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SECRETION OF AN ESTERASE FROM THE CULTURED SUSPENSION CELLS OF *MARCHANTIA POLYMORPHA*

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Key Word Index—*Marchantia polymorpha*; liverworts; cultured cells; secretion; esterase.

Abstract—An esterase in the cultured suspension cells of *Marchantia polymorpha* was secreted into the culture medium in which the cells were growing. The esterase, which hydrolyses naphthyl acetate, was partially purified. It was a single polypeptide M_r 40k on a molecular sieve column and on SDS–polyacrylamide gel electrophoresis.

INTRODUCTION

Plant cell cultures such as *Nicotiana tabacum* [1, 2] and *Marchantia polymorpha* [2, 3] have the ability to hydrolyse acetates to the corresponding alcohols. The hydrolysis of acetates with cultured cells of *M. polymorpha* was fast [3], and this was presumed to a result of the secretion of esterases into the medium. Following various reports that peroxidases in cultured cells of carrot [4, 5], phosphatases in cultured cells of tobacco and carrot [6, 7], glucosidases in cultured cells of rice [8] and ascorbate reductases in cultu ed cells of pumpkin [9] can be secreted from plant cell cultures, we now provide evidence in this paper that an esterase is secreted from the cultured cells in *M. polymorpha.*

RESULTS AND DISCUSSION

The presence of esterase, peroxidase, alcohol dehydrogenase, catalase and NADH–cytochrome reductase in the culture medium of the suspension cultures of *M. polymorpha* and the cytoplasmic fraction of the cells was examined by procedures described in the Experimental section. The activities of esterase and peroxidase were observed in the culture medium, as shown in Table 1, but catalase (a marker enzyme of microbodies [10]), NADH–cytochrome reductase (a marker enzyme of mitochondria [11]), alcohol dehydrogenase (a cytosolic enzyme [12]) were not detected in the medium, although these were present in the cytoplasm.

Figure 1 shows the time course of the esterase activity and the total amount of proteins in the medium of the suspension cultures of *M. polymorpha.* The amount of protein increased with culture time and reached a maximum after a 10 day incubation. The esterase activity also increased with the increase in the amount of protein present in the medium.

The secreted esterase was partially purified by anion-exchange and molecular sieve chromatography; it was 17-fold pure against the culture medium (Table 2). The M_r of the esterase was estimated to be 40k by column chromatography with a TOSOH G-3000SW molecular sieve. SDS–polyacrylamide gel electrophoresis of the esterase showed one band at 40k, indicating that the esterase was a single polypeptide. This enzyme was maximally active at pH 6.5, but was not active at pH below 5 or above 8.5. The optimum temperature for the esterase activity was 40°. The enzyme in a 0.1 M phosphate buffer solution at pH 6.5 lost no activity during a 30-min pre-incubation at 40°, but 50% of the activity was lost in heating at 55°–60°.

EXPERIMENTAL

Plant material. Cultured cells of *M. polymorpha* [13] had been routinely subcultured every 2 weeks for more than 4 years using MSK-2 medium [14]. Suspension cultures were maintained by incubating about 5–8 g of the suspension cells in a 300 ml conical flask containing 100 ml fresh medium at 25° on a rotary shaker at 70 rpm under 3000 lux illumination.

Prepn of cytoplasmic fr. All procedures were performed at 4°. The cultured cells of *M. polymorpha* were sepd from the medium (35 ml) by filtering through nylon mesh. The mass of cultured cells (41 g) was washed with H_2O and homogenized in 30 ml of Tris–HCl buffer (50 mM; pH 7). The homogenates were squeezed through gauze and then filtered through a Toyo No. 2 filter paper. The filtrate (34 ml) was used as a cytoplasmic fr.

Assay of enzyme activities. The cytoplasmic fr. or culture medium (0.2 ml) was added to 2 ml of KPi buffer (0.1 M; pH 6.5) containing 3.3 mM 4-aminoantipyrine and 4.25 mM naphthyl acetate dissolved in a small

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Table 1. Total enzyme activities of esterase, peroxidase, catalase, NADH–cytochrome reductase and alcohol dehydrogenase in the culture medium and cytoplasmic fraction

Fraction	Volume (ml)	Esterase (nkat)	Peroxidase (nkat)	Catalase (nkat)	NADH–cytochrome reductase (ΔA_{550} min^{-1})	Alcohol dehydrogenase (ΔA_{340} min^{-1})
Culture medium	35	77	283	0.0	0.4	0.0
Cytoplasm	34	108	4080	117	146	0.3

Table. 2. Purification of the esterase from the culture medium of the suspension cells of *M. polymorpha*

Step	Protein (μg)	Activity (nkat)	Specific activity (nkat μg^{-1})	Purification (fold)
Culture medium*	2200	4500	2.0	1.0
Ammonium sulphate ppt.	305	1040	3.4	1.7
DEAE-Toyopearl	45	593	13	6.5
TOSOH G-3000SW XL	14	467	33	17

*1.1 l of the medium was used.

