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INVOLVEMENT OF SUCROSE SYNTHASE IN SUCROSE CATABOLISM*

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Key Word Index—*Zea mays*, Gramineae, maize, sucrose synthase, invertase, sucrose breakdown, UDPG pyrophosphorylase, ATP pyrophosphohydrolase

Abstract—Maize scutellum slices incubated in water utilized sucrose at a maximum rate of 0 12 μmol/min per g fr wt of slices When slices were incubated in DNP, there was a three-fold increase in the rate of sucrose utilization Sucrose breakdown in higher plants can be achieved by pathways starting with either invertase or sucrose synthase (SS) Invertase activity in scutellum homogenates was found only in the cell wall fraction, indicating that SS was responsible for sucrose breakdown *in vivo* SS in crude scutellum extracts broke down sucrose to fructose and UDPG at 0 39 μmol/min per g fresh wt of slices The UDPG formed was not converted to UDP + glucose, UMP + glucose-1-P, UDP + glucose-1-P or broken down by any other means by the crude extract in the absence of PPi In the presence of PPi, UDPG was broken down by UDPG pyrophosphorylase which had a maximum activity of 26 μmol/min per g fr wt of slices Levels of PPi in the scutellum could not be measured using the UDPG pyrophosphorylase phosphoglucomutase glucose-6-P dehydrogenase assay because they were too low relative to glucose-6-P which interferes in the assay An active inorganic pyrophosphatase was present in the scutellum extract which could prevent the accumulation of PPi in the cytoplasm ATP pyrophosphohydrolase, which hydrolyses ATP to AMP and PPi, was found in the soluble portion of the scutellum extract The enzyme activity was increased by fructose-2,6-bisP and Ca^{2+} In the presence of both activators, enzyme activity was 1 1 μmol/min per g fr wt of slices, a rate sufficient to supply PPi for the breakdown of UDPG These results indicate that sucrose breakdown in maize scutellum cells occurs via the SS UDPG pyrophosphorylase pathway

INTRODUCTION

Sucrose breakdown in higher plants can follow two pathways The first starts with the hydrolytic breakdown by invertase to yield glucose and fructose This appears to be the more common pathway in those plant organs where the conversion of sucrose to starch or cell wall does not occur or occurs at a relatively slow rate [1] In plant tissues there are cell-wall-bound invertases, which can take part in the translocation mechanism in some plant organs [2], and soluble invertases, which presumably are linked to the mobilization of sucrose stored in vacuoles [2, 3] The second pathway for sucrose breakdown starts with sucrose synthase (SS), yielding fructose and nucleoside diphosphoglucose (NDPG) [4] The available evidence strongly suggests a relationship between SS and tissues involved in starch or cell wall synthesis [5–7] In these tissues, the synthetic reactions utilizing NDPG would control sucrose breakdown However, if SS is involved in tissues where sucrose is converted entirely to glycolytic intermediates, there must be a system by which the NDPG produced is broken down to form G-1-P or glucose

Maize scutellum cells rapidly catabolize internal sucrose with the uptake of O_2 and the production of CO_2 and ethanol in quantities that indicate that all the sucrose carbons were metabolized through glycolysis, alcoholic fermentation and the Krebs cycle [8] This paper deals with the mechanism of sucrose breakdown in maize scutellum cells It is shown that invertase activity in scutellum homogenates is limited to the cell wall fraction, leaving SS as the only endogenous enzyme capable of breaking down sucrose Then it is shown that UDPG pyrophosphorylase and ATP pyrophosphohydrolase are present in the maize scutellum cells and that their activities are great enough to account for *in vivo* rates of breakdown of UDPG to G-1-P and UTP

