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INVERTASE AND MALTASE IN THE FREE SPACE OF THE MAIZE SCUTELLUM*

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Key Word Index—*Zea mays*; Gramineae; maize scutellum; invertase; maltase; free space.

Abstract—When maize scutellum slices were incubated in solutions of sucrose or maltose, there was a release of glucose into the bathing solution. The pH optima for glucose release were 2.5 for sucrose and 3.5 for maltose. From measurement of rates of glucose uptake into slices in the presence or absence of sucrose, it is calculated that glucose uptake will introduce errors of 3–9%, depending on the sucrose concentration, in estimates of free-space sucrose-hydrolase activity at pH 2.5. At their respective pH optima, maltose was hydrolysed at a rate 2.5 times that of sucrose. When frozen-thawed slices were used the same pH optima were obtained, but rates of hydrolysis were increased. Raffinose and melezitose also were hydrolysed with pH optima of 2.5 and 3.5, respectively. α-Methyl glucose was not hydrolysed. A 60-min HCl treatment (pH 2) of scutellum slices destroyed 69% of the sucrose-hydrolase activity and 100% of the maltose-hydrolase activity. In contrast, sucrose uptake and sucrose synthesis from exogenous fructose were not affected by HCl treatment. It is concluded that there are two hydrolases, acid invertase and maltase; that they are either on or outside the plasmalemma (in the free space); and that they are not necessary to the disaccharide uptake processes either by supplying exogenous hexose or by acting as transporters.

INTRODUCTION

Acid invertase activity located at the cell surface (in the free space) appears to be widely distributed in plants [1]. One obvious role for this enzyme is to supply glucose and fructose to cells that can take up hexose but not sucrose (e.g. immature sugarcane internodes [2]). But free-space invertase activity also is present in many plants that do not require sucrose hydrolysis for uptake of sucrose carbon [1], and it may be that a supply of exogenous hexose is also advantageous to these plants. It has been suggested that free-space invertase might act as a transfructosylase or transglucosylase in cell wall synthesis [3], but there is no evidence that the enzyme has a role other than to hydrolyse sucrose.

When slices of the maize scutellum are incubated in sucrose solutions, there is a slow release of glucose into the bathing solution in quantities far too small to account for the uptake of sucrose carbon [4]. This paper reports a study undertaken to determine if the enzyme causing glucose release is a free-space invertase and if it plays a direct role in sucrose uptake. A free-space maltase was present [5], and some of its properties are compared with those of the invertase.

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RESULTS AND DISCUSSION

Effect of pH *on rates of hydrolysis of exogenous sucrose and maltose*

Fresh scutellum slices (0.5 g) were incubated in buffered 50 mM solutions of sucrose or maltose over the pH range 2 to 7 (for sucrose) or 2.5 to 6 (for maltose). Portions of the bathing solutions were removed for glucose analysis 1 and 31 min after adding the slices, and the increase in glucose during this period was used to calculate disaccharidase activity. Two kinds of control flasks were run: in one, scutellum slices were omitted to correct for acid hydrolysis in the pH range 2 to 3 and, in the other, disaccharide was omitted to correct for glucose leakage from the slices over the entire pH range. Acid hydrolysis of sucrose occurred at a rate *ca* 65% of the rate of enzymic hydrolysis at pH 2; the acid hydrolysis rate dropped to 15% of the enzymic rate at pH 2.5, and it was negligible at pH 3. Acid hydrolysis of maltose was negligible at pH 2.5. Glucose leakage from the slices was greatest at pH 5 (0.6 μmol/0.5 g/0.5 hr) and was only one-third as great at pH 2.5.

