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Ionic liquids affect the adsorption of liposomes onto cationic polyelectrolyte coated silica evidenced by quartz crystal microbalance

Filip Duša [a], Suvi-Katriina Ruokonen [a], Ján Petrovaj [a], Tapani Viitala [b,*], Susanne K. Wiedmer [a,*]

[a] Department of Chemistry, P.O. Box 55, 00014 University of Helsinki, Finland
[b] Centre for Drug Research, Division of Pharmaceutical Biosciences, Faculty of Pharmacy, P.O. Box 56, 00014 University of Helsinki, Finland

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ABSTRACT

The worldwide use of ionic liquids (ILs) is steadily increasing, and even though they are often referred to as "green solvents" they have been reported to be toxic, especially toward aquatic organisms. In this work, we thoroughly study two phosphonium ILs; octyltributylphosphonium chloride ([P_{8444}]Cl) and tributyl(tetradecyl)phosphonium chloride ([P_{14444}]Cl). Firstly, the critical micelle concentrations (CMCs) of the ILs were determined with fluorescence spectroscopy and the optical pendant drop method in order to gain an understanding of the aggregation behavior of the ILs. Secondly, a biomimicking system of negatively charged unilamellar liposomes was used in order to study the effect of the ILs on biomembranes. Changes in the mechanical properties of adsorbed liposomes were determined by quartz crystal microbalance (QCM) measurements with silica coated quartz crystal sensors featuring a polycation layer. The results confirmed that both ILs were able to incorporate and alter the biomembrane structure. The membrane disrupting effect was emphasized with an increasing concentration and alkyl chain length of the ILs. In the extreme case, the phospholipid membrane integrity was completely compromised.

1. Introduction

Ionic liquids (ILs) are salts which typically are in melted state at temperatures below 100 °C. Their main benefits are a negligible vapor pressure, an ability to dissolve a large number of compounds, and the possibility to design on-demand ILs by combining different cations and anions [1]. Due to their low vapor pressures, ILs have often been referred to as "green solvents" because this enables an easier handling and storage of ILs [2]. Many recent studies, however, suggest that ILs possess a considerable toxicity, especially in aquatic environments [1,3,4]. ILs are frequently used in a large number of applications, yet there is still a considerable lack of information about their interaction with living organisms. Therefore, further characterization of ILs in this regard is of paramount importance.

Phosphonium-based ILs are a class of compounds where the phosphorus atom in the cation is typically bound to four alkyl substituents. In this work we investigate lipid membrane interactions with two commercially available phosphonium chloride ILs with long alkyl chains, namely, octyltributylphosphonium chloride ([P_{8444}]Cl) and tributyl(tetradecyl)phosphonium chloride [P_{14444}]Cl (Fig. 1). Recent applications include the use of phosphonium ILs for nucleation and electrodeposition of metals [5,6], for analyte separations in analytical chemistry [7,8], in liquid–liquid extractions [9,10], and for selective capture and detection of arsenic [11]. Because cations of the ILs used in this study are amphiphilic we first evaluated whether these form micelles. The critical micelle concentration (CMC) is a very important characteristic of an amphiphilic compound. The CMC is reflected as a sudden change in solution properties when micelles are formed [12]. The formation of micelles may affect the way the compounds interact with biomembranes. There are several methods for CMC determination, such as conductivity, NMR diffusion, surface tension, fluorescence spectroscopy, and others (see Ref. [13]). CMC values in this study were determined with fluorescence spectroscopy and surface tension analysis.

The biomembrane is one of the key cellular structures, which protects a cell from the external environment. Thus, any alteration or disruption of the biomembrane can have serious or even lethal consequences for the organism. It has recently been reported that imidazolium based ILs can induce membrane fusion [14] and alter membrane permeability [15]. Much in the same way as certain amphipathic α-helical peptides, positively charged surfactants, and other peptides [16–21]. Therefore, further knowledge about the

* Corresponding authors.
E-mail addresses: tapani.viitala@helsinki.fi (T. Viitala), susanne.wiedmer@helsinki.fi (S.K. Wiedmer).

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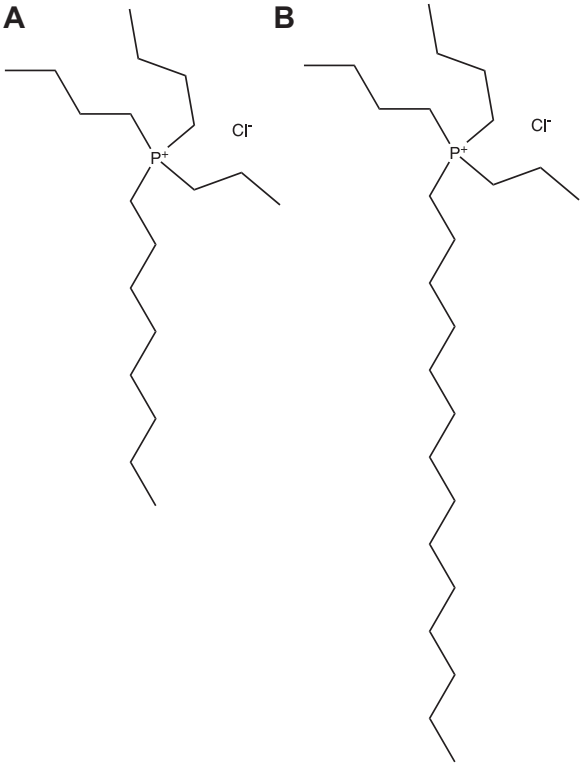

Fig. 1. Structure of ionic liquids (A) $[P_{8444}]Cl$ and (B) $[P_{14444}]Cl$.

behavior of phosphonium ILs in regard to their interactions with biomembranes can bring light onto possible benignity or toxicity of particular ILs.

