Antimicrobial peptides: Alternatives to combat bacterial infections

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Due to the increasingly growth of antibiotics resistance, antimicrobial peptides (AMPs) with multiple modes of action have been considered as the alternatives to combat pathogens. Here, we discussed two recent research publications about the development of AMPs. The first example published on *J. Pept. Sci.* demonstrated the *N*-terminal modification with lipid acid significantly improved the cationic AMPs' antibacterial and antifungal activities with secondary structure changes. The other research article combined *in silico* computational screening tool and experimental activity test for AMPs optimisation. Both the chemical modification and *in silico* optimisation would assist the potent antimicrobial agents development.

ANTIBIOTIC RESISTANCE

The discovery and introduction of penicillin are developed by Sir Alexander Fleming and Sir Howard Florey in 1940s, which has started the golden era of antibiotics (Fleming, 1929;Florey, 1953). After penicillin, various kinds of conventional antibiotics have been discovered or developed to save millions of patients all over the world. Antibiotic has been well-accepted as one of the most advanced therapeutic medicine in human history. However, the antibiotic resistance is becoming one of the three greatest threats to public health reported from World Health Organisation (WHO), which is accelerated by the widely overuse of antibiotics in humans and animals. Such significant increasing of bacterial multi-drug resistance has led the global calls for the development of novel antimicrobial agents (Laxminarayan et al., 2013). Furthermore, the existence of antimicrobial peptides (AMPs), as host defence peptides, has been first postulated by Ehrlich more than 100 years ago and confirmed as a small cationic protein in the mid-1960's (Ehrlich, 1891;Zeya and Spitznagel, 1968). Due to the potency and various modes of action, AMPs have rapidly emerged as an important alternative to small molecule antibiotics. Due to the advanced development of physical and chemical strategies, researchers are focusing on the modifications of short AMPs for the potential alternatives to combat nosocomial infections. This short article will focus on a pair of recent publications that imply more clues for the development of more potent antibiotic agents.

FATTY ACIDS AND AMP

Lipopeptides, consisted of linear or cyclic peptides and covalently linked fatty acids moiety, have shown great potential to fight against multi-drug resistant pathogens. This combination of fatty acids with cationic AMPs could change the conformation of AMPs secondary structure in presence of bacterial membranes (Chu-Kung et al., 2004). Their mechanism of lipopeptides has been extensively investigated with the FDA approved antibiotic, daptomycin. The daptomycin could partly insert its lipid tails into bacterial membrane, followed by self-aggregation on the membrane and led bacterial membrane depolarization (Straus and Hancock, 2006). Here, the first example was published by Húmpola *et al.* in *J. Pept. Sci.* In their paper, they demonstrated the activity improvement after the modifications with unsaturated or saturated fatty acids on short AMP (named as A2), which was produced by *Lactobacillus plantarum* NRCI 149 from pineapple (Húmpola *et al.*, 2017).

Húmpola *et al.* showed that A2 peptide displayed an amphipathic structure predicted by Schiffer-Edmundson wheel projection. Figure 1 showed the hydrophilic face was composed with Lys and Gln residues. Then, different fatty acids with alkyl chains length from C8 to C20 were attached at the *N*-terminus (Ile residue) of the aforementioned decapeptide, A2 (Figure 1). Due to the properties of lipopeptides, their critical micelle concentrations (CMC) were analysed, which indicated the modified A2 analogues between hydrocarbon chains C8 and C12 had similar physicochemical parameter.

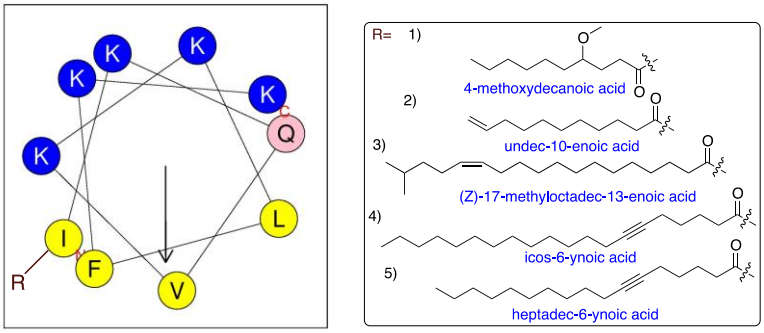

Figure 1. Schiffer-Edmundson wheel projection of A2 of Húmpola *et al.* with various fatty acids modification.

