Digestive proteases in four species of Polistes wasps

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Adults of Polistes exclamans, Polistes metricus, Polistes fuscatus, and Polistes annularis (Hymenoptera: Vespidae) were examined for digestive protease activity. All samples analysed were found to contain chymotryptic-like, trypsin-like, 'carboxypeptidase-A-like,' and 'carboxypeptidase-B-like' activities. The wasp chymotryptic enzyme has a serine and histidine in its active center and a specificity for aromatic amino acids and in these respects is similar to bovine chymotrypsin. The trypsin-like enzyme does not react like its bovine homologue, though low concentrations of this enzyme and the limit of sensitivity of the spectrophotometer may account for the differences. Enzyme comparisons are made between these Polistes species and three Vespid species.

Introduction

In colonies of social wasps the larvae are fed solid food (insect larvae or soft-bodied arthropods) by an adult female. In return, the larvae secrete a drop of salivary fluid which is subsequently swallowed by an adult (Maschwitz 1966). This phenomenon of fluid exchange between members of a colony has been termed trophallaxis (Wheeler 1918). Maschwitz (1966) determined that this secretion is both attractive and nourishing, containing carbohydrates in concentrations approximately four times that found in larval haemolymph. Amino acids and proteins are also present but at one-fifth their haemolymph concentration. One drop of this salivary gland secretion from a very large larva is sufficient to keep an adult alive for half a day (Maschwitz 1966). When a colony of Vespa orientalis was deprived of access to larval secretions, the queen died within a few days (Ishay and Ikan 1968).

The work of Ikan et al. (1968) indicated that the larvae of Vespa orientalis are the only members of the colony capable of protein digestion. However, proteolytic activity has since been identified in the midguts of both larvae and adults and in the larval saliva of Paravespula germanica by Spradbery (1973) and in the midguts of adult castes of Vespula germanica and Vespula maculifrons by Grogan and Hunt (1977).

The question of wasp nutrition appears to be a complicated one. Wasps are said to forage for two distinct food types: carbohydrates for themselves and protein for their larvae (Ikan and Ishay 1966). Floral nectars (Baker and Baker 1973, 1975), honeydew from Homoptera (Ehrhardt 1962), and insect haemolymph (Wyatt 1961) all of which are foods of adult social Hymenoptera, contain amino acids as well as carbohydrates. The larval saliva of social Vespidae contains sugars and free amino acids and so resembles these food sources (J. H. Hunt, personal communication). These readily available sources of free amino acids suggest that protein may be nonessential for adults.

There are indications, however, that protein in the form of solid food may be regularly ingested. In some species, cannibalism of larvae and pupae is not uncommon (Wilson 1971). In temperate zone Polistes, the dominant female devours eggs of subordinate females to assure that only her brood will be reared (Gervet 1964; Eberhard 1969). Bits of insect prey are malaxed by adults before being offered to larvae; whether adults are ingesting por-
tions of this food has not been determined. Indeed, whether the structure of the *Polistes* digestive system is capable of dealing with solid particles is uncertain (e.g. Eisner 1957).

**Objectives**

The primary objective of this research was to survey *Polistes* species commonly encountered in the St. Louis, Missouri, area for the presence and activity of digestive proteases. Differential abundance patterns of the enzymes between sexes were quantified. The enzymes were characterized in terms of their cleavage specificities in order to compare them with mammalian protease enzymes.

**Materials and Methods**

**Collection**

Colonies of *Polistes exclamans*, *Polistes fuscatus*, *Polistes metricus*, and *Polistes annularis* were collected during the late summer of 1976. Males were separated from females; no external morphological features differentiate queens from workers. Adults that eclosed from nests in the lab were held in isolation and their midgut contents examined separately.

**Nonspecific Protease Determination**

Wasps, held at −10°C for several minutes, were placed under a dissecting microscope and their abdomens were cut open dorsally to expose their midguts. The tip of a tuberculin syringe was inserted into the lumen of the extended midgut, and its contents were removed. The extract was diluted to 100 µl with phosphate buffer (pH 7.0) and held at 4°C for immediate analysis. Larval salivary secretions were collected by prodding the mandibular area of larvae with a capillary tube. Protease activity in such samples was assayed with Azocoll by modifying the procedure outlined by Calbiochem, La Jolla, CA, document 10-1455. The samples to be analysed were diluted to 0.5 ml in 0.1 M phosphate buffer (pH 7.5) with 500 mM NaCl. Absorbancy measurements were made at 254 nm and units of activity were defined as number of micromoles of substrate hydrolyzed per minute at pH 7.5 and 25°C (Folk and Shirmer 1963).

