Biochimica et Biophysica Acta 1818 (2012) 1142–1147

Contents lists available at SciVerse ScienceDirect

Biochimica et Biophysica Acta

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

Magnetically oriented dodecylphosphocholine bicelles for solid-state NMR structure analysis

Olga V. Nolandt [a], Torsten H. Walther [a], Stephan L. Grage [b], Anne S. Ulrich [a,b,*]

[a] Karlsruhe Institute of Technology (KIT), Institute of Organic Chemistry and DFG-Center for Functional Nanostructures, Fritz-Haber-Weg 6, D-76131 Karlsruhe, Germany
[b] Karlsruhe Institute of Technology (KIT), Institute of Biological Interfaces (IBG-2), P.O.B. 3640, D-76021 Karlsruhe, Germany

ARTICLE INFO

Article history:
Received 24 March 2011
Received in revised form 5 January 2012
Accepted 10 January 2012
Available online 17 January 2012

Keywords:
DMPC/DPC bicelles
Twin arginine translocation
Membrane protein
Solid-state NMR
PISEMA
Dodecylphosphocholine

ABSTRACT

A mixture of 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) with the short-chain detergent n-dodecylphosphocholine (DPC) is introduced here as a new membrane-mimetic bicelle system for solid-state NMR structure analysis of membrane proteins in oriented samples. Magnetically aligned DMPC/DPC bicelles are stable over a range of concentrations, with an optimum lipid ratio of q = 3:1, and they can be flipped with lanthanide ions. The advantage of DMPC/DPC over established bicelle systems lies in the possibility to use one and the same detergent for purification and NMR analysis of the membrane protein, without any need for detergent exchange. Furthermore, the same batch of protein can be studied in both micelles and bicelles, using liquid-state and solid-state NMR, respectively. The applicability of the DMPC/DPC bicelles is demonstrated here with the ^{15}N-labeled transmembrane protein TatA.

1. Introduction

Nuclear magnetic resonance (NMR) is a powerful tool for structural investigations of membrane proteins. Over the last few years various membrane-mimicking systems have been introduced to enhance the spectroscopic accessibility of these proteins. For example, planar bicelles are employed in solid-state NMR measurements as lipid systems for membrane protein reconstitution. The term "bicelles" denotes binary bilayered mixed micelles consisting of a long-chain lipid and a short-chain detergent [1], for which a bilayered discoidal shape [2] or a "Swiss Cheese" model [3] has been proposed. One of the first bicelle systems for structural investigation of membrane proteins by NMR consisted of the lipid DMPC mixed with either dihexanoylphosphatidylcholine (DHPC) or CHAPSO molecules as the short-chain detergent [1,4,5]. Meanwhile, stable bicelles have been achieved with a large variety of long-chain components, with chain lengths ranging from C12 to C18 [6], different degrees of saturation, and various head groups. For instance, DMPC [1], POPC [7], DMPG [8], TBBPC [9–11], cardiolipin [12], and sphingomyelin [13] have been used in bicelle preparations. The choice of the short-chain detergent has been limited to fewer molecules so far, such as DHPC, 6-O-PC, DH(7)PC and CHAPSO [14]. It has been recently demonstrated that Triton X-100 as a part of the bicelle mixture considerably improves the resolution of the NMR spectra [15].

Bicelles can assume different morphologies depending on temperature, lipid composition and concentration [16]. They can be magnetically oriented in the static field of an NMR spectrometer. Magnetic alignment of the concentrated lipid mixture occurs at temperatures above the gel-to-liquid crystalline transition temperature of the main (long-chain) component of the mixture, as readily validated using solid-state ^{31}P NMR [4]. Below the transition temperature mixed micelles are formed, which tumble isotropically in solution, giving rise to a single isotropic peak in a ^{31}P NMR spectrum. With increasing temperature this signal transforms into several separate resonances, which are shifted towards negative ppm values above the transition temperature. The presence of sharp upfield signals indicates that the main lipid component has formed bilayer-like assemblies that are uniformly aligned in the magnetic field. These bicelles adopt an orientation with their bilayer normal perpendicular to the magnetic field, and they can be flipped to a parallel alignment by the addition of paramagnetic ions [3,17] or the use of diphenyl lipids [9–11]. The ability of bicelles to orient in a magnetic field renders them particularly useful as a membrane-mimicking environment for determining the helix alignment in membrane proteins using solid-state NMR [6,18,19]. Various separated-local-field techniques, such as PISEMA and SAMPI4, make use of uniformly oriented

