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PROTEOLYTIC ENZYMES OF DESERT LOCUST SCHISTOCERCA GREGARIA (FORSKAL)

S.N.H. Naqvi, Z.I. Khan, S.A. Qureshi and N.A. Khan

P.C.S.I.R. Laboratories, Karachi 39

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Activity of proteolytic enzymes (peptic, catheptic and tryptic) was determined according to the method of Tomeralli *et al.*[41] in the alimentary canal of desert locust. Tryptic activity was found to be significant at pH 8.3. Protease activity was negligible in all the parts (whole and tissues) of the alimentary canal of the locust starved for 48 hr. It was significant in foregut, hepatic caecae and midgut of the locust starved for 12 hr. Protease activity was high in hepatic caecae and midgut tissue respectively. Aminopeptidase activity was significantly high in midgut and hepatic caecae while low in malpighian tubules. Dipeptidase activity was also significantly high in midgut, hepatic caecae and malpighian tubules while meagre in salivary glands.

The literature on the proteolytic enzymes of insects has been reviewed by various workers.[1-3] Study of these reviews and other available literature[4-13] on the enzymes of *Schistocera gregaria* (Forskal) reveal that no work has been done on the proteolytic enzymes of desert locust, although they play an important role in the digestion of proteins. However, the activity of these enzymes has been determined in *Locusta* by Khan.[14-16] Therefore, the study of proteolytic enzymes was undertaken to compare the present findings with those of Khan[14-16] and other workers in various insects.[17-39] Moreover, these studies were done in different parts of the alimentary canal, at different intervals in starved and normal (starved for 12 hr only) locusts, to understand the digestion of proteins in desert locust.

Materials and Methods

Enzyme Source.—Locusts reared in the PCSIR Laboratories, Karachi, according to the method of Akhtar *et al.*[40] were starved for 12 hr to evacuate the gut from food particles and 48 hr for studying the effect of starvation. Different parts of the alimentary canal of single,4-week old adult locust were taken for experiments. Insects were chilled for 10 min at –5°C and dissected in Ringer's solution. The alimentary canal was weighed and then homogenized in 4.0 ml phosphate buffer (pH 8.3) in a Teflon Pyrex tissue grinder for 5 min. The homogenate was centrifuged for 10 min at 3000 rev/min to give a clear supernatant, which was stored at –5°C in a freezer. Extracts of different parts which included foregut, midgut, hepatic caecae and hindgut having luminal contents, and others like salivary glands and malpighian tubules, were also prepared similarly. The same parts were cut longitudinally to wash the luminal contents in Ringer's solution and then immediately dried on filter paper. After drying and weighing each tissue was extracted with 1.0 ml of phosphate buffer (pH 8.3) as mentioned above.

Determination of Enzyme Activity.—Determination of peptic, catheptic and tryptic activities of the whole homogenate of the alimentary canal, was done by using the chromophoric protein derivative, azo-albumen as substrate, according to the procedure of Tomarelli *et al.*[41] Phosphate buffers with a pH range from 2.0 to 10.0 were used for the determination of the proteolytic activity (peptic, catheptic and tryptic). One ml of each buffer was mixed with 0.5 ml of the homogenate (from 5 ml homogenate of the whole gut), 1.0 ml of the substrate solution and a drop of toluene. The mixture was incubated for 6 hr at 38°C. One sample containing boiled homogenate and a blank were taken, along with the normal reaction mixtures for comparison. After incubation, undigested protein was precipitated by adding 5.0 ml 5% trichloroacetic acid to each sample. An aliquot (1.0 ml) was taken from this filtrate and mixed with 4.0 ml 0.5N NaOH to develop the colour. Optical densities were read at 440 mμ on the spectrophotometer and the corrected readings were converted into enzyme units, according to standard method.

Protease activity in different gut parts and in their tissues was determined according to Tomarelli *et al.*[41] by using homogenates of different parts and their washed tissues. Incubation was done for 0, 1, 3, and 6 hr at 38°C in all the samples.

For the determination of aminopeptidase and dipeptidase activity, 0.5 ml of the homogenate was prepared in the phosphate buffer and mixed with 0.5 ml of substrate (5% solution leucylglycyl glycine for aminopeptidase and 5% solution glycyl glycine for dipeptidase) and the mixture was incubated at 38°C for 6 hr. Samples of 3 μl were withdrawn from the incubating mixture at 0, 1, 3 and 6 hr by a microsyringe and were applied to Whatman chromatographic paper No. 1. Spots of the standard substrate, glycine and boiled extract were also similarly applied. Descending

paper chromatography was performed for 48 hr using n-butanol–acetic acid–water as solvent system (120:30:50 v/v). The colour of the spots was developed by dipping in solution of 0.25% ninhydrin.

