Raman spectroscopic study on the conformation of 11 S form acetylcholinesterase from *Torpedo californica*

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Vibrational Raman spectroscopy has been used to study the conformation of the 11 S form of acetylcholinesterase from *Torpedo californica*. Secondary structure analysis by the method of Williams [(1983) J. Mol. Biol. 166, 581-603] shows 49% α-helical structure, 23% β-sheets, 11% turns and 15% undefined structure. Secondary structure estimates obtained for this enzyme by Raman spectroscopy and circular dichroism have been analyzed.

Raman spectroscopy; Acetylcholinesterase; Secondary structure

1. INTRODUCTION

Vibrational spectroscopy has proven useful in the study of the structure of molecules involved in cholinergic neurotransmission. To date, studies have been conducted on the conformation of the chemical transmitter, acetylcholine and some of its analogues [1–4], as well as on postsynaptic membranes from the *Torpedo marmorata* electric organ enriched in acetylcholine receptor, and the isolated receptor molecule [5,6]. Continuing this approach an examination of the Raman spectra of acetylcholinesterase (AChE) was undertaken to identify specific features in the conformation of the enzyme and to provide a quantitative estimation of its secondary structure.

For polypeptides and proteins Raman frequen-

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cies of both the peptide amide backbone (main chain) and the side chains are revealed. Several of these frequencies have been shown to be sensitive to their molecular conformation or environment [7]. Further progress has been made by the development of methods to quantify secondary structure using the Raman spectrum [8–11]. The most recent is the method of Williams [10,11]. This method involves well-determined regions of the Raman spectrum, namely the amide 1 and amide III regions. Each amide group in a protein has Raman amide bands that depend on conformation, on whether it is hydrogen-bonded to water or to another amide [12] and on coupling between the residues [13,14]. Thus, although the amide bands in proteins are typically single broad bands, it is possible to deconvolute them into component lines. The experimental Raman spectrum of the analyzed protein is correlated with those of proteins of known structure. To accomplish this, singular value analysis for solving least-squares problems is used. At the end of the analysis, by summing the fractions of secondary structure in the known proteins, the structure of the protein under study can be estimated. Prior to determina-

tion of the secondary structure, the side chain bands are subtracted from the amide I region and the result is normalized [10]. Using this method we have made a quantitative estimation on the secondary structure of the 11 S form of AChE isolated from *T. californica*. Our results indicate a predominant α-helical structure (49%); the β-sheets are found to be 23% and the turns to be 11% of the defined structure. Some aromatic side chain vibrations have also been analyzed. Many of the tyrosine and tryptophan residues appear to be exposed to an aqueous environment.

2. MATERIALS AND METHODS

The 11 S form of AChE was prepared from *T. californica* electric organ using the method of Taylor and Jacobs [15].

The Raman instrument used here was a Coderg PH1 double monochromator with 1800 grooves/mm holographic gratings and an ITT TW 130 PMT cooled to $-30°C$. The slits were 6 cm^{-1}. Excitation was provided by an Ar$^+$ laser (Coherent Innova 90-3) tuned to produce 150 mW at sample

level at a wavelength of 488 nm. A thermostatted cell, described in [5], was used as sample holder; the volume of the sample was about 10 μl and the protein concentration about 20 mg/ml in phosphate buffer at pH 7.5. The temperature was controlled at 10°C. An Apple IIe microcomputer was used for control of the repetitive scannings. An average of 25 scans was collected for each spectrum. Data points were taken every 1 cm^{-1} with 1 s counting time. Subtraction of the buffer and water spectrum and the fluorescence baseline correction were made according to [10]. Smoothing of the spectra was achieved by Fourier transforms.

3. RESULTS

The Raman spectra and assigned frequencies of 11 S AChE in the spectral ranges 600–1800 cm^{-1} and 2500–3120 cm^{-1} are shown in fig.1. The subtracted and normalized spectrum of the amide I region and the same spectrum after subtraction of the side chain bands and normalization are given in fig.2.
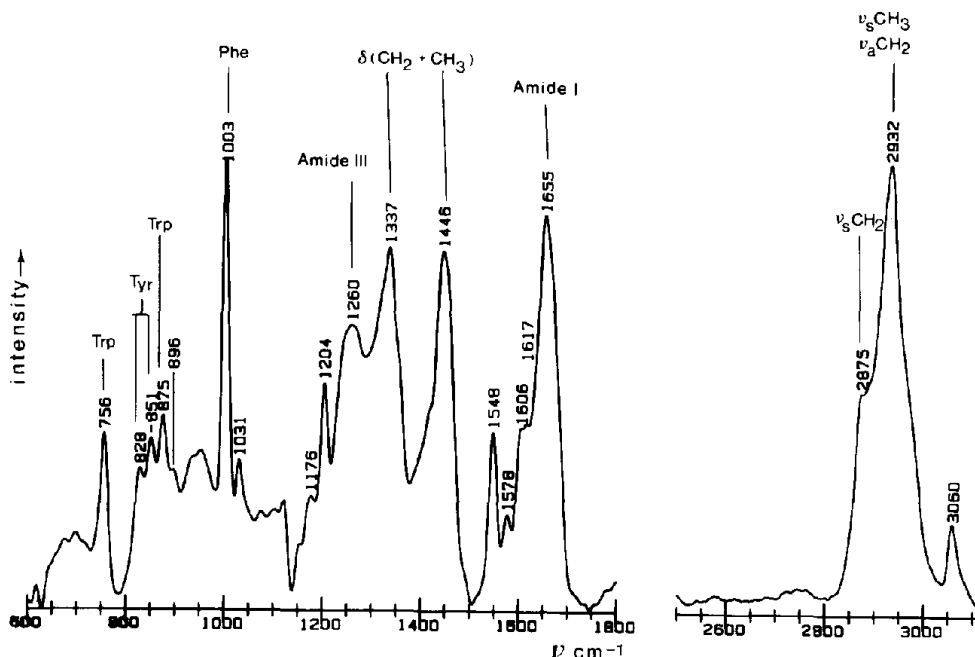

