Plasma Membranes and Disease

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Preface

When one defines a cell, that definition must include the cell's limiting boundary, the plasma membrane. Indeed, one can appropriately consider the plasma membrane as the first cellular organelle. However, it is an entity that has elaborated and diversified enormously during evolution, and it is now more correct to think of the plasma membrane of complex multicellular organisms as a set of organelles assembled at the cell periphery and concerned with interaction between cells, between cells and their fluid environment, and between the extra- and intracellular compartments. Among the critical functions incorporated in plasma membranes are development, maintenance and modulation of electrochemical gradients between the cell exterior and the cytoplasm, targeted concentration of enzymes, assurance of close topological associations between functional molecules, signal recognition and signal transduction.

The field of plasma membrane pathobiology has expanded rapidly in step with the important recent developments in basic membrane research and, not surprisingly, in view of the diversity of membrane functions, a large variety of membrane anomalies have been recognized as being responsible for numerous disease processes. On top of this comes the important involvement of the plasma membrane in many infectious diseases, as well as in a variety of therapeutic incursions.

My purpose in writing this book was to integrate existing basic biochemical, biophysical and molecular biological information on normal plasma membranes, as well as membranes involved in disease processes, and to present the material in a manner accessible to specialists in various aspects of membrane biology and in membrane-related pathobiology, as well as to a wider circle of graduate students and scholars in diverse fields of

biomedicine. I hope that the book will prove as helpful for the reader as it has been challenging to the author.

Boston, MA.
May, 1978

Donald F. Hoelzl Wallach

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Plasma Membrane Organization

INTRODUCTION

Plasma membranes are generally about 8 nm thick. They consist of two dissimilar, 2.5 nm polar lamellae, each bordering on an aqueous compartment, as well as on a central apolar layer. All cellular membranes contain both protein and lipid. Proteins generally make up two-thirds or more of the membrane mass. Plasma membranes also bear sugars linked covalently to membrane proteins (glycoproteins) and lipids (glycolipids).

Basic research on plasma membrane structure is undergoing rapid expansion and sophistication and this chapter is intended to draw attention to recent developments that have direct or indirect implications for plasma membrane pathobiology.

MEMBRANE LIPIDS

Composition—General Array

Individual membrane lipid molecules are very poorly soluble in aqueous media. Also, all but some minor membrane lipids are *amphipathic* molecules with a polar and/or charged terminus and an apolar one. This property causes the lipid molecules to aggregate in aqueous solvents and leads to the formation of bilayers with the polar residues oriented toward water and the

apolar moieties directed toward each other, Fig. 1.1. In cellular membranes, lipids are also organized in bilayer arrays and their apolar residues form part of the apolar membrane core.

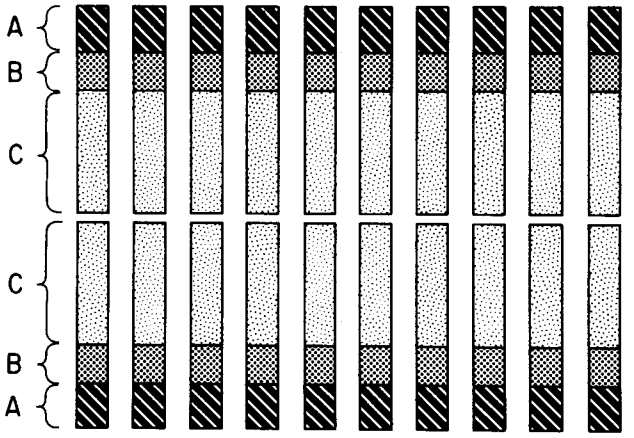


Fig. 1.1 Zones of differing polarity and hydrophobicity perpendicular to the plane of a lipid bilayer. A, Head group domain; polar and/or charged. B, Ester or amide region; intermediate polarity. C, Acyl chain region; apolar, hydrophobic.

