Chemistry and Physics of Lipids 215 (2018) 1–10

Contents lists available at ScienceDirect

Chemistry and Physics of Lipids

journal homepage: www.elsevier.com/locate/chemphyslip

Structural changes in cellular membranes induced by ionic liquids: From model to bacterial membranes

G. Bhattacharya[a], R.P. Giri[b], A. Dubey[c], S. Mitra[a], R. Priyadarshini[c], A. Gupta[c], M.K. Mukhopadhyay[b], S.K. Ghosh[a,*]

[a] Department of Physics, School of Natural Sciences, Shiv Nadar University, NH-91, Tehsil Dadri, G. B. Nagar, Uttar Pradesh, 201314, India
[b] Surface Physics and Material Science Division, Saha Institute of Nuclear Physics, Bidhannagar, Kolkata, 700064, India
[c] Department of Life Sciences, School of Natural Sciences, Shiv Nadar University, NH-91, Tehsil Dadri, G. B. Nagar, Uttar Pradesh, 201314, India

ARTICLE INFO

Keywords:
Cellular membrane
Ionic liquid
Lipid monolayer
Lipid bilayer
X-ray reflectivity

ABSTRACT

Ionic liquids (ILs) have generated considerable attention recently because of their cytotoxicity and application as antibiotics. However, the mechanism of how they damage cell membranes is not currently well understood. In this paper, the antibacterial activities of two imidazolium-based ILs, namely 1-butyl- 3-methylimidazolium tetrafluroborate ([BMIM][BF4]) and 1-ethyl- 3-methylimidazolium tetrafluroborate ([EMIM][BF4]) have been investigated. The activity of [BMIM][BF4] on gram negative bacteria *E. coli* is observed to be stronger compared with the short chained [EMIM][BF4]. To explain this observation, the effects of these ILs on the self-assembled structures of model cellular membranes have been investigated. The in-plane elasticity of a monolayer formed at air-water interface by 1,2-dipalmitoyl- sn-glycero- 3-phosphocholine (DPPC) lipids was reduced in the presence of the ILs. The x-ray reflectivity studies on polymer supported lipid bilayer have shown the bilayer to shrink and correspondingly exhibit an increase in electron density. The presence of a certain mol% of negatively charged lipid, 1,2-dipalmitoyl-*rac*-glycero-3-phospho-L-serine (DPPS), in DPPC mono- and bi-layers enhances the effect considerably.

1. Introduction

Ionic liquids (ILs) are molten salts consisting of organic or inorganic cations and anions with a melting point, normally below 100 °C (Benedetto, 2017; Hallett and Welton, 2011; Welton, 1999). These non-flammable and non-explosive solvents have potential applications in various fields (Plechkova and Seddon, 2008; Ye et al., 2001). These liquids are the versatile solvents as they dissolve both the organic and inorganic compounds (Wasserscheid and Welton, 2008). In solar power plant, these liquids are used as thermal storage media and the heat transfer fluids (Wu et al., 2001). They are being used as electrolytes in supercapacitors that enhance the capacitive performance (Lian et al., 2016; Zhong et al., 2015), as lubricant because of high viscosity (Mu et al., 2004) and as storage media for sustainable chemical waste management (Seitkalieva et al., 2017). They have also uses as biocatalysts (Egorova and Ananikov, 2018) and biocompatible stabilizers of proteins (Reslan and Kayser, 2018). In their recent paper, Benedetto and Ballone have described the applications of room temperature ILs in pharmacology, biomedicine and biotechnology (Benedetto and Ballone, 2018). These molecules possess high electrical conductivity and good thermal stability (Bonhote et al., 1996; Torimoto et al., 2010) and due to very low vapor pressure, hardly evaporate and, hence, do not greatly pollute the air (Earle et al., 1999; Holbrey and Seddon, 1999). However, these molecules are now under investigation in order to understand their adverse effects on marine and soil-based organisms.

