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PHOSPHODIESTERASES OF TOBACCO CELL CULTURES AND THE INTACT PLANT

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Key Word Index—*Nicotiana tabacum*; Solanaceae; tobacco; cell tissue culture; phosphodiesterase; phosphatase.

Abstract—Tobacco cells grown in suspension culture contained a higher specific activity of acid phosphodiesterase than tissues of the intact plant. The activity of the cells increased with age. The presence of alkaline phosphodiesterase both in the cultured cells and the intact plant was demonstrated, and the properties were partially characterized.

INTRODUCTION

In a previous report [1], the purification and properties of acid phosphodiesterase from cultured tobacco cells were reported. The enzyme has a pH optimum of *ca* 6, and is fully active in the presence of EDTA. The phosphodiesterase also shows pyrophosphatase activity, and hydrolyzes various phosphodiesters and pyrophosphate bonds. However, it does not hydrolyze RNA or DNA. The enzyme hydrolyzes the 5′-terminal methylated blocked structures of cytoplasmic polyhedrosis virus *m*RNA and tobacco mosaic virus RNA [2, 3]. It is probable that the acid phosphodiesterase is involved in regulation of the activity of *m*RNA in the tobacco cells by splitting the 5′-terminal blocked structure of the RNA. The enzyme can be easily purified to a homogeneous state, and so it may be a useful tool in studies on the structure and biological functions of 5′-terminal modification of nucleic acids.

In this report, changes in the acid phosphodiesterase activity during growth of the cells in culture, and comparison of the activity between the cultured cells and the intact plant, were investigated. The presence of alkaline phosphodiesterase in the cultured cells was also demonstrated.

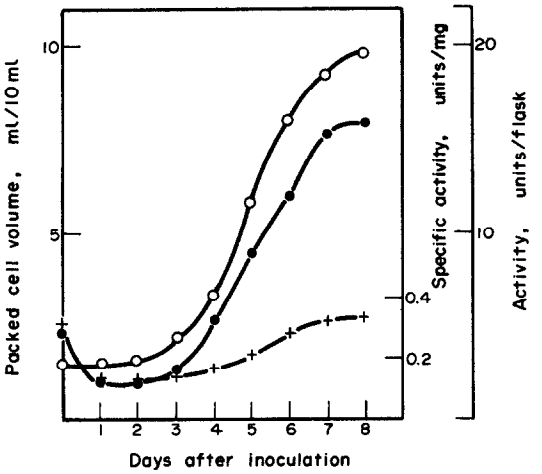

Fig. 1. Growth of tobacco cells in batch suspension culture and phosphodiesterase activity. Cells were grown in 1 l. of culture medium and samples were removed from the culture every day during the growth for measurement of packed cell volume and for extraction of enzyme. ●, Acid phosphodiesterase activity per culture flask (units/flask); +, Acid phosphodiesterase activity per mg extracted protein (units/mg); ○, packed cell volume (ml/10 ml).

RESULTS AND DISCUSSION

Acid phosphodiesterase activity during the growth of the tobacco cells

Changes in the activity of acid phosphodiesterase assayed with *p*-nitrophenyl thymidine 5′-phosphate as substrate during the growth of the tobacco cells in batch suspension culture for 8 days, are shown in Fig. 1. The enzyme activity, expressed per culture flask or per unit extracted protein, decreased at the first day of the culture, and then increased in accordance with the increase of packed cell volume and reached a maximum at day 8.

Comparison of acid phosphodiesterase activity between cultured cells and the intact plant

Analyses of enzyme extracts prepared from leaves and roots of the tobacco plant and from cultured cells, showed that the cultured cells contained higher sp. act. of acid phosphodiesterase than the intact plant (Table 1). This indicates that the cultured cells are superior to the intact plant as a source of the phosphodiesterase.

It has been reported that tobacco cells in culture were susceptible to infection with tobacco mosaic virus [4], but the cells achieve only a low level of infection and multiplication of the virus. The tobacco mosaic virus RNA lacking 5′-terminal blocked structure by the action of the tobacco acid phosphodiesterase has been shown to have no infectivity [3]. Therefore, it is probable that the low efficiency of infection with the virus to tobacco cells in culture is related to their high activity of the acid phosphodiesterase.

Table 1. Phosphodiesterase activities in different parts of tobacco

Plant part	Acid phosphodiesterase activity (m units/mg protein)	Alkaline phosphodiesterase activity (m units/mg protein)
Cultured cells	153	40.3
Leaf	24.7	5.3
Root	37.2	40.8

As described previously [1], acid phosphodiesterase activity of the extract from the cultured cells was separated into several fractions by DEAE-cellulose column chromatography. The analysis of the extract from the cultured cells by the column, previously equilibrated with 10 mM Tris–HCl buffer (pH 7.5), showed that *ca* 90% of the enzyme activity applied was passed through the column, and only 10% was adsorbed to the column. On the other hand, when the extract from leaves of the plant was applied to the identical column, 45% of the activity was passed through and the other 55% was retained to the column. These results suggest that cultured cells and leaves of the plant are also different in relative content of multiple forms of acid phosphodiesterase.

