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High anisotropy of flow-aligned bicellar membrane systems

Maxim Kogan, Bengt Nordén, Tamás Beke-Somfai*

Department of Chemical and Biological Engineering, Physical Chemistry, Chalmers University of Technology, SE-412 96 Göteborg, Sweden

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ABSTRACT

In recent years, multi-lipid bicellar systems have emerged as promising membrane models. The fast orientational diffusion and magnetic alignability made these systems very attractive for NMR investigations. However, their alignment was so far achieved with a strong magnetic field, which limited their use with other methods that require macroscopic orientation. Recently, it was shown that bicelles could be aligned also by shear flow in a Couette flow cell, making it applicable to structural and biophysical studies by polarized light spectroscopy. Considering the sensitivity of this lipid system to small variations in composition and physicochemical parameters, efficient use of such a flow-cell method with coupled techniques will critically depend on the detailed understanding of how the lipid systems behave under flow conditions. In the present study we have characterized the flow alignment behavior of the commonly used dimyristoyl phosphatidylcholine/dicaproyl phosphatidylcholine (DMPC/DHPC) bicelle system, for various temperatures, lipid compositions, and lipid concentrations. We conclude that at optimal flow conditions the selected bicellar systems can produce the most efficient flow alignment out of any lipid systems used so far. The highest degree of orientation of DMPC/DHPC samples is noticed in a narrow temperature interval, at a practical temperature around 25 °C, most likely in the phase transition region characterized by maximum sample viscosity. The change of macroscopic orientation factor as function of the above conditions is now described in detail. The increase in macroscopic alignment observed for bicelles will most likely allow recording of higher resolution spectra on membrane systems, which provide deeper structural insight and analysis into properties of biomolecules interacting with solution phase lipid membranes.

1. Introduction

Studies focusing on structure, dynamics, and insertion mechanisms of membrane associated or embedded molecules require appropriate membrane mimics to reduce the complexity of native membranes. Accordingly, commonly used solution phase lipid model systems include micelles, uni- and multilamellar vesicles, most often with a single lipid composition (Chan and Boxer, 2007; Nordén et al., 2010). For the past couple of decades a multi-compound lipid system known as bilayered micelles (Gabriel and Roberts, 1984) or bicelles (Sanders and Landis, 1995) has become increasingly used due to its versatile morphology and possibility to align in a strong magnetic field, making them ideal for structural investigations using NMR spectroscopy (Gabriel and Roberts, 1984; Katsaras et al., 2005; Nieh et al., 2004; Prosser et al., 2006; Sanders and Landis, 1995). Combining the features of detergents with high surface curvature and standard planar lipid bilayers, bicelles are excellent to reconstitute membrane proteins into lipid bilayers, while maintaining their functionality (Jiang et al., 2010; Johansson et al., 2009; McKibbin et al., 2007). By now several detailed reviews have appeared on their various properties and applications in structural biology and biophysics (Diller et al., 2009; Dürr et al., 2013; Johansson et al., 2009; Katsaras et al., 2005; Nieh et al., 2004; Poget and Girvin, 2007; Prosser et al., 2006; Zhao and Wu, 2012).

However, alignment of bicelles has so far been achieved with a strong magnetic field, which prohibited their use in other spectroscopic methods that require macroscopic orientation such as emission anisotropy, linear dichroism (LD) or FTIR-LD (Prosser et al., 2006). Recently we have reported that bicellar mixtures, composed of commonly used long-chain lipid dimyristoyl phosphocholine (DMPC) and short-chain lipid dicaproyl phosphocholine (DHPC) components (Prosser et al., 2006; Sanders and Schwonek, 1992), can be aligned by using flow-alignment in a Couette flow cell, which made recording LD spectra of various membrane probe molecules, including a membrane associated protein, possible (Kogan et al., 2011).

Abbreviations: CD, circular dichroism; DHPC, 1,2-dicaproyl-*sn*-glycero-3-phosphocholine; DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; FTIR-LD, Fourier transform infrared linear dichroism; LD, linear dichroism; LUV, large unilamellar vesicle; NMR, nuclear magnetic resonance; RetA, retinoic acid; Py, pyrene.

* Corresponding author. Tel.: +46 31 772 3029.
E-mail address: beke@chalmers.se (T. Beke-Somfai).

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Linear dichroism (LD) is a technique proved to be useful and efficient for probing membrane bound structure as well as orientation of macromolecules, e.g. various peptides (Ardhammar et al., 2002; Caesar et al., 2006; Damianoglou et al., 2010; Hicks et al., 2008, 2009; Svensson et al., 2011), proteins (Caesar et al., 2009; Esbjorner et al., 2007b; Reymer et al., 2009), amyloid fibrils (Kitts et al., 2011; Morris et al., 2013), and synthetic biomaterials (Hicks et al., 2010; Michl and Thulstrup, 1980, 1987; Thulstrup and Michl, 1980). Likewise, a variety of small chromophores and metal–organic complexes were also successfully described (Ardhammar et al., 1998, 1999, 2001; Jonsson et al., 2013; Matson et al., 2012; Svensson et al., 2007, 2008). Continuous advances in instrumentation enabled LD to be combined with flow techniques, which by today have led to the result that shear aligned phospholipid vesicles or liposomes are often used in LD studies as a membrane system (Hicks et al., 2012; Nordén et al., 2010). However, limitations associated with liposomes, such as high light scattering caused by size of the vesicles or low alignment of membrane surfaces due to the high curvature of the shear-deformed vesicles, makes more detailed spectra hard to obtain (Ardhammar et al., 2002). Therefore, the observed flow-alignment of bicelles may also be important for expanding the set of lipid morphologies commonly used in LD studies.

