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New and Notable

A New Phase in Phosphatidylcholines

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"The study of lipid bilayers has been and will continue to be greatly enriched by investigating how the structure and thermodynamic properties vary as the lipids are varied, including both naturally occurring and specifically synthesized lipids" (Sun et al., 1996). Although early biophysical investigations focused on systematic exploration of thermodynamic and structural properties of symmetric diacyl phospholipids, such investigations have become passé. There are many reasons for this: a desire to "get on" with the study of more complex systems, particularly lipid-protein systems; belief that the success of theoretical models in reproducing some aspects of lipid behavior reflects a detailed understanding of lipid thermodynamics; and the fact that "real" membranes are very complex structures in comparison to the simple "model" membranes. The study presented by Snyder et al. (1996) in this issue of the *Journal*, which evolved from an investigation of a more complex system (Mendelsohn et al., 1995), superbly illustrates both the limitations of our understanding of the forces that govern the assembly of lipid bilayers and some of the tools that can be used to improve this understanding.

In addition to the liquid crystal phase, disaturated phosphatidylcholines can form "ripple phases," tilted-chain gel phases, and "subgel phases" (referred to by Snyder et al. (1996) as G_r, G_d, and G_s, respectively). All three phases have been reasonably well characterized for a variety of lipids, and the transitions among these phases have been studied in detail. The recent investigations on the C(22) and C(24) lipids have now identified a new, "high-density" orthorhombic phase (G_o)(Sun et al., 1996; Snyder et al., 1996). Unlike the subgel phase, this new phase is fully hydrated. Although the new phase may be found even in di-C(18) phosphatidylcholine (PC), it is formed much more readily in C(22) and C(24) PCs. The stability of the new phase increases markedly with increasing chain length. The chains of lipids existing in this new ordered orthorhombic configuration are essentially perpendicular to the plane of the lipid bilayer, in contrast to those of the usual gel phase, which are tilted. Although there is little difference in chain conformational disorder between the new phase and the usual hydrated gel phase, the new phase demonstrates considerably greater orientational order of the lipid acyl chains.

The discovery of a new phase that can form even in such an extensively investigated lipid as C(18) PC pointedly highlights the limitations in our knowledge of even simple lipid systems. The most obvious limitation is in our understanding of the factors governing chain tilt in phosphatidylcholines. Mismatch between headgroup and acyl-chain region size has long been accepted as the explanation for chain tilt in gel phase phosphatidylcholines. Yet C(24) PC forms a tightly packed, apparently noninterdigitated, bilayer with acyl chains perpendicular to the bilayer normal. Why? We do not yet have a detailed mathematical model of the lipid bilayer that allows us to quantitatively account for this phenomenon. In retrospect, this should be no surprise. Our understanding of the details of factors promoting acyl-chain interdigitation, in either mixed-chain or symmetric-chain phospholipids is similarly weak. The qualitative understanding that we have developed to explain these phenomena is sufficiently strong to rationalize the headgroup packing necessary for a high-density orthorhombic phase such as that described by Sun et al. (1996) and Snyder et al. (1996), but not sufficiently strong to predict it.

Studies of how lipid "structure and thermodynamic properties vary as the lipids are varied" are important tools for improving these gaps in our knowledge. Two equally important tools that have also fallen out of favor are solvent replacement and theoretical investigations.

Studies on dehydrated lipids (Bush et al., 1980) and on lipids in which water has been replaced with other solvents (McDaniel et al., 1983; O'Leary and Levin, 1984) can significantly enhance our understanding of water-specific and solvation-specific effects on headgroup/acyl-chain relationships. Replacement of water with other solvents can induce changes in bilayer structure similar to those induced by changes in acyl-chain length, low-temperature incubation, or bilayer perturbants such as ethanol. Nevertheless, variation of solvent is even less frequently employed than variation of lipid structure in the attempt to fully understand lipid thermodynamics.

