THE SECONDARY STRUCTURE OF ACETYLCHOLINE RECEPTOR RECONSTITUTED IN A SINGLE LIPID COMPONENT AS DETERMINED BY RAMAN SPECTROSCOPY

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The structures of very few intrinsic membrane proteins are known in great detail because of difficulties in preparing three-dimensional crystals of membrane proteins suitable for high-resolution x-ray studies. Raman spectroscopy can determine secondary structural information without crystals and is thus a valuable tool for obtaining protein structure in the absence of crystals. By applying a newly developed method for analyzing the amide I vibrational region (1) to the first Raman spectrum of the purified acetylcholine receptor (AChR) from *Torpedo californica*, we have determined the percentages of six classes of secondary structure for the protein.

The one previous determination of the AChR secondary structure was made by circular dichroism (CD) measurement on a detergent-solubilized preparation (2). It is desirable to measure the secondary structure of the protein in a more membrane-like environment such as liposomes, but, because of excessive scattering, liposomes are not suitable for CD measurements. This scattering problem does not apply to Raman spectroscopy. We have reconstituted the protein in dielaidyl phosphatidylcholine (DEPC) for Raman study. Previous Raman studies of native membrane preparations of other systems (3, 4) failed to provide accurate measurement of the amide I region because of the overlap of the C═C stretching bands from unknown proportions and types of lipids. By reconstituting the receptor in membranes of only one lipid component, we are able to subtract the lipid contributions to the spectrum to an accuracy of better than ±3%.

The receptor was isolated from electroplax tissue of *Torpedo californica* by affinity chromatography (5) in excess cholate (Sigma Chemical Corp., St. Louis, MO) and DEPC (Avanti Biochemicals, Inc., Birmingham, AL) using acetylcholine as the affinity ligand. Lipid exchange was carried out while the AChR was attached to the affinity column. The cholate used to solublize the lipid-protein micelles was removed by dialysis. The resulting samples were pelleted by three or more slow freezing and thawing cycles. All Raman spectra shown here were collected at 20°C, which is 8°C above chain-melting temperature of pure DEPC.

Fig. 1 shows the Raman spectrum of the reconstituted receptor with approximately equal contributions to the spectrum by protein and lipid bands, indicating high receptor concentration in the bilayer. The largest peak in the amide I region (1,600 cm^{-1} to 1,710 cm^{-1}, Fig. 2 *a*) near 1,672 cm^{-1} is because of the *trans* C═C stretching mode of DEPC; we show the corresponding spectrum of the pure lipid in Fig. 2 *b*. The amide I band of proteins arises from peptide-group vibrations sensitive to local perturbations of the environment caused by different secondary structures. The total amide I band from a complex protein is a linear combination of bands from regions of different structures. Determination of structures from the amide I band in a multi-component system requires subtraction of all bands in this spectral region not arising from

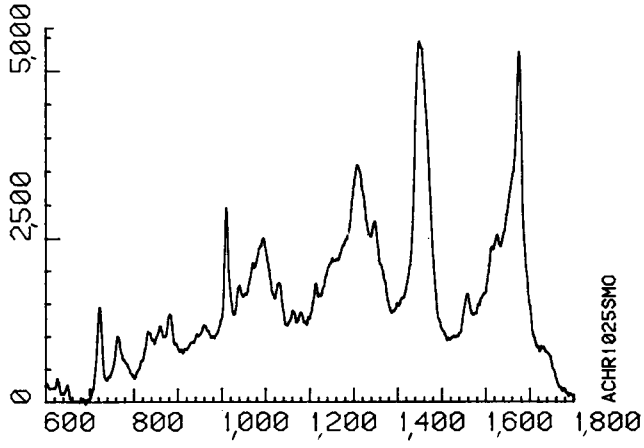

FIGURE 1 The spectrum of AChR reconstituted in DEPC at 20°C. Spectra were collected from samples held in melting point capillaries with ~200 mW of 5145-Å light. Multiscanning for at least 15 h was employed in all cases.

