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One-Bead-One-Peptide Library to Purify *Crotalus durissus terrificus* Phosholipase A2

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Introduction

Crotalus durissus terrificus (Cdt) is the only species of that genus in Argentina. Crotoxin represents more than 50% of the dry weight of its venom. This protein has two subunits: A (phospholipase A2, PLA2), and B (crotapotin). The venom protein profile differs according to geographical regions and contain several biologically active molecules [1,2]. Muller, et al [3] evaluated the antiviral activity of isolated toxins from Cdt and found that PLA2 strongly inhibits the yellow fever and dengue viruses growth in VERO E6 cells.

The aim of this work was to design a high performance purification method of Argentinean Cdt PLA2 by affinity chromatography with ligands selected from the screening of peptidic combinatorial libraries. Affinity chromatography is the better choice to purify proteins from complex mixtures like snake venoms. Short peptides, as affinity ligands, are stable and resistant to proteases and can be produced in high quantities and purity. The challenge is to find a peptide ligand with enough affinity to use in industrial-scale chromatography [4]. Divide-couple-recombine (DCR) method allows obtaining a library with all possible combinations of the amino acids in the form of "one bead-one peptide". Peptide ligands can be selected from library screening [5,6]. We have developed a rapid and non-expensive strategy for the identification of peptides contained on positive beads by using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS), and 4-hydroxymethylbenzoic acid (HMBA) as the linker in order to introduce a cleavage site to release the peptides from matrix before MS analysis [7,8].

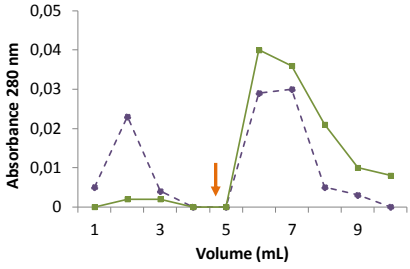


Fig. 1. Pure PLA2 chromatography on P1-Sepharose (--●--) (adsorption buffer: 20 mM sodium phosphate, pH 7.0) and on P2-Sepharose (-■-) (adsorption buffer: 20 mM sodium phosphate, pH 8.0). The arrow indicates the buffer change.

Results and Discussion

A combinatorial library containing the decapeptides XXXXXGGAGG where X= A, E, F, H, L, N, P, R, S, T, V or Y was synthesized on HMBA-ChemMatrix resin by the DCR method using Fmoc chemistry as previouly described [4]. The screening was carried out using pure PLA2 labeled with NHS-Biotin. Those peptidyl-beads with affinity for the protein were revealed using Streptavidin-Peroxidase and α-Chloronaphtol/H_2O_2. Those beads that turned to violet colour were isolated to release the peptides from HMBA-ChemMatrix resin using ammonia vapor. Then, the peptides were analyzed by MALDI-TOF MSMS to determinate their sequence. We sequenced 50 peptides and studied their amino acid frequences and moieties. Peptide 1 and Peptide 2 were selected to be immobilized in Sepharose.

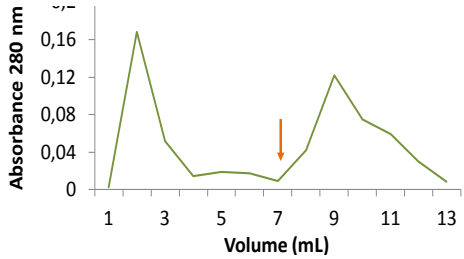
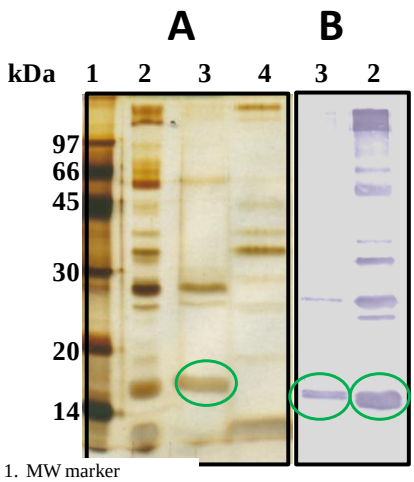


Fig. 2. Cdt whole venom chromatography on P2-Sepharose (adsorption buffer: 20 mM sodium phosphate, pH 8.0, buffer) and PLA2 purification. The arrow indicates buffer change.



1. MW marker
2. Cdt venom
3. Eluted fraction
4. Pass-through fraction

Fig. 3. (A) SDS-PAGE of Cdt's venom fractionation Silver staining and (B) Western Blot analysis of whole venom and elution fraction. The circle indicates the PLA2 band.

Pure samples of PLA2 in equilibrating buffer were loaded on columns (0.5 × 5 cm) filled with Peptide 1-Sepharose (P1) or Peptide 2-Sepharose (P2). The columns were washed with equilibrating buffer until the absorbance at 280 nm reached its initial value. The equilibrating buffers assayed were 20 mM sodium phosphate, pH 7.0, and 20 mM sodium phosphate, pH 8.0. The elution was performed with 100 mM sodium acetate buffer, pH 3.0, 0.25 M NaCl.

The best result for P1-Sepharose matrix was achieved by employing 20 mM sodium phosphate, pH 7.0, as the adsorption buffer and for P2-Sepharose by using 20 mM sodium phosphate, pH 8.0 buffer. A 97% of PLA2 adsorption was achieved with P2-Sepharose matrix, while the adsorption percentage with P1-Sepharose was only 70% (Figure 1). Therefore, P2-Sepharose matrix was used for further experiments.

A Cdt's whole venom dilution (100 µL of 5 mg/ml) in equilibrating buffer was loaded on a chromatographic column filled with P2 matrix, and

Figure 2 shows the chromatogram obtained. The PLA2 was adsorbed and eluted quantitatively. When Cdt venom was applied to P2 matrix, the SDS-PAGE analysis showed that the band corresponding to PLA2 only appears in the elution fraction (Figure 3 A). The identity was determined by Western Blot analysis performed with crotalic horse antivenom and rabbit antihorse IgG-peroxidase (Figure 3 B).

The results obtained indicate that affinity chromatography with peptide ligands are an efficient method to purify, in only one step, Argentinean Cdt PLA2. This matrix can be used to purify Cdt PLA2 for possible uses as antiviral against several tropical diseases.

Acknowledgements

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