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Alanine scanning analysis of the antimicrobial peptide lyp1987 using a template-based approach

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ABSTRACT

A preference for several amino acids is observed to occur at particular positions of cationic α-helical antimicrobial peptides (AMPs), which ensures the formation of amphipathic regions once they assume their correct secondary structure in membranes or membrane-mimicking environments and makes them active against pathogens. This study determined the effect of alanine mutations on the secondary structure and bioactivity of lyp1987 (GRLQAFLAKMKEIAAQTL-NH_2), a cationic α-helical AMP obtained from the venom of Lycosa poonaensis which exhibits broad range activity against Gram-positive and Gram-negative bacteria with micromolar minimum inhibitory concentrations (MIC). CD spectroscopy revealed no significant difference in the secondary structure, with all alanine-substituted analogs exhibiting predominantly α-helical structure in buffered 2,2,2-trifluoroethanol solution. Alanine substitution at Glu12 and Thr17 increased the activity of lyp1987 against Gram-positive and -negative bacteria, while alanine substitution at Lys9 increased its selectivity against Gram-positive bacteria. Further investigation can be done to determine positions and substitutions that will give less cytotoxic analogs.

Background

Most antimicrobial peptides (AMPs) are composed of up to 50 amino acids and have a net positive charge due to the presence of lysine and arginine and around 50% hydrophobic residues, which form separate domains in the secondary structure.(1) Studies have actually shown a preference for several amino acids to occur at certain positions in the primary sequence of naturally-occurring α-helical AMPs which form their amphipathic regions that are implied in their antimicrobial activity once they assume their correct secondary structure.(2-3)

The AMP lyp1987 (GRLQAFLAKMKEIAAQTL-NH_2), isolated from the venom of Lycosa poonaensis, is an 18-mer α-helical cationic AMP with an amidated C-terminus that exhibits broad range activity against bacteria, with MIC values of 15-20 µM against E. coli NBRC 3972, 75-100 µM against S. aureus NBRC 13276, and 2.5-5 µM against B. subtilis NBRC 3009.(4) It has a secondary structure containing three positively-charged residues (Arg2 and Lys9, and Lys11) effectively flanking a predominantly hydrophobic face which is observed and hypothesized to be involved in improved cell-penetrating ability of model AMPs, but might be implied in other undiscovered bioactivities of lyp1987.(5)

Methodology

Automated microwave-assisted solid-phase peptide synthesis of lyp1987 and alanine-monosubstituted analogs
Fmoc chemistry, DIC/Oxyma coupling agents, Rink amide MBHA resin

↓

HPLC purification
Gradient elution using 0.1% TFA in diH_2O and 0.1% TFA in acetonitrile
Up to ≥95% purity

↓

MALDI-TOF-MS
α-cyano-4-hydroxycinnamic acid as matrix

Antimicrobial assay
Resazurin-based broth microdilution assay using P. aeruginosa ATCC 27853 and S. aureus ATCC 6538

CD spectroscopy
In 0.2 M phosphate buffer (pH 7.0) and in 50% 2,2,2-trifluoroethanol

↓

Cytotoxicity assay
MTT-based colorimetric assay using HK2 (ATCC® CRL 2190) normal human kidney cell line
For lyp1987 and active analogs

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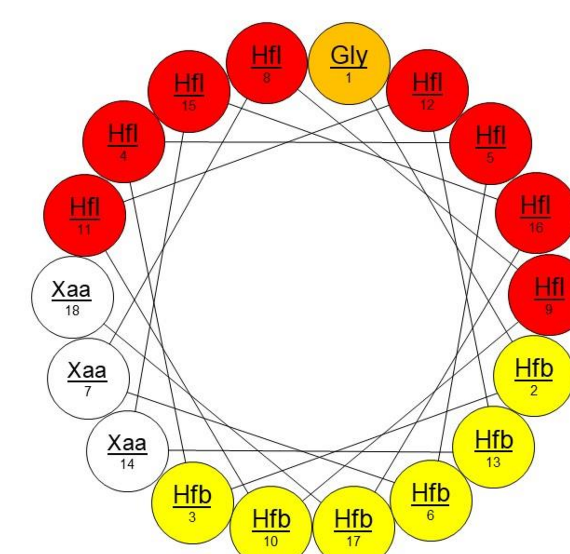
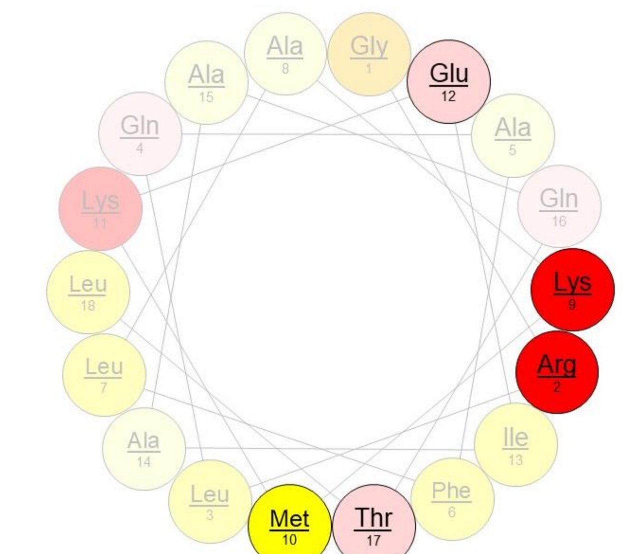
Analog Design



