

Immunologic comparison of phospholipases A present in Hymenoptera insect venoms

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A comparative study was made of the effect of anti-bee venom phospholipase A₂ serum and anti-bald face hornet venom serum on the activity of phospholipase A₂ present in yellow hornet, yellow jacket, bald face hornet, and honeybee venoms. Activity of phospholipase A₂ present in the venoms was measured titrimetrically using egg yolk dispersion as the substrate. Antiserum against pure bee venom phospholipase A₂ activity completely suppressed bee venom phospholipase A₂ activity but failed to inhibit phospholipase A₂ activity in yellow jacket, yellow hornet, and bald face hornet venoms. Anti-bald face hornet venom serum suppressed the activity of phospholipase A₂ present in the four insect venoms with the inhibitory effect decreasing in the order: bald face hornet > yellow hornet > yellow jacket > honeybee. A combination of immunoelectrophoresis and immunodiffusion techniques gave indication of common antigenic sites in a component of yellow hornet venom and a component of bald face hornet venom. The results suggest that IgG antibodies against pure bee venom phospholipase A₂ may not give any protection against the reaction(s) due to the phospholipase A₂ in yellow hornet, yellow jacket, and bald face hornet venoms, while antibodies against bald face hornet venom phospholipase A₂ may give some cross-protection.

Hymenoptera venoms are concentrated mixtures of pharmacologically and biochemically active agents. The action of these venoms in man has been the subject of many studies due to the serious allergic reactions caused by the venom components.¹⁻¹⁵ Many persons showed severe, even fatal bee sting reactions.¹⁶ The deaths caused by stinging insects far exceed the deaths from snake bites.¹⁷ The types of pathology responsible for these reactions are reported in the literature.¹⁸⁻²⁴ Barr²⁵ has reviewed the types of reaction, immunologic studies, characteristics of Hymenoptera venoms, and treatments of stings.

Besides a series of toxic peptides, Hymenoptera venoms generally contain such enzymes as hyaluronidase, phospholipase A, and phospholipase B.²⁶ Bee venom phospholipase A has been isolated,^{27, 28} well characterized,²⁹ and se-

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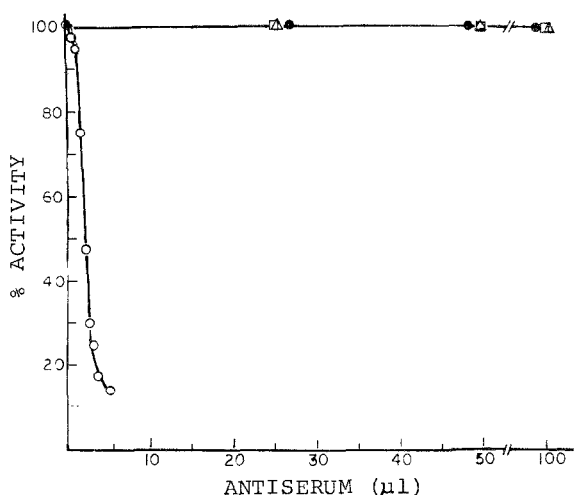


FIG. 1. Effect of antiserum against bee venom phospholipase A_2 on the activity of phospholipase A_2 present in the venom of the: ○, honeybee; □, yellow hornet; △, yellow jacket; ●, bald face hornet.

quenced.²⁷ Phospholipase A_2 is capable of producing a series of pharmacologic effects,^{30, 31} such as contraction of smooth muscles, destruction of mast cells, and lowering of the blood pressure with marked tachyphylaxis. This enzyme has been shown to be the most lethal component of bee venom, causing respiratory paralysis.³² Recently, several authors have reported that phospholipase A_2 is a major allergen of bee venom,³³⁻³⁵ but no studies have been made with phospholipase A_2 from other Hymenoptera venoms.

