Molecular dynamics simulation of the interaction of peptide analogues derived from the tick OsDef2 defensin with model Gram-positive and Gram-negative bacterial cell membranes

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Antimicrobial peptides (AMPs) are promising drug candidates to counteract the increasing antimicrobial resistance towards conventional antibiotics . The Os analogues, derived from the OsDef2 defensin from the soft tick *Ornithodoros savignyi,* have previously been shown to be active towards Gram-positive and -negative bacteria at micromolar concentrations[1] . AMPs are known to penetrate and damage plasma membranes which underlies their primary mechanism of action[2]. Therefore, details regarding the interaction of the Os analogues with the plasma membrane of bacteria is necessary to understand their functionality and for their successful development as antibacterial therapeutics. Molecular dynamics (MD) simulation was used to characterise the intermolecular interactions of four OsDef2 analogues (Os, Os3- 12, Os11-22 and Os-C) with model Gram-positive and Gram-negative bacterial cell membranes. The peptides were found to adopt mixed secondary structures consisting of beta and random coil elements upon interaction with the model membranes. The secondary structures of the peptides are similar in the presence of both model membranes, however, conformational flexibility is increased in the presence of Gram-negative model membranes. MD analyses revealed that all the Os analogues interact with Gram-negative and -positive model membranes within 200 ns. The peptides interact and penetrate the Gram-positive model membrane more readily than the Gram-negative model membrane. The shorter analogues, Os3-12, Os11-22 and Os-C, penetrate the model membranes through the N- termini, whereas Os interacts through the N-terminus and C-terminus. In addition, hydrogen bonding occurs predominantly through the positively charged Lysine and Arginine residues located in the N-termini of the peptides. Higher amount of hydrogen bonding was observed between Os analogues and the Gram-positive model membrane possibly due to the nature of the lipid headgroups. Generally, membrane-targeting peptides self-associate and reorganise to permeate the membrane[3]. The Os peptides frequently self-associated and assembled as dimers, trimers or even tetramers on the surface of the bacterial membranes with aggregation conceivably higher in Gram-positive model membranes. As expected, almost all the peptides had a disordering effect on the membrane lipid acyl chains upon peptide-membrane interaction. In conclusion our findings suggest that electrostatic forces and hydrogen bonding via the N-termini of the peptides dictates the extent of interaction/penetration of the peptides in the model membranes. The formation of larger aggregates and decreased conformational flexibility further facilitates the penetration of the Os peptides into the model membranes which leads to membrane disordering effects indicative of antibacterial activity. This information will aid in the design of second generation peptides with increased Gram-positive and Gram- negative targeting activity.

Keywords: antimicrobial resistance, antimicrobial peptides, tick defensin, molecular dynamics simulations, drug development

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