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Localization of Trypsin-like Enzyme and Aminopeptidase in the Midgut of the Adult Horn fly, *Haematobia irritans* (L.) and the Stable fly, *Stomoxys calcitrans* (L.) (Diptera: Muscidae), and Effect of the Anterior Midgut Homogenates on the Proteinases.[1)]

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Summary

In the horn fly and the stable fly, significant activity of a trypsin-like enzyme was detected in the lumen contents of anterior and posterior midguts, while a significant activity of aminopeptidase was found only in the lumen content of posterior midgut. The inhibitory effect of the homogenate of the anterior midgut on the activity of the trypsin-like enzyme extracted from the posterior midgut was not found in the horn fly and the stable fly.

Introduction

There have been a number of studies on the digestive physiology of haematophagous insects. Since two reviews were given by Gooding in 1972 and 1975, however, only a few investigations have been carried out for the stable fly (Lehane, 1976, 1977; DeLoach and Spates, 1979, 1980; Lee and Davies, 1979; Spates and DeLoach, 1980; Spates, 1981) and for the horn fly (Hori, 1981; Hori *et al.*, 1981, 1982), which are serious insect pests of cattle in Japan. Localization of digestive proteinases in the midgut has been investigated for the tsetse fly (Wigglesworth, 1929; Langley, 1966; Gooding, 1974, 1977; Gooding and Rolseth, 1976) and for the stable fly (Lehane, 1976). The anterior midgut of the tsetse fly contains trypsin inhibitor(s) (Gooding, 1974; Houseman, 1980). In the present work, the distribution of a trypsin-like enzyme (TLE) and aminopeptidase (AP) in the midgut of the horn fly, *Haematobia irritans* (L.), and the inhibitory effect of the anterior midgut on TLE and AP were investigated, and were compared with the case of the stable fly, *Stomoxys calcitrans* (L.).

Materials and Methods

Adult flies used. Horn flies and stable flies collected from cattle in the field and starved for 8 hr and for 15 hr, respectively,

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were used for the experiments.

Preparation of enzyme solution. In the experiment for determining the inhibitory effect of the anterior midgut (AM) on TLE and AP in the posterior midgut (PM), the midguts dissected out were cut at the point slightly behind the junction of AM and PM. Cutting at this point does not permit outflow of the content of AM including proteases. In the experiment for determining the localization of TLE and AP in the midgut, AM and PM were further divided into the lumen content and the epithelial tissue. The midguts were cut longitudinally on a slide glass in order to permit the fluid content to flow out of the lumen and then washed out twice. The contents and the wash were stocked as samples of the lumen contents. Then the tissues were washed in distilled water and stocked as samples of the gut tissues. Each sample was homogenized in 0.9% NaCl solution (3 guts/m*l* in the horn fly and 1 gut/m*l* in the stable fly). The homogenates were refrigerate-centrifuged for 20 min at 20,000 *g* and the resulting supernatants were used for assays of TLE and AP.

Assay for TLE and AP activities. TLE and AP activities were assayed according to the procedure used by Hori *et al.* (1981). Reaction mixtures were incubated for 10 min (TLE) or 30 min (AP) at 30°C. Then the reaction was stopped by adding 0.38 m*l* of 30% acetic acid. The optical absorbancy of the reaction mixture was measured at 410 nm with a Shimadzu UV-200 S spectrophotometer. Activities were expressed in OD units.

In the experiment on the inhibitory effect of AM, the following three kinds of reaction mixture were prepared; (1) 3.0 m*l* substrate solution + 1.5 m*l* homogenate of AM + 1.5 m*l* 0.9% NaCl solution, (2) 3.0 m*l* substrate solution + 1.5 m*l* homogenate of PM + 1.5 m*l* 0.9% NaCl solution and (3) 3.0 m*l* substrate solution + 1.5 m*l* homogenate of AM + 1.5 m*l* homogenate of PM. In addition to these, the substrate and enzyme controls were run at the same time.

All experiments involved 4 replicates of assays, only the experiment on the localization of enzymes of the horn fly involved 5.

Results and Discussion

Inhibitory effect of the anterior midgut on TLE and AP in the posterior midgut

In order to decide the part of the midgut to be used for analyses, the inhibitory effect of the anterior midgut on proteinases was tested. As shown in Table 1, it was found

Table 1. Effect of mixing of enzyme preparations from the anterior and posterior midguts on TLE and AP activities in two species of flies.

