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MINI-REVIEW

Principles of Membrane Stability and Phase Behavior Under Extreme Conditions

P. J. Quinn[1]

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Abstract

Biological membranes consist of a complex assortment of lipids and proteins. The arrangement of the components, particularly in regard to their lateral disposition in the plane of the membrane under physiological conditions, is dependent on the phase behavior of the different membrane lipids and the way that this behavior is modified by interaction with other membrane components and electrolytes in the aqueous medium. Irreversible phase separation of components within the membrane may result from exposure to extreme environmental conditions including temperature, pressure, or electrolyte concentration. The principles underlying the phase-mixing behavior of model membrane systems can be used to provide useful information about the factors that determine the stability of biomembranes under physiological and nonphysiological conditions. These data are reviewed and used to predict events that take place when membranes are exposed to environmental stress.

Key Words: Membrane lipids; phase separations; nonbilayer lipids; environmental stress on membranes.

Introduction

Membrane functions, particularly selective permeability and barrier properties, are perturbed by exposing organisms to environmental conditions that are, to varying degrees, outside of those conditions considered to be in the physiological range. Exposure of individual membrane constituents to extreme conditions is unlikely to affect physical structure or denature the components. Membrane lipids are relatively small molecules the conformation

[1]Department of Biochemistry, King's College London, Campden Hill, London W8 7AH, United Kingdom.

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of which depends on chemical structure and interaction with neighboring molecules. Proteins, however, undergo irreversible denaturation, but usually this process occurs at temperatures higher rather than that at temperatures lower than those of the physiological range. Electrostatic effects of electrolytes or pH on protein conformation appear to be largely reversible since common methods of purifying proteins involve salting-out procedures. This suggests that stresses on the membrane do not irreversibly alter the components of the membranes themselves, but rather alter the way that the components are arranged within the structure.

According to current dogma of membrane structure, a matrix formed by a fluid bilayer of amphipathic lipids serves to support the various membrane proteins that are either embedded within the matrix or attached to the surface (Singer and Nicolson, 1972). The components are not linked covalently together and the stability of the structure relies entirely on entropic forces and factors that govern the interaction between the constituents themselves and with the surrounding aqueous medium. There is said to be no intrinsic long-range order imposed by the lipid bilayer on the arrangement of membrane proteins although short-range order may arise from specific interactions between components. Long-range order, on the other hand, may be due to the interaction with cytoskeletal elements or other factors external to the membrane.

One of the most notable features of the lipid component of biological membranes is the highly complex assortment of different molecular species of lipid. In general, only a few lipid classes (designated by the type of polar group) are represented in each morphologically distinct membrane. The dominant lipid classes are those that carry no net charge, and there are generally smaller proportions of acidic lipids. Within each of the major lipid classes, there is usually an extensive array of molecular species that differ in the nature of the hydrocarbon substituent. In the case of fatty acyl substituents, these may differ in chain length, number or position of *cis*-unsaturated bonds, or the position of attachment to the glycerol backbone. The purpose of this highly complex lipid composition is not presently understood, especially since a wide variety of membrane proteins can be reconstituted into lipid bilayers of single molecular species of lipid, yet retain their original functions. Furthermore, the biochemical mechanisms that are responsible for preserving the proportion of the different lipid classes, the distribution of molecular species within each class, as well as the ratio of amphipathic lipid to nonlipid membrane components are presently unknown. Nevertheless, it may be rationalized that the complex nature of the lipid constituents is required for the dynamic functions of the membrane on the one hand, and its stability as a selective permeability barrier on the other.

Studies of the phase behavior of pure molecular species of lipid in aqueous systems over recent years have indicated that most membrane lipids fall into one of three categories when dispersed in pure form at physiological temperatures. Two are bilayer phases—gel and liquid-crystalline configurations, respectively, of the hydrocarbon chains—and the third is a nonbilayer phase referred to as hexagonal II. Secondly, the molecular species of membrane lipid do not mix ideally under physiological conditions and there is a tendency for the lipids to phase separate within the dispersion to form a mixed-phase system.

In this review, the factors that govern the phase behavior of membrane lipids are examined especially with regard to their ability to mix or form separate phases. The likely effect of the interaction of the lipid components with other membrane constituents on the phase behavior is discussed in the context of the arrangement of molecules within membranes when they are exposed to conditions outside of the normal physiological range.

