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Full Length Article

The effect of thiolated phospholipids on formation of supported lipid bilayers on gold substrates investigated by surface-sensitive methods

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ABSTRACT

Most of the model lipid membrane studies on gold involve the usage of various surface-modification strategies to rupture liposomes and induce lipid bilayer formation since liposomes with polar surfaces do not interact with bare, hydrophobic gold. In this study, a thiol-modified phospholipid, *1,2-Dipalmitoyl-sn-Glycero-3-Phosphothioethanol* (DPPTE) was incorporated into *phosphatidylcholine* (PC) based liposomes to form supported lipid bilayer (SLB) on gold surfaces without further modification. The binding kinetics of liposomes with different DPPTE ratio (0.01 to 100% mol/mol) and diameters were monitored by Quartz Crystal Microbalance with Dissipation (QCM-D). The dissipation change per frequency change, i.e. acoustic ratio, which is evaluated as a degree of the viscoelasticity, considerably decreased with the presence of DPPTE (from 162.3 GHz^{-1} for flattened PC liposomes to ca. 89.5 GHz^{-1} for 100% DPPTE liposomes) when compared to the results of two reference rigid monolayers and two viscoelastic layers. To assess the quality of SLB platform, the interpretation of QCM-D data was also complemented with Surface Plasmon Resonance. The optimum thiolated-lipid ratio (1%, lower thiol ratio and higher rigidity) was then used to determine the dry-lipid mass deposition, the water content and the thickness values of the SLB via viscoelastic modelling. Further surface characterization studies were performed by Atomic Force Microscopy with high spatial resolution. The results suggested that model membrane was almost continuous with minimum defects but showed more dissipative/soft nature compared to an ideal bilayer due to partially fused liposomes/overlapped lipid bilayers/multilayer islands. These local elevations distorted the planarity and led the increase of overall membrane thickness to ~7.0 nm.

1. Introduction

Supported lipid bilayer systems prepared by surface-mediated liposome fusion allow researchers to isolate and study one or few biological membrane components. These non-fouling biomimetic surfaces that resist cell/protein adhesion can be modified/functionalized by changing their lipid composition or incorporating membrane-associated proteins or molecules. Functionalization by different components helps to develop biosynthetic systems such as drug-screening platforms, membrane-based molecular biosensors, and then utilized in medical diagnostics, biomaterial improvement, and various other biomedical assays [1–8]. Taking the advantage of high stability and flat geometry, SLBs make long-term experimentation possible and allow the usage of surface sensitive characterization tools such as quartz crystal microbalance with dissipation monitoring (QCM-D), surface plasmon resonance (SPR), atomic force microscopy (AFM) and fluorescence recovery after photobleaching (FRAP) within aqueous environments [4,9–12].

Silica, mica and glass are well-established and most commonly employed solid supports for the preparation of SLBs since their hydrophilicity and negative charge at neutral pH provide liposomes a surface to adsorb, deform, flatten and finally rupture to form a bilayer [8]. In that case, the lipid layer is attached to the support by noncovalent bonds. These systems generally have high fluidity because of underlying water layer, but low stability due to weak interactions [13]. Liposomes with polar surfaces, on the other hand, neither interact with bare, hydrophobic gold surfaces [14], nor rupture on oxidized gold surfaces [15]. However, to take the advantages of the attractive properties (such as biocompatibility, electrical conductance) of gold [16,17], an efficient chemisorption method, gold-thiol bond chemistry is often used. For that, a self-assembled monolayer (SAM) is first formed onto the gold using this chemistry, and then a single phospholipid monolayer is attached on

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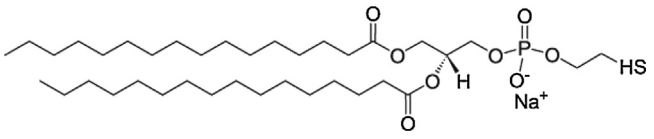

Fig. 1. Chemical structure of DPPTE [23].

that tightly packed and well-ordered SAM. These type of strategies yield higher stability, but might hamper the fluidity of SLB, and in turn may hinder the dynamics or electrochemical characteristics of the formed bilayers [18–21].

The majority of lipid membrane studies on gold surfaces involve the usage of various surface-modification strategies to induce lipid bilayer formation. These surface-based constraints can be eliminated by the direct formation of SLB on bare gold. In this study, a synthetic phospholipid functionalized with thiol-anchors at the lipid headgroups, *1,2-Dipalmitoyl-sn-Glycero-3-Phosphothioethanol* (DPPTE) (Fig. 1), was used together with *phosphatidylcholine* (PC) to enable spontaneous lipid bilayer formation on gold. In this way, the optimized ratio of DPPTE phospholipids can supply the sufficient stability by chemical adsorption to solid support, and PC components mainly give the fluidity to the constructed lipid adlayer. There are few examples of the usage of thiolated phospholipids within zwitterionic liposomes to avoid the need of a premodified gold surface [20,22]. Pera and Fritz [22] reported that lipid bilayer with DPPTE showed stronger mechanical coupling to the surface as to the physisorbed bilayers. Having used thiolated liposomes at three different ratios, bilayer formation had been detected by nanomechanical bending of cantilever sensors in AFM. Despite their well-grounded approach, the main limitation is the lack of real-time monitoring of lipid bilayer formation. It is an end-point application and supplied just a snapshot of the whole adhesion process. Further, lipid bilayer formations were characterized only through their mechanical properties. However, the characterization of the bilayers need a more detailed information concerning real-time monitoring of bilayer formation, the amount of adsorbed lipid mass on the surface, hydrodynamically coupled or mechanically trapped solvent (water content) within the bilayer. To our knowledge, the binding kinetics and characterization of this type of liposomes with both temporal and spatial resolution was thoroughly investigated for the first time by our study employing three different methods (QCM-D, SPR and AFM). For this purpose, different liposomes at varying DPPTE ratios and at different diameters were tested and the results were discussed for a better understanding.