Previous studies have indicated that morphology, orientation and related physicochemical parameters can vary for bicellar systems significantly depending on temperature, lipid composition, or lipid:water ratio (Katsaras et al., 2005; Kogan et al., 2011; Nieh et al., 2004). Thus, for the successful application of these systems in biophysical studies it is crucial to characterize flow alignment of DMPC/DHPC mixtures under varied conditions. In our initial study we have tested temperature dependence of a bicelle sample with DMPC/DHPC ratio of 3.2 for 3 wt% and 20 wt% lipid concentrations (Kogan et al., 2011). Here we aimed to carry out a more detailed systematic analysis on flow alignment of bicelle mixtures and used three commonly-employed molar ratios of DMPC and DHPC, 2.5, 3.2, and 4. We also varied the total weight percent of the lipids between 3 wt%, 5 wt%, 10 wt%, and 20 wt%. Due to the strong temperature dependence and complex morphology of these systems, each setup was investigated over a temperature range of 19–40 °C, which is broad enough to span the temperatures at which alignment might be observable.

Results indicate that bicelles can be flow-aligned in a wide range of investigated conditions. Based on the obtained data we present the change of macroscopic orientation factor throughout the investigated intervals, which may serve as a quick guide for strategic planning in future bicelle studies using flow-orientation.

2. Materials and methods

2.1. Materials

The lipids 1,2-dicaproyl-*sn*-glycero-3-phosphocholine (DHPC 6:0), 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC 14:0), 1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC) and 1,2-dioleoyl-*sn*-glycero-3-phosphoglycerol (DOPG) were obtained from Avanti Polar Lipids, Inc. (Alabaster, AL, USA). Pyrene (Py) and retinoic acid (RetA) were purchased from Sigma–Aldrich.

2.2. Preparation of DMPC/DHPC samples

Stock solutions of DMPC and DHPC in chloroform were mixed together at appropriate volumes calculated from following parameters: molar ratio, $q = [\mathrm{DMPC}]/[\mathrm{DHPC}]$, and lipid concentration in total weight percent of lipids, $l = 100\% \times (m(\mathrm{DMPC}) + m(\mathrm{DHPC}))/m(\mathrm{buffer})$. Rotary evaporation was used to prepare a dry lipid film, which was held for at least 2 h under vacuum to remove all the solvent traces. The dry lipids were solubilized in 10 mM sodium phosphate buffer at neutral pH.

Stock solutions of pyrene and retinoic acid (all dissolved in ethanol) were added directly to the lipid samples so that the final concentration of ethanol in the samples was less than 2% (v/v). Prior to all measurements the samples were repeatedly heated to 40 °C and vortexed, then cooled to 4 °C and vortexed, at least five times.

For DMPC/DHPC bicellar samples the total lipid concentrations were: 3 wt% (50 mM), 5 wt% (80 mM), 10 wt% (160 mM) and 20 wt% (320 mM). The concentrations of the added dyes were 50 μM: for both retinoic acid (RetA) and pyrene (Py), with probe-to-lipid ratios: 1:1000 (3 wt%); 1:1600 (5 wt%) 1:3200 (10 wt%) 1:6400 (20 wt%).

2.3. Preparation of large unilamellar lipid vesicles (LUVs)

A lipid mixture of DOPC/DOPG (molar ratio 60/40) in chloroform was slowly dried by rotary evaporation to form a lipid film. After at least 2 h under vacuum the film was dispersed in 5 mM potassium phosphate buffer (pH 7.4), containing 50 wt% sucrose. At least five freeze–thaw cycles (liquid nitrogen/40 °C) were performed. The uniform size of the LUVs in the sample was obtained by 21-times extrusion through polycarbonate filter with 100 nm pores using a hand-held extruder (Avestin, Inc.).

The DOPC/DOPG samples were prepared at lipid concentrations of 0.1% (1.3 mM). The concentration of the added RetA was 5 μM resulting in probe-to-lipid ratio of 1:250.

2.4. Sample orientation

The Couette flow cell was used for sample alignment according to the previously published methods (Wada, 1964, 1972). In short in the Couette cell the sample is placed in the narrow gap between the walls of a tube and a stationary rod. The spinning of the tube creates a shear gradient, G, which is related to the spin velocity according to:

$$\frac{G}{s^{-1}} = \frac{2\pi R_o \Omega}{R_o - R_i}, \tag{1}$$

where Ω is the rate of rotation in revolutions per second, R_o and R_i are the radius of the outer and the inner cylinders forming the gap.

To optimize sample consumption we used a custom made low-volume outer-rotation Couette cell with $R_o = 1.52$ mm and $R_i = 1.25$ mm giving a sample path length of 0.54 mm and a volume of ~100 μL. However, for some of the samples with high lipid concentration bubble and foam formation was observed at shear forces greater than 400 s^{-1} which resulted in signal disturbances and low signal to noise ratio. Therefore, to provide a standardized uniform comparison of all investigated DMPC/DHPC samples and at the same time maintain good alignment, a shear force of 350 s^{-1} was applied throughout this study unless noted otherwise.

For samples containing DOPC/DOPG liposomes we used a custom made outer-rotation cell, which could apply a stable shear force of 3100 s^{-1} commonly used for liposomal orientation. The total pathlength of the cell was 1 mm and the volume of the cell was 1.6 mL

2.5. Linear dichroism (LD) spectroscopy

Linear dichroism (LD) is defined as the difference in absorption of linearly polarized light parallel and perpendicular to a macroscopic orientation axis of the sample:

$$\mathrm{LD} = A_{\parallel} - A_{\perp}. \tag{2}$$

For samples containing LUV, as well as for bicellar lipid mixtures, orientation of the lipid bilayers is commonly achieved by a