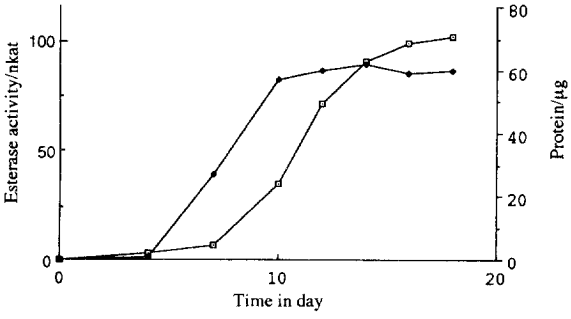

Fig. 1. Time courses of the esterase activity (—□—) and the amount of protein (—◆—) in the culture medium during growing of the cultured suspension cells.

amount of acetone. The mixt. was incubated at 35°. After 30 min, the reaction was stopped by addition of 36 mM $K_3Fe(CN)_6$ (2 ml). The enzyme activities were determined by measuring *A* 500 nm [15].

Peroxidase, alcohol dehydrogenase, catalase and NADH–cytochrome reductase activities were assayed according to the methods given in refs [16–19], respectively. The protein concn was determined with bovine serum albumin as standard by the method of ref. [20].

Time course of esterase activity. Cultured tissues of *M. polymorpha* (5 g) were transplanted to 100 ml of freshly prepd medium in a 300 ml conical flask and the cultures were incubated at 25° on a rotary shaker under illumination. At intervals, one of the cultures was filtered through nylon mesh. The protein concn and esterase activity of the cultured medium were determined as described above, and are shown in Fig. 1.

Purification of esterase. The culture medium (1.1 l) filtered from the suspension cultures was treated with $(NH_4)_2SO_4$. The protein pptd between 40–80% satn was collected and dissolved in 10 mM Tris–HCl buffer (0.5 ml; pH 7). The soln was subjected to a DEAE-Toyopearl column (1.5 × 30 cm) that had been equilibrated with the above buffer. After elution of the unabsorbed proteins, the proteins were eluted with 10 mM Tris–HCl buffer (pH 7) containing NaCl (0 to 0.5 M linear gradient) at *ca* 0.5 ml min^{-1}. Frs (2 ml each) were collected and then each fr. was subjected to assay. The active frs were filtered through a Millipore membrane (0.45 μm), and further purified by HPLC equipped with a TOSOH G-3000SW XL column equilibrated with 10 mM Tris–HCl buffer (pH 7) containing 0.3 M NaCl. The M_r of the esterase was estimated by the elution profiles on HPLC. Thyroglobulin (M_r 670k), ferritin (M_r 440k), catalase (M_r 232k), aldolase (M_r 158k), bovine serum albumin (M_r 67k), ovoalbumin (M_r 43k), chymotrypsin (M_r 25k) and RNase (M_r 13.7k) were used as standards.

SDS–PAGE of the esterase was run in a gel containing 12.5% (w/v) polyacrylamide and 0.1% (w/v) SDS by the method of ref. [21]. Prior to electrophoresis, the esterase was heated at 100° for 10 min in 0.01 M Tris–HCl buffer, pH 7, containing 2% (w/v) of SDS, 2% (w/v) of mercaptoethanol, and 5% (w/v) of glycerol. The gels were stained with Coomassie Brilliant Blue R. Phosphorylase B (M_r 97.4k), bovine serum albumin (M_r 67k), carbonic anhydrase (M_r 31k), trypsin inhibitor (M_r 21.5k) and lysozyme (M_r 14.4k) were used as standards.

Thermal properties. The optimum temp. on enzyme activity and the thermal stability were examined at pH 6.5 with naphthyl acetate as a substrate at 20–70°.

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