**Trypsin-like Determination**

Midguts were removed from live wasps by grasping the apex of the abdomen and pulling gently. The entire digestive tract was extracted as above. The reaction tubes containing 2.9 ml freshly prepared substrate of 0.5 mM hippuryl-L-arginine (HA) in 25 mM Tris–HCl buffer (pH 7.65) with 100 mM NaCl (Folk et al. 1960). Unit of activity was defined as number of micromoles hydrolyzed per minute at pH 7.65 and 25°C.

**Chymotryptic Determination**

Midguts were extracted as above. The reaction tubes contained 1 mM benzoyl-L-tyrosine ethyl ester (BTEE) in 50% methanol (w/w) to a final concentration of 0.5 mM. The rate of hydrolysis of BTEE is determined from the change in absorbancy at 256 nm. One unit is equivalent to 1 pmol of substrate hydrolyzed per minute at pH 7.8 and 25°C (Hummel 1959).

**Carboxypeptidase-A-like Determination**

Midguts were extracted in 10% LiCl by the methods described for trypsin. One-tenth millilitre enzyme extract was added to reaction tubes containing 2.9 ml freshly prepared substrate of 1 mM hippuryl-L-phenylalanine (HPA) in 25 mM Tris–HCl buffer (pH 7.5) with 500 mM NaCl. Absorbancy measurements were made at 254 nm and units of activity were defined as number of micromoles of substrate hydrolyzed per minute at pH 7.5 and 25°C (Folk and Shirmer 1963).

**Carboxypeptidase-B-like Determination**

Midgut extraction was as for carboxypeptidase A. One-tenth millilitre enzyme extract was added to 2.9 ml freshly prepared substrate containing 1 mM hippuryl-L-arginine (HA) in 25 mM Tris–HCl buffer (pH 7.65) with 100 mM NaCl (Folk et al. 1960). Unit of activity was defined as number of micromoles hydrolyzed per minute at pH 7.65 and 25°C.

**Enzyme Characterization**

Inhibitors of trypsin and chymotrypsin were added to the reaction mix: phenyl-methyl-sulfonyl fluoride (PMSF) (Gold 1965); N-α-p-tosyl-L-lysine chloromethyl ketone (TLCK) (Schoellman and Shaw 1963); and 1-1-tosylamine-2-phenyl-ethylchloro-methyl ketone (TPCK) (Shaw et al. 1965). One-tenth millilitre of inhibitor was added to a reaction tube to give a final concentration of 0.95 mM PMSF, 0.2 mM TLCK, or 0.2 mM TPCK. The enzyme was added to the buffer and preincubated for 20 min with PMSF and for 60 min with TLCK or TPCK prior to initiation of the reaction. In all cases where inhibitors were added, the reaction mix was decreased by 0.1 ml buffer to maintain a constant volume.

**Protein Determination**

Protein determination was carried out according to methods of Lowry et al. (1951).

**Results**

**Azocoll Protease Activity in Polistes spp.**

In the four species of *Polistes*, protease activity was found in the larval saliva and in the midgut extracts from both adults living in the active colonies and from adults that eclosed in the laboratory (Table 1). Crops of newly eclosed adults were found to contain fluid but no protease activity was detected in it. No protease activity was found in wing muscle (nondigestive tissue) that was used as a control for identification of possible lysosome activity.