RESULTS AND DISCUSSION

Utilization of endogenous sucrose by maize scutellum slices

Incubation of scutellum slices in water resulted in a continuous decrease in internal sucrose content (Fig 1) The highest rate of sucrose utilization occurred during the first hr of incubation when sucrose was utilized at 0 12 μmol/min per g fresh wt of slices The rate of sucrose utilization declined thereafter up to the end of the 5 hr incubation period When slices were incubated in 0 5 mM DNP, the rate of sucrose utilization increased about 3-fold to an average rate for the first hr of 0 34 μmol/min per g fr wt of slices (Fig 1) Only two enzymes (invertase and SS) are known that could catalyse the initial breakdown of sucrose, and therefore, their activities in the scutellum should be great enough to account for the rates of sucrose breakdown shown in Fig 1

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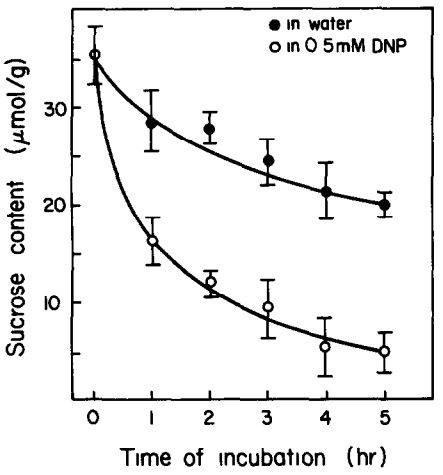

Fig 1 Utilization of endogenous sucrose by maize scutellum slices incubated in water or 0 5 mM DNP Each point is an average from three experiments

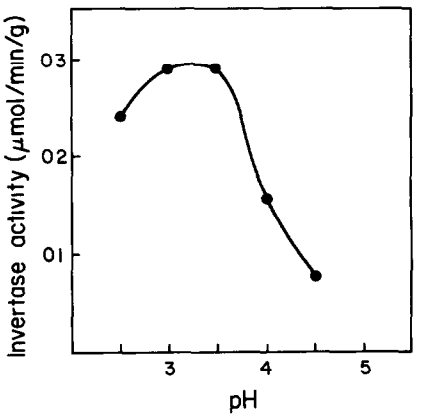

Fig 2 Effect of pH on the invertase activity of the 'solids' fraction of scutellum homogenates Each point is an average from two experiments

Enzymes involved in sucrose breakdown

Invertase Invertase activity was not present in the soluble or membrane fractions of scutellum homogenates at all pH values tested (2 5–8 5) In a previous paper from this laboratory [9], freeze-labile soluble invertase activity with pH optima of 6 and 7 was reported in scutellum homogenates It was not possible to reproduce these results despite repeated efforts including grinding with mortar and pestle instead of the Virtis homogenizer The membrane fraction was tested as a possible source of the invertase reported earlier, but it did not contain invertase activity A different maize cultivar was used in the present experiments, and this may explain the different results However, the presence in the scutellum of a cytosolic invertase is unlikely because incubation of slices in high concentrations of sugar alcohol or in mannose greatly inhibits the breakdown of cytosolic sucrose [8, 10], and these treatments should not inhibit invertase

The 'solids' fraction of the scutellum homogenates contained invertase activity with a pH optimum of 3 0–3 5 (Fig 2) The activity declined steeply on both sides of the pH optimum, and there was no activity above pH 5 A free-space invertase in scutellum slices was reported previously [9], and it was presumed to be located outside the plasmalemma and bound to the cell wall

Soluble invertases are known to bind firmly to cell wall material when the cell is disrupted [11, 12] The bonds formed between the soluble invertase and the wall material are believed to be of the ionic type or formed through a protein-tannin-wall complex since they can be broken by solutions of high ionic strength or by nonionic detergents [11, 12] To test if part of the wall-bound invertase activity corresponded to soluble invertase bound to wall material after the cell was broken, the 'solids' fraction was treated with solutions of high ionic strength and with detergents in an effort to solubilize any bound invertase

