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Small-scale structure in fluid cholesterol–lipid bilayers

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ABSTRACT

Cholesterol is the single most abundant molecule in animal plasma membranes, in the range of 20–30 mol%, where it is known to modulate the lipid-bilayer component of the membrane and lead to increased mechanical stability, lower permeability, larger thickness, and a distinct lateral organization. The phase equilibria of membranes with cholesterol and the associated large- and small-scale structure have turned out to be a particularly elusive problem. With the proposal that lipid domains and so-called 'rafts', characterized by high local levels of cholesterol in a liquid-ordered phase, are important for a wide range of cellular functions, an understanding and a quantitative assessment of the nature of these cholesterol-induced structures and their types of ordering have become urgent. Recent progress in neutron diffraction studies of lipid–cholesterol model membranes has now revealed details of the lateral ordering, and combined with earlier molecular model studies a picture emerges of the membrane as a locally structured liquid with small ordered 'domains' of a highly dynamic nature.

1. Introduction

Higher sterols are universally present in large amounts (20–30 mol%) in the plasma membranes of all eukaryotic cells: cholesterol in animals, ergosterol in yeast and fungi, phytosterols in plants, and, e.g., fucosterol in algae [1]. The early work by Nobel Laureate Konrad Bloch demonstrated that the biochemical pathway to cholesterol required the presence of molecular oxygen in order to remove a bottleneck between the precursor lanosterol and the streamlining of higher sterols [2]. By noting the coincidence in time of the occurrence of eukaryotes and the increase in oxygen pressure in the Earth's atmosphere, the evolutional path from prokaryotes to eukaryotes could be translated into the molecular 'evolution of a small molecule,' cholesterol [3].

The issue is then what is so special about cholesterol (or other higher sterols) [4]. It is known that some cellular functions have specific requirements for cholesterol in small amounts [5,6], but why do all plasma membranes of eukaryotes universally have very large amounts of cholesterol, in the order of 20–30 mol%, making cholesterol, by any comparison, the single most abundant molecule in plasma membranes? Another question is, what is it cholesterol can do which its biochemical precursor lanosterol cannot? In his work, Konrad Bloch pointed out the effectiveness of cholesterol to order fluid lipid bilayers, providing for low passive permeability and increased mechanical strength [2,7]. These effects have been demonstrated by a large body of literature for a wide range of model membranes [8].

In most cases biological function requires membranes to be in a fluid (or liquid) state in the sense that it has to allow for rapid diffusion of the lipid molecules and the imbedded membrane proteins. Membranes in liquid phases are generally thinner and less mechanically stable than their solid counterparts, which on the other hand do not allow for rapid diffusion. The cholesterol molecule tends to stabilize the membrane by ordering lipid acyl chains in liquid membranes because of its rigid steroid structure and an α-face that is molecularly smooth in contrast to lanosterol with three bumpy methyl groups decorating its α-face [9]. In solid membranes, cholesterol has the opposite effect due to packing constraints [10]. So considering ordering, cholesterol molecules prefer solid phases, while when it comes to packing, cholesterol prefers liquid phases. This duality in cholesterol's affinity for liquid and solid lipid bilayer phases was cast into a thermodynamic phase diagram in 1987 by the proposal that cholesterol in large amounts induces a new mesophase, the liquid-ordered (l_o) phase, that dominates the phase diagram in the range of physiological relevant concentrations of cholesterol, as shown in Fig. 1 [11]. By the introduction of this new mesophase, the traditional gel (solid) phase was renamed as the solid-ordered phase and the traditional liquid-crystalline (fluid) phase was renamed as the liquid-disordered phase. It is noteworthy that the phase diagram has an upper critical point. In the neighborhood of this point, critical fluctuations will prevail and the correlation length describing local dynamic domain formation can get very large [12,13]. The experimental observation of the liquid-ordered phase in

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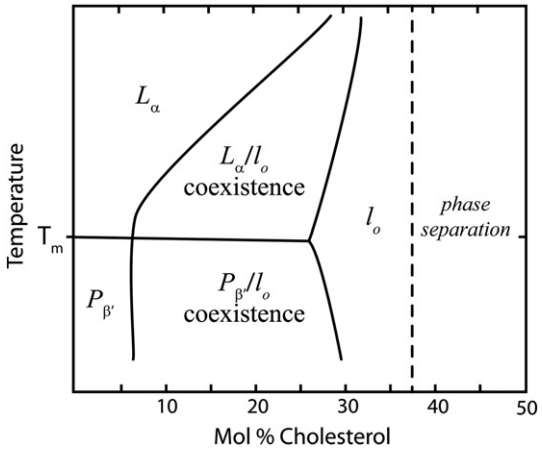

Fig. 1. Generic phase diagram of phospholipid/cholesterol bilayers, such as DMPC/cholesterol and DPPC/cholesterol, displaying a solid-ordered phase ($P_{\beta'}$), a liquid-disordered phase (L_α), a liquid-ordered phase (l_o), as well as the regions of two-phase coexistence [100]. At high levels of cholesterol, cholesterol separates out as a pure crystalline phase [10].

a cholesterol lipid binary mixture was for the first time reported in a landmark paper by Vist and Davis [14]. The quantitative determination of binary lipid–cholesterol phase diagrams has turned out to be very elusive, and only a few cases have been resolved and the same holds true for phase diagrams of binary mixtures of lipids with other higher sterols, with lipid–ergosterol being a notorious exception [15]. In contrast, a number of ternary phase diagrams have been determined for systems involving cholesterol and two different lipid species, usually a lipid with a high melting point (e.g., long-chain saturated phospholipids) and a lipid with a low melting point (e.g., sphingolipids or unsaturated phospholipids) [16]. The liquid-ordered phase is a unique phase in membranology and no other molecules but the higher sterols have been shown to be able to stabilize this type of phase. In this sense cholesterol (and other higher sterols) is something special [4].

