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Fast-tumbling bicelles constructed from native *Escherichia coli* lipids

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ABSTRACT

Solution-state NMR requires small membrane mimetic systems to allow for acquiring high-resolution data. At the same time these mimetics should faithfully mimic biological membranes. Here we characterized two novel fast-tumbling bicelle systems with lipids from two *Escherichia coli* strains. While strain 1 (AD93WT) contains a characteristic *E. coli* lipid composition, strain 2 (AD93-PE) is not capable of synthesizing the most abundant lipid in *E. coli*, phosphatidylethanolamine. The lipid and acyl chain compositions were characterized by ^{31}P and ^{13}C NMR. Depending on growth temperature and phase, the lipid composition varies substantially, which means that the bicelle composition can be tuned by using lipids from cells grown at different temperatures and growth phases. The hydrodynamic radii of the bicelles were determined from translational diffusion coefficients and NMR spin relaxation was measured to investigate lipid properties in the bicelles. We find that the lipid dynamics are unaffected by variations in lipid composition, suggesting that the bilayer is in a fluid phase under all conditions investigated here. Backbone glycerol carbons are the most rigid positions in all lipids, while head-group carbons and the first carbons of the acyl chain are somewhat more flexible. The flexibility increases down the acyl chain to almost unrestricted motion at its end. Carbons in double bonds and cyclopropane moieties are substantially restricted in their motional freedom. The bicelle systems characterized here are thus found to faithfully mimic *E. coli* inner membranes and are therefore useful for membrane interaction studies of proteins with *E. coli* inner membranes by solution-state NMR.

1. Introduction

Bacterial membranes are complex bilayers of lipids that form a protective barrier for the cell against the fluctuating outer environment. Beyond being a protective, semipermeable barrier, the lipids that constitute membrane bilayers have functional roles [1,2]. It has for example been shown that phosphatidylethanolamine (PE) is required for correct membrane insertion of LacY [3]. Also, PE, phosphatidylglycerol (PG), and cardiolipin (CL) are involved in membrane-related processes like protein translocation and cell growth [1]. Moreover, the lipid bilayer forms the matrix for integral membrane proteins and is sensed by peripheral membrane proteins. For instance, it has recently been shown that the transmembrane protein proteorhodopsin requires lipids of a specific acyl chain length for optimal function [4] and that the peripheral glycosyltransferases MGS [5–7] and WaaG [8] interact specifically with anionic species of the membrane.

The inner membrane of *Escherichia coli* consists mainly of three types of phospholipids: PE (60–80 mol%), PG (15–30 mol%), and CL (5–10 mol%) [2,9,10]. The lipid composition does not vary in response to changes in growth temperature [11,12] but the ratio of CL increases during the stationary phase [13]. Acyl chains can vary in length and degree of unsaturation depending on growth conditions. Under typical growth conditions, 16:0 acyl chains are the most abundant species (~40%) and about 50% of the acyl chains are unsaturated [14,15]. At the onset of the stationary phase a substantial fraction of double bonds is converted into cyclopropanated species, which are believed to increase the physical and chemical stability of cellular membranes [16–18]. PG is a bilayer-forming lipid while PE is not. In the presence of divalent ions, CL can form a hexagonal phase but it is bilayer-prone otherwise [19]. Therefore, *E. coli* inner membranes maintain considerable curvature stress, which is required for cellular viability [15]. The bilayer is maintained in a fluid, lamellar phase by modifications of the acyl chain composition in response to temperature changes [11].

Solution-state NMR methods have contributed to a detailed understanding of protein-membrane interactions. To render experiments interpretable, the size and complexity of biological membranes have to be reduced due to methodological limitations of solution-state NMR. Micelles and bicelles are membrane mimetic systems that are routinely employed to mimic biological membranes in NMR studies

Abbreviations: CL, cardiolipin; DHPC-d_{22}, tail-deuterated 1,2-dihexanoyl-*sn*-glycero-3-phosphocholine; DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; DMPG, 1,2-dimyristoyl-*sn*-glycero-3-phosphoglycerol; NOE, nuclear Overhauser enhancement; PC, phosphatidylcholine; PG, phosphatidylglycerol; PE, phosphatidylethanolamine; TLC, thin layer chromatography.