Predominant nature of amino acids of each position of an 18-mer α-helical AMP. Hfl = hydrophilic, Hfb = hydrophobic, Xaa = no specific amino acid



α-helical conformation of lyp1987, with positions of alanine monosubstitution highlighted.

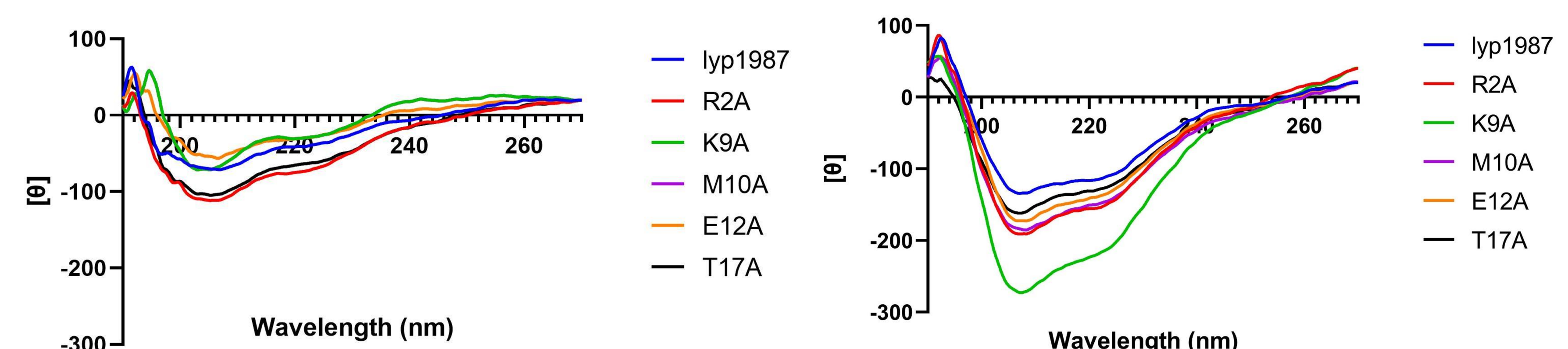
lyp1987	GRLQAFLAKMKEIAAQTL#
R2A	G**A**LQAFLAKMKEIAAQTL#
K9A	GRLQAFLA**A**MKEIAAQTL#
M10A	GRLQAFLAK**A**KEIAAQTL#
E12A	GRLQAFLAKMK**A**IAAQTL#
T17A	GRLQAFLAKMKEIAAQ**A**L#

Primary sequences of lyp1987 and synthesized analogs. Positions of alanine substitution for each peptide written in red, boldface and underlined. # indicates C-terminal amidation for all peptides.

Results

Peptide	MIC[a] (µM)		IC_{50}[b] (µM)
	P. aeruginosa ATCC 27853	S. aureus ATCC 6538	HK2 kidney cells
lyp1987	25	>200	41.14
R2A	>50	>200	n.d.
K9A	25	50	8.8
M10A	>50	>200	n.d.
E12A	6.25	50	31.6
T17A	12.5	100	30.6

Abbreviation: n.d.: not determined. [a]MIC refers to the lowest concentration causing ≥99% inhibition of microbial pathogens. Data presented as average MIC (n=3). [b]IC_{50} refers to the concentration causing 50% inhibition of cell viability. Data presented as average IC_{50} (n=3).



CD spectra of lyp1987 and selected analogs in 0.2 M phosphate buffer (pH 7.0) (left) and in 50% 2,2,2-trifluoroethanol in 0.2 M phosphate buffer (pH 7.0) (right).

Conclusions

- Arg2 and Met10 are essential to the antimicrobial activity of lyp1987.
- Alanine mutation at Lys9 increases its activity against Gram-positive bacteria.
- Alanine mutation at Glu12 and Thr17 increases its activity for both Gram-negative and -positive bacteria.
- lyp1987 can be further engineered to increase its antimicrobial activity and reduce its cytotoxicity.

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