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CELL WALL INVERTASES FROM SUGAR CANE

MARTA AMELIA VATTUONE, FERNANDO EDUARDO PRADO* and ANTONIO RODOLFO SAMPIETRO†

Instituto de Química Biológica, Facultad de Bioquímica, Química y Farmacia, Universidad Nacional de Tucumán, Chacabuco 461, 4000—S. M. de Tucumán, Argentina

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Key Word Index—*Saccharum officinarum*; Gramineae; sugar cane; invertase; cell wall; isoenzyme.

Abstract—Invertases in sugar cane leaf sheaths are firmly bound to the cell wall. They consist of three different enzymes distinguished on the basis of optimum pH, K_m and the response to inhibitors. This invertase complex differs from the single enzyme described for immature and mature stalk tissues.

INTRODUCTION

Two groups of invertases are present in sugar cane tissues [1]. One group consists of cell wall invertases [2, 3] and the other of intracellular enzymes [3]. Both are thought to have different physiological functions. Cell wall enzymes are involved in the translocation of sucrose from the apoplasm to the symplasm of sugar cane [2] and intracellular enzymes are probably involved in the mobilization of the sucrose stored in the cell vacuole [4, 5]. Most of this knowledge comes from studies of stalk tissues. Cell wall enzymes of other tissues have not been examined to date. In the present work a study has been made of the cell wall invertases in sugar cane leaf sheaths.

RESULTS

Effect of pH

Special care has been taken in these experiments because of the strong buffering capacity of the cell wall. Figure 1 shows a typical pH activity curve obtained with cell wall preparations from sugar cane leaf sheaths. Three pH optima were observed (*ca* pH 4, 5.5 and 7.5) and these were interpreted as independent isoenzymes.

Attempts to solubilize enzymes

Successive washing with buffers that were reported as good eluting agents for cell wall enzymes did not remove the invertase activity [6–10]. Similar lack of effect was observed in washing with 2% Tween 60 [11, 12] and when the cell walls were obtained by homogenizing the tissues in the presence of 0.8 mg of casein per mg of dry tissue.

Effect of substrate concentration

The enzymes working at pH 7.5 and 5.5 have a K_m of 1.3×10^{-2} M and 3×10^{-2} M respectively. The enzyme working at pH 4 (K_m *ca* 1.1×10^{-2} M) showed an inhibitory effect with excess substrate (Fig. 2).

Effect of various chemicals

Comparison of the inhibitory effect of various chemicals on the three activities showed strong differences (Table 1). These experiments appear to confirm that three activities occur in the cell wall from leaf sheaths.

DISCUSSION

Immature and mature tissues from sugar cane stalk possess invertase activity. However, on the basis of

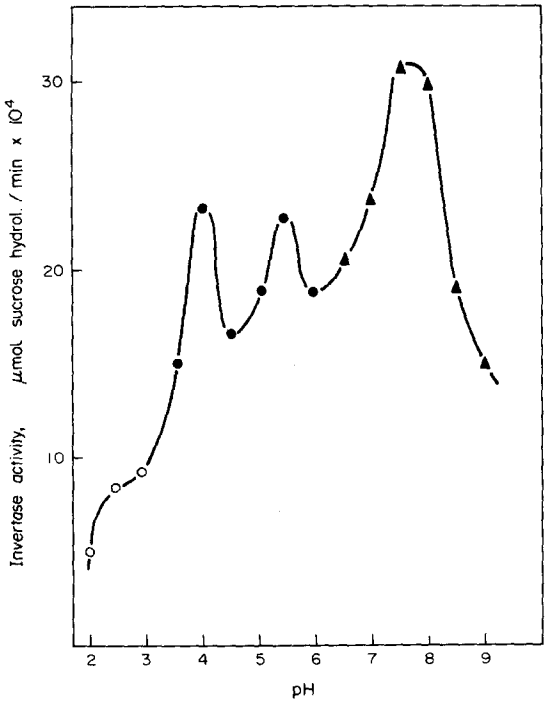

Fig. 1. Effect of pH on invertase activity. The pHs were carefully controlled. The reaction mixtures were as described in Experimental. —○——○— 0.2 M glycine–HCl buffer (pH 2–2.9); —●——●— 0.2 M NaOAc buffer (pH 3.56–5.95); and —▲- - -▲— Na Pi buffer (pH 6.5–9).

* Fellow of the Consejo Nacional de Investigaciones Científicas y Técnicas, Buenos Aires, Argentina.

† Career Investigator from the Consejo Nacional de Investigaciones Científicas y Técnicas, Buenos Aires, Argentina.

