Easy Strategy To Increase Salt Resistance of Antimicrobial Peptides

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Received 14 February 2011/Returned for modification 29 April 2011/Accepted 8 July 2011

The efficacies of many antimicrobial peptides are greatly reduced under high salt concentrations, limiting their development as pharmaceutical compounds. Here, we describe an easy strategy to increase salt resistance of antimicrobial peptides by replacing tryptophan or histidine residues with the bulky amino acids β-naphthylalanine and β-(4,4′-biphenyl)alanine. The activities of the salt-sensitive peptide P-113 were diminished at high salt concentrations, whereas the activities of its β-naphthylalanine and β-(4,4′-biphenyl)alanine-substituted variant were less affected.

Antimicrobial peptides play important roles in the host innate defense mechanism by interacting and permeabilizing microbial membranes (21). With an increase of antibiotic resistance, the potential for the development of antimicrobial peptides as novel therapeutic agents could overcome the problem of resistance (5).

The development of antimicrobial peptides has been hindered by several problems. One of these problems is salt sensitivity (13). The efficacy of human β-defensin-1 is greatly reduced in the presence of high salt concentrations in bronchopulmonary fluids in cystic fibrosis patients (4). Similar problems were observed in the clinically active histidine-rich peptide P-113, indolicidins, gramicidins, bactenecins, and magainins (12, 14, 17, 20). A number of studies have been reported on the design of salt-resistant antimicrobial peptides. However, most of them were focused on overall structure modifications, such as structure rigidity, helix stability, hydrophobicity, and amphipathicity (2, 3, 6, 12, 13, 15, 16).

Previously, we found that a novel Trp-rich peptide, Ac-KWRRWVRW1-NH2, designated Pac-525, demonstrated improved activity against both bacteria and fungi and showed reduced hemolytic activity (18). Nal-Pac-525, with all of its tryptophans replaced by the bulky amino acid β-naphthylalanine (7), was shown to have a higher antimicrobial activity than Pac-525 (19). More importantly, unlike Pac-525, the antifungal activity of Nal-Pac-525 against fluconazole-resistant fungal pathogens was not blocked by high-salt incubation conditions (17). However, Nal-Pac-525 has a high hemolytic activity that prohibits its further clinical application (Fig. 1). Thus, the advantages and disadvantages of bulky amino acid substitution to salt-resistant antimicrobial peptides remain to be elucidated. P-113 is a 12-amino-acid histidine-rich peptide derived from the saliva protein histatin 5. The anti-Candida activity of P-113 has been documented previously (14). Recently, a clinical trial involving HIV patients showed that P-113 has a good outcome for oral candidiasis therapy, but the activity of P-113 is restricted to a low-salt condition, limiting its application (8).

Based on the studies of Pac-525 and Nal-Pac-525, it is hypothesized that the substitution of tryptophan residues by the bulky amino acid β-naphthylalanine may generate a potent peptide with improved antimicrobial activity and salt resistance. β-Naphthylalanine residues may position themselves deeper into the bacterial and fungal cell membranes, making the peptide more efficient in disrupting the membranes, hence compensating the competition from the cations to the negatively charged microbial cell surface. However, the hemolytic activity of Nal-Pac-525 increases dramatically with the β-naphthylalanine substitutions (Fig. 1). These observations lead to the hypothesis that the replacement of the aromatic residues with bulky aromatic amino acids of salt-sensitive and low-hemolytic antimicrobial peptides may result in salt-resistant peptides with slight increases of their hemolytic activities.

The histatin derivative P-113, AKRHGYKKRFH-NH2, was

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† Published ahead of print on 18 July 2011.

FIG. 1. Hemolytic activities of P-113 (●), Phe-P-113 (□), Nal-P-113 (●), Bip-P-113 (■), Pac-525 (○), and Nal-Pac-525 (△). Melittin (▲) was used as a control.
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<th>NaCl concn (mM)</th>
<th>LYM broth</th>
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**TABLE 1.** MIC values for ampicillin, P113, Phe-P113, Nal-P113, and Bip-P113 in different concentrations of NaCl.

**TABLE 2.** MIC values for ampicillin, P113, Phe-P113, Nal-P113, and Bip-P113 in different pH conditions and concentrations of MgCl₂.

**Control**
used to test this hypothesis. We have designed and synthesized Nal-P-113, with His 4, 5, and 12 replaced by β-naphthylalanines (19). Bacterial and fungal strains from ATCC and clinical isolates (17) used in this study are listed in Tables 1, 2, and 3. The antibacterial activities of P-113 and Nal-P-113 were determined by the standard broth microdilution method of the National Committee for Clinical Laboratory Standards with Mueller-Hinton (MH) broth and LYM broth (14). The antimicrobial activities of P-113 and Nal-P-113 were determined in LYM broth medium (14) with different salt concentrations. The MIC value is the lowest concentration of peptide at which there was no change in optical density. All tests were measured in triplicate. The hemolytic activities of the peptides were determined from hemolysis against human red blood cells (hRBC) (18). The MIC values of Nal-P-113 were found to be more potent than those of P-113, and its activities are diminished under high-salt conditions (Tables 1 and 2). On the other hand, the MIC values of Bip-P-113 are found to be more potent than those of P-113, and Bip-P-113 still retains its antimicrobial activities under high-salt conditions (Tables 1 and 2). Even though Bip-P-113 has slightly higher hemolytic activity than Nal-P-113 (Fig. 1), it exhibits less than 5% hemolytic activity at its effective MICs. Certain antimicrobial peptides containing potent and broad-spectrum activities against various microbial pathogens are already in clinical trials (5). However, the antimicrobial activities of some agents were found to diminish under physiological and high-salt conditions (1, 15, 22). In this study, the β-naphthylalanine and β-(4,4′-biphenyl)alanine-substituted peptides, Nal-P-113 and Bip-P-113, have potent activity against microbial pathogens, including methicillin-, ampicillin-, and fluconazole-resistant strains. Moreover, the antimicrobial activity is no longer hindered by high salt concentrations.

We thank Chi-Cheng Peng, Hung-Lun Chu, and Cheng-Ying Lee for help in some of the experiments. This work is supported by grants from National Science Council, Republic of China.

REFERENCES


