

LIPID POLYMORPHISM AND THE ROLES OF LIPIDS IN MEMBRANES

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The reasons for lipid diversity in membranes are not understood. Here we review evidence supporting the proposal that factors related to the polymorphic capabilities of lipids provide a rationale for lipid diversity. In particular, the ability of lipids to adopt different polymorphic phases appears to be related to a generalized shape property, where lipids with a cylindrical geometry preferentially adopt the bilayer phase whereas 'cone' shaped lipids adopt the hexagonal H_{II} phase. Lipid diversity may then be considered to satisfy three demands. The first is obviously a need for bilayer forming lipids to provide the basic permeability barrier, whereas the second concerns a need for non-bilayer lipids and associated structures for fusion and related membrane contact phenomena to proceed. A third, and less obvious demand satisfied by non-bilayer lipids concerns the ability of lipids of different shapes to modulate the order in the hydrocarbon region when constrained to a bilayer organization. These possibilities are summarized in a metamorphic mosaic model of membranes.

Keywords: lipid polymorphism; lipid diversity; order parameters; fusion

I. Introduction

An early review on lipid polymorphism [1] opened with the statement that 'the reasons for the great variety of lipids in membranes and the relations between lipid composition and membrane function pose major unsolved problems in membrane biology'. Since that time a large amount of effort has been expended to further characterize the polymorphic properties of lipids, the factors influencing these phase preferences and the molecular basis of polymorphism. Here we address the question as to whether this is leading to an increased understanding of the roles of lipids in membranes and factors regulating membrane lipid composition.

The body of this review is divided into six sections. First, we present an overview of the lipid diversity problem. Subsequently, factors influencing the polymorphic phase preferences of lipids are reviewed, which leads into a discussion of the molecular basis of polymorphism. This sets the stage for §V, which concerns the functional roles of non-bilayer structures in membranes with particular reference to membrane fusion. Given the fact that non-bilayer lipids in membranes are normally constrained to exist in a bilayer environment, it is of interest to also consider their influence on bilayer properties. This is discussed in §VI. Finally, we present a metamorphic mosaic model of membranes which attempts to synthesize the insight gained from studies of lipid polymorphism to achieve a new framework for understanding the functional roles of lipids in membranes.

II. Rationales for Lipid Diversity

The erythrocyte membrane contains well over 100 different molecular species of lipids [2], and similar observations can be made for most other plasma and organelle membranes. The reasons for this diversity and factors regulating changes in lipid composition in response to external variables are not understood. It is likely that the lipid composition of membranes reflects to a large extent some basic, physical property of the component lipids. Two such basic properties are first, the gel (frozen) and liquid crystalline (fluid) phase properties of bilayer lipids and, second, the ability of (liquid crystalline) lipids to adopt a variety of structures in addition to the bilayer phase (lipid polymorphism). The question we address here is whether factors related to the gel-liquid crystalline properties of lipids or factors related to the polymorphic phase properties of lipids give more basic insight into possible rationales for lipid diversity.

Satisfactory rationales based on such physical properties should ideally satisfy four criteria. First, lipids exhibiting the physical properties in question must obviously be present in membranes. Second, it must be possible to modulate these physical properties by biologically relevant variables to achieve appropriate regulation. Third, modulation of these physical properties must potentially account for a large proportion of observed properties of lipids in membranes. Finally, the rationale should allow definition of some well defined parameter that is a conserved quantity in membranes.

Rationales of lipid diversity based on fluidity arguments are based on observations that membrane lipids provide a fluid bilayer structure, and that acyl chain composition and headgroup type can strongly influence the gel (frozen) or liquid crystalline (fluid) nature of this bilayer (for review see Ref. 3). The presence of different lipids could then provide appropriate fluidity characteristics. Further, local domains of appropriate lipid composition could possibly modulate local fluidity and thereby protein function. Difficulties with this rationale have been summarized elsewhere [1,4,6] and include the fact that lipids preferring the gel state at physiological temperatures are not present in the large majority of biological membranes. Second, there is little evidence to support the contention that local domains of differing fluidity (as opposed to order, see below) can be achieved in membranes in response to stimuli such as ionic strength, pH, divalent cations or proteins. Third, the possibility of departures from bilayer structure, as are clearly required for membrane mediated processes such as fusion, is not addressed by the fluidity rationale. The fourth difficulty is fundamental, and concerns the loosely defined nature of the fluidity parameter. As emphasized elsewhere [4–6] this leads to considerable confusion. For example, it is commonly assumed that more saturated lipids or the presence of cholesterol makes membranes less 'fluid'. This is not necessarily the case. Strictly speaking, membrane fluidity is the reciprocal of the membrane viscosity, which in turn is inversely proportional to the rotational and lateral diffusion rates (D_R and D_l , respectively) of membrane components [7]. Thus, a linear relation between membrane fluidity and D_R and D_l would be expected, which is not observed.

Incorporation of cholesterol into phosphatidylcholine model membranes (at temperatures above the acyl chain gel to liquid crystalline transition temperature) has little or no influence on the lateral diffusion rates observed [8,9], and can actually increase the rotational diffusion rates [10]. The major influence of cholesterol is to increase the order in the hydrocarbon [11,12].

A rationale of lipid diversity based on the polymorphic properties of lipids appears to offer a more acceptable framework, as detailed in this review. First, an appreciable proportion (30 mol% or more) of membrane lipids either adopt non-bilayer structure in isolation or induce such structure in mixed lipid systems. Second, as pointed out in the next section, the polymorphic phase preferences of lipids can be modulated by a wide variety of physiologically relevant factors, leading to satisfying possibilities for regulation. Third, membrane mediated events requiring local departures from bilayer organization are clearly included in this framework. Finally, as indicated in §III and §VI, the polymorphic phase properties of lipids give insight into 'shape' properties of lipids which in turn offer insight into basic packing properties of such lipids in bilayers. Such packing properties, particularly as they relate to the order in the hydrocarbon, may represent conserved, regulated quantities in membranes.

