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ISOLATION OF PHOSPHOLIPASE A_2 FROM *AGKISTRODON BILINEATUS* VENOM

Venom produced by the Mexican mocassin, *A. bilineatus*, contains phosphomonoesterase, phosphodiesterase, 5'-nucleotidase, esterases, thrombin-like, L-amino acid oxidase, protease, phospholipase A_2, and NAD nucleosidase activities (Tu et al., 1967; Denson et al., 1972; Sifford and Johnson, 1978; Brunson et al., 1978). Of these enzymes, phospholipase A_2 (PhL-A_2) was chosen in this work for possible isolation. This choice of PhL-A_2 for isolation was due primarily to its heat stability and to its distribution in the eluates obtained by ion exchange chromatography of the crude venom as evidenced previously (Sifford and Johnson, 1978).

Assay procedures with minor modifications (Sifford and Johnson, 1978) included phospholipase A_2 using the clearing of an egg yolk suspension (Marinetti, 1965), phosphomonoesterase and phosphodiesterase (Richards et al., 1965), esterase (Tu et al., 1965), 5'-nucleosidase (Lo et al., 1966; Ging, 1956), and L-amino acid oxidase (Paik and Kim, 1965). Hyaluronidase was assayed according to the turbidimetric procedures of Kass and Seastone (1944).

A 450 mg sample of crude venom (Sigma) was separated on Concanavalin A covalently bound to Sepharose 4B gel (Con A) into glycoproteins (anthrone reagent positive) and nonglycoproteins (anthrone reagent negative) by employing the methods of Iscove et al. (1974) and Aspberg and Porath (1970). In fractionations by ion exchange chromatography employing DEAE Sephadex A-50, the methods of Cheng and Ouyang (1967), Ouyang et al. (1971), and Johnson and Sifford (1978) were used. Proteins were desalted by using Sephadex G-10 columns at 4°C. Sephadex G-75 and G-50 columns were used to separate PhL-A_2 from higher molecular weight molecules.

An immunizing schedule was prepared according to Ownby et al. (1979). Preimmune serum was obtained from approximately 12 month old New Zealand white rabbits. An immunizing dose was prepared by dissolving 17 mg of lyophilized *A. bilineatus* crude venom in 20 ml of sterile physiological saline. A 0.5 ml aliquot of this solution was then mixed with 0.5 ml of Freund's complete adjuvant. Injections of 0.5 ml then were made subcutaneously into each thigh. Booster injections were prepared by mixing 0.5 ml of *A. bilineatus* venom (0.8 mg/ml) and 0.5 ml of Freund's complete adjuvant. One week later, subcutaneous injections of 0.5 ml of the solution were made in each shoulder. Four weeks after the booster injections, antiserum via heart puncture was collected and stored at −20°C.

Rabbit antiserum for the purified phospholipase A_2 fraction was prepared by injecting an immunizing dose containing 0.3 ml of purified enzyme (0.1 mg/ml) and 1.0 ml Freund's complete adjuvant. Subcutaneous injections of 0.65 ml of this solution were made into each thigh. One week later, booster injections of the same dose were administered into each shoulder. Four weeks later, antiserum was collected and stored at −20°C.

Immunoelectrophoresis methods outlined by Campbell et al. (1963) and Garvey et al. (1977) were employed to determine PhL-A_2 purity. Dodecyl Sulfate-Polyacrylamide gel electrophoresis procedures of Weber and Osborn (1969) were used by Dr. Collis Geren (University of Arkansas at Fayetteville) to assay crude venom and fraction samples.

A. bilineatus crude venom contains nonglycoprotein and glycoprotein enzymes (Fig. 1). The larger nonglycoprotein fraction (Fraction I), comprising approximately 80% of the crude venom proteins, contained numerous enzyme activities. These included PhL-A_1, phosphomonoesterase, phosphodiesterase, 5'-nucleosidase, hyaluronidase, TAMEase, BAEEase, and L-amino acid oxidase activities.

Fraction I, obtained by Con A chromatography, was pooled, lyophilized, and then desalted with Sephadex G-10. Fractionation of this desalted nonglycoprotein fraction by ion exchange chromatography (DEAE Sephadex A-50) yielded three large fractions and several minor fractions. PhL-A_2 activity was concentrated in the second major fraction (Fig. 2). PhL-A_2 activity (14,000 units/mg) in this fraction was much higher than that of the crude venom (234 units/mg).

The PhL-A_1-containing fraction obtained by chromatography with DEAE Sephadex A-50 was divided into two samples. Even-numbered tubes (42 through 66) were pooled to from one sample while odd-numbered tubes (41 through 67) formed the other sample. These samples, after lyophilization and desalting, were fractionated with Sephadex G-75. In both instances, PhL-A_2 activity (24,400 units/mg) was observed only in the low molecular weight fraction (Fig. 3).