The predominant membrane lipids are the glycerophospholipids (Fig. 1.2). Except for lysophospholipids these bear two fatty acid chains, most commonly 18–20 carbons long, one saturated and one unsaturated to varying degrees. These fatty acid chains link to the glycerol backbone via an ester (most common) or ether linkage. In the most common glycerophospholipids, phosphatidylcholine (lecithin), phosphatidylethanolamine, phosphatidylserine and phosphatidylinositol, the glycerol moiety is coupled via a phosphate diester linkage to choline, ethanolamine, serine or inositol, respectively. A major phospholipid, sharing the phosphorylcholine residue with lecithin but differing in its apolar moiety, is sphingomyelin. Lecithin and sphingomyelin bear no net negative charge at neutral pH, phosphatidylethanolamine is nearly electroneutral, phosphatidylserine bears one net negative charge and inositol phospholipids bear one or more negative charges. Phospholipids are normally asymmetrically represented at the two polar layers of cellular membranes.

Cholesterol is a second major membrane lipid (Fig. 1.2). In plasma membranes it occurs in nearly equimolar ratios with phospholipids. There is

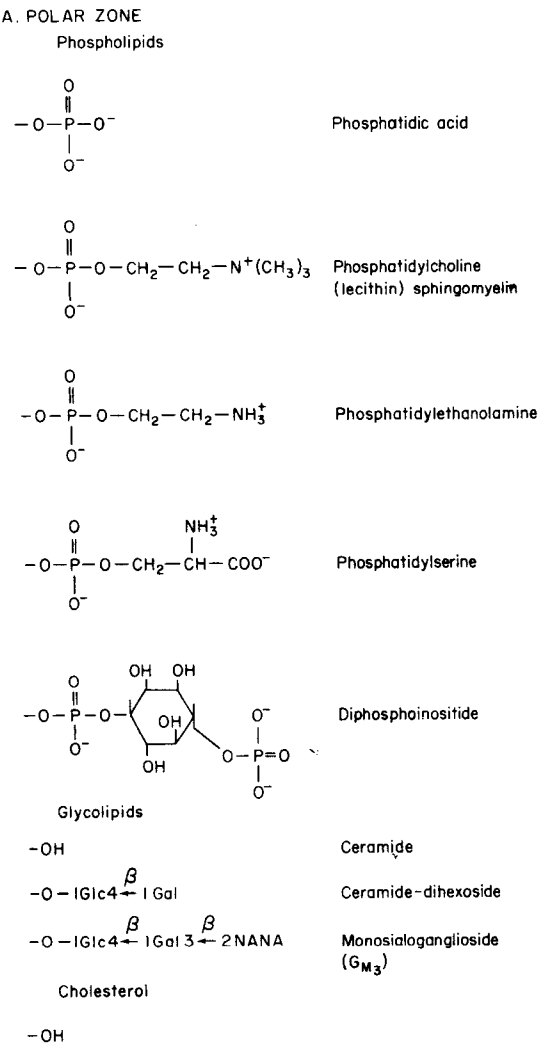
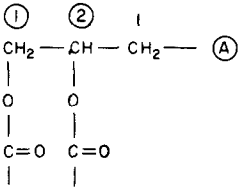


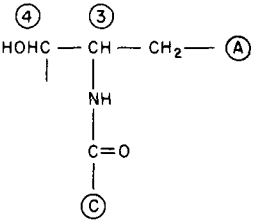
Fig. 1.2 Structures of the major membrane lipids. Zones A, B and C refer to Fig. 1.1.

B. INTERMEDIATE ZONE

Glycerophospholipids



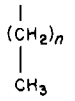
Sphingolipids — Sphingomyelin; Sphingoglycolipids



