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A GLYCOPROTEIN PROTEINASE IN *AGKISTRODON BILINEATUS* VENOM

Snake venoms are noted for their wide variety of proteolytic enzymes. The Mexican mocassin *Agkistrodon bilineatus* is a pit viper whose crude venom is no exception (Sifford and Johnson, 1978). The goal of this work was to determine if *A. bilineatus* venom contains glycoprotein proteases.

Assay procedures for protease, phosphomonoesterase, phosphodiesterase, 5'-nucleotidase, phospholipase A₂, N-benzoyl-L-arginine ethylesterase (BAEEase) and p-toluenesulfonyl-L-arginine methyl esterase (TAMEase), thrombin-like, L-amino acid oxidase and NAD nucleosidase were the same as those used in previous works (Sifford and Johnson, 1978; Brunson et al., 1978). Hyaluronidase activity was measured by the turbidimetric method of Kass and Seastone (1944).

Separations of crude venom into proteins positive to the anthrone reagent (glycoproteins) and nonglycoproteins were performed using Concanavalin A (Con A) covalently bound to Sepharose 4B gel as described by Iscove et al. (1974) and Asperg and Porath (1970). The glycoprotein fraction was desalted with a column (1 × 90 cm) of Sephadex G-10 at 4°C. This desalted fraction was lyophilized and then fractionated by ion exchange chromatography (DEAE Sephadex A-50). In this procedure the methods of Cheng and Ouyang (1967) and Ouyang et al. (1971) including the modifications by Sifford and Johnson (1978) were used. Sephadex G-100 at 4°C was then used to separate molecules according to their molecular weight. Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis separations were performed by Dr. Collis Geren, University of Arkansas at Fayetteville, according to the procedures outlined by Weber and Osborn (1969).

Antisera in New Zealand white rabbits were developed according to Ownby et al. (1979). Immuno-electrophoresis procedures (Campbell et al., 1963; Buchler Instruments Manual, 1964; Garvey et al., 1977) were used to determine the purity of the glycoprotein protease enzyme.

Fractionation of the crude venom with Con A yielded two fractions; Fraction I composed of nonglycoproteins and Fraction II composed of glycoproteins (Fig. 1). The glycoprotein content of the crude venom was calculated as 17.4%. Enzyme activities of Fraction II were determined. Mean enzyme-specific activities included 11.79 μ moles/min/mg for phosphomonoesterase; 3.54 μ moles/min/mg for phosphodiesterase; 83 units/mg for phospholipase A₂; 2.0 μ moles/min/mg for 5'-nucleotidase; 0.01 PU_{mg} for protease; 66 TRU and 332 NF units for hyaluronidase; 0.04 units/mg for NADase; 70 units/mg for BAEEase; 20 units/mg for TAMEase; and 1.5 μ oles/hr/mg for L-amino acid oxidase. Thrombin-like activity was not observed in Fraction II.

Fraction II was pooled, lyophilized, desalted, and applied to a DEAE Sephadex A-50 column. This fractionation yielded several minor fractions and one major fraction, Fraction P₁ (Fig. 2).

Protease activity was present in Fraction P₁. This fraction was lyophilized and 5 ml of distilled water added. Fractionation of 1 ml aliquots with Sephadex G-100 yielded a fraction (P₂) having protease activity along with low NADase, TAMEase, and BAEEase activities (Fig. 3). Assays for phosphomonoesterase, phosphodiesterase, phospholipase A₂, 5'-nucleotidase, hyaluronidase, and L-amino acid oxidase in the fraction were negative.

Disc electrophoresis of the crude venom produced 12 fractions. Fraction P₂, containing the glycoprotein protease, contained 5 components (Fig. 4). The trace activities of NADase, BAEEase, and TAMEase in Fraction P₂ may account for these discs.

Seven precipitin arcs were produced with crude venom as the electrophoretically separated antigens and with the crude venom antiserum in the trough (Fig. 5). By using Fraction P₂ in one well and crude venom in the other well as the electrophoretically separated antigens and crude venom antiserum in the trough, one precipitin arc (A) was produced by Fraction P₂ and the antiserum. The crude venom antigens again produced seven precipitin arcs (Fig. 6). By using Fraction P₂ in one well and crude venom in the other well as the electrophoretically separated antigens and Fraction P₂ antiserum in the trough, one precipitin arc was produced against Fraction P₂ and the crude venom (Fig. 7). Although this arc is probably due to the glycoprotein protease more data are required for substantiation.

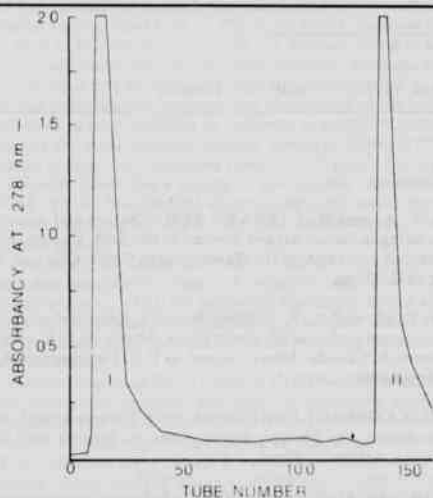


Figure 1. Chromatography of *Agkistrodon bilineatus* crude venom (450 mg) on a Concanavalin A Sepharose 4-B column (2.5 × 15 cm) at 4°C by two stage elution. The arrow indicates the start of the second stage elution (using α -methyl-D-mannoside). Eluates (5 ml/tube) were collected at a flow rate of 17 ml/hr.

