Lipid phase coexistence and membrane fluidity favor membrane insertion and pore-forming ability of sticholysin I, a highly cytolytic toxin from a sea anemone

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Sticholysin I (StI) is a cytolysin produced by the sea anemone *Stichodactyla helianthus*, with high affinity for sphingomyelin (SM)-containing membranes. However, the role of SM content, membrane structure and its dynamics on StI’s activity is not clearly understood. To gain insight into the role of SM on the interaction with membrane and pore-forming ability of StI, we examined its binding to monolayers and vesicles of phosphatidylcholine (PC) with different SM content. Furthermore, the effect of acyl chain length and unsaturation, two features related to membrane fluidity, was also evaluated. This study revealed that StI preferentially binds and penetrates -with faster kinetic- liquid-expanded films with high lateral mobility. This would explain the increase in StI’s activity with decreasing acyl chain length or increasing unsaturation. To study the role of lipid microdomains we performed studies in model lipid systems of SM, PC, and sterols promoting or not lateral domain formation. The presence of sterol favored toxin-membrane association and pore forming ability in the order cholesterol > ergosterol ≥ cholestenone > the control binary mixture PC:SM, even though lipid domain borders did not function as preferential binding sites for StI. Evidence obtained by atomic force microscopy and polarized total internal reflection fluorescence microscopy showed that StI reduces line tension promoting lipid mixture that could be a strategy to create a more suitable environment for N-terminal insertion and pore-formation. Altogether these results suggest that the physico-chemical properties of the membrane hydrophobic core resulting from phospholipid packing and lipid microdomain are important for toxin-membrane association and oligomerization.

Keywords: sticholysin, membrane fluidity, lipid phase separation