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Polyelectrolyte-supported lipid membranes

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Abstract

In this work, we report the influence of the electrostatic interaction between lipid bilayer membranes and their solid polyelectrolyte multilayer support on the properties of the membrane. All involved sample preparation steps were carried out as convenient adsorption procedures from aqueous solutions. The lipid fluidity within the membrane as well as the surface coverage of the support could be tailored via the electrostatic interaction strength between the lipid bilayer and the supporting polyelectrolyte cushion. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Lipid bilayer membranes; Polyelectrolyte multilayers; Self-assembly; Neutron reflectometry; FRAP; Impedance spectroscopy; Surface plasmon spectroscopy

1. Introduction

Among the variety of concepts for supported model membranes, e.g. Refs. [1–4], the introduction of the electrostatic interaction to a polyelectrolyte-supported charged model membrane is expected to be a promising concept towards the goal of simulating a biological membrane in order to study its complex properties [5]. All preparation techniques involved in this approach are based on self-assembly processes, leading to a convenient water-based technique to build up a model membrane [6,7]. In the following, the results of structural and functional investigations on these assemblies are reported.

2. Experimental

The polyelectrolyte multilayers consisted of poly(ethyleneimine) (PEI, $M_W \approx 50–60$ kg/mol, 50 wt.% aqueous solution, Aldrich), poly(4-styrenesulfonicacid) sodium salt (PSS, $M_W \approx 70$ kg/mol, Aldrich) and poly(allylamine) hydrochloride (PAH, $M_W \approx 50–60$ kg/mol, Aldrich) and were prepared via the adsorption process from solutions described by Decher et al. [6] on functionalized Au or SiO_x substrates. The lipids used for the preparation of the bilayers were dimyristoyl-L-α-phosphatidylglycerole (DMPG, negatively charged in aqueous solution), dimyristoyl-L-α-phosphatidylcholine (DMPC, zwitterionic) and nitrobenzoxadiazole-C_4-L-α-phosphatidylcholine (NBD-C_4-PC) as a dye for the fluorescence measurements. Lipid mixtures were prepared in order to dilute the charge density of the lipid bilayer.

Uni-lamellar lipid vesicles (ULV) were prepared via the extrusion technique [8]. The sizes of the formed vesicles were determined via dynamic light scattering technique (DLS) [9]. The ULVs were floated onto the swollen polyelectrolyte support [7]. All aqueous preparation steps were carried out in MilliQ water or in buffer solution (pH 5.4 for impedance spectroscopy), respectively.

The polyelectrolyte-supported bilayers were investigated by means of time-dependent surface plasmon spectroscopy (SPS) [10,11], neutron reflectometry (NR) [12], impedance

Table 1
Hydrodynamic radius R_H of the liposomes prepared via the vesicle extrusion technique measured via dynamic light scattering

Lipid system	R_H (nm)
DMPC	48.4 ± 0.5
DMPG	18.0 ± 2.0
DMPC/DMPG (10:1)	53.0 ± 2.0
DMPC/DMPG/NBD-PC (10:1:0.1)	52.0 ± 1.0

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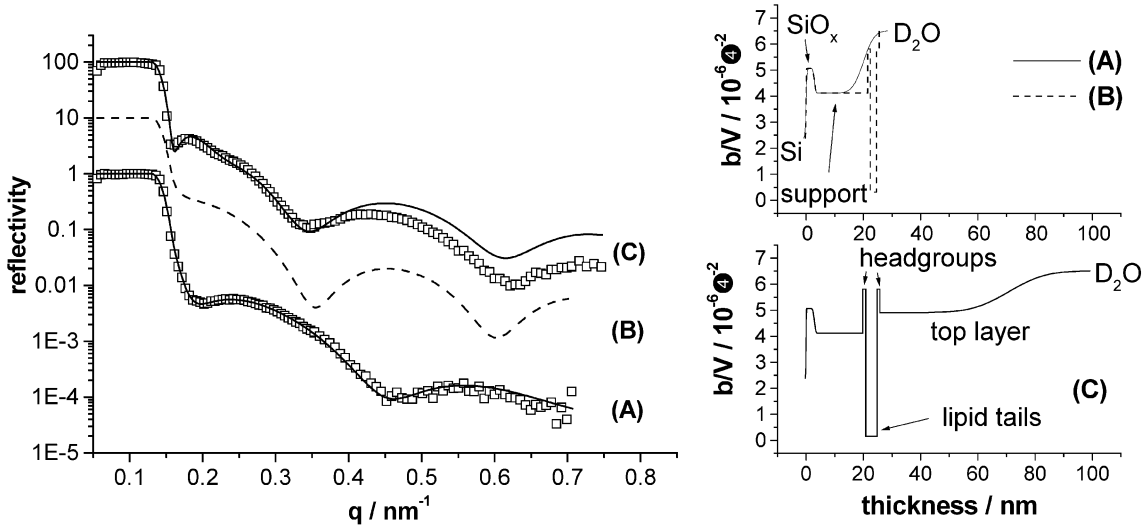