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[20,21]. Micelles are detergent aggregates that do not form bilayers and have been shown to distort structure and function of some membrane proteins [22–28]. In contrast, bicelles contain a lipid bilayer and therefore they more closely mimic biological membranes as compared to micelles [21,29]. They are thus widely used NMR-accessible membrane mimetics.

Bicelles are characterized by their q-value, which is the molar ratio of lipids to detergent. For q-values in the range 0.25 to 1 small, isotropic bicelles are formed. In an idealized model isotropic bicelles are aggregates in which lipids form a disk-shaped bilayer stabilized by detergents in the rim [30]. For $q < 0.25$ the spatial segregation of detergents and lipids is most likely lost [31,32], while for $q \gg 1$ large bilayer structures are formed, which under appropriate conditions orient in magnetic fields [33–35]. The simplest bicelles consist of a single bilayer-forming lipid type, e.g. 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC), and a detergent-like molecule, e.g. 1,2-dihexanoyl-*sn*-glycero-3-phosphocholine (DHPC), which has shorter acyl chains than DMPC [36,37]. In order to emulate specific properties of natural membranes other lipid species can readily be introduced into bicelles. For instance, to simulate the characteristic negative charge of bacterial membranes, fast-tumbling as well as high q-value bicelles have been enriched with anionic lipids such as 1,2-dimyristoyl-*sn*-glycero-3-phosphoglycerol (DMPG) or 1,2-dimyristoyl-*sn*-glycero-3-phosphoserine (DMPS) [38–42]. Using synthetic lipids, up to around 40% DMPG has been introduced into fast-tumbling bicelles with $q = 0.5$. Moreover, bicelles that contain glycolipids abundant in plant membranes can also be produced and it was shown that up to 30% of either mono- or digalactosyl-diacylglycerol could be incorporated into fast-tumbling bicelles while maintaining the bicelle morphology [43]. Finally, the chain length and degree of unsaturation of lipids in bicelles can be varied [4,31,44], also in aligned bicelles with high q-values [45].

It is not possible to prepare fast-tumbling bicelles (with q-values of 0.5 or lower) with high anionic surface charge density or head-group compositions similar to that of *E. coli* of purely synthetic lipids composed of one single type of acyl chain. Therefore, natural lipids extracted directly from *E. coli* provide a means to fine-tune the lipid composition for studies of peptides and proteins in a realistic lipid environment. Here, we present an in-depth analysis of the dynamics and composition of lipids that were extracted from two strains of *E. coli* membranes in small isotropic bicelles. These new types of bicelles are sufficiently small to conduct solution-state NMR experiments while they at the same time provide a natural environment to investigate protein-membrane interactions [7,40,46]. While the inner membrane of strain 1 (AD93WT) has a typical *E. coli* inner membrane composition (~75% PE, 20% PG, 5% CL), strain 2 (AD93-PE) cannot synthesize PE and its inner membrane consists of about 80% PG and 20% CL [15]. The latter construct can be of specific interest when investigating the role of PE in protein-membrane interactions. Moreover, we investigated the effect of growth temperature on the lipid composition and on the dynamic properties of the corresponding lipid mixtures in bicelles.

2. Experimental procedures

2.1. Materials

Tail-deuterated 1,2-dihexanoyl-*sn*-glycero-3-phosphocholine (DHPC-d_{22}) was purchased from Avanti Polar Lipids (Alabaster, AL).

