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Diffusion measurements of water, ubiquinone and lipid bilayer inside a cylindrical nanoporous support: A stimulated echo pulsed-field gradient MAS-NMR investigation

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Abstract

Stimulated echo pulsed-field gradient ^{1}H magic angle spinning NMR has been used to investigate the mobility of water, ubiquinone and tethered phospholipids, components of a biomimetic model membrane. The diffusion constant of water corresponds to an isotropic motion in a cylinder. When the lipid bilayer is obtained after the fusion of small unilamellar vesicles, the extracted value of lipid diffusion indicates unrestricted motion. The cylindrical arrangement of the lipids permits a simplification of data analysis since the normal bilayer is perpendicular to the gradient axis. This feature leads to a linear relation between the logarithm of the attenuation of the signal intensity and a factor depending on the gradient strength, for lipids covering the inner wall of aluminium oxide nanopores as well as for lipids adsorbed on a polymer sheet rolled into a cylinder. The effect of the bilayer formation on water diffusion has also been observed. The lateral diffusion coefficient of ubiquinone is in the same order of magnitude as the lipid lateral diffusion coefficient, in agreement with its localization within the bilayer.

Keywords: MAS-NMR PFG-STE; Tethered phospholipid bilayers; Nanoporous anodic aluminum oxide support; Oriented model membrane; Ubiquinone; Diffusion constants

1. Introduction

Customized biomimetic membranes are often used to study basic cellular function. Many reactions in membranes depend on the lateral motion and the fluid dynamic properties of all membrane components. The success of the development of model membranes involves the formation of a fluid single lipid bilayer allowing for a compartment mimicking the natural permeable barrier of the cell. Solid supported lipid membranes constitute a powerful approach to provide information on molecular processes occurring in biological membranes or some understanding of membrane–protein interactions as these models facilitate large transmembrane protein insertion [1–5]. These samples are highly suitable for biophysic studies due to their stability and robustness. One promising strategy consists of anchoring the lipid bilayer at one end to a spacer arm linked to a solid support [6,7].

Recently, a growing interest has emerged in a particular support: the anodic aluminium oxide support [8–14]. In the first report, the main interest in this support lies in the large surface area and a high volume concentration of membrane components as in naturally occurring membrane stacking in chloroplasts or mitochondria [8]. For the following reports, the interest was rather from an NMR

Abbreviations: AAO, anodic aluminum oxide; DOPE, 1,2-dioleoyl-sn glycero-3-phosphatidylethanolamine; EggPC, egg yolk L-α-phosphatidylcholine; HR-MAS, high resolution magic angle spinning; MLVs, multilamellar vesicles; NMR, nuclear magnetic resonance; PEG, polyethylene glycol; NHS, N-hydrosuccinimide; PET, polyethylene terephtalate; PFG-STE, pulsed-field gradient stimulated echo; POPC, 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphatidylcholine; SUVs, small unilamellar vesicles

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spectroscopy point of view, namely alignment of the sample in the magnetic field to enhance spectral resolution [9–14]. Indeed, the lipid bilayer, with (or without) protein embedded, covers the inner cylindrical pore surface of this support commonly used as filters. This supported model membrane seems to be an alternative approach to the various model membranes for structural solid-state NMR spectroscopic studies of complex membrane proteins coated on glass plates or bicelles [15–17].

Solid-state NMR also provides a study of molecular motions covering a large range of dynamic processes occurring in biological membranes [18]. This technique seems to be well suited for a non-invasive study; indeed, it does not require a fluorophore probe nor a planar surface as in the case of Fluorescence Recovery After Photo bleaching. In some NMR approaches, lateral diffusion can be extracted from exchange spectroscopy [19] and with higher precision by using a spherical support [20]. Another type of experiment consists of using pulsed-field gradients combined with stimulated echo sequences [21]. A series of bipolar gradients can be used to produce higher effective gradient strengths [22]. In a recent approach [23], diffusion measurements have been obtained by the combination of pulsed-field gradient experiments with magic angle spinning which allow for high spectral resolution. Advantages of PFG-STE solid-state NMR has been demonstrated on magnetically aligned bicelles [24].