Coating of surfaces with phospholipids is often a method of choice for studying interactions between model biomembranes and various compounds [22,23]. Furthermore, it is possible to obtain a deeper understanding about the mechanism of how compounds influence the membrane structure by observing changes in the properties of phospholipid layers adsorbed on solid surfaces [19,24]. Advanced quartz crystal microbalance techniques (QCM), i.e., impedance based QCM or QCM with dissipation monitoring, are powerful tools for determining various properties (e.g., thickness, mass, and viscoelasticity) and interaction dynamics of adsorbed layers. One of the main advantages of advanced QCM techniques is that they enable fast and label-free detection of compounds interacting with the sensor [25]. The resonance frequency of a quartz crystal sensor is dependent on the mass of the adsorbed layer, whereas the change in energy dissipation of the sensor (ΔD) provides information about viscoelastic properties of the adsorbed layer. In this regard, advanced QCM can be considered as a biosensing device where the resonance frequency and energy dissipation of the sensor with a bound biological structure (e.g., lipid bilayer/liposomes) will change upon addition of ILs interacting with the system [25,26]. Advanced QCM can also provide highly relevant information about disruption of lipid bilayers [27], interaction with membrane active moieties [28], binding dynamics [26], and lipid packing density [29].

Zwitterionic and anionic phospholipids are the most abundant components of biomembranes. In this work, zwitterionic egg L-α-phosphatidylcholine (eggPC) and negatively charged 1-palmitoyl-2-oleyl-*sn*-glycero-3-[phospho-rac-(1-glycerol)] (POPG) were used for preparation of liposomes. These liposomes were used in the form of large unilamellar vesicles with approximate diameters of 130 nm. Such a system was used in our previous study, where interactions between liposomes and ILs were investigated with capillary electromigration techniques [30]. In order to better understand the interactions observed in our previous study, this work focused on the characterization of ILs and their influence on liposomes. It has already been shown that liposomes bind to silica surface, and based on their composition, size, concentration, and experimental conditions they can either adsorb as intact liposomes or rupture to form phospholipid bilayers on the silica surface [21,31,32]. Increase in porosity of such silica surfaces has also been reported to increase the kinetics of vesicle rupture and bilayer formation [33]. The liposomes used in our study were highly negatively charged due to 25 mol% of anionic POPG. Since the silica surface of the QCM sensor was negatively charged at the used pH of 7.4 (the pK_a value of the silanol groups is approximately 4.9, meaning almost complete dissociation), it was necessary to apply a fast and reliable coating method with cationic layer. This prevented unwanted interactions with the cationic ILs, which would eventually result in the formation of a 'dynamic' IL coating. Furthermore, it ensured fast liposome binding to the sensor surface. Positively charged polyelectrolyte coatings can be utilized to screen off the surface interactions between positively charged compounds and the negatively charged silanols [34]. Polybrene, a widely used cationic polyelectrolyte for coating of silica [35], was employed in this work. The same polyelectrolyte was also utilized in our recent capillary electrophoresis study of interactions between the same liposome-ILs systems with common pharmaceutics [30]. In this work, we will demonstrate that QCM is a highly suitable technique for analysis of interactions between phosphonium ILs and phospholipid membranes, providing new insight into the possible mechanism of ILs toxicity. Moreover, the QCM methodology will also be used to bring complimentary information on trends and phenomenon observed in our previous study [30].

2. Materials and methods

2.1. Chemicals

POPG (sodium salt) was purchased from Genzyme Pharmaceuticals (Liestal, Switzerland), eggPC (Egg, Chicken) was purchased from Avanti Polar Lipids (Alabaster, AL, USA). Polybrene (hexadimethrine bromide) was purchased from Fluka (Buchs, Switzerland). Hydrogen sodium phosphate, hydrogen peroxide (H_2O_2; 50% weight in H_2O), ammonium hydroxide (NH_4OH; 30% weight in H_2O), and 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulphonate (CHAPS) (purity of 98%) were purchased from Sigma (Darmstadt, Germany). Dihydrogen sodium phosphate monohydrate and HPLC-grade methanol were from Mallinckrodt Baker (Deventer, The Netherlands). The pH calibration solutions (7.01 and 10.01) were purchased from Merck (Darmstadt, Germany). Sodium hydroxide (1.0 M) was from FF-Chemicals (Yli-Ii, Finland) and chloroform from Rathburn (Walkerburn, UK). Distilled water was further purified with a Millipore water-purification system (Millipore, Molsheim, France). Sodium dodecyl sulfate (SDS, purity of 99%) and pyrene (GC-grade; purity of 99%) were obtained from Merck (Darmstadt, Germany) and Fluka (Sigma-Aldrich, Switzerland), respectively. 1-Ethyl-3-methylimidazolium acetate [emim][OAc] was purchased from Iolitec GmbH (Heilbronn, Germany). $[P_{8444}]Cl$ and $[P_{14444}]Cl$ were provided by Cytec Industries (Woodland Park, NJ, USA).

2.2. Buffer preparation

Sodium phosphate buffer was prepared by mixing disodium hydrogen phosphate and sodium dihydrogen phosphate to yield an ionic strength of 10 mM and a pH of 7.4. The buffer solution was filtered through a 0.45-μm polytetrafluoroethylene syringe filter