Abstract

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Title of the thesis: *Studies on the interaction of antimicrobial peptides and protein with phospholipid membranes.*

Lipid bilayer is the basic building block of all biological membranes in which cholesterol, proteins and other bio-active molecules are embedded in it. Biological membranes are complex and regulated by various proteins and cholesterol. Therefore, it is often useful to study the artificial lipid bilayer as model system of biological membrane in order to gain insights into the structure and functions of the membranes. In this thesis, we have studied systematically the interaction of antimicrobial peptides with phospholipid membranes in view of understanding the mechanisms of antimicrobial activity. Antimicrobial peptides (AMPs) are promising and emerging materials for the development of drug therapeutics, such as antibiotics which may replace the conventional antibiotics. AMPs are known to target the bacterial membrane, and create defects such as pores, leading to disruption of the membrane. The large unilamellar vesicle (LUV), prepared from extrusion method, were used to study the interaction of antimicrobial peptides with phospholipid membrane. However, giant unilamellar vesicles, owing to their large size (10-50 μm), show the evidence of transmembrane pores induced by AMPs under phase contrast microscope. The calcein release experiment on LUV exposed to AMPs underpins the evidence of transmembrane pores. On the other hand, a variety of experimental techniques such as dynamic light scattering, zeta potential, fluorescence spectroscopy, isothermal titration calorimetry were employed in order to gain insights into thermodynamics and binding kinetics of AMPs with membranes. We compare binding affinity of AMP to PG, PE and PC as PG ≫ PE> PC. Weaker affinity toward neutral phospholipids suggest that interactions of AMPs with lipids are primarily governed by negatively charged lipids, which are indeed major constituents of bacterial membranes. The membrane-membrane interactions, induced by AMP are also evidenced from DLS measurement. The intrinsic binding constant of AMP and other thermodynamical parameters were determined from ITC thermogram using surface partition model. In this thesis we also studied the interaction of a membrane protein KMP-11 (which is believed to be responsible for leishmaniasis) with phospholipid membrane. In the last part of the thesis, we intend to established a methodology to detect lipid phase state and dependence on chain saturation upon interaction of lipid with AMPs based on fluorescence properties of a lipophilic dye nile red. The present study will definitely reinforce the therapeutic applications. Nevertheless, our study provides important insights into the various biophysical techniques to investigate lipid peptide interaction.

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