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Origins of extreme boundary lubrication by phosphatidylcholine liposomes

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ABSTRACT

Phosphatidylcholine (PC) vesicles have been shown to have remarkable boundary lubricating properties under physiologically-high pressures. Here we carry out a systematic study, using a surface force balance, of the normal and shear (frictional) forces between two opposing surfaces bearing different PC vesicles across water, to elucidate the origin of these properties. Small unilamellar vesicles (SUVs, diameters < 100 nm) of the symmetric saturated diacyl PCs DMPC (C_{14}), DPPC (C_{16}) and DSPC (C_{18}) attached to mica surfaces were studied in their solid-ordered (SO) phase on the surface. Overall liposome lubrication ability improves markedly with increasing acyl chain length, and correlates strongly with the liposomes' structural integrity on the substrate surface: DSPC-SUVs were stable on the surface, and provided extremely efficient lubrication (friction coefficient $\mu \approx 10^{-4}$) at room temperature at pressures up to at least 18 MPa. DMPC-SUVs ruptured following adsorption, providing poor high-pressure lubrication, while DPPC-SUVs behavior was intermediate between the two. These results can be well understood in terms of the hydration-lubrication paradigm, but suggest that an earlier conjecture, that highly-efficient lubrication by PC-SUVs depended simply on their being in the SO rather than in the liquid-disordered phase, should be more nuanced. Our results indicate that the resistance of the SUVs to mechanical deformation and rupture is the dominant factor in determining their overall boundary lubrication efficiency in our system.

1. Introduction

Liposomes are closed, largely-spherical vesicles of colloidal dimensions, consisting of phospholipids (PL) that form a self-closed bilayer membrane. Due to their composition and structure they are biocompatible, and thus serve as a useful tool for biomedical applications including drug delivery systems, signal enhancers in medical diagnostics, vaccines, gene delivery systems and solubilizers for different substances [1–3]. Liposomes and phospholipid bilayers are also an important model system in basic studies of biological membranes [4], as phospholipids are the dominant components of such bilayer membranes and are responsible for many of their unique physicochemical and biochemical properties [5,6]. PLs have been conjectured to lubricate articulating cartilage [7–10] in the classical boundary lubrication mode, where the surfactant alkyl tails are exposed at each surface and slide past each other [7]. Recently, however [11], it was shown that, for boundary lubrication between self-assembled layers of polar surfactants under water, the slip-plane shifted from the mid-plane between the surfactant layers to the headgroup/substrate interface. This is in contrast to classical boundary lubrication [12,13] (which was assumed also for PLs on cartilage [7–10]), and resulted from the hydration of the polar-head-groups, and the consequent hydration lubrication acting at that interface [11,14].

Several studies of lubrication by PL layers or multilayers, and by surface-attached liposomes have since been reported [15–19]. Low friction was demonstrated with 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC) bilayers using lateral force microscopy [15]. In another study, friction properties of liquid phase phosphatidylcholine (PC) bilayers were examined by means of a tribometer. A friction coefficient of $\mu = 0.035$ was demonstrated in Tris buffer and the importance of mechanical stability under shear or normal load for good lubrication performance was emphasized [16]. The importance of lubricant stability was emphasized in another work where 1,2-dioleoyl-*sn*-glycero-3-phosphatidylcholine (DOPC) bilayers were studied. In this work it was also demonstrated that liquid phase bilayers are less efficient lubricants than gel phase bilayers [17].

