Peptide stapling - application in antibacterial therapies

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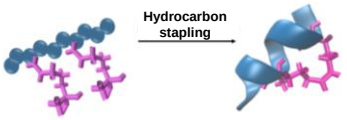
Introduction

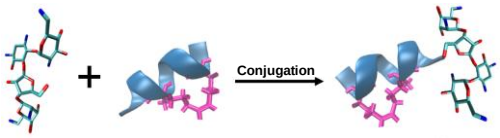
Membrane-active peptides (MAPs) are short chains of amino acids that interact with cell membranes, primarily targeting lipid bilayers and inducing changes in their structure. MAPs have wide-ranging applications in medicine and biotechnology, particularly in antimicrobial therapies (**antimicrobial peptides**, AMPs), anticancer therapies, and drug delivery (**cell-penetrating peptides**, CPPs). Their ability to disrupt cell membranes makes them particularly promising against pathogens that are resistant to traditional antibiotics. We have proposed to stabilize the biologically active structures of selected MAPs with a peptide stapling technique, enhancing their proteolytic stability and antibacterial activity without increasing their hemolytic activity or toxicity to other human cells. Additionally, to enhance the effectiveness of some stapled peptides, we conjugated them with **aminoglycosides**.

Goal of our reaserch

We employed a **hydrocarbon stapling technique**, a specific method of peptide stapling that involves creating hydrophobic bridges composed of hydrocarbons between amino acid residues within the peptide. This process introduces synthetic amino acids containing alkene groups (unsaturated hydrocarbons) into precise positions (*X* and *X + 4*) in the peptide chain.

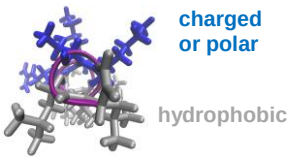


As aminoglycosides, we chose **amikacin** (AMK) and **neomycin** (NEO). We proposed two methods for conjugating peptides with AMK and NEO: a non-cleavable triazole ring formed through a 'click chemistry' reaction, and a disulfide bond, which is cleavable in the bacterial cytosol.



Results

Hydrocarbon staples were introduced into the sequences of several peptides: **anoplin**, an AMP with low antibacterial activity; **(KFF)$_3$K**, a CPP which facilitates the transport of molecules into the bacterial interior; and three desi9gned peptides rich in lysine and leucine residues (**KAL**, **KAL2** and **KAL3**). All selected peptides exhibited an amphipathic nature, a positive net charge, and a propensity to form a helical structure in a lipid environment.



Amphipathic helical structure of a peptide

The staples were inserted into the peptides in a manner that preserved their amphipathicity, hydrophobicity, and net charge.

Secondary structure

The secondary structures of the peptides were determined by **circular dichroism (CD)** spectroscopy in phosphate buffer and in the presence of various lipid membrane environments, including micelles and liposomes. Unlike the unmodified peptides, the stapled analogs exhibited a stable **α-helical structure** in phosphate buffer, as indicated by minima at 208 nm and 222 nm in the CD spectrum. Furthermore, all peptides adopted a helical structure in each lipid environment.

Proteolytic stability

The proteolytic stability of the tested peptides was investigated in **trypsin** (for anoplin analogs), **chymotrypsin** (for (KFF)$_3$K analogs), or **human blood serum** (for peptides rich in lysines and leucines). All modified peptides exhibited increased proteolytic stability.

Antibacterial activity and hemolysis

Antibacterial activity was examined against gram-positive (*Staphylococcus aureus*) and gram-negative (*Escherichia coli*) bacterial strains. Peptides containing the hydrocarbon staple demonstrated greater antibacterial activity than the unmodified linear ones, with only a slight increase in hemolytic activity.

Peptide	Sequence	MIC [μM]		Hemolysis [%] at 16 μM
		E. coli K12 MG1655	*S. aureus* ATCC 29213	
anoplin	GLLKRIKTLL	32	>64	0.7
anoplin [2-6]	GS_5LKRS_5KTLL	4	16	2.8
anoplin[5-9]	GLLKS_5IKTS_5L	16	4	29.8
(KFF)$_3$K	KFFKFFKFFK	32	>32	0.35
(KFF)$_3$K[2-6]	KS_5FKFS_5KFFK	2	16	2.3
(KFF)$_3$K[5-9]	KFFKS_5FKFS_5K	2	8.6	2.0
KAL	KALKKLLAKWL	16	64	0.4
KAL[2-6]	KS_5LKKS_5LAKWL	4	8	4.6
KAL[3-7]	KAS_5KKLS_5AKWL	4	8	0.8
KAL2	KALAKLLKKWL	32	>64	0.8
KAL2[2-6]	KS_5LAKS_5LKKWL	2	4	10.8
KAL2[3-7]	KAS_5AKLS_5KKWL	2	4	2.5
KAL2[6-10]	KALAKS_5LKKS_5L	2	4	0.2
KAL3	KALKKLLKAWL	8	>64	0.4
KAL3[2-6]	KS_5LKKS_5LKAWL	2	4	15.0
KAL3[3-7]	KAS_5KKLS_5KAWL	2	4	4.4
KAL3[6-10]	KALKKS_5LKAS_5L	2	4	2.1
NEO-anoplin	NEO-GLLKRIKTLL	8	16	3.4
NEO-anoplin[2-6]	NEO-GS_5LKRS_5KTLL	8	8	3.9
AMK-anoplin	AMK-GLLKRIKTLL	16	32	4.3
AMK-anoplin[2-6]	AMK-GS_5LKRS_5KTLL	4	16	3.2
NEO-SS-anoplin[2-6]	NEO-SS-GS_5LKRS_5KTLL	8	4	2.7
NEO-SS-anoplin[2-6]	NEO-SS-GS_5LKRS_5KTLL	8	4	21.4

S_5 - (S)-2-(4'-pentene)alanine

Conclusions

The hydrocarbon stapling technique **stabilized the active α-helical structures** of modified peptides, which was crucial for enhancing their antibacterial activity

Stapled peptides, compared to unmodified ones, **exhibited up to 8-fold enhanced antibacterial activity** against *E. coli* and *S. aureus* strains, along with **improved proteolytic stability**, while **showing no hemolytic or cytotoxic effects**.

AMG-peptide conjugates demonstrated only slightly enhanced antimicrobial activity compared to their unconjugated counterparts, particularly against resistant strains. No synergistic effects were observed between the unconjugated segments.

Overall, the hydrocarbon stapling technique proved to be the most effective modification tested for enhancing the antibacterial activity of MAPs.

References

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