A chitinase system from *Carcinus maenas*

The presence of chitinase activity in crustacean hypodermis is suggested by the partial resorption of chitin during moulting and by the observations of RENAUD[1] and KUHN AND TIEDMAN[2]. Using the crab *Maia squinado*, RENAUD[1] showed that extracts of the hypodermis released aminosugars from fragments of the integument, while KUHN AND TIEDMAN[2] described the hydrolysis of N-acetyl-β-glucosaminides by a lobster hypodermis preparation. Evidence of chitolytic enzymes in the hypodermis of the shore crab *Carcinus maenas* has now been obtained; the tissue is readily available and presents a convenient source of chitinase and N-acetyl-β-glucosaminidase.

Acetone-dried powders were prepared from the hypodermal tissue underlying the carapace of *C. maenas*, about 1 g of powder being obtained from 10 crabs. The powder could be stored at −14°, and retained both chitinase and N-acetyl-β-glucosaminidase activities for over a year. Enzymic extracts were prepared by mixing acetone powder with water (1:10, v/v) for 30 min with occasional stirring. The suspension was centrifuged (2,000 rev./min for 5 min) and the green-brown solution, after dialysis against distilled water (cellophane), was stored at −14° or 0° under toluene. Such preparations retained activity for over 6 months.

For use as a substrate in assays of enzymic activity, chitin was prepared in the following way: 368 mg of finely powdered chitin[3] were dissolved in 6 ml conc. HCl, and the viscous yellow solution diluted with 200 ml water. The precipitated chitin was collected by centrifugation, washed with 50 ml water, redissolved in HCl and precipitated as before. After two more washings, the chitin was suspended in water and thoroughly dispersed in a Potter-type homogenizer; it was then dialysed against running tap water (cellophane) for 40 h, and thrice against distilled water. The concentration of chitin in the suspension was determined on a dry-weight basis by drying (100° for 3 h), and weighing the solid obtained by centrifugation of a known volume of the suspension. The freshly shaken suspension contained 6.48 mg dry weight of chitin/ml and had an apparent extinction of 3.41 at 420 mμ (see ref. 4). The extinctions were measured in a Unicam SP 600 spectrophotometer using 1-cm cells. Using appropriate dilutions of this suspension, a linear relationship was established between the apparent extinction and chitin concentration over the range 0–1.3 mg/ml. The chitin content of incubation mixtures could thus be calculated.

The hydrolysis of phenyl and methyl N-acetyl-β-glucosaminides by the dialysed extracts in the presence of acetate or citrate buffer (pH 4.3) at 30° was followed. After incubation, proteins were removed by heating at 100° for 1 min and centrifuging; N-acetylglucosamine in the supernatants was estimated by the method of REISSIG, STROMINGER AND LELOIR[5]. It was found (Fig. 1) that the phenyl derivative was hydrolysed approximately 1.5 times faster than the methyl glycoside. The effect of pH on initial reaction velocities was investigated, and in a number of determinations (*e.g.*, see Fig. 2) optima between 4.3 and 4.8 were observed.

Chitinase activity was measured by incubating enzyme preparations with colloidal chitin and observing both the decrease in turbidity and the appearance of N-acetylglucosamine. The incubation mixtures consisted of 0.1 *M* phosphate buffer (pH 6.9), 0.1 ml; colloidal chitin suspension, 0.2 ml; enzyme preparation, 0.1 ml; toluene, 0.5 ml. At intervals, two tubes were withdrawn, their contents diluted to

3.0 ml, and after mixing, the apparent absorbancies at 420 mμ were measured. After heating at 100° for 1 min, proteins and unchanged chitin were removed by centrifugation, and the N-acetylglucosamine in the supernatants was determined. The results of one experiment are shown in Fig. 3. The hydrolysis products were examined by

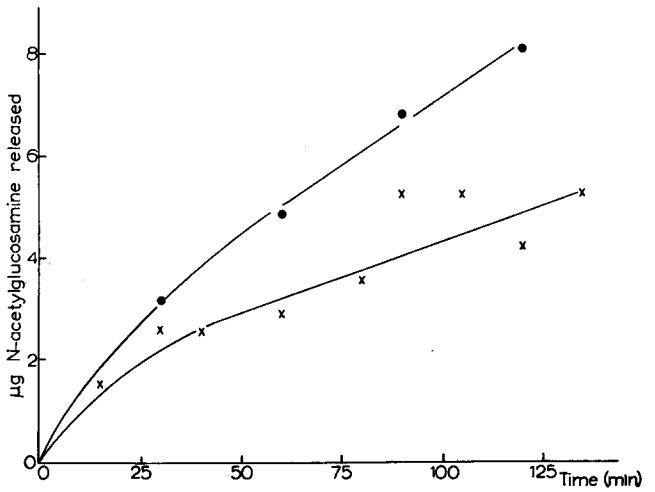

Fig. 1. Hydrolysis of glucosaminides. The incubation mixtures contained 0.1 ml dialysed extract, 0.2 ml of 0.067 *M* acetate buffer (pH 4.3), and 0.094 μmole of substrate, the final volume being 0.4 ml. X, methyl N-acetyl-β-glucosaminide; ●, phenyl N-acetyl-β-glucosaminide.