Theoretical investigations, such as those carried out by Nagle (1973, 1975, 1976) and others in the early 1970s, assisted greatly in understanding the intra- and intermolecular interactions giving rise to the gel-to-liquid crystalline phase transition. These studies also significantly increased the understanding of the forces governing bilayer assembly in general. Although considerable recent attention has gone to modeling simple bilayers using complex tools such as molecular dynamics simulations and to modeling complex multicomponent bilayers, lit

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tle attention has been given to developing improved models of tightly packed lipid assemblies. The recent works by Sun et al. and Snyder et al. suggest that further work on such "simple" systems is clearly justified.

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Revisiting the Ionic Selectivity of Na^+ Channels

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Voltage-dependent Na^+ channels are responsible for the generation and propagation of the action potential in many types of excitable cells. Na^+ channels discriminate effectively between the two most common monovalent cations in biological systems, Na^+ and K^+, preferring Na^+ by a ratio of 30:1 (Favre et al., 1996). Rapid membrane depolarization is effected by Na^+ channels through simple positive feedback: the channels open in response to small membrane depolarizations, and as a result the membrane potential further depolarizes approaching the Na^+ reversal potential. This selectivity for Na^+ over K^+ is vital to Na^+ channel function.

The question of how channels differentiate among the cations present in physiological solutions is not trivial. Na^+ channels are faced with a dual problem. First, they must distinguish between Na^+ and K^+, two ions with identical charge and only slightly different size. Second, they must allow Na^+ permeation while excluding Ca^{2+}, an ion of similar size but different charge. In this issue of *Biophysical Journal*, Favre et al. (1996) address in detail the molecular mechanisms underlying these selectivities, and suggest that in Na^+ channels, a single residue serves a "sentry" role to prevent Ca^{2+} permeation and is also crucial in the discrimination between Na^+ and K^+.

At the molecular level, Na^+ channels belong to the S4 family of voltage-gated cation channels (Noda et al., 1984). These ion channels are formed by four homologous domains arranged around a central conduction pore. Although in some cation channels the four domains are actually identical subunits, in Na^+ and Ca^{2+} channels the domains are homologous repeats within a single protein. The ion conduction pathway is lined by the four P-region segments located between the fifth and sixth putative transmembrane regions in each domain (MacKinnon, 1995). In both Na^+ and Ca^{2+} channels, the P-regions contain numerous charged residues. The pseudosymmetry of these channels dictates that when a charge appears at the same position in all four domains, the pore will contain a "ring" of charge. The initial illustration of the importance of these rings came from work localizing the tetrodotoxin and saxitoxin binding sites (Noda et al., 1989; Terlau et al., 1991). Neutralization of charged residues in either of two rings had large effects on both toxin affinity and single-channel conductance.

Subsequently, Heinemann et al. (1992) showed that one of these rings is critical in determining the differences in permeation properties between Na^+ and Ca^{2+} channels. As expected of residues that influence ionic selectivity, the residues in this ring differ between channels: in Na^+ channels, they are DEKA (one residue contributed each by domains I-IV), whereas in Ca^{2+} channels they are EEEE. Heinemann et al. mutated the Na^+ channel to make it similar to the Ca^{2+} channel by altering the residues in domains III and IV. The resulting mutant Na^+ channels displayed conduction properties that resembled those of Ca^{2+} channels. The DEEA and DEKE channels were permeable to both Na^+ and K^+. Furthermore, the double mutant DEEE channel was blocked by low Ca^{2+} concentrations, whereas it conducted current at high Ca^{2+} concentrations. Although the Heinemann et al. work stimulated substantial research into the question of permeation in Ca^{2+} channels (Yang et al., 1993; Ellinor et al., 1995), the problem of selectivity in Na^+ channels has only been addressed recently (Chiamvimonvat et al., 1996; Favre et al., 1996).

In the current study, Favre et al. use site-directed mutagenesis to examine the contribution of each charged DEKA residue in terms of its effect on two different permeation characteristics: exclusion of Ca^{2+} and discrimination between monovalent cations. They expressed the mutant channels in *Xenopus* oocytes and assessed the relative permeabilities to different ions by measuring the change in reversal potential with exchanges of the bath solution.

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