Interaction between Sticholysin II and Lipid Membranes. A Spin Label Study

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Sticholysin II (StII), a cytolysin from the sea anemone *Stichodactyla helianthus,* belongs to the highly homologous family of actinoporins. StII forms pores in biological and model membranes, where its putative receptor is considered to be sphingomyelin (SM). Cholesterol has also been implied in protein-membrane interaction. Pore formation is proposed to involve a sequence of steps: binding to the membrane surface, dissociation of the N-terminal region (which comprises the first ten hydrophobic residues and an amphipathic a-helix) from the body of the protein, and interaction with the bilayer interface. These steps would be followed by penetration of the first ten residues into the membrane hydrophobic core, and oligomerization into a tetramer. A toroidal pore would be formed, lined by the polar and charged residues of the amphipathic a-helix and the phospholipids headgroups, possibly negatively charged and/or inducers of positive curvature. We investigated the interaction between StII and vesicles of variable lipid composition (SM:PC 50:50, SM:PC:Chol 50:15:35, SM:PC:DOPE 50:35:15, SM:Chol:DOPE 50:35:15, and SM:DOPE 85:15) by means of electron paramagnetic resonance (EPR) spectra of membrane-bound spin-labeled derivatives of stearic acid (SASL) carrying the nitroxide moiety at carbons 5, 7, 12, and 16. Spectral changes were found to depend both on lipid composition and on the label position. The effects were greater for bilayers containing cholesterol. In addition, the effects were more pronounced in spectra of nitroxides located closer to the water-lipid interface. These results are in agreement with the proposed model of the toroidal pore. Moreover, the data support the hypothesis that cholesterol plays a role in pore formation.
Supported by FAPESP, CNPq, CAPES.