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Biomimetic antibiotics based on amphiphilic peptoids and their self-assembly

March 2022

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U.S. DEPARTMENT OF ENERGY

Prepared for the U.S. Department of Energy
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Abstract

Bacterial infections pose a serious threat to mankind. Inspired by antimicrobial peptides (AMPs) and their membrane-disruption mechanism, there is immense interest in the design and development of synthetic mimetics for antibacterial applications, overcoming the intrinsic drawbacks of AMPs (e.g. susceptibility to proteolytic degradation). Herein, by exploiting the self-assembly and pore-forming capabilities of sequence-defined peptoids, we discovered a new family of low molecular weight peptoid antibiotics that exhibited excellent broad-spectrum activity and high selectivity toward a panel of clinically significant Gram-positive and Gram-negative bacterial strains. Tuning peptoid sidechain chemistry and structure enabled us to tune the efficacy of antimicrobial activity and study the structure–activity relationship. These findings offer a novel approach to identify new families of antimicrobial peptoids and correlate the pore-forming capability of self-assembling peptoids with their antimicrobial activities. Because peptoids are highly robust and biocompatible, we exhibit these peptoid-based antibiotics will be significant for combating the emerging drug resistance.

Summary

In this project, we have synthesized a number of amphiphilic peptoids, and tested them for antibacterial activity. We found some peptoids exhibit good antibacterial activities against a variety of Gram-positive and Gram-negative bacterial (some are up to 3 and 12.5 μg mL−1 minimum inhibitory concentrations against Gram-positive MRSA and Gram-negative *E coli*, respectively). We found that star-shaped amphiphilic peptoids have much better antibacterial activity than linear peptoids do. We also tested the critical micelle concentration (CMC) for some of these peptoids and studies their self-assembly behaviors. We found some of antibacterial sequences can self-assemble into nanofibers at a concentration above CMC. These results indicate that exploiting the self-assembly and pore-forming capabilities of alkyl-containing peptoids is an effective way to identify new peptoid sequences that are highly effective for antimicrobial activities. Low molecular weight peptoids identified here exhibited excellent broad-spectrum activity and high selectivity toward a panel of clinically significant Gram-positive and Gram-negative bacterial strains, including vancomycin-resistant *E. faecalis* (VREF, ATCC 25922), methicillin-resistant *S. aureus* (MRSA, ATCC 33591), methicillin-resistant *S. epidermidis* (MRSE, RP62A), *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 27853), and *K. pneumoniae* (ATCC 13383).

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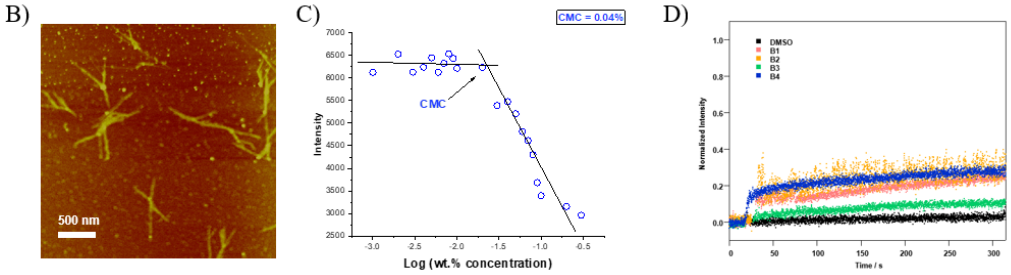
1.0 Introduction

The emerging bacterial resistance caused by the misuse antibiotics have now grown into global public health threat. To address the current situation of combating bacterial resistance, tremendous efforts have been made to identify and isolate antimicrobial peptides (AMPs) from nature systems, because in living organisms, these produced natural host defense peptides exhibit broad-spectrum antimicrobial activities and bacteria can rarely develop resistance toward them. By having α-helical- or β-sheet-like structures and positively charged surfaces, it is generally believed that these peptides are able to interact with negatively-charged membranes of bacteria and then their hydrophobic residues can insert into the nonpolar core of bacterial cell membranes, resulting in the disruption of bacterial cell membranes. Inspired by these peptides and their unique cell membrane disruption mechanisms, extensive research has been done to develop amphiphilic peptides for antimicrobial activities.[1-5] However, the currently available natural AMPs and developed peptide-based antibiotics have significant drawbacks, such as susceptible to proteolytic degradation and high cost of production. These drawbacks have stimulated the development of sequence-defined synthetic molecules as AMP mimetics for antimicrobial applications.[6, 7] Peptoids, which are poly-N-substituted glycines,[8] have received particular attention because they are sequence-defined, biocompatible, and highly stable.[8, 9] Moreover, they can be cheaply and efficiently synthesized through a sub-monomer synthetic method, and several hundred commercially available amines can be used to attain large sidechain diversity.[10] While peptoids have been developed as antibiotics by having the cationic feature and helical structure[11-13], the current peptoid sequences suitable for efficient antibiotics are so limited, and the chemical diversity of the cationic pendant groups is currently limited to a few positively charged ammonium, guanidinium, and imidazolium residues, none channel forming peptoids have been reported. In this project, by taking advantage of the self-assembly and pore-forming capabilities of amphiphilic peptoids, we developed a novel approach for effective discovery of antimicrobial peptoid sequences, we found that channel-forming peptoids induce a better efficacy in killing bacteria.

