Review letter

MEMBRANE PROTEINS AND MEMBRANE STRUCTURE

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1. Introduction

Recently, the distinctive characteristics of membrane proteins have been recognized and established experimentally. This knowledge has led us to develop a model of membrane structure, represented diagrammatically in figs. 1 and 2, in which most of the proteins of the membrane penetrate into and form an intrinsic part of the membrane continuum. It is our view that these proteins are usually organized into functional units or complexes, and that interactions between different complexes are important to the structure of the membrane. Some of the concepts which are central to the model are discussed in this review.

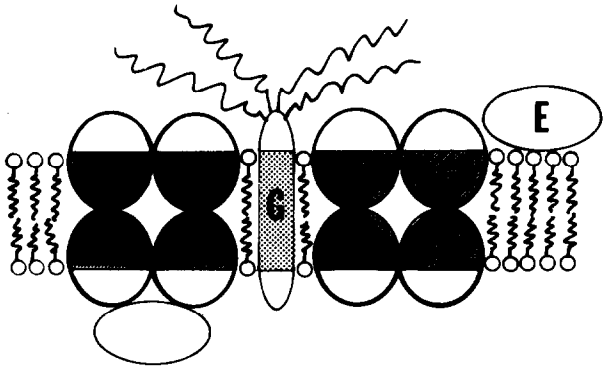

Fig. 1. A schematic cross-sectional view of a hypothetical plasma membrane to show: i) The localization of intrinsic (I) and extrinsic (E) proteins. ii) The amphipathic nature of intrinsic proteins. The polar portion of the molecules (unshaded) is exposed to the aqueous medium while the nonpolar portion (shaded) is in contact with the hydrocarbon tails of the lipid. iii) The organization of the majority of proteins into complexes which span the membrane. The size of complexes isolated from most membranes is in the range of 100,000–200,000 daltons. The subunit polypeptides generally have molecular weights between 10,000 and 50,000 daltons. Association of proteins into complexes allows the possibility of channels through which solutes may traverse the membrane. iv) The spanning of the membrane by glyoproteins (G) as single polypeptides.

2. The lipid of membranes is organized into a bilayer

The evidence that the lipid in membranes is organized into a bilayer, with the polar head groups exposed to the aqueous phase on both sides of the

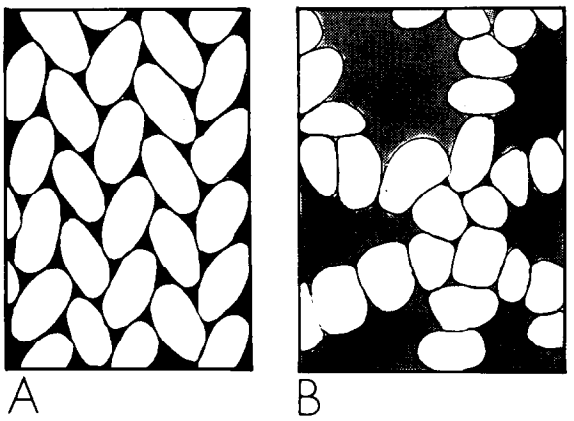

Fig. 2. Protein domains in membranes. A) A representation of the lattice structure of membranous cytochrome oxidase showing the inter-complex associations. B) A schematic representation of the non regular protein domains of most membranes. As a result of these protein–protein interactions, the lipid is not continuous but isolated into discrete pockets.

membrane, and the hydrocarbon tails forming the hydrophobic interior of the bilayer, has been summarized in several recent reviews [1–3] and will not be covered here.

3. There are two broad classes of membrane protein

Membrane systems contain two broad categories of proteins, which differ in their position with respect to the lipid bilayer and therefore in their mode of interaction with the lipid.

Intrinsic [4] or integral [5] membrane proteins penetrate into and sometimes completely through the interior of the bilayer and have, therefore, a predominantly hydrophobic interaction with lipid.

Several examples of intrinsic proteins have been clearly defined. Evidence has been obtained from electron microscopy, and more recently from X-ray diffraction studies, that rhodopsin penetrates deeply into the lipid bilayer in the rod outer segment [6–9]. Electron microscopic evidence has been interpreted to indicate that cytochrome oxidase penetrates completely through the lipid bilayer [10], and the observation that the major glycoprotein of the human red cell membrane can be chemically labelled from both sides of the membrane supports the concept that this protein also spans the lipid bilayer [11]. These, and indeed all intrinsic membrane proteins, can only be liberated from their respective membranes by reagents which disrupt hydrophobic interactions. They are insoluble in aqueous solution in the absence of detergent or other solubilizing agents, although in some cases polymerization occurs which yields a water soluble aggregate. Further, intrinsic membrane proteins have the unique ability to recombine with lipid to form membranes.

