Antibacterial and non-cytotoxic peptide derived from the p-BthTX-I

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INTRODUCTION

Based on antimicrobial activity of the bothropstoxin-I (BthTX-I) we synthesized and characterized peptide derived from the C-terminal region of BthTX-I (p-BthTX-I, sequence: KKYRYHLKPFCKK) and its disulfide-linked dimeric form, obtained via air oxidation $(p\text{-BthTX-I})_2$. p-BthTX-I and $(p\text{-BthTX-I})_2$ showed antimicrobial activity against both gram-negative and gram-positive bacteria, including multidrug-resistant strains and were not active against *C. albicans*, erythrocytes, epithelial cells, or macrophages, showing a possible specificity against prokaryotic cells. In addition, the dimeric form of the peptide was more active than the monomer. After serum incubation, our results showed that the dimeric peptide are completely degraded after 25 min. However, mass spectrometry showed that the main degradation product was a stable peptide that had lost four lysine residues on its C-terminus region ($des\text{-}Lys^{12}/Lys^{13}\text{-}(p\text{-BthTX-I})_2$). Antibacterial activities were evaluated against a variety of bacteria and exhibited similar or better activity than the $(p\text{-BthTX-I})_2$. Aiming to analyze if the dimerization position could alter peptide activity, two others peptides were synthesized. The first was dimerized through a glutamic acid residue at the amino terminal [$E(p\text{-BthTX-I})_2$] and another was dimerized through of the C-terminal lysine residue without the Lys^{12} and Lys^{13} residues ($des\text{-}Lys^{12}/Lys^{13}\text{-}(p\text{-BthTX-I})_2K$).

RESULTS

SPPS

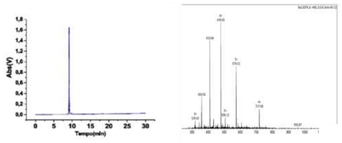
KKYRYHLKPFCKK
KKYRYHLKPFCKK

Dimeric native peptide - $(p\text{-BthTX-I})_2$

serum incubation

KKYRYHLKPFC
KKYRYHLKPFC

$des\text{-}Lys^{12}/Lys^{13}$ - $(p\text{-BthTX-I})_2$



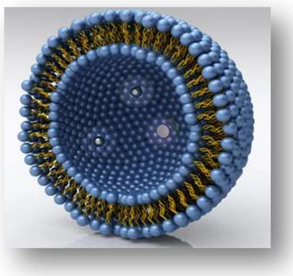
E
KKYRYHLKPFCKK
KKYRYHLKPFCKK

$E(p\text{-BthTX-I})_2$

KKYRYHLKPF
KKYRYHLKPF
K

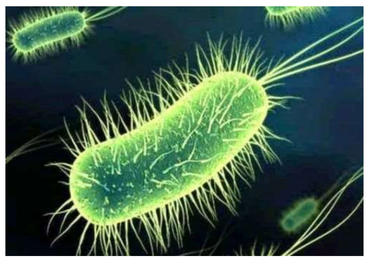
$des\text{-}Lys^{12}/Lys^{13}\text{-}(p\text{-BthTX-I})_2K$

Biological activities



Peptides did not induced CF release.
The mechanism of action of peptides is not through membrane permeabilization.

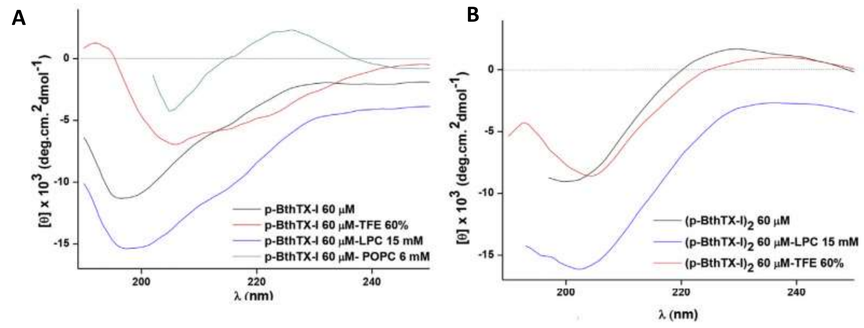
HC50 > 128 µmol/L. The average value of the peptide concentration that produced 50% hemolysis.
The peptides did not present activity against *C. albicans*, erythrocytes, epithelial cells, or macrophages in the



