

Features

Talking Point

Topography of integral membrane proteins: hydrophobicity analysis vs. immunolocalization

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Immunolocalization studies of acetylcholine receptor (AChR) topography have led to models that are disconcertingly at odds with earlier theoretical predictions. Does this discrepancy mean that the prediction methods are flawed, or that some of the experimental tests have unrecognized pitfalls?

Theoretical predictions of transmembrane protein folding are now made whenever the sequence of a membrane protein is reported. They are based mainly on two distinct rationales. The most widely used approach is to scan the sequence for hydrophobic regions of adequate length to span the non-polar region of a lipid bilayer as α -helices¹. The transmembrane domains of bacteriorhodopsin and the three integral proteins of the photosynthetic reaction center, the only membrane proteins known at sufficient structural resolution, are entirely comprised of α -helices. To a good approximation, these helices are successfully predicted using hydrophobicity analysis^{1,2}.

An additional rationale is based on the widely held view that proteins defining aqueous channels must contain transmembrane polar residues. When pores are imagined to be comprised of α -helices (alternative secondary structures are conceivable, but presumed to be exceptional), modeling the structure includes a search for laterally amphipathic helices, the polar sides of which are predicted to line the channel. Such helices would not necessarily be revealed by hydrophobicity analysis.

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Models and tests of acetylcholine receptor topography

Two distinct theoretical proposals for the folding of the acetylcholine receptor (AChR) have been put forward (see Fig. 1, a and b). The AChR is a transmembrane cation-conducting channel formed from the pseudosymmetrical arrangement of five homologous subunits ($\alpha_2\beta\gamma\delta$) that almost certainly share a common transmembrane folding pattern with externally exposed N-termini^{3,4}. On the basis of hydrophobicity analysis, a four-helix model was initially proposed, in which the transmembrane domain of each subunit consists of four hydrophobic α -helices, numbered I–IV (Fig. 1a, Refs 5–7). The five-helix model (Fig. 1b, Refs 8,9) includes an additional highly charged amphipathic helix (helix A).

The four-helix model predicts that the C-termini of the subunits should lie in the external medium, while models including additional, polar segments can predict either orientation. Identifying the correct model would have wide-ranging implications for the structure and mode of folding and assembly of the AChR and of other homologous channel-forming complexes such as the glycine and GABA_A receptors^{10,11}.

Examination of the binding of antibodies directed against identified regions of the subunits' sequences indicated that the C-termini lie in the cytoplasm, which appeared to disprove the four-helix model¹². Further experiments actually suggested that hydro-

phobicity analysis was highly unreliable in identifying transmembrane segments: according to the latest model based on immunolabeling data (Fig. 1c), the most hydrophobic of the proposed helices (helix IV) would actually reside in the cytoplasm, while two hydrophilic segments (M6 and M7), one of them non-helical, would span the bilayer^{13,14}.

Aqueous channels with hydrophobic walls?

We have sought to re-examine these conclusions using a biochemical approach. Membrane-impermeant reagents were used to reduce a disulfide bridge located at the C-terminus of the *Torpedo* AChR δ -subunit. The data unambiguously showed that this bridge faces the external medium^{15,16}. The use of reconstituted vesicles permitted the design of rigorous controls not practical with native vesicles. It represents, however, a non-trivial difference between the biochemical and immunological experiments. Functional and structural data indicate however that a re-arrangement of the AChR molecule upon reconstitution is very unlikely¹⁵. Indeed, similar, although less well-controlled reduction experiments on native vesicles also suggest an external location for the bridge^{17,15}.

If the subunits' C-termini are extracellular, models (b) and (c) in Fig. 1 are incorrect, whereas model (a) remains a viable possibility. Model (a) predicts that the aqueous channel is lined by some of the hydrophobic helices. This would be consistent with some physiological properties of the AChR (Ref. 4) and with recent biochemical experiments showing that compounds purported to block the channel can be used to covalently label helix II^{18–21}. In addition, electrophysiological measurements on chimeric AChR indicate that a sequence segment comprising helix II and the loop between helices II and III modulates the properties of the channel²². It is worth noting that hydrophobic helices I–III are relatively well conserved in the glycine, GABA_A and nicotinic acetylcholine receptors, while helix A is not²³.