In contrast to intrinsic membrane proteins are proteins which do not penetrate the lipid bilayer, but are held at the surface of the membrane by predominantly electrostatic interactions. These proteins have been called *extrinsic* [4] or peripheral [5] membrane proteins and can constitute as much as 50% of the total protein, as in the membrane of beef erythrocyte [12]. Examples of extrinsic proteins include cytochrome *c* of the mitochondrial inner membrane [13], spectrin of the red cell membrane [12], and the basic protein of myelin [14], all of which can be removed from their respective membranes by reagents which disrupt electrostatic interactions. These and all extrinsic proteins are characteristically soluble in aqueous solution, once liberated from the membrane, and will not re-form membranes upon the addition of lipid.

4. Intrinsic proteins are characteristically amphipathic

The unique environment of intrinsic membrane proteins, partially buried in the hydrocarbon interior of the lipid bilayer and partially exposed to the surrounding aqueous medium, demands that these proteins be bimodal or amphipathic. For thermodynamic reasons which have been discussed extensively by Singer and his collaborators [5, 15, 16], these proteins must have an asymmetric distribution of polar and nonpolar groups about their surface, such that charged groups are exposed to the aqueous phase and not to the hydrophobic interior of the bilayer. It is not surprising, therefore, that most intrinsic membrane proteins have a lower polarity than extrinsic proteins and non-membrane proteins in general [17, 18]. Presumably, a reduction in the number of polar groups allows the asymmetric distribution required of the amphipathic proteins. This is not however the only way of achieving a bimodal character. Strittmatter and his associates have shown that both cytochrome b_5 [19] and cytochrome b_5 reductase [20], which are considerably more polar than the majority of intrinsic proteins [17], have in fact an asymmetric distribution of polar and non-polar groups along their polypeptide chain. Thus, a very "hydrophobic tail" is organized, which must penetrate the hydrophobic interior of the lipid bilayer and anchor the more polar and globular functional part of these molecules to the interior of the membrane. An asymmetric distribution of polar and non-polar amino acid residues has also been identified in the polypeptide chain of the major glycoprotein of the human red cell membrane [21]. However, in this case, a central portion of the polypeptide chain is non-polar, with the two terminal portions of the chain being composed of predominantly polar groups. This is in keeping with its proposed position with respect to the bilayer, penetrating completely through the bilayer, with extensions into the aqueous phase at both surfaces of the membrane. Other

methods of increasing the stability of intrinsic proteins within the hydrocarbon phase may exist. For example, evidence has been presented recently that some intrinsic membrane proteins, including the Folch-Lees proteolipid of myelin [22] and a proteolipid for sarcoplasmic reticulum [23], have a considerable amount of fatty acid which is covalently bound to some of the polar residues of these molecules, thereby reducing the net charge on the protein and at the same time increasing the hydrophobic character of the molecule.

5. Intrinsic proteins are generally organized into complexes

The functional sectors of the mitochondrial and bacterial electron transfer chains [24–27], the ATPases concerned in active translocation of Ca^{2+} [28] or Na^+ and K^+ [29], have all been isolated as lipoprotein complexes, which are capable of generating membranes *de novo* when the depolymerizing reagent used in their isolation is removed. These complexes are tightly linked sets of proteins and, since the integrity of this tight association is required for activity, it follows that the complex is the form of the *in vivo* organization of these membrane components. In fact, membranes generally can be depolymerized into lipoprotein units, whose size would suggest an aggregate of proteins [30]. In one case, the erythrocyte membrane, the association of proteins has been studied in some detail. Bifunctional aldehydes have been used to link closely associated polypeptides [31, 32]. At low concentrations of reagent, the intrinsic proteins were all cross-linked into discrete sets with the exception of the glycoproteins, which are highly charged molecules, a property unfavorable to close association with other proteins. These glycoproteins appear to be free floating in the lipid and they are separated with the lipid when red cell membranes are treated with chloroform–methanol solutions [33].

