transients (C.A.T.) (RIDL 34–12B, 400

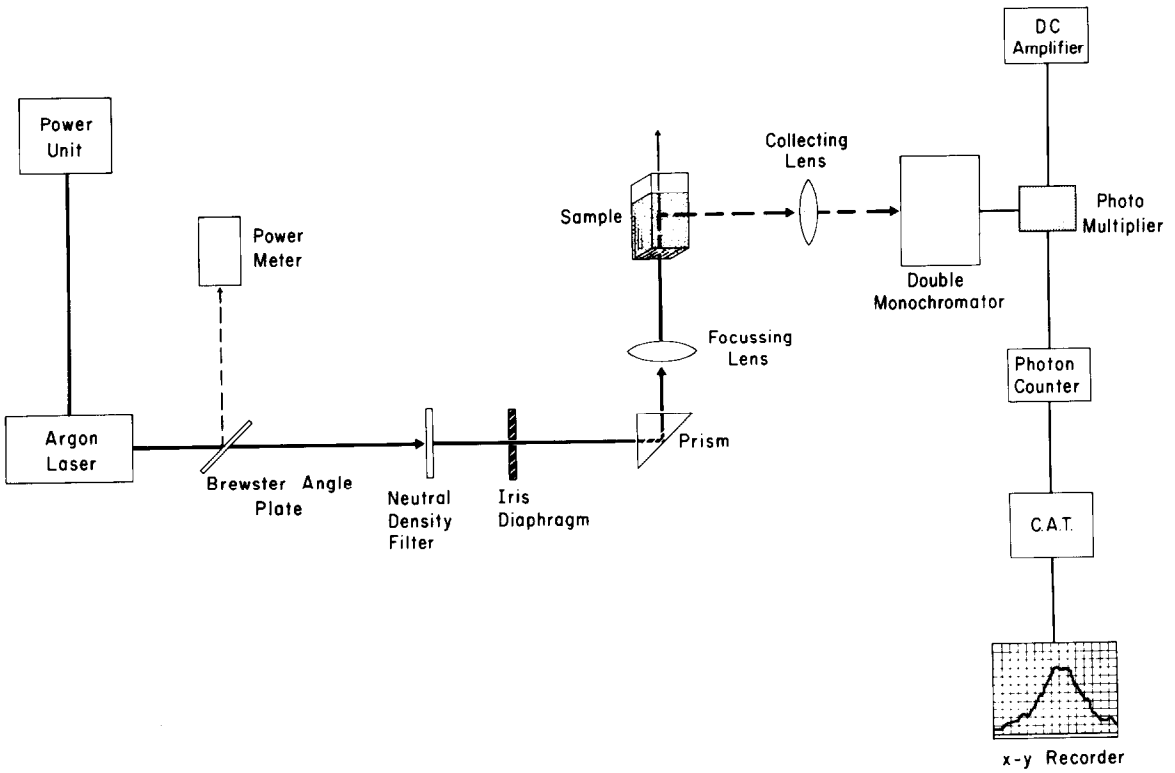

Fig. 1. Schematic representation of the laser Raman spectrograph. See text for details.

channel), and an X-Y recorder (Mosely 7000A). Typical dark counts are 20 counts/sec. The net counting rates of the Raman bands are 500 to 10,000 counts/sec.

In the presence of solutes with appreciable Rayleigh or Mie scattering, the laser beam diffuses more than with pure solvent and this reduces the photon flux in the irradiated volume. We avoid such intensity losses by adjusting the laser power to maintain the Raman scattering of the solvent within better than ± 3% in the irradiated volume. Solvent and solution are scanned alternately at 20–80 $cm^{-1} min^{-1}$ and the solvent spectra subtracted from the solution spectra by the C.A.T. Spectra are recorded after one, two, three and four subtractions. A number of spectra are also recorded directly. The frequency precision of recorded Raman scattering peaks is ± 2 cm^{-1}. However, because the subtraction procedure introduces a certain degree of statistical error, the widths and shapes of the subtracted bands, as well as their relative heights are subject to greater uncertainty.

