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Biolubrication from Phospholipid Membranes: Correlation between nanomechanical resistance and tribological properties

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Abstract

The analysis of joint molecular interfaces has led to the identification of lipid bilayers adsorbed on the joint rubbing surfaces.
Nanostructural physical techniques such as lipid deposition by vesicle fusion method or by co-adsorption of lipid-detergent micelles and atomic force microscopy are used to reproduce and characterize the nano-mechanical resistance of supported phospholipid bilayers forming biological rubbing surfaces.
We have studied the mechanical and tribological properties of DOPC fluid bilayers in different solutions. We observed a clear correlation between membrane resistance (probed by AFM force spectroscopy) and the tribological properties of lipid bilayers (using our homemade biotribometer). The latter allows simultaneously the measurement of the friction coefficient and the visualization of surface degradation by fluorescence microscopy.
In both buffered and buffered saline solution, phospholipid bilayers are more resistant and present better lubricant properties. The addition of salt improves the resistance to indentation but not to shear.
Our study thus showed that good mechanical stability of the bilayers is essential and suggested that the low friction coefficient is ensured by the hydration layers between adjacent lipid bilayers, with an important role of ions.

Keywords: Phospholipid bilayers; biolubrication; friction coefficient; nanomechanics, atomic force spectroscopy

1. Introduction

Phospholipids, together with proteins, are the major components of biological membranes and play vital roles in many biological processes. Supported phospholipid bilayers (SPB) composed of phospholipids adsorbed to a planar solid support are widely used as models to investigate the properties

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of these membranes and associated processes such as molecular recognition, enzymatic catalysis, cell adhesion and membrane fusion [1-3]. SPB are also important for a number of applications including biosensors design, solid surfaces and biomaterials biofunctionalization, protein crystallization and DNA immobilization [2,4,5]. In particular, phospholipids' layers are found in the synovial fluid and appear to play a key role in joint lubrication in controlling and reducing frictional forces between biological surfaces [6-9].

Beside synovial joints, phospholipids' layers are also present elsewhere within the human body in tissues including pleura, pericardium and peritoneum where they provide effortless sliding of the tissues. While for load bearing synovial joints, a deficiency in the lipidic multilayers is associated with osteoarthritis, as phospholipids' layers perform multiple roles (in the peritoneum, they represent the lubricant/release agent preventing surgical adhesions, and allow membrane semipermeability for peritoneal dialysis), in each human body site, the prophylactic use of exogenous phospholipid layers may have potential clinical applications [10,11].

The physical and chemical properties of biological membranes are of critical importance to understand specific membrane function. The stability of SPB is a major concern for the use of such layers in these various applications.

The atomic force microscopy (AFM) provides an important way to measure this stability and to observe the nature of bilayer defects. Atomic force spectroscopy gives valuable experimental information about the interaction forces and mechanical behavior of the studied systems with nanometric and nanonewton resolution through the force-distance curves. Thus, a quantitative measurement of the force at which the jump occurs can shed light on basic information concerning cell membrane nanomechanics as well as interaction forces between neighboring lipid molecules in the membrane. Therefore, this force is closely related to membrane stability [12].

In order to better understand the molecular mechanisms responsible for the lubricating ability of phospholipids bilayers, it is essential to vary different physico-chemical parameters (temperature and phase of the lipid bilayer, ions, pH, viscosity of the buffer, etc.), their preparation method, the roughness of the support surface and to measure the structure and the mechanical properties of the contact (lipid packing, water layers, resistance of the bilayers to friction or normal load, lipid mobility and mobility of the fluid around the bilayer, etc.).

Thus, the aim of the present work is to study in more details these DOPC fluid bilayers and to investigate the influence of the lubricant composition on their tribological properties in order to better understand the molecular mechanisms responsible for lubrication by phospholipid bilayers.