Although many workers^{2, 8, 36} have suggested that there are antigens common to the venom in whole body extracts of some members of Hymenoptera, a significant difference in the antigenic characters of pure venom, venom sac extracts, and whole insect extracts has been shown by O'Connor and Erickson.³⁷ Lichtenstein and associates³⁸ have preferred the use of appropriate Hymenoptera venoms over whole body extracts for the immunization against venom sensitivity because they believe that the antigens usually responsible for systemic reactions are the venom components and the antibody produced by whole body extracts would not give sufficient protection against all antigenic venom components. A comparative study of binding to reaginic antibodies of bee venom and bee venom phospholipase A_2 shows that the phospholipase A_2 is one of the main components binding to the reaginic antibodies.³⁹ Mellitin has also been reported as a possibly important allergic component in honeybee venom.⁴⁰ Hyaluronidase has also been shown to be a potent allergen^{34, 41, 42} (RAST inhibition studies with highly purified hyaluronidase will be reported elsewhere). Therefore, a study of the antigenic properties and immunologic comparison of individual venom components would be of great importance.

Antigenic properties of bee venom phospholipase A_2 have been shown by several workers.⁴¹⁻⁴³ A quantitative comparison of inhibition of phospholipase A_2 activity by sera of normal men and beekeepers has been made by Mohammed and

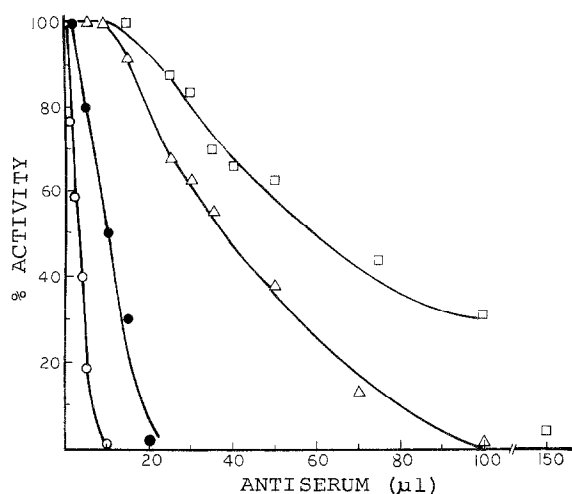


FIG. 2. Effect of antiserum against bald face hornet venom on the activity of phospholipase A_2 present in the venom of the: $\circ$, bald face hornet; $\bullet$, yellow hornet; Δ , yellow jacket; $\square$, honeybee.

El Karemi.⁴⁴ IgG antibodies against phospholipase A_2 have been found in beekeepers' sera and a desensitized patient's serum.³⁸ Very little is known about the immunologic comparison of phospholipase A present in various Hymenoptera insects. We have observed a complete inhibition of bee venom phospholipase A_2 activity by the serum of a rabbit immunized with pure bee venom phospholipase A_2 . This observation prompted us to carry out a comparative study on the inhibitory effect of homologous and heterologous antisera on the activity of phospholipase A present in various stinging insect venoms.

MATERIALS AND METHODS

Venoms of the following species were used in this work: (1) Honeybee (*Apis mellifera*), (2) yellow hornet (*Vespula arenaria*), (3) yellow jacket (*Vespula vulgaris*),* and (4) bald face hornet (*Vespula maculata*).

Bee venom was purchased from Charles Mraz. The venoms of yellow jacket, yellow hornet, and bald face hornet were milked from the insects in the field using the electrical stimulation method of Benton and associates.⁴⁵ Antiserum to bald face hornet venom was prepared in a rabbit. Bee venom phospholipase A_2 , prepared by the method of Shepherd and associates²⁸ was used to produce monospecific antiserum in rabbits. All other chemicals used were analytical grade reagents.