QCM-D allows real-time, *in situ* monitoring and label-free qualitative and quantitative assessment in SLB characterization. It provides data on adsorption kinetics using the frequency change in the sensor (Δf) that reflects mass deposition, and also senses energy losses from the system into the surrounding environment as a result of the soft or loose nature of the adlayer by measuring changes in the dissipation factor (ΔD) [4,11,24,25]. Hence, the rigidity/stiffness or viscoelasticity/softness of the adsorbed film and structural differences between different adsorbed systems (intact liposomes or bilayer/multilayer formation etc.), or structural changes in the same film during the actual adsorption process can be determined by the evaluation of both signals [26]. Furthermore, the quantitative results on deposited mass and layer thickness can also be extracted using multiple frequency overtones/harmonics of crystals' resonance frequency since these different harmonics probe the adlayer at different depths that are inversely proportional to the frequency of the wave [27]. Higher overtones are qualitatively more related for the processes closer to the sensor surface whereas lower overtones are representative for the liquid-film interface [28].

SPR is an optical technique based on the changes in surface plasmons confined onto the metal surface that are extremely sensitive to changes in refractive index in close vicinity of the surface. Thus, SPR can provide information about adsorption behavior of liposomes on surface, the properties of the resulting films and quantify adsorbed masses in real-time [29,30]. AFM, on the other hand, is a very powerful technique that provides information on the local organizations, topographical changes and biomechanical properties of the adlayer [10,31]. In this study, to obtain a comprehensive understanding on water content, and roughness of the adlayer, QCM-D data was combined with SPR and AFM results.

2. Materials and methods

2.1. Materials

L-α-phosphatidylcholine (Egg, Chicken PC, 840051), *1,2-Dipalmitoyl-sn-Glycero-3-Phosphothioethanol (Sodium Salt)* (DPPTE, 870160P), and liposome extrusion accessories (610000) were purchased from Avanti Polar Lipids (USA).

SPR sensor chips (chromium (1 nm) and gold (50 nm) coated BK7 glass slides) and AT-cut gold coated quartz crystals with a fundamental frequency of 5 MHz and a diameter of 14 mm were purchased from NanoDev (Turkey) and Q-Sense (Sweden), respectively. We used commercial silicon cantilevers (Nanosensors, type: PPP-NCL-50 & PPP-NCHR) with resonance frequencies of 163080 Hz and 335710 Hz and spring constants of 50 N/m and 42 N/m, respectively. The radius of curvature of tips were less than 10 nm.

Purified water was used throughout the experiments with a minimum resistivity of 18.2 MΩ cm. Phosphate buffered saline (PBS) (0.01 M, pH 7.4, containing 137 mM NaCl) was prepared and filtered through 0.2 μm filters, and degassed before each QCM-D and SPR experiments.

2.2. Preparation of unilamellar liposomes

Phosphatidylcholine (PC) and *1,2-Dipalmitoyl-sn-Glycero-3-phosphothioethanol* (DPPTE) lipids were dissolved separately in chloroform at +4 °C (2.5 mg/mL), aliquoted and stored at −20 °C. To prepare thin lipid film layer, 40 μL of lipid solution with different molar PC/DPPTE ratios (0.01 to 100%) was poured into a round-bottom flask. Chloroform was evaporated by manually rotating the flask and using a dry nitrogen stream in a fume hood. The formed thin lipid film was left overnight at nitrogen atmosphere at +4 °C to ensure the removal of all solvent residues. The dried lipid film layer was hydrated by phosphate buffered saline (PBS, 0.01 M, pH 7.4) to a final lipid concentration of 0.02 mg/mL. The flask was agitated vigorously for 10–15 min until a clear solution was obtained. To obtain unilamellar liposomes, hydrated and vortexed lipid solutions were extruded 21 times through a polycarbonate membranes with three different pore sizes (50, 100 and 200 nm). The extrusion method yielded normal distributions with mean diameter of 92 ± 3, 142 ± 7 nm and 174 ± 3 nm, respectively, measured by dynamic light scattering (DLS) (Malvern, Zetasizer).

The obtained unilamellar liposomes were collected in a falcon tube and stored at +4 °C overnight to be used on the following day. Liposomes were produced freshly for each adsorption kinetic measurements and used within the 24 hours of preparation.

2.3. Preparation of sensor surfaces

Immediately before each use, clean QCM and SPR gold-coated surfaces were rinsed with ultrapure water, and dried under nitrogen gas. After each measurement, the crystals were cleaned with H_2O:NH_3(25%):H_2O_2 (30%) (5:1:1) solution for 5 min in 75 °C to remove the thiol residuals from the gold surfaces, then they were