After 1 hr incubation of the 'solids' fraction of scutellum homogenates with the solutions listed in Table 1, all the invertase activity remained in the insoluble part Little or no invertase activity was lost from the 'solids' fraction, and activity was absent from the treatment solution after overnight dialysis When the 'solids' fraction was treated with borate buffer, there was a large increase in invertase activity (Table 1) Borate treatment of the wall fraction of grape berry homogenates also resulted in an increase in invertase activity [11] Solutions similar to those of Table 1 have been used successfully in extracting soluble invertase from the cell wall fraction of sultana grape berries [11], Jerusalem artichoke tubers [12] and Japanese morning glory callus tissue [13] Presumably, the invertases solubilized in this way were cytoplasmic or vacuolar in origin

Table 1 Attempts to solubilize bound invertase from the 'solids' fraction of maize scutellum homogenates

Initial invertase activity* in 'solids' fraction	Treatment of 'solids' fraction (60 min, 30°)	Soluble invertase in treatment soln after dialysis†	Invertase activity* remaining in 'solids' fraction
0 290	1 M NaCl	ND	0 278
0 290	20 mM borate, pH 8 5	ND	1 020
0 290	5% Tween 20	ND	0 300
0 290	2% Carbowax 4000	ND	0 280

*Activity at pH 3 in μmol sucrose hydrolysed/min per g fr wt of scutellum slices Values are averages from two experiments

†Activity tested at pH 3 and 6 ND, Not detectable

Even though a soluble invertase, free or bound, was not found, it still was possible that soluble invertases were inhibited, inactivated or became strongly bound to cell debris in the crude scutellum extract To test these possibilities, an extract containing invertase activity from the maize seedling root-shoot axis was added during preparation of the scutellum extract The root-shoot extract was prepared in the same way as the scutellum extract used in the soluble invertase assay (see Experimental), and it contained invertase activity over a wide range of pH with optimum activity at pH 4 5–5 0 When the root-shoot extract was added during grinding the scutella, recovery of the added soluble invertase activity in the final dialysed extract was 88–105 % over the pH range 4 5–7 0

From the invertase experiments, it is concluded that the scutellum invertase is bound to the cell wall and that scutellum cells do not contain soluble or membrane-bound invertases Therefore, catabolism of endogenous sucrose must require SS

Sucrose synthase The effect of pH on the activity of scutellum SS in the breakdown direction is shown in Fig 3 The breakdown activity of SS was maximum over a broad range of pH from 6 to 8 5 The activity decreased more steeply on the acid side and showed the highest activity at pH 6 5 Similar observations have been reported for SS from other tissues [14] At the pH of the cytoplasm of maize scutellum cells (7 5–8 0) [15], the activity of SS at saturating substrate levels was 0 39 μmol/min per g fr wt of slices, and this rate is sufficient to account for the rates of sucrose utilization in water or DNP (Fig 1) SS breakdown activity at cellular levels of UDP (0 85 mM) [Echeverria and Humphreys, unpublished] was about 0 34 μmol/min, which is just sufficient to account for the rate of sucrose utilization in DNP (Fig 1) The activities of SS with TDP, ADP and GDP were less than the activity with UDP, and were insufficient to account for the rate of sucrose utilization in DNP (Table 2)

UDPG phosphorylase Breakdown of sucrose by SS produces fructose and NDPG Fructose can enter glycolysis directly, but the glucose moiety in NDPG must be converted to free glucose or glucose phosphate When UDPG and orthophosphate were incubated with the scutellum extract (pH 8, 30°) in the absence of PP_i, UDPG was not broken down Therefore, neither hydrolysis nor phosphorolysis of UDPG occurred However, a UDPG phosphorylase that converts UDPG + P_i to UDP + G-1-P has been found in potato tuber [16] The potato enzyme is stimulated ten fold by μM fructose-2,6-bisP (F-2,6-bisP) The scutellum extract was assayed for UDPG phosphorylase in the absence and presence of 2 μM F-2,6-bisP at pH 7 and 8, but the production of G-1-P could not be detected The addition of NaF was without effect When known amounts of G-1-P were included in the assay, recovery was 95 % or better

