

The use of Arginine Analogues in the Preparation of Short Cationic Antimicrobial Peptides

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Introduction

Cationic antibacterial peptides (CAPs) is a promising class of molecules in the battle against microorganisms that have developed resistance towards conventional antibiotics¹. Peptides based on truncated lactoferricin has proven to be very efficient antimicrobial agents and a minimal antibacterial motif, consisting of two units of hydrophobic bulk and two cationic charges, has been defined. Thus, very small di- and tripeptides (Figure 1) can be designed with a high antibacterial activity towards Gram-positive bacteria.

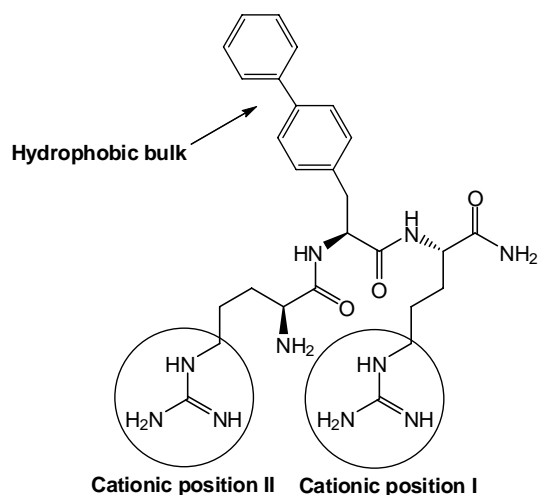


Fig. 1 General structure of a short cationic antibacterial peptide (**001a**) derived from lactoferricin. Hydrophobic bulk is provided by the central amino acid and the positive charges via the guanidine groups and the N-terminal.

A recent study described how these compounds are digested by trypsin and suggested a range of design principles to counteract this degradation in an attempt to prolong the half-life of the peptides². So far, those guidelines only deal with the size and placement of the hydrophobic elements and little is yet known about the role of the cationic contributions provided by the two arginine residues. These arginines are generally taken for granted in these structures and there should be ample room for structural alterations.

The current project is aimed at investigating, in more detail, the role of arginine in these peptides. The following aspects will be evaluated:

- Design of analogues
- Incorporation of analogues/ease of preparation
- Effect on antibacterial potency
- Effect on proteolytic stability
- Cytotoxicity

Results and Discussion

Biphenyl alanine (**Bip**) was used throughout the study to provide a central hydrophobic amino acid residue. It is commercially available and has previously successfully been incorporated in antibacterial peptides¹.

Lysine (**Lys**) was the first obvious alternative to Arginine (**Arg**) as it also represents a basic, naturally occurring amino acid. It has previously been used to produce peptides with somewhat inferior bactericidal potency³.

The two basic phenyl propanoic acid derivatives **Ana** and **Gnp**, were included to establish the effect of adding hydrophobic bulk in the direct vicinity of the cationic charge and not as a separate amino acid residue or as an end-capping. The following four amino acids were combined to generate a small library of candidate compounds (Figure 2).

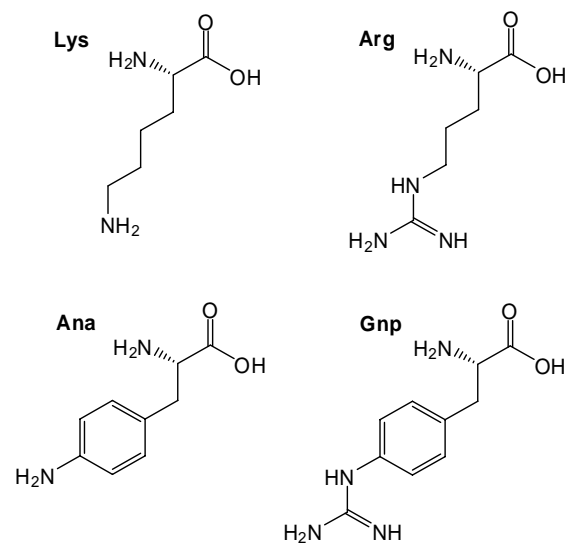


Fig. 2 Structures of the basic amino acids employed in the study to generate variety

Microwave irradiation was employed in conjunction with the general Fmoc-SPPS (Solid Phase Peptide Synthesis) methodology on Rink amide resin to generate the peptide library. None of the alternative amino acids called for any alterations in the chemistry employed to prepare the CAPs.