The solvent is distilled water passed through a water purification system supplied by Hydro Services and Supplies, Inc. (Durham, N.C.). Poly-L-lysine (mol. wt. 130,000) and poly-L-glutamate (mol. wt. 110,000) were obtained from Pilot Chemicals (Watertown, Mass.). Standard procedures are used to prepare conformational isomers of poly-L-lysine [5] and poly-L-glutamate [6]. The polypeptide conformations are checked by infrared spectroscopy, using films on AgCl plates and a Perkin-Elmer Model 221 spectrophotometer. The polypeptide concentrations are about 1 mg/ml. Since the illuminated sample volume is less than 0.1 μl, our spectra originate from less than 1×10^{-7} g of solute (6×10^{-10} moles of C = O).

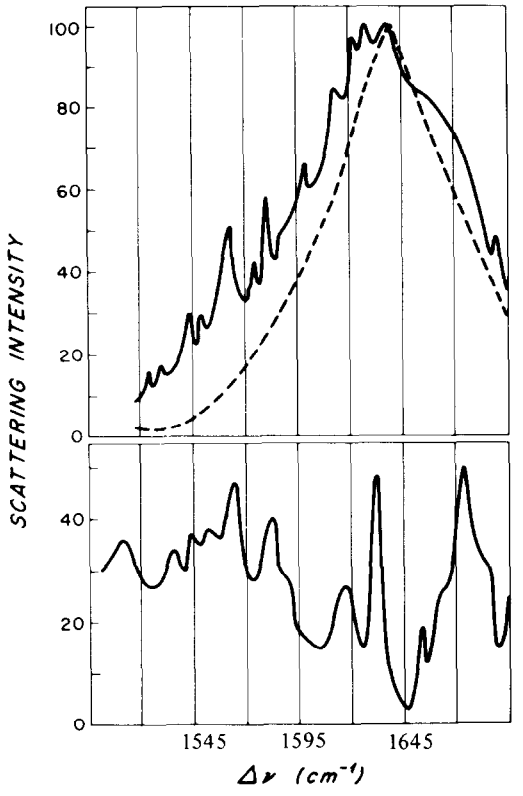

Fig. 2. *Upper panel:* Raman spectrum of water (- - -) and spectrum of an aqueous solution of poly-L-lysine (1 mg/ml) in the antiparallel β-conformation (——). The spectra have been normalized for comparison by setting the intensity of the 1637–1640 cm^{-1} band to an arbitrary value of 100. The water spectrum was obtained with a single sweep. The poly-L-lysine spectrum is a summation of four scans; the scattering intensities due to the polymer are thus four times greater than with a single sweep.

Lower panel: Raman spectrum of a 1 mg/ml solution of β-structured poly-L-lysine, with the water contribution subtracted out. The spectrum is the result of four subtraction manipulations. The greater apparent bandwidth compared with the unsubtracted spectrum, as seen in the greater width of the argon emission peak at 1585 cm^{-1}, is due to the statistical uncertainties in the subtraction procedure.

