

Allergens in bee venom

III. Identification of allergen B of bee venom as an acid phosphatase

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Allergen B previously isolated from honeybee venom and shown to be a mildly acidic protein consisting of polymers of a chain of 49,000 d is shown to have acid phosphatase activity. Allergen B is homogeneous by several criteria. No acid phosphatase, alkaline phosphatase, or esterase activity was found in any other allergen or fraction of bee venom. Acid phosphatase activity was also found in yellow jacket venom and extracts of venom sacs from bumblebees and paper wasps.

Five distinct allergens have been identified and isolated from honeybee venom. The first allergen identified was phospholipase A¹; later hyaluronidase,² melittin,² allergen B,³ and allergen C³ were found to have significant allergenic activity. Since these five substances make up virtually all of the protein part of bee venom and since the smaller molecular weight substances such as apamin appear to have much less, if any, allergenic activity,^{2, 4} these substances should account for almost all of the allergenic activity of the venom. In the near future it should be possible to have completely standardized preparations of venom available, since the concentration and native state of each of the components can be measured by immunologic and biochemical methods. In this study, it is shown that allergen B is an enzyme with acid phosphatase activity providing an additional criterion for evaluating bee venom preparations to be used for diagnosis and therapy.

MATERIALS AND METHODS

Bee venom and allergens

Bee venom was obtained by electrical stimulation and purchased from Champlain Valley Apiaries, Middlebury, Vt. Venoms collected in 1971, 1974, 1975, and 1976 were tested. An additional sample of bee venom was purchased from the Sigma Chemical Co. Bee venom sacs were obtained from Davis Insect Venoms, Oshawa Ontario, and

were extracted by crushing with a glass rod in water. All of the bee venom preparations gave similar gel filtration patterns and all the venoms and the venom sac extract contained the known enzyme activities of bee venom. Hyaluronidase, phospholipase A, and melittin were prepared by gel filtration on Sephadex G-75 and ion exchange on carboxymethyl Sephadex-25.^{2, 5} Allergens B and C were prepared by gel filtration on Sephadex G-200, isoelectric focusing, and solid-phase immunoadsorption with the use of guinea pig anti-allergen B coupled to CNBr-activated Sepharose.³

Yellow jacket venom obtained by electrical stimulation and yellow jacket, paper wasp, and bumblebee venom sacs were purchased from Davis Insect Venoms.

Antisera

Antisera to the various antigens were prepared by multiple intradermal injections in Freund's complete adjuvant. Both New Zealand white rabbits and DHC/BA inbred guinea pigs were used.

Hyaluronidase assay

Hyaluronidase was determined with the use of Worthington Biochemical Co. radial diffusion plates. A secondary standard was used to determine National Formulary units.

Acid phosphatase

Acid phosphatase was determined by hydrolysis of 1 mg/ml p-nitrophenylphosphate in 0.1 M sodium citrate-0.001 M MgCl₂, pH 5.0. Alkaline phosphatase was determined in 0.1 M NaHCO₃-0.001 M MgCl₂, pH 9.8. Gels were stained for acid phosphatase by the method of Allen and associates.⁶

Other procedures

The procedures for assaying for phospholipase A and melittin were previously described.² The methods for

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radioallergosorbent test (RAST), inhibition of RAST, polyacrylamide gel electrophoresis (PAGE), sodium dodecyl sulfate (SDS) electrophoresis, and isoelectric focusing were described by Hoffman and Shipman² and Hoffman and co-workers.³ The criteria for selection of allergic sera were previously elaborated.^{2, 3}

RESULTS

Allergen B, a substance consisting of polymers of a polypeptide chain of 49,000 d, was isolated from bee venom and shown to be a homogeneous preparation by immunodiffusion with rabbit and guinea pig antisera, PAGE, SDS-PAGE, and isoelectric focusing. These data were presented by Hoffman and co-workers.³ All of the various fractions and purified allergens previously isolated from bee venom^{2, 3} were tested for acid phosphatase, alkaline phosphatase, and nonspecific esterase activity. None of the fractions showed any strong alkaline phosphatase or esterase-hydrolyzing α -naphthyl acetate activities. Acid phosphatase activity was found only in allergen B and G-75 fraction 1, which contains allergen B. No acid phosphatase activity was found in allergen C. Fig. 1 illustrates an immunodiffusion experiment showing the precipitin bands and acid phosphatase stain for fraction 1, allergens B and C, and hyaluronidase. Only allergen B stains for acid phosphatase. The quantitative results of testing the various allergens and fractions are shown in Table I.

