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WALL-BOUND α-GLUCOSIDASE OF SUSPENSION-CULTURED RICE CELLS

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Key Word Index—*Oryza sativa*; Gramineae; suspension-cultured cells; wall-bound α-glucosidase.

Abstract—Wall-bound α-glucosidase showed similar properties to other α-glucosidases produced by suspension-cultured rice cells except for weak soluble-starch hydrolysing activity. The wall-bound enzyme could not be solubilized from wall pellets with high salt concentrations, detergents or a combination of 8 M urea and 0.1 M sodium sulphite. Three carbohydrates susceptible to α-glucosidase digestion were also contained in the wall pellets. They were composed mainly of α-linked glucose residues, but gave a negative iodine test. One of them is maltotetraose, the others are polysaccharides.

INTRODUCTION

α-Glucosidase (EC 3.2.1.20) 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]. In addition, it is reported that two membrane-bound α-glucosidases are involved in the processing reaction in the biosynthesis of the oligosaccharide portion of *N*-linked glycoprotein [3]. However, there are few reports on wall-bound α-glucosidase. On the other hand, many wall-bound carbohydrases are reported, some, or all, of which are involved in cell wall autolysis [4–13]. Although wall-bound α-glucosidase may also be involved in cell wall autolysis as other wall-bound carbohydrases, a carbohydrate susceptible to α-glucosidase digestion is not found in cell walls.

When rice cells are grown in R-2 medium [14] with shaking, they produce four α-glucosidases and secrete one of them into the culture medium. The other two enzymes can be extracted from the cells with buffer and NaCl [15] and will be involved in the breakdown of starch with α- and β-amylases. After the solubilization of the two enzymes, the cell wall pellets contain much α-glucosidase activity and carbohydrate susceptible to α-glucosidase digestion. Thus, the fourth enzyme, which is not solubilized with NaCl, may be firmly bound to the cell wall and involved in its metabolism. The present study was undertaken to determine the properties of wall-bound α-glucosidase and carbohydrate susceptible to digestion by the enzyme, which were located in rice cell wall pellets.

RESULTS AND DISCUSSION

Occurrence of wall-bound α-glucosidase

The washed cell wall pellets (P-I) obtained after extraction of the soluble fraction with NaCl showed high α-glucosidase activity (Table 1). P-I was treated with Driselase, a crude fungal cellulase preparation, and pancreatic α-amylase (Sigma) to remove cellulose and starch.

Table 1. Activity of four α-glucosidases produced by rice cells

	α-Glucosidase activity (units/g wet wt of cells)
Culture filtrate	0.29
Buffer soluble fraction	0.57
NaCl soluble fraction	0.51
Cell wall pellets	8.87

Driselase is composed of cellulase, xylanase and laminarinase, in a ratio of 100:51.3:8.6, respectively. Although 69.1% of the carbohydrates in P-I were digested by Driselase, most of the α-glucosidase activity remained in the pellets (P-II). α-Amylase also released 52.9% of the carbohydrates from P-II, but the α-glucosidase activity in P-II was left intact as in the Driselase treatment. After extensive treatment with their enzymes, the cell wall pellets (P-III), in which 16.4% of the carbohydrates in P-I remained, contained glucose, arabinose, xylose and galactose (Table 2). Thus, P-III will contain noncellulosic glucan, xyloglucan and arabinogalactan, which are polysaccharides of cell wall, suggesting that α-glucosidase is firmly bound together with their polysaccharides. P-III was used as an α-glucosidase preparation to determine the enzymatic properties. Extensive treatment of P-I with detergents, Triton X-100, deoxycholic acid and Nonidet P-40, did not release any α-glucosidase activity from P-I indicating that contamination with membrane components was essentially absent in P-I. Sorghum grain α-glucosidase, which is extensively crosslinked by disulphide bonds, is also insoluble in water. However, since the insolubility of the enzyme is due to the disulphide bonds the enzyme is effectively solubilized from the grain by splitting the bonds with a combination of 8 M urea and 0.1 M sodium sulphite [16]. α-Glucosidase in P-I is

Table 2. Glycosyl compositions of cell wall pellets

Glycosyl residue	Wt % of total sugars I*	II*	III*	IV*	V*
Glucose	54.4	93.1	95.7	99.0	100
Arabinose	21.4	1.6	2.7	0.3	0
Xylose	17.5	3.4	1.6	0.7	0
Galactose	6.7	1.9	tr	tr	0

I*, cell wall pellets obtained after treatment with Driselase and α-amylase.
II*, precipitate obtained after neutralization of cold alkali-solubilized fraction.
III*, Fraction 1.
IV*, Fraction 2.
V*, Fraction 3.

