On the Orientation of Lipids in Chloroplast and Cell Membranes

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Abstract

The widespread recognition of the corpuscular nature of membrane ultrastructure demands re-evaluation of established concepts of their molecular organization. Many aspects of membrane physiology, composition, and metabolism provide support for the proposal that most membranes consist of two-dimensional polymers of lipoprotein subunits. Such a model allows the activity, specificity, and adaptability attributed to biological membranes. Evidence which supports this corpuscular model for membranes and some inadequacies of the bimolecular lipid leaflet model are pointed out.

The lamellae of plant chloroplasts are membranes which clearly consist of subunits (quanta-somes). Their four surfactant lipids and pigments comprise 50% of the lipoprotein subunits. In each of these surfactant lipids there is found a limited and specific group of fatty ester components. This phenomenon suggests that the hydrocarbon chain of the fatty esters may specifically complement certain hydrophobic amino acid sequences in the membrane protein. The protein, then, would determine the sites where the lipid will be most firmly bound. It is proposed that the lipids of membrane subunits are bound by hydrophobic association of the hydrocarbon chains of the lipids with complementary hydrophobic regions within the interior of the protein. The resulting two-dimensional lipoprotein aggregate would possess the strongly anionic charged groups of the phospholipids on its surface. Metabolically-driven alterations in conformation of such a flexible lipoprotein ion exchange membrane allows a consistent interpretation of biological membrane transport phenomena.

Introduction

Function of the Living Cell requires remarkable specificity, efficiency and adaptability of interfaces known as membranes. The lipid-protein character of cell membrane has long been recognized. The structural relationships of the lipid molecules and their protein matrix have not been open to direct observation. As a result it has not been possible to interpret physiological function of membranes in terms of molecular structure.

An assembly of circumstantial evidence for the hydrophobic association of lipids and protein in chloroplast (1,2) and mitochondrial membranes (3) engenders the suggestion that most, if not all, membranes may be two-dimensional aggregates of lipoprotein subunits. This proposal is at variance with the classical bimolecular lipid membrane concept reviewed by Brady and Trams (4), but possesses several common attributes. Some of the limitations of the lipid bilayer model for membrane structure are described in the following paragraphs.

The Lipid Bilayer Model

The lipid bilayer model for the structure of membranes of cells was suggested by Davson and Danielli (5) on the basis of observed lamellar micellar aggregation (6) of natural surfactant lipids. Dramatic observations of lipid lamellar aggregation in myelin figures have been made by Stoeckenius (7,8). Lipid bilayer membrane stability and physical properties have been studied extensively (9,10). In order that these structures be involved in performance of membrane functions a very special matrix of superficial protein would be required (11). Its charge distribution must be roughly complementary to that of the adjacent anionic lipid layer and its reaction to molecular stimuli (ATP, cations, substrate approach, etc.) must result in specific alterations of the lipid layer such as production of holes, channels of appropriate charge gradients, and variations of the hydrophobic barrier. Such proteins would be essentially cationic in order to control lipid orientation most directly. The specific associations of cytochrome c with phospholipids provides strong evidence for this type of interaction (12) with basic proteins. Membrane proteins, however, are not basic proteins, and if anything, contain major amounts of the acidic amino acids (13-15).

Lipid monolayer studies have shown that branching, unsaturation, and short chains reduce film stability (16). Myelin is an example of a very stable natural lipid bilayer membrane structure. O'Brien (17) observed that when the Schwann cell is incapable of elongation of the C18 fatty acids, the myelin it produces is poorly organized. C18 and C16 acids are probably characteristic of the Schwann cell plasmalemma or cell membrane and therefore differ from the saturated C14 chains of lipids in normal myelin. Even though the "cell membrane" of the Schwann cell and its myelin layers are contiguous, one cannot conclude that they are identical. There must be a gradual transition from cell membrane lipoprotein to the lipid bilayer myelin as the lipid components of myelin are synthesized in the cell. The active functional membranes of most cells contain large amounts of unsaturated or branched chain acids. These are not the types which one would expect to find in stable lipid bilayer membranes.