Alkaline phosphodiesterase in the cultured cells

The presence of alkaline phosphodiesterase that is active at alkaline pH in the presence of metal ion has been demonstrated in several plants [5–7]. The alkaline phosphodiesterase activity was also detected in the extract from the cultured cells as shown below.

The enzyme extract was chromatographed on Sephadex G-200, and the result is shown in Fig. 2. The column fractions were assayed using *p*-nitrophenyl thymidine 5′-phosphate as substrate at pH 5 in the presence of EDTA, and at pH 9 in the presence of $MgCl_2$. Three peaks of the enzyme activities were observed as shown in Fig. 2, and designated as I, II and III.

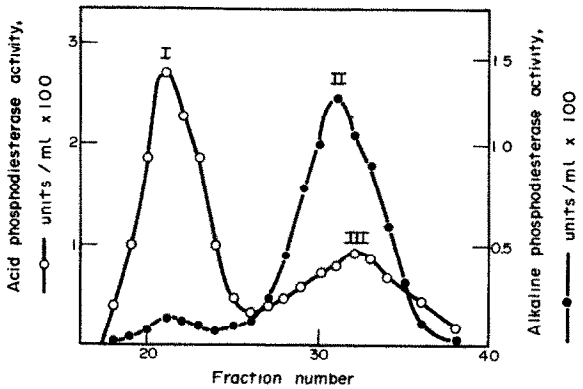

Fig. 2. Chromatography of phosphodiesterases from cultured tobacco cells on a Sephadex G-200 column (1.6 × 70 cm). The column was equilibrated and eluted with 10 mM Tris–HCl buffer (pH 7.5) containing 0.2 M NaCl. Phosphodiesterase activities were assayed with 0.5 mM *p*-nitrophenyl thymidine 5′-phosphate as substrate in 0.1 M acetate buffer (pH 5) containing 10 mM EDTA (○), and in 0.1 M Tris–HCl buffer (pH 9) containing 10 mM $MgCl_2$ (●).

The elution position of peak I corresponded to the acid phosphodiesterase that was previously reported [1]. The enzyme also showed slight activity at pH 9.

The enzyme in peak II was alkaline phosphodiesterase. The fractions represented by peak II showed optimum activity of phosphodiesterase at about pH 9. The activity was inhibited by the addition of 10 mM EDTA or 10 mM 2-mercaptoethanol to the reaction mixture. The enzyme showed little activity toward *p*-nitrophenyl thymidine 3′-phosphate. The mw of the alkaline phosphodiesterase was tentatively estimated to be 70000 to 80000 by the gel filtration on Sephadex G-200 using human γ-globulin, bovine serum albumin and ovalbumin as standard marker proteins. As shown in Table 1, the sp. act. of alkaline phosphodiesterase in the extract from cultured cells was similar to that from roots, and *ca* 7 times as high as that from leaves.

Peak III was distinct from peak II in elution position. And the enzyme activity in peak III was not inhibited by the addition of 10 mM EDTA. Therefore, the activity in peak III is not due to alkaline phosphodiesterase. The enzyme in peak III showed *ca* 25 times higher activity toward *p*-nitrophenyl phosphate than *p*-nitrophenyl thymidine 5′-phosphate. The elution profiles assayed with both substrates exactly coincided. Therefore, peak III is considered to be due to the acid phosphatase. Acid phosphatase further purified by DEAE-cellulose and hydroxylapatite column chromatography also showed slight activity toward *p*-nitrophenyl thymidine 5′-phosphate.

EXPERIMENTAL

Tobacco cells (*Nicotiana tabacum* cv Bright Yellow) were grown in suspension as described [1]. The tobacco plants were grown in a greenhouse for 3 months after germination. Enzyme extracts from the cells or tissues of the plant were prepared by homogenization in 10 mM Tris–HCl buffer (pH 7.5) containing 14 mM 2-mercaptoethanol and 10% (w/v) Dowex 1 × 8 chloride (200–400 mesh). The homogenate was centrifuged at 15000 *g* for 20 min and the supernatant was dialyzed against 10 mM Tris–HCl buffer (pH 7.5), and the dialyzate was used for expts. Acid phosphodiesterase activity was assayed with *p*-nitrophenyl thymidine 5′-phosphate as substrate as described previously [1]. Alkaline phosphodiesterase activity was assayed in a mixture that contained 0.5 mM *p*-nitrophenyl thymidine 5′-phosphate, 0.1 M Tris–HCl buffer (pH 9) and 10 mM $MgCl_2$ in a total vol. of 1 ml. After incubation for 15 min at 30°, the reaction was stopped by the addition of 2 ml of 10 mM EDTA in 0.1 N NaOH. The *A* at 400 nm was measured. One unit of the enzyme activity is defined as the amount that catalyzes the formation of 1 μmol of product under the conditions. Acid phosphatase activity was assayed as described [1]. All the other methods were as described previously [1].

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