Inhibition study

Varying amounts of antiserum were added to aliquots of enzyme or crude venom having a standard total amount (0.375 μ moles of fatty acid released per minute) of phospholipase A_2 and the mixture was incubated at 37° C for 15 min. The activity measurement of each mixture was carried out in a microreaction vessel by automatic titration of the fatty acid liberated with 0.005 M NaOH using Radiometer (Copenhagen) pH stat model TT11/SBR2/ABU12/TTA31 at temperature 37° C and pH 8.0. The method of de Haas and associates⁴⁶ was followed

*About 80% of yellow jackets found in the Buffalo area are *Vespula vulgaris* or a *vulgaris* variant, showing close similarity with *V. maculifrons*.

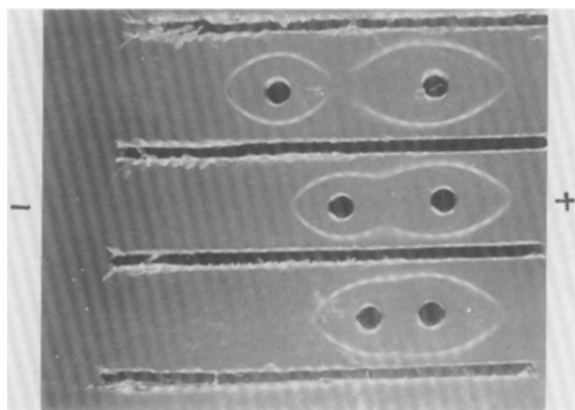


FIG. 3. Combination of immunodiffusion and immunoelectrophoresis. Identification of the precipitin arc is due to phospholipase A_2 in bee venom. The wells on the right contained crude honeybee venom which was electrophoresed at 20 mA for 30 min. The wells on the left contained pure bee venom phospholipase A_2 . The troughs contain an antiserum against bee venom phospholipase A_2 prepared in a rabbit.

for the assay. Crude egg yolk was used as the substrate. One egg yolk, washed several times in order to remove the white completely, was dispersed in 200 ml of double-distilled water using a Waring blender at high speed for 60 seconds. Ten milliliters of the assay medium contained 5.0 ml of the egg yolk dispersion, 1.3×10^{-3} M deoxycholate, 3×10^{-3} M CaCl_2 , and 0.5% bovine serum albumin.

Immunodiffusion

The technique of Heremans,⁴⁷ a combination of immunoelectrophoresis and immunodiffusion, was used in order to determine components showing lines of identity. By varying the distance between two wells containing the components (antigens), the various lines of identity or partial identity between the precipitin lines of two venom components could be established. Agarose (2%) gel, prepared in 0.03 M barbiturate buffer (pH 8.2), was poured on glass plates. Three wells were cut at a standard distance from the edge of the plate and equal volumes of venom or phospholipase A_2 were applied in respective wells. The electrophoresis was done in 0.03 M barbiturate (Na^+) buffer (pH 8.2), using a flatbed (Shandon) apparatus. Two strips of Whatman No. 1 filter paper were used to bridge the gel with the buffer in the electrode compartments. The time and current (ma/running tube) used in each experiment varied. After electrophoresis, troughs were cut on both sides of each well and antiserum was applied. New wells were cut at varying distances from the original wells. A standard amount of pure bee venom phospholipase A_2 , 5 μl of 2.0 mg/ml protein solution (specific activity of 312 μmoles of fatty acid released/mg of protein/min), was applied in each of the new wells and the agarose plate was placed in a humidor overnight for the diffusion to take place.

RESULTS

Anti-bee venom phospholipase A_2 serum completely inhibited the activity of bee venom phospholipase A_2 (Fig. 1). The same antiserum did not show a detectable inhibitory effect on the activity of the phospholipases A_2 present in yellow hornet, yellow jacket, and bald face hornet venoms even when 50-fold of the amount required to give 50% inhibition of the same quantity of bee venom phospholipase A_2 activity was added. Anti-bald face hornet serum suppressed the phospholipase A_2 activity of the four venoms and the effects were measured