The fatty acid components of lipids of the bilayer membrane form a relatively liquid phase like those in a soap micelle or crystal. The nature of the hydrocarbon chain of each class of complex lipid, therefore, could have little specific interaction with other lipids or with the adjacent protein. It appears that the lipid bilayer model could demand no specificity in the nature of the hydrocarbon chains of the lipids. In natural cell membranes, as we shall see, this is not the case, and hydrocarbon chains of each type of lipid are rather specifically selected.

Electron microscopy supported the lipid bilayer membrane model as long as stained sections were the primary subjects for examination. The "three layer"
pattern of two adjacent dark lines bordering an unstained central line has come to be recognized as "the unit membrane" (18,19). It is the result of osmium or manganese accumulation at the interface between the charged groups of the phospholipids and the adjacent proteins. Stoeckenius (8) has reviewed the evidence in support of this deduction. Coordination complexes of the heavy atom with lipid phosphate ionic groups and protein nitrogen sp³ ligands may well account for the observed strict localization of bound metal in the stained membrane. The distance between these stained regions in a lipid bilayer membrane being a liquid phase, would be much less than twice the length of a C₁₈ fatty acid or 30-35Å. The spacing in phospholipid myelin figures has been established by Stoeckenius (8) as 38 to 40Å for the lipid bilayer repeating unit. The observed separation is not this but up to 140Å in some cell membranes. Such a disparity could hardly result from a mistaken presumption of location of the metal in the stained membrane. It seems reasonable to suspect that some membranes may have structures different from that of myelin.

The Corpuscular or Lipoprotein Subunit Membrane Model

Appearance of Membrane Subunits

Shadowed surfaces of many cell membranes reveal a mosaic-like array of repeating units (20). In Halobacterium halobium (21,22) these units are 140Å in diameter (Fig. 1 and approximately equal to the thickness of the cell membrane as estimated from electron micrographs of osmium-stained sections. In Mycoplasma lariavii the membrane structures have been estimated to be 80Å in thickness by Razin et al. (23). In all cases the sections of these obviously corpuscular membranes stain with osmic acid to give the typical "unit membrane" pattern of two parallel black lines. Chloroplast lamellae also possess subunits. The freeze-etch electron microscopic technique of Mühlethaler (24) gives the most striking picture of membrane surfaces. This method is based upon the fracture of frozen cells along membrane surfaces. Water is sublimed from the freshly cleaved surface to reveal the lipoprotein subunits in bold relief. These are shadowed by metal evaporation and the replica is reproduced by the electron microscope. The image suggests that chloroplast membrane surfaces are a mosaic of identical subunits. Many intracellular membranes appear to possess structure suggesting that they are formed by aggregation of identical subunits. By entirely independent analytical method, low-angle X-ray scattering analysis, Kreutz (25) revealed a periodicity of electron density in the plane of chloroplast lamellae. These 38Å repeating units are, surprisingly, revealed in electron micrographs of stained sections as shown in Figures 2 and 3 (2). Whether these represent discrete lipoprotein subunits or not is debatable but highly suggestive. On such bases, Sjöstrand has proposed a globular structure for the plasmalemma (26).

Lipid-Protein Association

Both natural surfactant lipids and proteins may be considered as being aggregated by hydrophobic interactions ¹ (27). These result from the very large net entropy increase as the hydrophobic side chains of the polypeptide or the hydrocarbon chains of the surfactant lipids are transferred from the water phase where of necessity they direct orientation of surrounding water molecules. Kauzmann (28) pointed out that as a hydrophobic structure is removed from the water phase, the organization of the surrounding water molecules is diminished with a concomitant increase of entropy. Aggregation of phospholipids and proteins involve related equilibria of vertical equilibria (Fig. 4) and are driven by the very large TAS factor involved. It was clear from the myoglobin structure

¹ The term "hydrophobic association" is preferred to the widely used "hydrophobic bonding" since no covalent or hydrogen bonding is implied.