Bismaleimidohexane-Stapled Anoplin Analogs

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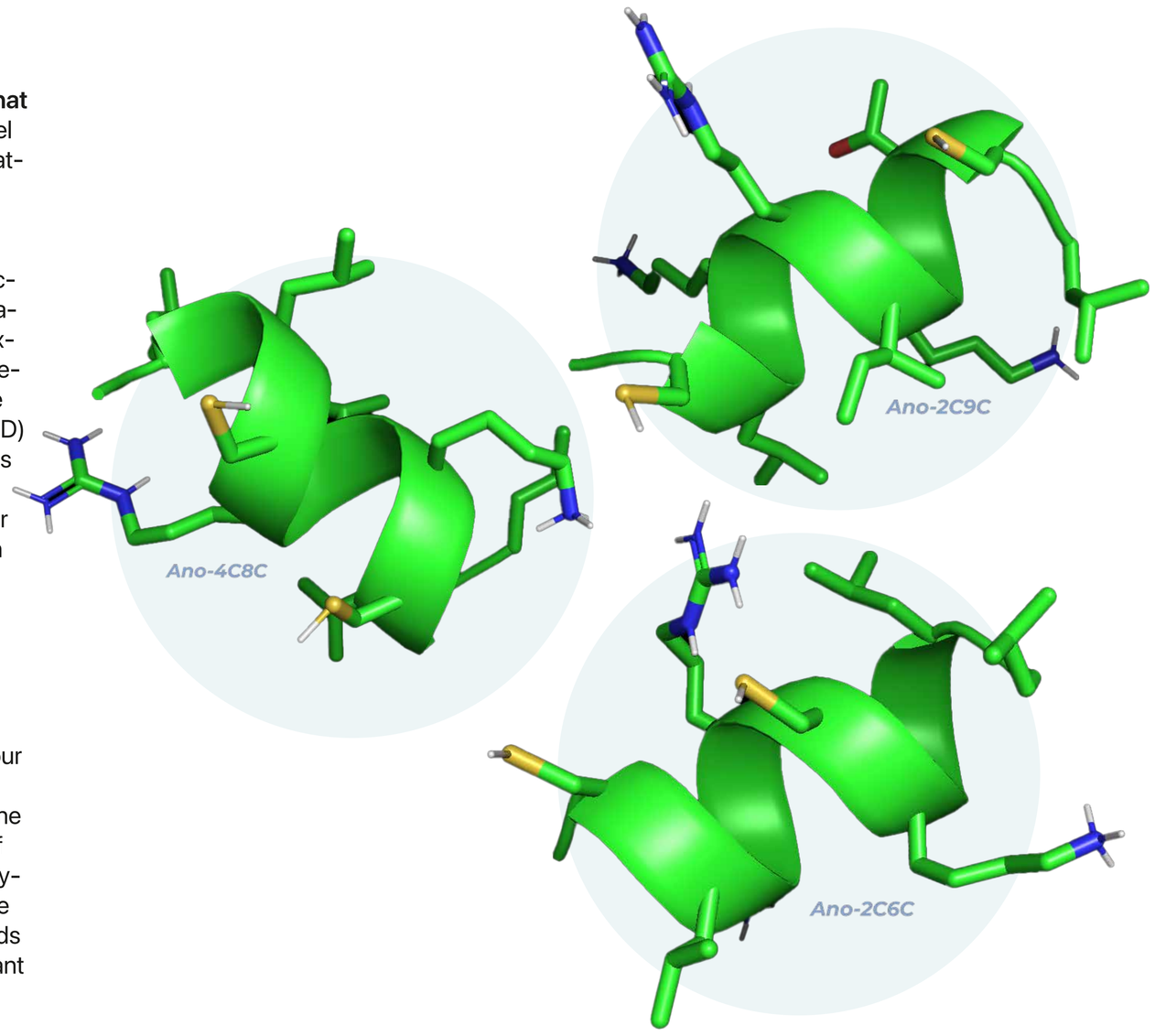
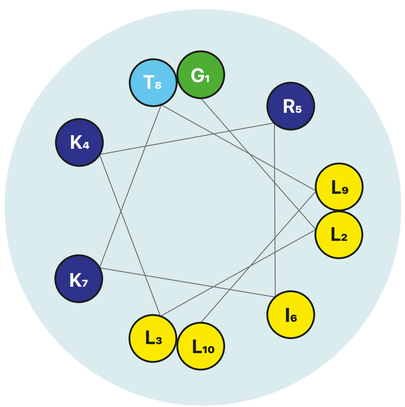
Abstract