Corrected rates of disaccharide hydrolysis as a function of pH are shown in Fig. 1. Maximum rates of hydrolysis occurred at pH 3.5 with maltose and pH 2.5 with sucrose. At their respective pH optima, maltose was hydrolysed at a rate 2.5 times that of sucrose. When frozen-thawed slices were used, the pH optima were the same as those obtained with fresh slices, but

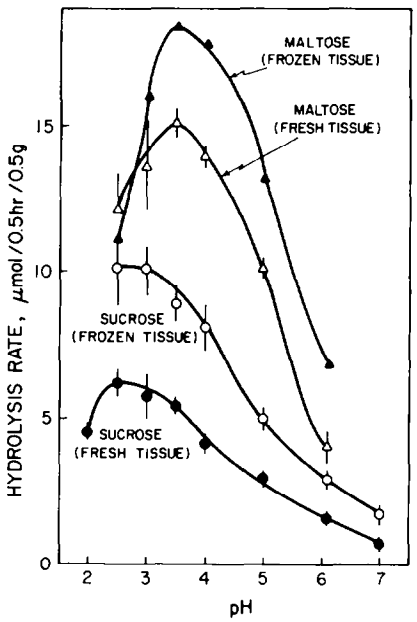

Fig. 1. Effect of pH on hydrolysis of sucrose and maltose. Sugar solutions (50 mM) were buffered with 25 mM Na citrate (pH 2.5 to 5), MES (pH 6.1) and ADA (pH 7). The pH 2 flask contained HCl (no buffer). Points are averages from 4 to 6 experiments and the standard deviations are indicated. Points for maltose (frozen tissue) are averages from 2 experiments.

the maximum rates of hydrolysis increased *ca* 65% for sucrose and 23% for maltose. Frozen-thawed slices were washed for 55 min before use in the disaccharide hydrolysis assay (see Experimental), and the wash solutions were not tested for disaccharidase activity.

The effect of buffer on sucrose hydrolysis was tested in the pH range 3 to 5. When buffer was omitted (pH adjusted with HCl) and fresh slices were used, rates of sucrose hydrolysis very similar to those shown in Fig. 1 were obtained. With frozen-thawed slices, sodium acetate buffers gave the same results as sodium citrate. Acetate buffer was not used with fresh slices because it caused large amounts of sucrose to leak from the slices.

In one experiment (fresh slices with sucrose at pH 4), both glucose and fructose were measured and were found to be produced in equal amounts (from 3 replicates: 4.0 ± 0.2 μmol glucose/0.5 hr and 3.9 ± 0.2 μmol fructose/0.5 hr) which indicates that the enzyme was acting as a hydrolase not a transferase.

Invertases in plant homogenates are known to bind to cell wall preparations, e.g. [6]. The binding is reversible and depends on ionic strength and pH. It is possible, therefore, that slicing released intracellular disaccharidases from the scutellum and that some of the released intracellular enzymes were bound to the cell walls and these gave the activities shown in Fig. 1. To test the latter possibility, disaccharidase activities were measured using whole scutella and scutellum slices prepared from the same crop of seedlings and using 100 mM disaccharide (instead of 50 mM) to increase the rate of substrate penetration into the scutellum. In two experiments, average rates of hydrolysis obtained with whole scutella were 91% (sucrose, pH 2.5) and 80% (maltose, pH 3.5) of those obtained with scutellum slices.

Substrate specificity

Substrates for invertase (β-fructofuranoside fructohydrolase, EC 3.2.1.26) must possess an unsubstituted β-D-fructofuranosyl residue [7]. Therefore, raffinose is hydrolysed whereas melezitose is not. The substrate specificities of higher plant α-glucosidases (excluding α- and β-amylases, neither of which attack maltose) are not well known [8]. A partially purified α-glucosidase from alfalfa hydrolysed maltose but not α-methylglucose. The alfalfa preparation also hydrolysed sucrose, but from the pH optima (4.5 for maltose; 6 for sucrose) it was concluded that invertase was present as an impurity [9]. Yeast α-glucosidase hydrolyses maltose, sucrose, and more slowly melezitose and α-methyl glucose, but it does not hydrolyse raffinose [10].