2. Material and methods

2.1. Material

As fluid phospholoipid, we used (DOPC) 1,2-dioleoyl-sn-glycero-3-phosphatidylcholine wich was solubilized to 1mM together with 1% (wt %) NBD-PC, 1-palmitoyl-2-{6-[(7-nitro-2-1,3-benzoxadiazol-4- yl)amino] hexanoyl}-sn-glycero-3-phosphocholine in chloroform/ ethanol (9/1, v/v). NBD-PC which has a tail anchored fluorescent part is used to visualize the bilayer homogeneity by fluorescence microscopy.

As lubricants we used: ultrapure water {W} pH 5.5, TRIS buffer {T} pH 7.2, saline 150mM NaCl {S} pH 5.8 and buffered saline solution TRIS, 150mM NaCl {TS} pH 7.2.

Resistance to nano-indentation is measured by an AFM (force spectroscopy mode) while friction and bilayer degradation under shear are measured with our biotribometer.

2.2. Preparation of fluid phase phospholipid bilayers.

We used an 8 mm radius convex soft HEMA lens (Corneal Industrie, Annecy, France) and a flat borosilicate glass plate as the surfaces on which lipid bilayers were deposited for tribological measurements. HEMA has a ~1 nm rms roughness and a Young modulus of about 1.5 MPa [13], When swollen in saline solution (150mM NaCl, actual pH 7.2), the HEMA lens contains 25% water (wt %) and has mechanical and physicochemical properties similar to those of articular cartilage [14].
Borosilicate glass surfaces are transparent, rigid and very flat. They can therefore be used for fluorescence microscopy, AFM imaging, AFM nano-indentation as well as tribological experiments.

SPB were prepared by co-adsorbing DOPC and DDM surfactant (cmc = $1.67.10^{-4}$ mol/l) on glass and HEMA lens surfaces from mixed micellar solutions according to Tiberg et al. [15] using one and two incubations. SPB were conserved in ultrapure water or 15mM Tris buffer pH 7.2 and used within a day to prevent bilayers to develop defects over time.

2.3. Atomic force microscopy

Measurements were carried out with a commercial AFM (Multimode NanoScope III, Veeco Instruments, Santa Barbara, CA) equipped with a liquid J-scanner. Force plots were acquired using V-shaped silicon nitride tips. Individual tip radii were found to be 20-40 nm. Thousands of approach-retraction cycles were performed at several locations of the lipidic bilayer and the cantilever deflection was recorded versus the Z-piezo position of the AFM (Fig.2A). The tip-sample approaching velocity was set for all force curves at 400 $nm \cdot s^{-1}$ so that the effect of the velocity on the breakthrough force could be totally neglected. Jump distances, breakthrough and adhesion forces were automatically calculated using our own *C++* code.

2.4. Experimental setup for tribological measurements

A homemade biotribometer (Fig.1A) permitting in situ visualization of the contact was used to measure the frictional forces between the two contact surfaces: a compliant soft HEMA lens and a flat borosilicate glass plate, each surface being covered with one DOPC bilayer as previously described [13-14]. An upright epifluorescence microscope (Leica DMLM) equipped with a CCD camera (Leica DC350F) was used to view the contact through the glass body. This observation was performed in situ during friction and under white and blue light to visualize the centering of the contact area (see the border of the contact area, arrow in Fig.1B) and the bilayer integrity respectively (Fig.1B).

An eddy current position sensor measured the deformation of the flexible blades holding the tank, and permitted calculating the tangential force. An average normal pressure of 0.3 MPa (realistic in the context of joint biolubrication) was imposed, resulting in a contact area diameter of about 2 mm independent of the bilayer type. The friction coefficient μ was defined as the ratio between the tangential force (once the surfaces slide against each other) and the normal load. Constant 0.6 mm/s sliding velocity and 6.9 mm sliding amplitude for 50 min (about 150 back and forth cycles) were imposed and controlled. These severe conditions (i.e., low value of speed and high contact load) permitted simultaneously a good visualization of the contact by the optical microscope and to impose a lubrication regime of boundary type, characteristic of biological contacts that often operate under similar conditions [16-17].

