THE ESTIMATION OF PROTEIN SECONDARY STRUCTURE BY LASER RAMAN SPECTROSCOPY FROM THE AMIDE III′ INTENSITY DISTRIBUTION

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1. Introduction

The determination of protein secondary structure in aqueous solution has undergone an important advance [1]. It is now possible to estimate fractions of bi-H-bonded and mono-H-bonded helix, antiparallel β-sheet, parallel β-sheet, and β-turn to within ±5% of X-ray diffraction estimates with ~70% confidence, whereas before, using circular dichroism (CD) [2] or Raman spectroscopy [3], only fractions of total helix and β-sheet were estimated, to within ~8%. This improvement has come about mostly through the generation of computer-subtracted difference laser Raman spectra, and specifically through the subtraction of the water spectrum from the amide I spectrum of proteins.

Here we present evidence that this general approach can be applied to the amide III′ region of the laser Raman spectrum of proteins in D_2O as well, and that estimates of the structure of solvent exchanged regions of the protein can thereby be obtained.

When a protein is dissolved in D_2O, exposed amide N–H groups exchange with the solvent, and the amide III vibrations shift to a lower frequency from between 1200 and 1320 cm^{-1} to what are termed the amide III′ vibrations between 900 and 1000 cm^{-1}. While several investigations have pursued analyzing the amide III region of the vibrational spectrum for protein secondary structure determination [3–7] little has been done with the amide III′ region.

We believe that there are some practical advantages to using the amide III′ over the amide III region for structural studies:

(i) Bands due to helix in the amide III region are not very intense relative to the corresponding non-helical bands (R. W. W., A. K. D., W. L. P., in preparation).

(ii) CH deformation modes are strong in the amide III helix region.

The amide III′ region seems to be less affected by these kinds of problems. Both helical and β-sheet polypeptides show strong amide III′ bands in D_2O. The C–C stretching bands in this region are also conformationally sensitive, and may constructively reinforce the structurally corresponding amide III′ bands.

Here, the spectrum of each protein in D_2O was subtracted from the corresponding spectrum of the protein in H_2O so that bands unaffected by deuteration of the amide nitrogen are removed. The resulting difference spectrum represents the true contours of the amide III′ spectrum.

Four of these amide III′ difference spectra [6] were then used to calculate the spectra of helix and β-sheet by a procedure similar that in [2] for CD spectra. We have performed these same calculations on the water-subtracted amide I spectra of these proteins in H_2O, a method developed more fully in [1] so that a direct comparison can be made between the results of these two methods.

2. Materials and methods

Lysozyme was obtained from Miles, 3 X cryst. lot 7058. Ribonuclease A was from Sigma, lot 92C-8008. Concanavalin A was from Sigma, lot 115C-7060. These proteins were dissolved in H_2O and D_2O. fd Phage were grown as in [8] and prepared in 0.01 M borate, pH and pD 8. Solvent was subtracted from

Raman spectra and difference spectra were computed as in [6,8].

Reference spectra of 3 types of structure were computed from four equations of the form:

$$FhIh + FbIb + FuIu = \text{'}I \text{ observed'}.$$

'*I* observed' is the experimental Raman intensity at a fixed frequency; *Ih*, *Ib* and *Iu* are the reference intensities for helix, β-sheet, and undefined; and *Fh*, *Fb* and *Fu* are the fractions of structure types. *Fh*, *Fb* and *Fu* were taken from references e, g, h and i as shown in table 1 for the four proteins. Using the same equation with each *I* known, *Fh*, *Fb* and *Fu* were computed for each protein from 15 equations, one for each five wavenumbers between 930 and 1000 cm^{-1} for the amide III′ region, and between 1630 and 1700 cm^{-1} for the amide I region. The amide III′ region used here was selected to avoid exchange sensitive tryptophan bands at <930 cm^{-1} and >1000 cm^{-1}.

The LLSQF subroutine from the IMSL library was used to solve the over-determined systems of equations on the Amdahl 470 at Washington State University.

3. Results

The computed spectra for helical, β-sheet and undefined structures shown in figs.1,2 show that the bands due to helix and β-sheet are better resolved in the amide III′ than in the amide I region. The band for undefined structure is not well defined in the amide III′ region, however.

We compare the estimates of secondary structure obtained from these reference spectra to the estimates obtained by other methods in table 1. While the estimates derived from the amide III′ region do not agree as well with the X-ray estimates from references g, h and i as do the estimates obtained from the amide I region, they compare favorably with the Raman estimates obtained in [3] and with the CD estimates.