From these results, we draw the tentative conclusion that phosphorolysis of UDPG does not occur in the scutellum Although the scutellum extract did not contain UDPG phosphorylase activity, it did contain high levels of UDPG pyrophosphorylase activity, which could make the glucose in UDPG available for glycolysis

Pyrophosphorylases The predominant pyrophosphorylase activity in the scutellum extract was UDPG pyrophosphorylase (Table 3) The activity of this enzyme, 17 05 μmol/min per g fr wt of slices at pH 8, was in excess of that necessary for sucrose catabolism and greatly surpassed the pyrophosphorylase activities with ADPG, TDPG and GDPG (Table 3) Similar observations have been reported for pyrophosphorylases from many plant

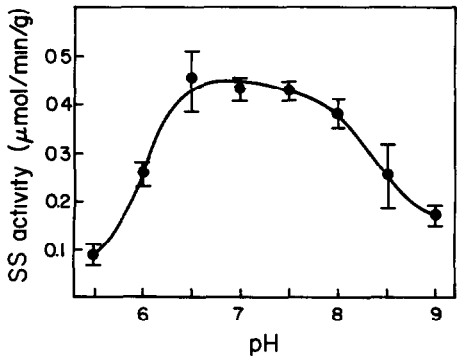

Fig 3 Effect of pH on the breakdown activity of sucrose synthase Each point in an average from three experiments

Table 2 Specificity of sucrose synthase in the breakdown direction for nucleoside diphosphates

Nucleoside diphosphate	SS activity*	% of activity with UDP
UDP	0 390	100
TDP	0 195	50
ADP	0 110	28
GDP	0 078	20

*Activity at pH 8 in μmol fructose produced/min per g fr wt of scutellum slices Results are averages from two experiments

Table 3 Enzymes involved in sucrose breakdown

Enzyme	Location	pH optimum	Activity, pH 8*
Sucrose synthase	soluble	6 5	0 39
Invertase	wall bound	3 0–3 5	ND
Invertase	soluble	—	ND
Pyrophosphorylases			
UDPG	soluble	9 0	17 05
TDPG	soluble	8 5	0 54
GDPG	soluble	8 5	0 23
ADPG	soluble	8 5	0 02
ATP pyrophosphohydrolase	soluble	8 0	0 25
ATP pyrophosphohydrolase + 2 μM F-2,6-bisP and 0 3 mM Ca^{2+}	soluble	8 0	0 87
Inorganic pyrophosphatase	soluble	8 0	3 55

*Activities in μmol/min per g fresh wt of slices Activities at pH 8 are given as an estimate of the *in vivo* rates, and are averages from two or more experiments ND, Not detectable

tissues (e g [17]) Optimum pH for the pyrophosphorylase activities in the scutellum extract was in the range of 8 5–9 0 Similar results have been obtained with pyrophosphorylases from both dicots and monocots (e g [18, 19])

The higher activities of UDPG pyrophosphorylase and of SS using UDP as the glucosyl acceptor (Tables 2 and 3) indicate that UDP is the nucleoside diphosphate involved in the sucrose breakdown system of maize scutellum cells Degradation of UDPG by scutellum extracts occurred only in the presence of PPi Levels of PPi in scutellum extracts could not be measured by the UDPG pyrophosphorylase phosphoglucomutase G-6-P dehydrogenase method because the levels of PPi were low relative to those of G-6-P which interferes in the assay The high inorganic pyrophosphatase activity of the scutellum extract (Table 3) indicates that PPi would not accumulate in the cytoplasm, and it appears that PPi production is required during UDPG utilization Many synthetic reactions (e g fatty acid thiokinase) produce PPi, but if these reactions served as major sources of PPi for UDPG pyrophosphorolysis, they would have to be temporally in step with sucrose breakdown It appears more likely that the direct hydrolysis of ATP to AMP + PPi would be the source of PPi

