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A single-step purification of bothropstoxin-1

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Abstract

Bothrops venoms are complex mixtures of components with a wide range of biological activities. Among these substances, myotoxins have been investigated by several groups. Bothropstoxin-1 (Bthtx-1) is a phospholipase A_2-like basic myotoxin from *Bothrops jararacussu*. The purification of this component involves two chromatographic steps. Although providing a pure material, the association of these two steps is time consuming and a single-step method using high performance chromatography media would be useful. In the present study, we describe a single-step purification method for Bthtx-1. *Bothrops jararacussu* venom was dissolved in 1 ml buffer. After centrifugation, the supernatant was injected into a Resource-S cation exchange column connected to an FPLC system and eluted with a linear salt gradient. The complete procedure took 20 min, representing a considerable time gain when compared to a previously described method (Homsi-Brandenburgo MI et al. (1988) *Toxicon*, 26: 615-627). Bthtx-1 purity and identity, assessed by SDS-PAGE and N-terminal sequencing, resulted in a single band with a molecular mass of about 14 kDa and the expected sequence of the first 5 residues, S-L-F-E-L. Although the amount of protein purified after each run is lower than in the previously described method, we believe that this method may be useful for small-scale purifications.

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- Bothropstoxin-1

Bothrops venoms are extremely complex mixtures of toxins, enzymes and peptides, possessing a wide range of biological activities (1). Myotoxins derived from phospholipases A_2 have been isolated from venoms of several species (2,3). Some of these are devoid of catalytic activity (2-4). These nonenzymatic myotoxins exhibit high specificity for skeletal muscle and induce severe myonecrosis (2). Characteristically, these toxins display high isoelectric points (above pH 8) (2,3,5,6), molecular masses of approximately 13.5 kDa (5,6) and amino acid substitutions in the calcium-binding loop (4,5), which presumably abolishes the calcium-binding capacity. Homsi-Brandenburgo et al. (2) isolated bothropstoxin-1, the main myotoxic component of *Bothrops jararacussu* venom. Their purification protocol involved gel filtration on Sephadex G-75 followed by cation exchange using SP Sephadex C-25. Since then, other researchers (5,6) have employed

similar procedures for the isolation of these non-catalytic myotoxins. Although the above method (2) yields a pure product, it is quite time consuming. In the present study, we describe a single-step purification for bothropstoxin-1.

Bothrops jararacussu venom (25 mg) (Instituto Butantan) was dissolved in 1 ml 50 mM sodium acetate, pH 5. After centrifugation, the supernatant was injected into a 1-ml Resource-S cation exchange column connected to a dual pump FPLC system (Pharmacia Biotech, Uppsala, Sweden). Buffers A and B consisted of 25 mM sodium phosphate buffer, pH 7.8, and 25 mM sodium phosphate buffer, pH 7.8, containing 2 M NaCl, pH 7.8, respectively. Flow rate was 2.5 ml/min. After an initial 10-ml wash with 7.5% B buffer (0.15 M NaCl), elution of bound fractions was performed using a linear gradient (slope = 1%/ml) for 25 ml. The column was then washed with 10 ml of B buffer, followed by 10 ml A buffer to wash NaCl out of the column.

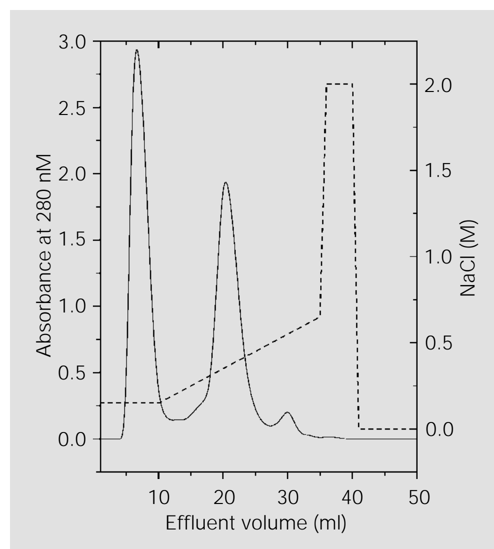

Figure 1 - Ion-exchange chromatography of 25 mg B. jararacussu venom dissolved in 1 ml 50 mM sodium acetate, pH 5, on a 1-ml Resource-S column (6.4 x 30 mm), bead size: 15 µm. A buffer: 25 mM sodium phosphate, pH 7.8. B buffer: 25 mM sodium phosphate buffer, pH 7.8, containing 2 M NaCl. Flow rate: 2.5 ml/min. The gradient is indicated by the dashed line. The procedure was carried out at room temperature.

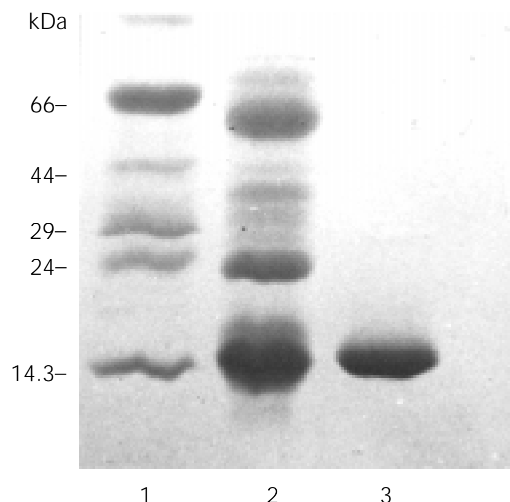

Figure 2 - SDS-PAGE (15% 100 x 80 x 0.75 mm gel) of molecular weight markers (lane 1), crude Bothrops jararacussu venom (lane 2) and purified bothropstoxin-1 (lane 3). Twenty-five µg of each sample, dissolved in 20 µl sample buffer was applied to each well. The current was fixed at 20 mA and, following the run, the gel was stained with Coomassie blue R-250.

Aliquots of crude venom, molecular weight standards and purified bothropstoxin-1 were submitted to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) under reducing conditions (7). The purified myotoxin was also submitted to N-terminal sequencing using automated Edman degradation.

The protein was sequenced in an Applied Biosystems 473 A protein sequencer from the Center of Protein Sequencing, Amino Acid Analysis and Peptide Synthesis of the Department of Biochemistry, Institute of Chemistry, University of São Paulo.

Ion-exchange chromatography resulted in three major peaks (Figure 1). The first was eluted before the gradient was applied, the second was eluted in approximately 0.42 M NaCl and the third in 0.56 M salt. The major peak (peak 2) was dialyzed against deionized water and freeze-dried.

SDS-PAGE (Figure 2) of the purified fraction resulted in a single band with a molecular mass near 14 kDa. However, contaminating phospholipases may have co-migrated with bothropstoxin-1. Several proteins with molecular weights ranging from 12.9 to 15.5 kDa have been identified in *B. jararacussu* venom (2), so the appearance of a single band on SDS-PAGE was insufficient to demonstrate purity.

Edman degradation yielded a single se-

quence, NH_2-S-L-F-E-L, which was the same as that reported for bothropstoxin-1 (2,5), indicating that the complete purification of bothropstoxin-1 was achieved by the method described.

The proposed method reduced purification time by roughly 140-fold when compared to the method of Homsi-Brandenburgo et al. (2), but dialysis or desalting may be required after purification. Although the amount of protein purified per run is low, due to the small column employed, this method could be readily scaled up for larger amounts of materials.

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