Table 3. Substrate specificity of wall-bound α-glucosidase

	Relative rate of hydrolysis (%) Wall-bound α-glucosidase	*A. awamori* α-glucosidase
Maltose	100	100
Cellobiose	—	0.0
Isomaltose	33.8	28.9
Maltotriose	46.8	—
Maltotetraose	45.0	50.5
Amylose	12.9	1.2
Soluble starch	9.4	0.8
Fraction 1	—	0.6
Fraction 2	—	0.7
Fraction 3	—	45.2

different from sorghum α-glucosidase after solubilization. No α-glucosidase was solubilized from P-I after the treatment of P-I with a combination of 8 M urea and 0.1 M sodium sulphite for 2 hr at 0°. In addition, P-III was partially digested with pronase at 30° for 3 hr, resulting in the liberation of 20.6% of protein and no α-glucosidase from P-III. The results indicate that the α-glucosidase in P-I is not insolubilized via a S–S-linkage. The wall-bound α-glucosidase activity is the highest of the α-glucosidases in rice cells suggesting that the enzyme may play an important role in the cell walls (Table 1).

General properties of wall-bound α-glucosidase

The pH optimum of the enzyme was 4.0–4.5. After 20 hr preincubation at 30° with 50 mM McIlvaine's buffer, the enzyme was stable in the pH range 4.5–6.5.

The temperature optimum for the activity of the enzyme was at 50° after 15 min incubation. After 15 min preincubation with 50 mM acetate buffer, pH 5.0, at various temperatures, the enzyme was stable at temperatures up to 45°. The thermostability of the wall-bound enzyme was similar to those of the others of rice cells, although the higher thermostability of wall-bound enzymes was reported for invertases [17, 18] and β-galactosidase [12].

Substrate specificity

The enzyme activity on various substrates was examined and the relative rates of hydrolysis are listed in Table 3. The enzyme hydrolysed maltose, maltotriose and maltotetraose easily, but hydrolysed isomaltose, amylose ($\overline{Dpn}$. *ca* 18) and soluble starch more slowly. This soluble starch-hydrolysing activity of wall-bound α-glucosidase was less than half the activities of the others of rice cells [15]. This implies that wall-bound α-glucosidase may play different roles from the others of rice cells, which will be involved in the breakdown of starch. The wall-bound enzyme produced panose as the main α-glucosyl transfer product from 5% maltose as do the other glucosidase enzymes of rice cells [15].

Occurrence of carbohydrate susceptible to digestion by α-glucosidase

The washed pellets (P-IV) obtained after neutralization of the cold alkali-solublized fraction, in which 0.65% of carbohydrates in P-I remained, was easily hydrolysed by glucoamylase from *Aspergillus awamori* [19] and pancreatic α-amylase separately. The highest neutral glycosyl residue of P-IV was glucose and the content was 93.1% of the total glycosyl residues (Table 2). Since 81.5% of the glycosyl residues of P-IV are digested by glucoamylase and 93.1% by pancreatic α-amylase, the carbohydrates of P-IV are composed mainly of α-linked glucosyl residues. Wall-bound α-glucosidase also liberated glucose from P-IV suggesting that wall-bound α-glucosidase is located in the cell wall with its carbohydrates and is involved in the metabolism of these carbohydrates. When P-IV was treated with pronase, all the carbohydrates susceptible to α-glucosidase digestion were liberated into the supernatant indicating that their carbohydrates are associated with protein in the rice cell wall. The carbohydrates were subjected to gel filtration on Bio-Gel P-4 and P-100 (Bio-Rad) columns and divided into three fractions (Fractions 1, 2 and 3) (Figs 1 and 2). Each fraction was

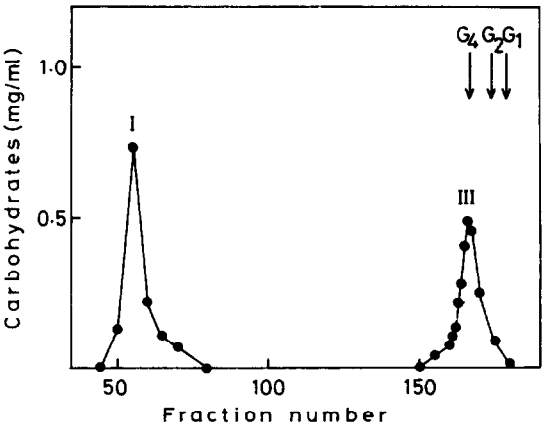

Fig. 1. Gel filtration chromatography of carbohydrates susceptible to α-glucosidase digestion on Bio-Gel P-4 after pronase digestion. Cell wall pellets were treated with pronase. After centrifugation and concentration of the solution, the supernatant was placed on the column (1.7 × 155 cm) and filtered with 0.15% acetic acid at a flow rate of 0.13 ml/min. The eluate was collected in 2.1 ml fractions and the fractions were assayed for total sugars by the pHOH–H_2SO_4 method. I, Fractions 1 and 2; III, Fraction 3; G_1, glucose; G_2, maltose; G_4, maltotetraose.