The low molecular weight PhL-A_2 fraction (tubes 16-22) obtained by Sephadex G-75 chromatography was pooled, lyophilized, desalted, and applied to a Sephadex G-50 column. This fractionation yielded a fraction free of the larger molecules (Fig. 4).

Immunoelectrophoresis and disc electrophoresis of crude venom samples indicated a complex mixture of proteins although the PhL-A_2 fraction obtained by the sequence of Con A-Sepharose 4B, DEAE A-50, Sephadex G-75, and Sephadex G-50 chromatography procedures was greatly purified (Figs. 5-8). Close examinations of both types of electrophoresis patterns, however, indicated trace contaminations. These contaminations could be due, in part, to TAMEase and L-amino acid oxidase since the distributions of these enzymes in *A. bilineatus* venom overlap PhL-A_2 after DEAE Sephadex A-50 chromatography (Sifford and Johnson, 1978). At present, work is directed toward purification of large amounts of PhL-A_2 in order that more enzyme characteristics may be obtained.

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

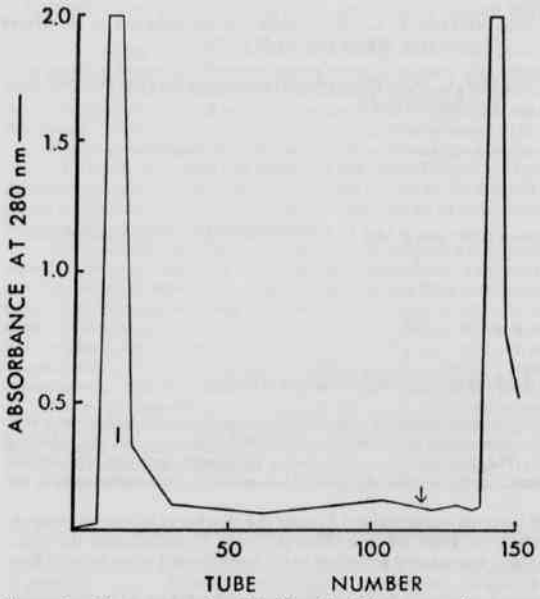

Figure 1. Chromatography of *Agkistrodon bilineatus* crude venom (450 mg) on a Concanavalin A Sepharose 4B column (2.5 × 15 cm) at 4°C by two stage elution. The arrow indicates the start of the second state elution (using α-methyl-D-mannoside). Eluates were collected at a flow rate of 17 ml/hr.

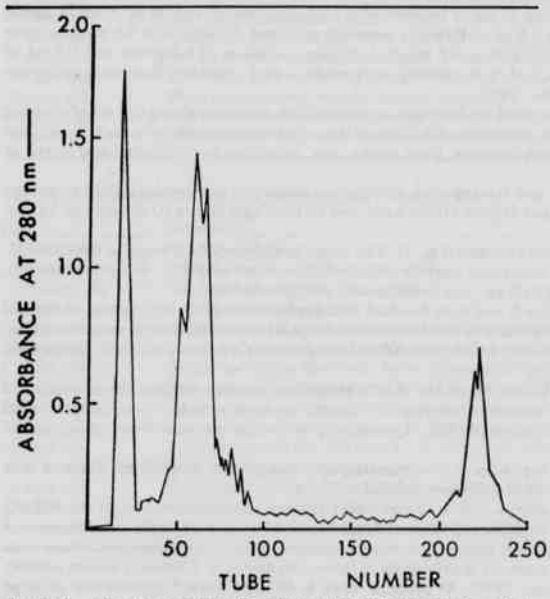

Figure 2. Chromatography of *Agkistrodon bilineatus* venom nonglycoproteins on a DEAE Sephadex A-50 column (2.5 × 56 cm) at 4°C by two stage elution with ammonium acetate buffer. Eluates of 5.0 ml each were collected at a flow rate of 17 ml/hr.

