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Identification and Synthesis of Epitopes from a *Phoneutria Nigriventer* Toxin to Produce Immunogens

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Introduction

The spider *Phoneutria nigriventer*, lives predominantly in Brazil and in the north of Argentina. Envenoming caused by *P. nigriventer* constitutes a medical emergency treatable with suitable antivenoms. The availability of venom, necessary for antivenom production is scarce, mainly because these spiders are difficult to capture and handle in the laboratory. New approaches are necessary for a more efficient and economical antivenom production to satisfy national and regional the demands. *P. nigriventer* venom is composed primarily by cysteine-rich peptide neurotoxins which possess the high stable inhibitor cystine knot (ICK) structural motif [1]. Among them, δ-ctenitoxin-Pn2a (UniProt: P29425), also called PnTx2-6, is one of the most toxic neurotoxins for humans and responsible for envenoming symptoms. Although high toxic, is a poor immunogen due to its high stability, what impairs its degradation in the antigen-presenting cells (APC) necessary to initiate the immune response [2].

The aim of this work was to identify PnTx2-6 epitopes and design and synthesize immunogens to complement the use of crude venoms in the production of antivenom serums in Argentina.

Results and Discussion

Epitopes of the neurotoxin Tx2-6 (δ-ctenitoxin-Pn2a) were identified with the "MHC-II Binding Predictions" tool of the "Immune Epitope Database Analysis Resource" [3,4]. The most antigenic zone, corresponding to the *C*-terminal, GYFWIAWYKLANCKK, was synthesized in solid phase using Fmoc/tBu chemistry and Rink amide-MBHA resin. The Cys was replaced by α-aminobutyric acid to avoid disulphide bonds formation. To increase its immunogenicity palmitic acid was added at the *N*- terminal. Also, branched peptides were synthesized using Fmoc-Lys(Fmoc)-OH. Synthesized peptides were analysed by electrospray ionisation mass spectrometry (ESI-MS). Table 1 shows the monoisotopic masses obtained with the deconvolution function Xtract for each peptide. All the peaks obtained in the mass spectrum corresponded to the synthesized immunogens, demonstrating the high purity of the synthesis. M+44 and M+88 corresponded to the incomplete decarboxylation intermediate Trp(CO_2H) during Trp(Boc) final cleavage which is easily hydrolysed when peptides are dissolved in water.

Table 1. Monoisotopic masses obtained in mass spectra.

Peptide	*Monoisotopic mass (M)*	*Monoisotopic masses obtained by deconvolution*		
Palm-GYFWIAWYKLAN-Abu-KKG-NH_2	2166.3	M	M+44	
(Palm-GYFWIAWYKLANAbuKK)$_2$-KG-NH_2	4500.6	M	M+44	M+88

Also, peptides were analysed by reversed-phase high-performance liquid chromatography (RP-HPLC) (Figure 1). The RP-HPLC analysis of the linear palmitoylated epitope demonstrated a purity higher than 85% while the branched palmitoylated peptide showed two peaks. Probably, the branched palmitoylated peptide exists in two forms that convert very slowly between both conformations.

Similar results were obtained with a branched palmitoylated peptide of the scorpion Tt1g neurotoxin, also synthesized in our laboratory.

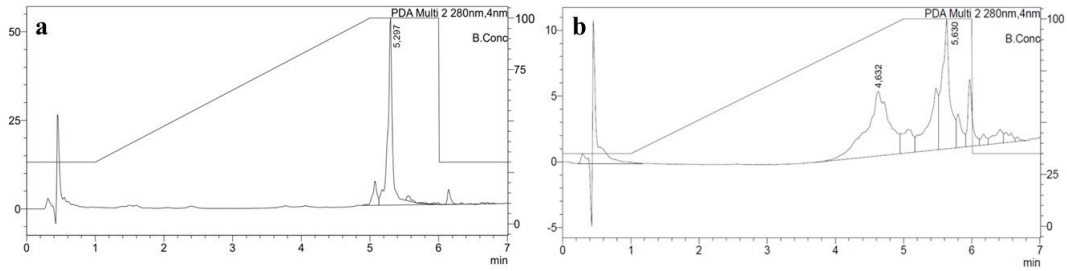

Fig. 1. RP-HPLC chromatograms. RP column (C18 3.5µm, 4.6x50mm). Solvent A: 0.045% TFA in H_2O, Solvent B: 0.036% TFA in acetonitrile: a) Palm-GYFWIAWYKLAN-Abu-KKG-NH_2, b) (Palm-GYFWIAWYKLAN-Abu-KK)$_2$-K-G-NH_2.

Currently, synthesized peptides are being 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 evaluate their immunogenicity and ability to generate neutralizing antibodies in horses to produce *P. nigriventer* antivenom.

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