Phytochemistry, 1975, Vol. 14, pp. 333–340. Pergamon Press. Printed in England.

MALTOSE UPTAKE IN THE *ZEA MAYS* SCUTELLUM*

THOMAS E. HUMPHREYS

Department of Botany, Agricultural Experiment Station, University of Florida, Gainesville, FL 32611, U.S.A.

(*Received* 14 *May* 1974)

Key Word Index—*Zea mays*; Gramineae; maize; scutellum; maltose transport; plasmalemma; maltase.

Abstract—Maltose transport in slices of the maize scutellum was demonstrated despite the presence of an active maltase situated at the cell surface. The maltase could be inhibited or destroyed by treatments (neutral pH during uptake, pretreatment in Tris buffer at pH 7·5, or in 0·01 N HCl) that allowed appreciable rates of maltose uptake to occur. Using Tris- and HCl-treated slices, it was found that at disaccharide concentrations of 50 and 100 mM, maltose and sucrose were taken up at very nearly the same rates. At sugar concentrations below 50 mM, sucrose was taken up at greater rates than maltose. The maltose content of the slices was directly proportional to the maltose concentration of the bathing solution, and about 4 hr were required for equilibration. From this, it is concluded that one way maltose enters the slices is by free or facilitated diffusion. However, endogenous maltose is utilized by the slices at rates that are much too low to account for the net rates of maltose uptake. Although the slices contain a high level of surface maltase activity, only a low level of endogenous maltase activity was found. This probably accounts for the slow utilization of endogenous maltose. Therefore, the existence of a specific maltose transport system is proposed; a system that contains a carrier saturable with maltose, but one that does not release free maltose into the cytoplasm.

INTRODUCTION

During germination of cereal grains the starch of the endosperm is degraded to soluble sugars by hydrolytic enzymes [1–3]. Glucose is generally considered to be the product of this hydrolysis that is absorbed by the scutellum [2]. Maltose (4-*O*-α-D-glucopyranosyl-D-glucose) and maltotriose are also present in the endosperm during germination, but presumably are converted to glucose by amylases and maltase (E.C. 3.2.1.20) [2,4]. However, two reports indicate that maltose can be taken up by the scutellum without first being hydrolyzed to glucose [5,6].

This paper reports on the maltose transport systems of the maize scutellum. Evidence suggesting that the primary transport system does not release free maltose into the cytoplasm is presented.

RESULTS

Sugar uptake

The release of large amounts of glucose into the bathing solution when scutellum slices were incubated with maltose [5] makes it impossible to obtain accurate measurements of maltose uptake. Glucose release was presumed to be caused by a maltase located at the cell surfaces; and, therefore, attempts were made to inhibit this enzyme. Inhibition was achieved by raising the pH of the bathing solution, by pretreating the slices in Tris buffer or in HCl.

The effects of pH on glucose production and sugar uptake are shown in Table 1. As the pH of the bathing solution was increased glucose production from maltose was inhibited to a greater

Table 1. Effect of pH on sugar uptake and glucose production by scutellum slices incubated with maltose*

Bathing soln pH	Glucose in bathing soln (μmol/10 ml)	Sugar uptake (as glucose) (μmol/hr g)
Control†	50	27
4·5	56	29
5·6	40	29
6·5	14	22
7·6	4	13

* Slices (1·0 g fr. wt) were incubated in H_2O at 30° for 1 hr, washed once with water and then placed in maltose (10 mM) plus K phosphate buffer (40 mM). Incubation was continued at 30° for 4 hr during which samples of the bathing solution were removed for maltose and glucose determinations. The maximum amounts of glucose found in the bathing solutions and uptake rates during the last 2 hr of the maltose incubation are given in this table.

† No buffer, pH about 4.

* Florida Agricultural Experiment Station Journal Series No. 5269.

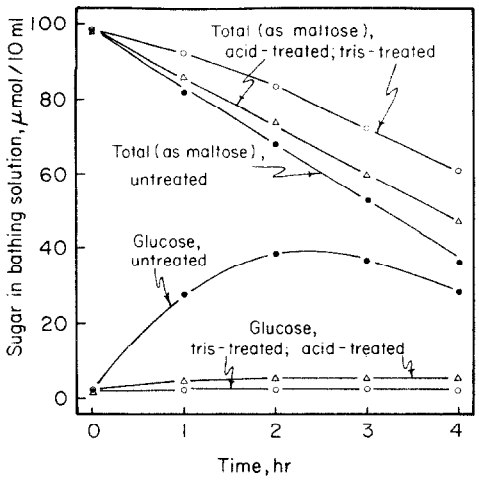

Fig. 1. Effect of Tris and HCl treatment of scutellum slices on sugar uptake and glucose appearance during incubation in 10 mM maltose. Slices (1·0 g fr. wt) were placed in flasks containing 10 ml of Tris buffer (0·06 M, pH 7·5) or HCl (0·01 M). The slices were incubated at 30° for 60 min (Tris) or 30 min (HCl). At the end of the incubation the bathing solution was removed and the slices were washed with 10 ml of H_2O. Untreated slices were incubated in H_2O for 60 min. Maltose uptake was measured as described in Experimental.