Part of midgut	Activity (A_{410})			
	TLE		AP	
	Horn fly	Stable fly	Horn fly	Stable fly
Anterior (AM)	0.257	0.103	0	0.001
Posterior (PM)	0.453	0.151	0.265	0.093
[AM] + [PM][a]	0.710	0.254	0.265	0.094
[AM + PM][b]	0.726	0.296	0.293	0.105

[a] The sum of the enzyme activity of AM and the enzyme activity of PM.

[b] The enzyme activity in the mixture of AM and PM.

that there was no statistically significant difference between the total activity of proteases (TLE or AP) in AM and PM and the activity of protease (TLE or AP) in the mixture of AM and PM in both horn fly and stable fly, suggesting that AM had no inhibitory effect on TLE and AP (at least on crude enzyme) activities in PM.

GOODING (1974) and HOUSEMAN (1980) found that the extract from AM inhibited the activity of trypsin extracted from PM in *Glossina morsitans*; the latter author confirmed that the inhibitor was of fly origin but not of serum origin. GOODING maintained, therefore, that the assay should be performed by separating AM from PM for a reliable determination of trypsin level in the midgut. Since the horn fly and the stable fly have no TLE or AP inhibitor in AM as shown in this investigation, the assay for TLE or AP can be performed in some cases without separating AM from PM.

Localization of TLE and AP in the midgut

The results are shown in Table 2. In both horn fly and stable fly, a significant activity of TLE was detected in the lumen contents of AM and PM but not in their epithelial cells. The activity was significantly higher in the posterior than in the anterior lumen in the horn fly, while it was not significantly different between them in the stable fly. In both species, on the other hand, a significant activity of AP was detected only in the lumen content of PM. LOTMOR (1949) stated that AM was a storeroom of blood ingested and PM was a place for blood digestion in the stable fly *Stomoxys calcitrans*. WIGGLESWORTH (1929) and LANGLEY (1966) reported that most of proteolytic activity was present in the middle region of the midgut (a part of PM) in the tsetse flies, and GOODING (1974, 1977) found that in *Glossina morsitans morsitans* trypsin, proteinase VI and VII, aminopeptidase, and carboxypeptidase A and B were mostly contained in PM with only a trace in AM. LEHANE (1976) confirmed that AM contained only 12% of total proteolytic activity of the whole midgut in *S. calcitrans* and suggested that this region may play no effective role in enzyme production. Most proteolytic enzymes seem to be synthesized in the opaque zone of PM and secreted into the lumen (LEHANE, 1976). AM as well as PM of the horn fly and the stable fly contained a relatively high activity of TLE. This relatively high activity in AM may be due to the fact that the midguts were cut at the point located slightly behind the junction of AM and PM when they were separated from each other, so that AM may include a minor part of the

Table 2. Localization of TLE and AP in the midguts of the horn fly and the stable fly

Part of midgut		Activity (A_{410})			
		TLE		AP	
		Horn fly	Stable fly	Horn fly	Stable fly
Anterior	Epithelial Cells	0.021	0.008	0.005	0.008
	Lumen content	0.120[a]	0.252	0.016[b]	0.006[c]
Posterior	Epithelial Cells	0.005	0.002	0.020	0.005
	Lumen content	0.305[a]	0.172	0.229[b]	0.179[c]

[a] Significant at 5% level (t-test) in TLE activity of lumen content between the anterior and posterior midguts.

[b,c] Significant at 5% level (t-test) in AP activity of lumen content between the anterior and posterior midguts.

opaque zone. Gooding (1977) indicated that AP was located only in PM and furthermore Gooding and Rolseth (1976) stated that AP was present in or on the PM-cells rather than in the gut lumen. In the horn fly and the stable fly, AP was present in the lumem of PM, indicating that AP may be synthesized in PM-tissue and secreted into the lumen. The presence of AP in PM-lumen in the horn fly and the stable fly might be more useful for blood digestion than in the tsetse fly, because AP in the lumen might be mixed more quickly and effectively with blood proteins and peptides and might have more functional advantage of rapid degradation of them than AP in or on the epithelial cells.

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ノサシバエ成虫の中腸における トリプシン様酵素とアミノペプチダーゼの分布

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ノサシバエとサシバエの前部中腸と後部中腸の腸管内に，有意な活性のトリプシン様酵素が検出された。一方アミノペプチダーゼは後部中腸の腸管内にだけ検出された。ノサシバエの前部中腸の抽出物中に，ツエツエバエでみられたようなトリプシン様酵素の活性を抑制する物質は検出されなかった。

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