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Review

Bicelles: A natural 'molecular goniometer' for structural, dynamical and topological studies of molecules in membranes

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ABSTRACT

Major biological processes occur at the biological membrane. One of the great challenges is to understand the function of chemical or biological molecules inside the membrane; as well of those involved in membrane trafficking. This requires obtaining a complete picture of the *in situ* structure and dynamics as well as the topology and orientation of these molecules in the membrane lipid bilayer. These led to the creation of several innovative models of biological membranes in order to investigate the structure and dynamics of amphiphilic molecules, as well as integral membrane proteins having single or multiple transmembrane segments. Because the determination of the structure, dynamics and topology of molecules in membranes requires a macroscopic alignment of the system, a new membrane model called 'bicelles' that represents a crossover between lipid vesicles and classical micelles has become very popular due to its property of spontaneous self-orientation in magnetic fields. In addition, crucial factors involved in mimicking natural membranes, such as sample hydration, pH and salinity limits, are easy to control in bicelle systems. Bicelles are composed of mixtures of long chain (14–18 carbons) and short chain phospholipids (6–8 carbons) hydrated up to 98% with buffers and may adopt various morphologies depending on lipid composition, temperature and hydration. We have been developing bicelle systems under the form of nano-discs made of lipids with saturated or biphenyl-containing fatty acyl chains. Depending on the lipid nature, these membranous nano-discs may be macroscopically oriented with their normal perpendicular or parallel to the magnetic field, providing a natural 'molecular goniometer' for structural and topological studies, especially in the field of NMR. Bicelles can also be spun at the magic angle and lead to the 3D structural determination of molecules in membranes.

1. Introduction

In the course of time, several models have been specially designed to investigate the structure and dynamics of integral membrane proteins in their natural membrane environment such as micelles [1,2], multilamellar vesicles [3], unilamellar vesicles [4] or mechanically oriented bilayers between glass plates [5,6]. Within the last 25 years a new membrane model system became

Abbreviations: DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (14:0/14:0); DPPC, 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (16:0/16:0); DCPC, 1,2-dicaproyl-*sn*-glycero-3-phosphocholine (6:0/6:0); DHPC, 1,2-diheptanoyl-*sn*-glycero-3-phosphocholine (7:0/7:0); DiOMPC, 1,2-di-*O*-tetradecyl-*sn*-glycero-3-phosphocholine (ether lipid) (14:0/14:0); DiOHPC, 1,2-di-*O*-hexyl-*sn*-glycero-3-phosphocholine (ether lipid) (6:0/6:0); TBBPC, 1-tetradecanoyl-2-(4-(4-biphenyl)butanoyl)-*sn*-glycero-3-phosphocholine (14:0/BB); DBBPC, 1-dodecanoyl-2-(4-(4-biphenyl)butanoyl)-*sn*-glycero-3-phosphocholine (12:0/BB); POPC, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (16:0/18:1); Menk, Tyr–Gly–Gly–Phe–Met (methionin enkephalin); *X*, lipid composition of long chain lipid = long chain lipid/total lipids (mole percent); *q*, long chain-to-short chain lipid (mole ratio); *h*, sample hydration = mass of water/total sample mass; SAXS, small angle X-ray scattering; NMR, nuclear magnetic resonance spectroscopy; MAS, magic angle sample spinning; S_{CD}, carbon deuterium bond order parameter; S_{mol}, molecular order parameter; EPR, electron paramagnetic resonance spectroscopy.

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very popular, representing an intermediate between the previously used lipid vesicles and classical micelles. These model systems are based on the pioneering work on 'bilayered micelles' [7]. Further improvement of this model and the resemblance to bile salt phosphatidylcholine, entailed a change in the terminology of these systems in 1995 to 'bicelles' [8]. Due to remarkable features such as their self-alignment in the magnetic field, they became a valuable tool especially for applications in the NMR field [9].

Bicelles are a mixture of aliphatic long chain lipids (between 12 and 18 carbons) and short chain lipids (6–8 carbons). Their morphology is fairly versatile depending on composition, temperature and hydration. The most recognized organization is a nanodisc with the long chain lipids present in majority in the disc plane and the short chain lipids mainly distributed in the torus of the disc. In this review we will focus on bicelles that are disc-shaped and that adapt a uniform and spontaneous alignment under static conditions in the magnetic field, B_0. Phase diagrams will be discussed, together with examples of molecules inserted into the membrane for either doping the membrane with electric charges or for structural and dynamical determination.

2. Morphology of bicelles

The morphology of bilayered lipid mixtures is fairly versatile and changes upon lipid composition, hydration and temperature. In the literature, several models for bicelles have been reported: disc-shaped, cylindrical 'wormlike' micelles or perforated lamellae [10–12]. For the latter, the structure would be composed of multilamellar sheets, oriented by the magnetic field, containing holes formed by short chain phospholipids. Neutron diffraction experiments have also been performed on such a lipid mixture in macroscopically confined sample geometry, suggesting that bilayered micelles can form two distinct oriented domains of perforated lamellae seemingly littered with defects [13–18]. By electron microscopy, disc dimensions of 30–100 nm diameter and 4–5 nm thickness have been measured for TBBPC/DCPC and DMPC/DCPC, DPPC/DHPC, DiOMPC/DiOHPC bicelles [11,19]. In this morphology, the long chain lipids are mainly present in the disc and the short chain lipids are present on the half torus. Devaux and Warschawski pointed out that the short chain amphiphile DCPC undergoes a rapid exchange between toroidal and planar regions, and hence proposed their "mixed bicelle model" [20]. The detergent-like short chain lipids play the role of stabilizing the edges of the bicelle nanodisc. In contrast to liposomes, bicelles do not have an aqueous inside. The properties are close to a liquid crystal phase with one or two-dimensional ordering.