III. Factors Modulating Lipid Polymorphism

The polymorphic phase preferences of lipids have been extensively reviewed elsewhere (see Refs. 4 and 13). Briefly, individual species of lipids found in membranes can adopt or induce either of the three structures (micellar, bilayer or hexagonal H_{II}) indicated in Fig. 1. Lipids adopting the micellar phase are minor components of membranes, however, a large proportion (see Table I) of membrane lipids either adopt the H_{II} phase on hydration or induce H_{II} structure in mixed lipid systems. The most notable examples are (eukaryotic) phosphatidylethanolamines (PEs) as well as cholesterol, which can induce H_{II} phase organization. Non-bilayer lipids such as PE can of course be stabilized in a bilayer structure by the presence of bilayer preferring lipids such as phosphatidylcholine (PC), phosphatidylserine (PS) or sphingomyelin (SPM). It is usually found that between 20 and 50 mol% of the bilayer preferring lipids are required to maintain a net bilayer organization when mixed with H_{II} preferring lipids such as PE.

The structural preferences of these pure and mixed lipid systems can be modulated by a wide variety of factors, including hydrocarbon unsaturation, temperature, headgroup size and ionization as well as hydration. As indicated elsewhere [1,4], this behaviour can be related to a generalized 'shape' property of lipids which stems from the work of Israelachvili and coworkers [14] who have examined the molecular properties of amphiphiles which form spherical and non-spherical micelles on dispersion in water. A basic packing property which has proved useful is a dimensionless shape parameter defined as $S = v/a_o l_c$. Here a_o is an 'optimum' area per molecule at the lipid-water interface, v is the volume per molecule and l_c is the length of the fully extended acyl chain. That the S parameter

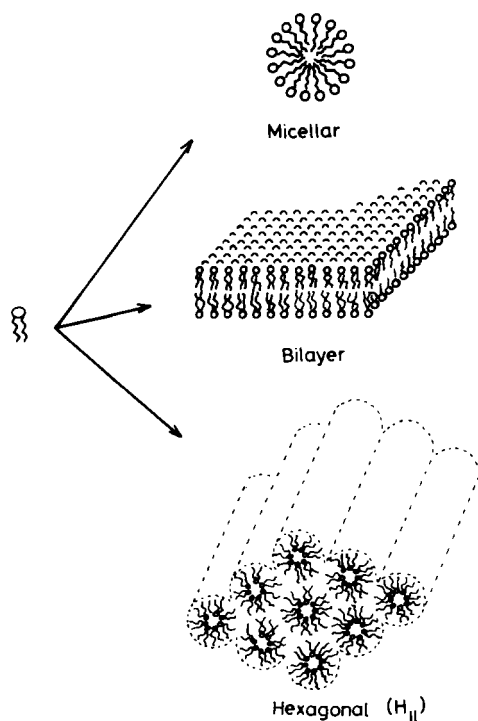


Fig. 1. Polymorphic phases available to lipids on hydration at concentrations above the critical micellar concentration. The aqueous pores of the hexagonal H_{II} phase have a diameter which lies in the range of 2 nm or larger.

TABLE I

PHASE PREFERENCES OF MEMBRANE LIPIDS FROM EUKARYOTES

Abbreviations: CL, cardiolipin; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PS, phosphatidylserine; SPM, sphingomyelin.

Bilayer	Hexagonal H_{II}
PC	
SPM	PE PS (pH < 3)
PG	
PI	
PA	PA (+ Ca^{2+}) PA (pH < 3)
CL	CL (+ Ca^{2+}) Cholesterol ^a Fatty acids

^aCholesterol and long-chain unsaturated fatty acids can induce the hexagonal (H_{II}) phase in some lipid mixtures.

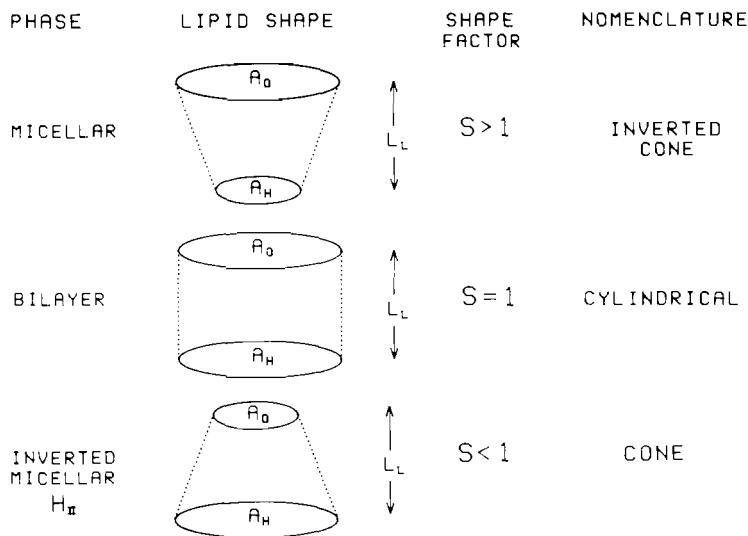


Fig. 2. Shape features exhibited by membrane lipids. a_o refers to the area subtended by the polar region, whereas a_h refers to the area at the hydrophobic interface. The shape factor $S = v/a_o l_c$, where v is the volume of the lipid molecule.

