Glycoprotein Proteinase in Agkistrodon bilineatus Venom

John D. Ruff
Arkansas State University

Bob D. Johnson
Arkansas State University

Dewey H. Sifford
Arkansas State University

Follow this and additional works at: http://scholarworks.uark.edu/jaas

Part of the Zoology Commons

Recommended Citation
Available at: http://scholarworks.uark.edu/jaas/vol34/iss1/48

This article is available for use under the Creative Commons license: Attribution-NoDerivatives 4.0 International (CC BY-ND 4.0). Users are able to read, download, copy, print, distribute, search, link to the full texts of these articles, or use them for any other lawful purpose, without asking prior permission from the publisher or the author.
This General Note is brought to you for free and open access by ScholarWorks@UARK. It has been accepted for inclusion in Journal of the Arkansas Academy of Science by an authorized editor of ScholarWorks@UARK. For more information, please contact scholar@uark.edu, ccmiddle@uark.edu.
A GLYCOPROTEIN PROTEINASE IN AGKISTRODON BILINEATUS VENOM

Snake venoms are noted for their wide variety of proteolytic enzymes. The Mexican moccasin 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 esteresterase (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 Jacobowitz 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 Owby 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 A2; 2.0 μmoles/min/mg for 5'-nucleotidase; 0.01 PU mg for proteinase; 66 TRU and 332 NF units for hyaluronidase; 0.04 units/mg for NADase; 20 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 P2 (Fig. 2).

Protease activity was present in Fraction P2. This fraction was lyophilized and 5 ml of distilled water added. Fractionation of 1 ml aliquots with Sephadex G-100 yielded a fraction (P3) having protease activity along with low NADase activity. BAEEase and TAMEase 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 traces.

Seven precipitin arcs were produced with crude venom as the electrophoretically separated antigens and with the crude venom antigen 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 antigen 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 antigen 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.
LITERATURE CITED


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.


We thank Dr. Collis Geren for his work with the gel electrophoresis. Dr. L. W. Hinck for his assistance with the immunological procedures, Karl Landberg for his assistance throughout this work, and Mrs. Alice Chandler for typing the manuscript.

Figure 3. Chromatography of Fraction P2 (Fig. 2) on a Sephadex G-100 column (1 X 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 (P2).

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

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

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

Arkansas Academy of Science Proceedings, Vol. XXXIV, 1980 131