The action of the scutellum disaccharidases on the trisaccharides, raffinose and melezitose, and on α-methyl glucose was tested. Fresh scutellum slices were incubated with 50 mM solutions of the sugars buffered at pH 2.5, 3.5 and 5 (Table 1). The increase in reducing sugars (expressed as glucose) in the bathing solution was used to calculate hydrolysis rates. Hydrolysis of raffinose was greater at pH 2.5 than at 3.5 or 5. The pH optimum for melezitose hydrolysis was 3.5. α-Methyl glucose was not hydrolysed.

The results of Table 1 and Fig. 1 indicate that there are two free space disaccharidases in scutellum slices: one is an invertase that hydrolyses sucrose and raffinose with a pH optimum of 2.5, the other is a maltase that hydrolyses maltose and melezitose with a pH optimum of 3.5. However, a single enzyme with two kinds of activities cannot be ruled out. The pH optimum of the scutellum invertase activity is much lower than those reported for invertases from other higher plants [1] or for invertases of yeasts and molds [7]. The pH optimum for scutellum maltase was also low, although a pH optimum of 3.9 has been reported for a wallbound maltase from maize leaves [11].

Glucose uptake as a factor complicating measurement of free space invertase

Since glucose uptake would cause free-space disaccharidase activity to be underestimated in fresh slices, rates of glucose uptake were measured at pH 2.5

Table 1. Substrate specificity*

Substrate	Reducing sugar, μmol glucose/0.5 hr/0.5 g		
	pH 2.5	pH 3.5	pH 5
Sucrose	10.6±0.3	—	5.2±0.5
Raffinose	5.5±0.5	4.3±0.2	3.4±0.2
Melezitose	4.1±0.8	5.8±0.4	3.4±0.3
α-Methyl glucose	0	0	0

*Sugar solutions (50 mM) contained 25 mM Na citrate at the pHs indicated. Values given are averages from 3 to 5 experiments followed by the standard deviation.

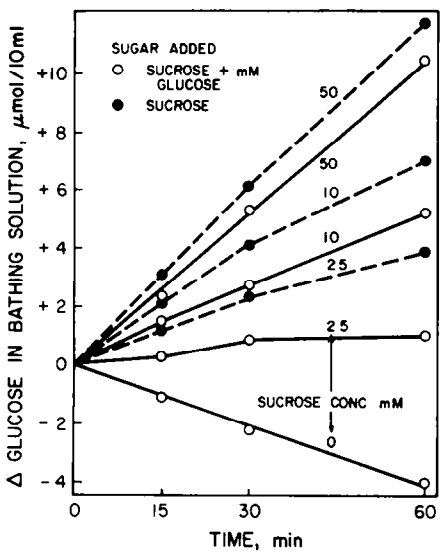

Fig. 2. Changes in the amounts of bathing solution glucose with time of incubation of slices in sucrose or sucrose plus glucose. Solutions were buffered with 25 mM Na citrate, pH 2.5. Points are averages from 2 experiments.

(25 mm citrate buffer) in the glucose concentration range found during invertase assay (≤ mM) and in the absence or presence of sucrose. The rate of glucose uptake was directly proportional to glucose concentration at least up to a concentration of 1 mM (2 μmol/0.5 hr/0.5 g). The glucose content of the bathing solution during incubation of slices in 50 mM sucrose increased at a constant rate during the first 30 min (Fig. 2), and, therefore, the average glucose concentration during the 30 min invertase assay at pH 2.5 was *ca* 0.3 mM (Fig. 1). At this concentration the amount of glucose taken up during the 30 min invertase assay would be 0.6 μmol, and this amount would cause invertase activity to be underestimated by only 9%. Rates of glucose uptake were constant from pH 5 to 7, but they declined below pH 5, and at pH 2.5 the rate of glucose uptake was 82% of the pH 5 rate. Therefore, at the higher pHs glucose uptake would cause about an 11% underestimate of invertase activity. However, 9–11% represents the maximum range of error because sucrose inhibits glucose uptake (Fig. 2). Slices were incubated in mM glucose plus increasing amounts of sucrose up to 50 mM. Control flasks contained sucrose alone. As the sucrose concentration was increased in the presence of mM glucose, the rate of glucose uptake decreased (Fig. 2). If glucose uptake and sucrose uptake were independent, the amount of glucose taken up should have been 2.7 μmol/30 min in the glucose (mM) plus sucrose (50 mM) flask and 0.6 μmol/30 min in the sucrose (50 mM) flask, a difference of 2.1 μmol. However, from Fig. 2, the experimentally determined difference was only 0.8 μmol. It is concluded that glucose uptake will introduce errors of 3 to 9%, depending on the sucrose concentration, in estimating free-space invertase activity at pH 2.5.