relates to a molecular shape property is easily realized from Fig. 2, which defines an additional parameter a_h as the cross-sectional area subtended at the hydrophobic end of the molecule. It is straightforward to show that for $a_o/a_h > 1$ that $S > 1$, for $a_o/a_h = 1$ that $S = 1$ and that when $a_o/a_h < 1$, $S < 1$. Using the language introduced previously [1], lipids which have a preferred shape corresponding to $S < 1$ are referred to as 'cone' shaped, whereas lipids where $S \approx 1$ are cylindrical and lipids where $S > 1$ have an 'inverted cone' shape. The relation between these shape properties and the geometry of the macroscopic lipid aggregate is clear, as lipids in a micellar phase must exhibit an inverted cone shape in order to satisfy geometric packing constraints, bilayer lipids must be roughly cylindrical, whereas lipids in the H_{II} (or inverted micellar) structures must have a net cone shape.

The shape concept of lipids might appear unduly simplistic. However, it has proven remarkably successful in providing a predictive framework for understanding the polymorphic phase preferences of lipid dispersions. Before detailing this success, it is important to realize that lipid shape is an inclusive phenomenological concept which lumps together a large variety of complex molecular forces. For example, a_o , the optimum cross-sectional area at the lipid-water interface, would be expected to be sensitive to the size of the lipid headgroup (large headgroup leading to large a_o values), the charge on the headgroup (electrostatic repulsion effects), the hydration of the headgroup (charged headgroups giving larger effective a_o values due to inter-headgroup (lower hydration, smaller a_o) and so on. Alternatively, a_h will be sensitive to factors which modulate the splay at the end of the hydrocarbon chains. Thus increased acyl chain unsaturation, increased temperature and increased

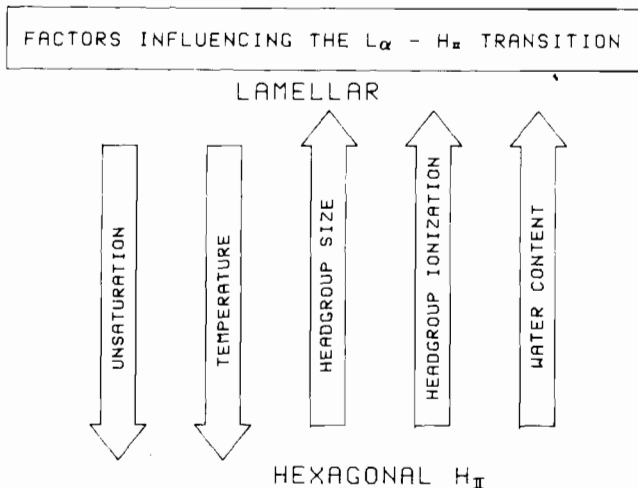


Fig. 3. Factors influencing the liquid crystalline bilayer (L_{α}) – hexagonal H_{II} phase preferences of membrane lipids. (Reproduced with permission from Ref. 46).

acyl chain length (for liquid crystalline lipids) would all be expected to increase the preferred value of a_h , leading to increased cone shape and possible H_{II} phase formation.

The features influencing lipid shape and their predicted influence on the L_{α} – H_{II} phase transition are summarized in Fig. 3. All of these factors modulate lipid polymorphism in the predicted manner. As reviewed elsewhere [1,4,13], for PE systems, increased acyl unsaturation leads to increased proclivity for H_{II} structure, and increased temperature induces bilayer to H_{II} transitions. The smaller headgroup of PE (as compared to PC) is consistent with H_{II} organization. The proclivity of unsaturated PEs for the bilayer phase at pH 9 [1,4,13] is consistent with deprotonation of the primary amine, resulting in a charged headgroup and thus a larger effective a_o . Similarly, protonation of the PS carboxyl and PA phosphate at lower pH values (pH < 4, see Refs. 15 and 16) leads to reduced inter-headgroup electrostatic repulsion, smaller a_o values and H_{II} structure. The ability of Ca^{2+} to trigger bilayer- H_{II} transitions in cardiolipin systems [17] as well as unsaturated PS-PE, phosphatidylglycerol-PE, phosphatidic acid-PE and phosphatidyl inositol-PE dispersions [4] can be rationalized on a similar basis, as can the ability of high salt concentrations to induce H_{II} organisation in PE-PS-cholesterol mixtures [18]. With regard to hydration, lower water content has been shown to reduce the T_{BH} in a variety of systems [19,20], most notably (egg) PC systems where H_{II} phase structure can be observed under conditions of low water content and high temperature [19]. Particularly graphic support for the shape concept comes from the prediction that appropriate mixtures of inverted cone shaped (micellar) lipids and cone shaped (H_{II} phase) lipids should assume a bilayer organization by virtue of shape complementarity arguments (see Fig. 4). Such behaviour is exhibited by detergent-PE systems [21] as well as lyso PC-cholesterol systems [22].

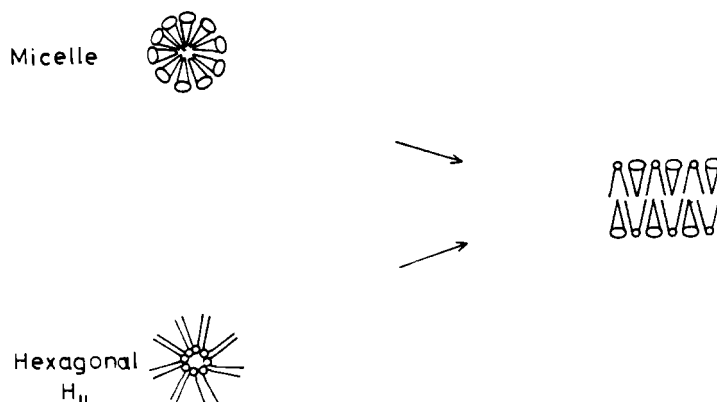


Fig. 4. Complementarity effects arising from the shape properties of lipids. Briefly, mixtures of cone shaped (H_{II} phase preferring) lipids and inverted cone shaped (micellar) lipids can exhibit bilayer structure (see text), a structure which is not adopted by either lipid species in isolation.

