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Non-bilayer lipid structures in model and biological membranes

B. de Kruijff, P. R. Cullis and A. J. Verkleij

Several lipid components of biological membranes preferentially adopt the hexagonal (H_{II}) phase on hydration. Moreover, mixed systems containing bilayer and H_{II} phase lipids often exhibit novel 'lipidic particle' structures which appear to arise from lipids in intrabilayer inverted micellar configurations. These non-bilayer structures may play dynamic roles in membrane mediated processes as diverse as membrane fusion and transbilayer 'flip-flop'.

Current models of biological membranes postulate a bilayer organization of the lipid component. This provides a semipermeable barrier between extra- and intracellular environments as well as a structural matrix with which proteins, fulfilling specialized functions, may be associated.

Two fundamental problems in membrane biology suggest that the resulting view of lipids as inert 'building blocks' of biomembranes is incomplete. First, a given biological membrane contains a vast variety of lipids. It is difficult to explain this diversity in terms of the requirements of a structural bilayer; requirements which could be satisfied by a single lipid species such as phosphatidylcholine. These difficulties are certainly compounded by the fact that certain membrane lipids form non-bilayer structures in isolation. Further, a second problem is posed by membrane-mediated processes such as fusion and 'flip-flop'. Clearly, neither of these phenomena can be reconciled with lipids remaining in a bilayer arrangement at all stages. It is against this background that we explore here the possibility that 'non-bilayer' lipids and associated structures are directly involved in such processes.

Lipid polymorphism

The ability of membrane lipids to adopt a variety of phases on hydration has been recognized for some time [1,2]. Given the remarkable implications inherent in the observation that non-bilayer lipids are present in membranes, it is surprising that these polymorphic capabilities have been largely ignored. Such a situation may be partially attributed to complexities of

X-ray analysis, as well as to a general preoccupation with bilayer systems, which provide a more obvious model for biomembranes.

Some of the technical difficulties in resolving these non-bilayer phases in more detail have been overcome by the application of ³¹P NMR [3,6] and freeze-fracture [7,8] techniques. In the case of ³¹P NMR, the lineshape obtained from model (liposomal) systems as well as biological membranes is a sensitive indicator of the phase(s) adopted by endogenous phospholipids. As shown in Fig. 1, an asymmetrical lineshape with a low-field shoulder is characteristic of bilayer phospholipids, whereas hexagonal (H_{II}) phase phospholipids exhibit a narrower lineshape with reversed asymmetry. Alternatively, lipids in structures that allow isotropic motion give rise to narrow symmetric signals, a situation experienced by some of the phospholipids in the sample of Fig. 1. Freeze-fracture, on the other hand is unique in giving detailed structural information about discrete, differentiated loci in the sample.

The application of ³¹P NMR, X-ray and freeze-fracturing techniques to fully hydrated preparations of individual membrane lipids have shown that as a rule either the hexagonal (H_{II}) phase or the bilayer phase is preferred. Important examples of H_{II} phase lipids include unsaturated phosphatidylethanolamines [3–5,9–11], monoglucosyldiglyceride [12,13], as well as phosphatidic acid [14] and cardiolipin [8,15] in the presence of Ca²⁺. In addition, lipids such as cholesterol [4,5,11] and unsaturated fatty acid [6] can induce the formation of hexagonal phases from bilayer systems.

An important feature of these phase preferences is that transitions between the H_{II} and the bilayer phase can occur. Factors triggering such transitions include temperature variation and changes in divalent cation concentration. For example, in the case of unsaturated phosphatidylethanolamines, bilayer → H_{II} transitions occur as the temperature is increased

