Alca1, a cyclotide extracted and isolated from *Allexis cauliflora* (Violaceae): Synthesis and biological activities

Olivier Ndogo Eteme[1], Letícia Oliveira Catarin Nunes[2], Marcela Nunes Argentin[3], Norival Alves Santos-Filho[2], Ilana Lopes Baratella da Cunha Camargo[3], Eduardo Festozo Vicente[1]

[1]*School of Science and Engineering, Sao Paulo State University (Unesp), Tupã 17602-496, São Paulo, Brazil.*
[2]*São Paulo State University (Unesp), Institute of Chemistry, Araraquara, 14800-900, São Paulo, Brazil.*
[3]*University of São Paulo, São Carlos Institute of Physics, São Carlos, 13566-590, São Paulo, Brazil.*
eduardo.vicente@unesp.br

https://doi.org/10.17952/37EPS.2024.P2059

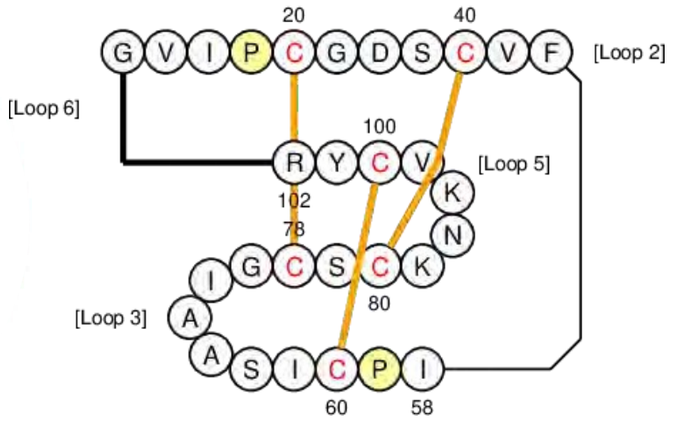
INTRODUCTION

Bacterial resistance to conventional antibiotics is a serious public health problem. The research of molecules with bactericidal activity is always current event with increase of multi-resistance. Over the past decade, small proteins known as antimicrobial peptides (AMPs), natural compounds produced by all prokaryotic and eukaryotic cells have shown promising results in overcoming the growing problems of antibiotic resistance. In parallel, there are the so-called cyclotides, which are cyclic peptides, rich in cysteines and originated from plants, showing potent antimicrobial activities with a very reduced toxicity. They are considered promising molecules for peptide drug design, due to their stable circular structure.

OBJECTIVES

In this work, the Alca1, cyclotide from the plant *Allexis cauliflora* (Fig. 1), were fully synthesized, purified, characterized, and the antibacterial activities were evaluated against multi-resistant bacteria and biofilm growth.

Figure 1. *Alexis cauliflora* (left). On the right, primary structure of cyclotide Alca1, showing the three disulfide brigdes between the cysteine residues. Also, the scheme provides the loops' location in the cyclotide.

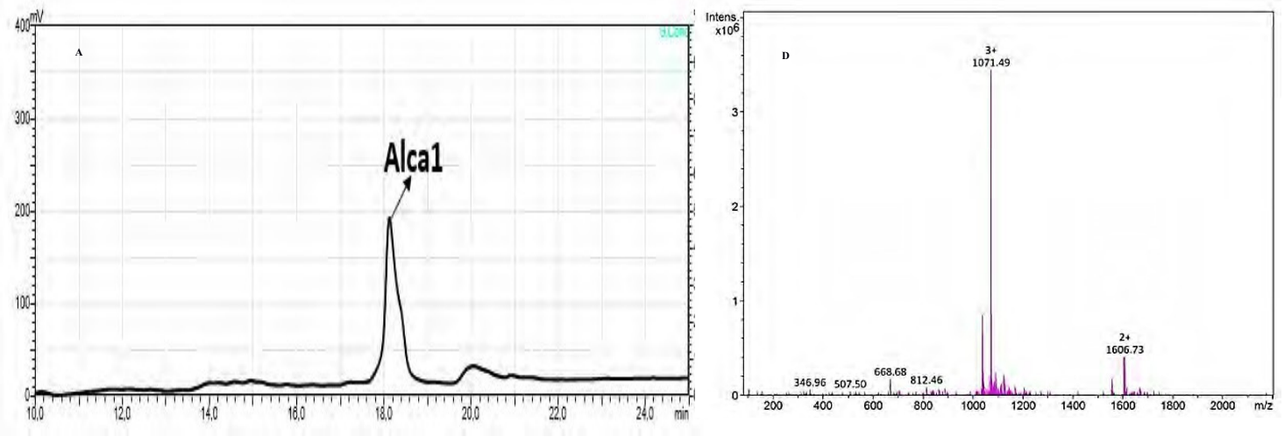


METHODOLOGY

The methodology are fully described in Akcan and Craik (2013) [1]. Synthesis of cyclotide Alca1 were evaluated for the first time in our lab and was achieved employing the solid phase peptide synthesis (SPPS) method using Fmoc chemistry, with a final yield of 40%. The synthesis reaction was monitored by combined HPLC and LC-MS methods, with the evaluation of the antibacterial property of cyclotide Alca1 from *Allexis cauliflora*. The antibacterial test was perform using microdilution method on many species of bacteria, such as *Staphylococcus epidermidis* (ATCC 35984), *S. aureus* (ATCC 25923), *S. aureus* (ATCC 8095), *Enterococcus faecalis* (ATCC 29212), *E. faecium* (ATCC 700221), *Klebsiella pneumoniae* (ATCC 700603), *Escherichia coli* (ATCC 25922), *Acinetobacter baumannii* (ATCC 19606) and *Pseudomonas aeruginosa* (ATCC 27853). Also, for hemolytic essays, a volume of 100 µL of the erythrocyte solution were added to the tubes containing the peptide dilutions and incubated at 37ºC for 1 hour. The tubes were then centrifuged at 500 g for 5 minutes. The supernatants were pipetted into microplates for later reading at 540 nm in a microplate reader.

