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Glycoprotein Proteinase in Agkistrodon bilineatus Venom

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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 A_2, N-benzoyl-L-arginine ethylesterase (BAEEase) and p-toluenesulfonyl-L-arginine methylesterase (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 Aspberg 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). Immunoelectrophoresis 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 μmoles/min/mg for phosphomonoesterase; 3.54 μmoles/min/mg for phosphodiesterase; 83 units/mg for phospholipase A_1; 2.0 μmoles/min/mg for 5'-nucleotidase; 0.01 PU^{gas}_{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 μoles/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 P_1 (Fig. 2).

Protease activity was present in Fraction P_1. This fraction was lyophilized and 5 ml of distilled water added. Fractionation of 1 ml aliquots with Sephadex G-100 yielded a fraction (P_2) having protease activity along with low NADase, TAMEase, and BAEEase activities (Fig. 3). Assays for phosphomonoesterase, phosphodiesterase, phospholipase A_2, 5'-nucleotidase, hyaluronidase, and L-amino acid oxidase in the fraction were negative.

Disc electrophoresis of the crude venom produced 12 fractions. Fraction P_2, containing the glycoprotein protease, contained 5 components (Fig. 4). The trace activities of NADase, BAEEase, and TAMEase in Fraction P_2 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 P_2 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 P_2 and the antiserum. The crude venom antigens again produced seven precipitin arcs (Fig. 6). By using Fraction P_2 in one well and crude venom in the other well as the electrophoretically separated antigens and Fraction P_2 antiserum in the trough, one precipitin arc was produced against Fraction P_2 and the crude venom (Fig. 7). Although this arc is probably due to the glycoprotein protease more data are required for substantiation.

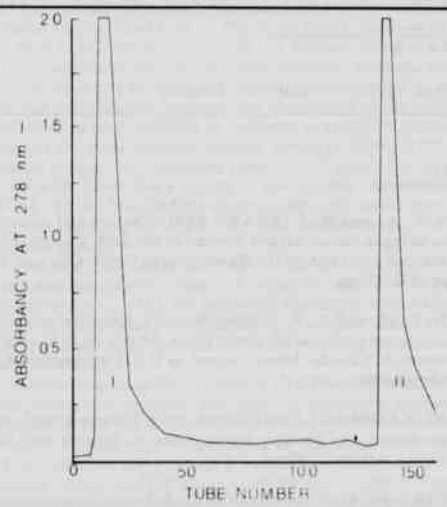

Figure 1. Chromatography of *Agkistrodon bilineatus* crude venom (450 mg) on a Concanavalin A Sepharose 4-B column (2.5 × 15 cm) at 4°C by two stage elution. The arrow indicates the start of the second stage elution (using α-methyl-D-mannoside). Eluates (5 ml/tube) were collected at a flow rate of 17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

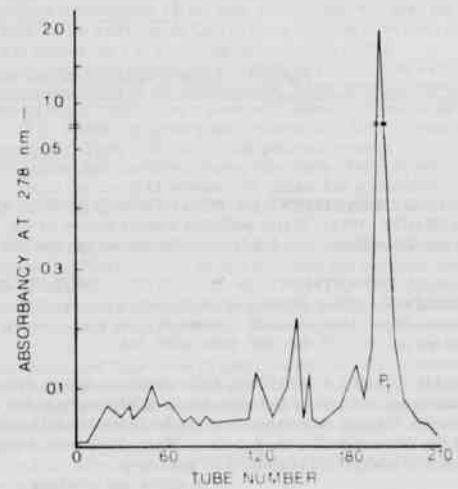

Figure 2. Chromatography of *Agkistrodon bilineatus* venom glycoproteins on a DEAE Sephadex A-50 column (2.5 × 56 cm) at 4°C using an ammonium acetate buffer gradient. Eluates of 5.0 ml/tube were collected at a flow rate of 17 ml/hr.

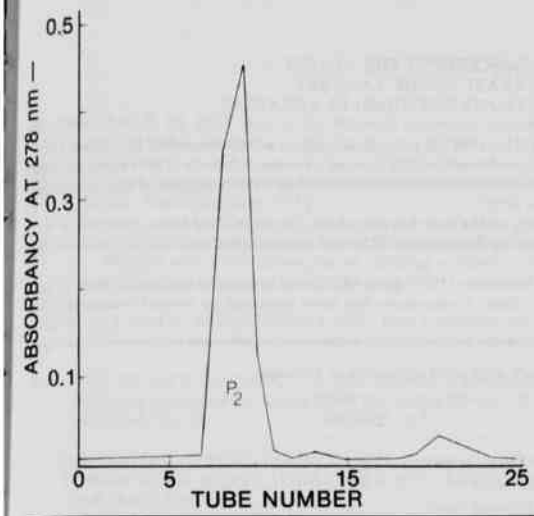

Figure 3. Chromatography of Fraction P_1 (Fig. 2) on a Sephadex G-100 column (1 × 90 cm) at 4°C. Eluates of 4.0 ml/tube were collected at a flow rate of 16 ml/hr.

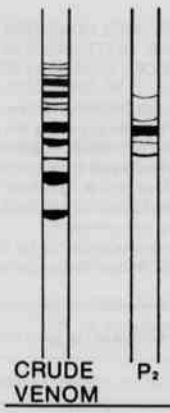

Figure 4. Comparison of patterns obtained by Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis of *Agkistrodon bilineatus* crude venom and the proteinase containing fraction (P_2).

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 (P_2) and *A. bilineatus* crude venom.

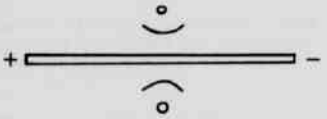

Figure 7. Immunoelectrophoretic patterns obtained by reacting anti-Fraction P_2 serum with electrophoretically separated proteinase containing fraction (P_2) and *Agkistrodon bilineatus* crude venom.

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