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Liquid disordered–liquid ordered phase coexistence in bicelles containing unsaturated lipids and cholesterol

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ABSTRACT

Magnetically orienting bicelles are often made by combining the long chain phospholipid 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) with the short chain phospholipid 1,2-dicaproyl-*sn*-glycero-3-phosphocholine (DCPC) in buffer. These bicelles orient with their bilayer normals perpendicular to the external magnetic field. We have examined the phase behaviour of DMPC/DCPC bicelles and the effects of cholesterol and the unsaturated phospholipid 1,2-dipalmitoleoyl-*sn*-glycero-3-phosphocholine (DPoPC) as a function of temperature using static solid state ^{2}H nuclear magnetic resonance spectroscopy. As expected, cholesterol has an ordering effect on the long phospholipid chains and this is reflected in the phase behaviour of the bicelle mixtures. Liquid disordered–liquid ordered, fluid–fluid phase coexistence is observed in DMPC/cholesterol/DCPC bicelles with cholesterol mole fractions of 0.13 and higher. DPoPC/DMPC/cholesterol/DCPC bicelles also exhibit two fluid phase coexistence over a broad range of temperatures and compositions. Bicelles can provide a useful medium in which to study membrane bound peptides and proteins. The orientation parallel to the magnetic field is favourable for studying membrane peptides/proteins because information about the orientation of relevant molecular bonds or internuclear vectors can be obtained directly from the resulting ^{2}H spectra. Lanthanide ions can be used to flip the bicelles to have their bilayer normals parallel to the external magnetic field. Yb^{3+} was used to flip the DPoPC/DMPC/cholesterol/DCPC bicelles while Eu^{3+} was found to be ineffective at flipping bicelles containing cholesterol in the present work.

1. Introduction

Biological membranes are made up of many different lipids and proteins which are organized in a two dimensional bilayer structure. In many animal cell membranes cholesterol is a critical component because of its ability to affect the physical properties of the membrane [1]. Mixtures of lipids comprised of two long chain phospholipids, one with saturated acyl chains and one with unsaturated acyl chains, with cholesterol often exhibit the coexistence of two fluid membrane phases. These phases are the liquid disordered (ℓ_d) and liquid ordered (ℓ_o) phases and this phase coexistence can occur over a broad range of temperatures and sample compositions making them a useful mimetic for domain-forming membranes. Previous investigations of this kind of phase behaviour, specifically ℓ_d–ℓ_o coexistence in ternary mixtures of 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC), 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC), and cholesterol, using ^{2}H Nuclear Magnetic Resonance (NMR) have been reported by Davis et al. [2–4] and Veatch et al. [5,6].

^{2}H NMR is frequently used for studies of lipid phase behaviour because the deuterium quadrupolar splittings are sensitive to molecular motion and orientational order [7]. Static ^{2}H spectra are significantly simpler for oriented samples than for powder samples in which all orientations occur with equal probability. Samples can be aligned either mechanically (by depositing lipid bilayers on glass plates for example) or magnetically (making use of the magnetic susceptibility of mixtures of long and short chain lipids) [8–10]. Magnetically aligned lipid bilayers (bicelles) are advantageous as a membrane mimetic since these oriented samples can give more signal for the same sample volume because there is no substrate needed to align them.

Long chain 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) combined with short chain 1,2-dicaproyl-*sn*-glycero-3-phosphocholine (DCPC) (often referred to as DHPC (1,2-dihexanoyl-*sn*-glycero-3-phosphocholine)) forms bicelles which are often used either with soluble proteins for solution-state NMR experiments, or with membrane peptides or proteins which are embedded into the bicelles for solid-state NMR experiments [11–15]. Though DMPC/DCPC bicelles are most widely used, bicelle mixtures can be made from various types of long chain lipids and short chain lipids/detergents or modified lipids [16,17]. ^{31}P and ^{2}H NMR are used to characterize the phase behaviour of bicelles with long chain phospholipids of various lengths or types and different short chain phospholipids or detergents [11,14,15,

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18–21]. The morphology of the particles or structures forming these orientable phases is an interesting subject and has been the object of considerable discussion [14,20,22,23]. Details of the structures formed depend on the lipid composition, the long to short chain ratio, q, the water concentration and the temperature. For values of q near 3 and water concentrations similar to those used in this study it is generally felt that the orientable phase consists primarily of perforated bilayers but that there may be a population of disk shaped particles as well. The essential feature for the purposes of this study is that the structures orient well in the magnetic field and be large enough to support two phase coexistence with domains large enough to be studied by NMR. This requires that the particles be roughly 200 nm or larger in dimension.

The effect of a polyunsaturated lipid (1-palmitoyl-2-linoleoyl-*sn*-glycero-3-phosphocholine (PLiPC)) and cholesterol on DMPC/DCPC bicelles has been investigated by Minto et al. [24]. They found that cholesterol increases the minimum alignment temperature, while the PLiPC decreases the minimum temperature for alignment of the bicelles. Cho et al. [25] reported two phase coexistence in POPC/DMPC/cholesterol/DCPC bicelles using lateral diffusion measurements by ^{1}H magic angle spinning NMR. Two fluid phase coexistence within aligned lipid samples is of interest for the investigation of peptides and proteins in these types of systems. In the present work, the phase behaviour of bicelles containing cholesterol is investigated and the coexistence of the ℓ_d and ℓ_o fluid phases is directly observed using static ^{2}H NMR. In the next section we present the experimental procedures used to prepare and study these systems. This is followed by a description of the orientational order and phase behaviour in the presence of cholesterol and/or unsaturated phospholipids. We observe a broad range of ℓ_d–ℓ_o phase coexistence. We also find that at high cholesterol concentrations in the DMPC/DCPC bicelles there is a significant fraction of the sample which is in an isotropic phase over the entire temperature range studied. However, the bicelles made with DMPC/DPoPC/DCPC and cholesterol possess a large ℓ_d–ℓ_o coexistence region with little or no isotropic phase, making them more suitable for studies of peptide or protein partitioning between the ℓ_d or ℓ_o phases. We also find that there may be some bicelles formed which are physically isolated and unable to exchange molecules with the rest of the sample. This may affect their suitability for studies of partitioning between the phases. We conclude with a summary of the results and suggestions for the use of these mixed bicelles for studying membrane peptides and proteins.