These experiments revive the idea

that the formation of an aqueous channel might not require the existence of strongly hydrophilic transmembrane segments; an assembly of relatively hydrophobic helices may suffice, provided some hydrogen-bonding residues line the channel's walls. Such helices would be individually stable in a transmembrane position, and thus identifiable by hydrophobicity analysis. The folding of AChR subunits during biosynthesis would likely proceed in a manner similar to that of bacteriorhodopsin or other non-channel forming proteins²⁴, and no extensive rearrangement would need to occur upon subunit assembly.

How reliable are theoretical and immunological approaches to membrane protein folding?

Our study is not consistent with the δ -subunit C-terminus being exposed to the cytoplasm. Recent immunologi-

cal²⁵ and biochemical²⁶ observations cannot be reconciled with M6 and M7 crossing the membrane. The conflict of these data with earlier immunolocalization results raises the issue of possible pitfalls. It is conceivable, for instance, that treatments used to open vesicles in immunoprecipitation protocols perturb the folding or packing of the AChR, leaving in doubt the significance of changes in the accessibility of a given epitope. Furthermore, the specificity of the antibodies is, of necessity, assayed on proteolytic fragments of denatured subunits or on synthetic peptides; while localization is done on the native oligomer. In those electron-microscopy experiments in which antibody concentrations are given, labeling is conducted at micromolar concentrations, two to three orders of magnitude above the concentrations used for testing immunospecificity by blotting or precipitation. If the epitope that is

recognized in these tests is sterically inaccessible in the native membrane, antibodies may bind to alternative (incorrect) sites with lower affinities. Examples of such cross reactions with anti-AChR monoclonal antibodies are known¹⁵.

The degree of accuracy that theoretical and immunological approaches provide in defining the transmembrane domains of integral membrane proteins remains open to question. What is apparent from the study of the acetylcholine receptor is that at least one of these approaches has significant shortcomings. Continued examination of this issue is worthwhile given the widespread use of the methods and the interest in clear interpretation of sequence data in terms of membrane protein topography.

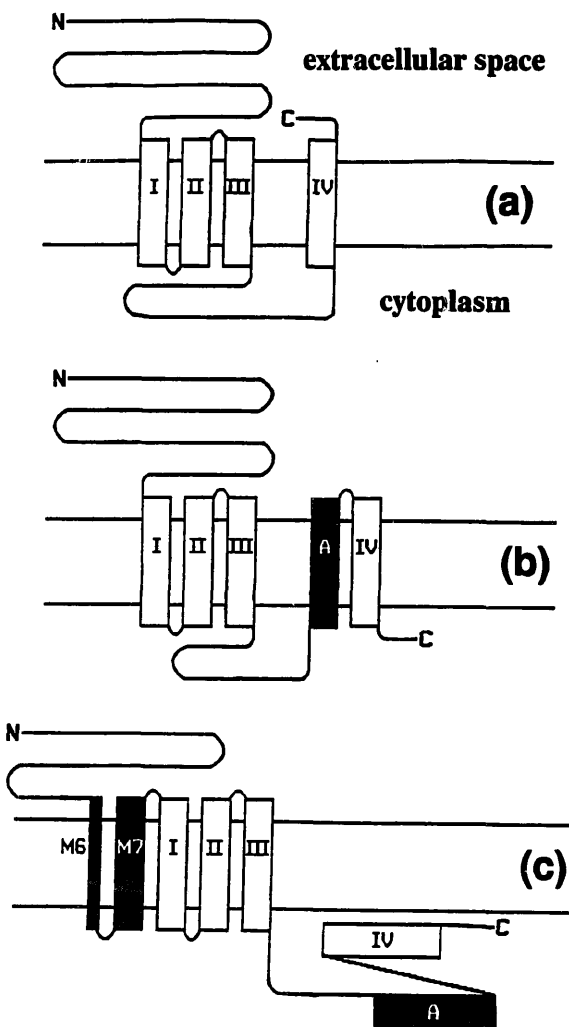


Fig. 1. Three models for the transmembrane folding of the acetylcholine receptor (AChR). See text for details. Adapted, with permission, from Ref. 15.

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