**Digestive Protease Activity of Polistes Midguts**

Chymotryptic and tryptic activities were found in male and female *P. exclamans*, *P. metricus*, *P. fuscatus*, and unsexed samples of *P. annularis* (Table 2). The predominant enzyme was chymotrypsin-like. Chymotryptic activity exceeded trypic activ-
TABLE 1. General protease activity in larval and adult Polistes

<table>
<thead>
<tr>
<th>Species</th>
<th>Age</th>
<th>Location of activity</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. annularis</em></td>
<td>Larvae</td>
<td>Saliva</td>
<td>+</td>
</tr>
<tr>
<td>Adults</td>
<td>Midgut extract</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td><em>P. fuscatus</em></td>
<td>Larvae</td>
<td>Saliva</td>
<td>+</td>
</tr>
<tr>
<td>Adults*</td>
<td>Midgut</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Wing muscle</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Newly eclosed</td>
<td>Midgut</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Crop</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td><em>P. metricus</em></td>
<td>Larvae</td>
<td>Saliva</td>
<td>+</td>
</tr>
<tr>
<td>Adults</td>
<td>Midgut</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td><em>P. exclamans</em></td>
<td>Larvae</td>
<td>Saliva</td>
<td>+</td>
</tr>
<tr>
<td>Adults</td>
<td>Midgut</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Newly eclosed</td>
<td>Midgut</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Crop</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

*Adults as referred to above have been with the colony for their adult life.
Newly eclosed adults were isolated in the laboratory.

ity in the same species by from 8.9 to 15 times. It exceeded 'carboxypeptidase-A-like' activity by from 7.4 to 44.2 times and exceeded 'carboxypeptidase-B-like' activity from 11.3 to 22.3 times. Its specific activity in males was from 1.1 to 2.8 times greater than that for females. The reaction rate for the chymotryptic enzyme was linear for 5 min in male *P. exclamans* (Fig. 1A), 3 min for male *P. metricus* (Fig. 2A), and 2 min for *P. fuscatus* (Fig. 3A). The reaction rates for females of these three species were linear for 7 min (Figs. 1B, 2B, 3B).

The trypsin enzyme specific activities were low in all species (Table 2). Activity rates appear to be linear (Figs. 1C, 1D, 2C, 2D, 3C, 3D).

Very few midguts of *P. annularis* were available. Specific activity values were higher for the chymotrypsin-like enzyme than for the trypsin-like enzyme (Table 2).

Unsexed samples of *P. exclamans* possessed 'carboxypeptidase-A-like' activity (Table 3) that was between chymotryptic and trypsin activities in abundance. 'Carboxypeptidase-B-like' activity was also found in the midgut of unsexed *P. exclamans* but at very low concentrations (Table 3). 'Carboxypeptidase-A-like' specific activity in female *P. metricus* (Table 3) was only 16% of the chymotryptic specific activity noted in conspecific females, but it was greater than trypsin specific activity by a factor of 1.5. 'Carboxypeptidase-B-like' specific activity in that species (Table 3) was one-tenth that of chymotrypsin-like enzyme and about equal to that of the trypsin-like form. 'Carboxypeptidase-A-like' enzyme was studied in *P. fuscatus* males (Table 3). Amounts of 'carboxypeptidase-B-like' enzyme were so low that it was impossible to determine the content with any degree of accuracy.

Effects of Inhibitors on Endopeptidases from *P. metricus, P. exclamans, and P. fuscatus*

To compare wasp chymotryptic activity with bovine chymotrypsin, three inhibitors were used. PMSF inhibited the enzyme in all samples. The range of inhibition was from 90 to 84% for males during the period of linearity (Figs. 1A, 2A, 3A) and from 68 to 60% for females (Figs. 1B, 2B, 3B). These results indicate that the activity of the *Polistes* chymotrypsin-like enzyme has a serine residue in its active center and in this respect is similar to bovine chymotrypsin.

Figures 1A, 1B, 2A, 2B, 3A, and 3B show that inhibition by TPCK in all cases exceeded 90% dur-

TABLE 2. Midgut chymotrypsin and trypsin specific activities in adult castes of four *Polistes* species.

