PHOSPHOLIPASE A_2 FROM THE VENOM OF THE SPIDER Eresus niger

B. U. Atakuziev, F. Nuritova, and P. B. Usmanov

UDC 547.993

A phospholipase A_2 with a molecular mass of 12-14 kDa has been isolated from the venom of the spider Eresus niger by three-stage column chromatography. Asparagine has been identified as the N-terminal amino acid.

At the present time, a whole series of neurotoxins blocking nerve-muscle transmission in various animal species have been isolated from spider venoms. We have found previously that the venom of the spider Eresus niger causes a block of nerve-muscle transmission in vertebrates, acting presynaptically, and possesses a phospholipase activity (10 μM × min^{-1} × mg^{-1}) similar to that of the presynaptic toxins of snake venoms [2]. In view of this, it seemed important to isolate the components of the E. niger venom responsible for its neurotoxicity and to establish the link between neurotoxicity and phospholipase activity.

In the present communication we consider the isolation and physicochemical characterization of the phospholipase A_2 from the venom of the spider Eresus niger.

A solution of the venom in 0.05 M ammonium bicarbonate buffer was subjected to ion-exchange chromatography on a column of DEAE-Toyopearl 650 equilibrated with the same buffer. Part of the venom [components (I)-(III)] was not adsorbed under these conditions and issued in the free volume. The subsequent desorption of the protein components was effected with a linear molarity gradient (0.05-0.5 M, pH 8.2) of ammonium bicarbonate buffer. Five components (IV)-(VIII) were obtained.

When 1 M ammonium bicarbonate buffer with pH 8.2 was fed to the column it was possible to obtain another fraction – (IX). Thus, with the aid of ion-exchange chromatography on DEAE-Toyopearl 650 the whole venum had been separated into nine components (I)-(IX) (Fig. 1). A determination of phospholipase activities showed that fraction (VIII) contained practical-

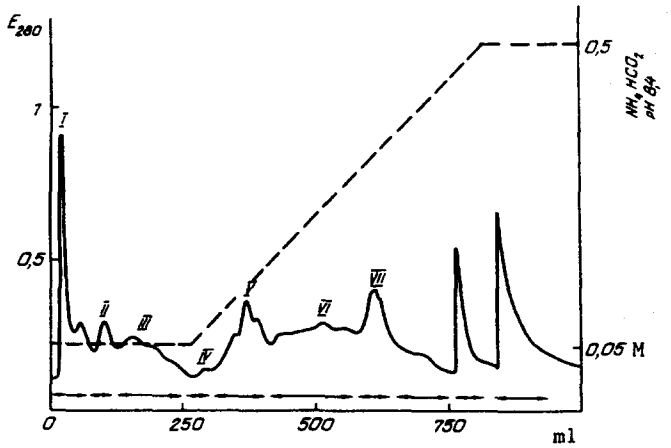

Fig. 1. Ion-exchange chromatography of the venom of the spider Eresus niger on a column of DEAE-Toyopearl 650 in a molarity gradient of ammonium bicarbonate buffer (column 2 × 10 cm, rate of elution 50 ml/h, volume of the gradient 1 liter). I-IX are combined fractions.

Institute of Physiology, Academy of Sciences of the Uzbek SSR, Tashkent. Translated from Khimiya Prirodnykh Soedinenii, No. 4, pp. 557-560, July-August, 1991. Original article submitted July 2, 1990; revision submitted January 25, 1991.

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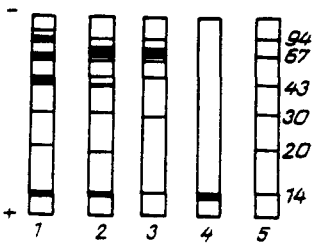

Fig. 2. Electrophoresis in PAAG in the presence of 0.1% of SDS and 6 M urea of the whole venom and its fractions: 1) whole venom of the spider E. niger; 2) fraction (VIII); 3) fraction (VIII-4); 4) phospholipase A_2; 5) marker proteins; the figures show their molecular masses, kDa: 94 – phospholipase B; 67 – bovine serum albumin; 43 – ovalbumin; 30 – carboanhydrase; 20 – trypsin inhibitor; 14 – lactalbumin.

ly all the phosphilipase A_2 activity of the whole venom (17 $\mu M \times min^{-1} \times mg^{-1}$). The results of comparative electrophoretic investigations of the E. niger venom and its fractions obtained after ion-exchange chromatography revealed their heterogeneity (Fig. 2).

The following stage of the separation included the gel filtration of fraction (VIII) on a column of Sephacryl S-300 (Fig. 3). As a result we obtained six fractions (VIII-1)-(VIII-6). A determination of phospholipase activities showed that only fractions (VIII-3) and (VIII-4) possessed enzymatic activity, fraction (VIII-4) having the highest activity (38 $\mu M \times min^{-1} \times mg^{-1}$). The weak activity of fraction (VIII-3) was apparently due to an insufficiently complete separation of this fraction from fraction (VIII-4), in which essentially all the enzymatic activity of the whole venom was concentrated. When fraction (VIII-4) was passed through a column of Sephacryl S-300 that had previously been calibrated with such proteins as dextran blue (2,000,000 Da), albumin (67,000 Da), myoglobin (17,000 Da), and cytochrome C (12,300 Da) under identical conditions it was found that fraction (VIII-4) issued from the column in a volume of eluate located between the volumes corresponding to the myoglobin and cytochrome. On the basis of these facts it may be concluded that the molecular mass of this component was 17,000-12,300 Da (see Fig. 3).