Phase Diagrams of Lipid Mixtures

The thermodynamic parameters by which the mixing of lipids in lamellar phases may be described are usually evaluated from data that describe equilibria between liquid and solid phases of each lipid in a bilayer arrangement. For example, the liquidus and solidus curves in an experimental phase diagram may be fitted simultaneously by using an appropriate thermodynamic method (Lee, 1977, 1978; van Dijck *et al.*, 1977). This approach requires careful attention to details of solid-phase mixing of lipids, including the possibility of multiple solid phases, even in cases where the mixing of the lipids that occurs in the liquid phase may be of greater interest from a biological standpoint. The need for care in the determination and analysis of phase diagrams has been recently emphasized due to the recognition that solid phases of many pure phospholipids are highly complex. The polymorphism has been reported to exist in the solid phases of saturated phosphatidylcholines (Silvius *et al.*, 1985; Lewis and McElhaney, 1985), branched-chain phosphatidylcholines (Mantsch *et al.*, 1985), *trans*-unsaturated phosphatidylcholines (Silvius, 1986), phosphatidylethanolamines (Wilkinson and Nagle, 1984), phosphatidylglycerols (Wilkinson and McIntosh, 1986), and monogalactosyldiacylglycerols (Blaurock and McIntosh, 1986; Quinn and Lis, 1986; Mannock *et al.*, 1985). Future studies will probably reveal that other lipids exist in several stable subgel phases. The existence of multiple phases influences the behavior when such lipids are mixed with other lipid species, particularly if the second species exhibits a single solid phase or a different set of solid phases (Mansourian and Quinn,

1986). The literature, unfortunately, is replete with examples of experimental conditions that have failed to account for these complicating effects of multiple solid phases in the construction of phase diagrams, and caution must be exercised in interpreting such data. Another complication that arises in the determination of the complete phase diagrams of lipid mixtures is that the rate of equilibration of lipids between various solid phases may be measured in terms of days or even weeks. One reason for this is the relatively low rates of lateral diffusion of lipids in gel-state bilayer phases. In other situations, the kinetics of formation of certain types of solid phase, such as subgel or crystalline phases, are inherently slow (Ruocco and Shipley, 1982). In view of these factors, it is essential to determine the composition of mixtures that are close as possible to their equilibrium conditions; this may require prolonged incubation or storage under designated conditions to achieve the equilibrium state.

Phase Separations in Phospholipid Mixtures

Studies of phospholipid mixtures consisting of the same phospholipid class, but with differing hydrocarbon chain components, have been reported. Phase separations in binary lipid systems of this type are not convincing unless the chain lengths of the hydrocarbon substituents differ by more than two methylene groups. A peritectic phase behavior has been characterized in aqueous dispersions of mixtures of 1,2-dimyristoyl- and 1,2-distearoylglycerophosphocholine by using densitometry (Schmidt and Knoll, 1986). The phase boundaries of this system are presented in Fig. 1. It was found that, up to a value of 0.17 distearoyl-/dimyristoylphosphatidylcholine, as the molar fraction of the distearoyl derivative is increased there is a monotonous, somewhat asymmetric increase in volume, exhibiting typical phase behavior; solidus and liquidus lines are of different shape. If the value of the molar fraction of the distearoyl derivative is between 0.3 and 0.7, a biphasic increase in volume is easily observed and is reproducible. The increase in volume at 23°C, which represents the peritectic temperature, is progressively reduced if the molar ratio is increased from 0.3 to 0.7, and there is no change of volume if the molar fraction of the distearoyl-containing lipid is 0.70.

Other studies of binary mixtures of phosphatidylcholines containing different hydrocarbon chain lengths dispersed in excess water have shown that the size of vesicles formed is greatly influenced by the composition of the mixture and the temperature at which the lipids are dispersed (Gabriel and Roberts, 1984, 1986). Phase separations in these mixtures can be induced under isothermal conditions by ultrasonic irradiation in a manner that also depends on the particular temperature and composition (Bakouche *et al.*, 1986).