Acid phosphatase activity was found in bee venom collected by one supplier in each of four different years, in venoms from another commercial source, and in extracts of bee venom sacs obtained from a third source.

Yellow jacket venom and extracts of venom sacs from yellow jackets (*Vespula spp.*), bumblebees (*Bombus spp.*), and paper wasps (*Polistes exclamans*) were all tested for acid phosphatase and all found to be positive, as is shown in Table II.

The acid phosphatase activity of allergen B was not inhibited by rabbit or guinea pig antibodies raised against it, but absorption of a mixture of allergen B and C with insoluble anti-B completely removed acid phosphatase activity (Table I).

DISCUSSION

Allergen B of honeybee venom is shown to possess substantial acid phosphatase activity. Thus this allergen along with phospholipase A and hyaluronidase can be assayed by biochemical means. Since both hyaluronidase^{2, 7} and allergen B³ are somewhat labile, assaying their activity levels may provide a means for evaluating the state of denaturation of the venom preparation. Biochemical assays are much easier to reproduce in various laboratories than immunologic

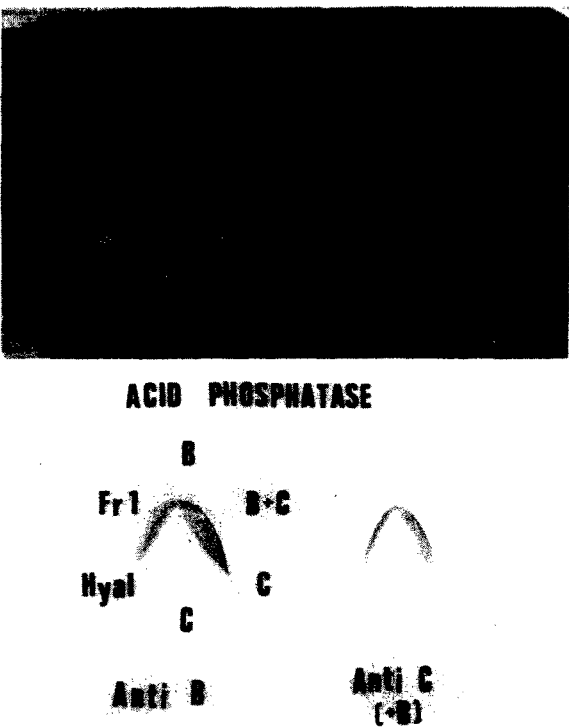


FIG. 1. Immunodiffusion of bee venom G-75 fraction 1, allergens B and C, and hyaluronidase with guinea pig antisera against allergen B (left) and allergen C containing a small amount of anti-B (right). The patterns above show the precipitate lines and those below are the same experiment stained for acid phosphatase. Only the B-anti-B lines stain for acid phosphatase.

TABLE I. Acid phosphatase activity in bee venom fractions

Parameter	$\mu\text{M units/OD}_{280}^*$
Allergen B	2.0
Allergen C	0.00
Hyaluronidase	0.00
Phospholipase A	0.00
Melittin	0.00
Fraction 2 (G-75)	0.00
Fraction 3 (G-75)	0.00
Allergen B + C	1.2
Allergen B + C absorbed insoluble anti-B	0.00

*Optical density per centimeter at 280 nm.

assays and do not require standardization of human and animal antisera. Four of the five known allergens of bee venom can now be determined by simple, reliable, and reproducible methods. Only allergen C remains with no known biologic activity.

The finding of acid phosphatase activity, along with phospholipase⁸ and hyaluronidase (manuscript in preparation) activities in venoms from *Bombini* and *Vespoidea* indicate that there may be a biochemical as

TABLE II. Acid phosphatase activity in insect venoms*

Venom	Concentration	$\Delta OD_{410}/10 \text{ min}$
Yellow jacket	34 OD ₂₈₀ /ml	0.13
Yellow jacket sac extract	10 sac/ml	0.06
Paper wasp sac extract	10 sac/ml	0.09
Bumblebee sac extract	5 sac/ml	0.20

*Each extract, 10 μ l, added to 1 ml of substrate and buffer.

well as an immunologic relation between Hymenoptera venoms.

It should be possible in the near future to have completely standardized preparations of bee venom and its constituent allergens for both diagnosis and immunotherapy.

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