The shape concept structure offers excellent correlation between theory and experiment. However, it should be noted that the shape parameter S is not a well defined, measurable quantity (see §VI) as the lipid shape may vary according to the environment. The curvature parameter introduced by Gruner and coworkers [29,30] provides a quantitative measure of the tendency of a lipid to form non-bilayer structures, as indicated in § V.

IV. Mechanisms and Dynamics of Bilayer- H_{II} transitions

The shape hypothesis offers a rationale for why lipids adopt H_{II} or bilayer structures, however, it does not address the mechanisms whereby transitions between these structures proceed or the dynamics involved.

An initial observation relating to mechanisms of bilayer- H_{II} transitions indicated that the inverted cylinders characteristic of the H_{II} organization form parallel to the planes of closely apposed bilayers [23]. Concurrently, the presence of structures which are apparently intermediate between bilayer and H_{II} structure was observed by freeze-fracture techniques [24]. These 'lipidic particles' are commonly observed in mixtures of bilayer and H_{II} preferring lipids and can be largely interpreted as inter-bilayer inverted micelles joining two closely apposed bilayers (see Fig. 5b). As these particles can often be observed to line up and apparently coalesce into the inverted tubes characteristic of the H_{II} phase [25,25] there is strong circumstantial evidence that such inverted micelles are intermediaries in bilayer- H_{II} transitions.

These proposals are supported by the elegant thermodynamic analysis presented by Siegel [27]. Briefly, Siegel calculates the probability of formation of an inverted micellar intermediate (IMI) as the product of the probability of a local density fluctuation of lipids (providing sufficient lipid to form the IMI) and the probability that these inverted micelles form between bilayers that are both closely apposed

and have the necessary lipid density. Although the numerical estimates derived must be viewed with caution due to the assumptions made regarding intermediate structures and the activation energies required to generate these structures, the conclusions are of interest. This applies particularly to the calculated short lifetime of IMIs in bilayer- H_{II} transitions of PE systems, which would render detection difficult. This is consistent with experimental observations. In a subsequent analysis [28] Siegel proposes that inverted micellar structures represent the primary point defects in bilayer- H_{II} transitions, but that inverted cylinder formation may not result from fusion of interbilayer inverted micelles. Rather, he suggests that the rapid evolution of H_{II} structure relies on generation of line defects which form between bilayers as initially coalesced IMIs diffuse away from each other. Aggregation of these line defects can then result in H_{II} structure.

Theoretical work on forces driving bilayer- H_{II} transitions has been performed by Gruner and coworkers [29,30] who introduced the equilibrium curvature concept as a quantitative measure of the preferred phase structure. Briefly, this approach views each monolayer independently. Monolayers for which the preferred area at the lipid-water interface is less than that swept out by the acyl chains will tend to curl to form cylinders with an (equilibrium) radius of curvature R_0 . The 'bending' energy for a given radius R is then given (to least significant order in $1/R$) by $E = k_c (1/R - 1/R_0)^2/2$. The tendency of the monolayers to assume cylinders of radius R_0 is opposed by the need to fill the intercylinder spaces, which requires stretching of the acyl chains. Thus transition from bilayer to cylindrical geometry involves a competition between hydrocarbon stretching and monolayer curvature forces.

V. Functional Roles of Non-bilayer Structure

The ability of lipids to adopt non-bilayer organization naturally suggests that such structures may play regulated roles in membrane mediated phenomena. Lipid diversity could then be partly justified by the need for appropriate lipids to form such structures. Two points may be made. First, as indicated above, bilayer- H_{II} transitions occur as interbilayer events, and it is therefore likely that expressions of non-bilayer structure are primarily related to inter-membrane contact phenomena. Second, it is unlikely that the H_{II} phase per se is of biological utility. Local discrete departures from bilayer organization (e.g. inter-bilayer inverted micelles) would be more compatible with maintenance of membrane integrity.

Membrane fusion is an interbilayer event for which there is now strong evidence for the involvement of intermediate non-bilayer lipid structures. Conceptually, this proposal is appealing as irrespective of the particular factors initiating fusion, a local departure from lipid bilayer structure at the fusion interface must occur during some stage of the fusion event. As reviewed elsewhere [31] there is also very strong experimental evidence in model systems to support a role of non-bilayer structure in fusion. First, all factors known to promote H_{II} phase formation in multilamellar vesicle (MLV) dispersions will promote fusion between unilamellar vesicles with