Results

Since the preliminary experiments showed the presence of proteolytic enzymes, experiments were conducted to find the nature of the enzymes, whether peptic, catheptic or tryptic. This was done by using buffers of different pH in the Tomeralli *et al.*[41] procedure. The data obtained have been plotted in Figs. 1 and 2 as the units of enzyme activity against pH. Figure 1 shows the maximum activity between pH 8.0 and 8.5. However, for finding out exact optimum pH, experiments were repeated using phosphate buffers of 8.0, 8.1, 8.2, 8.3 and 8.5 pH. The optimum pH was as 8.3 (Fig. 2). Figure 1 indicates that peptic type of enzyme is absent, whereas there is an insignificant peak in the catheptic range as compared with the tryptic peak. The tryptic type of enzyme seems to be prominently active in the alimentary canal of the desert locust.

Protease.—Protease activity was quantitatively determined in different parts of the alimentary canal as a whole and in their washed tissues, in the locusts, starved for 48 and 12 hr at different incubation periods. The data are given in Tables 1 and 2 respectively. Activity of the enzyme is almost absent in the 48-hr starved locust except the weak activity in the hepatic caecae and midgut. Faint activity in the tissues of these parts indicate that the above-mentioned activity may be due to the lumenal contents of these parts (Table 1).

In the locust starved for 12 hr and fed on cabbage and glucose (9:1), the enzyme activity was strong in hepatic caecae and midgut (whole), moderate in foregut (whole) while weak in hindgut (whole) and caecal tissue and negligible in other parts. However, among the tissues it is highest in caecal tissue which indicates the possible enzyme secretory function of this tissue (Table 2). Although the activity was maximum after 6 hr incubation but the major part of the substrate was hydrolysed in 3 hr. The rate of hydrolysis is faster during the first 3 hr as compared with the later 3 hr (Table 2).

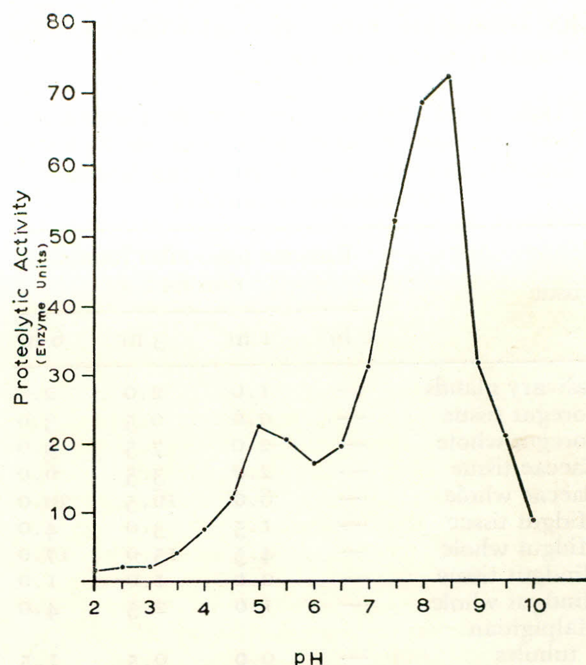

Fig. 1.—The proteolytic activity in the homogenate of the alimentary canal of desert locust.

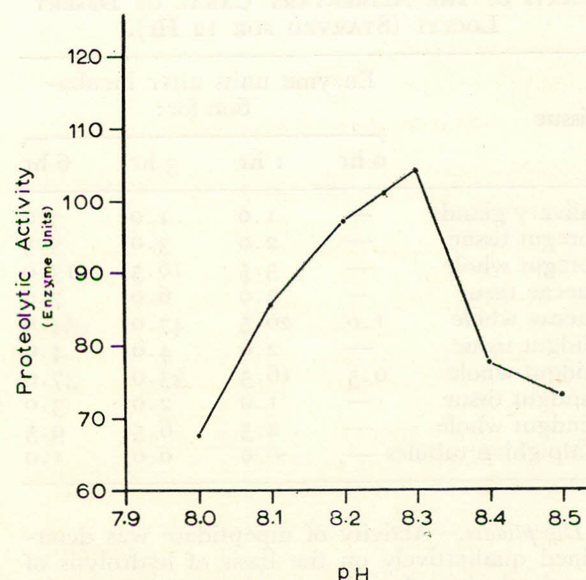

Fig. 2.—The optimum pH for tryptic activity in the homogenate of the alimentary canal of desert locust.

