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Computer-aided design, structural dynamics analysis, and *in vitro* susceptibility test of antibacterial peptides incorporating unnatural amino acids against microbial infections

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ABSTRACT

Background and objective: Antibacterial peptides (ABPs) are essential components of host defense against microbial infections present in all domains of life. The AMPs incorporating unnatural amino acids (*u*ABPs) exhibit several advantages over naturally occurring AMPs based on factors such as bioavailability, metabolic stability and overall toxicity.

Methods: Computer-aided modeling and *in vitro* susceptibility test were combined to rationally design short *u*ABPs with potent antimicrobial activity. In the procedure, peptide characterization and machine learning modeling were used to develop statistical regression predictors, which were then employed to guide the molecular design and structural optimization of *u*ABPs, to which a number of commercially available unnatural amino acids were introduced.

Results: An improved *u*ABP population was obtained, from which several promising candidates were successfully prepared and their antibacterial potencies against three bacterial strains *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli* were measured using broth microdilution assay. Consequently, four *u*ABPs with hybrid structure property were determined to have high potency against the tested strains with minimum inhibitory concentration (MIC) of <50 μg/ml.

Conclusions: Molecular dynamics (MD) simulations revealed that the designed *u*ABPs are amphipathic helix in solution but they would largely unfold when spontaneously embedding into an artificial lipid bilayer that mimics microbial membrane.

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1. Introduction

Infectious diseases caused by new or previously unrecognized microorganisms are a major problem worldwide. Although the term became part of the journalist's lexicon in the 1990s, emerging infectious diseases have long been recognized as an important outcome of host–pathogen evolution. Because infections may have severe public health consequences, they are a focus of both the popular press and the scientific research [1]. In the past 70 years, antibiotics have been essential in the fight against infectious diseases and have been a contributing factor in the rise in life expectancy. However, with the growing microbial resistance to conventional antimicrobial agents over the past decades, the need for unconventional therapeutic options has become urgent. The World Health Organisations' 2014 report on global surveillance of antimicrobial resistance reveals that antibiotic resistance is no longer a prediction for the future; it is happening right now, across the world, and is putting at risk the ability to treat common infections in both the community and the hospitals [2]. Discovery of new classes of therapeutic strategies to combat antibiotic resistance has long been a great attraction in the medicine community. Many efforts have been directed toward finding alternative antibiotics unaffected by resistance mechanisms.

Antibacterial peptides (ABPs) are a unique and assorted group of molecules produced by living organisms of all types, considered to be part of the host innate immunity. These peptides demonstrate potent antimicrobial activity and are rapidly mobilized to neutralize a broad range of microbes, including viruses, bacteria, protozoa, and fungi. More significantly, the ability of these natural molecules to kill multidrug-resistant microorganisms has gained them considerable attention and clinical interest [3]. ABPs are membrane active polypeptides with important functions in the innate host-defense system of many organisms, which can target and then destabilize the cell membrane of a variety of Gram-negative and Gram-positive bacteria. Although ABPs have become as a promising alternative to traditional antibiotics for treatment of bacterial diseases, many potential problems should be solved before they can be put in clinic and commerce, including low bioavailability, high production costs, toxicity against eukaryotic cells, susceptibility to proteolytic degradation and the development of allergies to these peptides [4]. In recent years, incorporating with unnatural amino acids has been developed as a new and promising strategy to improve the metabolic stability and pharmacokinetic profile of ABPs. ABP incorporating with unnatural amino acids (*u*ABPs) has several advantages over naturally occurring peptides based on factors such as bioavailability, metabolic stability and overall toxicity [5].

Most currently existing methods cannot incorporate unnatural amino acids into natural peptides and often miss potent candidates because they are nonspecific and sensitive to physical conditions. In addition, although a number of experimental techniques such as peptide array have been used to successfully determine antibacterially preferable motifs, given the massive number of unnatural amino acid combinations, the accuracy of the motifs is still limited by the coverage of all possible peptides [6–8]. Attempts have been made to overcome such experimental issues by computer-aided approaches as linguistic model [9] and quantitative structure–activity relationship [10], which are benefited from the proliferation of known natural and synthetic AMP data and have thus become standard tools in the quest to develop novel peptide agents for treating bacterial infections [11–14]. Recently, Xiong et al. have successfully combated multidrug resistance in microbial infections by targeting bacterial functional proteome [15] and by using designed antimicrobial peptoids [16,17]. In the present study, we described a synthetic protocol to rationally develop *u*ABPs by integrating computational modeling and experimental assay. We also performed structural analysis and dynamics simulation to investigate the intermolecular interaction behavior of several potent *u*ABPs with an artificial lipid bilayer that mimics microbial membrane.

2. Materials and methods

2.1. *Molecular dynamics simulation*

Molecular dynamics (MD) simulations of *u*ABP interaction with microbial membrane were performed in GROMACS package [18] using the CHARMM27 force field [19]. The peptides were constructed as random structures and then equilibrated with MD simulations in implicit water environment. An artificial lipid bilayer made up of POPC lipids [20] surrounded by TIP3P water molecules [21] was set, and the initial position of equilibrated peptides was immersed in the water layer and parallel to the lipid bilayer. Long range electrostatic interactions were addressed by particle mesh Ewald summation [22], with a real space cutoff of 1.0 nm, and the LINCS algorithm [23] was employed to constrain bond lengths. Pressure control and temperature control were achieved by weak-coupling Berendsen schemes using coupling times with isotropic pressure coupling for the ensemble. A time step of 2 fs was employed and each simulation was run for 150 ns [24].

2.2. *Machine learning regression*

2.2.1. *ABP sample set*

A total of 1491 ABPs with natural amino acid composition and determined biological activity were compiled by Yan et al. [25]. These peptide samples were retrieved from the APD2 database [26]; they have only natural amino acid types, possess excess positive charges, and exhibit antibacterial activity against both Gram-positive and Gram-negative bacteria. The antibacterial potency is expressed as minimum inhibitory concentration (MIC), the lowest concentration of a specific peptide that will inhibit the visible growth of a variety of microorganisms after incubation [27].

2.2.2. *Structural parameterization*

Both local and global descriptors were used to characterize peptide structures. Three amino acid descriptors *z*-scale [28], *T*-scale [29] and NNAAIndex [30] covering unnatural amino acid types were employed as local characterization to parameterize peptides. The *z*-scale was derived by principal component analysis (PCA) of basic physicochemical properties such as