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Interaction of native and modified clupeine with Gram-negative model membranes.

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Clupeine, a cationic antimicrobial peptide found in the sperm cells of fish, is of interest as a food additive because of its antimicrobial activity against several foodborne pathogens. However, it has previously been shown that non-specific binding of clupeine to anionic molecules reduces its antimicrobial activity. It has also been shown that the overall positive charge of the native peptide can be reduced by blocking 10% of its arginine residues with 1,2-cyclohexanedione (CHD) to form CHD-treated clupeine. CHD-treated clupeine retains antimicrobial activity but it is not known if the modes of interaction against Gram-negative bacteria remain the same as the native peptide. The focus of the present study was to investigate the effect of charge reduction on peptide membrane interactions by comparing the effect of native and CHD-treated clupeine on *Escherichia coli* (*E. coli*) model biomembranes.

It was hypothesized that the reduction in charge would result in different interactions with model monolayers composed of *E. coli* PE, phosphatidylethanolamine, 79 mole%:PG, phosphatidylglycerol, 17 mole%: and CL, cardiolipin, 4 mole%), and model bilayer membranes composed of DPPC,: PE:PG:CL (79:17:4 mole%).

Peptide interaction with the model biomembranes were studied using Neutron Reflectometry (NR) and X-ray reflectometry (XRR) and symmetric bilayers were deposited on silicon blocks applying the Langmuir-Blodgett and Langmuir Schaefer techniques. Some lipid mixing was observed in the inner tail region ($\sim 69 \pm 0.24\%$ DPPC (1,2-dipalmitoyl (d62)-sn-glycero-3-phosphocholine) and $\sim 24 \pm 0.02\%$ PE:PG:CL); and in the outer tail region ($\sim 24 \pm 0.02\%$ DPPC and $\sim 56 \pm 0.01\%$ PE:PG:CL). Native and CHD-treated clupeine were not able to cross the model PE:PG:CL:DPPC bilayer biomembrane, however, CHD-treated clupeine showed increased interactions with the lipid head group. In spite of the different interactions observed in the model systems, a more comprehensive understanding of the safety and toxicology of both peptides is required before they can be used for food applications.

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