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Role of end-Tags in the enhancement of Antiendotoxin activities of antimicrobial peptides

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Antimicrobial peptides (AMPs) serve the first line of defense against invading pathogens and act by disrupting bacterial membrane integrity. Gram-negative bacteria cell wall is composed of two layers: the inner phospholipid membrane and the outer membrane with a highly conserved unique lipid called lipopolysaccharide (LPS; endotoxin). LPS acts as a permeability barrier against a number of bactericidal agents. Furthermore, LPS is well known as a potent inducer of the immune system when it is released to blood and often causes septic shock syndromes in human. Therefore, a potent antimicrobial agent shall not only possess antimicrobial activity but also have the ability to neutralize LPS and decrease its toxicity. Previously, we have designed a short tryptophan-rich peptide S1. However S1 has a low antimicrobial activity at high salt concentrations. We have developed an easy strategy to boost salt resistance and serum stability of short antimicrobial peptides by adding the non-nature bulky amino acid β-naphthylalanine to their C-termini. Herein, we have extended this study to characterize the anti-endotoxin effects of β-naphthylalanine end-tagged short antimicrobial peptides. Based on structure from transfer NOE (tr-NOE) and paramagnetic relaxation enhancement studies, we have found that S1-Nal-Nal positions itself deeply into LPS micelles, making the peptide more efficient in disrupting the LPS micelles than S1. Furthermore, the antiendotoxin activities of S1-Nal-Nal and S1 were compared based on *in vitro* and *in vivo* assays. The structural results will be used to help us design more potent antimicrobial peptides for clinical application in the future.