Mean and min-max (for the error bar) values of both initial and final friction coefficient (i.e., just after the beginning and after 50 min of friction, respectively) were calculated.

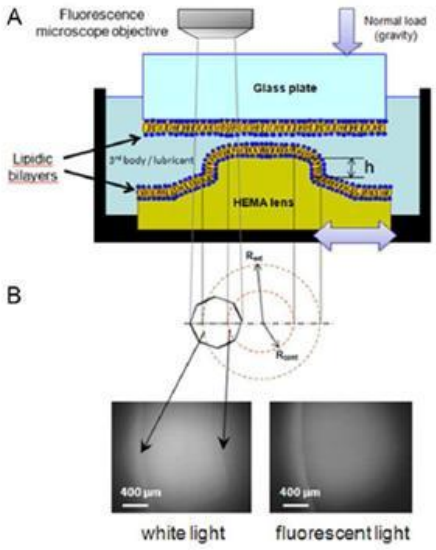

Fig. 1. Experimental setup for tribological measurements; (A) Ex vivo contact model; (B) Contact visualization using white and fluorescent light: Initial control image (before starting sliding the surfaces) of the contact border; DOPC-NBD SPB on each contact surface.

3. Results

3.1. Nano-mechanical properties of SPB

We have investigated the nano-mechanical resistance to indentation of DOPC bilayers by AFM force spectroscopy. We have recorded thousands of AR curves on SPB up to a maximal load of 20 nN. A crude estimate of the corresponding pressure exerted by the tip is obtained by dividing the measured force at rupture with a geometrical contact area $A=2\pi R\,\varepsilon$ when the bilayer is indented by a distance ε. By taking $\varepsilon=h/2$ ~2 nm (half of DOPC bilayer thickness), R~30 nm for the tip radius used in this sudy, we obtain the range of maximal pressure investigated P=60~ 240 MPa much higher than the physiological value.

We have listed in Fig.2 the statistics of nano-indentation experiments in ultrapure water (pH 5.5), saline solution (150 mM NaCl, pH 5.8), Tris buffer solution (pH 7.2), and Tris saline buffer (150 mM NaCl, pH 7.2). Each color corresponds to a different experiment with a different bilayer:

In ultrapure water, SPB with either one or two incubations were puncturated in 100% of the cases. However, one can note a clear difference between the two conditions: the distribution of breakthrough forces is peaked at about 0.375 nN in the case of one incubation (Fig.2B) while breakthrough forces are higher with a second broad peak between 1 and 3 nN in the case of two incubations (not shown). The jump distance distribution is independent of the tip used. It shows a large peak centered at 4.75 nm and 4.35 nm for one (Fig.2C) and two incubations (not shown) respectively. In a saline solution, the force