2. Materials and methods

Chain perdeuterated 1,2-dimyristoyl-d_{54}-*sn*-glycero-3-phosphocholine (DMPC-d_{54}), 1,2-dipalmitoleoyl-*sn*-glycero-3-phosphocholine (DPoPC), and 1,2-dicaproyl-*sn*-glycero-3- phosphocholine (DCPC) were obtained from Avanti Polar Lipids Inc. (Alabaster, AL) in powder form and used without further purification. Cholesterol was purchased from Sigma Aldrich (St. Louis, MO). Lanthanide salts were used to change the orientation of some bicelle samples; $YbCl_3$ and $EuCl_3$ were also purchased from Sigma Aldrich (St. Louis, MO).

2.1. Powder samples

Multilamellar dispersions of lipids were prepared by mixing the appropriate quantities of the dry, powdered lipids and cholesterol in ethanol until completely dissolved in a round-bottomed flask. The solvent was removed by lyophilizing overnight. The dry mixture was then carefully scraped from the flask and weighed. 50 mM phosphate buffer (pH 7.0) was added at a ratio of 4:3 (lipid weight to buffer volume) and the mixture was stirred alternately by hand using a glass rod and gentle centrifugation until the mixture was homogeneous. The sample was transferred into a small glass sample tube which was sealed using silicone to prevent any water loss during the experiments. Details of this sample preparation technique are discussed by Davis et al. [2].

2.2. Bicelle samples

Bicelle samples were made in a manner very similar to the powder samples. Important differences are that these samples include the short chain lipid, DCPC, such that the desired mole ratio (q) between the long chain lipid and short chain lipid is obtained, and the amount of buffer in the sample is significantly higher for bicelle samples than typically used for the multilamellar dispersions. DMPC-d_{54}, DPoPC, and cholesterol were weighed out as dry powders, while the DCPC was added as an appropriate volume of a 2.5 mg/mL (DCPC/ethanol) stock solution due to its highly hygroscopic nature. The lipids were dissolved in ethanol, the solvent was removed by lyophilizing overnight, then the dry mixture was scraped from the round-bottomed flask. Buffer was added such that the final ratio of buffer weight/total hydrated sample weight (w/w) was 0.6. Bicelle samples were hydrated with 50 mM phosphate buffer except in cases where the bicelles were to be flipped to have their normals oriented parallel to the magnetic field with the use of lanthanide ions. In these cases, the buffer was mixed with lanthanide salts giving the final dry sample/lanthanide molar ratio of 10:1 when the weight ratio of buffer/total hydrated sample is maintained as 0.6. All samples were transferred into small glass tubes which were sealed with silicone to ensure that the water content was constant throughout the experiments.

2.3. Experimental setup

^{2}H NMR experiments were performed on Bruker BioSpin (Milton, ON) spectrometers at 76.77 MHz, 92.15 MHz, or 122.84 MHz using a quadrupolar echo pulse sequence [7]. Home-made coils were used and the 90° pulses were optimized and were kept as short as possible in order to minimize any artefacts [26]. The 90° pulse lengths used were 2.25 μs at 76.77 MHz, 1.70 μs at 92.15 MHz, and 2.75 μs at 122.84 MHz, the echo delay was 40 μs at 76.77 and 92.15 MHz, and 25 μs at 122.84 MHz. In the quadrupolar echo pulse sequence, the delay prior to acquisition was set such that some points before the top of the echo were recorded. The signal was manually phase corrected in the time domain and then the data points were shifted so that one point was precisely at the top of the echo. The points before the top of the echo were then removed. This is an important process which results in symmetric spectra with a flat baseline [27]. The temperature for each NMR probe was calibrated using $Pb(NO_3)_2$ [28,29], and the corrected temperatures are presented here. The melting points of DPPC-d_{62} and DMPC-d_{54} were used as references for the calibrations.

2.4. Moment analysis

Moment analysis of deuterium spectra is a quantitative way to compare the molecular order of the phospholipids [30]. The nth moment of a spectrum can be defined as follows

$$M_n = \frac{1}{A}\int_{-\infty}^{\infty} |\omega'|^n f(\omega') d\omega' \tag{1}$$

where $\omega' = \omega - \omega_0$, ω_0 is the Larmor frequency and $f(\omega')$ is the function describing the lineshape of the spectrum. The area of the spectrum, A, is calculated as

$$A = \int_{-\infty}^{\infty} f(\omega') d\omega'. \tag{2}$$

The spectra are symmetric and the moments for the two halves of the spectra (from the negative frequency limit to the central frequency ω_0 and from ω_0 to the positive limit) are each calculated and the average is taken.

The first moment is of particular interest here because it is directly proportional to the average carbon-deuterium bond order parameter,