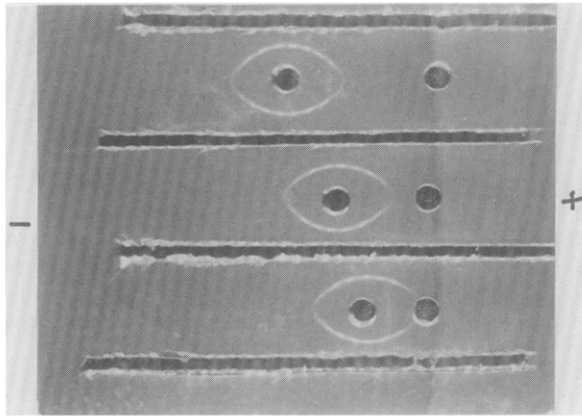


FIG. 4. Diffusion of an antiserum against bee venom phospholipase A_2 and yellow jacket venom. The wells on the right contained crude venom of the yellow jacket. All other conditions are as in Fig. 3. Note no perturbation of the bee venom phospholipase A_2 precipitin arc occurred with yellow jacket venom.

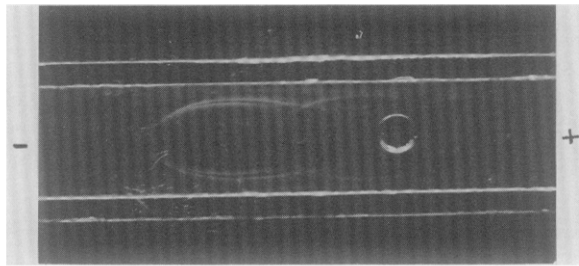


FIG. 5. Immunoelectrophoretic pattern of bald face hornet venom. The trough contains an antiserum against bald face hornet venom prepared in a rabbit. The electrophoresis was done at 24 mA for 45 min. Note at least seven precipitin arcs.

(Fig. 2). The amount of antisera required to inhibit 50% of the phospholipase A_2 activity varied with the species, increasing in the order: bald face hornet > yellow hornet > yellow jacket > honeybee. Three microliters of anti-bald face hornet venom serum reduced the activity of bald face hornet phospholipase A_2 to 50% while 21-fold more of the same antiserum was needed for 50% reduction of bee venom phospholipase A_2 activity.

A clear line of identity was observed between the precipitin lines of purified bee venom phospholipase A_2 and crude bee venom when they were allowed to diffuse against the specific antiserum to bee venom phospholipase A_2 (Fig. 3). Specific antiserum to bee venom phospholipase A_2 , diffused against crude yellow jacket venom, having pure bee venom phospholipase A_2 in the adjoining wells, gave no precipitin lines (Fig. 4). Crude venoms of yellow hornet and bald face hornet also did not show any precipitin lines when diffused against specific antiserum to bee venom phospholipase A_2 .

Immunoelectrophoresis of bald face hornet venom against antiserum to bald face hornet venom was done in order to determine the antigenic properties of the

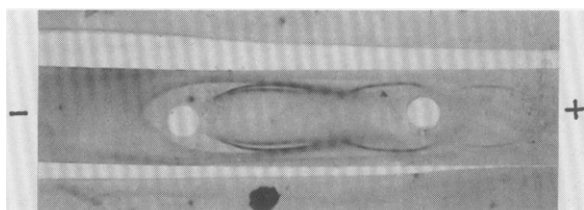


FIG. 6. Combination of immunodiffusion and immunoelectrophoresis. Well on the right contains bald face hornet venom. Well on the left contains pure bee venom phospholipase A_2 . The troughs contain anti-bald face hornet venom serum from a rabbit. Conditions of electrophoresis are as in Fig. 5. Note no precipitin line was formed with the pure bee venom phospholipase A_2 .

