PHOSPHOLIPASES A OF THE VENOM OF THE CENTRAL ASIAN COBRA

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Phospholipase A (E.C. 3.1.1.4) has been found in the venom of the Central Asian cobra; the enzyme has been isolated from this venom by Sephadex gel filtration and ion-exchange chromatography in the electrophoretically homogeneous state [1, 2]. It has also been shown that the venom of the cobra contains several phospholipases A [3]. The present paper gives information on the purification and isolation of the phospholipases A from cobra venom.

By gel filtration on Sephadex G-75 in 0.05 N acetic acid we separated the cobra venom into three fractions (Fig. 1). Various enzymes were found in the first, high-molecular-weight fraction: ATPase, 5-nucleotidase, hyaluronidase, and cholinesterase [4]. In the second fraction we found phospholipase A, and in the third – low-molecular-weight substances – peptides of different sizes and amino acids.

Disk electrophoresis of the second fraction showed the presence of 10-11 components migrating towards the cathode. Consequently, the subsequent purification of the phospholipase A was performed by chromatography on CM-cellulose cation-exchange resin. The second fraction (0.05 M acetic acid) was adsorbed on CM-cellulose equilibrated with a 0.05 M ammonium acetate buffer, pH 4.7. For elution we used a linear pH gradient of 0.05 M ammonium acetate buffer (4.7-5.8). With this procedure, phospholipase AI was eluted in fraction B and another phospholipase, AII, was found in fraction D desorbed from the ion-exchange resin with a 0.1 M solution of ammonium acetate, pH 6.0-6.1 (Fig. 2). Consequently, it may be assumed that the cobra venom contains two phospholipases A differing in the charge of the molecules. This is confirmed

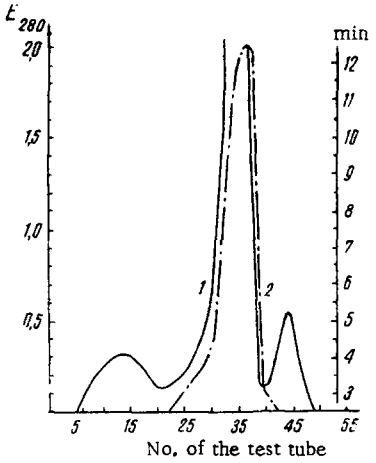

Fig. 1. Results of separation of the venom of the Central Asian Cobra on Sephadex G-75; 1) protein; 2) phospholipase A activity.

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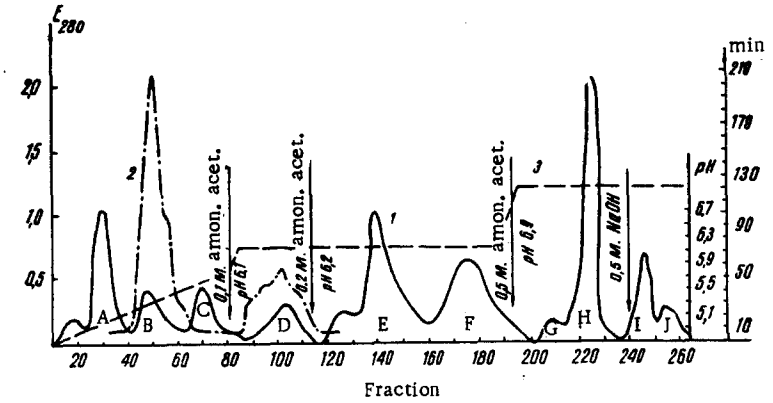

Fig. 2. Results of the separation of fraction II on CM-cellulose; 1) protein; 2) phospholipase A activity; 3) pH.

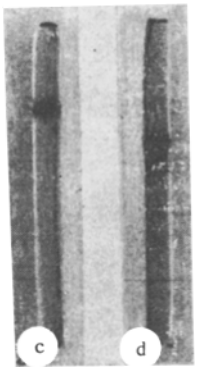

Fig. 3. Electrophoregrams (b) and (d) of fractions B and D with phospholipase A activity.

by the different rates of migration of the first and second phospholipases A in an electric field (Fig. 3b, d). The results of rechromatography on CM-cellulose and CM-Sephadex, and also of disk electrophoresis showed that both the phospholipases isolated are individual substances according to these criteria (see Fig. 3b, d). Thus, in addition to the phospholipase AI that has been described previously [1] we have obtained another component with phospholipase activity from cobra venom (fraction D).

To determine the molecular weights of the phospholipases A that we isolated, they were passed through a column of Sephadex gel G-75 (24 × 500 ml) prepared according to Andrews [5] and calibrated with proteins having known molecular weights: cytochrome C (12,500), ribonuclease (13,000), and α-chymotrypsinogen (25,000). The free volume of the column (V_0 = 78 ml) was determined with the aid of dextran blue. Elution was performed with 0.05 M tris-hydrochloride buffer (pH 7.5) containing 0.1 M potassium chloride. The rate of elution was 30 ml/h. By this method for the first phospholipase we obtained a figure for the molecular weight of 11,000 ± 1000 which corresponds to the molecular weights given in recent papers on the phospholipases A from the venoms of other snakes [6-9]. The molecular weight of the second phospholipase A was higher (18,000 ± 1000) and corresponded more to the molecular weight of the complex of phospholipase A with its specific inhibitor – a peptide with a molecular weight of ~ 5000 [9]. In view of the relatively low activity of the phospholipase AII (fraction D), the presence of such an inhibitor in it is possible (this will be investigated separately).

EXPERIMENTAL

The venom of the Central Asian cobra was obtained from the labroatory of the ecology of venomous snakes of the Institute of Zoology and Parasitology of the Academy of Sciences of the Uzbek SSR. Samples of venom dried over calcium chloride (collected in 1969-1971) were used.

The venom was fractionated on Sephadex G-75 (columns 25 × 500 mm) followed by the ion-exchange chromatography of the active fraction on CM-cellulose (columns 15 and 300 mm). The eluent (volume 3-4 ml) was collected in a KhKOV-1 automatic fraction collector. The amount of protein was determined from the absorption at 280 nm, and the fractions were combined according to the protein "peaks", concentrated on a rotary evaporator, desalted on Sephadex G-25, reconcentrated, freeze-dried, and investigated later.

The phospholipase A activity was determined from the time of inhibition of the coagulation of egg yolk and was expressed in minutes [10]. The purity of the protein fractions was checked by rechromatography on CM-cellulose and CM-Sephadex C-50, and also by disk electrophoresis according to Davis [11]. The moledular weights were determined by Andrews' method [5].

CONCLUSIONS

Two phospholipases A with molecular weights of 11,000±1000 and 18,000±1000, consisting of individual compounds according to rechromatography and disk electrophoresis, have been isolated by filtration through Sephadex G-75 gel and by ion-exchange chromatography on CM-cellulose from the venom of the Central Asian cobra.

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