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CELL WALL INVERTASES OF SUGAR CANE

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Abstract—A study of the cell wall invertases in the different organs of a sugar cane cultivar has been undertaken. The enzymes could not be separated from the cell wall. They are compared on the basis of optimum pH, K_m, the effect of various chemicals and the substrate specificities of the preparations. According to the results each organ appears to possess a set of cell wall invertases with predominance of a different activity in each case.

INTRODUCTION

Plant organs are recognized as different because of differences in structure, but these differences suppose that some different metabolic or biosynthetic pathways are involved in their formation. This observation does not mean, necessarily, a functional distinction after development. However, differences in composition of storage organs compared to other parts of the plant have been known from a prescientific age, when only stalks of sugar cane, or certain parts of medicinal or edible plants were used. These differences reflect, in biochemical terms a distinctive occurrence of metabolic pathways, and a different expression of the genome in each tissue. However, the extension of these differences is unknown at present. Thus it is of interest to know if the enzymes involved in the basic physiology of a plant are different in each organ of the plant. Cell wall invertases (β-D-fructofuranosidase fructohydrolases, EC 3.2.1.26) from sugar cane are thought to participate in the apoplasmic–symplasmic translocation of sucrose [1, 2]. This translocation is a basic physiological process that may be similar in the whole plant. Based on differences found in the literature [1, 3, 4] and in differences observed for apex and callus cell wall invertases [5] our laboratory has postulated the existence of differences in the cell wall invertases from each organ of the sugar cane [5, 6]. The present work is a comparative study of the cell wall invertases from each organ of a variety of sugar cane.

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RESULTS

Enzyme yields

Table 1 shows the amount of plant material used and the enzyme yield of each organ. Whatever the tissue, invertase activity occurs in each cell wall preparation.

Occurrence of firmly bound invertases in the cell walls

A whole cell wall preparation of each tissue was successively washed with buffers of different composition. The components of these buffers have been successfully used to release cell wall enzymes [7–13]. Table 2 shows the effect of the washings on the cell wall invertases. Differences in the enzyme recovery after each buffer treatment may be due to the action of the components of the buffers, the removal of substances occurring in the cell wall, the lability of the enzyme and the structure of the cell wall being conducive to a partial retention of certain components of the solutions even after several washings. Such is the case with inorganic phosphate [5]. Table 3 shows the elution of proteins and sugars during successive washings with buffers 1–4. The distinctive action of each buffer can be appreciated in the elution of proteins from root, blade, mature and immature stalk. The differential action of each buffer on sugar elution is also observable in immature stalk, blade and sheath tissues. However, none of these eluates showed invertase activity. Consequently, the invertases appear to be firmly bound to the cell wall of the sugar cane tissues.

Influence of pH and of substrate concentration

Table 4 shows the optimum pH and K_m of the cell wall invertases occurring in each tissue. Most tissues possess invertases with an optimum pH of *ca* 7.3–7.5 which is coincident with the optimum at pH 7.4 found

Table 1. Enzyme yield by each organ of sugar cane

Tissue	Fresh tissue (g)	Enzyme activity: Total (units)	Enzyme activity: Sp. act. (10^{-3} × units/g fr. wt)
Immature stalk	230	0.500	2.17
Mature stalk	470	0.207	0.44
Leaf sheaths	130	0.434	3.34
Leaf blades	315	2.025*	6.43
		2.070†	6.57
Root	40	0.570	14.25

*Enzyme determination at pH 3.55.
†Enzyme determination at pH 4.9.

Table 2. Attempts to release invertases from cell walls*

Buffer	Stalk: Mature	Stalk: Immature	Leaf: Sheath	Leaf: Blade		Root
1	0.192	0.549	0.489	1.440†	1.620‡	0.312
2	0.078	0.619	0.460	1.275	1.815	0.218
3	0.073	0.659	0.559	1.215	1.305	0.269
4	0.060	0.712	0.685	1.336	1.626	0.309
Tween	0.316	0.671	0.528	1.505	1.867	0.570

*Cell wall bound enzymes (total units).
†Enzyme determinations at pH 3.55.
‡Enzyme determinations at pH 4.9.
The composition of the buffers and the procedures are described in the Experimental.

for apex cell wall invertase of this sugar cane variety [5]. Invertases with optima at pH *ca* 3.3–4.9 occur in leaf blades and roots. These values are close to the observed optimum, pH 3.3, for callus cell wall invertases [5]. The K_ms (1 mM) of most of these tissues were of the same order as the K_m (2.85 ± 0.05 mM) of the acid invertase from callus cultures. The K_m (10 mM) of the invertase from leaf sheaths was of the same order of the K_m (42.5 ± 0.02 mM) as that determined for apex cell wall invertase of this variety of sugar cane [5] and for cell wall invertases from leaf sheaths of the variety CP 48-103 [4]. However, the differences, even among very close values of K_m, were significant at the level of 1%. Inhibitions by excess of substrate as is found with a cell wall invertase [4] from the variety CP 48-103 were not observed.

