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SHORT ANALOGUES OF CATHELICIDINS PMAP-36 AND BMAP-27: STRUCTURE-ACTIVITY RELATIONSHIP AND STABILITY TO PROTEOLYSIS

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1 – Introduction

Due to the widespread use of antibiotics, bacterial antibiotic resistance has become a major global health problem in recent years. PMAP-36 and BMAP-27 are natural antimicrobial peptides that belong to the cathelicidin family and have broad-spectrum antibacterial activity. The mechanism of action of these peptides hampers the development of bacterial resistance, making them promising for the development of new drugs.

Poor stability in the cellular environment is a limitation for the use of these molecule, but it can be increased by inserting D-configured amino acids into the peptide sequences.

2 – Aim

We present the synthesis of peptide sequences derived from PMAP-36 and BMAP-27 containing all-L and all-D amino acids,

Aim:

- to understand whether the shortened analogues PMAP36(12-24) and BMAP27(1-18) retained the helical structure of the original peptides and thus their antimicrobial activity
- to investigate their stability in proteolytic medium and their cytotoxicity in cells.

List of the synthetized peptides and of their physico-chemical properties

	Name	Peptide Sequence
	PMAP-36	H-GRFRRLRKKTRKRLKKIGKVLKWIPPIVGSIPLGCG-NH_2
a	L-PMAP36(12-24)	Ac-KRLKKIGKVLKWI-NH_2
b	D-PMAP36(12-24)	Ac-krlkkigkvlkwi-NH_2
	BMAP-27	H-GRFKRFRKKFKKLFKKLSPVIPLLHLG-NH_2
c	L-BMAP27(1-18)	Ac-GRFKRFRKKFKKLFKKLS-NH_2
d	D-BMAP27(1-18)	Ac-grfkrfrkkfkklfkkls-NH_2

3 – Synthesis

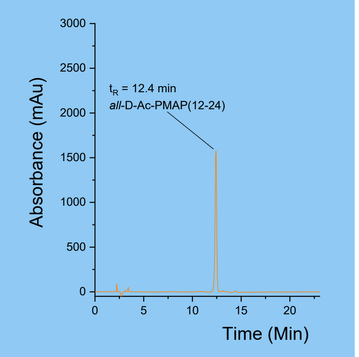
SPPS
- Fmoc/tBu
- Activation: DIC/Oxyme
- Cleavage: TFA/TIS/H_2O (95:2.5:2.5)

PURIFICATION
- Authomatic flash chromatography (column: C18, 100 Å, 12g cartridge)

CHARACTERIZATION
- analytical HPLC
- ESI-MS

	Theoretical MW $[M+H]^+_{calcd}$	Experimental MW $[M+H]^+_{exp}$	Retention time* t_R (min)
a	1651.1	1651.1	13.5
b	1651.1	1651.1	12.4
c	2383.5	2383.1	11.4
d	2383.5	2383.1	14.7

*Retention time refers to 10-40%%B (CH_3CN/H_2O, 9:1) over 30 min gradient for **a** and **b** and 15-45%B over 30min gradient for **c** and **d**.



HPLC profile of peptide **b** (10-40%%B over 30 min

Biological Activity

- D-peptides are as active as L-peptides. In some cases, they are even more active
- D-peptides are not toxic at the concentrations required for bacterial activity

Proteolytic resistance

- The two L-peptides are rapidly degraded by both trypsin and human serum.
- In contrast, D-peptides are degraded at a much slower rate and remain intact for at least 24 hours.

Conformation

- Short PMAP and BMAP analogues retain the helical structure of the native peptide
- NMR confirms the helical propensity of the two shortened peptides: long range NOEs signals are observed

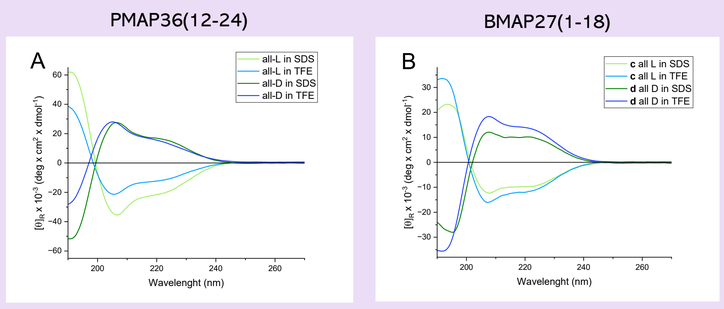
CONCLUSION

The designed short peptides, which contain D-amino acids, are

→ capable of maintaining both the conformation and the activity of the native peptides

→ stable in physiological proteolytic environments

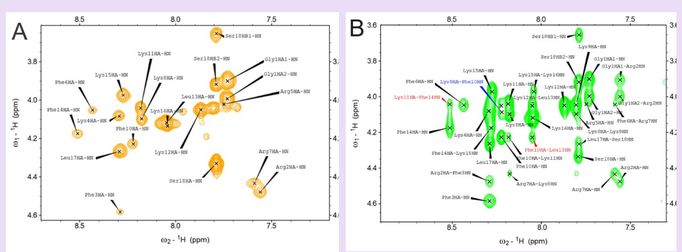
4 – Conformation



CD spectra of (**A**): L-PMAP36(12-24) (**a**) and D-PMAP36(12-24) (**b**). (**B**): L-BMAP27(1-18) (**c**) and D-BMAP27(1-18) (**d**). The spectra were taken in SDS (blue) and TFE (green), at 25°C.