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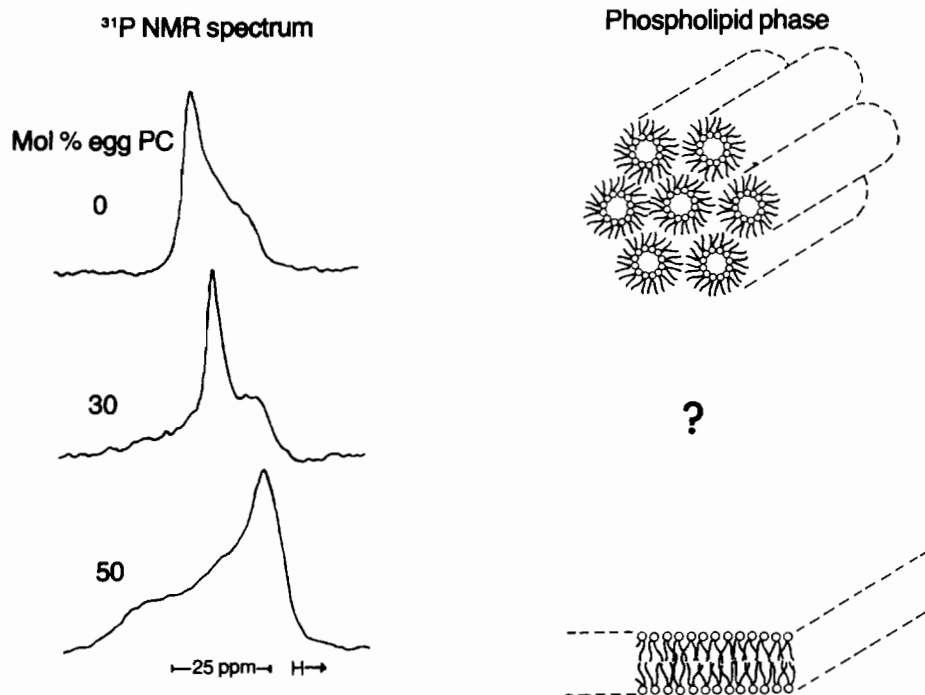


Fig. 1. ^{31}P NMR spectra of different phospholipid phases. Hydrated soya phosphatidylethanolamine adopts the hexagonal H_{II} phase at 30°C . In the presence of 50 mol % of egg phosphatidylcholine only the bilayer phase is observed. At intermediate (30%) phosphatidylcholine concentrations an isotropic component appears in the spectrum.

through a characteristic value (T_{BH}) which is sensitive to the fatty acid composition [3–5,11]. It is intriguing to note the low heat content of this transition (<1 Kcal/mol) [7,11] implies a low energy barrier between these very different macroscopic structures.

Phases allowing isotropic motion

As biological membranes contain a mixture of bilayer and non-bilayer lipids, the behaviour of mixed lipid systems consisting of H_{II} phase and bilayer phase phospholipids is of particular interest. Fig. 1 demonstrates the ability of (equimolar) egg phosphatidylcholine to stabilize bilayer structure in the presence of soya phosphatidylethanolamine, which adopts the H_{II} configuration in isolation (Fig. 1). Unexpected behaviour is revealed at intermediate phosphatidylcholine concentrations, however. As previously mentioned, a narrow spectral component is observed, indicating isotropic motional averaging. This spectral feature is not restricted to phosphatidylethanolamine–phosphatidylcholine dispersions, but also is exhibited by a wide variety of systems composed of mixtures of bilayer phase lipid and hexagonal (H_{II}) phase lipids. These include phosphatidylcholine–cardiolipin– Ca^{2+} systems, phosphatidylcholine–monoglycosyldiglyceride mixtures as well as phosphatidylcholine – phosphatidylethanolamine–cholesterol systems [13].

The potential importance of this ‘intermediary’ lipid structure to biomembrane structure and function is indicated by the

fact that hydrated preparations of the total lipids extracted from the rod outer segment [16] and *E. coli* [17] membranes show major isotropic averaging spectral components. The organization of this intermediary phase is therefore of particular interest. However, a variety of lipid structures available to phospholipids can give rise to narrow symmetric ^{31}P NMR spectra characteristic of isotropic averaging, including small bilayer vesicles, micelles, inverted micelles or other lipid phases such as cubic or rhombic. It is therefore fortunate that freeze-fracture techniques give less equivocal indications of the structures present.

