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Cellulase of marine mollusc *Littorina sp.*

Little information is available on the various glycosidases in marine invertebrates but in recent years interest in them has been increasing due to their application to the study of biopolymers[1-3].

Recently, data on cellulases from marine invertebrates have been communicated by YOKOE AND YASUMASU[4] on their relative activity, by OKADE, NISIZAWA AND NISIZAWA[5] on a cellulase from Dolabella sp. and by HULTIN AND WANNTORP[6] on cellulase from the sea urchin *Paracentrotus lividus* Lk. In the present paper it is shown that an extract of hepatopancreas from the marine mollusc Littorina sp. contains several glycosidase activities. The purification of one of them—homogenic cellulase (β-1,4-glucan 4-glucanohydrolase, EC 3.2.1.4)— is described.

CM-cellulase activity was measured viscometrically. For rapid determination (column profile), the flow time of 10 ml of 0.5% CM-cellulose and 0.5 ml of enzyme solution was measured after incubation of the solution for 10 min at 37° and the value ($1/\eta_{sp}$) where η_{sp} is the specific viscosity was calculated. The specific activity was estimated in HULTIN[6] viscometric units per mg of protein using suitably diluted enzyme. Protein was determined colorimetrically[7] using bovine serum albumin as a standard.

Molluscs were collected in autumn on the Japanese sea coast and kept for some months (at 4°) without any loss of activity. The hepatopancreas of molluscs was homogenized and extracted with 2 vol. ice water. After centrifugation (10 min, 5000

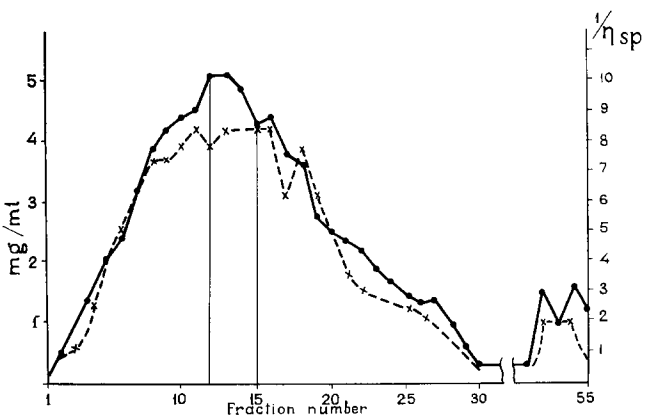

Fig. 1. Gel filtration on Bio-Gel P-30. ●—●, protein, mg/ml; ×— — —×, CM-cellulase activity. For conditions, see text.

rev./min), the supernatant was precipitated by 3 vol. cold (−10°) acetone. The precipitate was separated by centrifugation and then diluted with 1 vol. water (buffer); the insoluble part was removed. The preparation obtained showed both the CM-cellulase and the cellobiase as well as laminarinase, alginase, pectinase, fucoidinase, and maltase activities. Assay procedures were: for laminarinase, the measurement of reducing sugar, produced from laminarin[8]; for alginase and pectinase, the viscosi-

metrical measurement under conditions identical to those described above but using 0.1% substrates; for fucoidinase and maltase, the identification of mono- and oligosaccharides in the reaction mixture by paper chromatography. There was no enzyme acting upon native cellulose. CM-cellulase in solution does not undergo any changes upon freezing or at room temperature for some days. Loss of activity was observed after heating to 60° and more. The pH optimum of the preparation was within the range pH 5–6. At a final concentration of 5 mM, Ag^{+}, Hg^{2+} and Fe^{3+} caused a sharp decrease of cellulase activity; Ca^{2+}, Co^{2+} and Mg^{2+} slightly increased it.