ATP pyrophosphohydrolase This enzyme hydrolyses ATP to AMP and PPi ATP pyrophosphohydrolase activity was found in the soluble fraction of scutellum homogenates, and it could be the source of PPi for the conversion of UDPG to G-1-P during sucrose catabolism The activity of ATP pyrophosphohydrolase was calculated by measuring the formation of hexose phosphates in a reaction mixture containing ATP, UDPG, UDPG pyrophosphorylase, $MgCl_2$, NaF and crude enzyme extract at pH 8, which was determined to be the pH optimum Because of the presence of inorganic pyrophosphatase in the extract, the measured activity (0 25 μmol/min per g fr wt of slices) was an underestimate (Table 3) When F-2, 6-bisP and Ca^{2+} were added to the reaction mixture, higher rates were obtained The maximum rate (0 87 μmol/min) was obtained with 2 μM F-2,6-bisP and 0 3 mM Ca^{2+} (Fig 4) When AMP was measured as an indication of ATP pyrophosphohydrolysis, the activity also was increased (to 1 1 μmol/min) by F-2,6-bisP and Ca^{2+}, indicating that Ca^{2+} and F-2,6-bisP acted directly on ATP pyrophosphohydrolase When ADP was substituted for ATP in the reaction mixture, AMP was not formed, indicating that AMP was produced by splitting pyrophosphate from ATP

When AMP formation was measured, Mg^{2+} (required for UDPG pyrophosphorylase) could be omitted from the reaction mixture Using AMP measurement, there was no increase in ATP pyrophosphohydrolase activity when Mg^{2+} was substituted for Ca^{2+} Considerable activity was obtained in the absence of added Ca^{2+}, even after dialysis of the extract against EDTA (Fig 4) Apparently, the enzyme binds Ca^{2+} very tightly as evidenced by the experiments of Fig 4 where Ca^{2+} activation was obtained in reaction mixtures that contained both EDTA (introduced with the scutellum extract) and ATP at concentrations considerably greater than that of Ca^{2+} The effective Ca^{2+} concentration for enzyme activation probably would be better obtained by incubating the extracts in Ca^{2+} solutions followed by dialysis

ATP pyrophosphohydrolase was first described in snake venoms [20] It has been found in cell membrane

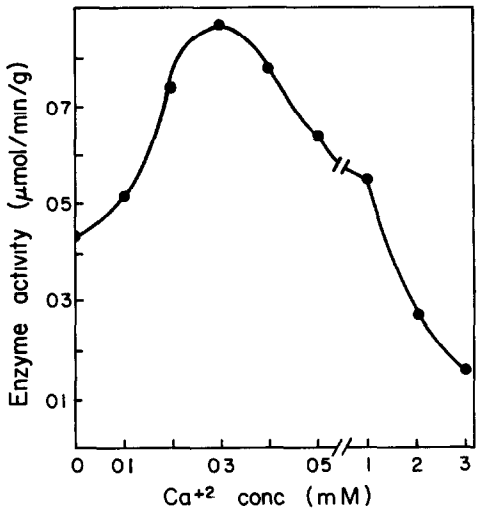

Fig 4 Effect of Ca^{2+} concentration on the activity of ATP pyrophosphohydrolase Enzyme activity tested in the presence of 2 μM F-2,6-bisP after overnight dialysis of the scutellum extract in EDTA Each point is an average from two experiments

fractions of rat liver cells [21] and rat erythrocytes [22] and in the soluble fraction of *Physarum polycephalum* homogenates [23] ATP pyrophosphohydrolase preparations from the plasma membrane of rat liver [21] and from the soluble fraction of *P polycephalum* [23] required Ca^{2+} for activity As with the fungal enzyme, scutellum ATP pyrophosphohydrolase activity was confined to the soluble fraction, the membrane fraction having no activity This is thought to be the first report of ATP pyrophosphohydrolase in higher plants