The indiscriminate use of antimicrobials has led to the emergence of pathogenic organisms that have developed resistance strategies to these drugs. This necessitates the development of novel antimicrobial agents to add to our current therapeutic arsenal. **Anoplin (GLLKRIKTLL-NH2)** is a cationic, **α-helical antimicrobial peptide (AMP)** that was first isolated from the solitary spider wasp, *Anoplius samariensis*, by Konno and colleagues (2001). It **exhibits broad-spectrum antibacterial activity and low hemolytic activity**. The ease and economy in the synthesis of the short peptide chain, and the manipulability of the peptide structure for chemical modification make anoplin a practicable candidate for developing antibacterial agents. In this study, cysteine-substituted anoplin analogs were synthesized by solid-phase peptide synthesis (SPPS), and stapled with bismaleimidohexane (BMH) via the formation of thioether linkage between the sulfhydryl groups of substituted cysteine residues with the terminal maleimides of BMH. The secondary structure of the peptides and the effect of the BMH-stapling on the antibacterial activity were investigated. The circular dichroism (CD) spectra indicated the helical characteristics of the peptides. The peptides showed variable activities against Staphylococcus aureus and Escherichia coli. The BMH-stapled anoplin analog, Ano-4C8C(BMH), exhibited a more potent activity against S. aureus than anoplin. To the best of our knowledge, this study is the first report on the use of BMH for stapling cysteine-substituted anoplin analogs, and the potential of the novel BMH-stapled peptides as antibacterial agents.

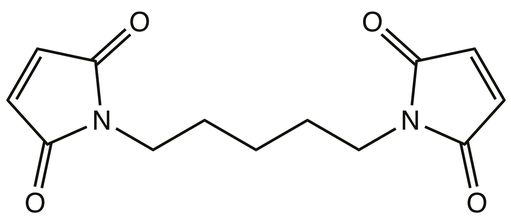


Introduction



- **Anoplin** is **an amidated decapeptide** with a positive four **(+4) charge**. It is randomly coiled in water and in buffer solutions but folds into an α-helical structure in membrane mimicking solvents, TFE and SDS. The amphipathicity of anoplin is apparent from its helical wheel diagram. The hydrophobic amino acids, leucine and isoleucine are on one side of the α-helical axis while the hydrophilic amino acids are on the other side (Konno, et al., 2001). This is important in the mechanism of action of anoplin.

- **Bis(maleimido)hexane** (BMH) is a homobifunctional crosslinker with a non-cleavable, six-atom spacer arm between terminal maleimides. It finds application in protein labelling and bioconjugation.



Objectives

1. Synthesize anoplin and the cysteine-substituted anoplin analogs by SPPS; and staple the cysteine-substituted analogs with BMH.
2. Characterize the secondary structure of the anoplin and the BMH-stapled analogs.
3. Evaluate the antibacterial activity of anoplin and the BMH-stapled analogs.

Methodology

Synthesis
- Solid Phase Peptide Synthesis (SPPS)
- BMH Stapling

Purification
- High-Performance Liquid Chromatography (HPLC)

Characterization
- Mass Determination: MALDI TOF
- Secondary Structure Determination: Spectroscopy
- MIC Determination: Broth Microdilution Assay

Results and Discussion

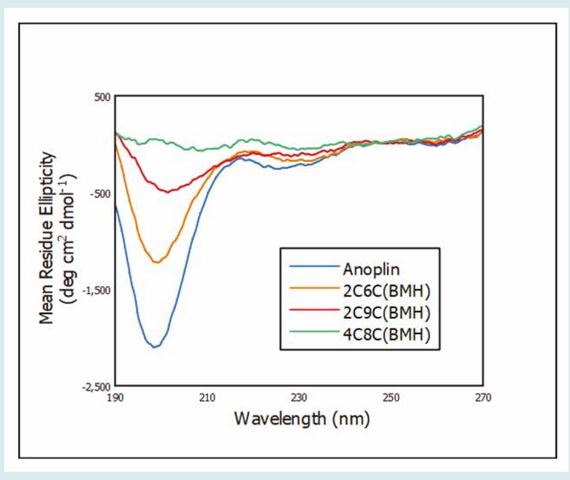
A. Mass Spectrometry Data and Percentage Yields of the Stapled Peptides