2.2. Purification of lipids from AD93WT and AD93-PE

Lipids were purified from AD93WT and AD93-PE strains according to the protocol described earlier [7,15]. AD93WT and AD93-PE strains were grown at 30 °C from an overnight culture for 24 h in 2× LB with appropriate antibiotics (25 μg/ml kanamycin and 34 μg/ml chloramphenicol for AD93WT and 25 μg/ml kanamycin for AD93-PE) and 20 mM $MgCl_2$ in the case of AD93-PE. Cells were subsequently collected and re-suspended in 20 mM PIPES, 1 mM EDTA, 150 mM NaCl, and 0.0002% NaN_3 buffer at pH 7.4. Lipids were extracted with chloroform:methanol 2:1 (v/v) and were subsequently washed in the aforementioned buffer to remove divalent ions (in particular Mg^{2+}). The chloroform phase was then collected and purified with adsorption chromatography on a silica gel column. Diacylglycerol and fatty acids were eluted with chloroform and phospholipids were subsequently eluted with methanol. All lipid fractions were concentrated to about 20 mg/ml and kept at −20 °C in a 2:1 (v/v) chloroform:methanol solution. Membrane lipid compositions were checked by thin layer chromatography (TLC) using a separation on Silica Gel 60 plates (Merck, Germany) with chloroform/methanol/acetic acid 85:25:10 (v/v/v) as the mobile phase. All lipids were spotted by iodine staining.

2.3. Growth curves

Cell cultures of AD93WT were grown under the above conditions at 20 °C, 30 °C, and 37 °C and AD93-PE cell cultures were grown at 30 °C. For each condition two cell cultures were grown with a delay of 12 h. The OD_{600} of culture 1 was measured in regular intervals during hours 1–12 and 22–33 of the growth cycle. The OD_{600} of culture 2 was measured in regular intervals during hours 13–21 of the growth cycle.

2.4. Bicelle preparation

The two bicelle types analyzed in this study were: 1. AD93WT bicelles made from AD93WT lipid extract and 2. AD93-PE bicelles made from AD93-PE lipid extract. Moreover, lipids were also extracted from AD93WT cells grown at either 20 °C or 37 °C, in addition to cells grown at 30 °C as described above. The bicelles produced from these lipids are referred to as 20 °C or 37 °C AD93WT bicelles. Assuming a typical lipid composition [15] and average length of the acyl chains of 16.5 carbons [11] average molecular weights of 730 g/mol for AD93WT and 880 g/mol for AD93-PE lipids were calculated. Bicelles were prepared by the removal of the chloroform:methanol solvent under a stream of N_2 gas and lipid films were rehydrated in 50 mM of pH 7.4 phosphate buffer in 100% D_2O to obtain a final concentration of 50 mM lipids. Following the addition of 100 mM of DHPC-d_{22} the sample was subjected to three cycles of freezing at −20 °C and thawing at room temperature to yield $q = 0.5$ bicelles. One should note that since up to 10 mM DHPC is free in solution, the effective q-value is always higher [35]. To reference 1H spectra and as an indirect reference for 31P and 13C spectra [47] 50 μM of 4,4-dimethyl-4-silapentane-1-sulfonic acid was added to the sample.

2.5. NMR spectroscopy

2.5.1. Peak assignment and lipid composition

Experiments to assign ^{13}C, ^{1}H, and ^{31}P peaks in AD93WT and AD93-PE bicelle spectra (natural abundance 1D-^{13}C, 1D-^{1}H, 1D-^{31}P, ^{13}C-HSQC, and ^{31}P heteronuclear experiments) were conducted on a Bruker Avance 600 MHz spectrometer equipped with a triple resonance (^{1}H, ^{13}C, ^{31}P) probe-head. ^{31}P and ^{13}C resonance frequencies were 243 MHz and 151 MHz, respectively. Assignments were obtained from standard literature values [48–50]. Moreover, natural abundance 1D-^{13}C and ^{1}H spectra of synthetic PE, PG, and CL dissolved in chloroform aided the assignment. The spectra of the synthetic lipids in chloroform are shown in Fig. S1A (^{1}H) and B (^{13}C) in the Supplementary material. The lipid composition was calculated from peak integrals in ^{31}P spectra. The composition of chain modifications was obtained from peak integrals in ^{13}C spectra. To ensure that differences in relaxation behavior of acyl chain carbons did not influence the peak integrals, the amount of a specific acyl chain modification is reported with respect to the respective peak integral of 30 °C AD93WT bicelles and not relative to the total acyl chain composition. Variations in lipid concentrations were corrected for by multiplying with a concentration factor obtained