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Choice of cuvette material can influence spectroscopic leakage and permeability experiments with liposomes

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ABSTRACT

Liposome solute permeability experiments are widely performed to gain information about lipid membrane characteristics. Spectroscopic methods are often used for this purpose, usually monitoring the leakage of a self-quenching fluorescent dye (e.g., carboxyfluorescein, CF) from the liposomes. Hereby, we investigate the effect of liposome-cuvette interactions, a seldom considered detail, on the results obtained from liposomal permeability experiments. The spontaneous leakage of CF from liposomes with different surface properties and phase states is followed using quartz and polystyrene cuvettes, and the results are compared. It is shown that for most lipid compositions the leakage profiles vary notably between different cuvette materials. Reproducibility of the measurements also varies depending on the cuvettes used, with polystyrene providing with more robust results. To explain these observations, the interaction of liposomes with polystyrene and quartz-like surfaces was characterized with the help of the quartz crystal microbalance with dissipation monitoring (QCM-D). Our results show that, while liposomes seldom interact with polystyrene, quartz-liposome interactions are almost unavoidable and have a large impact on the leakage experiments mainly via two mechanisms: i) the rupturing of liposomes on the cuvette surface causing a fast release of encapsulated CF, and ii) the disruption of adsorbed liposomes caused by magnetic stirring. Depending on their composition, the liposomes interact in different ways with quartz, affecting thus the extent of each proposed mechanism. The experiments demonstrate the importance of considering the cuvette material when planning and conducting spectroscopic experiments with liposomes.

1. Introduction

Phospholipid vesicles, i.e., liposomes, are widely used as simplified biological membrane models and can also be used as carriers for hydrophilic solutes in pharmaceutical applications. For most applications, knowledge of the membrane overall properties is desired. Given that the permeability of solutes through the membrane is tightly coupled to membrane physical properties such as its fluidity, order, and amount of defects, studies of membrane permeability are often used to characterize membrane stability (e.g., Lande et al., 1995; Shimanouchi et al., 2009; Agmo Hernández et al., 2011, 2015; Angelini et al., 2011; Maherani et al., 2013; Hays et al., 2001; Cócera et al., 2003).

A widely used way to determine membrane permeability towards solutes is to study the release of a self-quenching fluorescent hydrophilic compound from liposomes. Carboxyfluorescein and calcein are dyes commonly used for this purpose. By encapsulating high concentrations of the dye within liposomes, the release of the molecule can be monitored (Weinstein et al., 1977). The technique is based on the fact that the fluorescence is quenched at high dye concentrations, while, at sufficiently low concentrations, there is a linear correlation between concentration and fluorescence intensity (Chen and Knutson, 1988; Shimanouchi et al., 2009). Besides studies on liposome stability, dye leakage experiments are also widely used to study how the composition of the surrounding solution may affect the membrane (Gadras et al., 1999; Barbet et al., 1984), and how lipid membranes interact with, e.g., surfactants (Memoli et al., 1999; de la Maza and Parra, 1997; de la Maza et al., 1998; Agmo Hernández et al., 2015; Eriksson et al., 2018), peptides (Mazzuca et al., 2010; Rex and Schwarz, 1998; Wessman et al., 2010), and other relevant molecules (Piel et al., 2007; Pajean and Herbage, 1993; Kayalar and Düzgüneçs, 1986; Engelke

Abbreviations: CF, carboxyfluorescein; CL, cardiolipin; DPPC, 1,2-dipalmitoyl-sn-glycero-3-phosphocholine; IMM, inner mitochondrial membrane; PBS, phosphate buffered saline; POPC, 1-palmitoyl-2-oleyl-sn-glycero-phosphocholine; POPE, 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine; QCM-D, quartz crystal microbalance with dissipation monitoring; SDS, sodium dodecyl sulfate; Soy-PI, soy L-α-phosphatidylinositol; Soy-PS, soy L-α-phosphatidylserine

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et al., 2001). CF and calcein leakage studies are also of great importance to characterize liposomes designed for drug delivery, as information about the encapsulation properties of the liposome and how they can be affected by components in serum is thus accessible (Hashizaki et al., 2006; Silvander et al., 1998).

A broad search in the literature, however, illustrated that the material of the cuvette used during CF leakage experiments is seldom reported. In the above examples, only a few reports (Hays et al., 2001; Hashizaki et al., 2006; Silvander et al., 1998; Eriksson et al., 2018) precisely report the cuvette material. Given that for CF and calcein both the emission and excitation wavelengths lie in the visible range, where most cuvette materials show good transparency, it is likely that quartz, glass and plastic cuvettes are used without distinction. In cases where the cuvette material is reported, it very often consists of quartz, which is generally considered to be superior to plastics due to its high chemical stability, low surface roughness, enhanced transparency, etc. Also, the surface adsorption of surfactants, peptides, and other molecules of interest is likely to be diminished or avoided by using quartz cuvettes, while plastic cuvettes are the less preferred option from this perspective, especially if the plastic material is hydrophobic.