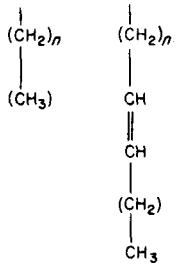
Cholesterol — None

C. APOLAR ZONE

Glycerophospholipids



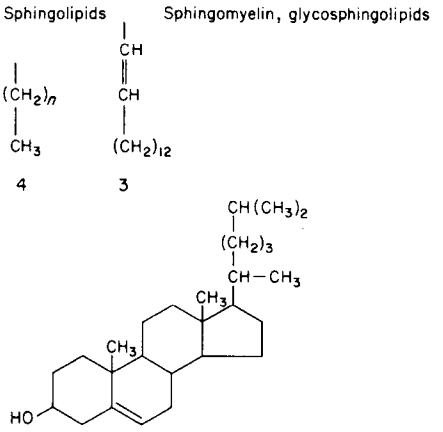
Lysophospholipids



Phospholipids

2

Fig. 1.2—*continued*



Notes: In glycerophosphatides position 1 is usually occupied by a saturated acyl chain and position 2 by an unsaturated one. In sphingolipids the intermediate segment contains the amide linkage and hydroacyl group of sphingosine. The apolar segments portion of sphingomyelin consist of the $(CH_2)_{14}CH_3$ chain of sphingosine and the fatty amide chain (usually C_{24}).

Fig. 1.2—*continued*

less cholesterol in intracellular membranes. (Normal mitochondrial membranes probably contain no cholesterol.) The hydroxyl headgroup of cholesterol lies at the polar surfaces of the membrane and the apolar acyl chain extends into the apolar core. Some of the cholesterol of plasma membranes appears segregated into clusters; the rest is intercalated between other lipids.

The sphingoglycolipids (Fig. 1.2) constitute a third important membrane lipid category. Their apolar segments are identical to that of sphingomyelin, but a sugar residue is substituted for phosphorylcholine. The saccharide moieties can be neutral or when they include sialic acid residues, negatively charged (gangliosides). Sphingoglycolipids are most concentrated in the outer layers of plasma membranes, with their sugar moieties extending into the extracellular aqueous phase and their apolar chains forming part of the apolar membrane interior.

Phase Transition—Phase Segregation

Small changes in temperature or ionic conditions can bring about large alterations in the structure of lipid assemblies, e.g. bilayers, without modifying covalent linkages. These alterations are important because of their effects on lipids *per se* and on membrane proteins.

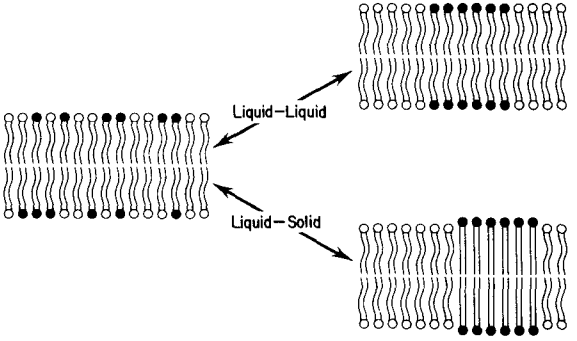


Fig. 1.3 Phase segregation and phase transition in a model lipid bilayer. Left: The bilayer composed of two phospholipids both in the liquid–crystalline state. Upper right: Segregation of the two types of phospholipid both still in the liquid–crystalline state. Lower right: One of the phospholipid classes (●) has shifted into the crystalline state (liquid–crystal⇌crystal phase transition), through, e.g. a drop in temperature. The crystalline phase of this phospholipid has also segregated from the liquid–crystalline phase of the second phospholipid (○). The greater thickness of the crystalline bilayer is indicated. Lipids can be shifted between phases (a) by changing temperature or (b) without temperature change by (i) altering the ionic composition of the aqueous environment or (ii) insertion of apolar proteins. Phase transitions that occur at *constant temperature* are the important phenomena in the membranes of animal cells.

Pure glycerophospholipids, or sphingolipids, in bilayer arrays can exist in two temperature-dependent types of physical state (Fig. 1.3): (a) *crystalline* phases, where the polar groups and acyl chains are in ordered, two-dimensional crystalline arrays and (b) *liquid–crystalline* phases, where the polar groups are in two-dimensional crystalline arrays, but the hydrocarbon chains form a disordered, liquid (fluid) continuum. A transition between these states, i.e. a *phase transition*, can be detected in numerous ways, including differential scanning calorimetry, Raman spectroscopy and nuclear magnetic resonance spectroscopy.