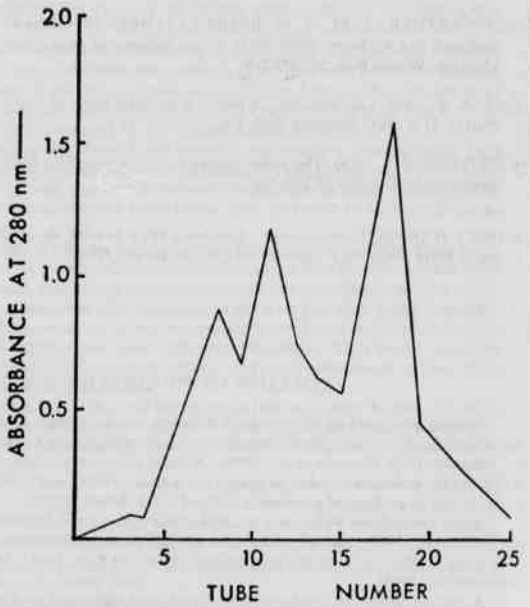

Figure 3. Chromatography of the phospholipase A_2 containing fraction, collected from DEAE A-50 ion exchange chromatography, on a Sephadex G-75 column (1 × 100 cm) at 4°C. Eluates of 4.0 ml/tube were collected.

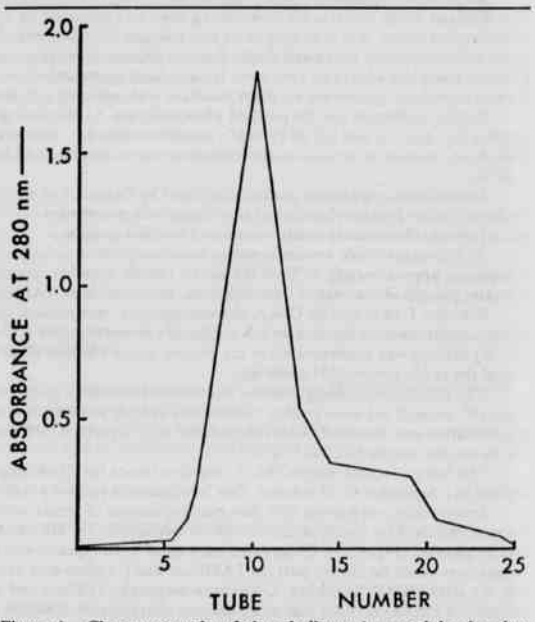

Figure 4. Chromatography of phospholipase A_2 containing fraction, from Sephadex G-75 chromatography, on a Sephadex G-50 column (1 × 100 cm) at 4°C. Eluates of 4.0 ml/tube were collected.

Figure 5. Immunoelectrophoretic patterns obtained by reacting anti-crude *Agkistrodon bilineatus* venom serum with electrophoretically separated crude venom.

Figure 6. Immunoelectrophoretic patterns obtained by reacting anti-crude *Agkistrodon bilineatus* venom serum with electrophoretically separated phospholipase A_2 containing fraction and crude venom.

Figure 7. Immunoelectrophoretic patterns obtained by reacting anti-phospholipase A_2 fraction serum with electrophoretically separated phospholipase A_2 containing fraction and *Agkistrodon bilineatus* crude venom.

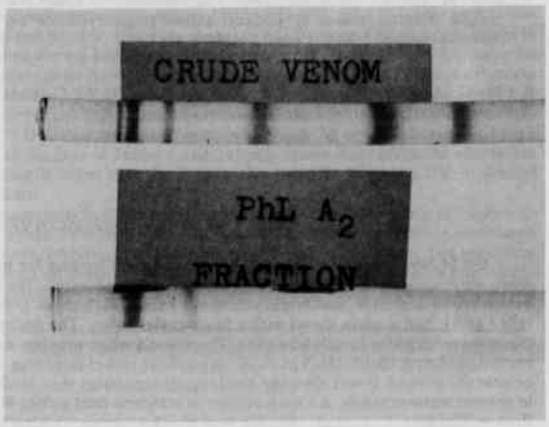

Figure 8. Patterns obtained by Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis of *Agkistrodon bilineatus* crude venom and the phospholipase A_2 containing fraction.

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SAGE THRASHER *(OREOSCOPTES MONTANUS)*, A NEW STATE RECORD

On 24 November 1979, Cheryl Lavers and I were looking for birds in the Farville area, about 6 miles NE of Jonesboro, Craighead County, when we discovered a Sage Thrasher *(Oreoscoptes montanus)*. The bird was rather Mockingbird-like *(Mimus polyglottos)* in shape, plain gray-brown crown and back, and bright yellow eye with a black pupil. Other characters differed from a Mockingbird in that it was smaller and shorter tailed and it had a white throat with a black malar stripe. The underparts, with a ground color of warm buff, were densely streaked with black chevrons arranged in length-wise rows. There was a white wingbar, and a dark tail with white outer corners. The bill was slender, very slightly de-curved, and dark bluish-black in color, as were the rather short legs. The bird ran along the ground in open places, or under brush and stayed on or near the ground. It was observed catching, decapitating, then swallowing ground crickets (*Acheta* sp.). We have both observed Sage Thrashers in several western states. An examination of standard field guides (Peterson, R. T. 1961. A field guide to western birds. Houghton Mifflin Co., Boston; Robbins, C. S. et al. 1966. Birds of North America. Golden Press, N.Y.) further confirmed our identification. A description and slides of the bird have been sent to Charles Mills, Curator, the Arkansas Audubon Society, and Dr. Douglas James, Department of Zoology, University of Arkansas at Fayetteville. This is a first recorded instance of the Sage Thrasher in Arkansas.