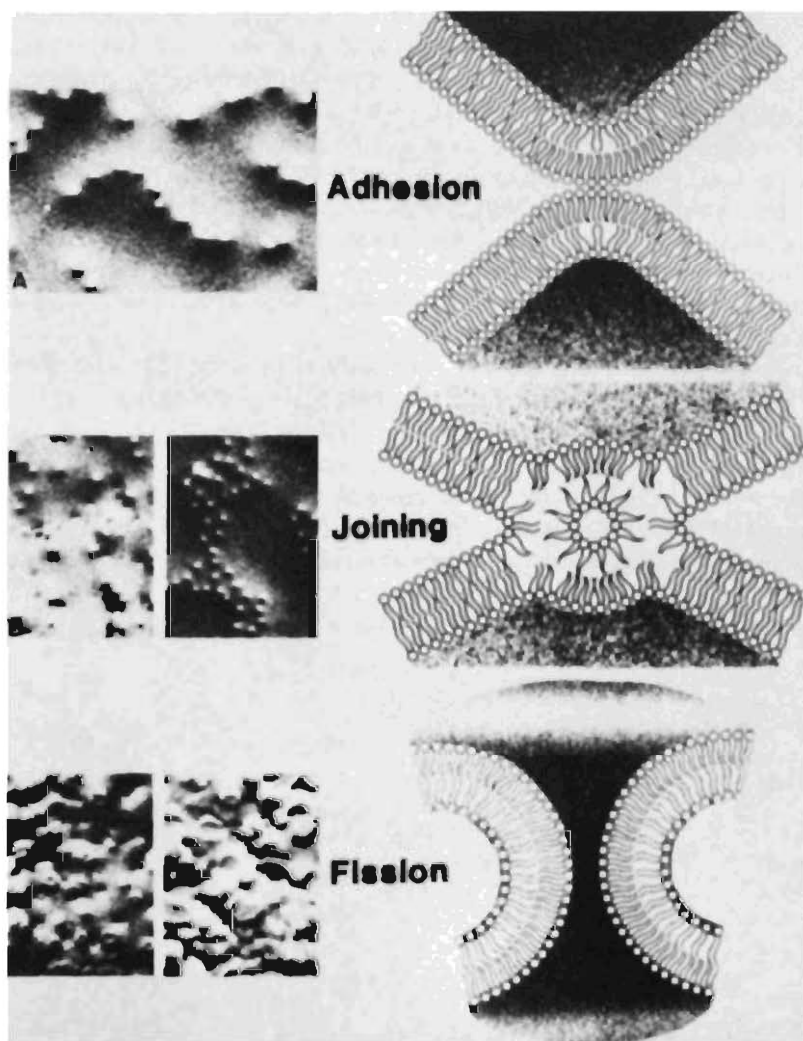


Fig. 5. Lipidic particle morphology and their interpretations. (Reproduced with permission from Ref. 26).

similar lipid composition (such fusion proceeds to eventually form the large H_{II} phase aggregates). Initiating factors include Ca^{2+} [32], low pH [32], higher temperatures [26], dehydration and 'fusogenic' lipids such as oleic acid and glycerol mono-oleate [33,34]. The fact that proteins can modulate lamellar- H_{II} transitions [35] suggests that fusogenic proteins may operate similarly. A second point is that observation of fusion in these model systems is often associated with the appearance of lipidic particles, which has led to the proposal [25] that inverted micelles are intermediate structures in fusion events as illustrated in Fig. 5. This proposal is contentious, however, as lipidic particles as detected by freeze-fracture appear to occur after the fusion event [36], and fusion can be observed in model systems where there is no clear H_{II} phase preferring component [37]. These difficulties are further discussed by Wilschut and Hoekstra, this volume [38].

Some of these problems have recently been analyzed by Siegel [39] who examined the possible roles of inverted micellar intermediates in membrane-membrane interactions and fusion from a theoretical viewpoint. He concludes that formation of inverted micelles (IMIs) is likely to be a primary contact event occurring between unilamellar membranes. Their formation can lead to three consequences. These include exchange of outer monolayer lipid between vesicles (see Fig. 6c), vesicle leakage or vesicle fusion. In particular, he considered temperature induced fusion

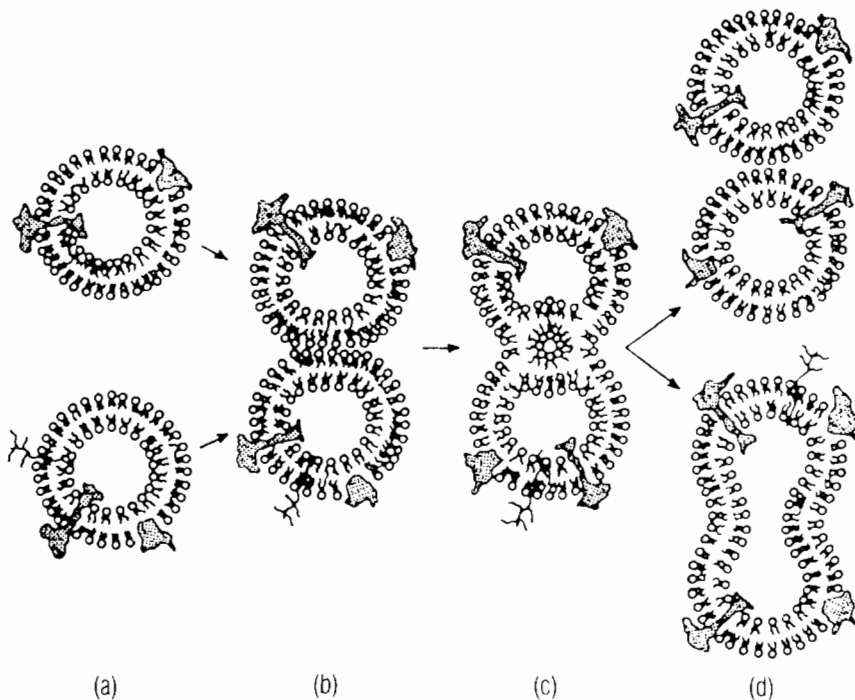


Fig. 6. A model of membrane fusion proceeding via an inverted micellar intermediate.

