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Identification and Synthesis of Immunogenic Peptides to Produce *Tityus* Antivenom

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Introduction

Several endemic scorpions belonging to the genus *Tityus* sp. can cause envenoming in humans in Argentina. The administered antivenoms used for accidental scorpion stinging treatments is produced by immunizing horses repeatedly with captured arachnid venoms extracted by electrostimulation of their telson. This classical antiserum production used since its description by Césaire Auguste Phisalix and Albert Calmette in 1894, depends on an extremely dangerous and low-yielding venom harvest, hampering national demands met. Furthermore, due to the high content of horse proteins injected, adverse reactions such as serum sickness has been reported [1].

Tityus trivittatus is one of the main scorpions of medical importance in South America. Beta-mammal Tt1g neurotoxin, a Cys-rich peptide, has been described as the responsible for the intoxication symptoms caused by its sting to humans [2]. Tt1g, as well as others Cys-rich peptides usually found in arachnids, has low immunogenicity because, its high stability and its digestion is difficult in antigen-presenting cells, a key step to trigger the adaptive immune response [3].

In this work, the epitopes from Tt1g were identified, and immunogenic peptides were designed and chemically synthesized to supplant and/or improve the traditional method of obtaining scorpion venom currently used in South America.

Results and Discussion

The "MHC-II Binding Predictions" tool from "Immune Epitope Database Analysis Resource", was used to identify the Tt1g epitopes [4]. Its most immunogenic fragment, LPNWVKVWERATNRC, corresponding to its *C* terminus, was synthesized by solid phase solid phase peptide synthesis (SPPS) with Fmoc/tBu chemistry using Rink amide-MBHA resin. Cys was replaced by α-aminobutyric acid to avoid Cys bonds formation. To increase the immunogenicity of the selected epitope, the *N*-terminal was palmitoylated and linear and branched peptides, using Fmoc-Lys (Fmoc)-OH, were synthesized. The purity and identity of the synthesized epitopes were assessed by electrospray ionization mass spectrometry (ESI MS) and RP HPLC.

All the peaks obtained in the mass spectra corresponded to the synthesized immunogens (Figures 1A and 2A), demonstrating their high purity. The RP-HPLC analysis of the linear palmitoylated epitope indicated a purity higher than 85% (Figure 1B). On the other hand, the branched palmitoylated peptide chromatogram showed two peaks (Figure 2B), probably due to the presence of two conformations in slow interconversion or the formation of micelle in aqueous medium that disassemble in organic medium. Similar results were obtained with a branched palmitoylated epitope from the spider Tx2-6 neurotoxin, also synthesized in our laboratory.

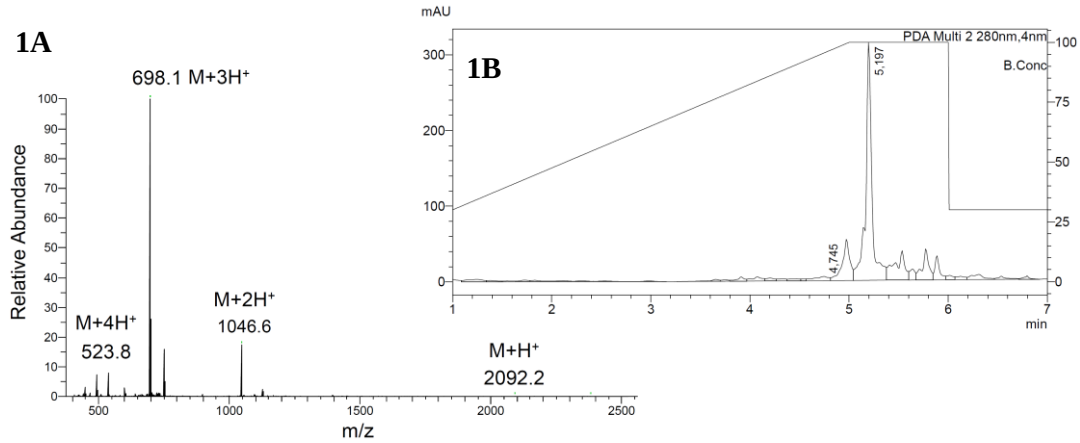

Fig. 1. Palm-LPNWVKVWERATNR-Abu-NH_2 (MW: 2091.58; monoisotopic mass: 2091.26 u). 1A) ESI MS; 1B) RP-HPLC analysis. RP column (C18 3.5µm, 4.6x50mm). Solvent A: 0.045% TFA in H_2O, Solvent B: 0.036% TFA in acetonitrile.

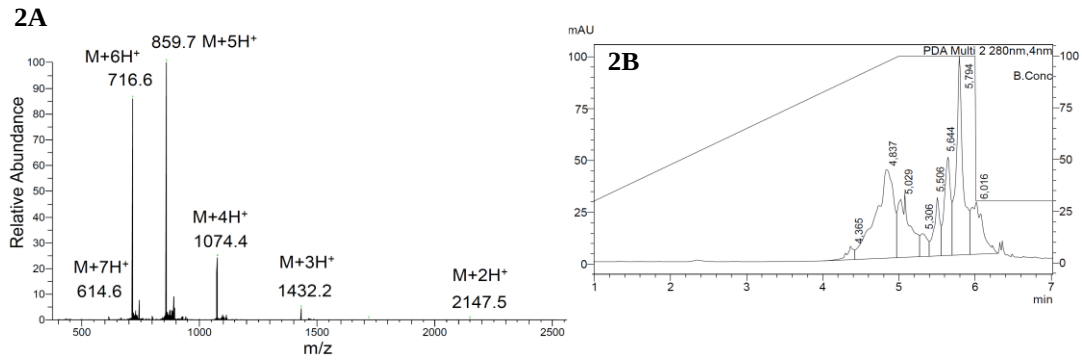

Fig. 2. (Palm-LPNWVKVWERATNR-Abu)$_2$K-NH_2 (MW: 4294.31; monoisotopic mass: 4292.58 u. 2A) ESI MS; 2B) RP-HPLC analysis. RP column (C18 3.5µm, 4.6x50mm). Solvent A: 0.045% TFA in H_2O, Solvent B: 0.036% TFA in acetonitrile.

Synthesised peptides are currently tested *in vitro* and afterwards they will be assayed in the *Instituto Nacional de Producción de Biológicos, Administración Nacional de Laboratorios e Institutos de Salud Dr. Carlos Malbrán* to assess their capacity to produce *T. trivittatus* antivenom suitable for large-scale production to meet national and regional demands.

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