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A SINGLE STEP PURIFICATION METHOD FOR BOTHROPSTOXIN I

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Bothrops venoms are extremely complex mixtures of proteins and peptides with a wid range of activities. Among these substances, myotoxins, which occur in several species have been investigated by several groups. Bothropstoxin I (BthTX-1), is a phospholipas A_2 like basic myotoxin from *Bothrops jararacussu*. It was first purified by Homs Brandenburgo *et al*, (*Toxicon*, 26, 7) and since then, several authors have adopted th same purification schedule which includes a Sephadex G-75 gel filtration followed by convex gradient elution on Sephadex SP C-25. Although providing a pure material, th association of these two steps is time consuming (around 48 hours) and a single ste method on more perfomant chromatographic media could be useful. In the present wor we describe a single step purification method for BthTX-1. *Bothrops jararacussu* venor (50 mg) was dissolved in 1 ml pH 5 50 mM sodium acetate. After centrifugation, th supernatant was injected in a 1 ml Resource-S (Pharmacia) column connected to a du pump FPLC system. Buffer A and B were respectively 25 mM sodium phosphate and 2 mM sodium phosphate 2 M NaCl, both at pH 7.8. The flow rate during the whole run wa of 3.5 ml/min. After an initial 10 ml wash with 7.5 % B, elution was started with a line gradient (slope=1%) for 30 ml. The column was then regenerated with 10 ml 100% buffer followed by a 10 ml A buffer wash. The complete procedure took about 20 minute representing a 144 folds time gain. BthTX-1 purity and identity were checked by SDS PAGE, and isoelectric focusing, all of the assays resulting on a single band with molecular weight around 14 kDa and an isoelectric point of around 8.3. Although th amount of protein purified after each run is lower than in the previously describe method, we believe that this method may be useful for low scale purification purposes.

SUPPORTED BY CAPES

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