Aminopeptidase.—Activity of aminopeptidase was determined qualitatively in different parts of the alimentary canal (whole and their washed tissues) of the desert locust, starved for 12 hr. Activity was determined chromatographically on the basis of hydrolysis of leucylglycyl glycine. As evident from Table 3 activity was found to be absent in salivary glands, foregut (whole and tissue) and hindgut (whole and tissue). However, slight indication of activity was found in salivary glands after 3 hr incubation and in hindgut (whole) after 6 hr incubation. Strong activity was found in hepatic

caecae and midgut (whole and tissue) increasing with incubation time. In malpighian tubules the activity was weak (Table 3).

Table 1.—Protease Activity in Different Parts of the Alimentary Canal of Desert Locust, Starved for 48 Hr after Various Incubation Intervals.

Tissue	Enzyme units after incubation for: 0 hr	1 hr	3 hr	6 hr
Salivary glands	—	1.0	2.0	2.0
Foregut tissue	—	0.0	0.5	3.0
Foregut whole	—	2.0	7.5	9.0
Caecae tissue	—	2.0	3.5	6.0
Caecae whole	—	6.0	16.5	20.0
Midgut tissue	—	1.5	3.0	4.0
Midgut whole	—	4.5	15.0	17.0
Hindgut tissue	—	0.0	1.0	1.0
Hindgut whole	—	1.0	2.5	4.0
Malpighian tubules	—	0.0	0.5	1.5

Table 2.—Protease Activity in Different Parts of the Alimentary Canal of Desert Locust (Starved for 12 Hr).

Tissue	Enzyme units after incubation for: 0 hr	1 hr	3 hr	6 hr
Salivary glands	—	1.0	1.0	3.0
Foregut tissue	—	2.0	3.0	3.5
Foregut whole	—	5.5	10.5	13.0
Caecae tissue	—	3.0	6.0	7.0
Caecae whole	1.0	20.5	47.0	54.0
Midgut tissue	—	2.0	4.0	4.0
Midgut whole	0.5	16.5	33.0	37.0
Hindgut tissue	—	1.0	2.0	3.0
Hindgut whole	—	2.5	6.5	9.5
Malpighian tubules	—	0.0	0.0	1.0

Dipeptidase.—Activity of dipeptidase was determined qualitatively on the basis of hydrolysis of glycyl glycine, by the paper chromatography technique. As evident from Table 4 activity of the enzyme was absent in foregut (whole and tissue) and hindgut (whole and tissue) except for slight indication in hindgut (whole). Activity was strong in midgut (whole and tissue) and caecae (whole and tissue) increasing with incubation period. It was moderate in malpighian tubules and weak in salivary glands. Activity was stronger in the whole midgut and hepatic caecae as compared to their tissues. It was highest in midgut. Moreover the hydrolysis occurred earlier in midgut, the major part being hydrolysed in initial 1 hr (Table 4).

Table 3.—Aminopeptidase Activity in Different Regions of the Alimentary Canal of *Schistocerca gregaria* (Forskal).

Source of homogenate	Hydrolysis of leucylglycyl glycine at different intervals at 38°C: 0 hr	1 hr	3 hr	6 hr
Salivary glands	—	—	?	—
Foregut tissue	—	—	—	—
Foregut whole	—	—	—	—
Caecae tissue	—	+	++	+++
Caecae whole	—	+	+++	+++
Midgut tissue	—	+	++	+++
Midgut whole	—	+	++	+++
Hindgut tissue	—	—	—	—
Hindgut whole	—	—	—	?
Malpighian tubules	—	+	+	+

Index to symbols: —, nil; ?, doubtful; +, low, ++, moderate; +++, high.

Table 4.—Dipeptidase Activity in Different Regions of the Alimentary Canal of *Schistocerca gregaria* (Forskal).

Source of homogenate	Hydrolysis of glycyl glycine at different interval at 38°C: 0 hr	1 hr	3 hr	6 hr
Salivary glands	—	—	+	+
Foregut tissue	—	—	—	—
Foregut whole	—	—	—	—
Caecae tissue	—	+	++	++
Caecae whole	—	—	++	+++
Midgut tissue	—	+	++	++
Midgut whole	—	++	+++	+++
Hindgut tissue	—	—	—	—
Hindgut whole	—	—	?	—
Malpighian tubules	—	+	++	++

Index to symbols: —, nil; ?, doubtful; +, low; ++, moderate; +++, high.