F-2,6-bisP has been reported in mung bean seedlings [24] and in leaves of pea and spinach [25] at concentrations of 0 3 to 1 0 mM F-2,6-bisP stimulates the activity of PPi-dependent phosphofructokinase from a wide range of leaf extracts [26] and from pea seedlings [24]

CONCLUSIONS

The data presented in this paper indicate that sucrose breakdown in maize scutellum cells occurs via the sucrose synthase UDPG pyrophosphorylase pathway as follows

$$\text{sucrose} + \text{UDP} \leftrightarrow \text{UDPG} + \text{fructose} \quad (1)$$

$$\text{UDPG} + \text{PPi} \leftrightarrow \text{UTP} + \text{G-1-P} \quad (2)$$

$$\text{ATP} \rightarrow \text{PPi} + \text{AMP} \quad (3)$$

The active inorganic pyrophosphatase of scutellum cells would prevent accumulation of PPi Therefore, primary control of the entire pathway would result from control of reaction (3), and this may involve F-2,6-bisP and Ca^{2+}

EXPERIMENTAL

Preparation of plant material Tissue slices were prepared from scutella of 3-day-old maize seedlings (*Zea mays* L cv DeKalb XL 80) as previously described [15]

Sucrose utilization by scutellum slices Groups of slices (0 5 mm or less in thickness, 0 5 g) were placed in 50 ml beakers and incubated in 10 ml of 10 mM $CaCl_2$ for 30 min at 30° in a

gyrotory H_2O bath Then (time 0) the slices were washed once in H_2O and incubated in H_2O or 0 5 mM DNP, the solns being replaced every 30 min until termination of the experiment At time 0 and each hr thereafter, groups of slices were killed in boiling 80% EtOH for sucrose analysis Procedures for the preparation of EtOH extracts and methods for sucrose analysis have been described [27]

Preparation of scutellum enzyme extracts For SS assay, scutellum slices (*ca* 1 5 mm in thickness, 10 g) were homogenized in a Virtis '60 K' homogenizer for 2 min in 100 ml of soln containing 100 mM Tris–HCl buffer pH 8 7, 30 mM cysteine-HCl and 20 mM EDTA The resulting homogenate was strained through two layers of cheesecloth and centrifuged in the cold at 21 000 *g* for 20 min The supernatant fraction was dialysed overnight in 4 l of 5 mM Tris–HCl pH 8 7 For soluble invertase assay, extracts were prepared as for SS assay except Pi buffer pH 7 was used For NDPG pyrophosphorylase, UDPG phosphorylase and ATP pyrophosphohydrolase assays, scutellum extracts were prepared as for SS assay except the soln contained 100 mM Epps buffer pH 8, 5 mM 2-mercaptoethanol and 5 mM EDTA The dialysis soln (4 l) contained 5 mM Epps pH 8 For some ATP pyrophosphohydrolase expts, 5 mM EDTA was added to the dialysis soln

Isolation of membrane fraction Membrane fractions were obtained after centrifugation of scutellum homogenates in a discontinuous gradient of 3 5 ml of 45% (w/w) sucrose and 1 ml of 34% sucrose in 5 mM Tris–HCl pH 7 2 as described in ref [28] Scutellum homogenates were prepared by homogenizing 10 g of slices in a Virtis '60 K' homogenizer for 2 min in 100 ml of soln containing 250 mM sucrose, 3 mM EDTA, 2 mM mercaptoethanol and 5 mM Tris–HCl pH 7 2 The homogenate was strained through two layers of cheesecloth and dialysed overnight against H_2O

