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Studies on proteolytic activity in *Gastrothylax crumenifer*. A Rumen Fluke of sheep *Ovis ovis*

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Abstract. Biochemical studies revealed the existence of proteolytic activity in *Gastrothylax crumenifer* with two peaks at pH 3·0 and at pH 6·0. The higher activity occurred at pH 3·0. The effect of various chemicals on the proteolytic enzyme is discussed.

Keywords. Proteolytic activity ; *Gastrothylax crumenifer*.

1. Introduction

The occurrence of proteolytic enzyme activity has been reported in some of the digenetic trematodes such as *Schistosoma mansoni* (Timms and Bueding 1959); *Fasciola hepatica* (Pennoit De Cooman and van Grembergen 1942; Halton 1963; Thorsell and Bjorkman 1965; Thorpe 1967; Howell 1973); *Philophthalmus burrili* (Howell 1971); *Haplometra cylindracea* (Halton 1963) and Strigeids (Erasmus and Ohman 1963). The present study was carried out to determine proteolytic activity in *Gastrothylax crumenifer* in relation to different pH levels and the effect of various chemicals on the enzyme activity.

2. Materials and methods

Live specimens of *G. crumenifer* were washed several times with Hedon–Fleig medium rinsed with distilled water drained as completely as possible on a Whatman No. 1 filter paper 10% (W/V) homogenates were prepared in ice-cold glass distilled water using a glass homogenizer. Cooling was provided by a beaker of ice and water during the homogenization. The crude homogenates were centrifuged at 1,500 g for 20 min at 4° C. The supernatant was used for further analysis.

Proteolytic activity was estimated by the method of Anson's colorimetric method as adopted by Yeshodha *et al* (1976) with slight modification. 2·5% egg albumin was used as substrate. To study the effect of pH on the enzyme activity, phos-

phate buffer was used to give a range of pH values from 2 to 9 by adding diluted acid and alkali. The assay medium consisted of 2·5 ml of substrate and 1 ml of supernatant and was incubated at 37° C for 30 min. Immediately after the incubation period 7·5 ml of 5% trichloroacetic acid was added to stop the reaction and the medium was warmed on a boiling water bath and cooled to room temperature. Then the mixture was centrifuged and the supernatant was taken for further analysis. To 2·5 ml of supernatant 5 ml of 0·5 N sodium hydroxide and 1·5 ml of twice diluted phenol reagent were added. The mixture was mixed for exactly 2 min to develop the blue colour. The products of enzymatic hydrolysis of the substrate were estimated colorimetrically with a Bausch and Lomb Spectronic 20 spectrophotometer at 660 nm. A control was run in an identical manner except that the trichloroacetic acid solution was first added to the egg albumin solution and then the enzyme extract was added to the mixture. Enzyme activity is expressed as μg tyrosine/100 μg protein/30 min at 37° C. All the experiments were run with 5 samples each at least in duplicates. The data shown on the graphs represent the average of five readings.

To assess the effect of chemicals the following investigations were carried out only at pH 3·0 and 6·0. Various chemicals such as mercuric chloride, cysteine, EDTA, potassium cyanide, lead nitrate, sodium arsenate, manganous chloride, triton x–100, sodium fluoride and iodoacetamide were dissolved in phosphate buffer solution to a final concentration of 0·1 M in the incubation mixture.

3. Results

It is evident from the data (figure 1) that two peaks of proteolytic activity in *G. crumenifer* occurred, one at pH 3·0 and the other at pH 6·0. The maximum activity (40·9 μg tyrosine) was observed at pH 3·0 and the minimum (6·5 μg tyrosine) at pH 5·0.

3.1. *Effect of chemicals*

The effect of various chemicals on proteolytic activity in *G. crumenifer* was studied only at the different peaks of enzyme activity observed, e.g., at pH 3·0 and 6·0.

3.2. *Activation at pH* 3·0

It has been observed (figure 2) that cysteine was a powerful activator of proteolytic enzymes in *G. crumenifer* at pH 3·0. Manganous chloride also showed a similar, though less intense, activating effect. EDTA showed a moderate effect. Triton x–100 seems to be a weak activator.

3.3. *Inhibition at pH* 3·0

It was found that mercuric chloride and sodium arsenate completely inhibited the proteolytic activity in *G. crumenifer* (figure 2). Sodium fluoride also seemed to be a strong inhibitor. Moderate inhibitory effect was shown by lead nitrate and a weak effect was shown by iodoacetamide.