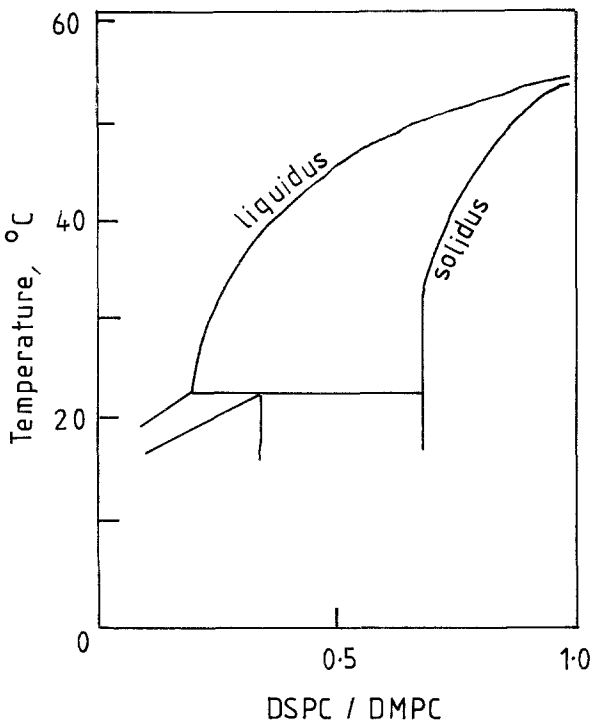


Fig. 1. The phase diagram of binary mixtures of 1,2-dimyristoyl-(DMPC) and 1,2-distearoyl-glycerophosphocholine (DSPC) in excess water. Data from Schmidt and Knoll (1986).

Phase separations are said, in these systems, to be created by a crystallization of the higher-melting-point components within the plane of the bilayer and their segregation into gel phase domains that are distinct from the remaining fluid lower-melting-point components as the dispersion is cooled. At temperatures greater than the gel to liquid-crystalline phase transition temperature of the higher-melting-point component, the two lipids probably are mixed in the fluid bilayer. The extent of phase separation that occurs during the cooling process is known to depend on the rate at which the dispersion is cooled and this is presumably a function of the aggregation of localized domains of gel phase lipid into extensive regions devoid of the fluid component.

Mixed-Lipid Classes

A number of studies of mixtures of phospholipids with the same acyl chains, but differing in polar head group, have been reported (Chapman *et al.*, 1973; Blume and Ackerman, 1974; Mendelsohn and Koch, 1980;

Arnold *et al.*, 1981; Silvius and Gagne, 1984a). The general conclusion from these studies is that phosphatidylserines and phosphatidylethanolamines are completely miscible, while mixtures of phosphatidylcholines with phosphatidylserines or phosphatidylethanolamines, in general, display solid-state immiscibility. Mixtures of lipids that differ in both chain length or saturation as well as polar group structure also tend to exhibit solid–solid phase separation; in certain instances, fluid–fluid phase separation can also be observed. As a general rule, the deviations from ideal mixing tend to be greater in the solid state of the mixture and at low concentrations of the higher-melting-point component.

There are a number of studies of binary mixtures of dipalmitoyl derivatives of glycerophosphorylcholine and glycerophosphorylethanolamine dispersed in excess water. The temperatures at which a transition from the gel phase to the liquid-crystalline phase of these two phospholipids occurs are different: 41°C for the choline phosphatide and 64°C for the ethanolamine phosphatide. Clearly separated solidus and liquidus phase boundaries can be detected using spectroscopic and other techniques, and the coexistence of phase-separated domains implies that there is nonideal mixing of the components (Lee, 1975; Shimshick and McConnell, 1973; Tenchov, 1985).

Surface-Charge Effects

It has been reported that mixtures of dimyristoyl derivatives of glycerophosphoric acid and phosphatidylcholine are completely miscible in the liquid-crystalline lamellar phase although there are more *gauche* conformers in the acidic lipid compared with the zwitterionic lipid (Kouaouci *et al.*, 1985). On addition of calcium ions, a rapid and extensive phase separation occurs (Graham *et al.*, 1985). Domains of phase-separated dimyristoylphosphatidylcholine adopt packing characteristics that are typical of the pure lipid, but a small proportion of phosphatidylcholine that is trapped within the phosphatidic-acid-rich domain (~ 20 mol %) displays quite different features. It appears that this component is packed more tightly to form a complex of phospholipid with calcium in a so-called cochleat phase. The latter phase is highly ordered and does not appear to undergo a cooperative thermotropic phase transition between 5 and 100°C. The clusters of trapped dimyristoylphosphatidylcholine do not melt cooperatively if the phase-separated mixture is heated, but become fluid as the dimyristoylglycerophosphoric acid that is not complexed with calcium melts at ~ 50°C.

Charge-shielding effects have also been shown to influence the phase behavior of mixtures of zwitterionic and acidic phospholipids in the presence of calcium ions. Calcium-induced phase separations between dielaidoyl and dimyristoyl-containing phosphatidylserines and similar derivatives of

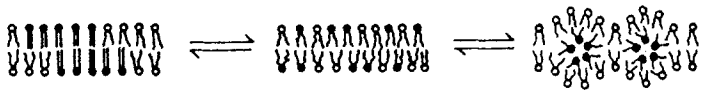
phosphatidylcholine in binary mixtures have been reported (Silvius and Gagne, 1984b). Thus, limited lateral separation into phases could be detected on addition of calcium to mixtures in the fluid phase that were otherwise completely miscible. At higher temperatures, mixtures that contained $< 40\%$ or $> 70\%$ phosphatidylserine in phosphatidylcholine did not show evidence of phase separation when they were at equilibrium in the presence of calcium, while two domains could be distinguished at intermediate concentrations. If the mixture was cooled, extensive immiscibility of solid phases occurred in this system.

