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Abstract

Author: Marta Majewska, M.Sc. Eng.
Supervisor: Prof. Włodzimierz Kutner, Ph.D., D.Sc.
Auxiliary supervisor: Dr. Piotr Pięta, Ph.D. Eng.
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Title: Electrochemical, spectroscopic and atomic force microscopy studies of mechanisms of antimicrobial peptides interactions with model biological membranes

The present thesis aims to resolve mechanisms underlying the antimicrobial activity of membrane-active peptides towards model biological membranes under biologically relevant conditions. The motivation for the research originates from the problem of antibiotic resistance in bacteria, one of the biggest threats to global health and food safety nowadays. Potential alternatives to conventionally available antibiotics are antimicrobial peptides (AMPs). It was initially hypothesized that their mode of action is membrane disruption through pore formation. However, with time, more complicated mechanisms have been proposed, and the mode of action of various AMPs remains unrecognized.

Knowledge of AMPs structure and its correlation with the mechanism of action is utilized to design novel antimicrobial agents for clinical applications. In the present study, two peptides were investigated, a newly isolated BacSp222 bacteriocin produced by *S. pseudintermedius* strain 222 and LL-37 human cathelicidin. Experiments were performed using a model biological membrane comprised of a phospholipid bilayer deposited on a solid substrate, either gold electrode or mica. The goal was to find the secondary structure, orientation, and localization of AMPs in the phospholipid bilayer. Simultaneously, the effects of the AMP on phospholipid conformation and orientation were unraveled. Three complementary experimental techniques were used, including atomic force microscopy (AFM) for direct visualization of the membrane surface, electrochemical impedance spectroscopy (EIS) to characterize changes in membrane electrical parameters, e.g., capacitance and resistance, and polarization-modulation infrared reflection-absorption spectroscopy (PM-IRRAS) performed on electrode-supported phospholipid bilayer for determination of conformational and orientational changes of analyzed molecules. These surface-sensitive techniques operate under an electrochemical setting, which provides biologically relevant conditions by mimicking transmembrane potential. The present research provides a novel insight into understanding the relations between LL-37 and BacSp222 and multicomponent model membranes.

Moreover, an attempt to screen the toxicity of nitrophenols, airborne pollutants hypothesized to interact with biological membranes, is presented. Similarly as for AMPs, surface-sensitive techniques were involved in revealing the activity of these pollutants on model biological membranes. Finally, the mechanical study of the model membrane in a ripple phase is discussed. Apparently, the formation and mechanical properties of the ripple phase in a supported single bilayer lipid membrane have uniquely been described.