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α-GLUCOSIDASES OF SUSPENSION-CULTURED SUGAR-BEET CELLS

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Key Word Index—*Beta vulgaris*; Chenopodiaceae; sugar-beet; cell suspension culture; α-glucosidases

Abstract—Four α-glucosidases have been fractionated from sugar-beet cells. They are divisible into two groups in terms of soluble starch-hydrolysing activity. One group hydrolyses soluble starch at a faster rate than maltose. The enzymes of this group are not detected during the phase of starch accumulation in cells, but undergo a marked increase with the decrease of starch content. A possible role for these enzymes is in the metabolism of starch in the cell. Another group hydrolyses soluble starch only very weakly. The former group are highly active in the release of α-1,3-linked glucose from nigerose.

INTRODUCTION

The enzyme α-glucosidase (EC 3 2 1.20) is ubiquitous in higher plants. It has been suggested that α-glucosidase forms a part of the non-phosphorolytic pathway for the breakdown of starch, and functions in seed germination by hydrolysing the oligosaccharides produced by α- and β-amylases [1, 2]. However, α-glucosidases from rice seeds, germinated green gram and sugar-beet seeds readily hydrolyse soluble starch, liberating glucose [3–6]. In higher plants, an α-glucosidase that hydrolyses soluble starch weakly is found only in sugar-beet seeds [5]. The *in vivo* significance of glucose production from soluble starch in plant tissue by α-glucosidase is not yet clear.

The α-glucosidases from sugar-beet seeds hydrolyse soluble starch at a faster rate than maltose [5, 6], as if they are glucoamylases. This strong hydrolysing activity suggests that, like glucoamylase, the enzymes may even hydrolyse starch to glucose without the preceding action of α- and β-amylases in the plant tissue. On the other hand, another type of α-glucosidase, which hydrolyses soluble starch only very weakly, has also been isolated from sugar-beet seeds at the same time [5]. The two enzymes from sugar-beet seeds exhibit very different properties, particularly in their soluble starch-hydrolysing activity. They may be located in a different part of the plant tissue and play different roles there. Therefore, sugar-beet seeds offer an excellent system with which to examine the physiological function of α-glucosidase.

Plant tissue cultures are often useful in studying the various physiological phenomena of higher plants. However, there are no reports on α-glucosidase in cultured sugar-beet cells.

When sugar-beet cells are grown in the medium of Murashige and Skoog [7] with shaking, they produce some α-glucosidases in the cell. A fraction of them can be extracted from the cells with buffer and most of them with sodium chloride. This paper deals with the partial purification and properties of the α-glucosidases produced by suspension-cultured sugar-beet cells.

RESULTS AND DISCUSSION

Isolation of four α-glucosidases

Fractions I and II were extracted from the cells with buffer and sodium chloride, respectively, (see Experimental). The α-glucosidase activity of Fraction I was much lower than that of Fraction II (Fig. 1) In other words, most of the α-glucosidase in the cell required sodium chloride to be solubilized, suggesting that α-glucosidase in sugar-beet cells is bound mainly to the cell wall via ionic interactions. In cultured cells of rice [8] and soybean [9], however, the total α-glucosidase activity of Fraction I is similar to that of Fraction II. Therefore, the distribution of α-glucosidases in sugar-beet cells appears to be different from that of the enzymes in rice and soybean cells.

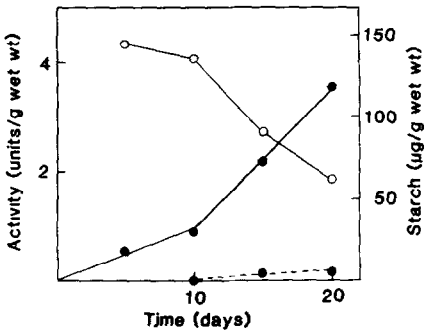

Fig. 1. Changes in the α-glucosidase activity and the starch content in cultured sugar-beet cells. Sugar-beet cells grown in the liquid medium of Murashige and Skoog were harvested at periodic intervals. ● --- ●, α-Glucosidase activity of Fraction I; ●—●, α-glucosidase activity of Fraction II; ○—○, starch content.

