Biochimica et Biophysica Acta 1848 (2015) 2910–2917

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamem

Analysing DHPC/DMPC bicelles by diffusion NMR and multivariate decomposition

Johannes Björnerås [a,*], Mathias Nilsson [b,c], Lena Mäler [a]

[a] Department of Biochemistry and Biophysics,The Arrhenius laboratory,Stockholm University,10691 Stockholm,Sweden
[b] School of Chemistry,University of Manchester,Oxford Road, M13 9PL,United Kingdom
[c] Department of Food Science,University of Copenhagen,Rolighedsvej 30,DK-1958 Fredriksberg C,Denmark

ARTICLE INFO

Article history:
Received 19 May 2015
Received in revised form 6 August 2015
Accepted 1 September 2015
Available online 2 September 2015

Keywords:
NMR
Diffusion
SCORE
DHPC
DMPC
Bicelles

ABSTRACT

Mixtures of lipids and detergents are known to form bicelles at certain parameter ranges, but many uncertainties remain concerning the details of the phase behaviour of these mixtures and the morphology of the formed lipid assemblies. Here we used nuclear magnetic resonance (NMR) diffusion data in combination with the multivariate processing method speedy component resolution (SCORE) to analyse mixtures of 1,2-dihexanoyl-*sn*-glycero-3-phosphocholine (DHPC) and 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) with the relative concentration q = [DMPC]/[DHPC] = 0.5 at total lipid concentrations ranging from 15 to 300 mM. With this approach we were able to resolve the heavily overlapping mixture spectra into component spectra and obtained reliable diffusion coefficients for lipid concentrations in the range 15 to 300 mM, although at high concentrations (250–300 mM), non-negativity constraints or overfactoring was required to successfully decompose the data. At 50–300 mM total lipid concentration, the radii estimated from the diffusion coefficient of DMPC indicate assemblies of the appropriate bicelle size, although small size variations exist, while at lower concentrations the morphology appears to change to larger assemblies. Taken together, the results suggest that for q = 0.5 DMPC/DHPC mixtures there is a relatively broad concentration range above 50 mM where bicelles may reliably be assumed to adopt the 'classical' bicelle morphology. The study clearly demonstrates the usefulness of our approach for accurately determining physical properties of complex mixtures such as bicelles. Both reliable diffusion coefficients and chemical shifts can be derived from overlapping data. This should prove useful for analysing the behaviour of other, more complex, lipid mixtures.

1. Introduction

Biological membranes are extremely complex systems, composed of many different types of lipids, packed with proteins, and with physical properties spanning large scales of length, time, and energy. Overall experimental investigations of the sub-molecular properties and interactions of all the components in a biological membrane in a native state is at present not possible, and the current in vitro biomembrane models have been constructed from fragmented information based on reductionist studies with various biophysical and biochemical methods on different parts of the system. In such studies it is common to use membrane mimetics, controllable lipid/detergent/polar solvent systems in which it is feasible to investigate, e.g., membrane protein structures [1–3], lipid segregation in a bilayer [4,5], or translocation of molecules through a lipid bilayer [6]. A group of such mimetic systems is the bicelles, which are complexes of detergents and bilayer-forming lipids, ideally in the form of bilayer patches whose edges are stabilised by the detergents, or bilayer sheets perforated by detergent-stabilised holes. Bicelles were introduced in the late 1980s [7–10], and have since been established as a valuable tool, first and foremost in nuclear magnetic resonance (NMR) spectroscopy studies of lipid bilayers and bilayer-associated biomolecules [11–13]. The phase behaviour of a bicelle lipid–detergent system depends on the physico–chemical properties of the molecules in the mixture and their absolute concentrations, the ratio between lipids and detergents – the q-value – as well as on other parameters such as temperature, ionic strength and pH. For example, it has recently been demonstrated that the size of bicelles increase with temperature [14], although certain properties, such as segregation of the two components, have been shown to be independent of temperature [14]. Losonczi and Prestegard showed that ionic strength was important for the stability of aligned bicelles [15], while Struppe et al. demonstrated that aligned DMPC/DHPC bicelles are stable at a pH of around 4 to 7 [16]. Moreover, certain high q-value bicelles have the tendency to spontaneously align with an external magnetic field, and the morphology of such phases have been extensively investigated [9,17–19]. Depending on their detailed properties, such aligned bicelles

Abbreviations: cmc, critical micelle concentration; DHPC, 1,2-dihexanoyl-*sn*-glycero-3-phosphocholine; DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; DOSY, diffusion-ordered spectroscopy; SCORE, speedy component resolution.

