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Master of Science HES-SO in Life Sciences

Biological activities and Titanium Surface Binding of Temporin-SHa via DOPA Functionalization

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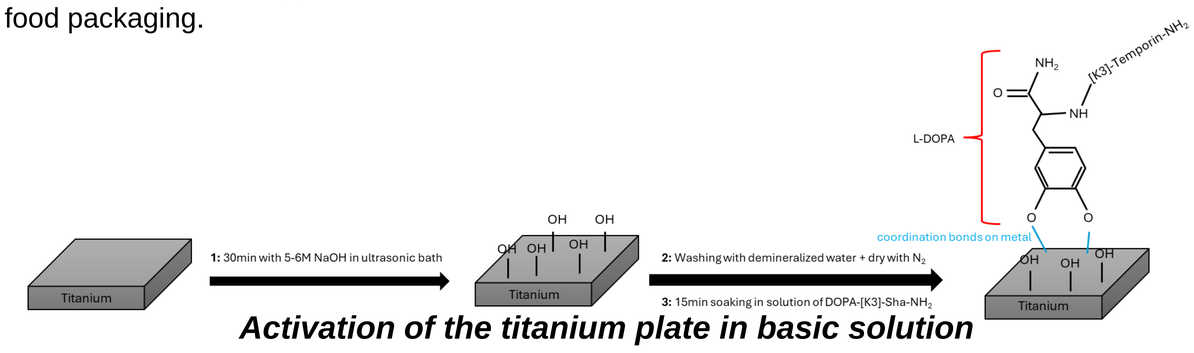
DESCRIPTION

The growing threat of antibiotic-resistant bacteria highlights the need for new antimicrobial strategies. Antimicrobial peptides (AMPs) stand out as promising solutions due to their broad-spectrum efficacy, high specificity, and low toxicity. One of the more recent advancements in this field is the green binding of AMPs to surfaces, which enhances their stability and performance while prioritizing environmental sustainability.

Antimicrobial peptides (AMPs) kill bacteria through mechanisms such as membrane disruption, cell wall synthesis inhibition, and interference with intracellular processes. When immobilized on surfaces, they effectively prevent bacterial adhesion and biofilm formation, addressing key challenges in medical device-associated infections.[1]

Green binding involves sustainable methods for attaching antimicrobial peptides (AMPs) to surfaces, employing non-toxic solvents and energy-efficient processes. This eco-friendly approach has broad applications across fields such as biomedical devices, water treatment, and food packaging.



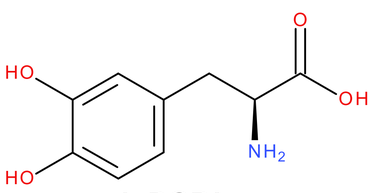
Activation of the titanium plate in basic solution

The AMP used for this project was the Temporin-SHa. Temporin-SHa is a short antimicrobial peptide originally isolated from the skin of the frog. It exhibits potent activity against a wide range of bacteria, including multidrug-resistant strains, by disrupting bacterial membranes.[2]

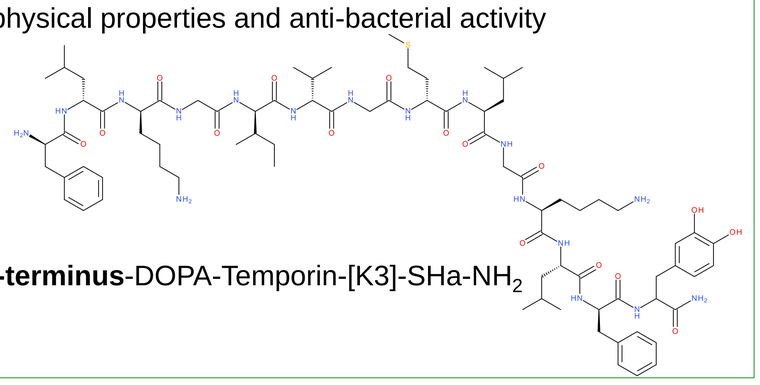
Inspired by mussel adhesive proteins, L-DOPA enables strong, stable binding of antimicrobial peptides to titanium via catechol groups in eco-friendly conditions.[1]

OBJECTIVES

- Develop L-DOPA chemistry, make a protocol for the deprotection and handling in the context of peptide chemistry
- Immobilization of peptide onto titanium surfaces, using low environmental impact methods. For example, with L-DOPA.
- Characterization of the products: physical properties and anti-bacterial activity