Fig.1. Raman spectrum of 11 S form of acetylcholinesterase from *T. californica*. Recording conditions are given in the text.
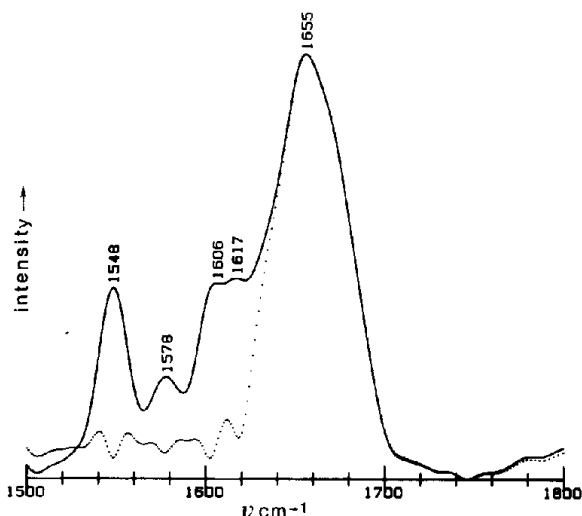
Fig.2. Amide I region of acetylcholinesterase spectrum: (——) after subtracting buffer and fluorescence background; (···) after subtracting aromatic ring vibrations.

Analysis of certain Raman vibrations of the AChE spectrum known to be sensitive to structure reveals the following structural information.

3.1. Peptide backbone

The amide I band centered near 1655 cm^{-1} is very strong and broad. The amide III region also appears as a strong band at 1260 cm^{-1}. The frequencies and intensities of these bands indicate the predominance of an α-helical structure in the native protein [16].

A quantitative estimation of structural components of the peptide backbone of the AchE molecule, derived from the analysis of the amide I and amide III regions, is given in table 1. Estimates

Table 1

Method	Structure types (%)							
	H_o	H_d	H_t	β_p	B_{ap}	β_t	T	U
Raman amide I	37.1	11.9	49	0.9	22	23	11.3	15.5
amide III			42				30 (19.2)	(7.8)
CD^a			33	9		14	23	17	26

a From [17]

H_o, ordered α-helix; H_d, disordered α-helix; H_t, total helix; β_p, parallel β-sheets; β_{ap}, antiparallel β-sheets; β_t, total β-sheets; T, turns; U, undefined

obtained by circular dichroism (CD) spectroscopy on identical enzyme samples [17] are also listed in table 1. The CD analysis of structure is based on a procedure developed by Hennessey and Johnson [18] which is mathematically identical to the method used here.

3.1.2. Analysis of the Amide I band

Williams made a comparative study between the correlation coefficients and standard deviations for spectroscopic (Raman and CD) and X-ray diffraction estimates on the secondary structure (see table 1 in [10]). Using the X-ray determinations of Levitt and Greer [19] to analyze the secondary structure by the Raman amide I band, he obtained a very high correlation between X-ray and Raman evaluations [10]. Smaller correlation coefficients were found when the structure of components was estimated by the CD method which is based on the X-ray determination of Chang et al. [20]. When the Raman analysis was repeated using the X-ray determinations of Chang et al., the results were similar to that of CD analysis [10]. Thus, the Raman estimates of secondary structure from the amide I region, using the criteria of Levitt and Greer, appear to be more accurate than those obtained from the analysis of CD spectra. This is especially true for β-sheets and turns [10].

Interestingly, the value of 23% which we found for the total β-sheet structure of AChE is the same as that given by CD spectroscopy (see table 1). Differences are seen, however, in the estimation of β-parallel and β-antiparallel structures: the β-sheets are predicted by Raman to be almost entirely antiparallel, whereas the CD estimate indicates 9% of parallel structure.

