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Note

Purification of acidic phospholipases from Indian cobra (*Naja naja naja*) venom*

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Phospholipases are useful materials in the study of the structure and function of biomembranes[1,2], and snake venoms are the richest sources of phospholipases[3]. Phospholipases of venoms show several pharmacological and pathophysiological activities[4]. Several isoenzymes of phospholipase A, as many as fourteen in *Naja naja* venom, have been reported[5–7]. Earlier, several workers attempted to purify different forms of phospholipase A using multi-step procedures involving heat treatment, gel filtration, electrofocusing and multiple ion-exchange column chromatography[6–8]. However, they failed to separate different forms of phospholipase from cobra venom. Evans *et al.*[9] purified three isoenzymes of phospholipase A from *Naja nigricollis* venom on a carboxymethyl-Bio-Gel A column.

Recently, we reported the separation of nine isoenzymes of phospholipase A from cobra (*Naja naja*) venom on a CM-Sephadex C-25 column[10]. Most of the phospholipase A activity was recovered in the non-retained fraction[11]. In this paper, we discuss the electrophoretic separation and purification of three or more acidic isoenzymes of phospholipase A from the non-retained fraction of cobra venom.

EXPERIMENTAL

Indian cobra (*Naja naja naja*) venom (batch No. 114) was purchased from Haffkine Institute (Bombay, India). Cm-Sephadex C-25 (4.5 mequiv./g) and acrylamide from Sigma (St. Louis, MO, U.S.A.) was used. The non-retained acidic fraction was obtained from cobra venom as described earlier[10]. Electrophoresis was carried out at pH 8.3 on 5% polyacrylamide disc gels using a constant current of 3 mA per gel for about 120 min[12]. Two of the gels were stained for proteins with Amido Black 10B. Non-stained gels were sliced (4 mm each) and each slice was eluted with 1 ml of 0.1 *M* phosphate buffer of pH 7.3. Aliquots were screened for phospholipase A activity by the semi-quantitative haemolytic assay method of Boman and Kaletta[13]. Colorimetric and densitometric measurements were made in a Bausch and Lomb Spectronic-20 and a Biochem Model M 77 densitometer, respectively.

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RESULTS AND DISCUSSION

The non-retained acidic fraction on electrophoresis at pH 8.3 on 5% polyacrylamide disc gel gave nine Amido Black 10B-positive protein bands and the densitogram recordings at 640 nm gave nine peaks (Fig. 1). The screening of the aliquots of the gel slice extracts for phospholipase A showed the presence of at least three isoenzyme forms (Fig. 1), which were named PL-1a, PL-1b and PL-1c. The aliquots containing the activities were pooled, dialysed against water and lyophilized. Each lyophilized protein, on re-electrophoresis on 5% polyacrylamide disc gel at pH 8.3, gave a single Amido Black 10B-positive band (Fig. 2). Thus the acidic isoenzymes of phospholipase A from cobra venom were purified by electrophoresis.

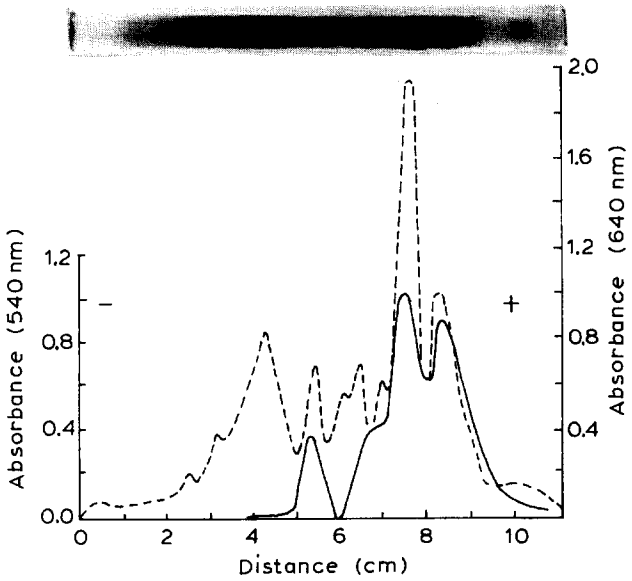

Fig. 1. Electrophoretic separation of phospholipase A: ---------, densitogram of non-retained fraction; ———, phospholipase A activity.

Recently, electrophoretic techniques have been applied to the purification of enzymes and proteins[14,15]. Grossman and Liefländer[16] resolved the purified acetylcholinesterase from *Naja naja atra* into 7–10 forms by electrophoresis and isoelectrofocusing. We have described a highly reproducible electrophoretic method for isolating and purifying the acidic phospholipases of cobra venom. Earlier attempts, despite the tedious multiple steps, failed, probably because of concentration-dependent association–dissociation of phospholipases[17] or protein–protein interactions in venoms[18,19]. Thus the electrophoretic method appears to be easier and more useful for the isolation and purification of acidic phospholipases of cobra venom.

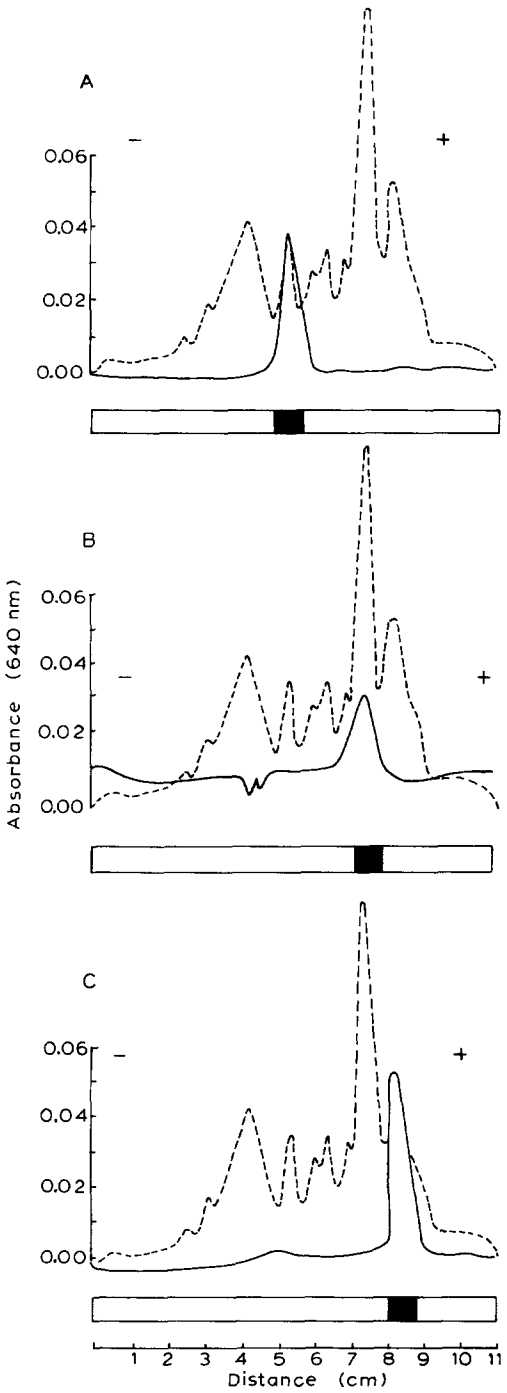

Fig. 2. Densitometric recordings obtained by re-electrophoresis of phospholipase A isoenzymes. (A) PL-1a; (B) PL-1b; (C) PL-1c. ---------, Densitogram of non-retained fraction; ———, densitogram of the corresponding isoenzymes.

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