Abbreviations: Tat, Twin-arginine translocation; DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; DMPG, 1,2-dimyristoyl-*sn*-glycero-3-[phospho-*rac*-(1-glycerol)]; DH(7)PC, 1,2-diheptanoyl-*sn*-glycero-3-phosphocholine; DHPC, 1,2-dihexanoyl-*sn*-glycero-3-phosphocholine; DPC, n-dodecylphosphocholine; 6-O-PC, 1,2-di-O-hexyl-*sn*-glycero-3-phosphocholine; CHAPSO, 3-(cholamidopropyl) dimethylammonio-2-hydroxyl-1-propanesulfonate; POPC, 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine; TBBPC, 1-myristoyl-2-[4-(4-biphenyl) butanoyl]-*sn*-glycero-3-phosphocholine

* Corresponding author at: Karlsruhe Institute of Technology (KIT), Institute of Organic Chemistry and DFG-Center for Functional Nanostructures, Fritz-Haber-Weg 6, D-76131 Karlsruhe, Germany.
E-mail address: anne.ulrich@kit.edu (A.S. Ulrich).

0005-2736/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbamem.2012.01.007

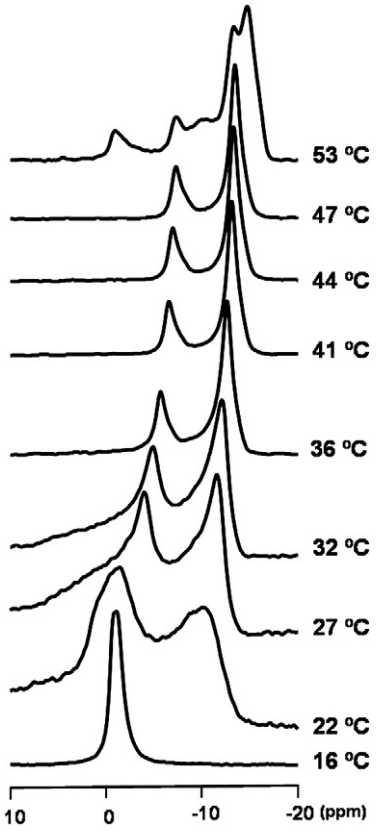

Fig. 1. ^{31}P NMR spectra of DMPC/DPC bicelles (20% total lipid, q = 3.2) at different temperatures.

samples and have been successfully applied in elucidating the structures of membrane proteins [20–25]. Amongst the different ways of preparing oriented membrane samples that are necessary for these experiments, bicelles have lead to the highest spectral resolution [26]. However, the use of bicelles is restricted to proteins that are compatible with one of the available bicelle compositions, into which they need to be reconstituted. In particular, the narrow choice of short-chain detergents that are suitable for bicelles has limited the range of applications of this type of sample preparation.

The aim of this work was to extend the range of possible short-chain components for bicelle systems, and thereby to increase the options for reconstituting membrane proteins. As an addition to the existing bicelle systems, we suggest here the robust DMPC/DPC mixture for studying membrane proteins by both solid-state NMR as well as liquid-state NMR. Dodecylphosphocholine (DPC) is a "lipid like" zwitterionic detergent that is often used to isolate and purify membrane proteins under mild conditions [27–30], and it often serves as a membrane-mimetic micellar environment for liquid-state NMR studies of membrane proteins [31–34]. Micelles and bicelles formed with this detergent could therefore provide an opportunity to reduce the number of necessary detergent exchange steps in sample preparation protocols involving DPC. In this contribution we demonstrate and characterize the ability of the DMPC/DPC bicelles to orient in the magnetic field similar to established bicelle systems. The suitability of this system for liquid-state and solid-state NMR analysis of membrane proteins is then validated using the pore forming subunit $TatA_{2-45}$ of the Twin-arginine protein translocation system from *Bacillus subtilis* as an example [21,23,33].