Influence of chemicals on invertase activities

Table 5 shows the behaviour of cell wall invertases from sugar cane with respect to various chemicals. This Table has been elaborated as a base to establish further differences among the invertases.

Glycosidase activities of the cell wall

Table 6 shows some sugars attacked by the cell wall glycosidases of the different tissues. Trehalase activity has been reported to be associated with the cell wall from apex tissues of this sugar cane variety [5]. As shown in Table 6 most of the tissues possess a cell wall trehalase confirming the occurrence of a bound form of this activity. Until this study only soluble trehalases were reported and studied in higher plants. Accepting that the action on β-methylfructoside is a case of invertase specificity, Table 6 provides further evidence of the differences among the cell wall invertases from different tissues.

DISCUSSION

In previous papers [5, 6], we have postulated differences among the invertases of the various organs of the sugar cane. The examination of the apex and callus obtained *in vitro* from apex of the sugar cane cv Tuc 68-19 [5] showed differences in the cell wall invertases. These results suggested that the invertases may be different in each tissue. The present examination of the remaining tissues has led to the conclusion that each organ of the sugar cane possesses cell wall invertases. However, it is difficult to assess whether an enzyme is part of the cell wall or an artifact of homogenization. The enzymes may be

Table 3. Protein (mg) and sugar (μmol) recovered from sugar cane plant tissues by treatment with buffers 1–4

	Stalk				Leaf				Root	
	Mature		Immature		Blade		Sheath			
Buffer	Sugar	Protein	Sugar	Protein	Sugar	Protein	Sugar	Protein	Sugar	Protein
1	3.82	44.0	1.89	4.00	8.50	27.0	1.60	5.30	0.48	1.10
2	2.36	8.50	1.12	4.90	2.60	7.20	0.50	4.20	0.51	4.40
3	1.10	1.40	0.57	1.59	2.60	8.00	0.41	0.70	0.31	2.30
4	0.46	2.80	2.80	12.50	3.10	10.80	0.61	8.70	0.36	4.80

A whole preparation of each tissue was used in these experiments. The composition of the buffers is described in the Experimental.

Table 4. Optimum pH and K_m of the invertases occurring in sugar cane cell walls

Tissue	Optimum pH	K_m (mM)
Immature stalk	7.50	7.67 ± 0.03
Mature stalk	7.30	2.87 ± 0.01
Leaf sheaths	7.50	1.19 ± 0.1
Leaf blades	3.55	65.8 ± 0.01
	4.90	4.85 ± 0.03
Root	3.30	3.22 ± 0.01

bound to the cell wall at the time of cell disruption by ionic interchange, by formation of a cell wall–tannin–protein [12, 13] complex or complexes with nucleic acids [14], by complexes with Ca^{2+} or similar ions or by adsorption. Any of these processes will result in an enzyme which is removable from the cell wall by a suitable treatment. These possibilities were considered in the preparation of buffers 1–4. Further, the cell wall preparations were washed with Tween 60, but even this treatment did not solubilize the invertases, minimizing the possibility of such an artifact. Thus, each sugar cane tissue possesses firmly bound cell wall invertases, and these enzymes may be occluded into the cell wall or may be linked through covalent bonds. Although the literature shows differences among the cell wall invertases from sugar cane leaf sheath [4], stalk [1–3] apex and callus [5], these studies were performed with different varieties of sugar cane, and some tissues were never examined. A global study within a variety and in the same period of growth was lacking. Consequently, the study of the cell wall invertases from variety Tuc 68-19 [5] was pursued. A group of cell wall enzymes with optimum pH *ca* 7.3–7.5 was detected. The K_ms of this group of neutral invertases were significantly different. Furthermore, the effect of various chemicals on the enzyme activities were also different in each case. Thus, each tissue appears to have, at least, one predominant distinctive isoenzyme. Each one of the pH curves showed zones of minor activity which may be caused by various overlapping invertases. The presence of two pH optima in cell wall preparations from leaf blades and of three optima in the cell wall invertases from leaf sheaths of the variety CP 48-103 [4] supports the hypothesis of the occurrence of a group of isoenzymes in each cell wall.