The phase behavior of binary mixtures of unsaturated derivatives of phosphatidylcholines and phosphatidylethanolamines dispersed in aqueous media containing calcium have been investigated by X-ray diffraction methods (Tamura-Lis *et al.*, 1986). In particular, the extent of the observed phase-separation behavior that occurs in phospholipids that contain mixed acyl chains was examined and compared with that of mixtures of the two phospholipids in which the acyl chains were identical. In the mixed acyl-chain phospholipids, no phase separation was observed either in water or in medium that contained calcium in concentrations up to 1 M. It was concluded that at least two lipid species are generally necessary for calcium-induced phase separation to occur. Moreover, the separation is not solely a segregation of the two lipid species into separate structures, but a consequence of the free energy of mixing having two minima, corresponding to different separations of the components of the bilayer. Studies of mixtures containing saturated acyl chains have also been published (Cunningham *et al.*, 1988).

Lateral phase separations between phospholipids and sulfatides (Viani *et al.*, 1986) and gangliosides (Masserini and Freire, 1986) have been observed. Likewise, transbilayer phase separations in highly sonicated vesicle systems consisting of phospholipids and sphingomyelin have been reported and the effects have been ascribed to the precise chemical configuration of the head group of the respective molecules (Kumar and Gupta, 1985).

Bilayer and Nonbilayer Phase Separations

Since virtually all biomembranes are comprised of a mixture of polar lipids, some molecular species of which will form bilayer structures when dispersed in excess dilute salt solutions at the growth temperature and others that form hexagonal-II structures under these conditions, studies of the factors that govern the phase behavior of mixtures of these two lipid types have direct biological relevance. Figure 2 summarizes the general principles of phase mixing and separation that have been established on the basis of such studies.



Low temperature
Saturated fatty acid chains
High water activity
Low ionic strength
Neutral/high pH

High temperature
Unsaturated fatty acid chains
Low water activity
High ionic strength
Low pH

Fig. 2. Factors influencing the balance between formation of bilayer and nonbilayer structures in mixtures of bilayer-forming (○~) and hexagonal-II-forming (●~) polar lipids in aqueous systems.

One system that has been examined in some detail is that of the chloroplast membrane of higher plants (Quinn and Williams, 1983). Two galactolipids dominate the lipid composition of this membrane. Monogalactosyldiacylglycerol represents more than half, by weight, of the total polar lipids of the chloroplast and, of the remainder, digalactosyldiacylglycerol forms approximately half of the remaining polar lipids. Phase behavior of each of these lipids when dispersed in pure form has been well characterized by X-ray diffraction (Sen *et al.*, 1983), freeze-fracture electron microscopy (Quinn and Williams, 1983), differential scanning calorimetry, and monomolecular film techniques (Sen *et al.*, 1981).

The native lipids from chloroplasts, in general, contain polyunsaturated fatty acyl chains. The monogalactolipid forms a reverse hexagonal phase and the digalactosyl-containing lipid forms a bilayer phase in excess water at temperatures between -10 and 80°C. ^{2}H-NMR (nuclear magnetic resonance) and low-angle X-ray diffraction methods have been employed to examine the phase behavior of binary mixtures of monogalactosyl- and digalactosyl-containing lipids (Brentel *et al.*, 1985). The mixtures that were examined consisted of monogalactosyl- and digalactyosyldiacylglycerols, codispersed in water, in molar ratios of 1 : 2, 1.2 : 1, and 2 : 1. Figure 3 shows the phase properties over the temperature range 10–40°C and for water contents up to 14 mol ^2H_2O/mol lipid for the dispersion in which the molar ratio of the lipids was 2 : 1 as they generally occur in the chloroplast membrane. At low water content, the hexagonal-II phase dominates the system. As the concentration of water is increased, a reversed cubic liquid-crystalline phase gradually replaces the hexagonal-II phase and, in turn, is replaced by a liquid-crystalline lamellar phase in excess water. With increasing proportions of monogalactosyldiacylglycerol, the nonlamellar regions of the phase diagram tend to increase. The reversed cubic liquid-crystalline phase was characterized as a bicontinuous cubic phase structure.