Rates of free-space invertase activity were determined in the sucrose concentration range of 2.5–50 mM. With these data (corrected for glucose uptake) K_ms for sucrose of 5.5 mM (pH 2.5) and 2.9 mM (pH 3.5) were calculated. Previously, a K_m of 2.1 (pH 4) was found for an invertase present in the insoluble residue of scutellum slices [5], and, apparently, this was the free-space invertase. A decreased affinity for sucrose at pHs below 4 also has been found for yeast invertase [12], although the yeast enzyme has optimum activity in the pH 4 to 5 range.

Effect of HCl *treatment on disaccharidase activity, sucrose uptake and sucrose synthesis*

As ap Rees [1] has pointed out, the appearance of glucose in the bathing solution does not necessarily indicate the presence of a free-space invertase because the following sequence of events would give the same result: sucrose uptake, sucrose hydrolysis by an endogenous invertase, leakage of glucose into the bathing solution. These processes are known to occur in the maize scutellum. Sucrose is actively taken up by the scutellum slices without prior inversion [4, 13], the scutellum contains a soluble invertase [14], and hexoses readily penetrate the scutellum plasmalemma apparently by free diffusion through pores [15, 16]. However, it appears unlikely that the invertase assayed in the experiments of Fig. 1 was intracellular rather than in the free space. First, the very low pH optimum for sucrose hydrolysis was essentially unchanged after the slices were frozen and thawed (a treatment that disrupts both plasmalemma and tonoplast as shown by EM photographs [17]), indicating that the results of Fig. 1 reflect the effect of pH on hydrolysis and not on transport of sucrose into and hexose out of the slices. Second, optimum activities of soluble invertase in scutellum extracts occurred at pH 6 and 7 (Fig. 3). The highest activity occurred at pH 6, but this was less than 50% of the presumed free-space activity at pH 2.5 and less than 25% of that in frozen-thawed slices (Fig. 1). Invertase activity (assayed in the pH range 5.5 to 7.5) was not released when frozen scutellum slices were thawed in H_2O nor was it released when frozen-thawed slices were ground in buffer at pH 7 (see Experimental). Apparently, the invertase found in extracts of fresh scutella (Fig. 3) was destroyed by freezing and thawing or was bound to the insoluble residue (cf. Fig. 1.).

Results of experiments with HCl-treated slices support the idea that invertase and maltase occur in the

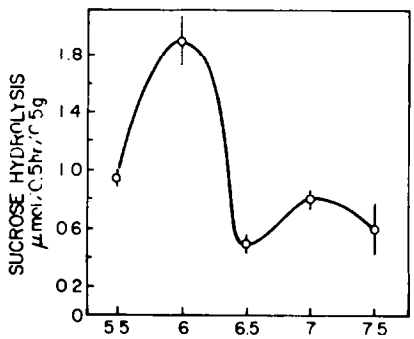

Fig. 3. Effect of pH on invertase activity of scutellum extracts. Sucrose solutions (50 mM) were buffered with 40 mM MES (pH 5.5 to 6.5) and phosphate (pH 7 and 7.5). Points are averages from 5 experiments and the standard deviations are indicated.

