Lipids

Bilayers and nonbilayers: structure, forces and protein crystallization

Editorial overview

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Abbreviations

cryoEM cryoelectron microscopy
PC phosphatidylcholine
PE phosphatidylethanolamine

Much of modern structural biology is focused, quite rightly, on proteins and nucleic acids. The methodologies underlying macromolecular crystallography, multidimensional NMR, solid-state NMR, cryoelectron microscopy (cryoEM) and so on have developed and, in some areas at least, converged to permit structural descriptions of increasingly complex biological assemblies. Nowadays, high-resolution X-ray crystallographic studies of medium-sized proteins seem limited primarily by the ability to develop an efficient expression system and by the ability to grow ‘diffraction-quality’ crystals; the combination of old and new phasing methods, coupled with access to X-ray synchrotron sources, seems to take care of the rest. Isotope-labeling NMR methods can provide solution structures of proteins, with the molecular weight ‘limit’ increasing yearly, and the issue of structure in crystal versus structure in solution can be addressed. The tremendous successes in the area of protein structure determination have led some investigators to more ambitious structural projects. Functional macromolecular assemblies of increasing complexity are now being addressed and the technique of cryoEM has taken its place in the arsenal of structural methods. In favorable cases, the resolution limit of cryoEM and electron crystallography is beginning to overlap with that of high-resolution X-ray methods. Furthermore, cryoEM and X-ray crystallography are showing signs of symbiosis; cryoEM for initial X-ray phasing, mapping high-resolution crystal structures onto lower resolution cryoEM pictures and so on. And so it goes for increasingly complex structures of proteins, with those residues in the α-helical assembly having now become joined by the multistrand β-barrel alternative.

Somewhat lost in this impressive forward progress are structural studies of the lipid compartment of membranes, in which the above-mentioned functional membrane proteins are arranged. The importance of phospholipids to membrane structure was emphasized some 75 years ago, but only with the combination of facile methods for the isolation of pure phospholipids from natural sources and the development of new synthetic methods for different phospholipid classes could systematic and detailed studies of their structure and properties begin. In Chapman’s laboratory, the properties of different phospholipid classes were defined and important parameters, such as bilayer thickness, lipid hydration, hydrocarbon chain melting and bilayer dynamics, were defined using a combination of X-ray diffraction, scanning calorimetry and spectroscopic methods [8,9]. My own efforts to produce three-dimensional crystals began in Chapman’s laboratory and ultimately, but not without many difficulties, the first crystal structure of a membrane phospholipid, phosphatidylethanolamine (PE), was described in 1974 [10]. The crystal structure of phosphatidylethanolamine (PC) was described by Pearson and Pascher in 1979 [11], but despite these initial successes, the list of solved crystal structures of membrane phospholipids remains quite short (see the review by Pascher et al. [12]). At least, we do have high-resolution structural pictures of the phospholipid conformation and bilayer organization for the crystalline state. Meanwhile, Luzzati’s laboratory, having described the polymorphism of soaps and detergents, turned its attention to biological lipids. Using low-angle X-ray diffraction methods, Luzzati and his colleagues were able to show that, for hydrated phospholipids, a plethora of structures exists for both ‘solid-chain’ gels and the more biologically relevant ‘melted-chain’ liquid-crystal regime [13]. For the latter, lamellar (bilayer), hexagonal, cubic and so on phases were identified and their structural parameters defined. For the
important bilayer phase, methods were developed to determine parameters such as the bilayer repeat distance, lipid and water bilayer thicknesses, and lipid area at the lipid–water interface. Corresponding structural parameters for the other phases could be determined. These nonbilayer phases, particularly the hexagonal and cubic varieties, have been implicated as possible intermediates during membrane–membrane fusion events accompanying cellular secretion and endocytic processes, in virus–membrane interactions and so on.

For phospholipid bilayers, a significant advance was made when methods to phase the bilayer X-ray reflections were developed by Wilkins and colleagues [14]. This allowed the electron density distribution across the bilayer to be derived. At the resolution available, four main zones could, in some cases, be identified: the electron-rich polar head-group region; the methylene chain region; the electron-deficient region corresponding to the chain terminal methyl groups at the bilayer center; and the water region between adjacent bilayers. Again, parameters corresponding to these different structural zones could be calculated, as could the important lipid area parameter. Several investigators have continued this structural approach (see examples by McIntosh [15], Nagle [16], White [17], Shipley [18] and their colleagues) and, through the application of some new theoretical approaches, structural descriptions of both the bilayer gel and the bilayer liquid-crystal phases of membrane phospholipids at ‘medium resolution’ are available.