Mixtures of these two chloroplast galactolipids have also been examined by freeze-fracture electron microscopy (Sen *et al.*, 1982a). Mixtures of

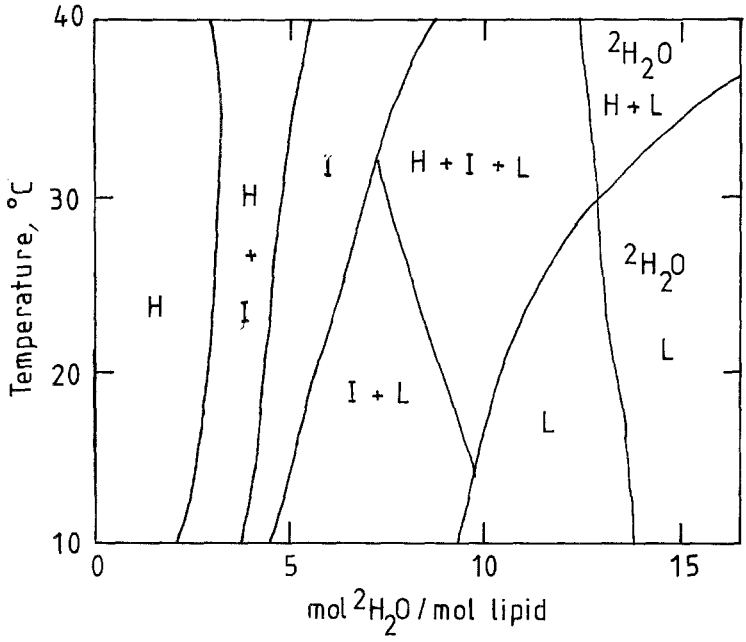


Fig. 3. A composition–temperature phase diagram of a 2 : 1 molar ratio mixture of monogalactosyl- and digalactosyldiacylglycerols dispersed in ^2H_2O: H, hexagonal-II; I, reversed cubic; and L, lamellar. All chain configurations are disordered liquid crystalline. Data from Brentel *et al.* (1985).

phospholipids that form bilayer and nonbilayer configurations have also been reported by Verkleij (1985). Direct evidence for phase separations between the bilayer and nonbilayer lipid components can be observed directly by the freeze-fracture technique, and the manifestation of phase separation varies from coexistence of bilayer and hexagonal-II domains, to tubular inverted micelles sandwiched within the lamellae of a bilayer, to inverted micellar structures within the bilayer matrix, and to other types of structure the precise configuration of which is uncertain. The extent of phase separation appears to depend on temperature, activity of water, and extent of unsaturation of the hydrocarbon chains of the nonbilayer lipid component (Sen *et al.*, 1982b; Gounaris *et al.*, 1983c)

Effect of Proteins on Lipid Phase Behavior

Intrinsic or integral proteins are operationally defined as those proteins requiring detergents to extract them from membrane lipids. As a consequence

of this direct interaction with the lipid hydrocarbon, intrinsic membrane proteins have a pronounced effect on the phase behavior of lipids present in the membrane matrix. Extrinsic proteins are also likely to affect membrane lipid phase behavior, one of the most obvious effects being interactions between cytochrome *c*, an extrinsic protein of the inner mitochondrial membrane, and cardiolipin, an important lipid component of this membrane (de Kruijff and Cullis, 1980). In this system, it was reported that addition of cytochrome *c* to cardiolipin model biomembranes specifically induces an hexagonal-II phase and possibly an inverted micellar structure of the phase-separated lipids in these systems.

Suppression of Nonbilayer Lipid Phases

Spectroscopic studies of reconstituted model membranes containing intrinsic membrane proteins have shown that the presence of protein causes a disordering of the lipids with which they are in contact compared with bulk lipids in the fluid phase (Rice and Oldfield, 1979). The explanation for this effect is provided by the fact that the hydrocarbon chains of the lipids must occupy voids created between and around the amino acid side chains of the intrinsic proteins in contact with the hydrophobic domain of the lipid. Indeed, because of the bulkiness of the hydrocarbon domain of nonbilayer-forming lipids, it could be argued that this feature is responsible for a preferential interaction between such lipids and the intrinsic membrane proteins. This is consistent with the observation that, when intrinsic membrane proteins are intercalated into exclusively bilayer-forming lipids, there is an increased leakage of ions across the membrane (van der Steen *et al.*, 1982), which can be prevented by including nonbilayer-forming lipid in the membrane–lipid mixture (van der Steen *et al.*, 1983). Accordingly, the nonbilayer lipid preferentially interacts with the intrinsic protein to seal the protein–lipid interface against leakage of solutes across the membrane.

