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A comparative study of the effect of cholesterol on bicelle model membranes using X-band and Q-band EPR spectroscopy

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ABSTRACT

X-band and Q-band electron paramagnetic resonance (EPR) spectroscopic techniques were used to investigate the structure and dynamics of cholesterol containing phospholipid bicelles based upon molecular order parameters (S_{mol}), orientational dependent hyperfine splittings and line shape analysis of the corresponding EPR spectra. The nitroxide spin-label 3-β-doxyl-5-α-cholestane (cholestane) was incorporated into DMPC/DHPC bicelles to report the alignment of bicelles in the static magnetic field. The influence of cholesterol on aligned phospholipid bicelles in terms of ordering, the ease of alignment, phase transition temperature have been studied comparatively at X-band and Q-band. At a magnetic field of 1.25 T (Q-band), bicelles with 20 mol% cholesterol aligned at a much lower temperature (313 K), when compared to 318 K at a 0.35 T field strength for X-band, showed better hyperfine splitting values (18.29 G at X-band vs. 18.55 G at Q-band for perpendicular alignment and 8.25 G at X-band vs. 7.83 G at Q-band for the parallel alignment at 318 K) and have greater molecular order parameters (0.76 at X-band vs. 0.86 at Q-band at 318 K). Increasing cholesterol content increased the bicelle ordering, the bicelle-alignment temperature and the gel to liquid crystalline phase transition temperature. We observed that Q-band is more effective than X-band for studying aligned bicelles, because it yielded a higher ordered bicelle system for EPR spectroscopic studies.

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1. Introduction

Cholesterol is an essential component of biological membranes (Yeagle, 1985). The composition of cholesterol varies in the range of 10–50 mol% in a variety of different biological membranes (Pasenkiewicz-Gierula et al., 2000). Cholesterol is needed for proper cell growth, function and stability (Yeagle, 1985; Kurad et al., 2004). It is also implicated in many diseases like heart disease, stroke, and Alzheimer's disease etc. (Borroni et al., 2003; McMullan and McElhaney, 1996). Therefore, studies focusing on cholesterol–lipid interactions are needed to better understand the effect of cholesterol on the organization of the membrane. Different techniques have been employed in the past to study the effect of cholesterol on model membrane systems such as electron paramagnetic resonance (EPR) spectroscopy, nuclear magnetic resonance (NMR) spectroscopy, fluorescence, X-ray diffraction, differential scanning calorimetry (DSC), Fourier transform infrared (FTIR) spectroscopy, neutron diffraction and thermal analysis, molecular dynamics simulation studies etc. (Yeagle, 1985; Kurad et al., 2004; Nusair and Lorigan, 2005; Lu et al., 2004; Dave et al., 2005; Aussenac et al., 2003; McMullen et al., 1993; Leonard et al., 2001; Chiu et al., 2002; Chong, 1994; Collado et al., 2005; Hofsä et al., 2003; Howard and Opella, 1996; Jedlovszky and Mezei, 2003; Kessel et al., 2001; McMullen et al., 1994; Seelig, 1976; Shin et al., 1990; Smondyrev and Berkowits, 1999; Rowe and Neal, 2003).

Magnetically aligned phospholipid bilayers are an excellent model membrane system for NMR and EPR spectroscopic studies (Nusair and Lorigan, 2005; Lu et al., 2004; Aussenac et al., 2003). Aligning bicelles in the magnetic field offers several advantages to increase both the spectral resolution and the signal-to-noise ratio revealing unique structural and dynamical information when compared to unoriented samples (Aussenac et al., 2003). Bicelles are formed by mixing a long-chain phospholipid, such as dimyristoyl phosphatidylcholine (DMPC) with short chain detergent such as dihexanoyl phosphatidylcholine (DHPC) (Vold and Prosser, 1996). The magnetic alignment of the bicelles depends on several factors such as the magnetic susceptibility anisotropy tensor ($\Delta\chi$) of the phospholipids, the strength of the magnetic field, the molar ratio of the long and short chain phospholipids (q-ratio), the temperature of the system and the types of the lanthanide ions used (Prosser et al., 1998a,b). The sign and magnitude of $\Delta\chi$ plays a major role in the alignment of the bicelles (Sanders et al., 1994; Mironov et

Abbreviations: DMPC, 1,2-dimyristoyl-sn-glycero-3-phosphocholine; DHPC, 1,2-dihexanoyl-sn-glycero-3-phosphocholine; HFS, hyperfine splitting; Cholestane, 3-β-doxyl-5-α-cholestane.