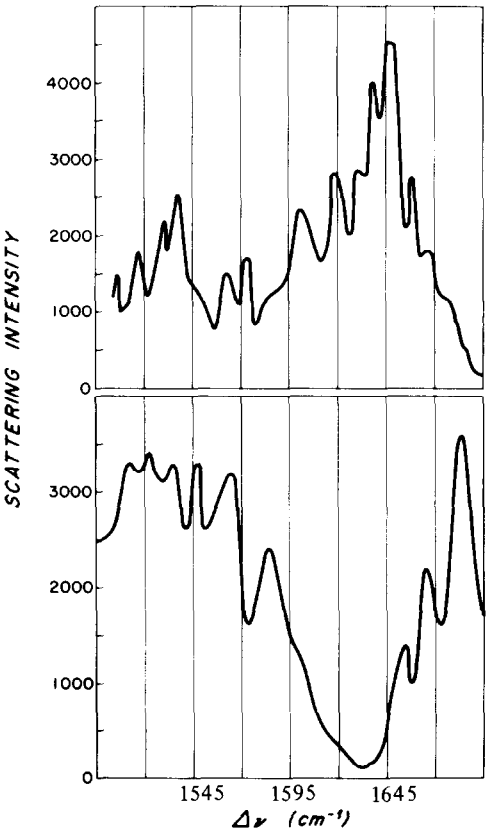

Fig. 3. *Upper panel:* Raman spectrum of α-helical poly-L-lysine.

Lower panel: Spectrum of "unordered" poly-L-lysine. See text for details.

3. Raman shifts of polypeptides in the region of 1500–1700 cm^{-1}

The upper panel of fig. 2 compares the Raman spectrum of an aqueous solution of β-poly-L-lysine with that of water, which gives a broad band maximal at 1640 cm^{-1}; the 1637 cm^{-1} band in the unsubtracted β-poly-L-lysine spectrum is probably due to water. The spectra of dilute solutions of β-structured poly-L-lysine demonstrate considerable fine structure superimposed on the water band, particularly below 1645 cm^{-1}. The band at 1585 cm^{-1} is an argon emission line; this can be removed with a filter, but we retain it as a wavelength and resolution standard. The lower panel of fig. 2 shows a spectrum of a solution of β-structured poly-L-lysine with the water contribution eliminated by the C.A.T. to bring out the net Raman shifts of polypeptide. The prominent peaks at 1631 and 1672 cm^{-1} presumably correspond to the 1630–1640 cm^{-1} and 1680–1690 cm^{-1} C = O stretching bands, typical of the infrared of polypeptides in the *antiparallel* β-pleated sheet

structure [6–9]; the 1672 cm^{-1} Raman scattering band is proportionally more intense than the 1680–1690 cm^{-1} infrared peak. The spectra of the β-structured polymers and those of all polypeptides studied demonstrate much detail between 1520 and 1580 cm^{-1} (Amide II, N-H bending region); this is also a characteristic of their infrared spectra [6–9].

Fig. 3 compares the spectrum of "unordered" poly-L-lysine with that of the α-helical form; both spectra have been corrected for scattering by water. "Unordered" poly-L-lysine has prominent bands at 1653, 1665 and 1683 cm^{-1}; α-helical poly-L-lysine exhibits little scattering above 1660 cm^{-1}, but has a major band at 1647 cm^{-1}. The strong 1631 cm^{-1} band seen with β-structured poly-L-lysine is lacking.

The Raman spectra of poly-L-glutamate in "unordered" and α-helical form are shown in fig. 4.

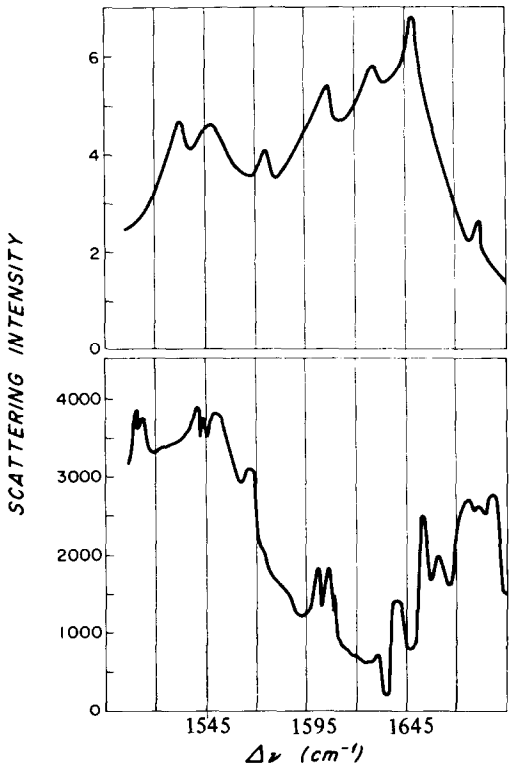

Fig. 4. *Upper panel:* Raman spectrum of α-helical poly-L-glutamate.
Lower panel: Spectrum of "unordered" poly-L-glutamate. See text for details.

