REPLACING L-AMINO ACID WITH D-AMINO ACID RESIDUES ON AN ULTRASHORT CATIONIC LIPOPEPTIDE TO IMPROVE ANTIMICROBIAL ACTIVITY

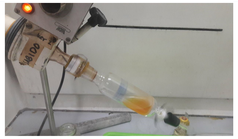
Patricia Joy Sabido (Student), Mara Esguerra (Adviser), Riel Carlo Ingeniero (Teacher)

Introduction

There is an urgent need to develop new antimicrobial agents due to the growing resistance of bacteria to commercial antibiotics. Lipopeptides are good drug candidates because their unique mechanism of destroying bacterial membrane makes them less susceptible to bacterial resistance. Recently, a number of ultrashort lipopeptides with cationic ornithine residues were designed and tested [1]. The tripeptide myr-(L-Orn)$_3$-NH$_2$ was found to have the best antimicrobial activity. Previous studies have shown that peptides with D-residues were more stable than those with L-residues [2]. This project aimed to evaluate the effect of incorporating D-amino acid residues into a known antimicrobial lipopeptide. The target compound, myr-(D-Orn)$_3$-NH$_2$ and its enantiomer, myr-(L-Orn)$_3$-NH$_2$, were synthesized, purified, characterized and tested for their anti-microbial activities against *E. coli* and *S. aureus.*

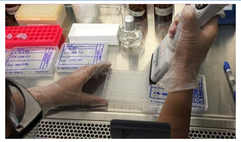
Methodology

Solid Phase Peptide Synthesis



Purification & Characterization

Antimicrobial Activity for MIC



Deprotect Rink resin with 20% piperidine → Couple amino acid to make tripeptide → Attach myristic acid at N-terminal → Cleave lipopeptide from resin with TFA → Purify sample by preparative HPLC → Determine HPLC of crude sample → Obtain mass using mass spectrometry → Quantify lipopeptides with fluorescamine → Prepare agar media for plates → Broth dilution assay of *E.coli* and *S.aureus* → Determine minimum inhibitory MIC

Results

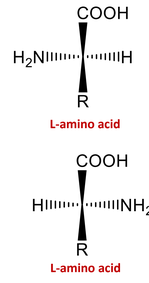


Fig 1. Structures of L (top) and D- (bottom) amino acids

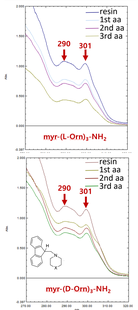


Fig 2. UV spectra of L (top) and D- (bottom) lipopeptide depro-tection solution

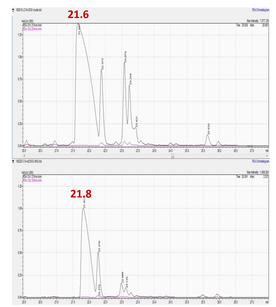


Fig 3. HPLC chromatogram of crude L (top) and D- (bottom) lipopeptide using inertsil analytical column

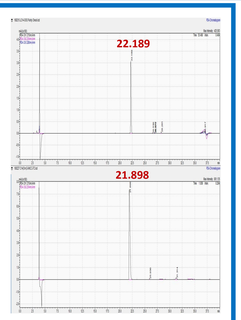


Fig 4. HPLC chromatogram of L (top) and D- (bottom) lipopeptides after purification by preparative HPLC

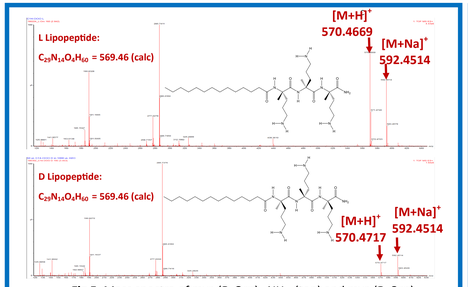


Fig 5. Mass spectra of myr-(D-Orn)$_3$-NH$_2$ (top) and myr-(D-Orn)$_3$-NH$_2$ (bottom) after purification by reverse phase preparative HPLC

