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PHOSPHORYLASE ISOENZYMES IN *POLYTOMA UVELLA*

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Abstract—Starch phosphorylase isoenzymes in growing cultures of *Polytoma uvella* were isolated by gel electrophoresis and DEAE-cellulose chromatography. Two forms (A and B) were found. These were not detected in the lag phase of the cultures but both forms were present in the log and early stationary phase. Initially form A was more prominent than form B. In the early stationary phase, form A decreased and only form B could be found in the older cultures.

INTRODUCTION

Multiple forms of starch phosphorylases have been demonstrated in a variety of higher plants [1–12] and their presence has been correlated with starch metabolism in most of these plants [2–4, 6, 8, 10, 11]. However, except for the observation of multiple forms of starch phosphorylase in some blue-green and green algae [13, 14], little information is available as to the presence or function of phosphorylase isoenzymes in colourless algae.

Polytoma uvella, a colourless alga, produces large amounts of starch, particularly when the cultures are in their late log and early stationary phase [15]. To understand starch biogenesis in *P. uvella*, the present communication reports the occurrence of phosphorylase isoenzymes in this alga at various stages of culture growth.

RESULTS AND DISCUSSION

When homogenates of *Polytoma uvella* cells taken at different stages of culture growth were subjected to polyacrylamide gel electrophoresis and DEAE-cellulose chromatography, no phosphorylase activity was detected on gels which contained extract from 48 hr cell cultures (Fig. 1), although some phosphorylase activity was detected in the crude extract (Table 1). It is possible that at 48 hr, when the cultures are in the lag phase, there were not sufficient cells available to obtain enough enzyme protein for detection. However, it had earlier been demonstrated that very little starch production takes place during lag phase and the enzyme activity is generally very low [15]. When cells were taken from 60 hr cultures and the extract subjected to gel electrophoresis, two phosphorylase enzyme bands were observed. The slower moving band (A) being much more prominent than the faster moving band (B) (Fig. 1). When extracts of cells in their early stationary (80 hr) and late stationary (120 hr) phase were electrophoresed, the faster moving phosphorylase isoenzyme (band B) was more prominent than the slower moving one (band A) and in 120 hr the latter isoenzyme could not be detected (Fig. 1). Similar patterns were observed on partial ammonium sulfate purification of extracts from 48, 60, 80 and 120 hr cultures and subsequent chromatography of the homogenate on DEAE-cellulose columns (Table 1). Apart from confirming the gel electrophoresis observations, it was also noted that fraction A, which was eluted from the column after fraction B, was much more active in the cells taken from 60 hr cultures. The activity of this fraction decreased in cells taken from 80 hr cultures and was not detected in 120 hr old cultures, whereas the activity of fraction B was prominent in the latter cultures (Table 1).

In *P. uvella* rapid starch synthesis takes place when the cultures are in their late log and early stationary phase,

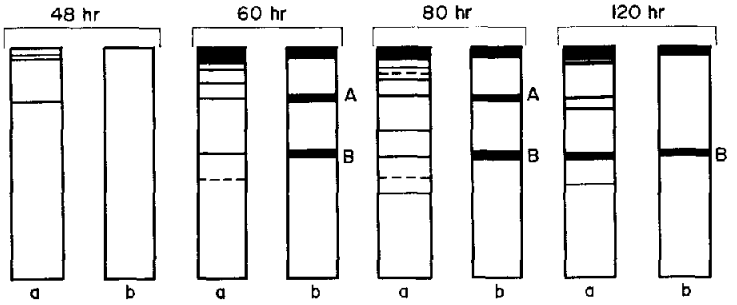

Fig. 1. Polyacrylamide gel electrophoresis. Performed by employing a 7% gel. Tris–HCl (50 mM, pH 8.3) was used as electrode and gel buffer. The gels were cut into halves; one half was stained with Coomassie blue (a) and the other (b) was incubated in a reaction mixture to test for phosphorylase activity. Details of enzyme assay are described in the text.