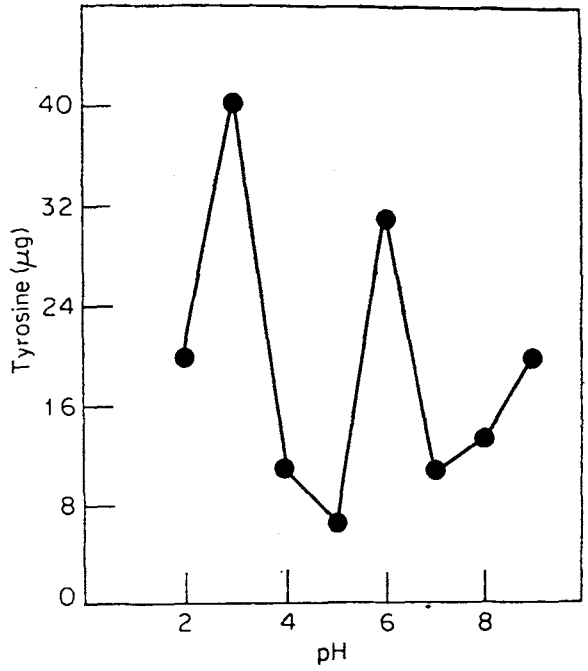
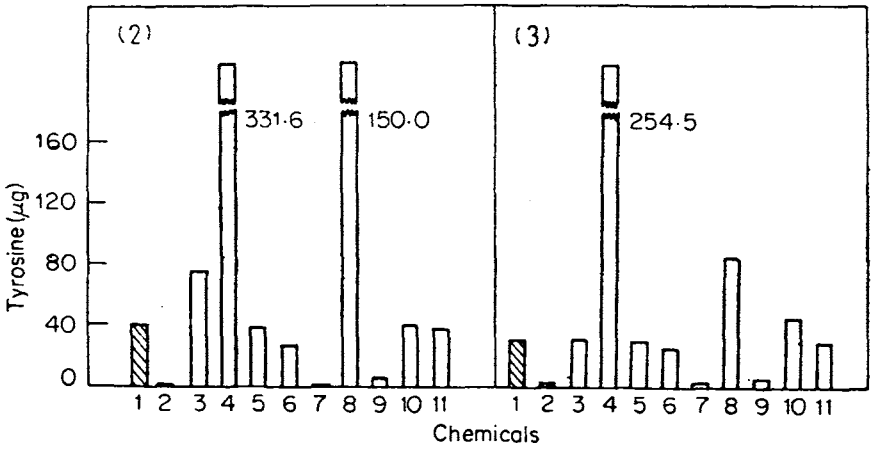


Figure 1. The proteolytic activity (μg tyrosine/100 μg protein/30 min at 37° C) in *G. crumenifer* at various pH ranges (pH 2 to pH 9).



Figures 2 and 3. The effects of activators and inhibitors on proteolytic activity in *G. crumenifer* at pH 3·0 and 6·0. 1. Untreated (control). 2. Mercuric chloride. 3. EDTA. 4. Cysteine. 5. Potassium cyanide. 6. Lead nitrate 7. Sodium arsenate. 8. Manganous chloride. 9. Sodium fluoride. 10. Triton x-100. 11. Iodoacetamide.

3.4. *Activation at pH* 6·0

Strong activating effect on proteolytic enzyme was shown by cysteine (figure 3). Manganous chloride also showed a strong effect but not to the extent of cysteine. Triton x-100 showed a little and EDTA a very little effect.

3.5. *Inhibition at pH 6·0*

The results (figure 3) clearly indicate that mercuric chloride and sodium arsenate were strong inhibitors of proteolytic enzymes in *G. crumenifer* at pH 6·0. Sodium fluoride also seemed to be a strong inhibitor, though less intense than the mercuric chloride and sodium arsenate. Lead nitrate showed a moderate effect. A weak inhibitory effect was shown by potassium cyanide and iodoacetamide.

4. Discussion

In the present study, the maximum activity of proteolytic enzymes from *G. crumenifer* was observed only in the acid range. Two peaks of proteolytic activity, one at pH 3·0 and the other at pH 6·0, with a higher activity at pH 3·0 occurred. The presence of proteolytic activity in *G. crumenifer* is supported by the work of previous authors which have shown similar proteolytic activity in the homogenates of *Fasciola hepatica* (Pennoit De Cooman and van Grembergen 1942; Howell 1973) and in *Schistosoma mansoni* (Timms and Bueding 1959) and in *Haplometra cylindracea* (Halton 1963). The existence of two peaks of proteolytic activity in *G. crumenifer* may be due to the possible occurrence of more than one enzyme form.

The proteolytic activity in *G. crumenifer* was activated by EDTA, cysteine, manganous chloride and triton x-100. On the other hand, mercuric chloride, lead nitrate, sodium arsenate, sodium fluoride, potassium cyanide, iodoacetamide inhibited the proteolytic activity. So far, the effect of chemicals on proteolytic activity in helminths has not been detected by biochemical methods, though some authors demonstrated it histochemically. Howell (1971) however observed inhibition of proteolytic activity in the gastrodermis of *Philophthalmus burrili* by mercuric chloride.

The protease in *G. crumenifer* may have a digestive function. The ability to split ingested protein can be deduced from the evidence for proteolytic activity in *G. crumenifer*.

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