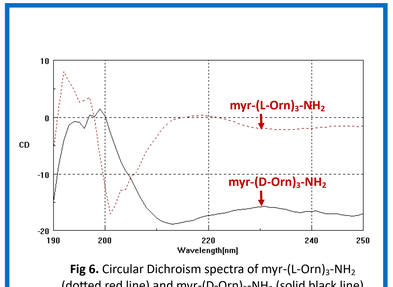


Fig 6. Circular Dichroism spectra of myr-(L-Orn)$_2$-NH$_2$ (dotted red line) and myr-(D-Orn)$_3$-NH$_2$ (solid black line) in water

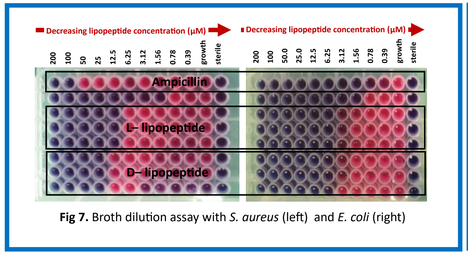


Fig 7. Broth dilution assay with *S. aureus* (left) and *E. coli* (right)

Table 1. MIC values in μM of the lipopeptides and ampicillin against *E.coli* and *S. aureus* .

MIC	*E. Coli*	*S. aureus*
Myr-D-(Orn)$_3$NH$_2$	25.0	12.50
Myr-L-(Orn)$_3$NH$_2$	12.5	6.25
Ampicillin	50.0	1.56

Conclusion

The target compounds, myr-(D-Orn)$_3$-NH$_2$ and its enantiomer myr-(L-Orn)$_3$-NH$_2$, were successfully made using solid phase peptide synthesis. Reverse phase preparative HPLC effectively purified the compounds. Mass spectometric analysis confirmed that the desired lipopeptides were obtained. Furthermore, CD analysis distinguished the enantiomers. Finally, both lipopeptides exhibited better antimicrobial activity against *S.aureus* than *E.coli.* Although the L-lipopeptide was slightly more active than the D-lipopeptide, the results verify that these antimicrobial agents kill bacteria through non-specific intractions. With the added value of being more stable, myr-(D-Orn)$_3$-NH$_2$ could prove to be an excellent drug candidate.

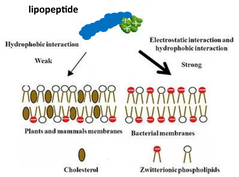


Fig 8. Mechanism of action of lipopeptide

Recommendations

The researchers learned that developing a drug entails a lot of work. It is recommended that the stability of the L and D lipopeptides be checked. Furthermore, the activity of the lipopeptides against more resistant strains of bacteria and its antifungal activity could be tested. Finally, to test the viability of the lipopeptide as a drug, it is the hemolytic ability of the two lipopeptides could also be checked.

Acknowledgments

This research was supported by the Philippine Science High School Main Campus. I would like to thank the following researchers from the University of the Philippines, Diliman: H. Candaza, A. Petate, R. de Boda of the Peptide Synthesis Laboratory; and, P. Torres and M. Villena of the Analytical Services Laboratory.

Bibliography

[1] Lohan, S., Cameotra, S. S., & Bisht, G. S. (2013) Systematic Study of Non-Natural Short Cationic Lipopeptides as Novel Broad-Spectrum Antimicrobial Agents. Chemistry Biology and Drug Design, 82(5), 557-566.
[2] Hong, Sung Yu, et al. “Effect of D-Amino Acid Substitution on the Stability, the Secondary Structure, and the Activity of Membrane-Active Peptide.” Biochemical Pharmacology, vol. 58, no. 11, 1999, pp. 1775–1780., doi:10.1016/s0006-2952(99)00259-2.