free space of the scutellum. Slices were treated for 30 or 60 min in H_2O or HCl (pH 2) and then were used for disaccharidase assay either fresh or after freezing and thawing. Frozen-thawed slices were washed 55 min before use in the assay, but the wash solutions were not tested for activity. HCl treatment decreased both invertase and maltase activities, but maltase was the more sensitive (Table 2). A 60-min HCl treatment destroyed 69% of the invertase activity and 100% of the maltase activity when fresh slices were used and 64% of the invertase activity and 91% of the maltase activity when frozen-thawed slices were used. After 30 min in HCl, invertase activity in fresh slices decreased 43% (Table 2), but, when invertase activity was measured in HCl at pH 2, the activity was only 27% less than the maximum (Fig. 1). Apparently, the drop in invertase activity when the pH was lowered from 2.5 to 2 (Fig. 1) was a result of enzyme destruction rather than an effect of pH on the catalytic reaction.

In contrast to the effects of HCl on free-space invertase and maltase, the ability of the slices to take up sucrose was not impaired by HCl treatment (Table 3). Maltose also is taken up after HCl treatment [5]. HCl treatment also had no effect on rates of sucrose synthesis from exogenous fructose (Table 3). Sucrose synthesis at a rate of 35 μmol/hr/g fr. wt would require 70 or 105 μmol of ATP/hr, depending on whether or not sucrose phosphate was an intermediate. Scutellum slices contain *ca* 0.5 μmol ATP/g [18]; and, therefore, the rates of sucrose synthesis shown in Table 3 would require that tissue ATP turn over 2.3 to 3.5 times per min for the 2 hr period of the experiment. The results of Table 3 indicate that HCl treatment does not injure the plasmalemma (sucrose and fructose uptake) or the cytoplasm (sucrose synthesis), and, therefore, the enzymes catalysing hydrolysis of sucrose and maltose (Table 2) must be either on the plasmalemma (peripheral proteins [19]) or outside the plasmalemma. Furthermore, the results indicate that these hydrolytic enzymes are not necessary to the disaccharide uptake processes either by supplying exogenous hexose of by acting as transporters [20].

The increases in invertase and maltase activity after freezing and thawing (Fig. 1, Table 2) were too large to have been artifacts resulting from underestimation of enzyme activity in fresh slices because of glucose uptake. Furthermore, the absolute increase in disaccharidase activity following freezing and thawing was only *ca* 50% as great in HCl-treated slices as it was in H_2O-treated slices (Table 2), indicating that some of the enzymes responsible for the increased activity were on or outside the plasmalemma. Perhaps sucrose does not penetrate rapidly enough into some parts of the free space (e.g. along the outside of the plasmodesmata) for full enzyme activity to be attained in fresh slices. Alternatively, if the disaccharidases are peripheral proteins, freezing and thawing may dislodge them or reorient them on the plasmalemma increasing their activity.

Table 2. Effect of HCl treatment on disaccharidase activity*

Treatment	Slice preparation	Hydrolysis rate, μmol/0.5 hr/0.5 g Sucrose	Maltose
H_2O, 30 min	Fresh	6.1±0.8	15.1±0.5
	Frozen-thawed	9.6±1.5	18.5±0.2
HCl, 30 min	Fresh	3.5±0.5	1.8±0.5
	Frozen-thawed	5.8±0.5	3.8±0.7
HCl, 60 min	Fresh	1.9±0.1	0
	Frozen-thawed	3.5±0.2	1.6±0.2

*Sugar solutions (50 mM) contained 25 mM Na citrate at a pH of 2.5 for sucrose and 3.5 for maltose. Values given are averages from 4 to 6 experiments followed by the standard deviation.

Table 3. Effect of HCl treatment on sucrose uptake and synthesis*

Treatment	Sucrose, μmol/hr/g Uptake	Synthesis
H_2O, 30 min	14.3±0.9	31.9
H_2O, 60 min	14.6±1.3	35.4
HCl, 30 min	13.8±1.1	34.8
HCl, 60 min	14.6±1.4	37.6

*The values for uptake are averages from 4 to 6 experiments followed by the standard deviation. Values for synthesis are averages from 2 experiments.

