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CONCANAVALIN A-NONBINDING ENZYMES OF CROTALUS SCUTULATUS VENOM

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ABSTRACT

Crotalus scutulatus venom was separated into two fractions by Concanaavalin A Sepharose 4B column chromatography. The Concanaavalin A-nonbinding fraction (F-I) exhibited phosophomonoesterase (orthophosphoric monoester phosphophydrase EC 3.1.3.2), phosphodiesterase, 5' nucleotidase (5' ribonucleotide phosphohydrolase EC 3.1.3.5), phospholipase A (phosphatidase 2-acetylhydrolase EC 3.1.1.4), hyaluronidase (hyaluronate glycosidohydrolase EC 3.2.1.d), N-benzoyl-L-arginine ethyl esterase, p-toluene sulfonyl-L-arginine methyl esterase, L-amino acid oxidase (L-amino acid: O2 oxidoreductase [deaminating] EC 1.4.3.2), and caseinolytic activities. Thrombin-like and NAD nucleosidase (5'-ribonucleotide phosphohydrolase EC 3.1.3.5) activities were not observed. DEAE Sephadex A-50 ion exchange chromatography by two stage elution of F-I yielded several fractions having proteinase activities. Proteinase activity was observed in the latter fractions of the first elution and in the fractions of the second elution.

INTRODUCTION

Previously we reported the separation of Crotalus scutulatus venom into two fractions by Concanaavalin A Sepharose 4B (Con A) affinity chromatography: Fraction I (F-I) consisting of non-binding proteins and Fraction II (F-II) consisting of binding proteins. Numerous enzyme activities were observed in the crude venom and F-II. The crude venom and the glycoproteins (F-II) which bound to the Con A were fractionated by ion-exchange chromatography, and the resulting fractions demonstrated a broad distribution of proteinase activities (Hinson et al., 1985). This study compares enzyme properties of Con A-nonbinding proteins (F-I) with those of the previously reported binding proteins of F-II and the crude venom.

MATERIALS AND METHODS

Lyophilized C. s. scutulatus venom was provided by Dr. H. L. Stahnke of Arizona State University, N-benzoyl-L-arginine ethyl ester (BAEE), p-toluene sulfonyl-L-arginine methyl ester (TAME), 5'-adenylic acid, bis-p-nitrophenyl phosphate sodium salt, beef plasma thrombin, B-NAD, bovine fibrinogen (F-4000), and bovine albumin Fraction V were purchased from Sigma Chemical Company; disodium p-nitrophenyl phosphate from Nutritional Biochemicals Corporation; Tris(hydroxymethyl)aminomethane, glycine, ammonium molybdate, hydroquinone, sodium sulfite, magnesium chloride, L-leucine, trichloroacetic acid (TCA), potassium cyanide, potassium hydrogen phosphate, and calcium chloride were purchased from Fisher Scientific Company; sodium hydrogen sulfite from J. T. Baker Chemical Company; hyaluronic acid from Worthington Biochemical Corporation; magnesium sulfate and sodium hydrogen phosphate from Mallinckrodt Chemical Works; casein from ICN Pharmaceuticals, Inc.; Sephadex G-25, Concanaavalin A-Sepharose (Con A), DEAE Sephadex A-50, and columns from Pharmacia, Uppsala I, Sweden. Phosphomonoesterase and phosphodiesterase (Richards et al., 1965), 5'-nucleotidase (Lo et al., 1966; Ginz, 1956), phospholipase A (Marinetti, 1965), N-benzoyl-L-arginine ethyl esterase (BAEEase) and p-toluene sulfonyl-L-arginine methyl esterase (TAMEase) (Tu et al., 1965; Schwert and Takenaka, 1955), thrombin-like (Sato et al., 1965), proteinase (Kunitz, 1947; Rich, 1965), L-amino acid oxidase (Paik and Kim, 1965), NAD nucleosidase (Colonick et al., 1951; Kaplan et al., 1951), and hyalurondase (Kass and Seastone, 1944) assay procedures included the minor modifications used in previous works (Sifford and Johnson, 1978; Hinson et al., 1985).

A column (2.5 x 15 cm) of Con A gel was used to separate the Con A-nonbinding proteins from Con A-binding proteins (Isacove et al., 1974; Asberg and Porath, 1970; Hinson et al., 1985). DEAE Sephadex A-50 ion exchange chromatography was accomplished by the methods of Cheng and Ouyang (1967), Ouyang et al. (1971), Sifford and Johnson (1978), and Hinson et al. (1985).