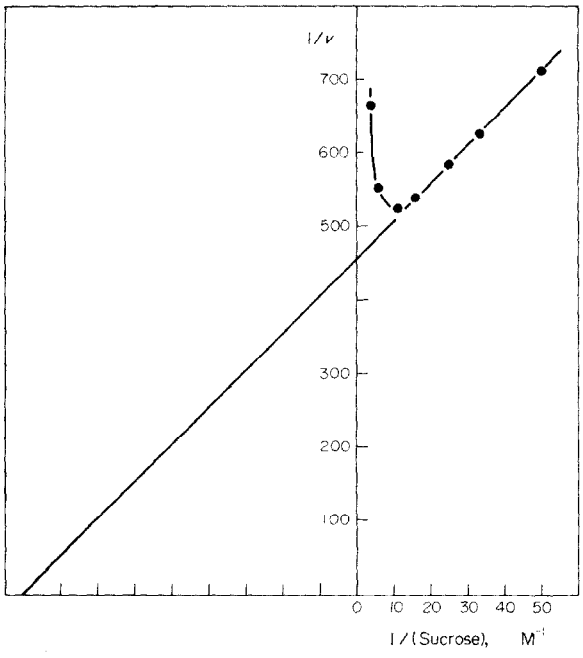

Fig. 2. Double reciprocal plot for sucrose hydrolysis by the cell wall invertase. Reactions were performed at pH 4.

differences in K_m and optimum pH the cell wall activity of mature tissues [2] appears to be different from the similar activity of immature tissues [3]. If changing physiological states in a tissue are accompanied by a change in the cell wall invertases, then one can expect that tissues with different physiological functions may also have differences in the cell wall invertases. The cell wall from leaf sheaths appears to have three different invertases. These enzymes were not removed from the cell wall with various buffers, indicating that the enzymes are not bound through electrostatic bonds. Tween 60, which was able to remove cell wall–tannin–protein complexes [11, 12] did not solubilize the invertases. The cell wall invertases were not displaced by homogenization in the presence of exogenous protein, although the amount of protein used during the homogenization was enough to saturate the cell wall adsorptive and ionic capacity. EDTA and 2-mercaptoethanol were also ineffective, suggesting that the enzymes were not bound through bivalent ions or –S–S– groups. Thus, these enzymes may be occluded into the cell wall or may be linked through covalent bonds.

In mature stalk an alkaline or neutral enzyme appears at maturity [3]. This enzyme is soluble and is thought to be soluble in the cytoplasm [1]. Thus, this paper reports for the first time the presence of a cell-wall-bound alkaline invertase in sugar cane. This enzyme is present in growing sugar cane with incipient stalk formation.

Table 1. Effect of chemicals on the activity of the invertases at the different optimum pHs

Effector	% Inhibition		
	pH 4	pH 5.5	pH 7.5
Heptamolybdate 5 mM	45	–	9
Ca^{2+} 5 mM	3	7	33
Tris 50 mM	16	26	66
Urea 200 mM	22	4	18
EDTA 10 mM	14	0	19
Mg^{2+} 10 mM	0	13	26
Hg^{2+} 1 mM	56	–	86
δ-Gluconolactone 100 mM	16	–	0
Co^{2+} 1 mM	2	20	28

EXPERIMENTAL

Plant material. 3- to 4-month-old sugar cane, cv CP 48-103, cultivated in the field at the Estación Experimental Agrícola de Tucumán was used.

Cell wall preparations. Leaf sheaths (300 g) were cut into small pieces and homogenized in 300 ml of 50 mM Na–Pi buffer, pH 7.5, 1 mM 2-mercaptoethanol (2-ME) and 5 μM $MnSO_4$. The homogenate was squeezed through 2 layers of cheese cloth and centrifuged at 270 g for 10 min. The pellet was resuspended in 10 ml of 50 mM NaOAc buffer, pH 5.5, 1 mM 2-ME, frozen and thawed ×3 to break cells, and homogenized in a Potter. This final step enhances the fluidity of the preparation. The suspension was centrifuged at 270 g. The pellet was resuspended in 10 ml of the last buffer and was centrifuged at 3020 g. This procedure was repeated ×3 and the resulting suspension constitutes the cell wall preparation. This preparation was adjusted to contain *ca* 4.5 × 10^{-2} enzyme units/ml at pH 4; 4.5 × 10^{-2} enzyme units/ml at pH 5.5 and 6 × 10^{-2} enzyme units ml at pH 7.5.

Attempts to solubilize cell wall invertases. Cell wall preparations were treated successively with the following solns: (1) 0.2 M Na–Pi buffer, pH 7.5, 1 M NaCl 1 mM 2-ME; (2) 0.2 M Na–Pi citrate buffer, pH 8.5, 1 mM 2-ME; (3) 0.2 M Na–Pi citrate buffer, pH 8.5, 1 M NaCl, 1 mM 2-ME, 30 mM EDTA. The cell wall suspensions were centrifuged at 3020 g and resuspended for 30 min in the corresponding buffer. Then, the suspension was centrifuged and the pellet washed and resuspended in the NaOAc buffer. Invertase activities were measured in the washed cell walls and, previous dialysis, in the buffers used for the elutions.

Assay of cell wall invertases. The reaction medium (100 μl) consisted of 20 μl 2 M sucrose, 50 μl cell wall suspension and 30 μl 0.2 M buffer of the desired pH. Reaction time was 30 min at 37°. The reactions were stopped by the alkaline–Cu reagent of ref. [13] and the reducing power was determined by the arsenomolybdate method [14]. Cell wall preparations were kept at −20°.

Enzyme units. One unit of enzyme was defined as the amount which hydroylses 1 μmol of substrate per min at 37° and at the optimum pH.

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