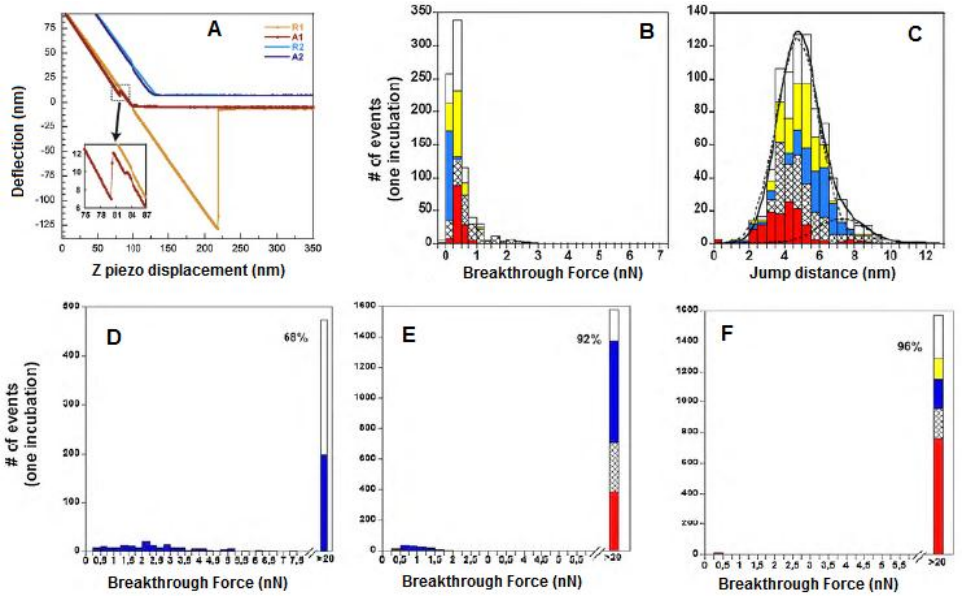

Fig. 2. Force spectroscopy by AFM experiments on a DOPC NBD SPB bilayer prepared by coadsorption method with one incubation. (A) Typical deflection-distance curves. The cantilever spring constant value is K = 0.08 N/m. Legend: A and R refers as approaching and retracting curves respectively, numbers as successive deflection–distance cycles. Cycles 1 exhibit a breakthrough feature with jump of about 4.6nm in the approaching curve at a force level of about 0.88nN and large adhesion peak of about 9.4nN in the retracting curves (see the enlarged region in the inset). Cycle 2 on the other hand does not display either jump or adhesion. (B, D, E and F) Histograms corresponding to the breakthrough force and (C) the jump distance. (A and C) in ultrapure water pH 5.5, (D) a saline solution (150mM NaCl, pH 5.8), (E) a Tris buffer (15mM Tris–HCl, pH 7.2) and (F) a Tris saline buffer (15mM Tris–HCl, 150mM NaCl, pH 7.2). Colours correspond to different experiments with different samples. In (C), the solid line is a fit with two Gaussians which are individually plotted as dotted lines.

curves are drastically changed and we observe that the bilayer resists to forces larger than 20nN with a frequency of 68% (Fig.2D). When the Tris buffer was used, only a few penetrations are observed either with one (Fig.2E) or two incubations (not shown). The penetration frequency is nearly zero in the Tris saline buffer with one (Fig.2F) or two incubations (not shown).

3.2. Friction coefficients and degradation of DOPC bilayers

We have measured the friction coefficient μ between hydrophilic surfaces (a convex lens in soft HEMA articulated against a flat borosilicate glass plate) each covered or not with a DOPC bilayer. We have also investigated the effect of buffer and salt. Results are summarized in Fig. 3A. For bare contact surfaces (without SPB), the initial friction coefficient is larger in ultrapure water (μ=0.125) than in saline (μ=0.11) or Tris solutions (μ~0.09, with or without salt). The final friction coefficient values after 50 min of friction follow the same tendency: a decrease when ions concentration and/or pH is increased. In ultrapure water, we even stopped the friction test after 20 min because a friction coefficient higher than 0.15 could damage glass surfaces. In the presence of SPB, the initial friction coefficient in ultrapure water (μ =0.075) is reduced as compared to bare surfaces, however, after 20 min, the friction test was