6. Complexes are associated into domains

There is considerable evidence of interactions between complexes to form protein domains. This long range ordering of proteins is most obvious in cases where a regular array results from the interactions of similar complexes, as for example in membranous cytochrome oxidase. In the oxidized form and over a narrow range of lipid to protein ratio (25–30% w/w), membranous cytochrome oxidase appears under the electron microscope, after negative staining, as a regular two dimensional array of protein complexes [10, 34]. Optically filtered micrographs indicate that each complex in the oxidized state is associated with its six nearest neighbors [35]. This array is unlikely to be a consequence of drying or fixation, because the order is stabilized in solution by glutaraldehyde only in the oxidized state and not when the enzyme is in the reduced from [36]. Glutaraldehyde itself cannot be responsible for the lattice arrangement, because this fixative and indeed all stain can be omitted in preparing the material for electron microscopy, without losing the lattice appearance [37]. The sensitivity of the lattice structure to the conformation of the complex is particularly interesting. The array is disrupted by reduction of the oxidase and also by exposure to pH conditions which diminish the activity of the enzyme [36].

The very regular arrangement of complexes in the cytochrome oxidase membrane is undoubtedly a special organization. However, long range but more irregular ordering of proteins into domains is indicated in many natural membranes. Most convincing are the freeze etched micrographs of some membrane which show large spots (interpreted as particulate aggregates, exposed during cleavage of the membrane along its hydrophobic interior) [38], closely apposed to form a network through the membrane [39–41].

It is clear that the stability of some membranes depends heavily on the associative bonds which link complex to complex within the protein domain. When the electrostatic repulsive forces between complexes are increased by alkalinization or by the removal of divalent counterions, many membranes can be induced to undergo fragmentation into lipoprotein units or into smaller vesicular membranes. For example, the mitochondrial membrane can be disrupted by sonication at high pH [42]; the *halobacterium* membrane is disrupted dramatically by dilution of the high salt medium [43]; the red cell membrane is disrupted extensively by chelation of divalent metal ions with 5 mM EDTA [44], succinylation of the proteins [45] and sonication at high pH [46]. These effects can all

be prevented by cross-linking with glutaraldehyde, thereby introducing new and stable protein–protein interactions into the membrane [46].

7. The interaction of protein and lipid affects the structure of both components

The requirement of lipid for activity of many membrane enzymes, and the dependence of their temperature characteristics and transport properties on the phase properties of membrane lipid, are clear indications of the effect of lipid on protein [47–50]. However, a reciprocal effect of protein on lipid has not so far been reported, although for the following considerations we think it likely.

Aggregates of intrinsic proteins and lipid micellar structures are in themselves both very stable entities, but when the two are mixed under the appropriate conditions, they combine to form membranes. One must conclude therefore, that a protein–lipid membrane continuum is the most stable organization of amphipathic proteins and lipid in aqueous solution. This is only likely if there is some effect of protein on lipid and of lipid on protein, i.e., a reciprocal stabilizing effect of the two components.

The maximum amount of lipid which can combine with protein to form a membrane appears to be defined for each membrane. When cytochrome oxidase and phospholipid are associated to form a membrane, up to 40% w/w of phospholipid is included in the membrane [51]. Above this amount no further lipid is incorporated into the membrane. Similarly there appears to be a well defined lipid to protein ratio in other membrane systems [52]. This precise ratio of components can only be explained by some important stabilizing relationship between protein and lipid, and/or a requirement for protein–protein interactions in the membrane, which could not be possible above a certain lipid to protein ratio.

8. Membrane models

The data now available on membrane proteins and protein–lipid interactions in membranes cannot be accomodated by the unit membrane model of membrane construction [53], which is superceded by the model we have presented and also by the mosaic model developed by Singer and his associates [5]. Our model and the mosaic model agree on some of the fundamental concepts such as the amphipathic nature of intrinsic membrane proteins; and the considerations developed in the early sections of this review apply equally to both models. However, we differ with Singer and his associates in the importance we place on protein–protein interactions and on the contributions these interactions make to the structure of the membrane. The data we have presented in this review are compatible with our model. Some of the information is difficult to rationalize by the mosaic model. Particularly the observation that there is an optimum amount of lipid which can be incorporated into membranes is not in keeping with the idea that proteins are "floating in a sea of lipid".

Almost all of the membranes we have considered, including the membranes of the chloroplast, mitochondrion, erythrocyte and *halobacteria*, can be adequately described by the picture of membrane construction we have developed. The extent to which our model explains the available data for the rod outer segment membrane and myelin has not yet been assessed.

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