The results of the electrophoretic investigation of the fractions obtained after separation by gel filtration on Sephacryl S-300 are shown in Fig. 2. It is not difficult to see that, in addition to a low-molecular-mass component, each fraction contained several high-molecular-mass components which, apparently, came from fraction (VIII-3), of higher molecular mass, during the separation of fraction (VIII-4) from the eluate issuing from the column.

The final stage of the work to obtain homogeneous phospholipase A_2 consisted in high-performance liquid chromatography of fraction (VIII-4) in a reversed phase on an Ultrapore RPSC column in a concentration gradient (from 0 to 60%) of acetonitrile. In this way it proved possible to separate fraction (VIII-4) into four components (1-4) (Fig. 4). A determination of phospholipase activities showed that only component (1), desorbed from the column at 33% acetonitrile, possessed enzymatic activity (30 $\mu M \times min^{-1} \times mg^{-1}$). The yield of phospholipase A_2 was ~2%.

Electrophoresis in PAAG in the presence of 0.1% of Na SDS, 6 M urea, and marker proteins revealed the presence of one band, located in the region of molecular masses of 12,200-14,000 Da, which corresponds to the molecular masses of phospholipases A_2 from other venoms of animal origin [3]. As an analysis of amino acid composition showed, that total proportion of hydrophobic amino acid residues was 36.7%, of polar residues 20.6%, of acidic residues 28%, and of basic residues 13.5%. Asparagine was identified as the N-terminal amino acid residue. At the present time the study of the mechanism of the action of phospholipase A_2 and of the fractions obtained is continuing, and its results will be published separately.

EXPERIMENTAL

Lyophilized venom of the spider Eresus niger was obtained from the Central Asian Zoological Combine.

Fractionation on columns of DEAE-Toyopearl 650 and of Sephacryl S-300 and also reversed-phase high-performance liquid chromatography on an Ultrapore RPSC column were conducted in accordance with the manufacturers' recommendations.

Electrophoresis in polyacrylamide gel (PAAG) was performed by Reisfeld's method [4] using Reanal instruments and reagents, and electrofocusing on a LKB Multiphore apparatus (Swe-

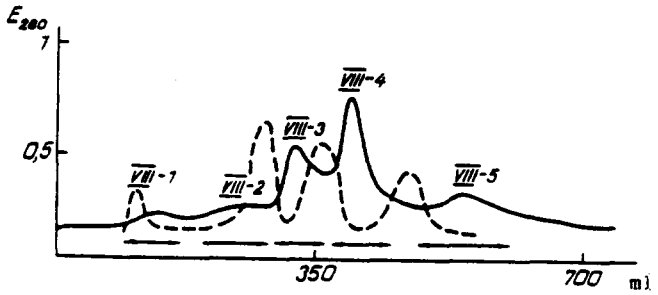

Fig. 3. Gel filtration of fraction (VIII) through Sephacryl S-300 (column 1 × 120 cm, 0.05 M ammonium bicarbonate buffer, pH 8.4, rate of elution 5 ml/min): (VIII-1)-(VIII-6) are combined fractions. The dashed line is the profile of the elution curve of the marker proteins: 1) dextran blue (2,000,000); 2) albumin (67,000); myoglobin (17,000); cytochrome C (12,300).

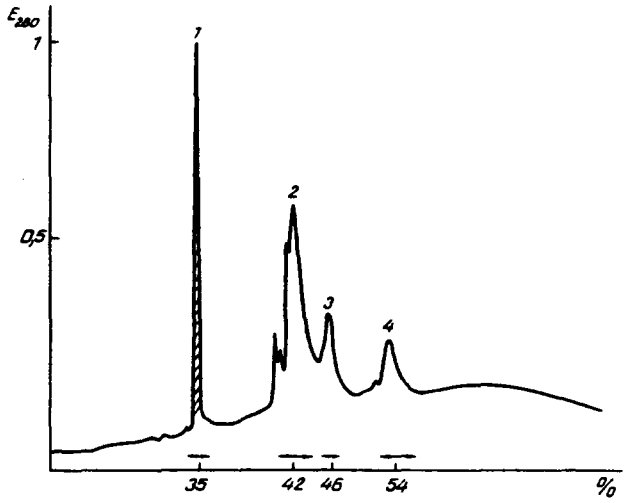

Fig. 4. Reversed-phase high-performance liquid chromatography of fraction (VIII-4) on an Ultrapore RPSC column (4.6 × 7.5 cm) equilibrated with 0.1% TFA. Elution was performed in a concentration gradient (from 0 to 60%) of acetonitrile over 60 min at the rate of 1 ml/min.

den) using standard plates. Phospholipase activities were determined by the potentiometric titration method [5]; the medium, with a total volume of 10 ml, contained 0.5% of Triton X-100, 10 mM $CaCl_2$, 150 mM NaCl, and 3 mM soybean phosphatidylcholine (Sigma, Type 11-S).

For amino acid analysis, a sample in glass ampul was covered with 6 N HCl and, after evacuation, was hydrolyzed at 110°C for 24 h. The N-terminal amino acid residue was determined by a method described previously [6].

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