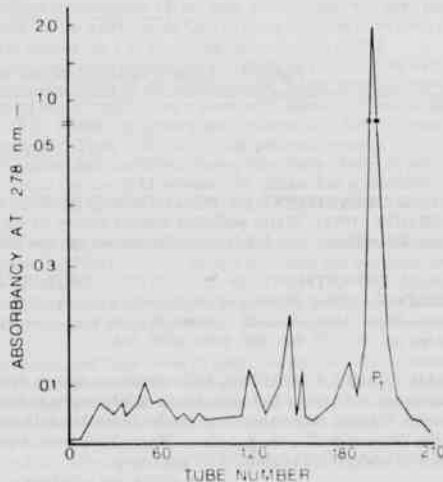


Figure 2. Chromatography of *Agkistrodon bilineatus* venom glycoproteins on a DEAE Sephadex A-50 column (2.5 × 56 cm) at 4°C using an ammonium acetate buffer gradient. Eluates of 5.0 ml/tube were collected at a flow rate of 17 ml/hr.

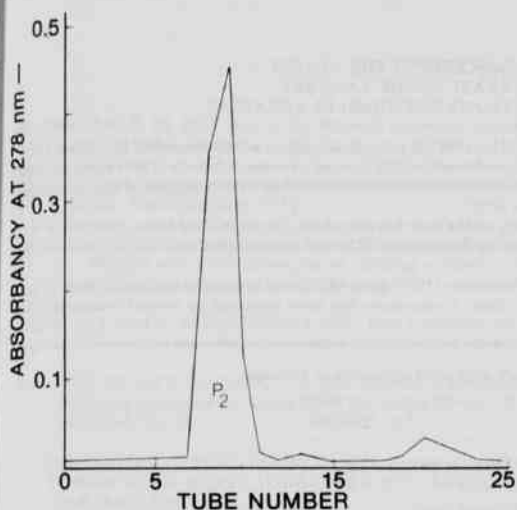


Figure 4. Comparison of patterns obtained by Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis of *Agkistrodon bilineatus* crude venom and the proteinase containing fraction (P₂).

Figure 3. Chromatography of Fraction P₁ (Fig. 2) on a Sephadex G-100 column (1 × 90 cm) at 4°C. Eluates of 4.0 ml/tube were collected at a flow rate of 16 ml/hr.

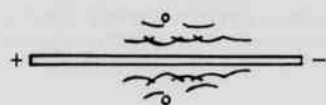


Figure 5. Composite slide of immunoelectrophoretic patterns obtained by reacting anti-crude *Agkistrodon bilineatus* venom serum with electrophoretically separated crude venom.

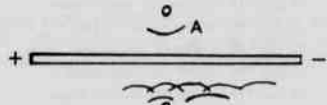


Figure 6. Composite slide of immunoelectrophoretic patterns obtained by reacting anti-crude *Agkistrodon bilineatus* venom serum with electrophoretically separated proteinase containing fraction (P₂) and *A. bilineatus* crude venom.

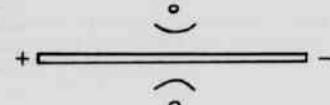


Figure 7. Immunoelectrophoretic patterns obtained by reacting anti-Fraction P₂ serum with electrophoretically separated proteinase containing fraction (P₂) and *Agkistrodon bilineatus* crude venom.

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LITERATURE CITED

- ASPERG, K. and J. PORATH. 1970. Group-specific adsorption of glycoproteins. *Acta Chem. Scand.* 24:1839-1841.
- BRUNSON, E. M., B. D. JOHNSON and D. H. SIFFORD. 1978. NAD nucleosidase of *Agkistrodon bilineatus* venom. *Amer. J. Trop. Med. and Hyg.* 27:365-368.
- BUCHLER. 1964. Routine Clinical Analysis using the Buchler Electrophoresis Apparatus. Buchler Instruments Division Nuclear-Chicago Corporation.
- CAMPBELL, D. H., J. S. GARVEY, N. E. CREMER and D. H. SUSSDORF. 1963. Immunoelectrophoresis. Pp. 149-155. *In Methods in Immunology, A Laboratory Text for Instruction and Research.* W. A. Benjamin, Inc., New York.
- CHENG, H. and C. OUYANG. 1967. Isolation of coagulant and anticoagulant principles from the venom of *Agkistrodon acutus*. *Toxicon* 4:235-243.
- GARVEY, J. S., N. E. CREMER and D. H. SUSSDORF. 1977. Immunoelectrophoresis. Pp. 328-336. *In Methods of Immunology, A Laboratory Text for Instruction and Research.* W. A. Benjamin, Inc., Reading, Mass.
- JOHN D. RUFF, BOB D. JOHNSON and DEWEY H. SIFFORD, Department of Biological Sciences and Department of Chemistry, Arkansas State University, State University, Arkansas 72467.
- ISCOVE, N. N., F. SIEBER and K. 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.
- 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.
- 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.
- OWNBY, C. L., W. M. WOODS and G. V. ODELL. 1979. Antiserum to myotoxin from prairie rattlesnake (*Crotalus viridis viridis*) venom. *Toxicon* 17:373-380.
- SIFFORD, D. H. and B. D. JOHNSON. 1978. Fractionation of *Agkistrodon bilineatus* venom by ion exchange chromatography. Pp. 231-242. *In Toxins: Animal, Plant and Microbial* (P. Rosenberg, ed.) Pergamon Press, Oxford and New York.
- WEBER, K. and M. OSBORN. 1969. The reliability of molecular weight determinations by dodecyl sulfate-polyacrylamide gel electrophoresis. *J. Biol. Chem.* 244:4406-4412.