In pure systems, crystal⇌liquid–crystal phase transitions can be brought about by very small temperature shifts. This is because such transitions are

highly cooperative, i.e. a state change in one acyl chain markedly facilitates that of other chains, with "cooperative units" of 50–200 chains. The critical transition temperature, T_c, depends on the nature of the acyl chain, e.g. length and unsaturation, as well as on the nature of the polar group, charge in particular. Thus, the T_c of dipalmitoyl (C16) lecithin lies at 41 °C, while that of lecithins with one saturated and one monounsaturated C16 chain is below 0 °C. Also, phospholipids with charged polar groups exhibit T_cs well below those characterizing uncharged, but otherwise identical phospholipids. For such lipids, a phase-transition brought about under given ionic conditions (e.g. pH, $[Ca^{2+}]$) by a temperature shift, can be induced *isothermally* by an appropriate change of ionic environment. In the case of negatively charged phosphatides, small amounts of Ca^{2+} can markedly raise T_c. In any event, for nearly all pure membrane glycerophospholipids containing, as is typical, at least one unsaturated acyl chain linkage, the T_cs will be well below physiological temperature. In contrast, native sphingomyelins exhibit broad, complex transitions, extending between 20 and 45 °C and containing several steps, including one centered near 37–38 °C. The T_c of neutral glycosphingolipids lies near 65 °C.

The thermotropic behavior of homogeneous lipid bilayers does not adequately represent that of mixed lipid membranes of *biomembranes* for the following reasons:

(1) Membrane phospho- and glycolipids are heterogeneous in *acyl chain* composition. Some favorable conditions (e.g. mixed chain phospholipids with identical polar groups) allow a single, cooperative phase transition with an intermediate T_c. However, in mixtures of lipids with sufficiently dissimilar hydrocarbon and/or polar residues, one class of lipid may form a liquid–crystalline phase under given conditions and the other a crystalline phase. Molecules of the second class will then associate to form crystalline islands in a liquid–crystalline continuum (or vice versa). This is *phase segregation* (Fig. 1.3). Segregation of dissimilar liquid–crystalline phases or even crystalline phases may occur.

(2) In plasma membranes, and other membranes containing *cholesterol*, phase changes are markedly modified (Fig. 1.4). This is because cholesterol modifies phospholipid interactions (a) to make phase transitions less cooperative, (b) to maintain an intermediate state of acyl chain "fluidity" above and below the normally sharp T_c, and (c) to allow chain mixing. Cholesterol thus acts as a "buffer" of cooperativity as well as of "fluidity", and allows the formation of a single phase out of a lipid mixture which might otherwise segregate into separate phases.

(3) Penetrating membrane proteins (see p. 12) can influence lipid crystal $\rightleftharpoons$ liquid–crystal transitions as follows (Fig. 1.4): (a) a shift, upward usually, in T_c and (b) a broadening, i.e. reduced cooperativity of the transitions. These

effects derive from the influence of penetrating proteins on surrounding acyl chains. Surface-located proteins (see p. 12) may also alter lipid phase behavior through ionic interactions with charged phospho- and glycolipids.

(4) In a few biomembranes, transition from the liquid–crystalline to the crystal state of the membrane phospholipids can induce segregation of the *membrane proteins.* As lipid crystallization extends two-dimensionally, penetrating proteins excluded from the growing crystalline phase are caused to aggregate. This process has been observed in the membranes of some microorganisms and the nuclear membranes of lymphoid cells.

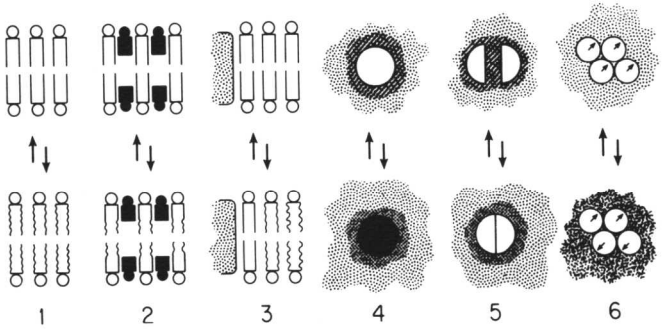


Fig. 1.4 Possible state transitions in a plasma membrane. (1–3, side views; 4–6, head-on views). 1. Crystal⇌liquid–crystal phase transition. 2. Same modified by cholesterol. 3. Same modified by penetrating protein. 4. Structural change of penetrating protein with modification of boundary layer. 5. Monomer⇌dimer transition plus boundary layer change. 6. Rotational reorientation (about an axis perpendicular to membrane plane) of multimeric protein subunits (plus boundary layer modification).