<table>
<thead>
<tr>
<th></th>
<th>Chymotrypsin</th>
<th>Trypsin</th>
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<tbody>
<tr>
<td></td>
<td>U/mg protein</td>
<td>U/mg midgut</td>
</tr>
<tr>
<td><em>P. exclamans</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>1.18</td>
<td>0.095</td>
</tr>
<tr>
<td>Male</td>
<td>1.34</td>
<td>0.12</td>
</tr>
<tr>
<td><em>P. metricus</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>0.68</td>
<td>0.05</td>
</tr>
<tr>
<td>Male</td>
<td>1.34</td>
<td>0.125</td>
</tr>
<tr>
<td><em>P. fuscatus</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>0.905</td>
<td>0.095</td>
</tr>
<tr>
<td>Male*</td>
<td>2.52</td>
<td>0.27</td>
</tr>
<tr>
<td><em>P. annularis</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>†</td>
<td>0.47</td>
<td>0.05</td>
</tr>
</tbody>
</table>

*Single samples.
†Unsexed.
FIG. 1. The chymotrypsin-like midgut activity of male (A) and female (B) and the trypsin-like midgut activity of male (C) and female (D) *Polistes exclamans*. The response is shown of the enzyme (●) to PMSF (△); to TLCK (○); and to TPCK (◊).

MINUTES

FIG. 2. The chymotrypsin-like midgut activity of male (A) and female (B); and the trypsin-like midgut activity of male (C), and female (D) *Polistes metricus*. The response is shown of the enzyme (●) to PMSF (△); to TLCK (○); and to TPCK (◊).

ing the period of linearity. For TPCK to be inhibitory, an enzyme must have a histidine in its active center and a specificity for aromatic amino acids. Both the *Polistes* and the bovine enzymes share these characteristics. TLCK was not an effective inhibitor of the wasp enzyme in either males or females (Fig. 1A, 1B, 2A, 2B, 3A, 3B). TLCK will inhibit an enzyme that has a histidine in its active center and a specificity for basic amino acids. This lack of inhibition represents an additional similarity between wasp and bovine enzymes.

Similar experiments were used to characterize the trypsin-like enzyme in *P. exclamans* (Figs. 1C, 1D), and *P. metricus* (Figs. 2C, 2D). The very low absorbance readings leave interpretation of the inhibition experiments open to question.

**Discussion**

Protease activity was found in larval saliva, in midguts of adults established in the colony, and in midguts of newly eclosed adults. Crop content of newly eclosed adults showed no proteolytic activ-
FIG. 3. The chymotrypsin-like midgut activities of male (A); of female (B); the trypsin-like midgut activity of male (C), and of female (D) *Polistes fuscatus*. The response is shown of the enzyme (●) to PMSF (△); to TLCK (○); and to TPCK (○).

TABLE 3. Midgut carboxypeptidase A and carboxypeptidase B specific activities in adult castes of two *Polistes* species. Enzyme activity values are averages of two replicates

<table>
<thead>
<tr>
<th>Carboxypeptidase A</th>
<th>Carboxypeptidase B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>U/mg protein</td>
</tr>
<tr>
<td><em>P. exclamans</em></td>
<td></td>
</tr>
<tr>
<td>Unsexed</td>
<td>0.159</td>
</tr>
<tr>
<td><em>P. metricus</em></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>0.110</td>
</tr>
<tr>
<td><em>P. fuscatus</em></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>0.057</td>
</tr>
</tbody>
</table>

The results summarized in Table 2 show differences in specific activity between species of *Polistes*. Differences in specific activity were also noted between castes of *V. germanica* and *V. maculifrons* (Grogan and Hunt 1977). A higher concentration of digestive enzymes was found in male *Polistes* than in females in the three species where sex differences were noted. Grogan and Hunt (1977) noted the reverse situation in *Vespula*.

The abundance of enzyme activity found in these studies differs from the results reported by Ikan et al. (1968) on *V. orientalis*. Pressure on the abdomen of a hornet was used by Ikan et al. (1968) to collect some of their samples. Such a technique might not result in the collection of fluid from the midgut digestive region but more probably from the crop, where digestion does not take place. Passage through the muscular proventriculus of the Hymenoptera appears to be one way (Eisner 1957), although regurgitation from the midgut may occur in other insects, for example, the cockroach (Morton 1967). A further discrepancy between this study...
and that of Ikan et al., probably arises from a difference in assay technique. The low specific activities obtained by Ikan are for total body protein assayed from whole body homogenates. The specific activity of enzymes as a percentage of total body protein would be much less than specific activity calculated on the basis of midgut protein. In light of their techniques, it is possible that even the low results presented in their tables have some positive significance.

The results of inhibition studies on the wasp trypsin-like enzyme are not easily interpreted and should be held in reserve until the enzyme can be further studied.

Acknowledgments

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