Fig. 1. Left-hand side: NR data of (A) the support without lipid, (C) DMPC/DMPG (10:1) on the support (4 PSS/4 PAH). The solid lines in (A) and (C) represent the model calculations of the data according to the parameters in Table 2. The doted line (B) represents a simulation of a lipid bilayer without an additional non-specific layer on top (see text). The curves were artificially shifted for clarity. Right-hand side: calculated profiles of the scattering length density b/V corresponding to the calculations (A): blank support, (B) dotted: theoretical profile without non-specific top layer and (C) the supported bilayer plus non-specific top layer.

spectroscopy (IS) [13,14], as well as fluorescence recovery after photobleaching (FRAP) [15].

3. Results and discussion

The swelling mechanism and structural properties of the polyelectrolyte support were reported earlier [16]. The polyelectrolyte support used for these studies consisted of 4×4 polyelectrolyte layers of alternating charge. The equilibrium swollen thickness in the aqueous environment was 17.1 ± 0.5 nm obtained from NR measurements (see below). This architecture was reported to provide an optimum support for lipid bilayers [17].

The crucial property of the lipids is their ability to form stable and uniform monolayers and bilayers. In the case of lipid mixtures, this ability is strongly related to their miscibility. The miscibility of lipids is predicable from the difference of the phase transition temperatures (T_m) of the lipids involved. Perfect miscibility occurs if $\Delta T_m = T_{m_1} - T_{m_2} = 0$. T_m values for the lipids used in this work were T_m (DMPC) = T_m (DMPG) = 23 °C [18]. According to these values, all mixtures of DMPC and DMPG were expected to be stable due to the perfect miscibility.

The sizes of the lipid vesicles prepared from lipid mixtures and pure lipids were investigated with DLS. The resulting hydrodynamic radii are summarized in Table 1. The DLS experiments yielded diameters of neutral liposomes and liposomes with diluted charge of twice the pore diameter of extrusion membrane. Completely charged liposomes were smaller, approximately 30% of the neutral vesicles diameter. The effect was discussed in the literature [19]. Due to their small size and thus their large mechanical surface tension due to the high curvature, the fully negatively charged DMPG liposomes were expected to fuse readily onto the 'attractive' polyelectrolyte support terminated by a positively charged PAH top layer.

SPS kinetics yielded the equilibrium adsorption time of the ULVs onto the swollen polyelectrolyte support (5–7 h for both types of vesicles). The thickness of the bilayer

Table 2
Parameters of the model calculations shown in Fig. 1

Calculation parameter	L_{SiO_x} (nm)	$b/V_{support}$ ($\times 10^{-6}$ Å^{-2})	$L_{support}$ (nm)	$\sigma_{support}$ (nm)		
Support (A)	2.9 ± 0.2	4.1 ± 0.2	17.1 ± 0.2	3.0 ± 0.2		
Calculation parameter	$b/V_{headgroup}$ ($\times 10^{-6}$ Å^{-2})	$L_{headgroup}$ (nm)	b/V_{tails} ($\times 10^{-6}$ Å^{-2})	L_{tails} (nm)	$b/V_{top\ layer}$ ($\times 10^{-6}$ Å^{-2})	$L_{top\ layer}$ (nm)
Supported bilayer (C)	5.8 ± 0.3	1.0 ± 0.2	0.2 ± 0.1	4.0 ± 0.2	4.9 ± 0.5	45.0 ± 0.5

L denotes layer thicknesses, b/V scattering length densities, and σ surface roughness.

formed via fusion of DMPC/DMPG (10:1) vesicles onto the support (4 PSS/4 PAH) was estimated by SPS. The Fresnel calculations resulted in a thickness of the adsorbed lipid layer of $L = 14.5 \pm 1.5$ nm. The thickness was significantly larger than the thickness of a DMPC bilayer reported in the literature ($L = 4.3$ nm, Ref. [20]), indicating that probably non-fused vesicles were left on top of or within the bilayer.

The NR experiments with the DMPC/DMPG (10:1) system resulted a similar structural picture (Fig. 1). The simplest model to simulate the experimental reflection data obtained from the supported bilayer included a distinct bilayer box profile plus a non-specific layer on top (profile C in Fig. 1; for simulation parameters, see Table 2). The top layer was approximated by the uniform layer with the average scattering length density b/V shown in Fig. 1 and Table 2. The thickness of the top layer was well defined by the visible modulation in the reflection data with a short period in q. The thickness of the top layer determined from the mentioned modulation was in the order of the radius of the fused liposomes. The modulation was strongly damped, indicating a rough interface towards the superstrate. The simulation without the additional non-specific top layer did not describe the data in a satisfying way (dotted curve B and profile B in Fig. 1).