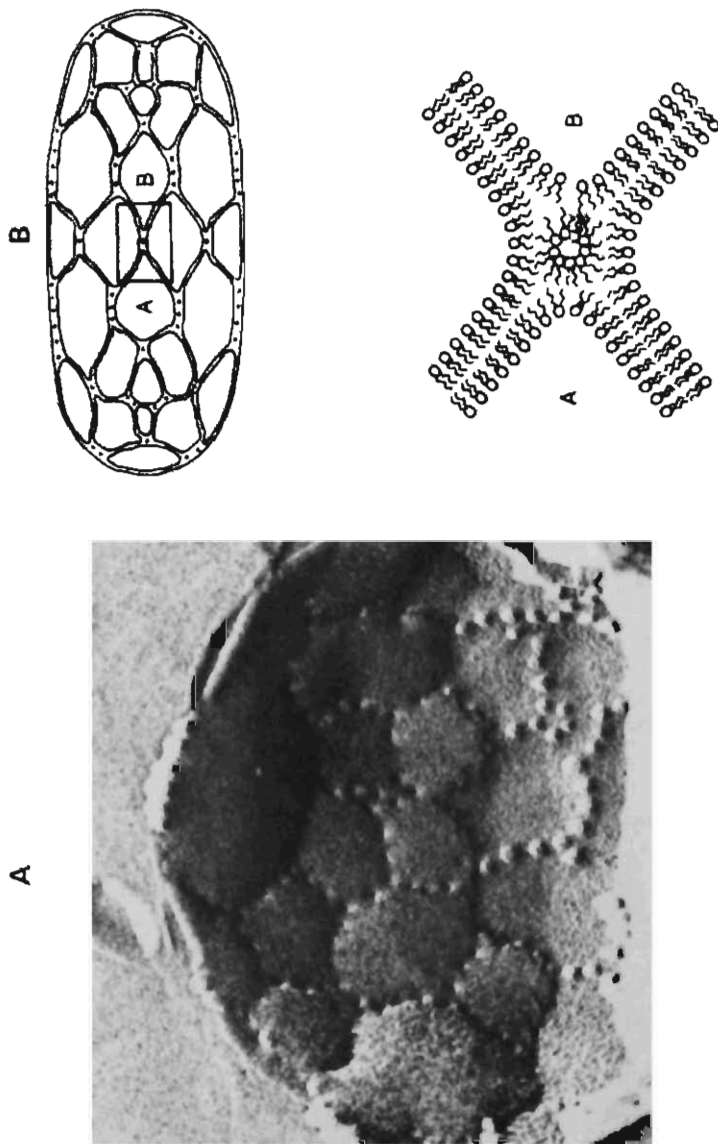


Fig. 7. (A) Freeze-fracture micrograph of aggregates produced on dialysis of sonicated vesicles (soya PE/egg PS, 5 : 1) against a pH = 3.0 buffer. (B) Honeycomb structure interpretation.

resulting from incubation in the region of the bilayer to H_{II} transition temperature T_{BH} . Briefly, at temperatures $T > T_{BH}$, vesicle rupture and leakage is most likely to occur as the inverted micelles coalesce to form tubes characteristic of H_{II} organization. Below T_{BH} , however, formation IMI can lead to fusion via formation of the fission intermediates of Fig. 6c. Siegel shows that the probability of the inverted micellar intermediate progressing to the 'fission' stage is likely to be very sensitive to the ratio $R = \bar{a}/a_o$ where \bar{a} is the cross-sectional area of the lipid headgroup in the lamellar phase and a_o is the corresponding quantity in the H_{II} phase. In particular, the probability of a completed fusion event is enhanced for smaller values of R (< 1.2). This may explain difficulties in observing fusion (as defined by mixing of internal compartments) in pure PE systems [39], which exhibit larger R values. Difficulties in observing inverted micellar intermediates may be attributed to the fact that only one would be required to initiate fusion, leading to detection problems.

Fusion obviously requires only a transitory intermediate event, however, work on model systems [4,23] indicates that the interbilayer attachments formed can be relatively stable in certain mixed lipid systems. This has led to the identification of 'honeycomb' structures such as indicated in Fig. 7. These and related observations suggest the possibility that membrane compartmentalization can occur within a continuous membrane structure. Such 'arrested fusion' allows unique opportunities for membrane morphology, and could be related to continuities between the inner and outer membrane of mitochondria and tight junction assemblies among others [26,40].

In summary, the results reviewed in this section strongly indicate a role for non-bilayer lipids and associated structures in membrane fusion and related phenomena. In unilamellar systems factors promoting inverted non-bilayer structures invariably promote fusion. Lipid-soluble fusogens which induce cell-cell fusion in vitro induce H_{II} organization in model systems. The lipid composition of biological membranes is compatible with such structural reorganization, and factors known to induce fusion in vivo can trigger non-bilayer organization in appropriate lipid systems. Finally, a firm theoretical basis appears possible. These general features contrast strongly with previous models of fusion events.

VI. Influence of Non-bilayer Lipids in a Bilayer Environment

The previous section emphasized roles of non-bilayer lipids as evidenced by their ability to assume inverted micellar or other non-bilayer structure. However, membrane lipids which form non-bilayer organization in isolation are usually constrained within a bilayer environment in biological membranes. Here we examine possible consequences for the properties of the bilayer.