Fraction I from sugar-beet cells was dialysed overnight against 20 mM acetate buffer, pH 4 5 and put on CM-cellulose column (bed volume = 20 0 ml, bed height = 10 cm) equilibrated with 20 mM acetate buffer, pH 4.5 The column was first eluted with the same buffer to wash off the unadsorbed protein, and then with 300 mM NaCl in the same buffer The eluate was used as an α-glucosidase preparation (E-I) to determine the enzymatic properties Fraction II from sugar-beet cells was subjected to gel filtration on a Sephacryl S-200 HR (Pharmacia) column and divided into three fractions (E-II-1, 2 and 3) (Fig 2).

General properties of the four α-glucosidases

The pH optima of the four α-glucosidases were found to be 4.5 (E-I, E-II-1), 4.0–5 0 (E-II-2), and 4 0 (E-II-3).

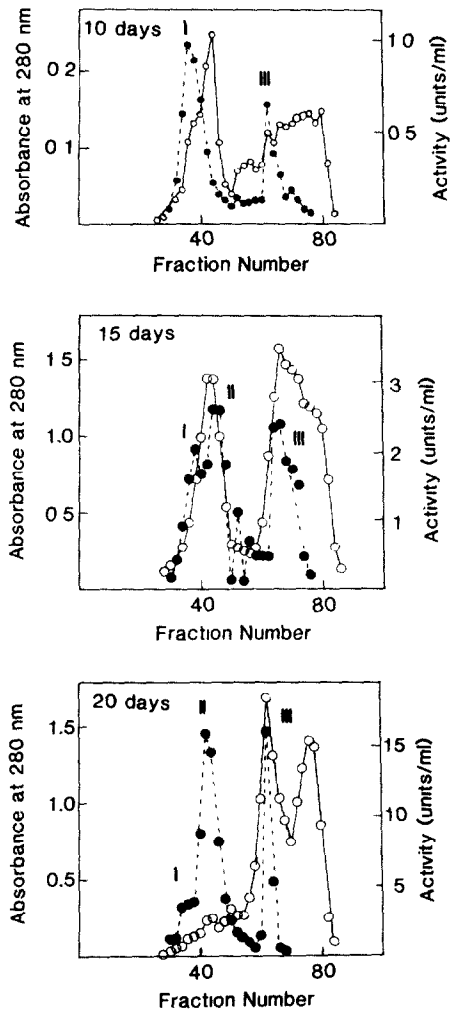

Fig 2. Gel chromatography on Sephacryl S-200 HR. Fraction II from sugar-beet cells harvested at periodic intervals was loaded on a Sephacryl S-200 HR column (bed volume = 236.5 ml, bed height = 93 cm) The column was eluted with 20 mM acetate buffer, pH 4 5, containing 150 mM sodium chloride The eluate was collected in 2 0 ml fractions ○—○, A_{280}, ●---●, α-glucosidase activity, I, E-II-1, II, E-II-2, III, E-II-3

After 20 hr preincubation at 30 with 50 mM McIlvaine's buffer, the four enzymes were stable in the pH range 4 0–7 0 The temperature optima of the four α-glucosidases were found to be 60° (E-I, E-II-2) and 55° (E-II-1, E-II-3) after 15 min incubation After 15 min preincubation with 50 mM acetate buffer, pH 4 5, at various temperatures, the four enzymes were stable at temperatures up to 60° (E-I) and 50° (E-II-1, E-II-2, E-II-3)

Substrate specificity

The activity of the four α-glucosidases on various substrates was examined and the relative rates of hydrolysis are listed in Table 1 E-I and E-II-2 readily hydrolysed maltose, nigerose, maltotriose, and soluble starch E-II-1 and E-II-3 also hydrolysed maltose, nigerose, and maltotriose, but hydrolysed soluble starch very weakly α-Glucosidases from *Aspergillus* species [10–12], *Bacillus cereus* [13] and sugar-beet seeds [5] hydrolyse soluble starch very weakly or not at all E-II-2 hydrolysed soluble starch at a faster rate than maltose This kind of α-glucosidase is found only in sugar-beet seeds [5, 6]

It is reported that two α-glucosidases are involved in the processing reaction in the biosynthesis of the oligosaccharide portion of *N*-linked glycoprotein [14] One of them releases α-1,3-linked glucose residues from lipid-linked saccharide intermediates E-I, E-II-2, and E-II-3 were highly active for release of α-1,3-linked glucose from nigerose suggesting that the three enzymes may be involved in the processing reaction in the biosynthesis of the oligosaccharide portion of *N*-linked glycoprotein (Table 1).