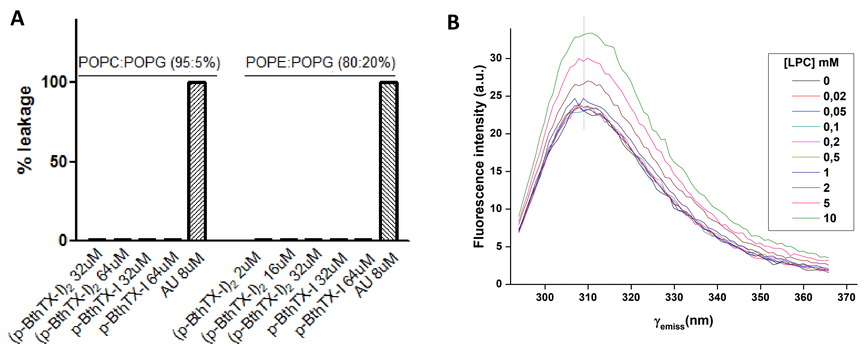
Peptides exhibited bactericidal activities against a variety of bacteria, including multidrug-resistant strains

Biological activities of the synthetic peptides.

Bacteria strains	p-BthTX-I MIC (µmol/L)	p-BthTX-I MBC (µmol/L)	$(p\text{-BthTX-I})_2$ MIC (µmol/L)	$(p\text{-BthTX-I})_2$ MBC (µmol/L)	Des-Lys^{12}, Lys^{13}-$(p\text{-BthTX-I})_2$ MIC (µmol/L)	Des-Lys^{12}, Lys^{13}-$(p\text{-BthTX-I})_2$ MBC (µmol/L)	$E(p\text{-BthTX-I})_2$ MIC (µmol/L)	$E(p\text{-BthTX-I})_2$ MBC (µmol/L)	$des\text{-}Lys^{12}/Lys^{13}\text{-}(p\text{-BthTX-I})_2K$ MIC (µmol/L)	$des\text{-}Lys^{12}/Lys^{13}\text{-}(p\text{-BthTX-I})_2K$ MBC (µmol/L)
S. epidermidis ATCC35984	128	512	16	64	32	32	32	>128	8	16
S. aureus ATCC25923	>512	N.D	512	512	128	256	>128	>128	32	128
E. faecalis ATCC29212	>512	N.D	512	>512	N.D	N.D	128	N.D	64	128
E. faecium HSJRP8	32	>512	8	>512	8	32	16	32	8	32
E. coli ATCC25922	>512	N.D	512	512	512	512	128	>128	32	34



CD spectra of p-BthTX-I (A) and $(p\text{-BthTX-I})_2$ (B) in diverse solutions



(A) CF release from POPC:POPG (95:5%) and POPE:POPG (80:20%) vesicles by the peptides at the specified concentrations. (B) Fluorescence spectra of $(p\text{-BthTX-I})_2$ in several LPC concentrations.

CONCLUSIONS

In this work, a new bactericidal peptide was synthesized based on the C-terminal region of the PLA_2 homologue BthTX-I. Our results showed that the dimerization of peptides are important for their activity as well as the existence of a Cys residue. Future studies are required to elucidate the mechanism of action of them. The peptides did not present activity against *C. albicans*, erythrocytes, epithelial cells, or macrophages, showing a possible specificity against prokaryotic cells, emphasizing the therapeutic potential of these molecules. The peptide ($des\text{-}Lys^{12}/Lys^{13}\text{-}(p\text{-BthTX-I})_2K$) exhibited the best activity, demonstrating that the position of dimerization is important for antimicrobial activity and the amino acids Lys^{12} and Lys^{13} are not essential for antibacterial activity of the peptide. Summarizing, our results demonstrate that the peptides analyzed are promising prototypes for new strategies to treat infections caused by multidrug-resistant bacteria and useful in design and generation of antimicrobial peptides.

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