Peptides	Monoisotopic Mass (Da)	Observed Mass[a] (Da)	% Yield	Net Charge	Retention Time[b] (min)
Anoplin	1152.8	1153.4	24.58	+4	19.9
Ano-2C6C(BMH)	1408.8	1409.9	9.29	+4	18.7
Ano-2C9C(BMH)	1408.8	1409.6	11.03	+4	19.3
ANO-4C8C(BMH)	1405.8	1406.8	11.32	+3	24.8

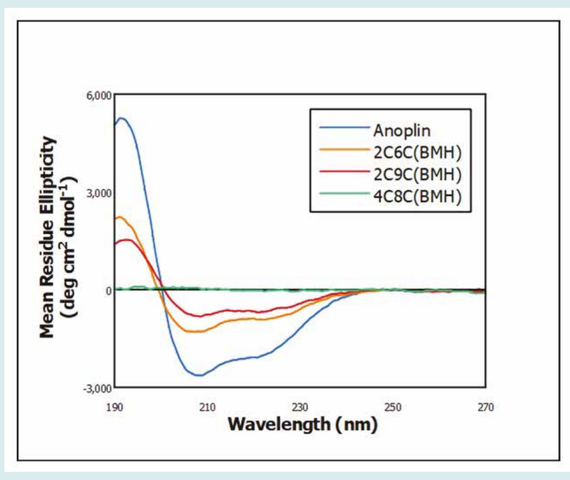
[a] Based on the [M+H]+ m/z
[b] from RP-HPLC in a Phenomenex Luna C18 analytical column with a linear gradient of 5-95% CH3CN/H2O/0.1% TFA

- The mass data from the MALDI analyses of the cysteine-substituted peptides indicate that they have been stapled by BMH. It is apparent from the retention time of the stapled peptides in RP-HPLC that the most hydrophobic among the peptides is Ano-4C8C(BMH), followed by anoplin and then Ano-2C9C(BMH) which has comparable hydrophobicity to that of anoplin. The increase in hydrophobicity of Ano-4C8C(BMH) is expected considering the decrease in the hydrophilicity of the peptide backbone coming from the mutation of lysine and threonine to cysteine, and the additional hydrophobicity conferred by the BMH staple.

B. CD Spectra



Phosphate Buffer



50% TFE

- The CD spectra of anoplin and the stapled peptides in buffer indicate that they are in a random coil conformation, exhibiting a negative band between 190 and 210 nm. In 50% TFE, anoplin folded into an α-helical structure, exhibiting the characteristic absorption at around 190 nm for the intense π-π* transition of the amide bonds, and the weak but broad absorbance at around 210 nm for the n-π* transition. Ano-2C6C (BMH) and Ano-2C9C(BMH) also adopted the helical structure exhibiting the same maxima at 190 nm and minima at around 210 and 220 nm. The CD spectra of Ano-4C8C(BMH) indicate that it did not form helical structure.

C. Minimum Inhibitory Concentrations (MIC) of BMH-stapled anoplin analogs against E. coli and S. Aureus

- Anoplin, with its broad spectrum antimicrobial activity, inhibited both the Gram–positive and Gram-negative bacteria. Ano-4C8C(BMH) inhibited S. aureus better than anoplin at a range of 5.5-11 μM. Ano-2C9C(BMH) selectively inhibited E. coli at a concentration of 22.7 μM.

Peptides	MIC (μM)	
	E. Coli ATCC 25922	*S. Aureus* ATCC 25923
Anoplin	13.9	28
Ano-2C6C(BMH)	>90.8	>90.8
Ano-2C9C(BMH)	22.7	>90.8
Ano-4C8C(BMH)	>22	5.5 - 11

Conclusions

- Anoplin and the cysteine-substituted analogs were synthesized by SPPS using an orthogonal group protecting strategy on a Rink amide resin.
- The BMH-stapled anoplin analogs were synthesized by stapling the cysteine-substituted peptides with BMH under ambient conditions.
- All peptides had a random coil conformation in phosphate buffer but took on the helical conformation in TFE solution, except Ano-4C8C(BMH) which did not exhibit the helical structure even in TFE.
- Ano-4C8C(BMH) exhibited better activity against S. aureus compared to anoplin.
- Ano-2C9C(BMH) was also found to be active against E. coli.

References

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