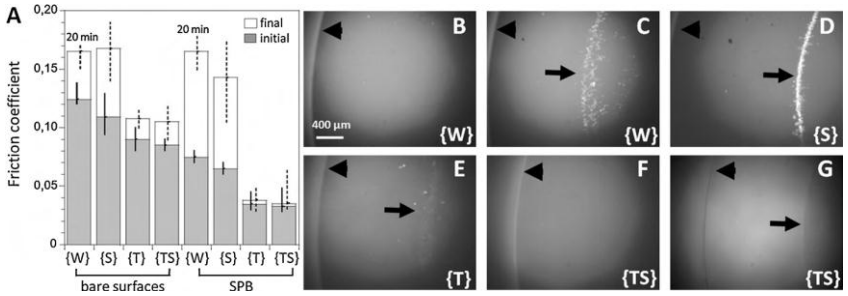

Fig 3. Effect of lubricant on the tribological behaviour of DOPC supported bilayers prepared with two incubations (A). Gray bars represent the initial value and white bars the final value after 50 min of friction (except two measurements stopped after 20 min due to excessive friction coefficient that may damage the glass surfaces). Error bars indicate the range between minimum and maximum measured values. In situ fluorescence (B–F) and white light (G) images of the recorded border of the contact area before (B) and after prolonged friction (C–G). The white light image (G) was recorded just before image (F) to identify the position of the border of the contact area. Abbreviations: *{*W*}*, water pH 5.5; *{*S*}*, saline solution pH 5.8 with 150mM NaCl; *{*T*}*, Tris buffer pH 7.2; *{*TS*}*, Tris saline buffer pH 7.2 with 150mM NaCl.

stopped as the value of μ reached the same very high value as bare surfaces. Strong bilayer degradation is visible by fluorescence microscopy (Fig.3C). In the saline solution, the initial friction coefficient with SPB in contact is sligthly smaller than in ultrapure water but again increased to μ=0.14 after 50 min of friction. We observed bilayer degradation (Fig.3D). In the other solutions, the situation is drastically changed. Both in Tris and Tris saline buffer, the friction coefficients are low and stable during prolonged friction (*i.e.*, μ=0.035) with little effect of salt if any.

This stability of the friction coefficient value in the Tris solution (Fig.3E) is accompanied by little bilayer degradation on the border of the contact region. In the Tris saline solution (Fig.3F), the bilayer fluorescence remains homogenous even in the border of the contact region (arrow in Fig.3G).

4. Discussion

We observe a positive correlation between resistance to normal indentation, low and stable friction coefficient and absence of bilayer degradation during shear.

In ultrapure water (pH 5.5), SPB are easily punctured, promptly damaged and their lubricant properties are lost after 20 min of friction (μ~0.15 as found for bare surfaces). This correlation also exists in a Tris buffer (pH 7.2) or in a Tris saline buffer (150mM NaCl, pH 7.2), as bilayers fully resist to indentation forces up to 20nN, exhibit low and stable friction coefficients (μ= 0.035) and are not or weakly damaged under shear. However, in a saline solution (150mM NaCl, pH 5.8), bilayers resist to normal indentation but their tribological properties are quickly degraded. The good resistance to indentation of DOPC bilayer in the saline solution is in agreement with a detailed force spectroscopy study that showed that the higher the ionic strength, the higher the force that must be applied with the AFM to penetrate the bilayer [12]. The authors explained the higher resistance to indentation as an increase in lateral lipid–lipid interactions (i.e., bilayer cohesion) promoted by ions (Na^+, Ca^{2+}) bound into the membrane.

Friction coefficient of fluid phase (DOPC) bilayers remains nearly one order of magnitude larger than the solid phase (DPPC) bilayers previously measured [13]. Even if they bear a similar charge in a given buffer, even if they resist to shear and normal stress, the mechanical response under high pressures of fluid bilayers is probably different than that of solid ones. The coupling between lipid mobility in the bilayers (which is different for solid and fluid ones) and surrounding liquid, along with localizing

precisely the slip plane may be the key steps toward the understanding of biolubrification mechanisms by lipid layers [18].

5. Conclusion

We have studied the mechanical and tribological properties of bilayers prepared by the co-adsorption method in different solutions.

We have shown that the frictional force between the sliding surfaces coated with fluid phospholipid bilayers strongly depends on the lubricant composition. The addition of salt improves the resistance to nano-indentation but not to shear. In both Tris and Tris saline buffer, bilayers are more resistant and present better lubricant properties.

The lubricant characteristics influence the tribological behavior in particular by modifying resistance to shear and load. These changes are representative of pathological conditions (such as arthritis and arthrosis [19,20,21]). Therefore the results of this work will develop the tribological analysis of joint diseases. Further studies must investigate in more detail the effect of the roughness of the substrate as well as the influence of the phospholipidic composition upon the tribological behavior of fluid phase phospholipid bilayers.

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