Fig. 1 presents the characteristic ^{31}P and ^{14}N NMR spectra as well as the images obtained by transmission electron microscopy for DMPC/DCPC systems ($X = 78\%$, $h = 80\%$, 100 mM NaCl) and TBBPC/DCPC systems ($X = 85.7\%$, $h = 80\%$, 100 mM NaCl). A reasonable monodispersity of discoidal nano-objects is observed for both systems; moreover, the sizes measured on the TEM images are in very good agreement with those obtained from ^{31}P NMR (^{14}N NMR) by directly integrating the area under the two (four) sharp peaks, assigned to the lipids in the plane and in the half-torus of the disc [11,19,21]. Among the advantages of the bicelle, the fact that the sample hydration can be varied almost at will (in the range 60–98%) is of particular interest. It must be mentioned here that magnetic alignment is a cooperative effect that is critically dependent on the viscosity/hydration of the sample. A good orientation can be obtained with moderate hydrations (70–90%). In comparison, parameters such as sample hydration, pH and salinity proved to be difficult to control with mechanically oriented samples between glass plates. In addition, the same well-hydrated bicelle sample can be used to perform MAS and wide-line NMR, to improve spectral NMR resolution and to determine the orientation of membrane proteins [22,23], *vide infra*.

3. Magnetic field orientation of discs

In earlier studies, CHAPSO, a mild zwitterionic bile salt derivative, has been used to form CHAPSO–DMPC mixtures [24]. They exhibit a magnetic alignment with the membrane normal perpendicular to the static magnetic field, B_0, over a wide range of compositions, pH, ionic strength and temperature. This magnetic orientation can be 90° flipped by adding amphiphilic aromatic hydrocarbons. Discoidal bicelles also show an alignment in high magnetic fields [25]. This is due to the anisotropic diamagnetic susceptibility of phospholipids, $\Delta\chi$ (difference between the parallel ($\chi_\parallel$) and the perpendicular ($\chi_\perp$) magnetic susceptibility to the long lipid axis: $\Delta\chi = \chi_\parallel - \chi_\perp$). Due to their small but measurable negative magnetic susceptibility, dialkanoylphospholipids are found to weakly align with their membrane normal perpendicular to the magnetic field. As a consequence, DMPC/DCPC bicelles align with their bilayer normal oriented perpendicular to B_0. Addition of unsaturated lipids such as POPC does not change the bicelle orientation [26].

Bicelle disc orientation can undergo a 90° flip by adding aromatic amphiphiles or paramagnetic lanthanide ions that have a positive $\Delta\chi$ and bind to the phosphatidylcholine headgroups [27–29]. Among lanthanide ions, Tm^{3+} has the largest positive $\Delta\chi$, allowing optimal alignment at a lower concentration compared to Eu^{3+}, Er^{3+} and Yb^{3+} [30,31]. The disadvantage of this approach is the uncontrolled interaction of lanthanides with molecules inserted into the membrane. Moreover, it is necessary to keep the amount of lanthanide ions to a minimum as they can interfere with spectroscopic experiments (line broadening) [27,30–32].

Changing the alignment of the bicelle normal from perpendicular to parallel is also possible by adding molecules with a large positive $\Delta\chi$, such as peptides [21] or amphiphilic aromatic compounds [33], taking advantage of the fact that phenyl rings have a strong positive $\Delta\chi$ [34,35]. Another approach has been to change the long chain phospholipid itself, by designing a modified phosphatidylcholine dodecanoyl-2-(4-(4-biphenyl)butanoyl)-*sn*-glycero-3-phosphocholine (DBBPC), containing a biphenyl unit in one of its acyl chains [36,37]. Mixtures of this new lipid DBBPC with DCPC can form bicelles, under specific conditions of temperature, hydration, and lipid composition, which align with their membrane normal parallel to the magnetic field; this is due to the large positive $\Delta\chi$ of the biphenyl unit. DBBPC/DCPC bicelles are stable for a ratio q [DBBPC/DHPC] ~6 and a temperature range from 10 to 54 °C [36]. More recently, the C12 aliphatic chain of DBBPC has been replaced by a C14 to mimic the chain length of natural membrane lipids closer, resulting in the phospholipid TBBPC (tetradecanoyl-2-(4-(4-biphenyl)butanoyl)-*sn*-glycero-3-phosphocholine) [19]. Freeze-fracture transmission electron microscopy images confirm that TBBPC/DCPC mixtures form discoidal nano-objects with an average diameter of 800 Å. The existence domains of TBBPC/DCPC have been established by ^{31}P and ^{14}N solid-state NMR (Figs. 1 and 2) and their specific alignment in the magnetic field has been characterized by small angle X-ray scattering (SAXS) [19]. It has also been shown that TBBPC/DCPC bicelles keep their orientation outside the magnetic field for several days.

4. Phase diagrams of magnetically oriented discs

Temperature–composition–hydration diagrams have been established to map out the different regions where bicelle discs are oriented by magnetic fields. ^{31}P solid-state NMR was proven to be