

1986

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Childs, C. K.; Hinson, Michael W.; Sifford, Dewey H.; and Johnson, Bob D. (1986) "Concanavalin A-Nonbinding Enzymes of *Crotalus scutulatus scutulatus* Venom," *Journal of the Arkansas Academy of Science*: Vol. 40 , Article 7.

Available at: <http://scholarworks.uark.edu/jaas/vol40/iss1/7>

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CONCANAVALIN A-NONBINDING ENZYMES OF *CROTALUS SCUTULATUS SCUTULATUS* VENOM

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ABSTRACT

Crotalus scutulatus scutulatus crude venom was separated into two fractions by Concanavalin A Sepharose 4B affinity chromatography. The Concanavalin A-nonbinding fraction (F-I) exhibited phosphomonoesterase (orthophosphoric monoester phosphohydrolase EC 3.1.3.2), phosphodiesterase, 5'-nucleotidase (5'-ribonucleotide phosphohydrolase EC 3.1.3.5), phospholipase A (phosphatidate 2-acylhydrolase EC 3.1.1.4), hyaluronidase (hyaluronate glycanohydrolase EC 3.2.1.d), N-benzoyl-L-arginine ethyl ester, p-toluenesulfonyl-L-arginine methyl ester, 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 scutulatus* crude venom into two fractions by Concanavalin 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-toluenesulfonyl-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, Concanavalin A-Sepharose 4B (Con A), DEAE Sephadex A-50, and columns from Pharmacia, Uppsala 1, Sweden.

Phosphomonoesterase and phosphodiesterase (Richards *et al.*, 1965), 5'-nucleotidase (Lo *et al.*, 1966; Ging, 1956), phospholipase A (Marinetti, 1965), N-benzoyl-L-arginine ethyl ester (BAEEase) and p-toluenesulfonyl-L-arginine methyl ester (TAMEase) (Tu *et al.*, 1965; Schwert and Takenaka, 1955), thrombin-like (Sato *et al.*, 1965), proteinase (Kunitz, 1947; Rick, 1965), L-amino acid oxidase (Paik and Kim, 1965), NAD nucleosidase (Colowick *et al.*, 1951; Kaplan *et al.*, 1951), and hyaluronidase (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 (Iscove *et al.*, 1974; Aspberg 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 1. Mean specific enzyme activities of *Crotalus scutulatus scutulatus* crude venom and the Concanavalin A Fractions I and II*.

Enzyme	Crude Venom****	Concanavalin A Fraction I (nonbinding proteins)	Concanavalin A**** Fraction II (binding proteins)
Phosphomonoesterase	7.67	5.8	49.7
Phosphodiesterase	1.73	0.2	26.2
Phospholipase A	200	250	500
Thrombin-like	0.0	0.0	0.0
5'-nucleotidase	1.8	0.03	8.7
Proteinase	0.068	0.12	0.23
Hyaluronidase**	120	42	25
NADase	0.0	0.0	0.0
TAMEase	850	970	710
BAEEase	2600	1620	2000
L-amino acid oxidase***	62	31	327

* 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 μ l/hr/mg.

**** Results from Hinson *et al.*, 1985.

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,

C. K. Childs, M. W. Hinson, D. H. Sifford, and B. D. Johnson

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 relyophilization, 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.

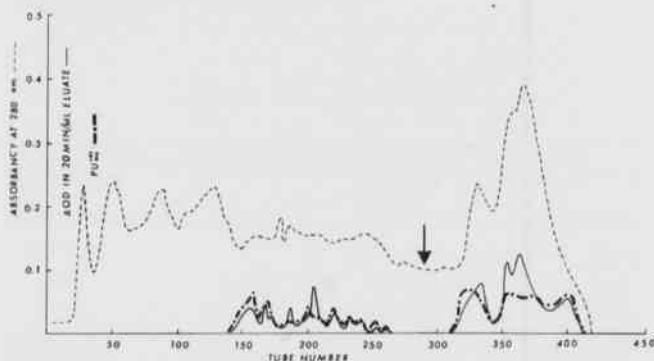


Figure 1. Distribution of proteinase activity in the eluates from DEAE Sephadex A-50 ion exchange chromatography of Fraction I (124 mg) from Concanavalin A-Sepharose 4B affinity chromatography of *Crotalus scutulatus scutulatus* crude venom. The ion exchange chromatography was performed on a column 2.5 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 (ΔOD) are indicated by ——. Specific activities of Fraction I proteinase (PU_{Mg}^{cas}) are indicated by - - -.