The subbression of nonbilayer lipid phase separations by the incorporation of intrinsic membrane proteins into hexagonal-II-forming lipids, such as dioleoylphosphatidylethanolamine, has been observed (Tarashi *et al.*, 1980). This suppression occurs in molar ratios of polypeptide to lipid on the order of 1 : 200, suggesting that the effect of the protein extends well beyond the lipids in direct contact with the hydrophobic domain of the polypeptide chain. Figure 4 provides an illustrative example of the effect of protein aggregation within the plane of the membrane composed of nonbilayer lipids on the phase behavior of the lipid. It can be seen that, when sialoglycoprotein of the erythrocyte membrane is incorporated into bilayers of dioloylphosphatidylethanolamine, the polypeptide is randomly oriented within the structure and embedded within a bilayer lipid matrix. Aggregation of the protein

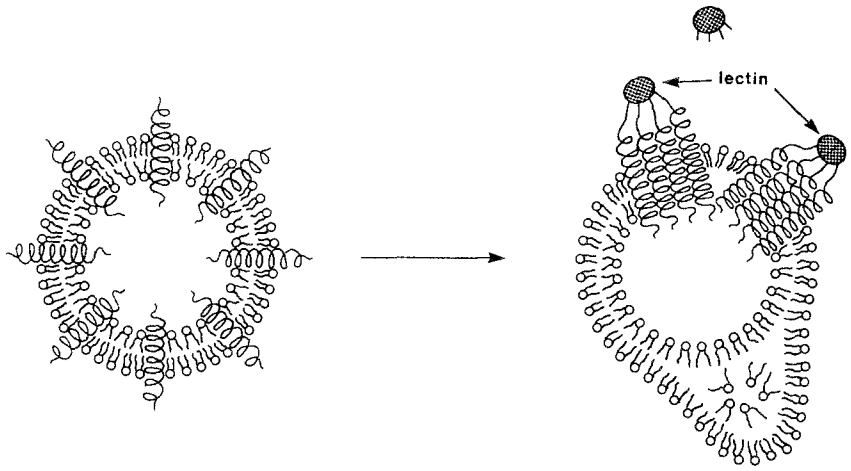


Fig. 4. The suspension of nonbilayer lipid phase separations by intrinsic membrane proteins and the creation of phase-separated nonbilayer lipid structures induced by lectin-mediated lateral aggregation of the proteins.

by addition of lectin causes phase separation of the polypeptide chains, leaving excess lipid that assumes nonbilayer configurations. The membranes in which protein aggregation occurs become leaky to solutes presumably because of the phase-separated lipid domains. Suppression of nonbilayer lipid phase separation can also be inferred in the case of retinol rod outer-segment disc membranes (de Grip *et al.*, 1979) and chloroplast thylakoid membranes (Quinn *et al.*, 1982; Gounaris *et al.*, 1983b) because there is no evidence for hexagonal II or other nonbilayer structures despite their presence in total polar lipid extracts of these biological membranes when dispersed in dilute salt solutions.

Exclusion from Gel Phase Bilayers

Gel phases are preferentially formed in lipids when cooled to low temperatures. They are defined by a sharp X-ray diffraction maxima centered around a spacing of 0.42 nm, implying a close association between lipid molecules mediated via van de Waal's interactions between the hydrocarbon chain substituents. Evidence for formation of gel phase lipid domains in biological membranes has been obtained from the appearance of sharp wide-angle X-ray diffraction spacings within membranes that show only a broad diffuse band at 0.46 nm characteristic of liquid-crystalline phase at the growth temperature (Tsukamoto *et al.*, 1980). The presence of gel phase bilayers can also be inferred from freeze-fracture electron microscopy of biological membranes equilibrated at low temperatures (Armond and

Staehelin, 1979; Brand *et al.*, 1979; Verwer *et al.*, 1979) where smooth regions devoid of membrane-associated particles are interpreted as gel phase lipid domains from which intrinsic membrane proteins are excluded.