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al., 2000). Normally the bicelles align with their bicelles normal perpendicular to the direction of the static magnetic field due to their negative $\Delta\chi$ value. However, at lower magnetic fields used in X-band and even for Q-band EPR, the phospholipid bilayers do not fully align at the perpendicular orientation without the addition of alignment reagents. The addition of Dy^{3+}which has a large negative $\Delta\chi$ value is needed for perpendicular alignment. Conversely, the addition of Tm^{3+} or Yb^{3+} with a large positive $\Delta\chi$ value flips the bicelle by 90°, such that the membrane normal is parallel to the direction of the static magnetic field (Prosser et al., 1998a,b; Cardon et al., 2001).

Previous studies in our lab have shown that magnetically aligned phospholipid bilayers doped with either Dy^{3+}, Tm^{3+} or Yb^{3+} have been successfully aligned at perpendicular and parallel orientations with respect to the magnetic field (Nusair and Lorigan, 2005; Lu et al., 2004; Cardon et al., 2001; Inbaraj et al., 2004; Tiburu et al., 2004). Nussair et al. and Lu et al. studied the effect of cholesterol on bicelles using EPR spectroscopy with a series of 5-, 7-, 12- and 16-doxyl stearic acid and cholestane spin probes and also with solid-state ^{2}H NMR spectroscopy (Nusair and Lorigan, 2005; Lu et al., 2004). Both of these EPR studies by Nussair et al. and Lu et al. were carried out at X-band.

The purpose of this paper is to have a comparative study of the effects of cholesterol on the bicelle model membrane systems using magnetically alignable DMPC/DHPC phospholipids at both X-band (9.5 GHz, 0.35 T) and Q-band (35 GHz, 1.25 T). EPR spectroscopy is highly sensitive to the rate of motion and degree of organization of the phospholipids due to exact matching of characteristic time scale of the nitroxide spin-label to the rates of molecular rotation of the lipids within the membranes (Moser et al., 1989). This paper attempts to effectively characterize a cholesterol-bicelle system using the corresponding molecular order parameters, hyperfine splitting values, and the change in the spectral line shapes of cholestane containing bicelles at different temperatures. The X-band vs. Q-band experimental results will be compared to better understand the effectiveness of the two magnetic fields and frequencies.

2. Materials and methods

2.1. Materials

1,2-Dimyristoyl-sn-glycero-3-phosphocholine (DMPC), 1,2-dihexanoyl-sn-glycero-3-phosphocholine (DHPC), and 1,2-dimyristoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy-(polyethylene glycol)-2000] (PEG2000PE) were purchased from Avanti Polar Lipids (Alabaster, AL). The cholesterol was obtained from Avocado Research Chemicals (Ward Hill, MA). Thulium (III) chloride hexahydrate, dysprosium (III) chloride hexahydrate, 3β-doxyl-5-α-cholestane [cholestane] and N-[2-hydroxyethyl] piperazine-N′-[2-ethanesulfonic acid](HEPES) were obtained from Sigma–Aldrich (St. Louis, MO). Deuterium-depleted water was obtained from Isotec (Miamisburg, OH). All phospholipids were dissolved in chloroform and stored at −20 °C prior to use. Aqueous solutions of N-[2-hydroxyethyl] piperazine-N′-[2-ethanesulfonic acid] (HEPES) buffer and lanthanide ions in deuterium-depleted water were prepared fresh each day.

