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Characterization of chemoselective surface attachment of the cationic peptide melimine and its effects on antimicrobial activity

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ABSTRACT

Antimicrobial peptides (AMPs) are promising alternatives to current treatments for bacterial infections. However, our understanding of the structural–functional relationship of tethered AMPs still requires further investigation to establish a general approach for obtaining consistent antimicrobial surfaces. In this study, we have systematically examined the effects of surface orientation of a broad-spectrum synthetic cationic peptide, melimine, on its antibacterial activity against Gram-positive and Gram-negative bacteria. The attachment of melimine to maleimide-functionalized glass was facilitated by addition of a single cysteine amino acid into the peptide sequence at the N-terminus (CysN) or C-terminus (CysC), or at position 13 (Cys13, approximately central). The successful attachment of the modified melimine was monitored using X-ray photoelectron spectroscopy and time-of-flight secondary ion mass spectrometry (ToF-SIMS) with principle component analysis. The ToF-SIMS analysis clearly demonstrated structural difference between the three orientations. The peptide density for the modified surfaces was found to be between 3.5–4.0 $\times 10^{-9}$ mol cm^{-2} using a modified Bradford assay. The ability of the surfaces to resist *Pseudomonas aeruginosa* and *Staphylococcus aureus* colonization was compared using fluorescence confocal microscopy. Reductions in total *P. aeruginosa* and *S. aureus* adhesion of 70% ($p < 0.001$) and 83% ($p < 0.001$), respectively, after 48 h were observed for the melimine samples when compared to the blank control. We found that melimine attached via the N-terminus was the most effective in reducing total bacterial adhesion and bacterial viability with two- and four times ($p < 0.001$) more activity than melimine attached via the C-terminus for *P. aeruginosa* and *S. aureus*, respectively. Furthermore, for Cys13, despite having the highest measured peptide density of the three surfaces, the higher concentration did not confer the greatest antibacterial effect. This highlights the importance of orientation of the peptides on the surface to efficacy. Our results suggest that the optimal orientation of the cationic residues is essential for maximum surface activity, whereby the optimal activity is obtained when the cationic portion is more available to interact with colonizing bacteria.

1. Introduction

Biomaterials are used extensively in a variety of medical devices and implants, such as catheters, stents, prosthetics, contact lenses and sensors. However, the increased use of biomaterials in these devices has coincided with an increase in nosocomial infections, to 2 million cases (2004) annually in the USA, resulting in 80,000 deaths [1]. Catheters, subcutaneous sensors and prosthetics account for about half of these cases of infections, despite sterilization and aseptic procedures [2,3]. Implant-associated infections present a significant economic burden on society, direct medical costs being estimated to be $3 billion annually in the USA [2]. Although less common than infections related to catheters, infections associated with surgical implants are generally more difficult to manage as they require a longer period of antibiotic therapy and repeated surgical procedures [1,3].

Numerous coatings for biomaterials have been developed to resist bacterial colonization. These are classed into two broad categories: "passive" and "active" [2,4]. However, all of the present coatings have certain disadvantages with their use, such as varying efficacy against bacterial species and antibiotic resistance. Therefore a new antimicrobial coating is needed.

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