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Mode of Action of Surface Bound Antimicrobial Peptides Melimine and Mel4 against Pseudomonas aeruginosa

Authors : Muhammad Yasir, Debarun Dutta, Mark Willcox

Abstract : Biomaterial-associated infections are a multi-billion dollar burden globally. Antimicrobial peptide-based coatings may be able to prevent such infections. The aim of this study was to investigate the mechanism of action surface bound peptides (AMPs) against Pseudomonas aeruginosa 6294. Melimine and Mel4 were covalently attached to glass coverslips using azido-benzoic acid. Attachment was confirmed using X-ray photoelectron spectroscopy. P. aeruginosa was allowed to attach to AMP-coated glass for up to 6 hours. The effect of the surface-bound AMPs on bacterial cell membranes was evaluated using the dyes DiSC3-(5), Sytox green, SYTO 9 and propidium iodide with fluorescence microscopy. Release of cytoplasmic materials ATP and DNA/RNA were determined in the surrounding fluid. The amount of cell death was estimated by agar plate counts. The AMPs were successfully covalently bound to the glass as demonstrated by increases in %nitrogen of 3.6% (melimine) and 2.3% (Mel4) compared to controls. Immobilized peptides disrupted the cytoplasmic membrane potential of P. aeruginosa within 10 min. This was followed by the release of ATP after 2 h. Membrane permeabilization started at 3 h of contact with glass coated AMPs. There was a significant number of bacteria (59% for melimine; 36% for Mel-4) with damaged membranes after 4 h of contact. At the 6 h time point, release of DNA occurred with melimine releasing 2 times the amount of DNA/RNA than Mel4 surfaces ($p < 0.05$). Surface bound AMPs were able to disrupt cell membranes with subsequent release of cytoplasmic materials, and ultimately resulting in bacterial death.

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