Enzyme assays (a) *Sucrose synthase* The activity of SS in the breakdown direction was tested with saturating levels of substrate in a reaction mixture containing 5 mM NDP, 50 mM sucrose, 5 mM NaF, 100 mM buffer and scutellum extract in a total vol of 0 5 ml The buffers were Mes pH 5 5–6 5, Mops pH 7 0, Epps pH 7 5–8 0, Bicine pH 8 5–9 0 and glycine pH 9 5 The reactions were carried out at 30° for 10 min and stopped by boiling for 1 5 min After cooling, the tubes were centrifuged at 1000 g for 10 min, and the supernatant fractions were analysed for UDPG (when applicable) and fructose (b) *Pyrophosphorylase* Enzyme activity was tested with saturating levels of substrate in a reaction mixture containing 80 mM buffer (same buffers as used in SS assay), 2 mM NDPG, 10 mM NaF, 2 mM PPi, 2 mM $MgCl_2$ and scutellum extract in a total vol of 0 5 ml The reactions were carried out in the same way as those for SS assay The supernatant fractions were analysed for G-1-P, G-6-P and F-6-P It was necessary to include F-6-P in the measurement because the extracts contained phosphohexoisomerase (c) *UDPG phosphorylase* Enzyme activity was tested in a reaction mixture that contained 30 mM Mops pH 7 0 or Epps pH 8 0, 5 mM UDPG, 5 mM KPi, 2 μM F-2,6-bisP and scutellum extract in a total vol of 1 ml In some expts, 10 mM NaF was added The reactions were carried out in the same way as those for SS assay The supernatant fractions were analysed for G-1-P, G-6-P and F-6-P (d) *ATP pyrophosphohydrolase* Enzyme activity was tested in a reaction mixture that contained 80 mM buffer, 10 mM ATP, 5 mM UDPG, 2 5 units UDPG pyrophosphorylase (Sigma type X), 2 mM $MgCl_2$, 2 μM F-2,6-bisP, 0 3 mM $CaCl_2$ and scutellum extract in a total vol of 0 5 ml Activity was calculated by measuring the production of hexose phosphates or AMP When AMP was to be measured, UDPG, UDPG pyrophosphorylase and $MgCl_2$ were omitted (e) *Invertase* Soluble invertase was tested in a reaction mixture containing 80 mM buffer, 100 mM sucrose and scutellum extract in a total vol of 1 ml For invertase activity present in the insoluble fraction, scutellum slices (10 g) were homogenized in 100 ml cold H_2O for 2 min in a Virtis '60 K' homogenizer The homogenate was strained through two layers of cheesecloth, and the solid portion was used to test for invertase activity Portions of the 'solids' fraction (0 5 g wet wt) were washed × 3 with cold H_2O, and then 3 ml of a soln containing 80 mM buffer and 100 mM sucrose was added The reactions were run in a gyrotory H_2O bath at 30° for 30 min, and were terminated by adding 1 ml of 1 M Pi buffer pH 7 and boiling for 2 min After cooling, the suspensions were centrifuged at 1000 *g* for 10 min, and the supernatant fractions were analysed for glucose The buffers were the same as those used in the SS assay except that citrate was used below pH 5 5

Solubilization of bound invertase Portions of the 'solids' fraction (0 5 g wet wt) prepared as described above were incubated for 60 min at 30° in 3 ml of the solns listed in Table 1, and then the suspensions were centrifuged at 1000 *g* for 10 min The pellets (remaining 'solids' fraction) were washed × 3 with H_2O and tested for invertase activity The supernant fractions were dialysed overnight in 4 l of H_2O at 5° and then tested at pH 3 and 6 for invertase activity

Sugar determinations Glucose was determined by the glucose oxidase method Samples for sucrose determination were incubated for 2 hr with and without invertase (Sigma, grade X) prior to glucose analysis Fructose was analysed by the Nelson–Somogyi method [29, 30]

Determination of AMP, UDP, UDPG and hexose phosphates These compounds were assayed enzymatically by the procedures described in ref [31]

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