Interaction of quaternary ammonium ionic liquids and bacterial cell membrane

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Introduction

The ionic liquids (ILs) still remain in interest of the many of scientific and industrial laboratories. They exhibit unique properties due to both ionic character and low melting point. They are characterized by nonflammability, high polarity and ionic conductivity (Markiewicz et al., 2014; Pernak et al., 2006). ILs also poses antimicrobial properties, e.g. growth inhibition, cyto- and genotoxicity. However, the studies that try to characterize ILs mostly are mechanistic and do not bring the answer how ionic liquids interacts with cell composites, e.g. proteins or cell membrane. It is very important because such compounds might influence of cell structures or the cell itself. Additionally, it turned out, that the quaternary ammonium salts can modify the electrokinetic potential of Gram-negative bacterial outer membrane. Thus, the question about possibility to adapt bacterial cells arises (Borkowski et al., 2016; Pernak, Sobaszekiewicz, & Mirska, 2003).

For the synthesis of ionic liquids, new precursors have been used lately, even those inspired by nature, e.g. theophylline. This compound can be found in leaves and beans of cocoa and its high concentration protects plants from pathogenic bacteria, fungi and insects. Such properties can suggest that it could be an active part of ILs, thus could influence on bacteria growth and viability. Increasing chain length causes the increase of toxicity, however this relationship is not true at chain length C18. The toxicity of C18T is significantly lower in relation to C16T and C14T. This phenomenon concerning a lot of ionic liquids comprising alkyl substituents is known as "cut-off" effect and is distinctly observed mainly at Gram-negative bacteria. It was also shown that some of the ILs can significantly modify surface charge of the external lipid membrane of Gram-negative bacteria which can cause the agglomeration of bacteria as well as the adsorption of negatively charged particles or compounds (Borkowski et al., 2017; Borkowski, Gutowski, Syczewski, Cłapa, & Czerwonka, 2018).

The aim of the presented studies was the adaptation of *Escherichia coli* to increasing concentration of

tetradecyltrimethylammonium theophyllinate. After adaptation, the bacteria were examined to recognition of changes in cell morphology, lipid and protein composition.

Materials and Methods

Strains of *E. coli* were cultivated in liquid tryptic soy broth (TSB, Sigma–Aldrich, Sigma-Aldrich Ltd., Poznań Poland). Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were estimated using the microtiter plates method, using sterile 96-well plates, and based on the measurements of dehydrogenase activity. The zeta (electro kinetic) potential of bacterial cells dispersed in TILs solution at concentration of 100 mg L⁻¹ liquid was determined using the laser Doppler velocimetry method. Fluorescence microscope – live/death staining method was performed by using propidium iodide and acridine orange dyes.

Results and Discussion

Our results showed how the Gram-negative bacteria can adapt to lethal concentration of quaternary ammonium ionic liquids. There are no doubts that the ionic liquids can affect the properties of bacterial outer membrane that can appear as the changes of electrokinetic potential or membrane permeability. The cultivation of bacteria with ionic liquids led to significant changes of membrane permeability and composition, thus influence on the cell morphology. Based on our experiments the main conclusion can be established. Bacteria can easily adapt to the increasing concentration of toxic quaternary ammonium ionic liquids.

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