In this report, we propose to examine by ^{1}H PFG-STE NMR the diffusion of the components of a tethered model membrane inside AAO. The diffusion of water, lipids and ubiquinone, a small diffusive molecule, a key component in the electron transport chains of mitochondrial and bacterial membranes, has been achieved. We have observed the effect of the cylindrical orientation of the lipids and the effect of the lipid bilayer formation through fusion of small unilamellar vesicles.

2. Materials and methods

2.1. Sample preparation

The AAO Anodisc 47 discs (Whatman, Maidstone, England) with a pore diameter of 200 nm and a thickness of 60 μm have a porosity of 80% and a pore density of 2.54×10^{9} pores per cm^{2}. The Anodiscs were cut into discs of about 3 mm diameter in order to stack 120 fully hydrated discs into a 4 mm MAS rotor. Amino groups were linked to the AAO surface by reaction with (3-aminopropyl) dimethylethoxysilane (Aldrich, Strasbourg, France). Then, the discs were dipped for 45 min in a 2.1-mM NHS-biotin solution in a phosphate buffer. After extensive rinsing, the supports were dipped for 10 min in a 40-mg/ml streptavidin (Sigma, Saint Louis, MO, USA) solution in a PBS buffer (phosphate buffer 0.01 M and NaCl 0.15 M). The supports were rinsed with an octylglucoside solution and then with the PBS buffer before mixing for 1 h with SUV mixture. The SUVs, composed of egg yolk L-α phosphatidylcholine, egg-PC, (Sigma, Saint-Louis, MO, USA) and 1,2-dioleoyl-*sn*-glycero-3-phosphatidylethanolamine, DOPE (Sigma, Saint Louis, MO, USA), in a molar ratio of 64.5/35% and containing 0.5% of 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylethanolamine-N-biotinyl (Avanti Polar Lipids, Alabaster, AL, USA) were obtained by sonication of the mixture at a concentration of 5 mM. The size of the vesicles was in the range of 30–50 nm. Fusion was promoted by treatment with a 30% (w/v) polyethylene glycol, PEG_{8000}, (Sigma, Saint Louis, MO, USA) solution. After rinsing the PEG, the final Tris buffer (pH 7.5) was introduced. The experiments with ubiquinone Q_{10} (Sigma, Saint Louis, MO, USA) were obtained from SUVs as mentioned above in a ratio of 1:2 (mol/mol) ubiquinone and phospholipids. For NMR diffusion measurements, the final buffer was replaced by deuterated water (99.9%, Merck, Germany).

For adsorbed phospholipids on a polymer film, 10 mg of EggPC dissolved in chloroform were spread on a polyethylene terephtalate, PET, strip. Chloroform was evaporated under vacuum in a closed vessel and then hydration was obtained with deuterated water in a saturated atmosphere for 2 days at 55 °C.

Multilamellar Vesicles (MLVs) were obtained by mixing 20 mg of EggPC in 100 μl of deuterated water. A homogeneous multilamellar dispersion was obtained by 4 cycles of freeze–thawing.

2.2. NMR experiments

The experiments were performed on a Bruker 500 Avance spectrometer (Wissembourg, France) with an 11.7 T field. The spectra were acquired at a resonance frequency of 500.13 MHz with a 4.2-μs 90° pulse and a 5-s delay between scans. The spectral width was 5 kHz and the number of acquisitions was 256. The 4-mm PFG-MAS probe with *z*-axis gradients was operated at a spinning rate of 5 kHz.

^{1}H NMR diffusion measurements were conducted using a PFG-STE sequence with sine-shaped bipolar gradient pulses of 1 ms duration and a longitudinal eddy current delay of 5 ms. Diffusion times were varied from 50 ms to 1 s. For each diffusion time, 16 different values of gradient strength varying from 0.01 to 0.60 T/m were used. At every gradient strength, 256 scans were acquired with a recycle delay of 5 s. Spectra were processed with an exponential multiplication equivalent to 3 Hz line broadening before Fourier transformation and were referenced to HDO. All measurements were performed at 30 °C.

The gradient strengths were calibrated on residual water (HDO) using a 20-μl deuterated water filled HR-MAS rotor with a spherical insert. The diffusion coefficient obtained for HDO, $(1.60 \pm 0.15) \times 10^{-9}$ m^{2} s^{-1} at 22 °C, was comparative to the value given by Bruker (1.872×10^{-9} m^{2} s^{-1} at 25 °C). The accuracy of the resonance intensities was within 5%.