2.0 Research

In this project, we identified a new family of low molecular weight peptoids that exhibited excellent broad-spectrum activity and high selectivity toward a panel of clinically significant Gram-positive and Gram-negative bacterial strains, including vancomycin-resistant *E. faecalis* (VREF, ATCC 25922), methicillin-resistant *S. aureus* (MRSA, ATCC 33591), methicillin-resistant *S. epidermidis* (MRSE, RP62A), *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 27853), and *K. pneumoniae* (ATCC 13383).

As shown in Figure 1E, while all peptoids are effective in killing bacteria with the μg scale MICs and a broad-spectrum activity, B4 with the highest ion-transport activity shows the lowest MIC against VREF and *P. aeruginosa*. These results suggest that the self-assembling and pore-forming capabilities of alkyl-containing peptoids can be used to identify peptoids that are active for antimicrobial activity, and a high ion-transport activity could lead to a low MIC in killing bacteria.



E)

MIC(μg/mL)				
	Gram+		Gram-	
	VREF	MRSE	K. pheumoniae	P. aeruginosa
B1	3~6	0.75~1.5	NA	6~12.5
B2	3~6	0.75~1.5	NA	6~12.5
B3	3~6	0.75~1.5	NA	6~12.5
B4	1.5~3	0.75~1.5	NA	3~6

Figure 1. A) Structures of peptoids B1-B4 (OMITTED). B) AFM image showing the nanofibers formed through the assembly of peptoid B4 at 5.0 mM in CH_3CN & H_2O (1:1). C) Fluorescence measurement showing B4 has a CMC of ~0.45 mM. D) The proton transport study using the HPTS assay showing the pore formation of peptoids B1 - B4, in which the B4 exhibits the highest ion transport efficiency. E) Antimicrobial activity of B1 - B4 against two Gram positive and two Gram negative bacterial strains.

Sequence modulation for improved antimicrobial activity. To further improve B4 for antimicrobial activity and develop a structure-function relationship, we further synthesized L1 (Figure 2A)

which is similar, antimicrobial results showed that L1 exhibited much lower MICs against both MRSA and *E. coli* bacteria (Figure 2B). We also synthesized B5. Interestingly, this second-generation of dendritic peptoids with four alkyl chains showed almost no activity against both MRSA and *E coli* bacterial strains. These results indicate that there is a valley of efficacy for design features – too many chemical features can significantly decrease the antimicrobial efficacy of peptoids. Because the previous studies by Barboiu et al.[14] showed that urea-induced hydrogen bond formation could significantly alter the intermolecular interactions which could be an important factor for antimicrobial activity.We further designed peptoid B7 to include hydrogen bonding groups. As shown in Figure 2B, attenuated efficacy in antimicrobial activity resulted, which could be due to the increased interactions among peptoids. [15] A close comparison of the antimicrobial activities induced by B2 and B4 shows that B2 is more efficient in killing *VREF* and *P. aeruginosa* while exhibiting same MICs for *MRSE* and *E. coli* (Figure 2). Because B2 and B4 are similar in structure, suggesting these design features could also influence the peptoid-based antimicrobial activity.

B)

		MIC(μg/mL)				
	MW	Gram+			Gram-	
		VREF	MRSA	MRSA	E.coli	P. aeruginosa
B4	1130.6	3-6	3	1.75-1.5	12.5	3-6
L1	821.1	NA	25	NA	>50	NA
B5	866.3	NA	NA		NA	NA
B6	1003.4	6-12.5	3-6	12.525	6-12.5	>25
B7	1375.0	6-12.5	12.5-25	>25	12.5-25	>25
B8	1115.7	NA	NA	NA	NA	NA
B9	722.9	NA	NA	NA	NA	NA
B10	863.16	NA	NA	NA	NA	NANA
B11	943.3	NA	3-6	NA	NA	NA

Figure 2. A) Structures of alkyl-containing peptoids obtained by modulating B4 (OMITTED). B) Antimicrobial activity of these modulated peptoids against three Gram positive and two Gram negative bacterial strains. – indicates that activity was not tested. NA shows no obvious activity over 50 μg/mL.

For that, we further synthesized B6, Figure 2B showed that B6 became less effective in killing all five bacterial strains. These results indicate that a correct number of features is important for peptoids to achieve a high efficacy for antimicrobial activity.

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