Table 5. Effects of some chemicals on cell wall invertases

		Invertase activity (%)					
	Concn	Stalk			Leaf		
Effector	(mM)	Mature	Immature	Root	Sheath	Blade	
Heptamolybdate	5	105.8	105.1	53.3	102.2	22.7*	100.0†
Hg^{2+}	1	14.0	8.8	53.9	1.3	34.7	34.6
Mg^{2+}	10	116.8	104.1	94.0	100.0	104.4	102.7
Tris	4	71.6	78.3	100.0	63.9	100.0	92.7
	50	59.6	51.2	100.0	27.2	100.0	89.6
EDTA	10	98.6	100.0	95.8	100.0	74.2	100.0
	30	97.0	105.8	95.8	117.8	75.0	100.0
Co^{2+}‡	1	100.0	100.0	113.8	83.4	97.3	119.2
Ca^{2+}‡	5	100.0	104.4	100.0	91.0	100.0	100.0
Urea	200	91.1	87.8	94.6	105.4	95.1	100.0
δ-Gluconolactone	100	100.0	102.4	87.4	87.4	94.2	100.0

*Determinations at pH 3.55.
†Determinations at pH 4.9.
‡These ions were proved using barbital buffer instead of phosphate buffer.

Table 6. Sugars hydrolysed by the cell wall enzyme preparations

Sugar	Concn (mM)	Tissue activity (%)					
		Root	Leaf			Stalk	
			Sheath	Blade		Immature	Mature
β-Methylfructoside	672	65.9	0.0	58.5*	9.6†	2.0	18.4
α-Methylglucoside	120	0.0	0.0	0.0	0.0	0.0	0.0
Trehalose	120	7.2	7.3	4.9	11.0	6.8	0.0
Melezitose	60	13.8	0.0	12.4	28.0	0.0	0.0
Sucrose	60	100.0	100.0	100.0	100.0	100.0	100.0

*Determinations at pH 3.55.
†Determinations at pH 4.9.

The K_m of the enzyme from apex tissue [5], optimum pH 7.4, differs significantly from the corresponding values of the neutral invertase group. Other groups of invertases showed optima pH *ca* 3.3–4.9. Here again the K_m values of these acid invertases are significantly different, and their behaviours towards the tested chemicals are also different. The callus enzyme appears to be very similar to the enzyme from root, but their K_m values were significantly different and the effects of chemicals were also different, supporting a distinction between these enzymes. According to these conclusions in each organ of sugar cane it is likely that a set of invertase isoenzymes occurs with the predominance of a different activity in each case. This would explain why callus cell wall contains an invertase seemingly distinct from those occurring in the plant.

EXPERIMENTAL

Plant material. 6-Month old sugar cane, cv Tuc 68-19, cultivated in the field at the Estación Experimental Agroindustrial de Tucumán was used.

Cell wall preparations. Mature and immature stalk, root, leaf sheath and blade were used as source of cell wall preparations. Cell walls were prepared as described in previous publications [4, 5].

Enzyme units. 1 unit of enzyme was defined as the amount which hydrolyses 1 μmol substrate/min at 37° at the optimum pH.

Enzyme assay. The reaction mixture consisted of 40 μl 0.2 M buffer, 10 μl 0.3 M sucrose and 50 μl cell wall suspension in a final vol. of 100 μl. The reactions were run at 37° for 30 min and stopped by addition of the alkaline–copper reagent of Somogyi [15]. The reducing power was determined by the method of Nelson [16]. NaPi buffer, pH 7.5 and NaOAc buffer, pH 3.3, 3.55 and 4.9 were used.

Attempts to solubilize the invertases. The cell wall suspension was treated successively with the following solns: (1) 0.1 M NaPi buffer, pH 7.5, containing 1 M NaCl and 1 mM 2-mercaptoethanol; (2) 0.2 M NaPi–citrate buffer, pH 8.5, containing 1 mM 2-mercaptoethanol; (3) 0.2 M NaPi–citrate buffer, pH 8.5, containing 1 M NaCl, 1 mM 2-mercaptoethanol and 30 mM EDTA; (4) 0.2 M sodium borate buffer, pH 8.5, containing 1 mM 2-mercaptoethanol. The suspensions of cell walls were centrifuged at 3020 *g* and resuspended for 30 min in 5 ml of the corresponding buffer. These suspensions were centrifuged and the pellets washed and resuspended in 5 ml buffer A (10 mM NaOAc buffer, pH 5.5, containing 1 mM 2-mercaptoethanol). The invertases were measured in the washed cell walls and in the dialysed washings. Similarly, samples of cell wall suspension were washed with 2% Tween 60. Proteins and total sugars were measured by the method of Lowry *et al.* [17] and the PhOH–H_2SO_4 reaction [18] respectively.

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