The lateral fluidity of similar DMPG/DMPC bilayers was measured via FRAP (Fig. 2, see below). The surface coverage of the lipid material (Fig. 3) was calculated from the membrane capacity obtained via IS [14]. FRAP showed a decreasing lateral lipid fluidity with an increasing charge density of the lipid mixture, whereas IS showed an increasing surface coverage, and thus, a decreasing membrane capacitance. These competing dependencies were closely related to the concentration of negatively charged lipid head groups in the bilayer and were interpreted as follows: the negatively charged head groups were attracted and immobilized by the positively charged top layer of the support. The electrostatic interaction hindered the lateral lipid diffusion

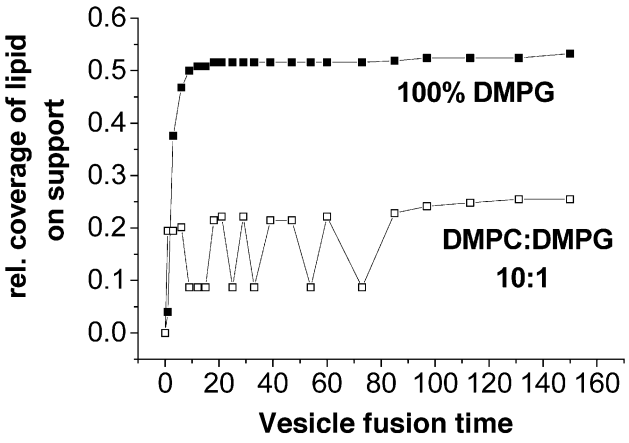

Fig. 3. Surface coverage calculated from membrane electrical capacity measured via IS.

but was, however, favorable for the formation of a stable and dense bilayer.

4. Conclusions

Lipid bilayer membranes were formed via the adsorption of vesicles prepared from lipid mixtures. The entire supported bilayer assembly was based on convenient adsorption processes from liquids. NR as well as SPS measurements indicated the existence of a layer on top of the bilayer, most likely formed by imperfectly enrolled vesicles.

Crucial membrane properties such as the lateral fluidity and membrane surface coverage appeared to be competitive with respect to the interaction strength between the charged membrane and support. Both properties could be tailored via the strength of the electrostatic interaction (simply by changing the concentration of the charged lipid in the mixture). However, the investigated membrane characteristics were not yet near to the ideal values reported in the literature. Further work is needed to find an optimum situation between the

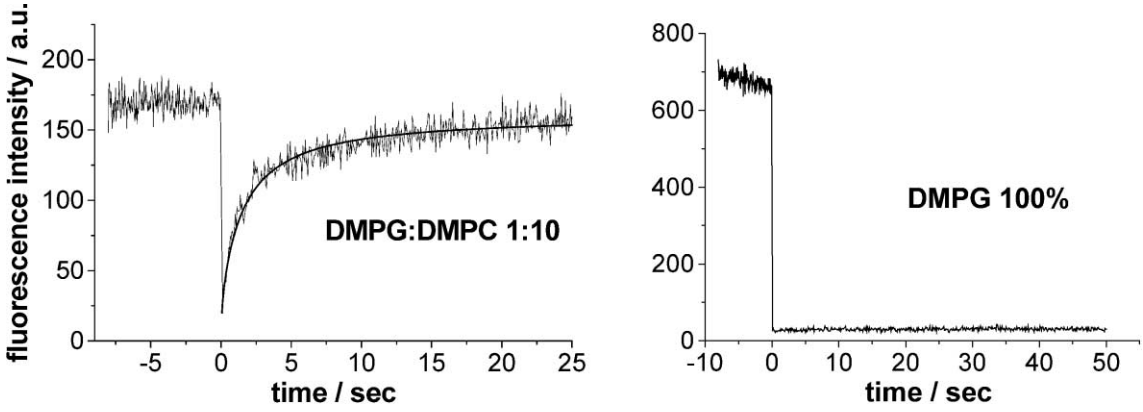

Fig. 2. Membrane fluidity dependent on electrostatic interaction strength between the support and bilayer measured via FRAP. Left-hand side ULVs consisting of DMPG/DMPC (1:10) at $T = 39$ °C. The solid line represents a fit of the data based on Fickian diffusion with a lateral diffusion coefficient $D = 0.80 \times 10^{-8}$ cm^2/s and a relative recovery of the fluorescence intensity of 89.7%. Right-hand side: pure DMPG ULVs.

competition between lateral fluidity and surface coverage of the lipid bilayer.

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