Influence of tryptophan insertion on the fibrillation capacity and antimicrobial efficacy of β-Hairpin Antimicrobial Peptides Dhanya Mahalakshmi Murali, Jian Xu, Dai Thien Nhan Tram and Pui Lai Rachel Ee

Department of Pharmacy and Pharmaceutical Science, National University of Singapore, Singapore



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Less active against Gram-Active against Gram-Negative

Positive

- Antimicrobial Peptides (AMPs) have gained attention for combating bacterial infections due to their unique mechanisms of action and low tendency to induce resistance.
- However, a major challenge in utilizing AMPs is their inherent selectivity, against either Gram-negative or Grampositive bacteria.
- To address this limitation, we modified our existing bacteria-responsive nanonet forming AMP: BTT1-3A by incorporating hydrophobic-aromatic tryptophan (W) at specific positions (1,3,5,11 and 13), named as 1W, 3W, 5W, 11W and 13W respectively.
- Since, tryptophan's bulky aromatic indole ring can enhance interactions with the thick peptidoglycan layer in the gram-positive bacterial membrane.

Expansion of Bacteria Spectrum

- The antibacterial efficacy of W-inserted peptides was assessed via the Minimum Inhibitory Concentration (MIC) assay, targeting model gram-positive strains; **S. aureus** and **E. faecalis**.
- The findings demonstrate that peptides **1W** and **13W** exhibit enhanced antibacterial activity, surpassing • the parent peptide and other W-inserted peptides by at least 2-fold.

K114 amyloidophillic assay evaluates the nanonet-forming ability of the modified peptides as it undergoes a red shift when it binds to amyloid-like fibrils.

2)

Both **IW and 3W show the highest** fluorescence intensity with LTA, similar to that of the parent peptide



Antifouling Ability

In clinical settings, the antifouling ability of peptides is crucial in preventing biofilms and improving the longevity and functionality of medical devices.

The antifouling ability of W-peptides was studied using confocal microscopy. E. faecalis biofilms were initiated in the presence of W-peptide and stained to perform a live/dead assay



- 2). Agglutination assay performed to evaluate the fibrillation capacity in the presence of bacteria \rightarrow bacteria form clusters in the peptide, of the reducing presence absorbance of the suspension.
 - 1W and 13W show the highest clustering for E. faecalis.

3)



40 μM BTT1-3A

E. Faecalis: Peptide-Free





Volume of bacterial cells in the presence of **W-peptide decreased by approximately 90%.**



Overall, it indicates a **decrease in bacterial adhesion and** trapped bacteria were observed on these solid surfaces.

Drug-Free Polymer with

Peptide-coated Polymer with bacteria incubated

t 0.5-

Polymer

Peptide

E. faecalis

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3)

Scanning Electron Microscope (SEM) showed an increase in the fibrillation tendency of both 1W and 13W peptides compared to the parent peptide at their MICs.



- Incorporating tryptophan into BTT1-3A significantly enhances its antimicrobial activity against Gram-positive bacteria while maintaining efficacy against Gram-negative bacteria.
- The strategic incorporation of tryptophan enhances fibrillation capacity against gram-positive compared to the parent peptide.
- Additionally, the modified W-peptides show potential antifouling ability and may act as an effective solution for preventing biofilm-related complications in medical settings.

Contact Information

National University of Singapore Email: phaeplr@nus.edu.sg

Department of Pharmacy	(A/Prof Rachel Ee)
18 Science Drive 4,	e0606578@u.nus.edu
Singapore 117543	Murali))

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