The antibacterial activity was tested against *Staphylococcus aureus* strain ATCC 25923 and methicillin resistant *Staphylococcus aureus* (MRSA) strain ATCC 33591 (Table 1) using standard methods.

Table 1 Peptide sequence and antibacterial activity

Peptide	Sequence	MIC ($\mu\text{g/mL}$)	
		<i>S.aureus</i>	MRSA
001a	Arg-Bip-Arg	200	50
001b	Lys-Bip-Arg	>200	200
001c	Gnp-Bip-Arg	75	50
001d	Ana-Bip-Arg	200	>200
002a	Arg-Bip-Lys	>200	>200
002b	Lys-Bip-Lys	>200	>200
002c	Gnp-Bip-Lys	>200	150
002d	Ana-Bip-Lys	>200	>200
003a	Arg-Bip-Gnp	75	50
003b	Lys-Bip-Gnp	100	50
003c	Gnp-Bip-Gnp	50	<25
003d	Ana-Bip-Gnp	150	100
004a	Arg-Bip-Ana	>200	>200
004b	Lys-Bip-Ana	>200	>200
004c	Gnp-Bip-Ana	150	75
004d	Ana-Bip-Ana	>200	>200

The peptide library was based around **001a**, a compound with limited bactericidal properties. The minimum antibacterial motif for this class of molecules is two charges and two units of hydrophobic bulk³, leaving **001a** short of one unit of bulk. More potent peptides have been designed by incorporating additional elements of hydrophobic bulk as a C-terminal capping group¹. This apparent shortage of hydrophobicity motivated the use of **Gnp** and **Ana** as arginine analogs in an attempt to establish if the hydrophobic units can be incorporated in the direct vicinity of the cationic charges while still being able to generate a working antibacterial compound.

Lys and **Ana** are poorer bases than **Arg** and **Gnp** and it is apparent that these compounds are less suited as cationic elements in these peptides when analyzing the results from the antibacterial study. The extra hydrophobic element is not enough to yield an effective peptide in this study since **Ana** is too weak a base to be protonated at physiological pH. Incorporation of **Lys** produced poor antibacterial peptides compared to peptides containing **Arg** in the same position. Peptide **003c** is the most potent peptide in this study, with two **Gnp** units providing both cationic charges and hydrophobic bulk (Figure 3). The two second most potent peptides, **001c** and **003a**, also indicate that where **Arg** and **Gnp** is positioned in this sequence is of less importance for the bactericidal effect.

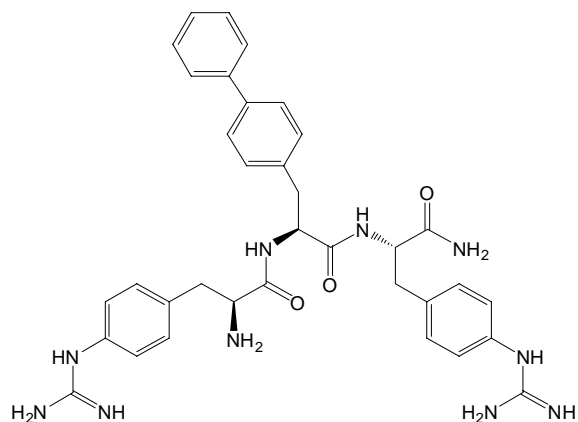


Fig. 3 Structure of peptide **003c**

These findings allows for a more versatile design of future antibacterial peptides as it indicates that the hydrophobic element does not necessarily has to be introduced as a separate building block. For the peptides presented here, studies of the cytotoxicity and the metabolic stability remain.

Acknowledgments

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References

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