Lipid particles

Freeze-fracture studies of lipid systems exhibiting ‘isotropic’ NMR spectra often reveal morphological features not previously encountered in pure lipid systems [13,16–19]. In particular, as shown in Fig. 2, numerous small (60–120 Å diameter) particles and pits are observed on the fracture faces. We have suggested that these particles (which are probably complementary to the pits) reflect inverted micelles sandwiched in between the monolayers of the lipid bilayer (see Fig. 2). In some cases these particles are organized in ‘strings’ (see for instance the MGDG/PC mixture) and the bilayers appear to bulge greatly. These systems possibly consist of a honeycomb network of vesicles in which the lipidic particles are located on the nexus of intersecting bilayers.

It is intriguing to see that these lipidic

particles are of a size comparable to that of the so-called intramembrane particles in freeze-fracture studies of biological membranes. These latter particles are interpreted to be the reflection of transmembrane proteins, the clearest evidence coming from labelling and recombination studies of the erythrocyte membranes. The intramembrane particles differ from the lipidic particles, in having no complementary pits on the opposite fracture face. This could possibly be explained by plastic deformation of proteins during fracturing [20]. Therefore, the presence of particles and complementary pits on the fracture face of a membrane could indicate, but not prove, the presence of lipidic particles (possibly inverted micelles).

Biological membranes

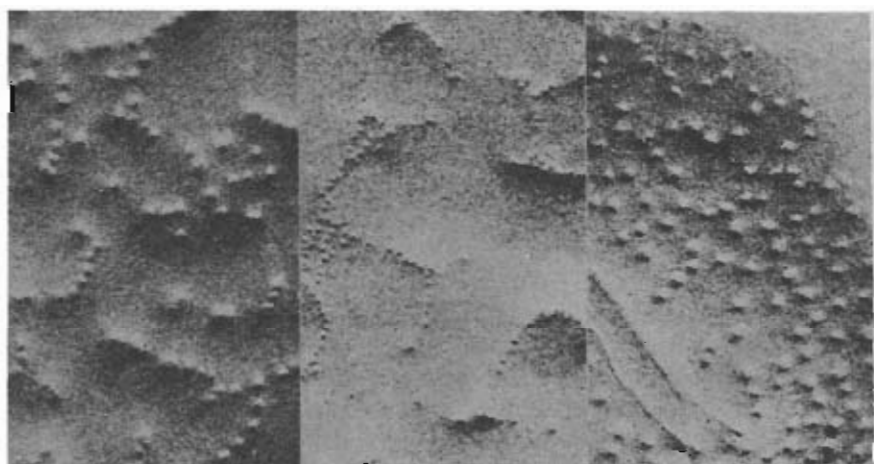
The data discussed so far clearly establish that lipids in biological membranes cannot, *a priori*, be assumed to be in a bilayer configuration. However, it is equally clear that the other preferred structure of membrane lipids, the hexagonal (H_{II}) phase, would not be expected to play a major role in biomembranes as it is difficult to imagine that such structures could maintain the permeability barrier vital to cellular integrity. The remaining possibility, that ‘intermediate’ structures such as lipidic particles can be present is somewhat more credible.

The human red blood cell membrane, perhaps the best characterized of all biological membranes, exhibits bilayer structure [21] as indicated by the ^{31}P NMR spectrum of Fig. 3 (a). This organization is very stable as bilayer structure is maintained even after extensive treatments with phospholipases and proteolytic enzymes. This stability could be related to the ability of the erythrocyte to withstand high mechanical stresses during passage through narrow capillaries, or it may reflect the relatively low metabolic activity associated with an insulating plasma membrane. In any event, this behaviour contrasts strongly with behaviour observed for metabolically active membranes such as the endoplasmic reticulum, as indicated by the ^{31}P NMR spectrum of Fig. 3 (b). This narrow lineshape, which is characteristic of rat, beef and rabbit liver microsomes [22,23] demonstrates isotropic motion for a large fraction of the endogenous phospholipids at 37°C . At lower (non-physiological) temperatures, the ^{31}P NMR spectrum increasingly adopts the bilayer shape, consistent with behaviour observed for model systems. It is interesting to note that the structure giving rise to isotropic motion is directly or indirectly induced by the pres-

ence of membrane protein (possibly cytochrome *P*-450 [23] as the extracted, hydrated lipids exhibit bilayer structure at 37°C. In summary, while at present freeze-fracture evidence for the presence of non-bilayer phases in the microsomal membrane is ambiguous, the ³¹P NMR data would be consistent with the existence of transient non-bilayer lipid structures, possibly inverted micelles at 37°C.

