Design of Short Synthetic β-sheet Forming Peptide Amphiphiles for Antimicrobial Applications

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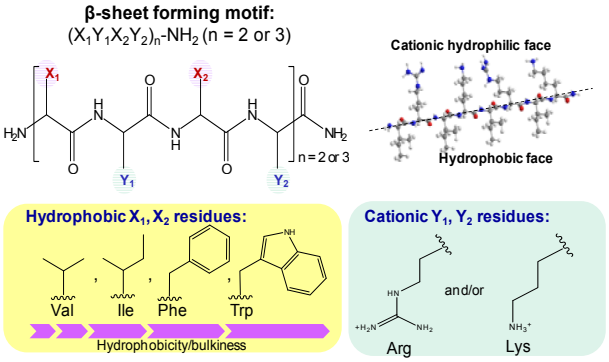
Statement of Purpose: Naturally occurring membrane-active antimicrobial peptides (AMPs) and their analogs have offered unprecedented opportunities to address the mounting problem of antibiotic drug resistance [1]. Although more than 1960 natural host defense AMPs have been identified to date, few AMPs are in clinical use owing to their high systemic toxicities. Other major challenges associated with the application of these AMPs as antimicrobial drugs lie in the high cost in synthesizing long peptide sequences, poor understanding of structure-activity relationships (SARs) and risk of compromising natural defenses. In our efforts to elucidate SARs and enhance the clinical applicability of AMPs, we designed a series of fully synthetic β-sheet folding peptide based upon a basic principle of having amphiphatic dyad repeats containing cationic and hydrophobic amino acids with high β-sheet folding propensities (Scheme 1). The antimicrobial and antibiofilm activities of the designed peptides were systematically evaluated in relation to their hemolytic activities.



Scheme 1. Design of synthetic β-sheet forming AMPs.

Methods: Peptides used in this study were synthesized by GL Biochem (Shanghai, China) with more than 95% purity. The secondary structure of the designed peptides in deionized water (DI water) and microbial membrane mimicking conditions (using 25 mM SDS micelles solution) were determined using circular dichroism (CD) spectroscopy. The minimum inhibitory concentrations (MICs) of the designed AMPs against *S. aureus* (ATCC29737), *E. coli* (ATCC25922) and *P. aeruginosa* (ATCC9027), and *C. albicans* (ATCC10231) were determined using the broth microdilution method. Hemolysis assay was performed as previously described using 4% v/v rat red blood cell suspension [2]. Selectivity index (SI) was calculated as HC_{10}/MIC. To investigate the antimicrobial mechanisms, *E. coli* and *S. aureus* suspensions (3.0×10^8 CFU/mL) were treated with an equal volume of peptide solution (125 mg/L) for 2 h, fixed, dehydrated using a series of graded ethanol, air-dried, and sputter coated with platinum for imaging using a FE-SEM setup (JEOL JSM-7400F, Japan). Cell viabilities and biomass of treated biofilms were determined using the XTT assay and crystal violet assay.

Results: Under microbial membrane mimicking environments, the designed peptides readily self-assembled into β-sheet secondary structures with characteristic CD spectra showing a maximum at ~ 200 nm and minimum at ~ 218 nm. The synthetic AMPs exhibited strong and broad spectrum antimicrobial activities against the four clinically relevant microorganisms tested, with geometric mean (GM) MICs ranging from 15.6 to 203.1 mg/L. By systematically varying the type of cationic amino acid residue while retaining Ile as the hydrophobic residue, it was found that the order of antimicrobial activities for n=2 repeat units was as follows: 4 Arg- > 2 Arg + 2 Lys- > 4 Lys-containing AMPs. Analyses of hemolytic activities showed that the 2 Arg + 2 Lys combination induced the least level of toxicity against rat red blood cells.

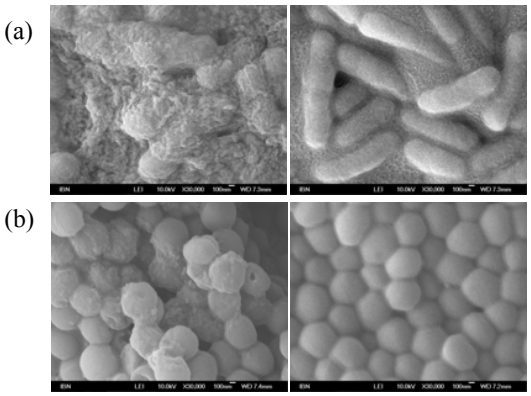


Figure 1. FE-SEM images indicating extensive membrane damage in (a) *E. coli* and (b) *S. aureus* mediated by representative Ile-containing AMP (n=2) (left) as compared to untreated control (right).

By varying the degree of hydrophobicity and bulkiness in the amino acid side chains while keeping the 2 Arg + 2 Lys cationic amino acid combination constant, we found that Ile residues confer the best antimicrobial effects with a high SI of 37.1. Among the peptides with n=3 repeat units, the valine-containing AMP demonstrated the lowest GM MIC value of 43 mg/L, with a high SI of > 58.1. Consistent with a membrane-lytic mechanism, treatment of microorganisms with the AMP was found to induce significant membrane damage and corrugation (Figure 1). In addition, the AMPs significantly inhibited the growth of sessile biofilm bacteria growth and led to a reduction in biomass ($p < 0.01$).

Conclusions: Taken together, our findings clearly demonstrated that the rationally designed synthetic β-sheet folding peptides are highly selective and have potential for use as broad spectrum antimicrobial agents to overcome the prevalent problem of multidrug resistance in a wide range of infectious disease related applications.

References:

[1] Hancock REW. Nat. Biotechnol. 2006; 24:1551-1557.
[2] Wiradharma N. Biomaterials 2011; 32:2204-2212.

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