ABSTRACT

Colicin U is a protein produced by bacterium *Shigella boydii.* It belongs to the group of pore-forming colicins. These colicins interact with receptors in the outer membrane of bacteria closely related to a producing colicinogenic strain. After interaction with the receptor, colicin is translocated across the outer membrane and periplasm to the cytoplasmic membrane where it forms pores. Consequently, the pore formation leads to membrane depolarization and cell death. In this thesis I decided to study the pore-forming properties of colicin U and its membrane topology.

It is shown that colicin U pores are formed by only one colicin molecule and they are voltage dependent. Using measurements with nonelectrolytes we estimated a theoretical inner profile of the pore and its inner diameter to be between 0.7 and 1 nm. Above that, a membrane topology of colicin U pore-forming domain (PFD) is studied. BLM measurements with biotinylated colicin U showed that a significant part of colicin's PFD was translocated to the opposite side of the membrane after the pore opening. The segment between substituted amino acids F463 and D486 was evidenced to be on the *trans* side of the membrane after the pore opening. Additionally, properties of peptide H1, which reflects a significant part of the first α-helix of colicin U PFD, is tested. This amphipathic α-helix is able to interact with the membrane of liposomes, even permeabilizing them. With respect to the location of the α-helix in the whole colicin structure, we suppose its important role in colicin U pore formation.