As they are not easily biodegradable, IL wastes are continuously accumulated in water bodies, causing the death of many aquatic organisms (Kulacki and Lamberti, 2008; Latała et al., 2005). Recent studies have pointed out the toxic effects of ILs on many other environmentally benign microorganisms and mammalian cell lines (Frade et al., 2007; Kumar et al., 2009; Stolte et al., 2006; Viboud et al., 2012). They are already known to show antimicrobial effects (O'Toole et al., 2012; Pernak et al., 2004). Recently, various biological assays and physiochemical methods have been employed to study the potential cytotoxicity of ILs over many prototypical model biological systems (Compton and Laszlo, 2002; DiCarlo et al., 2006; Laszlo and Compton, 2002; Ranke et al., 2007).

The ILs either damage the cellular membrane in their first step of interaction with a living cell or penetrate through the membrane to perturb the activity of organelles inside the cell. In both cases, the

* Corresponding author.
E-mail address: sajal.ghosh@snu.edu.in (S.K. Ghosh).

https://doi.org/10.1016/j.chemphyslip.2018.06.001
Received 19 March 2018; Received in revised form 16 June 2018; Accepted 18 June 2018
Available online 23 June 2018

molecules interact with the cellular membrane and cause structural changes. Greater understanding of this interaction is required to comprehend the effects of ILs on living organisms. Experimental techniques such as atomic force and transmission electron microscopy, and quartz crystal microbalance have been carried out along with molecular dynamic (MD) simulations to understand these interactions (Byrne and Angell, 2009; Evans, 2006, 2008; Gras et al., 2008; Kalhor et al., 2009; Portella et al., 2014). The MD simulations have suggested strong perturbation and physical disruption of the membrane structure, which may interfere the cell signaling (Bingham and Ballone, 2012; Cromie et al., 2009; Lim et al., 2014; Yoo et al., 2014, 2016). The strength of insertion of the ILs into the membrane is reported to depend on the concentration and hydrophobicity of the molecules (Yoo et al., 2014). Benedetto et al., have proposed a possible molecular level description of IL-bilayer interaction using a neutron reflectivity technique: by using 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) and 1,2-dimyristoyl-sn-glycero-3-phosphatidylcholine (DMPC) phospholipid bilayers supported on a silica substrate, they observed that though the global structure of the bilayers remains unaltered, there is an irreversible decrease in the bilayer thickness (Benedetto et al., 2014).

In our recent study (Bhattacharya et al., 2017), we used x-ray reflectivity (XRR) technique to shed light on the lipid organization in a soft supported bilayer. By using a membrane composed of zwitterionic lipid, 1,2-dipalmitoyl- sn-glycero- 3-phosphocholine (DPPC) and an ionic liquid, 1-butyl- 3-methylimidazolium tetrfluroborate ([BMIM][BF4]), we have also observed that the effective bilayer thickness and in-plane elasticity are reduced as a function of IL concentration. As mentioned above, the potential biological activity of ILs and their possible interactions with the living cell are not limited to the membrane but also to other cellular components (Benedetto and Ballone, 2016a, 2016b) which have been reviewed recently by Egorova et al. (Egorova et al., 2017). Even though there are some studies on the interaction of ILs with model cellular membranes, the exact molecular mechanism of this interaction is yet to fully understand.

In the present paper, the effects of imidazolium-based ILs, namely, 1-butyl- 3-methylimidazolium tetrafluroborate ([BMIM][BF4]) and 1-ethyl- 3-methylimidazolium tetrafluroborate ([EMIM][BF4]) on *E.coli* bacteria are investigated. The survival of these bacteria decays as a function of IL concentrations, both the ILs show antibacterial activities and [BMIM][BF4] exhibits stronger toxicity. Live and dead cell assay, and spot assay techniques are employed to study the lysing of the bacteria in presence of these ILs. To understand the mechanism of this lysing effect of ILs, here, two simplistic approaches have been adopted, where, the cellular membrane is modelled as; (i) lipid monolayer formed at air-water interface and (ii) lipid bilayer formed on a polymer cushion. It is to be noted that such systems have been described in our recent publication using only a zwitterionic lipid DPPC (Bhattacharya et al., 2017). In the current paper, we have extended the breadth and depth of the study with respect to two important parameters that are expected to control the interactions between ILs and lipid membrane; one being the electrostatics of the model membranes and the other being the hydrophobicity of the ILs. To closely mimic the cellular membrane, a negatively charged lipid 1,2-dipalmitoyl-*rac*-glycero-3-phospho-L-serine (DPPS) was added to DPPC. On the other hand, the hydrophobicity of the IL with same head group was modified by changing the chain length of an IL. The interaction of the ILs with self-assembled monolayers of DPPC with and without DPPS has shown the surface elasticity of the monolayers to decrease. X-ray reflectivity studies of lipid bilayer have provided the direct structural insights of the bilayer before and after the IL-membrane interaction. The results are found to be strongly correlated with the electrostatic condition of the lipid bilayers and the hydrophobic chain lengths of the ILs.