The liquid-ordered phase called the attention of the life science community (for a recent review, see [17]) in 1997 when Simons and Ikonen [18] proposed the existence of so-called rafts in biological membranes based on some ideas from an earlier work on lipid sorting in epithelial cells [19]. The early literature in the field of rafts was marred by the use of a definition of rafts as a certain detergent-resistant fraction of the membranes, and as pointed out by Heerklotz [20], the detergent is likely to introduce artifacts. The rafts were supposed to be small, molecularly organized units providing for some local structure in fluid biological membranes and hence furnishing platforms for specific biological functions [18,21–29]. These rafts were supposed to be enriched in cholesterol making them more ordered and thicker and hence appropriate anchoring places for certain acylated and/or hydrophobically-matched integral membrane proteins. The high levels of cholesterol in these rafts led to the proposal that rafts are local manifestations of the liquid-ordered phase, although in most cases the nature of the lipid ordering and the phase state were not established neither in cells nor in most model membrane studies.

Whereas the concept of dynamic local structures in disordered liquid systems, even in one-phase regions, is well known to physical scientists by terms such as structured fluids and microemulsions, the life-science community generally interpreted rafts as some kind of super-particles floating around in an otherwise structure-less liquid membrane. However, early work in the physical chemistry of lipid bilayers pointed to the possibility of dynamic heterogeneity [30–33] in thermodynamic one-phase regions of, e.g., binary systems. The source of dynamic heterogeneity is cooperative molecular interactions and thermal fluctuations that lead to density and/or compositional fluctuations in space and time. These fluctuations are best characterized by correlation functions or appropriate structure factors. From these functions a length scale can be extracted which is a measure of the range over which the liquid is correlated. This motional coherence was observed experimentally in the fluid phase of lipid membranes [34] using quasi-elastic neutron scattering. The length scale associated with this coherence length is probably the most relevant and quantitative description of a domain or a 'raft' in a liquid membrane.

While in most cases it is relatively easy to design experiments to determine large-scale domain structure and global phase separation in coexistence regions [29], it turned out to be much more involved to perform an experiment to measure the dynamic local structure and the associated correlation length in liquids where transient, nanometer-sized local structures are predicted. This has been particularly challenging for cholesterol-containing lipid membranes in the liquid-ordered phase, which is supposed to harbor the putative rafts. Lack of information on the nature of a possible small-scale structure even in simple liquid membranes with large amounts of cholesterol has had a significant impact on the progress in the scientific underpinning of the growing field of rafts in biological membranes [24,25,29,35–37]. In Section 2 we shall briefly review some of the existing experimental data pointing to the existence of lipid domains and small-scale structures in model membranes. Some insight into small-scale structure and dynamics of lipid–cholesterol membranes in the liquid-ordered phase has also been provided by model and computer simulation studies which we will review in Section 4.

Recently a break-through has been reported in the use of neutron diffraction to quantitatively assess small-scale structure in binary lipid–cholesterol membranes in the liquid-ordered phase [38,39]. The experiments revealed the existence of highly ordered lipid domains in equilibrium with a disordered matrix. The lipids in these domains were found to be in a liquid-ordered state and thought to be saturated with cholesterol molecules.

In the present short topical review we shall describe this break-through in Section 3 and in Section 5 discuss the results together with results from model studies described in Section 4 and thereby provide a status of small-scale ordered domains in membranes with high levels of cholesterol. We believe that the combined results from these experiments and the model simulations provide a much improved and solid underpinning for future work on rafts in biological membranes.

2. Lateral structure in fluid model membranes with cholesterol

It is interesting to note that membranes and the question of their internal molecular organization resemble a 'colloid-inside-a-colloid' problem. The lipid bilayer is an about 5 nanometer thin, self-assembled molecular aggregate, bound together by weak physical (colloidal) forces that are strongly renormalized by temperature. The in-plane organization of this free-standing soft and liquid interface in water is itself a question of physical forces that, influenced by the cooperative behavior and many-bodyness of the assembly, can stabilize dynamic and fluctuating local structures on varying length scales that also are subject to thermal renormalization [1,40].

The generic cholesterol–lipid binary phase diagram in Fig. 1 contains a region with phase separation between the two liquid phases, the liquid-disordered and the liquid-ordered phase. It has proved very difficult to measure the phase boundaries of this region and the nature of the coexistence region is still in dispute. Whereas there is a large body of literature on phase separation and lipid domains in a wide range of lipid membrane systems with cholesterol in cases where solid (gel, solid-ordered) phases are involved [16,41–45], and there is some work on systems involving coexistence between liquid-disordered (fluid, liquid-crystalline) and liquid-ordered phases [15,46–49], only a few studies (to be reviewed in Sections 3 and 4) address the question of local structure in lipid mixtures with cholesterol being in a single-phase liquid-ordered phase.