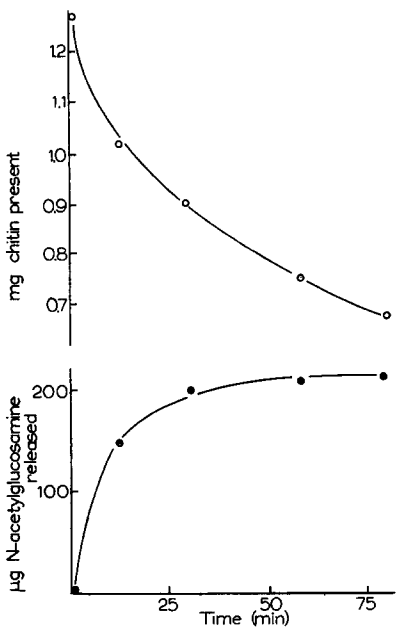

Fig. 2. Hydrolysis of colloidal chitin. Upper curve gives the fall in chitin content of the incubation mixtures, and the lower curve the increase in N-acetylglucosamine content over the same periods of time.

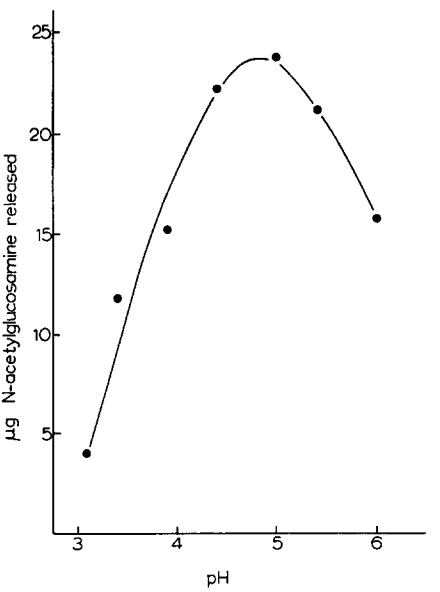

Fig. 3. The effect of pH on N-acetyl-β-glucosaminidase activity. The mixtures contained 0.1 ml of dialysed extract, 0.1 ml of 0.1 *M* citrate buffer and 0.1 μmole phenyl N-acetyl-β-glucosaminide, final volume being 0.4 ml. The incubation time was 30 min.

paper chromatography (phenol saturated with water) when N-acetylglucosamine appeared as the only substance giving a positive reaction with the Elson and Morgan spray reagent[6].

The results shown in Fig. 3 indicate that the release of N-acetylglucosamine from the chitin suspension stopped while the decrease in turbidity was still taking place. It therefore appears that possibly here, as in *Streptomyces griseus*[4], chitin breakdown may involve several enzymes, one of which causes depolymerization of the chitin chains, resulting in a lowering of the turbidities of chitin suspensions, another enzyme, which appears to be slowly inactivated at 37°, being responsible for the release of N-acetylglucosamine, either from the polysaccharides originally present, or from oligosaccharides produced enzymically. De-acetylation of the substrates was not observed.

Solutions of the *Carcinus* chitinase system have been used to demonstrate the release of N-acetylglucosamine from chitin samples obtained from several crustacean species by both the extraction method of BRACH[3], and the milder procedures of FOSTER AND HACKMAN[7].

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Pathways of triglyceride formation in adipose tissue

The assimilation of fatty acids by adipose tissue takes place predominantly by transformation into triglycerides, and the rate of uptake of the acids from the medium was found to be correlated with the esterifying capacity of the tissue[1]. The following reactions may contribute to the incorporation of fatty acids into triglycerides: (a) The formation of an acyl-CoA compound, which then interacts with α-glycerophosphate and finally is converted into triglycerides by a series of reaction steps, demonstrated in liver tissue[2,3]. The presence of the enzymes for this reaction sequence in adipose tissue has been demonstrated[4]. (b) The acyl-CoA compound formed may interact with diglycerides present in the tissue to form directly a triglyceride. (c) A lipase-activated exchange reaction between triglycerides and free fatty acids may introduce some of the radioactive fatty acid into the triglyceride[5].

It was of interest to determine which of these three possibilities was the dominant

Abbreviation: CoA, coenzyme A.