2.2. Sample preparation

The standard bicelle sample consists of 25% (w/w) DMPC/DHPC phospholipids (molar ratio 3.5:1) in HEPES buffer along with PEG200-PE, cholestane, and various amounts of cholesterol (0, 5, 10, 15 and 20 mol% with respect to DMPC). For bicelles with 10 mol% cholesterol, DMPC, DHPC, PEG2000-PE, cholesterol and the spin-label cholestane were mixed together in a flask at the molar ratios of 3.5/1/0.035/0.35/0.0196, respectively. Cholesterol concentrations were used from 0 to 20 mol% in 5 mol% increments. The chloroform in the flask was evaporated under a stream of nitrogen gas and the flask was placed under high vacuum overnight. The following day, 100 mM HEPES buffer at pH 7 was added to the flask so that the amount of lipid in the sample was 25% (wt%). The sample was vortexed and chilled on ice bath periodically until all of the lipids were solublized. In contrast, for the samples containing high cholesterol content (15 and 20 mol%) samples were vortexed at room temperature without ice and then kept in ice bath for 20 min until the sample became clear and homogeneous. All samples were then sonicated with a Fisher Scientific FS30 bath sonicator (Florence, KY) for about 30 min with the heater turned off and ice added to the bath. The sample was subjected to 4 freeze thaw (at 77 K in liquid nitrogen) cycles at room temperatures to homogenize the sample and remove air bubbles if any. Finally, at 0 °C (ice bucket), a 20 mol% (molar ratio to DMPC) of either $DyCl_3$ or $TmCl_3$ was added and mixed into the EPR sample. The total mass of each sample was approximately 200 mg. For X-band EPR experiments, 50 μL of sample was drawn into a 1 mm inner diameter capillary tube via a syringe. Both ends of the capillary tube were sealed with a Cristoseal (Fisher Scientific) and placed inside standard quartz EPR tubes (Wilmad, 707-SQ-250M) filled with the mineral oil. For Q-band measurements, the bicelles samples were placed in quartz capillaries, with an inner diameter of 0.3 mm (CV3040) from Vitro Com (Mountain Lakes, NJ). The ends of the capillaries were sealed with Cristoseal. The capillary with the sample was introduced into a Vitro Com quartz tube with a 1.5 mm i.d. (CV1518Q) and sealed at one end. The typical sample volume inside the Q-band EPR tubes was about 3–5 μL.

2.3. EPR spectroscopy

For X-band EPR studies, experiments were carried out on a Bruker EMX CW-EPR spectrometer consisting of and ER041xG microwave bridge and ER4119-HS cavity coupled with a BVT 3000 nitrogen gas temperature controller (temperature stability ± 0.2 K). Each spin-labeled EPR spectrum was acquired by taking a 42-s field scan with a center field of 3370 G, a sweep width of 100 G, a microwave frequency of 9.35 GHz, a modulation frequency of 100 kHz, a modulation amplitude of 1 G, and a microwave power of 2 mW.

For Q-band EPR studies, spectra were recorded at a microwave frequency of 34.18 GHz on a Bruker EMX Q-band EPR spectrometer consisting of an ER051QG microwave bridge and a TE01X-mode cylindrical Q-band cavity resonator (ER 5106) coupled with a CF935 dynamic continuous flow cryostat. Each spin-labeled EPR spectrum was acquired by taking a 42-s field scan with a center field of 12,120 G, a sweep width of 100 G, a modulation frequency of 100 kHz, and a microwave power of 2 mW.

2.4. Molecular order parameter (S_{mol}) calculations

The EPR spectra of a nitroxide spin label consist of three lines as a result of an unpaired $S = 1/2$ electron coupled to a ^{14}N ($I = 1$) nucleus. In a Cartesian coordinate system, the magnetic principal axes have the x-axis along the nitroxide N–O bond, the z-axis is along the 2p π orbital of the nitrogen and the y-axis is perpendicular to the xz plane. The order parameter S_{33} can be determined by measuring the resultant hyperfine splitting of the aligned spectra using the following equation:

$$S_{33} = \left[\frac{(A_{||} - A_{\perp})}{(A_{zz} - A_{xx})}\right]\left(\frac{a_N}{a_{N'}}\right) \quad (1)$$

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