A PROTEIN CHEMISTRY APPROACH TO THE MODELLING OF INTEGRAL MEMBRANE PROTEINS

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SUMMARY: The site of attachment of chemical probes and residues specifically modified by biological processes can be identified by protein sequencing. When such approaches are used with integral membrane proteins, the information is particularly valuable since it can help determine the disposition of the polypeptide chain in the bilayer, the number of transmembrane regions, the orientation of the individual helices within the bilayer and the approximate position of important functional sites. When combined with biophysical data on the relative position of these helices, some appreciation can be obtained of the 3-dimensional structure of the protein. This contribution illustrates the use of this strategy for the visual light receptor rhodopsin and the proton channel of the vacuolar H\textsuperscript{+}-ATPase. The resultant models are assessed in terms of the functional properties of these proteins and their evolutionary homologues.

INTRODUCTION

Although the intramembranous regions of most integral membrane proteins are likely to be largely α-helical, there are a number of critical pieces of information the absence of which renders meaningful representation of their structure very problematic. These vital elements of data are (i) the number of intramembranous helices, (ii) the packing arrangement of these segments and (iii) the orientation of the individual helices within the bilayer. The use of protein sequencing in conjunction with modification of particular amino acid side-
chains can go a long way towards providing definitive answers to some of these questions.

**RHODOPSIN:** The visual light-receptor rhodopsin is the most accessible member of a large family of receptors which on activation, stimulate guanine-nucleotide binding proteins. In the previous volume of this series, details were given of how the identification of the sites modified by succinic anhydride, sulphydryl reagents, diazodiiodosulphanilic acid, carbohydrate moieties and phosphate groups, and of the bonds cleaved by various proteases, were used to determine the number and approximate position of the transmembrane segments of the protein (Findlay, 1989; Findlay et al, 1988). These conclusions have been substantiated recently by the use of antipeptide antibodies with another member of the family the β-adrenergic receptor (Wang et al 1989). The approximate orientation of these intramembranous helices was determined by the exposure to modification of residues such as cysteine, tyrosine, tryptophan, histidine and lysine, by photosensitive probes which readily partition into the bilayer (Davison and Findlay, 1986). The general packing arrangement of these helices came from the work of Henderson and Unwin (1975) with bacteriorhodopsin, a protein of similar intramembrane volume to rhodopsin. Thus it was possible to attempt a 3-D representation of the protein using all this information together with a few local rules, such as no crossing-over of the extramembranous loops, close packing only of antiparallel segments, etc.

**Ligand binding site:** The ligand for rhodopsin, 11-cis retinal, forms a protonated Schiff's base with lysine. Following reduction to stabilize the covalent bond, the attachment point for the chromophore and with it the approximate location of the ligand binding site could be established (reviewed in Findlay