Furthermore, those saturated fatty acids modified A2 (C8 to C14) with similar CMC values displayed promising antibacterial activity against a panel of Gram-negative and Gram-positive bacteria. In addition, the monoenoic and methoxy substituted fatty acids modified analogues were more favoured to bacteria strains, including *P. aeruginosa* and *L. monocytogenes*. In contrast, the longer fatty acid (C20) modified A2 performed lower activity due to self-assembly micelles formation. On the other side, the methoxy substituted saturated fatty acid modification showed highly antifungal activity against all the *Candida* strains tested in this research article. Such improved antifungal activity might be due to the methoxy group increases the solubility and facilitate the peptide interaction with membrane. Moreover, the presence of double bond in monoenoic fatty acids modification showed significantly improvement for their antifungal activity. Such positive effects suggested that the *cis* double bond and the methyl branching moiety of the monounsaturated acid could disorder the fungal membrane.

Finally, the lipopeptide A2 analogues could adapt α-helical confirmations in presence of bacterial membrane model, DPPG. Such secondary structure changes enhanced the importance of stable structure for their antimicrobial activity. The fatty acid modification and the structural effects on cationic AMPs published by Húmpola *et al* will assist researchers in antibiotics field to develop more potent antibacterial agents.

IN SILICO OPTIMISED PEPTIDE LIBRARY AND AMP

In silico modelling approaches has been widely used for *in vitro* absorption, distribution, metabolism, excretion and toxicity screening. This strategy is capable to predict the most relevant pharmacokinetic, metabolic and toxicity endpoints for drug development (van de Waterbeemd and Gifford, 2003). Based on structure-activity relationship (SAR), Fjell *et al.* have created an artificial neural network for the potent AMPs *in silico* screening, which combines the quantitative SAR and machine learning techniques. Utilizing this artificial neural network, a library of 50 short AMPs has been predicted with highly activity accuracy (Fjell *et al*., 2009). Then, a more precise *in silico* AMP screening has been developed based on the aforementioned artificial neural networks, which use genetic algorithms (GA) methods to efficiently predict novel potent AMPs. Herein, the second example in this short article is reported by Godballe *et al.* in *Biopolymers (Pept. Sci.)*, who have efficiently combined *in silico* computational screening outcomes and experimental approaches for potent AMPs development (Godballe *et al*., 2016).

Godballe *et al.* demonstrated the experimental characterisation and visually mode of action of a small cationic AMPs library riched in Trp, which were optimised as GN peptide by the aforementioned GA *in silico* screening (Figure 2). Firstly, such small AMP library was tested with a panel of Gram-negative and Gram-positive bacteria, which displayed significant broaden antibacterial activity from 1.5 to 100 μg/mL. In addition, two of these peptides, GN-2 and GN-6 were chosen for the further effects study on cell size and DNA content with flow cytometry. It showed that the cell sizes were increased in presence of GN AMPs, which were consist with microscopy imaging analyses. Furthermore, such AMPs showed the morphological effects on bacterial cell with long and filamentous formation without cell lyses under microscopy.

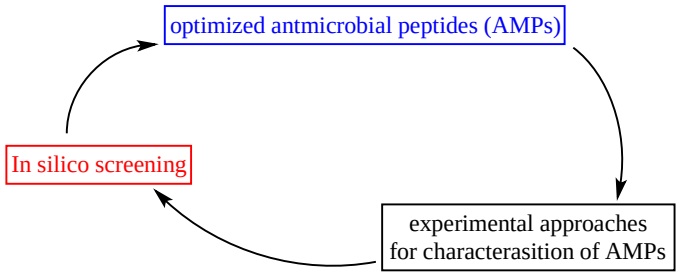

Figure 2. The *in silico* screening approaches with experimental strategy for potent AMPs development.

With the activity investigation of this *in silico* optimised GN peptide library, these peptides displayed very potent inhabitation for the bacteria growth. Furthermore, the microscopy and quantitative assays clearly showed the bacterial membrane disruption and fast membrane permeabilisation in presence of GN peptides, which implied their action of mode against pathogens. In summary, Godballe *et al.* demonstrated an effective strategy in combination of *in silico* computational screening with SAR prediction and experimental test to aid the potent AMPs development.

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