Discussion

The presence of a tryptic type enzyme has been reported in water scorpion *Laccotrephis maculatus* by Khan[36] with maximum activity at a pH 8.5 and in *Utetheisa pulchella* larva gut by Khatoon[38]

at a pH 8.0 whereas Champlain and Fisk[30] reported maximum activity at pH 7.9 for azo-albumen and 7.8 for azo-casein hydrolysis in *Stomoxys calcitrans*. In cockroach *P. americana* Powning *et al.*[26] reported optimum pH 7.1 for casein and 7.9 for gelatin hydrolysis. In the present case the optimum pH is 8.3 which indicates that tryptic activity in desert locust is similar to that of water scorpion and *U. pulchella*. However, the catheptic activity was not significant in desert locust as compared to tryptic activity. The presence of a tryptic type of protease in insects is confirmed by the present finding also. Thus it may be inferred that feeding habit has little effect on the optimum pH of the tryptic enzyme, especially when we consider the reports of Khatoon[38] and Shinoda[42] on *B. mori* and *U. pulchella*, respectively (both being phytophagous and lepidopteran[16] insect). It is perhaps the substrate or some other factor which plays a vital role in it.

Significant difference in the protease quantity in 48 hr and 12 hr starved adult locusts, indicates that protease activity decreases on starvation and increases on feeding. This was also pointed out by Schlottke[20–22] and Day and Powning[24] among various insects. Sufficient activity in the whole midgut and hepatic caecae, even in highly starved locust indicates that these are the protease secreting parts, whereas in other parts it is negligible.

The highest activity of protease in locust (12-hr starved) was found in hepatic caecae and then in midgut. This finding confirms the report of Khan[15] for *Locusta* and Day and Powning[24] for *Periplaneta* but not for *B. germanica*.[24] The variation between the activity in hepatic caecae and midgut is lesser than that in *Locusta*.[15] Significantly less activity in their tissues as against whole part indicates that the enzyme is synthesized in the epithelial tissues and is instantly poured into the lumen for hydrolysing proteins, present in these parts. Similar is the case in other phytophagous insects like *Tenebrio*,[31] *Locusta*[15] and *Utetheisa*.[38] This also points out an efficient digestion in *Schistocerca* especially in the light of the present data where, maximum part of hydrolysis occurs within 3 hr of incubation. Presence of faint activity in foregut and hindgut indicates a casual forward and backward flow of digestive juices in the alimentary canal.

The presence of aminopeptidase and dipeptidase activity in midgut and hepatic caecae (whole and tissue) confirms the reports of Lichtenstein,[44] Schlottke,[20] Dupsiva,[23] Khan[14] and Khatoon.[37] However, Khan and Ford[39] reported presence of aminopeptidase only. This difference may be due to the insect variation or the feeding habit. Their absence in foregut and hindgut of *Schistocerca* indicates that the hydrolysis of dipeptides and aminopeptides (polypeptides) occurs in the midgut and caecae region, as in the case of *Locusta*[14] and *Utetheisa*.[37] Consequently a "ferment chain" similar to vertebrates,[45] as pointed out by Schlottke,[22] does not seem to be present. The negligible quantity of protease in the tissue of midgut and caecae in comparison to the whole part, also suggest that digestion of proteins and peptides occur only in the midgut and caecae regions which are histologically similar. The concept of serial secretion of enzymes or the ferment chain seems to be incorrect in this case also.

The lumenal contents seem to possess more enzymatic activity as compared to the tissues because hydrolysis of the substrate occurs earlier in the whole part of the alimentary canal as pointed out by Khatoon.[37] Slight presence of these enzymes in the salivary glands may be for the primary hydrolysis of lumenal proteins. The presence of both these enzymes (amino and dipeptidase) in the malpighian tubules may be due to uncontrolled flow, because the malpighian tubules open lumenally at the junction of midgut and hindgut. This view is further supported by the slight presence of these enzymes in the hindgut.

Higher activity of protease in the caecae indicates that in this region the amount of polypeptides is higher as compared to in midgut. Thus it is probable that the minor amount of protease in the foregut, which reaches there due to uncontrolled flow, hydrolyses proteins into polypeptides. When they reach the hepatic caecae region they require more protease (aminopeptidase) to further hydrolyse them. Complete hydrolysis of proteins, polypeptides and dipeptides into amino acids take place in midgut due to the presence of protease, aminopeptidase and dipeptidase. Posterior to this region, the process of absorption starts and therefore these enzymes are absent in hindgut.

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