RESULTS AND DISCUSSION

Phosphomonoesterase, phosphodiesterase, 5'-nucleotidase, phospholipase A, BAEEase, TAMEase, proteinase, L-amino acid oxidase, and hyaluronidase activities were present in C. s. scutulatus crude venom and in the Con A-binding venom proteins of F-II. Mean activities of enzymes assayed in F-II, with the exceptions of hyaluronidase,

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Crude Venom</th>
<th>Concanaavalin A Fraction I</th>
<th>Concanaavalin A Fraction II</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phosphomonoesterase</td>
<td>7.67</td>
<td>5.8</td>
<td>69.7</td>
</tr>
<tr>
<td>Phosphodiesterase</td>
<td>1.73</td>
<td>0.2</td>
<td>26.2</td>
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<tr>
<td>Phospholipase A</td>
<td>200</td>
<td>250</td>
<td>500</td>
</tr>
<tr>
<td>Thrombin-like</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>5'-nucleotidase</td>
<td>1.8</td>
<td>0.03</td>
<td>0.7</td>
</tr>
<tr>
<td>Proteinase</td>
<td>0.086</td>
<td>0.12</td>
<td>0.23</td>
</tr>
<tr>
<td>Hyaluronidase**</td>
<td>120</td>
<td>44</td>
<td>25</td>
</tr>
<tr>
<td>NADase</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>TAMEase</td>
<td>850</td>
<td>970</td>
<td>710</td>
</tr>
<tr>
<td>BAEEase</td>
<td>2600</td>
<td>1620</td>
<td>2000</td>
</tr>
<tr>
<td>L-amino acid oxidase***</td>
<td>62</td>
<td>31</td>
<td>327</td>
</tr>
</tbody>
</table>

** All enzyme assays were performed at the optimum pH obtained by using the crude venom.
*** Hyaluronidase activity is expressed as turbidity reducing units/mg.
**** L-amino acid oxidase activity is expressed as μmol/h/mg.

TAMEase, and BAEEase, were greater than those in the crude venom (Hinson et al., 1985). These enzyme activities were also observed in the C. s. scutulatus Con A-nonbinding venom proteins of F-I. In F-I,
however, only phospholipase A, proteinase, and TAMEase activities were greater than those of the crude venom. Also, thrombin-like and NAD nucleosidase activities were not observed in F-I (Table 1).

The enzyme activities of F-I differed quantitatively from those observed in F-II by Hinson et al. (1985). The mean 5'-nucleotidase, phosphodiesterase, phosphomonoesterase, L-amino acid oxidase, phospholipase A, and proteinase activities of F-I were significantly lower than those observed in F-II. F-I BAEase activity was only slightly less than F-II BAEase activity. Hyaluronidase and TAMEase activities were slightly higher in F-I than in F-II (Table 1).

There was a broad distribution of proteinase activities in the C. s. scutulatus crude venom fractions obtained by DEAE Sephadex A-50 chromatography. These activities occurred in the first and latter fractions of the first elution and in the fractions of the second elution. And, when F-II was fractionated by DEAE Sephadex A-50, the proteinase activities were concentrated in the first fractions of the first elution (Hinson et al., 1985). In this work, F-I eluates were pooled, lyophilized, and desalted by using G-25 Sephadex. After rechromatography, this sample of Con A-nonbinding proteins was applied to a DEAE Sephadex A-50 column. After a two stage fractionation at 4°C using ammonium acetate buffer, the eluates were assayed for proteinase activity. Proteinase activity was observed in the eluates of the latter fractions of the first elution and in the fractions of the second elution (Fig. 1). This further substantiates that multiple proteolytic enzymes are present in C. s. scutulatus venom. Caseinolytic activities are present in the DEAE Sephadex A-50 fractions of the crude venom, the Con A-binding proteins, and Con A-nonbinding proteins.

Figure 1. Distribution of proteinase activity in the eluates from DEAE Sephadex A-50 ion exchange chromatography of Fraction I (124 mg) from Concanavalin A-Sepharose 4B affinity chromatography of Crotalus scutulatus scutulatus venom. The ion exchange chromatography was performed on a column 2.5 x 50 cm at 4°C by two stage elution. The arrow indicates the start of the second stage elution. Eluates of 3.25 ml each were collected with a flow rate of 17 ml/hr. Protein content estimated by absorbancy at 280 nm is shown by ---. Rates of substrate hydrolysis in 20 min per ml eluate measured by change in optical density (AOD) are indicated by -----. Specific activities of Fraction I proteinase (PF1 mg) are indicated by -----.  

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LITERATURE CITED