The reviews in this section by Nagle and Tristram-Nagle, McIntosh and Caffrey provide up-to-date accounts of developments in three areas: the structural description of bilayers; the use of X-ray diffraction methods to quantitate the forces operating between adjacent bilayers; and an attempt to understand the mechanism by which a nonbilayer cubic lipid phase can promote crystallization of membrane proteins (and perhaps other proteins). The review by Nagle and Tristram-Nagle (pp 474–480) focuses on the use of low-angle diffraction methods to derive accurate electron density maps (in this case, the one-dimensional bilayer profile) for phospholipid (mainly PC) bilayers in their different bilayer states. For the bilayer gel phase, quite good agreement is observed for the bilayer parameters (lipid thickness, chain tilt, lipid area, etc.) derived from the different studies. As the authors point out, however, for the perhaps more relevant chain-melted liquid-crystalline Lα phase, significant differences are reported, particularly for the molecular area of the phospholipid, whether derived by different X-ray diffraction methods or by other techniques (NMR, neutron diffraction, etc.). For the analysis of the electron density profiles, fitting approaches to locate the layer location of various component structural groups (headgroup, phosphate, –CH2– groups, –CH3 groups, water) have been used primarily by Nagle, White and their colleagues [16,17]. Finally, Nagle has made a significant contribution in considering the different types of bilayer structural disorder; for example, the disorder responsible for bilayer undulations is discussed and its importance in understanding interbilayer forces is stressed.

McIntosh, in his review (pp 481–485), continues this theme. He too has made many contributions to our understanding of lipid bilayer structure, principally through the use of X-ray diffraction methods but, in this review, he focuses on the use of this method to derive accurate measurements of the different forces operating between adjacent bilayers. Rand and Parsegian [19], in a seminal contribution, showed that the X-ray-derived bilayer parameters (specifically the water thickness as a function of water content, the latter regulated by changes in osmotic pressure) could be used to quantitate the interbilayer attractive and repulsive forces. McIntosh and his colleagues have built on this approach but, instead of using the ‘calculated’ water thickness (the so-called Luzzati method), prefer to use the electron density profiles. In this review, McIntosh focuses on the issue of the short-range interactions between adjacent bilayers. A variety of interbilayer forces are discussed, including hydration forces, entropic protrusion forces and undulation fluctuations. Most of the earlier studies by Rand and Parsegian, McIntosh and colleagues, as well as by Israelachvili and coworkers using a surface-force apparatus [20], focused on phospholipid membranes. In his review, McIntosh summarizes some of the conclusions from these studies. Recently, McIntosh has turned his attention to the interbilayer forces existing between glycolipid bilayer membranes, where the sugar moieties at the bilayer surface may contribute differently to the forces profile. Although, in terms of cell membrane fusion processes, it appears likely that the initial events may be protein-mediated, sooner or later the actual fusion of adjacent bilayers must take place. Without doubt, these incisive studies of bilayer forces will contribute to our understanding of the mechanism by which this crucial step of membrane fusion occurs.

Finally, Caffrey, in his review (pp 486–497), describes how a different lipid phase, the cubic phase, is proving to be a useful ‘medium’ for the crystallization of the typically refractile membrane proteins. The cubic phase is a member of the class of nonbilayer phases (also including rectangular, hexagonal and micellar phases) described originally by Luzzati [13] for a variety of simple (fatty acids, soaps and detergents) and complex (phospholipids, glycerides) lipids. The structures, symmetry and space groups of these different phases were determined, again using low-angle X-ray diffraction. For the cubic phase, there are several different space group possibilities and different lipids can form different cubic structures. The actual structure of the cubic phase, at least of the most common Ia3d variety, is now thought to be built from a highly curved bilayer matrix, with interpenetrating but noncontacting aqueous networks (see Figure 1 in Caffrey’s review). So, it is a phase with three-dimensional cubic symmetry, but
built with a bilayer motif. Caffrey has been at the forefront of the field of monoglyceride phase behavior and he has identified the phases exhibited by several different monoglycerides; the structures of the observed phases, including several different cubic phases, have been determined by low-angle X-ray diffraction. In 1996, a startling new observation was reported by Landau and Rosenbusch [21]; three-dimensional crystals of the membrane protein bacteriorhodopsin were grown from a mixed detergent/monoglyceride-containing system exhibiting a cubic mesophase structure! Apparently, in some cases, this cubic phase offers advantages over the more conventional detergent-alone medium for membrane protein crystallization, although the mechanism by which this phase might promote crystallization is not obvious at this stage. In his review, Caffrey presents some representative examples of cubic-phase-forming monoglycerides and their phase behavior, discusses how different additives (detergents, salts) might affect cubic phase structure and, finally, presents a possible mechanism by which membrane protein crystallization is facilitated. As Caffrey suggests, perhaps it is not the pure cubic phase that is crucial, but rather the coexistence of neighboring cubic (Ia3d) and lamellar (Lα) phases, both with the bilayer structural motif. Whatever the mechanism, this seems to be an exciting new weapon in the membrane protein crystallization arsenal and the systematic studies of glyceride phase diagrams will undoubtedly lead to a new class of crystallization screen.

Thus, structural studies of lipids continue to have intrinsic merit in terms of defining their structure and conformation in membranes. High- and medium-resolution pictures are available for phospholipid bilayers in their different crystalline, gel and liquid-crystalline states. Furthermore, these systematic X-ray diffraction studies of hydrated lipid systems have had significant impact on the seemingly unrelated fields of membrane forces and membrane protein crystallization. But are the latter two areas unrelated? I wonder.

References