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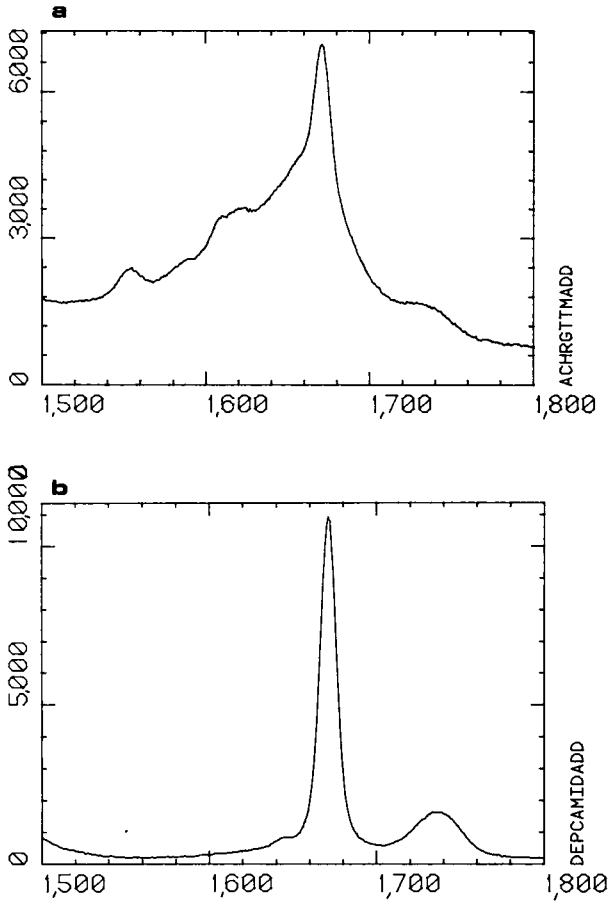

FIGURE 2 (*a*) The amide I region of the same sample as in Fig. 1. (*b*) The same spectral region of a DEPC dispersion under identical conditions. Note the contribution of the lipid to the spectrum in 2 *a*.

the protein in question. After the contributions of lipid and solvent were subtracted, several features still remained because of ring vibrations of aromatic amino acid side chains. These were deleted with a least-squares fitting procedure. The remaining curve (see Fig. 3) from 1,615 cm^{-1} to 1,710 cm^{-1} was fitted to a linear combination of amide I spectra from a reference set of proteins whose secondary structures were determined by x-ray crystallography. The coefficients obtained were used to generate the fraction of contributions of each of six major classes of secondary structures.

We find the following percentages of secondary structure (± 1 SD): 25% ± 4 ordered helix, 14% ± 4 disordered helix, 34% ± 5 anti-parallel β-strand, 0% ± 4 parallel β-strand, 15% ± 3 turn, and 10% ± 3 undefined. The total amount of helix is 38% ± 4 and β-strand is 33% ± 3. These values can be compared with the CD results of Moore et al. (2) of 34% for α-helix and 29% for the β-strand. Thus the secondary structure of AChR in detergent is similar to that reconstituted in lipids.

We have shown that it is possible to subtract the lipid contribution from the Raman spectrum of a lipid-protein complex and determine the protein conformation. Our estimates of the secondary structure content for the reconstituted receptor are consistent with the CD measurements on the protein in detergent micelles. The receptor has a good deal of helical character, but substantially less than that of the bacteriorhodopsin molecule, for example. Our results can be used to evaluate different models for the structure of AChR. X-ray scattering data (6) suggest that there are 80-Å long α-helices in the receptor. If we assume that all of the ordered helical content is in the 80-Å helices and we count only the 47 ordered residues in each helix, then there can be no more than 12 such helices in the complete receptor. This would constrain all other helices to be shorter than seven residues (disordered). Invoking the known strong sequence homology among the four types of subunits as evidence for structural homology, we conclude that there could be 0, 5, or 10 long helices per receptor molecule.

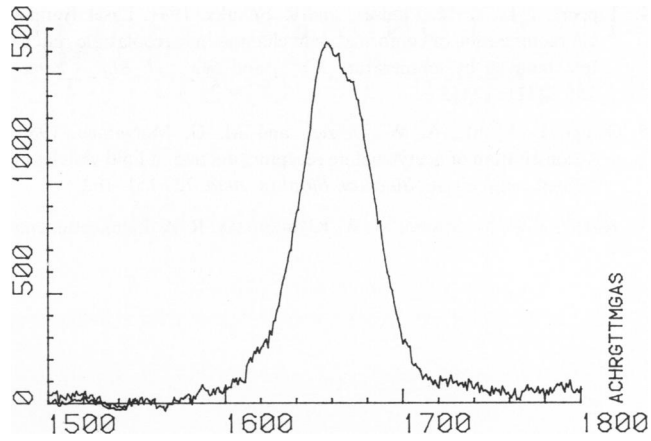

FIGURE 3 The amide I peak after subtraction of solvent, lipid, and amino acid residue bands.

Another application of our data is in establishing limits for the estimation of secondary structure directly from the primary sequence. The amino acid sequences of all four polypeptide subunits of the acetylcholine (AChR) have recently been determined by gene cloning methods (see, for example, reference 7). It is of interest to see how closely statistical methods such as that of Chou and Fasman (8) approach our results.

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Note added in proof: Under the sample preparation conditions described, no agonist-induced ion flux was observed. Further studies on viably reconstituted receptor are underway.

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