An important question concerning the stability of membranes at low temperatures is the question of which lipids segregate into the gel phase domains and which of the membrane lipids accompany the intrinsic membrane proteins. This question has been addressed by examination of the phase transition behavior of intact membranes compared with dispersion of total polar lipid extracts of these membranes (Mannock *et al.*, 1985). Such studies reveal that the midpoints of endotherms observed in biological membranes are considerably higher than the corresponding midpoint temperatures of total polar lipid extracts of the same membranes. One interpretation of these data is that, in the biological membrane, cooling generates gel phase domains from higher-melting-point lipids, allowing the lower-melting-point lipids to phase separate together with the membrane proteins into a separate domain within the bilayer structure. These lipids forming a gel phase at lower temperatures are prevented from undergoing a phase transition by their interaction with the protein. Their transition from gel to liquid-crystalline phase is observed, however, in the total polar lipid extracts in which the phase transition is unperturbed by any interaction with the protein.

Evidence for Lipid Phase Separations in Biomembranes

We have seen in the above discussion that phase separations of membrane lipids occur in model membrane systems and upon cooling biomembranes below the gel to liquid-crystalline phase transition temperature of the higher-melting-point lipids present in the membrane. It is known that a number of environmental factors are capable of generating phase separations in biomembranes, particularly the phase separation of nonbilayer lipid components. Such phase separations, for example, have been reported in a variety of different membranes subjected to environmental stress. Thus, nonbilayer structures are formed in the membranes of heat-stressed chloroplasts (Gounaris *et al.*, 1983b) and chloroplast thylakoids treated with 6 M guanidine thiocyanate (Machold *et al.*, 1977). Similar structures have also been observed in mitochondria following the addition of high concentrations of Mn^{2+} (van Venetie and Verkleij, 1982) and dehydrated retinol rod outer-segment disc membranes (Carless and Costello, 1982; Gruner *et al.*, 1982) and sarcoplasmic reticulum (Crowe and Crowe, 1982).

Most of these observations have been made by using freeze-fracture electron microscopy and the extent to which the membrane lipids have been phase separated cannot be accurately assessed quantitatively. It may be

expected, however, that the formation of phase-separated hexagonal-II lipid structures, which can be easily recognized by the technique, would require fairly extensive lipid phase separation. The phase separation of nonbilayer lipids into inverted lipid micelles sandwiched between the lipid bilayers, on the other hand, may be a more common form of lipid rearrangement. Because of the similarity to the membrane-associated particles commonly exposed on the membrane fracture face, it is difficult to distinguish lipid phase separation from the presumed intrinsic membrane protein–lipid complexes. Nevertheless, the presence of such phase separations would be expected to have rather drastic consequences on membrane barrier properties and the presumed asymmetric distribution of membrane lipids on either side of the structure. The creation, moreover, of the inverted lipid micellar structures would be a dynamic process such that their formation would only be transient. Under physiological conditions, phase separation of inverted phases is believed to play a vital role in the controlled fusion between membranes (Siegel *et al.*, 1988; Ellens *et al.*, 1988), but the factors responsible for regulating this process are presently unknown.

It is generally assumed that a biochemical process, mediated by enzymes, responsive to the phase properties of the membrane structure as a whole operates to preserve the balance between bilayer-forming and nonbilayer-forming lipid molecular species as well as the ratio of lipid to protein, which, as we have seen, also plays a role in the overall phase stability. There is evidence that these lipase enzymes are regulated by the form of presentation of the substrate so that they have low activity against substrate in a membrane bilayer, but readily attack substrate in inverted isotropic (but non hexagonal-II) arrangement (Dawson *et al.*, 1984).

Phase Separations at Higher than Physiological Temperatures

With the knowledge obtained from studies of model systems, it is possible to predict the consequence of temperature stress on the arrangement of constituents within biological membranes. Since the phase behavior of lipids will be determined by the precise chemical composition within each morphologically distinct subcellular membrane, it is expected that the steps leading to destabilization and disruption will take place at different temperatures in different membranes. Also, the ability to restore the membrane to a functional state upon relief of a temperature stress will depend on the ability of the normal homeostatic mechanisms to cope with the altered structure. Whether the cell survives would presumably depend on whether solutes can be redistributed to achieve physiological conditions, a factor that requires a restoration of selective permeability and adequate capacity of active transport systems.

Exposure of cells to temperatures greater than the physiological range will have the effect of disordering any domains of gel phase bilayer lipid that may exist under physiological conditions. It is generally assumed that the proportion of lipid in this form is relatively low. More profound effects can be predicted on the nonbilayer-forming lipids. Those that are already above the lamellar to hexagonal-II transition temperature are presumably constrained into a bilayer configuration by interaction with other membrane components. With increasing temperature, the tendency to form nonbilayer arrangements will increase, which would tend to override the prevailing constraints. Other molecular species of lipid would also pass through the lamellar to nonlamellar phase transition temperature and these would combine with the nonbilayer-forming lipids already present in the membrane to form discrete nonbilayer lipid domains. It is uncertain whether membrane proteins would be associated with such lipid structures, but, on the basis of the appearance of hexagonal-II domains in freeze-fracture replicas, there is no compelling evidence that they do. Furthermore, it is not known to what extent bilayer-forming lipids are excluded from the nonbilayer structures, but, because of their dynamic nature, it may be anticipated that they are fairly rigorously excluded.