ACKNOWLEDGEMENTS

We thank Mrs. Alice Chandler for typing the manuscript.

LITERATURE CITED

- ASPBERG, K., and J. PORATH. 1970. Group-specific adsorption of glycoproteins. *Acta Chem. Scand.* 24:1829-1841.
- CHENG, H., and C. OUYANG. 1967. Isolation of coagulant and anticoagulant principles from the venom of *Agkistrodon acutus*. *Toxicon* 4:235-243.
- COLOWICK, S. P., N. O. KAPLAN, and M. M. CIOTTI. 1951. The reaction of pyridine nucleotide with cyanide and its analytical use. *J. Biol. Chem.* 191:447-459.
- GING, N. S. 1956. Extraction method of colorimetric determination of phosphorus in microgram quantities. *Anal. Chem.* 28:1330-1333.
- HINSON, M. W., C. K. CHILDS, B. D. JOHNSON, and D. H. SIFFORD. 1985. Concanavalin A-binding enzymes of *Crotalus scutulatus scutulatus* venom. *Proc. Ark. Acad. Sci.* In press.
- ISCOVE, N. N., F. SIEBER, and L. H. WINTERHALTER. 1974. Erythroid colony formation in cultures of mouse and human bone marrow: Analysis of the requirement for erythropoietin by gel filtration and affinity chromatography on Agarose-Concanavalin. *A. J. Cell. Physiol.* 83:309-320.
- KAPLAN, N. O., S. P. COLOWICK, and A. NASON. 1951. *Neurospora* diphosphopyridine nucleotidase. *J. Biol. Chem.* 191:473-483.
- KASS, E. H., and C. V. SEASTONE. 1944. The role of the mucoid polysaccharide (Hyaluronic Acid) in the virulence of a group A hemolytic streptococci. *J. Exp. Med.* 79:319-329.
- KUNITZ, M. 1947. Crystalline soybean trypsin inhibitor. II. General properties. *J. Gen. Physiol.* 30:291-310.
- LO, T., Y. CHEN, and C. LEE. 1966. Chemical studies of Formosan cobra (*Naja naja atra*) venom. Part I. Chromatographic separation of components. *J. Chinese Chem. Soc. Ser. II.* 13:25-37.
- MARINETTI, G. V. 1965. The action of phospholipase A on lipoproteins. *Biochim. Biophys. Acta.* 98:554-565.
- OUYANG, C., J. HONG, and C. TENG. 1971. Purification and properties of the thrombin-like principle of *Agkistrodon acutus* venom and its comparison with bovine thrombin. *Thrombos. Diathes. haemorrh. (Stuttg.)* 26:224-234.
- PAIK, K., and S. KIM. 1965. pH substrate relationship of L-amino acid oxidase from snake venom and rat kidney. *Biochim. Biophys. Acta.* 96:66-74.
- RICHARDS, G. M., G. DUVAIR, and M. LASKOWSKI, SR. 1965. Comparison of the levels of phosphodiesterase, endonuclease, and monoesterases in several snake venoms. *Biochem.* 4:501-503.
- RICK, K. 1965. Trypsin. Pp. 806, *In Methods of Enzymatic Analysis*, 2nd ed. Academic Press, New York.
- SATO, T., S. IWANAGA, Y. MIZUSHIMA, and T. SUZUKI. 1965. Studies on snake venoms. IX. Separation of arginine ester hydrolase of *Agkistrodon halys blomhoffii* venom into three enzymatic entities: "Bradykinin releasing," "clotting," and "permeability increasing." *J. Biochem.* 57:380-391.
- SCHWERT, G. W., and Y. TAKENAKA. 1955. A spectrophotometric determination of trypsin and chymotrypsin. *Biochim. Biophys. Acta.* 16:570-575.
- SIFFORD, D. H., and B. D. JOHNSON. 1978. Fractionation of *Agkistrodon bilineatus* venom by ion exchange chromatography. Pp. 231, *In Toxins: Animal, Plant and Microbial*, P. Rosenberg, ed. Pergamon Press, Oxford and New York.
- TU, A. T., G. P. JAMES, and A. CHUA. 1965. Some biochemical evidence in support of the classification of venomous snakes. *Toxicon* 3:5-8.