* Corresponding author.
E-mail address: johannes.bjorneras@dbb.su.se (J. Björnerås).

http://dx.doi.org/10.1016/j.bbamem.2015.09.002
0005-2736/

may be used to study membrane proteins with solid-state NMR [20], or in solution to weakly orient water-soluble biomolecules, creating opportunities to extract structural information with NMR [21]. At lower q-values ($q \approx <1$), the lipid-detergent complexes will reorient rapidly on an NMR timescale; in this regime the assemblies are often referred to as isotropic bicelles [22] or fast-tumbling bicelles [23], and have been used to, e.g., study membrane interaction of peptides [24,25], or to solubilise membrane proteins for NMR studies [26].

The properties of such fast-tumbling bicelles have been investigated by several groups. Chou and co-workers showed that detergents exchange rapidly between assemblies, the size of which depends strongly on the q-value, and a population of free detergents; [27] van Dam et al. concluded that at $q = 0.5$, 1,2-dihexanoyl-*sn*-glycero-3-phosphocholine (DHPC) and 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) indeed formed disc-like objects, although smaller than predicted by the ideal bicelle model [28]. This finding was supported by using a combination of ^{31}P NMR, fluorescence, dynamic light scattering, and electron microscopy where the authors confirmed the segregation of lipids and detergents in DMPC/DHPC mixtures, as well as the discoidal shape of the assemblies, but concluded that the morphology appeared to change at phospholipid concentrations below ~130 mM [14]. Luchette and co-workers also determined from NMR and neutron scattering studies that disc-shaped objects were formed in DMPC/DHPC mixtures at q-values ranging from 0.2 to 1.0 [23]. Recently coarse-grained simulations were used to study the thermodynamic stability of bicelles [29]. Important questions still remain, however, such as the possibility of a co-existence of several types of assemblies for low q-value mixtures. The presence of such assemblies may be masked in NMR studies by the spectral similarities of the lipids, and scattering techniques may not be able to discriminate between species.

Diffusion NMR is a powerful way to analyse the relative sizes of species in solution [30]. The size information is typically probed through a pulsed field gradient stimulated echo experiment, where signal intensity is a function of gradient pulse strength and diffusion rate. The form of the signal decay for unrestricted diffusion follows some form of the Stejskal–Tanner equation [31,32] $S(g) = S_0 e^{-D\delta^2\gamma^2 g^2 \Delta'}$ where $S(g)$ is the signal amplitude, S_0 is the amplitude in the absence of diffusion, D is the diffusion coefficient, δ is the gradient pulse duration, γ is the magnetogyric ratio, g is the gradient amplitude, and Δ' is the diffusion time corrected for diffusion during the gradient pulses.

A popular way to process such data is diffusion-ordered spectroscopy (DOSY) [33,34], where each signal is fitted individually to the decay function and the result is presented as a 2D plot with chemical shift in one dimension and diffusion coefficient in the other. In its simplest, and most common form, each signal is fitted to a single exponential in the High Resolution (HR) DOSY approach. In HR DOSY, differences of 1% or less in diffusion coefficients can be measured, but that assumes that the resonances are well resolved in the spectrum. Unfortunately this is the exception rather than the rule, and when signals overlap the fitted diffusion coefficient is a compromise value of the species involved [35]. This is certainly the case for the bicelle mixtures, where most of the signals originating from DMPC and DHPC are almost perfectly overlapped. A logical extension is to fit a sum of exponentials (or even a distribution), but that demands high signal-to-noise ratio and the absence of systematic errors [36,37]. A better strategy is often to use multivariate decomposition of the experimental data. Several multivariate approaches are available [38–44], all of them in some way utilising the global properties of the data, i.e., that all spectral signals from a particular species should have the same diffusion behaviour. These methods are useful for decomposing data from heavily overlapped signals stemming from different molecular species, or from the same type of molecule in different environments. For the bicelle samples, most of the lipid spectra are typically overlapped to a large degree, which makes it difficult to measure diffusion for the two types of molecules, DMPC and DHPC, in the sample. The main advantage of the decomposition approach is that it gives both spectral information and diffusion coefficients for multiple components in a system with severely overlapping spectra. Such overlap effectively prohibits the use of the standard DOSY method, while some alternative approaches are non-trivial due to, e.g., the inherent uncertainties in fitting multi-exponential functions to noisy data [45].