The polymorphic phase preferences of membrane lipids discussed in §III provide certain insights. In particular, biological membranes are composed of mixtures of cylindrical (bilayer preferring) and cone shaped (H_{II} preferring) lipids. Assuming a planar bilayer, the distributed presence of cone shaped molecules will result in a smaller optimum hydrophilic membrane-water interface area as compared to the

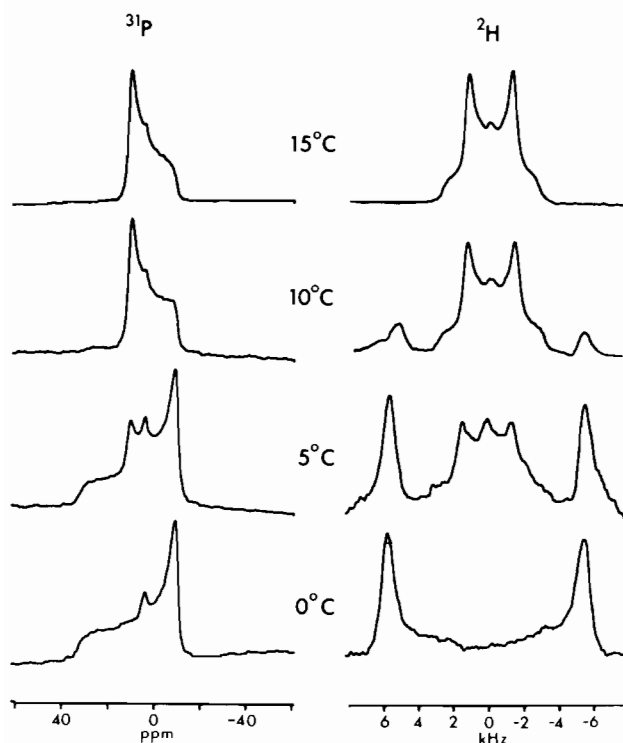


Fig. 8. ^{31}P - and ^2H -NMR spectra as a function of temperature of fully hydrated DOPE which is ^2H -labelled at the C_{11} position of the acyl chains ($[\text{C}_{11}\text{-}^2\text{H}_2]\text{DOPE}$). The ^{31}P -NMR spectra were obtained at 81.0 MHz in the presence of proton decoupling, whereas the ^2H -NMR spectra were obtained at 30.7 MHz. The change in the ^{31}P -NMR lineshape indicates the transition from lamellar to hexagonal H_{II} phase as the temperature is increased. (Reproduced with permission from Ref. 47).

optimum hydrophobic (inter-monolayer) interface. This will result in an increased surface free energy (and correspondingly increased surface tension) due to increased water contact with the hydrophobic region. In turn, this increased surface tension will lead to larger lateral compressions in the acyl chain region. Three predictions ensue. First, when a cone shaped lipid such as an unsaturated PE undergoes the bilayer- H_{II} transition the compression experienced in the bilayer phase should be significantly relieved and decreased order in the hydrocarbon should result. This can be tested employing ^2H -labelled species of PE and ^2H -NMR. In particular, in the absence of increased hydrocarbon disorder, a reduction in the quadrupolar splitting (ΔQ) by a factor of two or more should be observed for a ^2H -labelled lipid undergoing a bilayer- H_{II} transition. As shown in Fig. 8, ΔQ for $[\text{C}_{11}\text{-}^2\text{H}_2]\text{DOPE}$ actually decreases by a factor of 4.2. This markedly decreased hydrocarbon order in the H_{II} phase is in accord with previous results [41].

A second prediction is that the acyl chain order in a PC bilayer should be increased

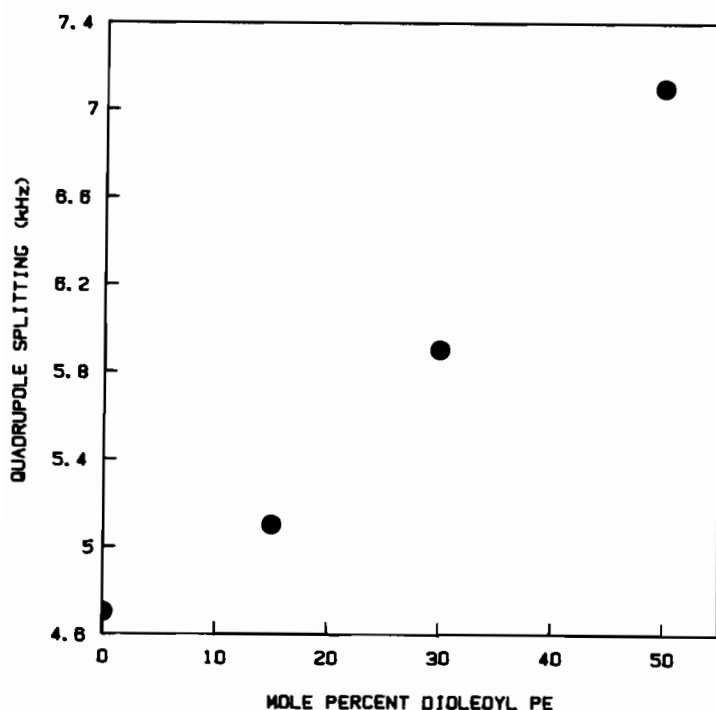


Fig. 9. Influence of increasing amounts of DOPC on the ^2H -NMR quadrupolar splitting (ΔQ) observed for $[11,11 - ^2\text{H}_2]\text{DOPC}$. The 30.7 MHz ^2H -NMR spectra were obtained at 30°C .

on titrating in an unsaturated (cone shaped) PE, assuming bilayer structure is maintained. That this is the case is shown in Fig. 9 where the quadrupolar splitting for ^2H -labelled DOPC (labelled at the 11 position of the acyl chain) is monitored as in the presence of increasing amounts of (unlabelled) DOPE. A dramatic increase in ΔQ from 4.7 to 7.1 kHz (at 30°C) is observed as the DOPE content is increased from 0 to 50 mol%, indicating significantly increased order in the hydrocarbon at the high PE contents. The lateral compression leading to increased hydrocarbon order parameters leads to the third prediction, which is that the permeability of the bilayer should be decreased at higher PE contents. This is by analogy to the effects of cholesterol which increases hydrocarbon order [11] and decreases membrane permeability in liquid crystalline PC bilayers [42]. The literature in this area is not extensive, however it has been noted that the K^+ permeability of egg PC liposomes decreases as the egg PE content is increased from 0 to 50 mol% [43].