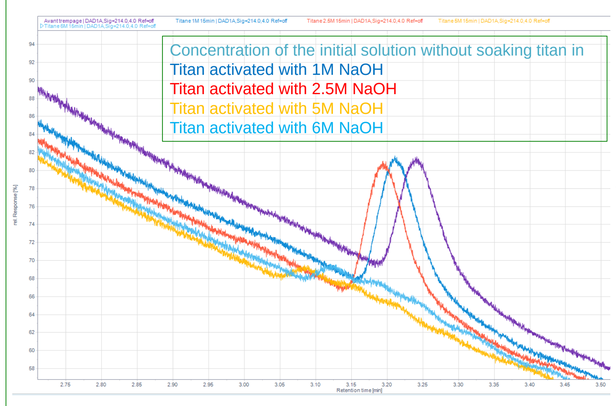


L-DOPA



C-terminus-DOPA-Temporin-[K3]-SHa-NH_2

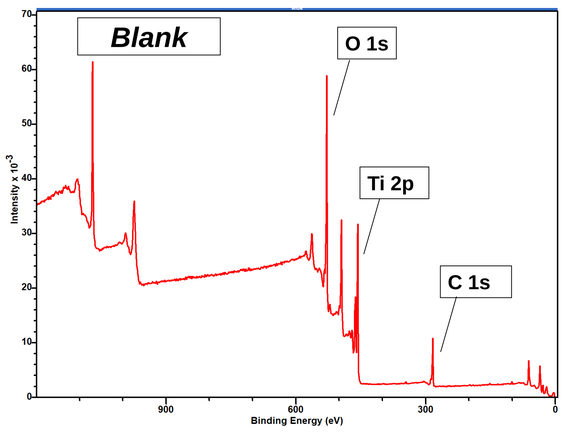
RESULTS

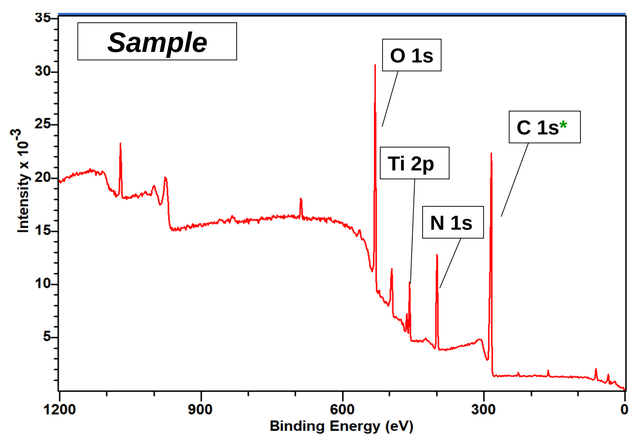


Peptide Binding Efficiency on Titan Plates by UV analysis @214nm

Titanium plates were pre-activated in NaOH solutions of different concentrations (1M, 2.5M, 5M, and 6M) for 30 minutes in an ultrasonic bath. The chromatogram compares peptide concentrations before and after soaking (15min) the plates.

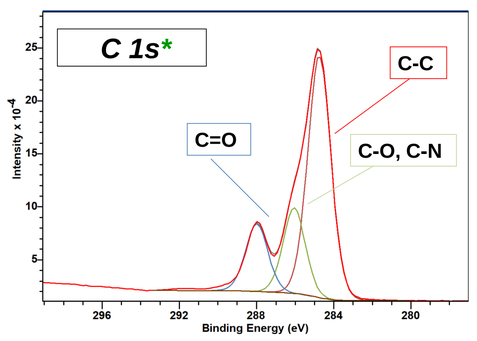
Purple Line: Initial peptide concentration before contact with titan plates.
1M and 2.5M NaOH (Blue and Red): Partial peptide binding observed, as peaks remain visible.
5M and 6M NaOH (Yellow and light Blue): Complete peptide binding achieved, indicated by the disappearance of peptide peaks.



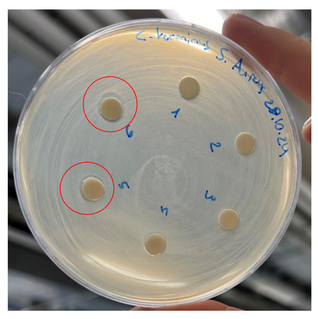


On the blank, only the titan plate is analysed (no presence of nitrogen, and small presence of carbon).

On the sample, DOPA-[K3]-Temporin-NH_2 is binded on the titan plate. The increased presence of nitrogen and carbon, when compared with the intensity of the blank peak, confirms the presence of the peptide on the plate.
Carbon peak is composed of different type of carbon binding, C=O, C-C, C-O and C-N. (see *Figure C 1s**)



XPS analysis



Observation of the biological activity of the C-terminus-DOPA-Temporin-[K3]-SHa-NH_2 from 0µM-10'000µM against *S.Aureus.*

Activity starts at the concentration of 7500µM of C-terminus-DOPA-Temporin-[K3]-SHa-NH_2.

Biological activity against S.Aureus with L-DOPA on the C-Terminus of the peptide

CONCLUSION

The use of specific conditions with basic solution is crucial for effective activation, enabling complete peptide binding to the titanium surface. L-DOPA plays a key role in facilitating this binding through its catechol groups, providing a versatile method for anchoring peptides to various surfaces. Its operation in aqueous conditions significantly reduces the need for toxic solvents, making it an environmentally friendly approach that aligns with the principles of green chemistry.
This strategy holds great potential for biomedical and industrial applications, particularly in the development of biocompatible coatings, regenerative medicine, and drug delivery systems.

1: Adhesive Antimicrobial Peptides Containing 3,4-Dihydroxy-L-Phenylalanine Residues for Direct One-Step Surface Coating, https://doi.org/10.3390/ijms222111915
2: Temporins: Multifunctional Peptides from Frog Skin: https://doi.org/10.3390/ijms24065426

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