Monitoring the Dynamic Interaction of the Peptide Protonectarina-MP with Zwitterionic and Anionic Vesicles

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The mechanisms of action of antimicrobial peptides (AMPs) have been thoroughly investigated since the AMPs are promising candidates for the development of novel antibacterial agents. Considering that the peptide Protonectarina-MP is reported to interact with cell membranes, we investigated the mechanism of interaction of this peptide both with zwitterionic (phosphatidylcholine) and anionic (phosphatidylcholine/phosphatidylglicerol: 70/30) vesicles. The peptides were manually synthesized in solid-phase, under two forms: with the C-terminus carboxyamidated and as carboxyl-free. The peptides and vesicles were incubated to form proteoliposomes up to 60 min at 25°C, and then diluted with D_2O; after 30 sec, the deuteration was quenched by the addition of formic acid to reduce the pH to 2.5 and the temperature was reduced to 0°C. The H/D exchange of proteoliposome suspension was directly infused into the mass spectrometer and analyzed by ESI-MS and MS/MS. It was determined that this peptide is positioned in parallel to the surfaces of both types of vesicles, keeping the N-terminal and C-terminal regions oriented outside of the membrane, while internal residues of the sequence tend to present a dynamic interaction, becoming in some times positioned within the hydrophobic core of the membranes, and outside of membranes in other times. Protonectarina-MP interacts with both types of membranes, however, Protonectarina-MP(-NH_2) interacted more intensively with the membranes than Protonectarina-MP(-OH). Thus, it is possible to speculate that the movement of the peptide on the membrane surface must generate a structural disturbance, which destabilize and cause a partial depermeabilization of the membranes. These results can explain the hemolytic and antibiotic activities presented by Protonectarina-MP(-NH_2).

Keywords: Proteoliposome; Anti-microbial peptide; H/D exchange; Mass spectrometry; Peptide–membrane interaction.

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