RESULTS

Figure 2. Chromatogram profile (left) and Alca1 peptide mass spectrometry spectra of the synthesized cyclotide. Retention time at 18,2 min and purity greater than 95%. The monoisotopic lion peak of Alca1 is 3,210.43 Da. From the analysis of the LC-MS spectrum, it was able to detect the peaks of the [M+2H] and [M+3H] ions, respectively: 1,606.73 and 1,071.49, which confirms the material obtaining.



RESULTS

Table 1. MIC and MBC results for Alca1.

Bacterial strains	MIC (mg/L)	MBC (mg/L)	MBC/ MIC	Activity*
S. epidermidis ATCC 35984	128	256	2	Bactericide
S. aureus ATCC 25923	256	512	2	Bactericide
S. aureus ATCC 8095	256	256	1	Bactericide
E. faecalis ATCC 29212	256	256	1	Bactericide
E. faecium ATCC 700221	64	128	2	Bactericide
K. pneumoniae ATCC 700603	256	256	1	Bactericide
E. coli ATCC 25922	64	512	8	Bactericide
A. baumannii ATCC 19606	64	128	2	Bactericide
P. aeruginosa ATCC 27853	>512	*N. R.*	*N. R.*	*N. R.*

**Activity defined by the CBM/CIM ratio. For results = 4 or < 4, the activity is considered bactericidal, above this value, the activity is considered bacteriostatic, according to Pankey and Sabath (2004). Activity not determined (N.D.) and N. R.: Test not carried out as the compound did not show activity against the strain.*

Figure 3: Alca1 hemolytic activity curve.

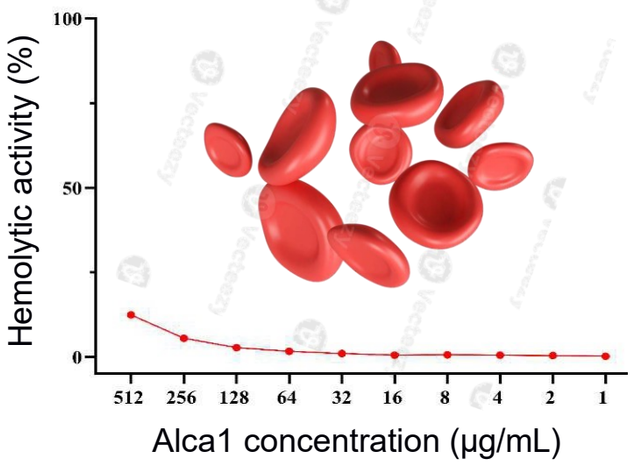


Table 2. Reduction of *S. epidermidis* ATCC 35984 biofilm by using the compound at a concentration of 512 mg/L.

Compound (512 mg/L)	Biofilm eradication ability (%)
Alca1	-26,2 ± 9,4

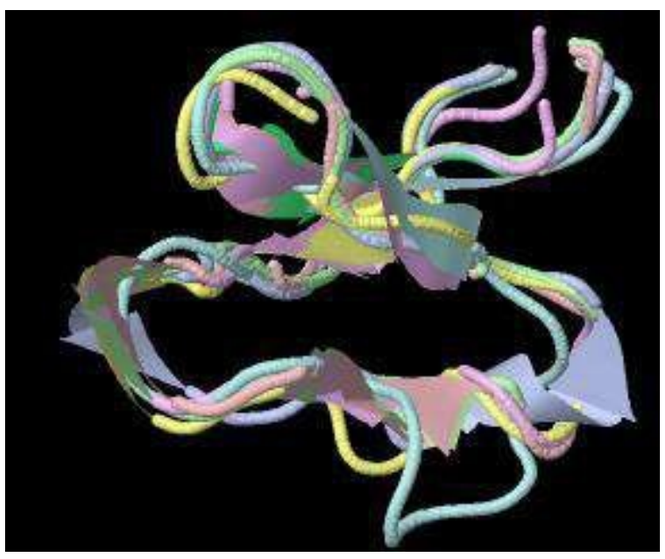


Figure 4. Overlay model. The superposition is carried out using the MaxSub algorithm. This algorithm attempts to find the maximum subset of atoms between two structures that can be superposed within a radius of 4.5 Å. Typically, one would choose as master either the top-ranked model or a model judged to be the most interesting based on some prior basic biological knowledge.

CONCLUSIONS

Therefore, the biological activities combined with its already reported high stability, the Alca1 antimicrobial cyclotide has demonstrated to be an excellent molecule for combating multi-resistant bacteria with low toxicity to erythrocytes.

REFERENCES

Akcan, M., & Craik, D. J. (2013). Synthesis of cyclic disulfide-rich peptides. *Peptide Synthesis and Applications*, 89-101.

ACKNOWLEDGEMENTS

FAPESP Grant process number 2021/06706-9 and 2022/02808-4