Studies on the preparation and on the properties of sea snail enzymes

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Introduction

The isolation and cultivation of somatic cells and protoplasts have recently become useful techniques in the genetic study and selective breeding of higher plants. Cocking (1960) has successfully isolated plant protoplasts using an enzymatic method involving cellulose. In our study we have been able to extract enzymes from the digestive system of the sea snail, *Turbo* sp., which can effectively degrade the cell walls of many marine algae.

Methods and materials

The sea snails were collected from the coastal area in Qingdao, China, and were starved for 2–3 days before use. After washing, the shells were broken and the digestive organs were removed. The procedure for preparing the enzymes is as follows:

Digestive organs of sea snail
↓ Homogenization and extraction in 0.1 M phosphate buffer, pH 7.2, at 4 °C. Centrifugation at 20 000 × g for 30 min.
Supernatant I
↓ 30% saturation Ammonium sulfate
Supernatant II
↓ 90% saturation Ammonium sulfate
Precipitate
↓ Dissolution in distilled water; desalting by dialysis
Enzyme solution
↓ Lyophilization
Sea snail enzymes

The obtainable yield of enzymes was rather high as shown in Table 1.

Results and discussion

Enzyme assays showed that the sea snail enzyme complex consists of cellulase, β-galactosidase, xylanase, amylase, alginase, and protease activities as shown in Table 2. The determination of these enzyme activities was based on their specific catalytic reactions (Bailey & Nevainen 1981).

Three enzyme fractions were precipitated from Supernatant I by the addition of increasing quantities of ammonium sulfate. After each addition, the solution was stirred overnight at 4 °C and then centrifuged. More ammonium sulfate was then added to the supernatant solution as indicated in Table 3. The sediment was dissolved in distilled water, and the enzyme activities were determined.

Disc polyacrylamide gel electrophoresis was performed according to Davis (1964). Following electrophoresis at 2 mA gel^{-1} for 3 h, one gel was sliced and each slice was assay for enzyme activities, while the duplicate gel was stained with the protein stain Amido Black. As seen in Fig. 1, approximately 14 protein bands were observed corresponding to the distribution of enzyme activities on the gel. The enzyme activities were indicated by appearance of optical density during exposure to the appropriate reaction systems: β-galactosidase, $A_{410\,nm}$; alginase, xylanase, amylase and cellulase, $A_{540\,nm}$.

The effect of the enzymes on the decomposition of the cell walls of vegetative cells of *Porphyra suborbiculata* is shown in Fig. 2. We used the *Turbo* enzymes to isolate individual cells from *Laminaria, Undaria* and *Gracilaria,* obtaining the same results.

When the individual *Porphyra* cells were cultured under controlled conditions, they grew into normal thalli.

Hydrobiologia 116/117, 319–320 (1984).

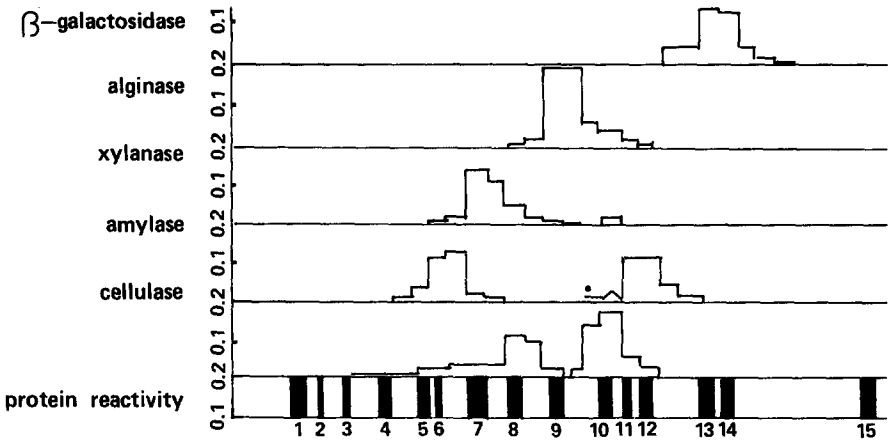

Fig. 1. Disc electrophoresis of sea snail enzymes.

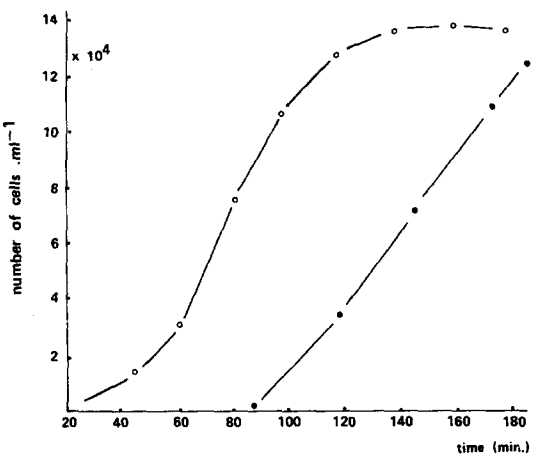

Fig. 2. The effect of enzymes on decomposition of the cell walls of *Porphyra* (O—O—, isolated cells; ·—·—, de-walled cells).

Table 1. The obtainable yield of sea snail enzymes.

No.	Date	Digestive organs (g wet wt)	Enzyme preparation (g)	Protein content mg g^{-1}	Yield (%)
1	18. i.1981	76	0.97	923	1.3
2	30.vii.1981	168	2.68	951	1.6
3	1.xii.1981	84	1.23	960	1.5

Table 2. Component activities of the sea snail enzyme complex.

No.	*Enzyme activity:* Cellulase	β-galactosidase	Xylanase	Amylase	Alginase	Protease
1	1.68	1 321	24.2	3.8	40.3	0.09
2	1.13	1 577	33.5	1.2	75.0	0.11
3	1.29	1 645	28.6	4.3	52.6	0.16

Table 3. The distribution of enzyme activities precipitated at increasing concentrations of ammonium sulfate.

Ammonium sulfate saturation	*Enzyme activities of sediment:* Cellulase	β-galactosidase	Xylanase	Amylase	Alginase
30–40	0.42	1 099	3.2	0.6	3.9
40–75	1.87	2 791	44.3	7.4	52.6
75–90	1.75	987	39.8	3.3	337.0

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