However, liposomes are known to interact with hydrophilic surfaces, including quartz (Johnson et al., 2002), in several ways. Thus, they may adsorb as intact liposomes or rupture forming supported lipid bilayers (Biswas et al., 2018; Richter et al., 2006; Johnson et al., 2002). On the other hand, interactions between liposomes and hydrophobic materials may result in the formation of adsorbed lipid monolayers on the surface (Agmo Hernández, 2013; Hellberg et al., 2002). To our knowledge, the potential interactions of liposomes with the cuvette material, and how these may affect the results of permeability experiments, are not normally considered when designing and interpreting leakage experiments

In a previous report (Eriksson et al., 2018), we found out that spontaneous leakage experiments performed with biomimetic liposomes in quartz or glass cuvettes were not reproducible. More robust results were obtained when using polystyrene cuvettes. It was hypothesized that the liposomes interacted significantly with quartz and glass, giving rise to the irreproducibility observed. Hereby, we explore this hypothesis and aim to explain the role of the choice of cuvette materials on the results obtained from spontaneous leakage experiments with liposomes of different compositions, surface properties, and phase states. We have chosen to focus on quartz and polystyrene as cuvette materials since they have diametrically opposite surface characteristics. Quartz cuvettes usually present a smooth surface profile and are hydrophilic, whereas polystyrene cuvettes have slightly higher surface roughness and are very hydrophobic. In order to explore whether differences between the leakage profiles on polystyrene and quartz arise due to liposome-surface interactions, we have characterized the latter with the help of the quartz crystal microbalance with dissipation monitoring (QCM-D).

2. Materials and methods

2.1. Chemicals

1-palmitoyl-2-oleoyl-sn-glycero-phosphocholine (POPC) was obtained as a kind gift from Lipoid Gmbh (Ludwigshafen, Germany). Cardiolipin (CL) from bovine heart sodium salt, 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoethanolamine (POPE), 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC), soy L-α-phosphatidylinositol (Soy-PI) sodium salt, soy L-α-phosphatidylserine (Soy-PS) sodium salt were bought from Avanti Polar Lipids (Alabaster, USA). Polyethylene glycol tert-octylphenyl ether (Triton X-100), cholesterol, and 5 (6)-carboxyfluorescein (CF) were purchased from Sigma-Aldrich (Steinheim, Germany). Chloroform (pro analysis) was from MERCK (Darmstadt, Germany). All aqueous solutions were prepared using deionized water (18.2 M Ω cm) obtained from a Milli-Q system (Millipore, Bedford, USA).

2.2. Liposome preparation

Lipid components were weighed or pipetted from stock solutions in chloroform and thereafter dissolved/diluted by adding extra chloroform. A lipid film was made by drying the mixture, first with a gentle stream of nitrogen and then additionally with a vacuum oven (Squaroid vacuum oven, Lab Instruments, IL, USA) overnight. The lipid film was then suspended in either a phosphate buffered saline (PBS, 10 mM sodium phosphate, 150 mM NaCl, pH = 7.4) or CF solution (100 mM CF, 10 mM sodium phosphate, pH 7.4), depending on the experiment performed. To create liposomes, the suspension was first freeze-thawed (freezing with liquid nitrogen and thawing with a water bath at 60 °C) and then extruded (Lipofast extruder, Avestin, Ottawa, Canada) 31 times through a 100 nm pore size filter (Whatman plc, Kent, UK). 15 freeze-thaw cycles were needed for CL containing samples while 5 cycles were enough for the other samples. The samples were stored 24 h in room temperature to reach an equilibrium state of the liposomes before starting experiments (Agmo Hernández et al., 2011).

2.3. Leakage measurements

Liposomes prepared in a CF solution were separated from the unentrapped dye by using a PD-10 gel filtration column (GE-Healthcare, Uppsala, Sweden) equilibrated with PBS. The samples were thereafter diluted to 12 μM to ensure that the fluorescence to CF free concentration relationship was in the linear range during the measurements. For POPE:CL (94:6) samples, the concentration was 120 μM to ensure enough amount of trapped CF. Directly after separation and dilution, the sample was transferred to the cuvette of choice and the experiment was started. Polystyrene (Kartell, Noviolio, Italy) and quartz cuvettes (Quartz SUPRASIL®, Hellma Analytics, Müllheim, Germany) 1 x 1 cm were used. The fluorescence signal was recorded with a SPEX fluorolog 1650 0.2 m double spectrometer (SPEX industries, Edison, USA) in the right-angle mode, with the excitation set to 495 nm and the emission to 520 nm.

Spontaneous leakage experiments were generally run over at least 4 h, measuring the fluorescence intensity every 5 s at 25 °C with continuous sample stirring, unless otherwise indicated. The degree of leakage over time ($x_{CF_{rel}}(t)$) was calculated by:

$$x_{CF_{rel}}(t) = \frac{I(t) - I_0}{I_{tot} - I_0} \quad (1)$$

where $I(t)$ is the time-dependent fluorescence intensity and I_0 is the intensity at the starting point of the experiment. I_{tot} is the maximum fluorescence intensity which was achieved by addition of 50 μl of 200 mM Triton X-100, which ensures complete liposome solubilisation. The experiments were repeated up to 8 times for each liposome composition to ensure that the trends observed are representative for each studied system. Unless otherwise stated, the leakage profiles presented were observed in all repetitions of the experiment.

The effect of magnetic stirring was investigated by incubating the samples with and without stirring and measuring their fluorescence intensity every half hour for a total period of two hours. The fluorescence intensity of a reference solution was monitored simultaneously to account for changes in the lamp intensity. Samples without magnetic stirring were gently agitated right before the measurements. The experiment was ended by adding 50 μl of 200 mM Triton X-100 in each sample to obtain I_{tot}. The degree of leakage was calculated by Eq. (1).

2.4. QCM-D characterizations

The Quartz Crystal Microbalance with Dissipation monitoring (QCM-D) technique was employed to study the adhesion and spreading of liposomes at different surfaces. A QCM-D E1 (Q-sense, Gothenburg,