The consequence of phase separation of nonbilayer lipid domains on membrane properties and stability have been referred to above. Thus, the selective permeability barrier properties are likely to be destroyed by inverted micellar-type structures in the bilayer, but not necessarily by hexagonal-II arrangements of the lipids which are effectively isolated by enclosure within a monolayer of lipid molecules. Furthermore, loss in membrane stability will be manifest as membrane fusion leading to vesiculation of the membrane. Loss in membrane protein function could be due to thermal denaturation or structural changes consequent on a change in lipid environment.

Temperatures Lower than the Physiological Range

The effect of cooling on membranes would be to convert lipids tending to form nonbilayer structures into a bilayer configuration, in which case those interactions that depend on nonbilayer phase behavior would be disrupted. This transition might be associated with discontinuities in Arrhenius plots of many membrane-bound enzymes, suggesting that interaction of such enzymes with nonbilayer-forming lipids reduces the energy of activation of their catalytic functions. There is no evidence that this change in lipid phase behavior is irreversible, but it should be emphasized that irreversible changes cannot be identified easily using conventional

methods of membrane isolation which invariably involve operations performed at temperatures of ~0–4°C.

Initial cooling above 0°C may also transcend the liquid-crystalline to gel phase transition temperature of some, so-called, high-melting-point lipids, and these lipids would tend to phase separate into gel phase bilayer domains. It is noteworthy that nonbilayer-forming lipids, in general, have a higher lamellar gel to liquid-crystalline phase transition temperature than bilayer-forming lipids with equivalent acyl chain composition. This means that nonbilayer-forming lipids are likely to dominate the gel phase regions of higher-melting-point lipids in membranes, and the bilayer-forming lipids will tend to segregate with the membrane proteins. This has important implications when membranes are rewarmed to physiological temperatures, as the nonbilayer lipids will not be constrained to a bilayer configuration by the proteins and hence will remain phase separated in the form of nonlamellar structures (Quinn, 1985).

Cooling below ice-freezing temperatures introduces new factors affecting phase behavior. First, the activity of water is reduced, which, in general, causes an increase in the lamellar gel to liquid-crystalline phase transition temperature. Thus, the size of the gel phase domains would be expected to increase considerably under these conditions although the components may be somewhat immobilized due to increases in viscosity and associated decrease in lateral diffusion rates required to produce phase separations. Secondly, zone-refining effects would tend to increase solute concentrations at the membrane surface, and charge shielding of acidic membrane lipids would tend to increase the gel to liquid-crystalline phase transition temperature of these lipids. This would cause the acidic lipids to phase separate to the gel phase domains. The effect of acidic lipids on imposing bilayer configurations on nonbilayer-forming lipids is likely to be an important function of these lipids, which are usually small, but ubiquitous, components of membranes.

The action of cryopreservatives, based on these effects of temperature on phase behavior of membrane lipids, may be predicted as an effect on the polar groups of membrane lipids that tends to preserve the phase behavior close to those prevailing at physiological temperatures. Thus, it may be expected that cryoprotective agents would act to lower the lamellar to nonbilayer phase transition temperature and the lamellar gel to liquid-crystalline phase transition temperature of nonbilayer-forming lipids, but have relatively little effect on the gel to liquid-crystalline phase transition temperature of bilayer-forming lipids. Furthermore, by reducing the temperature at which ice crystallizes formation of lamellar gel phases comprised of nonbilayer lipids may be prevented and the action of acidic lipids can be manifested at lower temperatures.

Conclusions

There is a considerable body of information on the phase behavior of synthetic lipids and increasing knowledge of these properties in molecular species of lipids found in biological membranes. Many studies have been published on lipid mixtures with the aim of understanding the factors governing mixing and phase separation. This review has aimed to collate information obtained to date to predict the effects of temperature and other environmental stresses on the organization of components within biological membranes. Naturally, the conclusions that can be drawn at this stage are of a rather speculative nature, but their value lies in providing a model that can be subjected to rigorous experimental examination. A more complete picture will largely depend on an increased understanding of the detailed factors involved in interaction between the individual membrane lipids as well as their interaction with membrane proteins and other nonlipid components.

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