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Friction and wear tests using cartilage on cartilage configuration with lubricating fluids containing vesicles composed of different phospholipids or their mixtures (both small unilamellar vesicles, SUVs, and multilamellar vesicles, MLVs) showed static and dynamic friction coefficients (at 37 °C) down to around 0.02 and 0.01, respectively [18]. Lower values of $\mu = 0.002$ were measured on soft (hydrogel) surfaces with adsorbed 1,2-dipalmitoyl-*sn*-glycero-3-phosphatidylcholine (DPPC) lipid bilayers in the gel phase at mean contact pressures of 0.3 MPa [19], while in the same study layers of DOPC (in their liquid phase) were shown to be poorer boundary lubricants (in Ref. [19] a review of several earlier friction studies with PL is given). This was attributed to the DPPC bilayers' better resistance due to being in the gel (or solid-ordered, SO) phase (in contrast to DOPC which was in its liquid disordered, LD phase) and to the hydration lubrication effect [20,21], that will be later discussed in more detail. A common feature of all these earlier studies [15–19] between opposing PL layers or multilayers was the low maximal mean pressure attained between the sliding surfaces, with none – as far as we can ascertain – exceeding about 1–2 MPa. This is notable because pressures in the major joints, where PLs have been conjectured to play a lubricating role [7,8,22], attain values of ca. 5 MPa in normal conditions, with maximal pressures in hips recorded up to ca. 18 MPa [23].

Very recently, it was discovered that SUVs of hydrogenated soy phosphatidylcholine (HSPC), attached as close-packed layers on solid (mica) substrates sliding past each other across aqueous media, could provide extremely efficient lubrication at physiologically-high pressures [24,25]. Friction coefficients as low as $\mu \approx 2 \times 10^{-4}$–$2 \times 10^{-5}$ were measured at pressures of up to at least 12 MPa, and attributed to the hydration lubrication mechanism arising from the highly-hydrated phosphocholine headgroups exposed at the outer surfaces of the HSPC vesicles. The persistence of the efficient lubrication up to high pressures was attributed to the smoothness and uniformity of the close-packed layers of the adsorbed vesicles, and in particular to the robustness to pressure of the liposomes. Much higher friction coefficients ($\mu = 0.065$) were measured for SUVs of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) liposomes [25], while at the same time the POPC vesicles showed evidence of rupture and squeeze-out from between the surfaces. HSPC (for which the SO-to-LD transition temperature for *unsupported*, or free-standing bilayers (FSBs), T_M(HSPC) = 53 °C) is in its SO phase at the room temperature of the studies, while POPC (T_M(POPC) = −3 °C) was in the LD phase (we discuss later the important issue of SO-to-LD phase transitions of *supported* bilayers, which is the relevant configuration in our study). It was conjectured, therefore [25], that surface-adsorbed layers of PC-SUVs would be highly-efficient lubricants up to high pressures when in the SO phase, but would be poor lubricants, particularly at higher pressures, if they were in their LD phase. However, whether this conjecture is correct, and indeed the entire issue of how the structure of PC phospholipids, and their corresponding vesicle structure and phase state, is related to efficient lubrication by surface-attached liposomes at high pressures, remains unknown. In the present study we endeavor to gain further insight into the origin of high-pressure lubrication by liposomes through a systematic study of a series of PC-SUVs, using a Surface Force Balance (SFB) in parallel with determination of their microscopic structure on the surface. We examine the lubricating behavior of surface-attached liposomes composed of three PC lipids with increasing acyl chain length: DMPC ($[C_{14}]_2$), DPPC ($[C_{16}]_2$) and DSPC ($[C_{18}]_2$). DPPC and DSPC are examined in the gel phase, and DMPC is studied in the SO phase and also in the mixed phase within the range of the SO-to-LD transition. Together with parallel examination of the structure of the surface-attached vesicles using atomic force microscopy (AFM) and cryo-scanning electron microscopy (cryo-SEM) we are able to shed strong light on the origins and determinants of friction reduction by surface-adsorbed PC vesicles.