Table 1. Starch phosphorylases from cultures of *Polytoma uvella*

Fraction	Age of culture (hr) 48		60		80		120	
	Total activity (units*/ml)	Sp. act. (units/mg protein)	Total activity (units/ml)	Sp. act. (units/mg protein)	Total activity (units/ml)	Sp. act. (units/mg protein)	Total activity (units/ml)	Sp. act. (units/mg protein)
Crude extract	0.50	0.04	9.63	0.36	18.13	0.71	15.71	0.58
0–90% $(NH_4)_2SO_4$ fraction	u.d.	u.d.	7.62	0.72	14.56	0.80	17.05	0.64
DEAE-cellulose chromatography:								
Fraction A	u.d.	u.d.	10.04	2.64	6.42	1.97	u.d.	u.d.
Fraction B	u.d.	u.d.	8.34	1.78	11.73	2.95	12.72	2.06

* One unit of enzyme was equivalent to the liberation of 1 μmol of orthophosphate/10 min at 30°.

that is, between *ca* 58–100 hr [15]. Phosphorylase isoenzymes have been implicated in starch synthesis [3, 4, 6, 8, 10, 14, 16, 17], particularly in the early stages of polysaccharide biosynthesis [3, 10]. This would suggest that the slower moving phosphorylase band A (Fig. 1) is probably associated with the rapid starch synthesis in *Polytoma*. Tsai and Nelson had previously found two phosphorylase isoenzymes in the endosperm of *Zea mays* [3]; one of these isoenzymes appeared only at the stage of rapid starch biosynthesis and was not found during seed germination, whereas the second isoenzyme was present through all stages of endosperm development and seed germination. Furthermore, it has been shown in other higher plant tissues that during the period of starch synthesis the electrophoretically slow moving isoenzymes are much more active [8, 10, 12]. Another indication that band or fraction A (Fig. 1 and Table 1) is involved in starch biosynthesis is the fact that in *P. uvella* the amylose varies with the age of the cultures, rising from 7.5% when the cultures are in their lag phase, to 14% in the late log and early stationary phase and then dropping to 6% in the late stationary phase [15]. Thus, it is conceivable that band or fraction A is involved in the synthesis of straight chain starch polymers in this organism. However, further studies are necessary to confirm whether phosphorylase isoenzymes are responsible for the synthesis of straight-chain polyglucosides since other enzymes, particularly adenosine diphosphate glucose: starch glucosyl transferase, have been shown to be important in starch synthesis [18–20]. The involvement of one of the phosphorylase isoenzymes with the synthesis of a straight-chain polyglucoside would also confirm earlier experiments which indicated that the amylose content in *P. uvella* was correlated with the P/Q enzyme ratio [15]. Thus, the decrease in one of the phosphorylase isoenzymes may result in increased branching of the polyglucoside due to Q-enzyme activity and thereby reducing the amylose content in the starch produced. That there is a relationship between P/Q ratio and amylose content has also been noted in developing corn endosperm and potato tubers grown in sterile cultures [21].

Finally, unlike some other higher plant tissues where more than two phosphorylase isoenzymes have been observed during starch synthesis [4, 7–10], only two isoenzymes were noted in *P. uvella* (Fig. 1 and Table 1). This is in agreement with the observations made for some blue-green and green algae [13, 14].

EXPERIMENTAL

P. uvella cells were cultured as reported in ref. [22]. Cells for analysis (from 6 l. of culture material) were harvested by centrifuging the suspension at 3000 *g* for 10 min, washed ×2 in cold Tris–HCl buffer (70 mM, pH 7.5) and finally resuspended in 10–15 ml of the above buffer.

Enzyme preparation. The cells, suspended in Tris–HCl buffer, were disintegrated by sonication at 0° and the homogenate centrifuged at 15000 *g* for 20 min. The pale yellow supernatant was used in electrophoresis expts. For DEAE-cellulose chromatography the proteins in the supernatant were precipitated by adding $(NH_4)_2SO_4$ to 90% satn. The ppt. was suspended in 10 mM Tris–HCl buffer and mixed by vigorous stirring. The mixture was centrifuged at 20000 *g* for 30 min. The clear supernatant was desalted by filtration through a column (2.5 × 30 cm) of Sephadex G-25 and loaded onto a DEAE-cellulose column (1.5 × 30 cm) previously equilibrated with 0.01 M Tris–HCl buffer (pH 7.5). The column was washed with 2 bed vols. of the above buffer and the enzyme eluted using a linear NaCl gradient.

Gel electrophoresis. Polyacrylamide gel electrophoresis was carried out according to ref. [16] using 7% acrylamide. Tris–HCl (50 mM pH 8.3) was used as electrode and gel buffer. As primer for phosphorylase action, 0.1% glycogen was polymerized with the gel [1]. Electrophoresis was performed at 5° by passing 6 mA current per tube. Bromophenol blue served as front indicator. Phosphorylase activity was demonstrated by incubation of the gels for 4 hr in 25 mM glucose-1-phosphate and 20 mM NaF in 0.1 M citrate buffer (pH 5.6). The presence of phosphorylases was demonstrated by staining the gels with I_2 reagent [23]. Protein in the gel slices was detected by staining the slices with Coomassie blue followed by destaining in 7% HOAc.

Enzyme assay. Phosphorylase activity was determined according to ref. [8].

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