Table 1
Percent of secondary structure as estimated from Raman spectra, CD and X-ray diffraction for 4 proteins

Protein	Structure type			Method	Ref.
	Helix	β-Sheet	Undefined		
Lysozyme	36/34	12/17	52/50	Raman	a
	32	9	59	Raman	b
	37/35	11/27	–	CD	c
	34	16	50	X-ray	g
	46	17	37	X-ray	d
	44	22	32	Raman	e
RNase A	13/17	49/35	39/48	Raman	a
	14	35	51	Raman	b
	22/25	47/44	–	CD	c
	18	38	44	X-ray	h
	23	30	48	X-ray	d
	19	40	38	Raman	e
Con A	6/3	50/57	44/40	Raman	a
	−1	48	53	Raman	b
	−1	49	–	CD	f
	3	57	40	X-ray	i
	2	59	39	X-ray	d
	0	55	39	Raman	e
fd Phage	76/76	6/6	18/18	Raman	a
	68	4	21	Raman	e

(a) This report, amide III′/amide I; (b) [3]; (c) [2], from 5 proteins/8 proteins; (d) [9]; (e) R. W. W., A. K. D., W. L. P., in preparation; (f) Computed from the CD spectrum in this laboratory by the method in [2]; (g) [10]; (h) [11]; (i) [12]

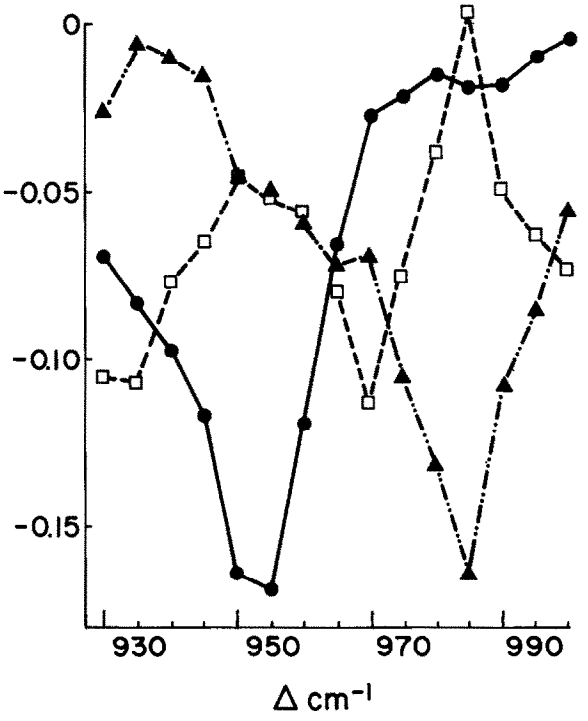

Fig.1. Amide III′ spectrum of helix (○), β-sheet (△) and undefined types of structure computed from the amide III′ difference spectra of 4 proteins. The *y* axis is the normalized difference spectrum intensity. The sum of the intensities is about −1.

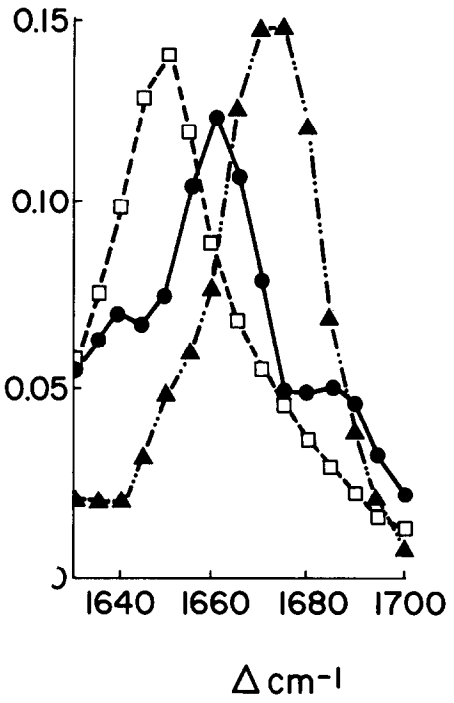

Fig.2. Amide I spectrum of helix, β-sheet and undefined (○)-types of structure computed from the water-subtracted amide I difference spectra of 4 proteins. The y axis is the normalized Raman intensity where the sum of the intensities is unity.

4. Conclusion

This report shows that the amide III′ region of the laser Raman spectrum can be used effectively to estimate the structure of D_2O exchanged proteins. This method would be useful in estimating the structure of protein domains that are exposed to and sequestered from the aqueous environment. For example, the structure of *trans*-membrane sections of intrinsic membrane proteins may be probed in this way. The secondary structure of the entire protein could be estimated from the amide I band, and the structure of the solvent exposed domains of the protein on the surface of the membrane could be estimated from the amide III′ difference spectrum. This information could lead to a quantitative estimate of the *trans*-membrane protein secondary structure. It remains, however, to be shown that this approach would work when other membrane components are present.

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