Among the multivariate decomposition approaches are the CORE [38] and more recently SCORE [46] methods that use an estimation strategy tailor-suited for NMR diffusion data, which is fitted with a pre-defined function (usually some form of exponentially decaying function), in an iterative loop. Among the advantages of the SCORE method are its speed and robustness [46].

In this study we therefore used the SCORE method [46], a multivariate decomposition algorithm based on the CORE method originally proposed by Stilbs [38], to investigate the behaviour of DHPC/DMPC, $q = 0.5$, mixtures as well as pure DHPC samples, at 25 °C and as a function of total lipid/detergent concentration. Although near the transition temperature, at 25 °C DMPC in DMPC/DHPC mixtures maintains a fluid phase [47], and several investigations of bicelles have been performed at this temperature [47–49]. Moreover, higher temperatures increase the risk of convection in the sample, requiring special precautions to be taken in order to avoid artefacts in the diffusion coefficient estimates [50,51]. Our results suggest that DMPC is located only in bicelles, and that the size of these bicelles is similar over concentrations between 50 and 300 mM, a wider region than has previously been suggested. There are no indications of a co-existing population of DHPC micelles, or any other particle species. Furthermore, SCORE decomposition of the data allows the estimation of chemical shift values for several nuclei in the lipid molecules, providing information of the chemical environment in different positions of the bicelles. Moreover, we demonstrate that accurate diffusion and chemical shift data can be obtained for individual bicelle components, without the need for, e.g., deuteration of lipids.

2. Experimental section

2.1. Preparation of bicelles and DHPC micelles

Unlabelled 1,2-dihexanoyl-*sn*-glycero-3-phosphocholine (DHPC) was used as the short-chained phospholipid detergent in the preparation of phospholipid bicelles, while 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) provided the bilayer part of the bicelles. All lipids were obtained from Avanti Polar Lipids (Alabaster, AL, USA) and used without further purification.

Fast-tumbling DMPC/DHPC bicelles with $q = 0.5$ (q equals the molar ratio of lipids and detergents, e.g. $q = [DMPC]/[DHPC]$), were produced by mixing DMPC (powder) with sodium phosphate buffer at pH 5.7 (prepared from a 100 mM buffer stock, lyophilised and resuspended in D_2O) giving an inhomogeneous slurry that was vortexed and subjected to an ultrasonic bath for a few minutes. A suitable amount of a 1 M deuterated aqueous solution of DHPC was added to the mixture to obtain a sample with a total lipid ($[PC] \equiv [DMPC] + [DHPC]$) concentration of 300 mM, and a buffer concentration of 20 mM. This mixture was subjected to several cycles of gentle heating from room temperature to 37 °C combined with vortexing and immersion in an ultrasonic bath, until a clear non-viscous solution was obtained. From this 300 mM mixture, a 500 μl NMR sample was prepared. Lower concentration samples were obtained by subsequent dilution of this sample with 20 mM deuterated sodium phosphate buffer.

For the DHPC micelle samples, a 1 M deuterated aqueous solution of DHPC was diluted with 100 mM deuterated sodium buffer stock solution and H_2O to obtain a sample with 150 mM DHPC in 20 mM buffer and the sample was subjected to vortexing. Lower concentration samples were prepared from this sample by dilution.

2.2. NMR spectroscopy

Translational diffusion measurements were carried out on a Bruker Avance spectrometer (Bruker Biospin, Fällanden, Switzerland), equipped