The major peak of the α-helical polymer occurs at 1647 cm^{-1}, as with α-helical poly-L-lysine. The lack of detail in the spectrum of "α-helical" poly-L-glutamate is in part due to the fact that at the pH used, 4.38, the polymer is still not totally α-helical [10]. Poly-L-glutamate in the "unordered" state (pH 8.16) resembles "unordered" poly-L-lysine, with major bands at 1653 cm^{-1} and about 1685 cm^{-1}. The doublet near 1600 cm^{-1} is attributable to side chain carboxylate; in the infrared, $-CO_2^-$ absorbs strongly near 1600 cm^{-1} [11].

4. Raman scattering and infrared absorption of polypeptides

Table 1 compares the Raman shifts of poly-L-lysine in the various conformations with established Amide I and Amide II infrared absorption bands [7, 8]. Both the Amide I and Amide II infrared absorption bands show corresponding Raman scattering peaks; this has been previously observed in Raman studies of certain proteins and polypeptides in the solid state [12–15] or in concentrated solution [12, 13]. The observed differences between the spectra are to be expected because some of the normal modes comprising the peptide vibrations are Raman-active but silent in the infrared. Additional discrepancies may relate to the fact that infrared data are obtained from solid samples or from D_2O solutions.

All conformations yield Raman peaks which correspond to major infrared absorption bands of these conformations. Thus, in the case of α-helix, both Raman and infrared exhibit major peaks near 1650 cm^{-1}. The 1672 cm^{-1} Raman band of β-structured poly-L-lysine may represent the infrared-inactive mode predicted for this conformation [7]. Solutions of the two polymers in the "unordered" state yield rich Raman spectra, but only one band (at 1653 cm^{-1}) corresponds to the infrared absorption maximum. The origin of the major scattering peaks at 1665 cm^{-1} and 1685 cm^{-1} and the basis for the differences between the polypeptides in this frequency region are to be elucidated.

Our results show that, in the case of model polypeptides in dilute aqueous solution, Raman spectroscopy can distinguish between polypeptides in α-helical, "unordered" and β-conformations. In its

Table 1
Raman and infrared frequencies (1500–1700 cm^{-1}) of poly-L-lysine in different conformations.

α-Helix[a,b,c]			Antiparallel-β[a,b,c]			"Unordered"[a,b,c]		
Raman	I.R.		Raman	I.R.		Raman	I.R.	
1517w	1516w	AII	1511w			1521m		
1537m	1535s	AII	1535w	1530s	AII		1535	AII
						1547		
			1564m			1565		
1600m								
1617m			1617m					
			1631vs	1636s	AI			
1639s	1650vs	AI			AI			
1647vs	1652m	AI			AI	1653vs	1656	AI
			1672vs		AI	1665vs		
				1685m	AI	1683vs		

[a] vs = very strong; s = strong; m = medium; w = weak.
[b] I.R. frequencies from ref. [6].
[c] AI = Amide I; AII = Amide II.

applicability to aqueous solutions and its ability to discriminate between α-helical and "unordered" states, Raman spectroscopy offers major advantages over infrared spectroscopy. Moreover, the usefulness of laser Raman spectroscopy in the analysis of peptide conformation is not restricted to the frequency region examined here. For example, skeletal vibrations of the peptide backbone should yield conformationally-sensitive scattering bands [13, 15–17] in frequency regions free of water interference. In addition, the region above 1700 cm^{-1} exhibits rich and conformation-dependent Raman scattering [17]. We will report on these matters subsequently.

Acknowledgements

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