Changes in the activities of the four α-glucosidases during growth of suspension-cultured sugar-beet cells

The activities of the four α-glucosidases from sugar-beet cells on different days of cell culture are shown in Fig 1 and Table 2 The α-glucosidase activity of Fraction II underwent a marked increase from days 10 to 20 of culture. However, the starch content of the cells decreased markedly. The enzyme activity of Fraction I was much lower than that of Fraction II The results imply that the α-glucosidase of Fraction II may be involved in the hydrolysis of starch α-Glucosidase of Fraction II was divided into E-II-1, E-II-2, and E-II-3 (Fig 2) E-II-2 was not detected in Fraction II obtained from 10-day-old cell cultures Subsequently, however, E-II-2 underwent a marked increase from days 10 to 20 of cell culture and showed the highest activity of the three fractions after 15 days (Table 2) The increase of E-II-2 resulted in an

Table 1 Substrate specificity of four α-glucosidases

	Relative rate of hydrolysis (%)			
	E-I	E-II-1	E-II-2	E-II-3
Maltose	100	100	100	100
Isomaltose	28 1	10 6	29 9	21 4
Nigerose	117 5	34 1	97 8	60 7
Maltotriose	108 2	74 0	113 4	71 4
Panose	32 9	22 8	31 8	31 0
Soluble starch	87 0	3 2	119 6	12 0

Table 2. Changes in four α-glucosidases during growth of sugar-beet cells

	Days of cell culture		
	10	15	20
E-I	0.01	0.13	0 15
E-II-1	0.34	0.31	0 19
E-II-2	trace	0.51	1 27
E-II-3	0.12	0.41	0.66

The activity was shown as units per g of cell

increase of the enzyme activity of Fraction II (Fig. 1). E-II-2 hydrolysed soluble starch at a faster rate than maltose (Table 1). Therefore E-II-2 in Fraction II is involved in the hydrolysis of starch

EXPERIMENTAL

Cell culture. The stem of sugar-beet (*Beta vulgaris* L. cv. Tsukisappu) was explanted onto the basal medium of Murashige and Skoog [7] containing 3% sucrose as the carbon source and 4.52 μM 2,4-dichlorophenoxyacetic acid as a growth regulator. The induced callus was carried through more than 30 transfers and then inoculated in the liquid medium of Murashige and Skoog. The sugar-beet cells were maintained in darkness for many generations and then used for the experiments. Suspension cells (*ca* 0.7 g wet wt) were inoculated into 125 ml of the medium in 500 ml conical flasks and grown with gentle shaking (88 strokes per min) at 25° in the dark The cells were harvested at periodic intervals and stored at −20°.

Preparation of fractions I and II. The cells were suspended in 25 mM acetate buffer, pH 4.5, and disrupted by sonication (20 kHz, model 5202 PZT, Ohtake Works, Tokyo) for 15 min at 0°. Complete disruption of the cells was confirmed by light microscopy. The homogenate was centrifuged at 8000 *g* for 10 min and the supernatant (Fraction I) was collected. The ppt of cell wall debris was washed, and suspended in 25 mM acetate buffer, pH 4.5, containing 2 M NaCl and stirred overnight. The suspension was centrifuged at 8000 *g* for 10 min and the supernatant (Fraction II) was collected. Fractions I and II were brought to 0.9 saturation with $(NH_4)_2SO_4$. Each ppt. was collected by centrifugation and dissolved in 25 mM acetate buffer, pH 4.5

Assay of α-glucosidase Maltose (2.8 μmol) was used, together with 50 mM acetate buffer, pH 4.5, and suitably diluted enzyme prepn in a total vol of 0.5 ml After 1 hr at 37°, the reaction was stopped by boiling for 5 min The amount of glucose formed was measured by the method of ref [15], as modified in [16] One unit of activity was defined as the amount of enzyme which liberates 1 μmol/hr of glucose from maltose under the conditions described above

Assay of starch Cell debris removed from Fractions I and II was treated with α-amylase (Sigma Chemical Co , pancreas type I-A) and then purified glucoamylase from *Aspergillus awamori* [12] Glucose liberated was determined by the glucose oxidase–peroxidase method as described above

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