Aliquots of the enzyme solution (600 mg protein) were layered on a Bio-Gel P-30 column (4.0 cm × 75 cm packed volume) pre-equilibrated with 0.01 M acetate buffer (pH 5.2) at 20°. Elution was carried out with the same buffer; fractions of 4 ml were collected. Some active cellulases of various molecular weights were eluted (Fig. 1). The active Fractions 12–15, including CM-cellulose, hydrolyzed cellobiose, laminarin and maltose. For further purification, the lyophilized fractions were placed on a Bio-Gel P-200 column (2.4 cm × 40 cm packed volume), pre-equilibrated with the same

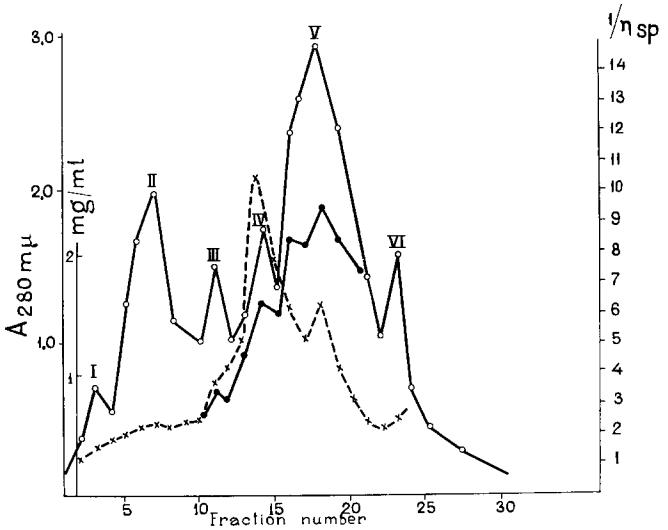

Fig. 2. Gel filtration on Bio-Gel P-200. ○—○, absorbance at 280 mμ; ●—●, protein, mg/ml; ×— — —×, CM-cellulase activity. For conditions, see text.

buffer; fractions of 5 ml were collected. Fig. 2 illustrates a typical elution pattern. Peak IV showed the highest cellulase activity. It did not hydrolyse laminarin and maltose. It had a low cellobiase activity. The final step of purification procedure involved repeated gel filtration on Bio-Gel P-200. In this step mercaptoethanol ($0.5 \cdot 10^{-3}$ M), Ca^{2+} and 5% saturated ammonium sulfate were added for stabilizing cellulase. The CM-cellulase thus obtained showed approx. 70-fold increase in specific activity. The exact calculation of activity increase was complicated because the initial activity of the preparation included the total activity of all the cellulases of the mixture.

The results of a typical purification are shown in Table I. The cellulase obtained

TABLE I

PURIFICATION OF CM-CELLULASE FROM HEPATOPANCREAS OF LITTORINA SP.

Purification step	*Total protein (mg)*	*Specific activity (Hultin units/mg protein)*	*Total enzyme units*	*Purification*	*Recovery of enzyme (%)*
Acetone precipitation	604	720	434 000	(1)	100
Column Bio-Gel P-30	72	4 010	288 000	5.6	66.4
Column Bio-Gel P-200 Peak IV	7.4	12 100	89 500	16.8	20.6
Repeated Column Bio-Gel P-200	0.7	50 800	35 560	70.6	8.2

migrated as a single band on disk electrophoresis in a polyacrylamide gel[9] and was shown to be homogeneous according to ultracentrifugation data.

The above mentioned action of metal ions for crude cellulase was also maintained for the purified enzyme, except Ag^+ which inhibited it at a final concentration of 10 mM.

The purification of other glucosidases is in progress.

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The effect of ^{60}Co γ-irriadation on the tryptic digestion of phosphorylase *b*

Tryptic digestion is a useful method for studying the steric conformational changes of proteins[1]. In the present work we investigated the effect of different allosteric effectors on the tryptic digestion of phosphorylase *b* (α-1,4-glucan:orthophosphate glucosyltransferase, EC 2.4.1.1) before and after ^{60}Co γ-irradiation. This represents a logical extension of our earlier experiments which indicated a higher radiation sensitivity for allosteric than for catalytic sites[2,3].

The procedures for the preparation and purification of rabbit muscle phos-