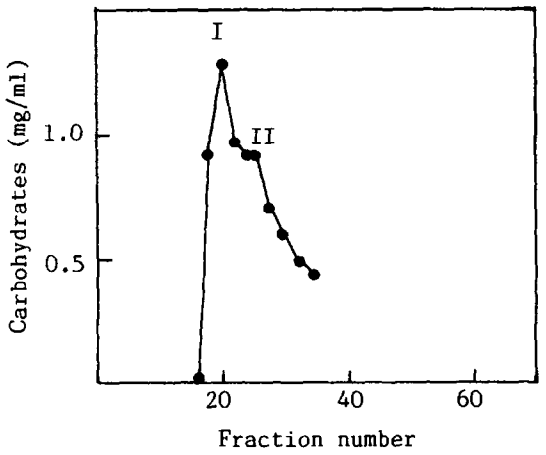

Fig. 2. Gel filtration chromatography of Fractions 1 and 2 on Bio-Gel P-100. Fractions 1 and 2 in Fig. 1 were subjected to gel filtration on a Bio-Gel P-100 column (1.7 × 62 cm). Chromatography was carried out by the same procedure as described in Fig. 1. I, Fraction 1; II, Fraction 2.

hydrolysed by wall-bound α-glucosidase. Fraction 3 emerged from the column in the same position as maltotetraose and the glycosyl residue was only glucose (Table 2). Moreover, Fraction 3 was hydrolysed by *Aspergillus awamori* α-glucosidase [19] at a similar rate to maltotetraose (Table 3). The results indicate that Fraction 3 is maltotetraose. α-Glucosidase from *A. awamori* hydrolysed Fractions 1 and 2 more slowly than amylose and soluble starch (Table 3). Since the enzymatic action of the α-glucosidase for the substrate was responsible for the polymerization, Fractions 1 and 2 will be larger polysaccharides than amylose (Dpn. *ca* 18) and soluble starch. Since Fractions 1 and 2 give a negative iodine test even in 10 times as much as the volume of soluble starch to give positive iodine test, they are not starch.

EXPERIMENTAL

Preparation of wall-bound α-glucosidase. Cell suspension cultures of rice (Norin No. 16) were cultured in R-2 medium [14]. Cells were suspended in 25 mM acetate buffer, pH 4.5 and disrupted by sonication for 15 min at 0°. Complete disruption of the cells was confirmed by light microscopy. The homogenate was centrifuged and the residue collected. The residue was washed × 3 with extraction buffer, suspended in buffer containing 2 M NaCl and stirred overnight. The suspension was centrifuged and the cell wall pellets collected.

Preparation of carbohydrate susceptible to α-glucosidase digestion. After treatment of cell wall pellets (P-I) with Driselase and α-amylase, the α-glucosidase preparation (P-III) was treated with 1 M NaOH at 0° for 30 min. The suspension was centrifuged and the supernatant collected. Using HOAc, the pH of the supernatant was adjusted to 6. The resulting pellets (P-IV) were collected by centrifugation and washed with deionized H_2O.

Assay of α-glucosidase. Maltose (13.9 μmol) was used, together with 50 mM acetate buffer, pH 4.5 and suitably diluted enzyme prepn in a total vol. of 2.5 ml. After 1 hr at 37°, the reaction was stopped by boiling for 5 min. Supernatant (0.5 ml) was pipetted out and the amount of glucose measured by the method ref. [20], as modified in [21]. One unit of activity was defined as the amount of enzyme which liberates 1 μmol/hr of glucose from 2.8 μmol of maltose in 0.5 ml of the reaction mixture under the standard conditions.

Analyses. Glucose and total carbohydrate were estimated by the glucose oxidase–peroxidase (as mentioned above) and PhOH–H_2SO_4 methods, respectively. P-I was subjected to Saeman hydrolysis [22]. Cell wall pellets were wetted with 72% H_2SO_4 and left at 30° for 45 min. The acid was then diluted with H_2O to give 2 N H_2SO_4 and heated at 100° for 4.5 hr. Other cell wall pellets (P-II–P-IV) were hydrolysed at 121° for 1 hr with 2 M TFA. Neutral glycosyl compositions were determined by GC as their TMSi derivatives. GC was carried out on glass columns (3 × 3000 mm) packed with 3% OV-1 on Chromosorb W (80–100 mesh); carrier gas, N_2 flow rate 40 ml/min; temp., 140–220° (5°/min).

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