Functional roles of non-bilayer lipid

As indicated in the introduction, two processes which are difficult to reconcile with purely bilayer structures are fusion and transbilayer 'flip-flop'. Experimental results and theoretical considerations are, however, clearly consistent with a role of non-bilayer structures in fusion processes, and, to a lesser extent, 'flip-flop' phenomena. For example, chemical fusogens which fuse erythrocytes also induce the hexagonal (H_{II}) phase in the erythrocyte membrane [6]. Alternatively, in model phosphatidylcholine-cardiolipin systems, Ca²⁺ (which induces the H_{II} phase for cardiolipin) induces vesicle fusion accompanied by the appearance of lipidic particles at the fusion interface [19]. Similarly, phospholipid 'flip-flop' could proceed via formation of transitory inverted micellar lipid configurations, which would be consistent with results obtained for the endoplasmic reticulum where phospholipid 'flip-flop' is fast at 37°C [24,25].



CARD/PC MGDG/PC PE/PC/CHOL

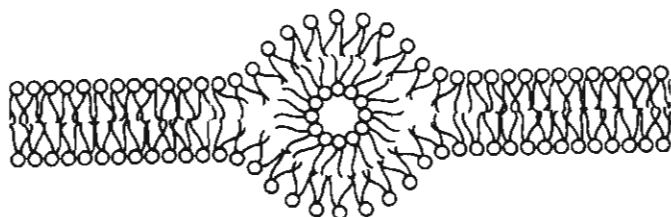


Fig. 2. Freeze-fracture micrographs of lipidic particles in cardiolipin-(Ca²⁺)-phosphatidylcholine [card/PC], monoglucosyldiglyceride-phosphatidylcholine (MGDC-PC), and phosphatidylethanolamine-phosphatidylcholine-cholesterol (PE/PC/CHOL) mixtures. As a model for these particles the inverted micelle is proposed.

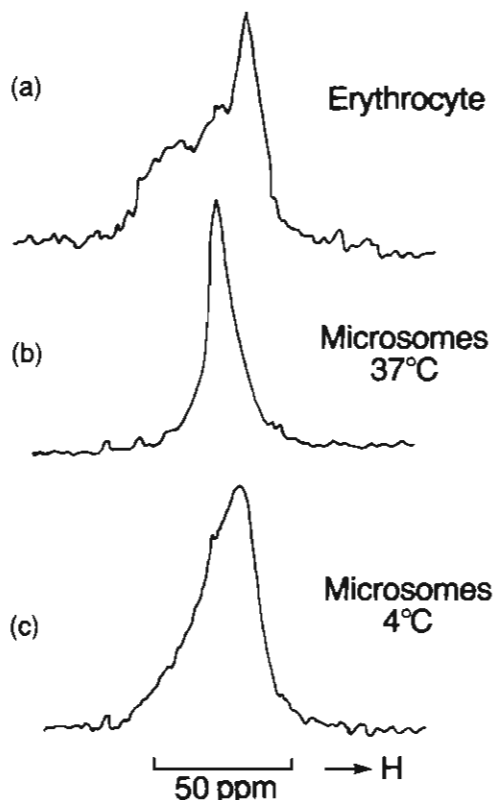


Fig. 3. ³¹P NMR spectra of various biological membranes.

Future research

We hope that this brief review indicates the potential importance of this exciting research area, which appears to be leading to a new appreciation of the possible roles of lipids in biomembranes. It must be emphasized, however, that this work is still

in a very early stage and answers to several basic problems remain obscure. In particular, evidence for the presence of non-bilayer lipid structures in biological membranes is ambiguous. Further, our understanding of the phase behaviour of membrane lipids remains still at a primitive level, as does our understanding of possible relationships between the functional abilities of a biomembrane and the bilayer or non-bilayer preferences of the endogenous lipids.

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