The proposal that the presence of cone shaped molecules in bilayers leads to increased surface free energies, higher surface tensions, lateral compression of acyl chains and decreased permeabilities has many interesting ramifications. For example,

it may be proposed that the presence of progressively more unsaturated PE in membranes renders the membrane progressively less permeable as long as bilayer structure is maintained. Modulation of lipid shape factors by binding of proteins or cations among other factors could lead to fluctuations in hydrocarbon order with associated regulatory possibilities. A fundamental attraction of this proposal is that it offers the possibility of relating lipid shape properties (as expressed by their polymorphic phase preferences in isolation) to the less ambiguous and measurable hydrocarbon order parameter in bilayers. In particular, in this work we have placed heavy emphasis on the lipid shape concept as it provides a most attractive predictive framework for understanding lipid polymorphism. Indeed, this success has led other authors [5] to suggest that an appropriate distribution of lipid shapes is a conserved quantity in membranes. However, the shape parameter remains relatively qualitative and does suffer certain ambiguities when applied to bilayer systems. For example, when a cone-shaped (H_{II} preferring) lipid is constrained in a bilayer environment, lateral compression of the acyl chains will result in a more cylindrically shaped molecule. Thus to talk of lipid shape as a conserved quantity is problematic. To express lipid shape properties in terms of their effects on hydrocarbon packing and order in membranes is more appealing.

In summary, the presence of H_{II} phase preferring PE in bilayer membranes increases the order in the hydrocarbon. It is expected that other H_{II} phase preferring lipids will exhibit similar effects resulting from increased surface tensions. This suggests that a major reason for lipid diversity in membranes which is expressed by their polymorphic capabilities may be directly related to establishing and regulating the order profile in the hydrocarbon. The concept that the order in the hydrocarbon is a basic, regulated and measurable quantity in membranes has, of course, been emphasized extensively elsewhere [44]. What has not been emphasized previously is that the physical properties (e.g. shapes) of lipids which dictate their polymorphic phase preferences can directly influence the hydrocarbon order when these lipids are constrained to a bilayer environment. A satisfying unification appears possible.

VII. The Metamorphic Mosaic Model of Membranes

In closing, it should be clear that studies of lipid polymorphism have led to fundamental new insight into the physical properties and functional roles of lipids in membranes. Lipid diversity can potentially be rationalized on the basis of requirements for non-lamellar as well as lamellar structures, and the presence of lipids with a variety of optimum shapes in bilayer membranes may establish appropriate hydrocarbon order and permeability properties. Furthermore, the fact that optimum shape properties can be modulated by a large variety of biologically relevant variables offers satisfying regulatory potential, both for generating non-bilayer structures and modulating hydrocarbon order. We believe this insight to be sufficiently profound to warrant a revised version of the Singer-Nicolson [45] fluid mosaic model. We have termed this model (see Fig. 10) the metamorphic mosaic model [23].

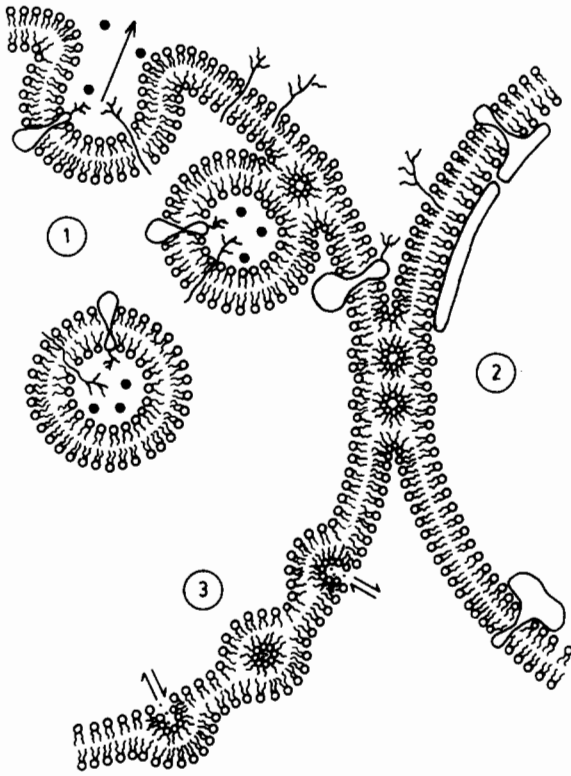


Fig. 10. A metamorphic mosaic model of biological membranes illustrating various aspects of membrane morphology and function potentially involving non-bilayer lipid structure. In region (1) an exocytotic fusion event proceeding via an intermediate inverted micellar or inverted cylinder organization is shown, whereas in region (2), inverted cylinder structure allows a stable semi-fused interbilayer connection to exist, possibly corresponding to tight junctions. In region (3) enhanced permeability to divalent cations is proposed to proceed via an inverted micellar intermediate, which may correspond to the ability of phosphatidic acid to act as a Ca^{2+} ionophore. Redrawn from Ref. 23.

Briefly, the term 'metamorphic' is introduced to acknowledge the ability of lipids to adopt a variety of structures (inverted micelles, cylinders and the H_{II} phase) in addition to the bilayer. The meaning of the term 'mosaic', which was originally coined to describe the mosaic nature of the protein intercalating into and through the bilayer, is extended to include the mosaic of lipids of different intrinsic shapes which make up the lipid bilayer.

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