In its normal range, the Sage Thrasher breeds in the sagebrush *(Artemisia)* deserts of western North America, coming as far east as western Oklahoma. It winters in the southern part of its range and Mexico, occurring as far east as central and southern Texas, with a small isolated winter colony in extreme southwestern Louisiana (A.O.U. check-list of North American birds, 1957). The species is seldom recorded as a vagrant. The following extralimital occurrences to the east of its range have been recorded: Florida, 2; Illinois, 2; Maryland, 1; New York, 2; North Carolina, 1; South Dakota, 1.

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SOME EFFECTS OF METHYL GREEN ON EXPRESSION OF DAMAGE INDUCED IN G1 *XENOPUS* CELLS BY ULTRAVIOLET LIGHT

Methyl green shows a high degree of specificity for DNA and is a component of many nuclear stains. Kurnick (1952) observed that this basic dye was readily bound by polymerized DNA; two amino groups of the dye binding to two phosphoric acid groups of DNA. Errera (1951) demonstrated that the affinity of DNA for methyl green is influenced by radiation and other agents which depolymerize DNA, or alter its internal configuration. Doudney and Haas (1958) showed that methyl green significantly influenced metabolic activities, such as DNA and RNA synthesis, in *Escherichia coli*. These results suggested that appropriate experiments, involving methyl green treatments coupled with germicidal UV exposures, might aid in describing the expression and repair of UV-induced lethal and mutational damage in prokaryotic cells. Experiments of this nature were carried out by investigators, such as Witkins (1961), which led to the notion that UV induces lesions in bacterial DNA that either are removed by repair systems or are converted to permanent structural changes during the first DNA synthetic period following the exposure. We report here an extension of such experiments to eukaryotic cells, in which some effects of methyl green on the repair of UV (254 nm)-induced damage in G1 phase *Xenopus* cells are examined.

Routine maintenance of log phase A8W243 *Xenopus* cultures, incubations, cell synchronizations, irradiations, mitotic index determinations, survival determinations (colony counts), and chromosome analysis have been described in detail (Griggs and Bender, 1973; Payne and Griggs, 1977; Griggs and Orr, 1979).

The basic dye used, methyl green, was obtained from Difco.

Figure 1 shows results of a series of mitotic index experiments performed to examine the effects of methyl green on progression of UV irradiated G1 cells through interphase and the first succeeding mitosis, (M1). These data described the appropriate time intervals for collection of the sets of mitotic cells analyzed for effects of methyl green on UV-induced aberration production (Table 1). Concentrations of the dye in the range 0.0 - 0.003 g/1 appeared to induce little delay in progression of the irradiated cells above that induced by the UV alone. The similarity in average height and width of these mitotic peaks indicated that the dye did not significantly reduce the number of irradiated cells that reached G1.

The average cell cycle for non-irradiated *Xenopus* cells was approximately 26 hours (not shown); eight hours G1, 13 hours S, three hours G2, and two hours M1. Payne and Griggs (1977) carried out autoradiographic studies which indicated that early G1 phase cells, exposed to low doses of UV (0 - 150 ergs mm^{-2} range), are not delayed in their progression through G1, but experience rather lengthy S phase delays. These facts, coupled with the data of Figure 1 and Table 1, indicate that chromatid aberration frequencies, resulting from UV exposure of early G1 cells, are significantly altered by methyl green only when the dye is in contact with the exposed cells as they pass through early S phase. Some relationship between the aberrant intracellular mechanism, by which methyl green augments chromatid type aberration production, and DNA synthesis is suggested by the fact that both mechanisms appear to function with peak efficiency in early S phase.

The data of Figure 2 indicate that a methyl green concentration of 0.003 g/1 has virtually no effect on the expression of UV-induced lethal damage, no matter where in the cell cycle the dye is applied. These data suggest that the mechanism which expresses UV-induced aberrational damage in *Xenopus* cells differs significantly from the mechanism which expresses lethal damage, supporting results of previous studies of the overlap of UV-induced lethal and aberrational lesions in *Xenopus* cells (Griggs and Orr, 1979; Payne and Griggs, 1977).