2. Materials and methods

2.1. Materials

1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) was purchased as a dry powder from Avanti Polar Lipids (Alabaster, AL USA). FOS-CHOLINE-12 or n-dodecylphosphocholine (DPC) was purchased as a dry powder from Anatrace (Maumee, OH, USA). The $TatA_{2-45}$ molecule is a part of the $TatA_d$ protein, which belongs to the Twin-arginine translocation system of *B. subtilis*. $TatA_{2-45}$, as described in [35], consists of 44 amino acids (from the 2nd to 45th amino acid of full-length $TatA_d$) and contains a transmembrane segment and an amphiphilic α-helix.

2.2. Protein expression and purification

The plasmid $pET28TatA_{1-45}$ [35] was transformed in *E. coli* strain BL21 (DE3) (Novagen, Merck, Darmstadt, Germany). The expression of ^{15}N-uniform labelled protein was performed in M9 medium, and purification was achieved by Ni-NTA affinity chromatography in the presence of the detergent N-lauroyl-sarcosinate (NLS) (Fluka, Sigma Aldrich, St. Louis, MO, USA). The His-tag was removed by CNBr cleavage, followed by a subtractive Ni-NTA affinity chromatography step. The protein sample was finally dialysed against water and lyophilised. The details of cloning, expression and purification have been described previously [21,23,35].

2.3. Sample preparation

Bicelle preparation for ^{31}P NMR: DMPC and DPC powder were dissolved in 200 µl of 10 mM phosphate buffer or water. We prepared bicelles with 20% and 40% total amount of lipid (including the DPC detergent, though for simplicity we denominate this as "total amount of lipid"), and with different values of q (molar ratio of DMPC to DPC) of 2.0, 2.4, 2.8, 3.2, and 3.6. The mixtures were vortexed alternatingly at 0 °C and 43 °C until the sample became clear in the cold. The bicelles were fluid at low temperature and viscous at high temperature. The pH of the bicelles was adjusted to pH = 6.8. The alignment direction of the bicelles was changed ("flipped") by adding the lanthanide $YbCl_3$ to a final concentration of 3 mM. Due to the high positive magnetic susceptibility of the lanthanide ions, which bind to the phosphate head groups of the lipids, the bicelles reorient to align their normal parallel to the magnetic field [3].

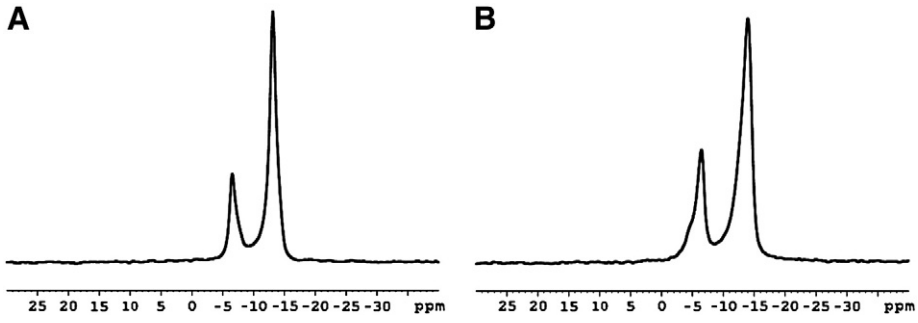

Fig. 2. Comparison of the ^{31}P NMR spectra of (A) DMPC/DPC and (B) DMPC/6-O-PC bicelles (20% total lipid, q = 3.2, T = 42 °C).