Lipid phase transitions can be detected in biomembranes and Raman spectroscopy has proved particularly useful for this. For example, Verma and Wallach (1976a), following both acyl chain stretching signals and acyl chain acoustical modes, discovered multiple thermotropic lipid transitions in erythrocyte membranes. As expected from the acyl chain unsaturation and high cholesterol content, the thermotropism of total extracted erythrocyte membrane *lipids* was restricted to a very broad transition, $T < 5\,^{\circ}C$. However, the *membranes* showed two distinct transitions. One, centered near $-4\,^{\circ}C$, was large but diffuse and extended between $\sim +10\,^{\circ}C$ and $\sim -20\,^{\circ}C$. The other, centered at $\sim +17\,^{\circ}C$, was smaller but sharper, possibly corresponding to Arrhenius plot discontinuities of several erythrocyte membrane functions. These results suggest that the lipids in erythrocyte membranes are segregated into domains of differing composition and behavior, but that most acyl chain transitions occur at subphysiological temperatures.

Exchange

The lipid components of various cells' plasma membranes can exchange with those of other cells both *in vivo* and *in vitro*. Much of the earlier literature on this topic has been reviewed by Wallach (1975; Chapters 3, 4, 5) and aspects of lipid exchange relevant to membrane pathobiology, therapy and toxicity are treated elsewhere in this volume.

Except in the case of the experimental or therapeutic use of liposomes (see, for example, Chapter 7, p. 265, exchange takes place dominantly via plasma lipoproteins as carriers of cholesterol and phosphatides, and albumin as carrier for free fatty acids and lysophosphatides.* Some exchange may also occur between colliding cells.

A number of specialized exchange mechanisms exist. The best studied of these, the low-density lipoprotein/high-density lipoprotein pathway for cholesterol exchange, is treated in Chapter 4 (p. 183). Also important, e.g. in lymphocyte activation (Resh, 1977), is the deacylation/reacylation cycle of phospholipids. This allows alteration of phospholipid acyl chain composition: phospholipids are cleaved, usually at the 2-position by membrane-associated or plasma phospholipase A:

$$\text{Phosphatide} \xrightarrow{\text{phospholipase A}} \text{lysophosphatide} + \text{fatty acid}$$

Both free fatty acid and lysophosphatide will bind to serum albumin. Such albumin-associated lipids can exchange passively with the membrane lipid pool. Diacyl phosphatides can be regenerated by membrane-associated acyl transferases in the reaction.

$$\text{Lysophosphatide} + \text{fatty acyl-CoA} \xrightarrow{\text{acyl transferase}} \text{phosphatide}$$

where the fatty acyl-CoA derives from a membrane or plasma fatty acid pool. The deacylation/reacylation reaction is a means for membrane lipid regeneration but its most important function may be in modulating membrane function.

Closely related to the deacylation/reacylation system is lecithin cholesterol acyltransferase, LCAT, an enzyme which catalyzes the reaction

$$\text{Lecithin} + \text{cholesterol} \underset{\longleftarrow}{\overset{\text{LCAT}}{\rightleftharpoons}} \text{lysolecithin} + \text{cholesterol ester}$$

The lysolecithin generated behaves as described, free cholesterol can

*Tall and Small (1977) have shown that the apoprotein of high density lipoprotein can complex with phospholipid molecules in aqueous dispersion causing clearing and formation of soluble disc-shaped (11 × 5.5 nm) phospholipid/protein complexes. They propose that the apoprotein, interacting with intact cells, might lead to selective modification of plasma membrane phospholipids.