The free-space maltase apparently has a role in the mobilization of endosperm starch. The maltase, besides hydrolysing maltose, may also attack higher maltooligosaccharides produced in the endosperm by α-amylase [21], and its action could prevent maltose inhibition of α-amylase [22]. The starchy endosperm contains little sucrose [23], and most of the sucrose available to the scutellum free-space invertase would be secreted by the cells of the aleurone layer [24]. Apparently, the free-space invertase has a very minor role during germination. The possibility was considered that the invertase played a role in sucrose transport across the plasmalemma, and that its hydrolytic activity became apparent only at very low pH. However, the results of Tables 2 and 3 indicate that it is not necessary for sucrose transport. The activity of the soluble invertase at pH 6 (Fig. 3) was high enough to account for rates of endogenous sucrose utilization in scutellum slices [25], but sucrose synthetase is also present in the scutellum and its activity is much higher than that of the invertase [14].

EXPERIMENTAL

Preparation and treatment of scutellum slices. Maize grains (*Zea mays* L. cv McNair 508) were soaked in running tap H_2O for 24 hr, then placed on moist paper towels, and grown in the dark at 24–25° for 72 hr. Scutella were excised and cut transversely with a razor blade into slices of 0.5 mm or less in thickness. Slices were washed in H_2O until washing was clear, blotted on filter paper, and weighed into groups of 0.5 g. Slices (80 to 90, 0.5 g) were 3–7 cells in thickness [17]. Slices were given an initial incubation in 10 ml H_2O or HCl (10 mM) for 30–60 min at 30°. When a 60-min incubation was used, the bathing solns were replaced with fresh H_2O or

HCl after 30 min. At the end of the initial incubation, slices were washed with 10 ml H_2O, incubated in a salt soln (KCl, $MgCl_2$, $CaCl_2$; each at 20 mM) for 25 min, and finally washed twice with 10 ml H_2O. Incubation in a salt soln minimized sugar leakage and replaced cations removed from free-space exchange sites by HCl treatment [26]. For some expts, slices were frozen at −4 to −5° at the end of the initial incubation above. Frozen slices were thawed with 10 ml H_2O and incubated in H_2O for 30 min at 30°. Then they were incubated in a salt soln and washed twice with H_2O as described above.

Preparation of scutellum extracts. Fresh, whole scutella (2.5 g) were ground in a mortar with 5 ml of a soln containing Na-Pi buffer (250 mM, pH 7), EDTA (10 mM) and cysteine (6 mM). The homogenate was strained through 2 layers of cheesecloth and centrifuged in the cold at 20 000 g for 20 min. The supernatant fraction was dialysed at 3° for 24 hr in 2 l. (soln changed after 12 hr) of 5 mM Na-Pi buffer, pH 7.

Sugar hydrolysis assay. Slices prepared as described above were placed in 10 ml of buffered sugar soln and incubated at 30°. Portions of the bathing soln were removed for sugar analysis at 1 min and at intervals thereafter (usually 31 min). Samples of the bathing solns were neutralized and frozen immediately. Dialysed scutellum extracts (0.3 ml) were added to 0.7 ml of a buffered sucrose soln. After 15 min at 30°, the reaction was stopped in a boiling water bath, the contents centrifuged and 0.2 ml of the supernatant soln removed for glucose analysis. The hydrolyses of maltose and sucrose were assayed using glucose oxidase. Trisaccharide and α-methyl glucose hydrolyses were assayed by measuring the increase in reducing sugars using Somogyi's adaptation of Nelson's method [27, 28].

Sucrose uptake and sucrose synthesis. Slices (0.5 g, treated in H_2O or HCl as described above) were incubated for 2 hr at 30° in 10 ml 5 mM sucrose (sucrose uptake) or 0.1 M fructose (sucrose synthesis) buffered at pH 5 with 25 mM MES. Uptake and synthesis were measured as described previously [13].

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