2. Experimental techniques

2.1. Liposome preparation

The lipids, 1,2-dimyristoyl-*sn*-glycero-3-phosphatidylcholine (DMPC, 14:0), 1,2-dipalmitoyl-*sn*-glycero-3 phosphatidylcholine (DPPC 16:0) and 1,2-distearoyl-*sn*-glycero-3-phosphatidylcholine (DSPC, 18:0), were purchased from Lipoid (Ludwigshafen, Germany). The main SO-to-LD transition temperatures of unsupported bilayers of these PCs are 24 °C, 41 °C and 54 °C respectively [26]. SUVs of each PC were prepared using standard approaches [25,27], both in our lab and by Y. Barenholz, R. Cohen and H. Kanaan in the Biochemistry Department of the Hadassah Medical School of the Hebrew University, Jerusalem. Briefly, lipids were dispersed in water, bath-sonicated for 5min and homogenized for 5 min at the appropriate temperature above the main phase transition of each lipid in order to obtain dispersed multilamellar vesicles (MLV). Next, the MLVs were progressively downsized using an extruder (Northern lipid Inc, Burnaby, BC, Canada) through polycarbonate filters having defined pore sizes starting with 400 nm (3 cycles), 100 nm (4 cycles) and ending with 50 nm (10 cycles). The PC-SUVs were characterized by dynamic light scattering (DLS) and subsequent to their adsorption on the surface, also by AFM and cryo-SEM. The DLS-derived dimensions of the solution-dispersed liposomes were in line with those estimated from AFM and cryo-SEM micrographs of the surface-attached vesicles (see later), though these latter had a spread of dimensions on the surfaces due to the adsorption process [25]. The water used throughout both for the liposome preparation and subsequent measurements was highly-purified (so-called conductivity) water from a Barnstead NanoPure system, with total organic content (TOC) ca. 1 ppb and resistivity 18.2 MΩ.

2.2. Preparation of liposome-coated surfaces

Liposome covered mica surfaces were prepared as follows. Freshly cleaved mica (mounted on cylindrical lenses for use in the SFB, see below) was placed in a 0.3 mM SUV liposome dispersion prepared with Barnstead purified conductivity water. Incubation was at room temperature, except for DMPC which was incubated at 20 °C (and at 28 °C in one experiment, as described below). After overnight incubation, the surfaces were rinsed to remove excess material by placing them in a beaker containing 300 ml water for 30 min, along with a delicate shaking motion. Surfaces prepared for SFB always passed the air–water interface, whereas for examination in the AFM, the surfaces did not pass an interface unless the effect of passing an interface was examined as described later. It is relevant for later understanding to note that, on withdrawal from the dispersion and subsequently from the rinsing beaker, the surfaces were partly coated by water in the form of wet patches separated by dry regions.

2.3. Atomic force microscopy (AFM)

Imaging of surfaces was carried out with an MFP-3D SA (AFM) instrument (Asylum Research, Santa Barbara, CA). Scanning in tapping mode in conductivity water was done using a silicon nitride V-shaped 115 μm long cantilever having a nominal spring constant of 0.35 N/m with a pyramidal silicon nitride tip (NP, Bruker).

2.4. Cryo SEM

Samples were plunged into liquid ethane using a custom made spring-loaded plunger. The size of the mica surfaces with the adsorbed liposomes was ca. 4 mm × 2 mm; they were generally covered by a water droplet which was gently imbibed by tissue prior to freezing. The frozen samples were mounted on a holder and transferred to a BAF 60 freeze fracture device using a VCT 100 Vacuum Cryo Transfer device (both instruments from Leica Microsystems, Vienna), etched at −80 °C for 2 h and coated with 3 nm Pt/C by double axis rotary shadowing. Samples were transferred to an Ultra 55 SEM (Zeiss, Germany) using the VCT 100 and were observed at a temperature of −120 °C at 2 kV using an in-lens secondary electron detector.

The electron microscopy studies were conducted at the Irving and Cherna Moskowitz Center for Nano and Bio-Nano Imaging at the Weizmann Institute of Science.

2.5. Surface Force Balance (SFB)

The technique and detailed procedures for the measurement of normal (F_n) and shear (F_s) forces between molecularly-smooth mica surfaces, half- silvered on their back side, in a crossed cylinder configuration (mean radius of curvature R), a closest separation D apart have been described in detail previously [28]. D is optimally measured to ±2–3 Å by monitoring the wavelength of optical interference fringes of equal chromatic order (FECO). The results presented here are based on nine separate experiments, each with several different contact points between the interacting