2. Materials and methods

2.1. Materials

1,2-dipalmitoyl- sn-glycero- 3-phosphocholine (DPPC), 1,2-dipalmitoyl-*rac*-glycero-3-phospho-L-serine (DPPS), 1-butyl- 3-methylimidazolium tetrfluroborate ([BMIM][BF4]), 1-eutyl- 3-methylimidazolium tetrfluroborate ([EMIM][BF4]), 3-aminopropyl triethoxysilane (APTES) and poly acrylic acid (PAA), all of analytical grade, were purchased from Sigma-Aldrich (USA). Methanol, chloroform, toluene of HPLC grade, were also purchased from Sigma-Aldrich (USA). All these chemicals are used in experiments without any further purification. Aqueous samples were prepared using de-ionized Millipore water (DI water) (resistivity ~ 18 MΩ cm).

2.2. Bacterial studies

2.2.1. Bacterial strains and culture conditions

E. coli MG1655 was used in the present study. Briefly, *E. coli* MG1655 cells were cultivated on Luria-Bertani agar plate at 37 °C. The cells from agar plate were grown overnight at 200 rpm in Luria-Bertani medium (Hi-Media, Mumbai).

2.2.2. LIVE/DEAD cell viability assay

To evaluate the antibacterial activity of the ILs, Live/Dead staining was performed using LIVE/DEAD® *Bac*Light™ Bacterial Viability Kit (L13152, Thermo Fischer Scientific). Cells were treated with 50 mM [BMIM] [BF4] and [EMIM] [BF4], respectively, for 12 h. Untreated cells were taken as control. After 12 h, cells were washed with 0.9% NaCl and stained for 15 min in dark according to manufacturer's protocol. Stained cells were observed by fluorescence microscopy using Nikon Eclipse Ti microscope equipped with Nikon DS-U3 camera using Plan Apo 100X/1.40 oil objective.

2.2.3. Spot assay

For spot assay, the overnight grown culture was diluted to an $O.D._{600}$ of 0.1 and cells were treated with 50 mM [BMIM] [BF4] and [EMIM] [BF4] whereas untreated cells were taken as control. Both treated and untreated cells were allowed to grow for 12 h at 37 °C and 200 rpm. After 12 h, serial dilutions of treated and untreated cells were performed and 5 µl was spotted on MacConkey agar (Hi-Media, Mumbai) plates. Plates were incubated overnight at 37 °C and photographed using Alpha imager (Protein Simple).

2.3. Pressure-Area isotherm

To record surface pressure-area isotherm, a Langmuir trough (KSV NIMA) with two movable Teflon barriers (dimension $54 \times 14.5 \times 0.75\ cm^3$) was used. Before each experiment, the trough was cleaned with DI water and chloroform for complete removal of any inorganic and organic contaminants. The interactions of [BMIM][BF4] and [EMIM][BF4] with zwitterionic lipid DPPC and a mixture of DPPC and negatively charged lipid DPPS were investigated by surface pressure-area isotherms and the in-plane elasticity was calculated from respective isotherms. To form the monolayer of DPPC at air-water interface, 0.5 mg ml^{-1} of lipid stock solution in chloroform was prepared. 150 µl of lipid solution was spread uniformly over the water surface by using a Hamilton syringe. After spreading, the barriers were kept stationary for 15 min for complete evaporation of chloroform. The temperature of trough was kept at 35 °C. The barriers were then compressed at a constant rate of 5 mm/min. For isotherms of DPPC/[EMIM][BF4], chloroform was used as common solvent to prepare homogenous solutions with a certain molar ratio. In order to find out the effect of membrane charge on membrane-IL interaction, an anionic lipid, DPPS was added to DPPC with a fixed molar ratio (DPPC: DPPS = 4:1) and a similar set of isotherm measurements were carried out. The in-plane