The estimation on the total α-helical structure of AChE by CD spectroscopy indicates a value of 33% which is not in close accord with the value of 49% obtained from Raman spectroscopy. The correlation is much improved when, instead of the value of the total α-helix (49%), the value of ordered α-helical structure (37%), is compared. Here, one might suppose that the disordered α-helix (12%) not observed in the CD determination could be included in the greater value for the undetermined structure estimated to be 26% by CD.

3.1.3. Analysis of the amide III band

While bands not sensitive to structural changes

overlap the amide III region (1200-1300 cm^{-1}), determination of the secondary structure provides independent evidence that supports the conclusions drawn from the amide I spectrum. According to the correlation coefficients from the comparison of Raman amide III and X-ray-derived estimates of secondary structure (see table VIII in [11]), only two classes of structure can be measured successfully: total α-helix (H_t) and total β-sheets (β_t).

Our estimation for the AChE molecule secondary structure indicates 42% of total α-helix and 30% of total β-sheets. For completeness, the fractions of turns and undefined structures are also included in table 1, but note that for these structure classes the correlation between X-ray and Raman spectroscopy is not sufficient [11]. It is noteworthy that the values for the total helix and for the total β-sheets determined from the amide I and amide III regions are similar and that the difference between them is small (7%). This difference could be due to the fact that, for the secondary structure determination based on the amide III region, fewer spectral points were used; also, this region contains another type of vibrations which are not subtracted.

Taking into account that standard deviations between X-ray and Raman determinations are about 5%, we can estimate that the Raman results obtained using the amide I and amide III regions yield similar values.

While estimates on the total β-sheet structure are the same for Raman and CD (table 1), there is an average difference of about 13% for estimations of the total helical structure, which is of the same order as the difference between 'undetermined' structures.

The argument for accepting the Raman estimates as better relies on the fact, as pointed out by the careful statistical analysis of Williams, that the overall agreement between Raman and X-ray estimates is the highest among the spectroscopic methods. The lower undetermined structure (on average about 11%) that we found also favors the estimates from the Raman method.

3.2. Tyrosine environments

The tyrosine doublet at 858 and 838 cm^{-1} is clearly resolved in the Raman spectrum of AChE (fig.1). The doublet which has been the subject of studies in Raman spectroscopy [21,22] is indicative of the state of tyrosyl residues in proteins. A surface tyrosine is considered to be 'normal' when the doublet intensity ratio (I_{850}/I_{830}) is about 1.25. In this case the phenolic OH group of the residue should be simultaneously acceptor and donor of moderate to weak hydrogen bonds [22]. The properties of tyrosines 'buried' in the hydrophobic region will vary from normal to those forming strong hydrogen bonds with negative acceptors. Thus, the ratio should vary from 1.25 to 0.3 [22]. In the case of the AChE molecule our investigation shows that the ratio I_{858}/I_{838} is 1.21. This means that most of the tyrosine residues reside on the surface of the protein 'exposed' to the aqueous medium.

3.3. Tryptophan environment

The state of tryptophan residues can also be discussed using the vibration sensitive to the environment situated near 1360 cm^{-1} [23-25]. It was pointed out that this vibration appears as a sharp peak when the tryptophans are 'buried' but that its intensity decreases when they become exposed. The absence of this vibration in the spectrum of AChE (fig.1) strongly suggests that the tryptophans are also exposed.

3.4. Other vibrations

The (C-H) bending vibrations of amino acid side chains appear as intense bands at 1337 cm^{-1} and 1446 cm^{-1} (δCH_2, δCH_3). Two (C-H) stretching vibrations are visible in the region 2800-3120 cm^{-1}, namely at 2875 cm^{-1} ($\nu_s CH_2$) and 2932 cm^{-1} ($\nu_a CH_2$, $\nu_s CH_3$) [26]. In the study of the interaction of the enzyme levan sucrase with Fe^{3+} [27] it was suggested that these vibrations, especially $\nu_a CH_3$, reflect the alteration in environment of the vibrating groups. The same conclusion has been made for the very strong phenylalanine vibration at 1003 cm^{-1}.

4. DISCUSSION AND CONCLUSION

The vibrations arising from the aromatic residues, together with the conformationally sensitive vibrations, can be very useful in studying conformational modifications of the interacting AChE molecule. The primary structure of acetylcholinesterase has been established [28] and the three intrasubunit and single intersubunit disulfide

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bonds assigned by isolation of the corresponding peptides [29]. The molecular forms of acetylcholinesterase found in *Torpedo* are either derived from a dimensionally asymmetric species containing a collagen-like unit or contain a covalently linked glycophospholipid. Thus, their structural analysis by crystallographic methods [30] may prove difficult. Therefore, methods for estimating secondary structure could provide important additions to our understanding of the structures of acetylcholinesterases.

Here, we have found 11 S AChE to exist in 49% α-helix, 23% β-sheets, 11% turns and 15% undefined structure. In addition, analysis of tryptophan and tyrosine vibrations reveals exposure of some of the aromatic residues to the solvent. It will be of interst to employ Raman methods to examine structural differences between the molecular forms of AChE and the conformational changes induced upon ligand binding.

ACKNOWLEDGEMENTS

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