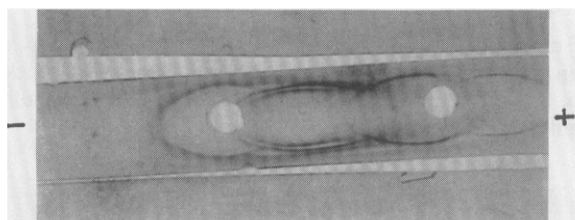


FIG. 7. Indication of common antigenic sites present in a component in yellow hornet venom and one of the components of bald face hornet venom. Left well contains yellow hornet venom serum from a rabbit. Right well contains bald face hornet venom. Electrophoresis of bald face hornet venom was performed at 24 mA for 45 min.

whole venom and at least seven precipitation arcs were formed, six of them moving in the direction of the cathode and one toward the anode (Fig. 5). No precipitin line formed between the antiserum to bald face hornet and bee venom phospholipase A_2 (Fig. 6). Indications of common antigenic sites in a component of yellow hornet venom and a component of bald face hornet venom could be seen (Fig. 7).

DISCUSSION

Studies based on whole insect extracts⁹ indicate that wasps and yellow jackets have two antigens in common while honeybees show one antigen in common with wasps and yellow jackets. Foubert and Stier² reported that animals sensitized to bee antigen demonstrated the least degree of heterologous sensitization. Settupane and Hopson⁴⁸ have suggested that honeybee venom is immunologically different from other Hymenoptera insect venoms. The results here show that a species-specific antiserum against bee venom phospholipase A_2 failed to inhibit the activity of phospholipase A_2 in venoms of yellow jacket, yellow hornet, and bald face hornet. This observation supports the concept that honeybee antigens are immunologically different from those of other insects. The results of immunodiffusion studies showed no formation of precipitation lines with venoms of yellow jacket, yellow hornet, and bald face hornet when diffused against an antiserum to bee venom phospholipase A_2 , supporting the results of inhibition studies. The slight inhibitory effect of antiserum against bald face hornet venom on the

activity of bee venom phospholipase A₂ also shows the immunologic difference of honeybee (apiid) venom from vespid venoms.

It is observed (Figs. 1 and 2) that the amount of antiserum required for 50% inhibition of phospholipase A₂ activity varied with the species. Studies on the action of antisera on homologous and heterologous snake venom phospholipase A activity shows such variations even in the closely related species of the same genus.⁴⁹ The variation in the amount of antiserum for 50% inhibition, or the failure of an antiserum to inhibit heterologous phospholipase A activity may be due to the species-specific variations in the antigenic sites of the enzyme molecule. This antigenic specificity of the various species is of considerable importance in the immunotherapy of systemic reactions. In the diagnosis and treatment of bee sting allergy, pure venom has been shown superior to whole body extracts³⁸ and the use of phospholipase A in following the therapy of honeybee venom-sensitive patients has also been suggested.⁵⁰ It is expected that immunization with a pure venom component would be more effective than a venom treatment in raising an antiserum against a particular component. However, it may be of greater importance to determine the degree of protection an antibody produced against phospholipase A present in one venom can give against possible allergic reactions due to the phospholipases A in venoms of other Hymenoptera. The results here suggest that the antibody to bee venom phospholipase A₂ may not give protection against the reaction caused by phospholipase A₂ present in vespid venoms. Although the antibody against phospholipase A₂ raised by immunization with bald face hornet (a vespid) venom can suppress the activity of phospholipase A present in other vespid and apiid (honeybee) venoms, its capacity to inhibit phospholipase activity of venoms decreased in the order: bald face hornet > yellow hornet > yellow jacket > honeybee. Our results suggest that a very high concentration of antibody to bald face hornet venom phospholipase A may give some protection against the reactions due to honeybee venom phospholipase A₂. The results also show that the antibody to bald face hornet venom phospholipase A may give more effective protection against yellow hornet venom phospholipase A₂ reactions than to reactions caused by yellow jacket phospholipase A₂. A real understanding of the venom action will be possible only if the properties of all its components are known. Components as yet unsuspected may be causing some of the serious reactions.

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