Circular Dichroism (CD) measurements in 100mM sodium dodecyl sulphate (SDS) and 2,2,2-trifluoroethanol (TFE). SDS was selected as a membrane-like environment, while TFE was chosen as a helix inducer solvent. The secondary structure of the D-peptides is the mirror image of that of the L-peptides

NMR 2D spectra were registered for all the four peptides. Long range NOEs typical of helical conformation were observed.



L BMAP(1-18): (**A**) Region of the TOCSY spectrum of peptide **c**, (**B**) Fingerprint region of the NOESY spectrum of peptide **c** . The αCH(i)→ NH(i+2) and αCH(i)→ NH(i+3) cross-peaks are highlighted in blue and red, respectively (600MHz, TFE d2, 308K).

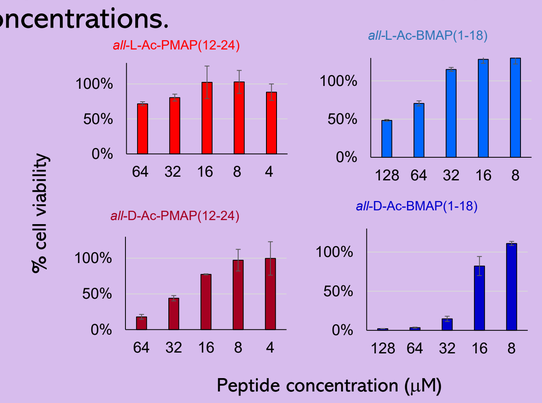
5 – Biological activity

Peptide	MIC (μM) E.coli ATCC 25922	S.aureus ATCC 25923	A.baumanni ATCC 19606	K.pneumoniae ATCC 700603	P.aeruginosa ATCC 27853	S.epidermidis ATCC 12228
all-L-Ac-PMAP(12-24)	4	>64	2	4	8	4
all-D-Ac-PMAP(12-24)	2	16	2	4	4	4
all-L-Ac-BMAP(1-18)	8	16	8	16	16	2
all-D-Ac-BMAP(1-18)	4	8	8	8	8	1

Antibacterial activity against Gram-negative and Gram-positive bacteria. *MIC, minimum inhibitory concentration is the lowest peptide concentration causing no visible growth after 18 h incubation in Mueller–Hinton broth at 37°C.

High antimicrobial activity was observed for L-PMAP(12-24) and D-PMAP(12-24) toward *E. coli, A. baumannii, K. pneumoniae, P. aeruginosa, and S. epidermidis.* L-BMAP(1-18) and D-BMAP(1-18) are active against *S. epidermidis*.

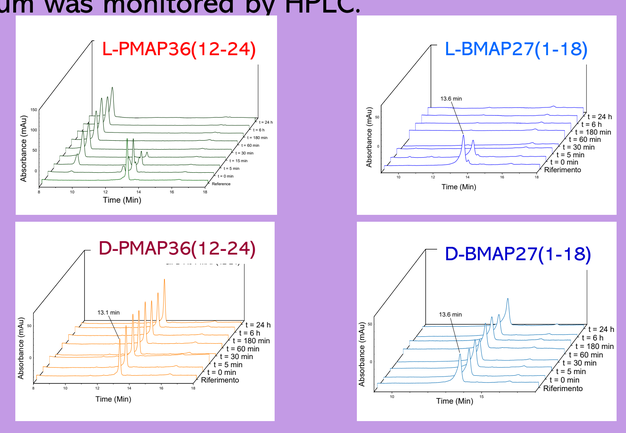
All-D peptides are more toxic than L-peptides only at high concentrations.



Cytotoxicity was measured by the MTT assay on human immortalized epidermal keratinocyte HaCaT cell line

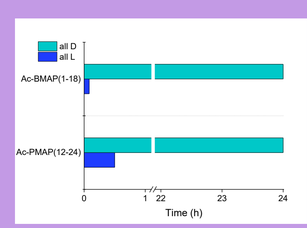
6 – Proteolytic resistance

The stability of the peptides in presence of trypsin or serum was monitored by HPLC.



HPLC profiles of peptides in Trypsin. Left, peptides **a** and **b**; right, peptides **c** and **d**. Gradient 5-65%B in 30mins. UV-vis detector at 214nm.

Proteolytic resistance to trypsin: peptides with all L-amino acids were degraded in less than half an hour, whereas peptides with all D-amino acids were not degraded at all.



Results of the trypsin degradation tests on the four peptides in human serum at 37°C.

A similar result is obtained from the degradation in human serum, where D-peptides are not degratated in the firs 24 hours.

REF. Biondi, B.; De Pascale, L.; Mardirossian, M.; Di Stasi, A.; Favaro, M.; Scocchi, M.; Peggion, C., Sci. Rep., 2023, 13,15132

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