extent than sugar uptake. The pH optimum (4–5) for glucose production (Table 1) is in the range usually found to be optimum for higher plant maltases [7]. Furthermore, sucrose is hydrolyzed at a much lower rate than maltose by scutellum slices [8], indicating that the hydrolase activity is that of a true maltase, not an invertase (E.C. 3.2.1.26).

Tris was tested for its inhibition of glucose release because intestinal maltase has been shown to be inhibited by Tris at an alkaline pH [9]. Intestinal maltase has a pH optimum near 7; and, therefore, the Tris inhibition of intestinal maltase was not an effect of pH. However, in testing the effect of Tris on the scutellum maltase it was necessary, because of the low pH optimum for the scutellum enzyme, to pretreat the slices with Tris at pH 7·5; the treated slices were then washed with water before placing them in maltose at *ca* pH 5 (Fig. 1). Tris treatment almost completely stopped glucose production. Therefore, the amount of sugar that disappeared from the bathing solution is a direct measure of maltose uptake (Fig. 1). Maltose uptake into Tris-treated slices increased with time of incubation in maltose until a steady state was obtained after 2 hr. Similar results were obtained at all maltose concentrations tested. Therefore, maltose uptake rates for Tris-treated slices were calculated from data obtained in the periods 2–4 hr or 2–5 hr after the start of the maltose incubation.

Previous work indicates that HCl pretreatment does not inhibit sucrose production when slices are incubated in fructose or sucrose [10]. Apparently, the cells are protected by the plasmalemma. However, a maltase situated on or outside the plasmalemma might be destroyed by the acid treatment. This was found to be the case (Fig. 1). After acid treatment, the release of glucose was almost completely stopped and maltose was taken up at a constant rate over the 4-hr experimental period. During the last 2 hr HCl- and Tris-treated slices took up maltose at nearly the same rate. Incubation in HCl removes considerable Mg^{2+} and small amounts of Ca^{2+} from the slices [11]. A 30-min incubation of the acid-treated slices in 0·05 M $CaCl_2$ or $MgCl_2$ had no effect on maltose uptake when slices subsequently were placed in maltose solutions.

Figure 2 shows the release of glucose from untreated slices and from Tris- and HCl-treated slices incubated in 20 and 100 mM maltose solutions. HCl treatment prevented glucose release during the entire 5-hr experimental period with both mal-

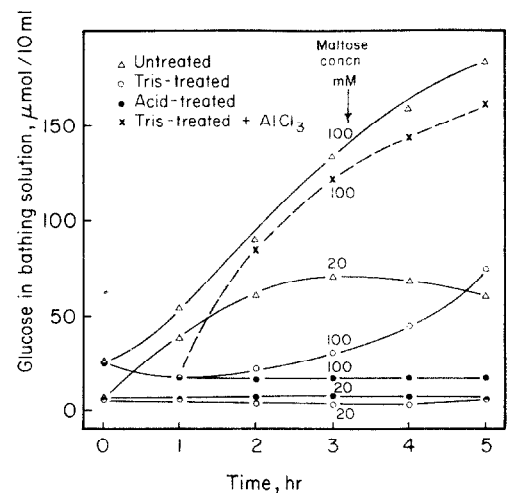

Fig. 2. Effect of maltose concentration on the release of glucose into the bathing solution by treated and untreated slices. Slices (1·0 g fr. wt) were placed in maltose solutions at time zero. After 1 hr the bathing solution was removed from one of the flasks containing Tris-treated slices in 100 mM maltose and was replaced with 100 mM maltose plus 20 mM $AlCl_3$. The glucose in the flasks at time zero was introduced as an impurity in the maltose. See footnote, Fig. 1 for preparation of Tris- and HCl-treated slices.

tose concentrations. Tris treatment prevented glucose release when 20 mM maltose was used. However, when 100 mM maltose was used, glucose was released at an increasing rate after the 1st hr until by the 5th hr the rate of glucose release from the Tris-treated slices approached that of the untreated controls. This is in line with the proposal [9] that Tris inhibits by binding to the active site of intestinal maltase in such a way that it is competitive with maltose. Tris inhibition of glucose production was released by $AlCl_3$ (Fig. 2). $AlCl_3$ also releases Tris inhibition of sucrose uptake in scutellum slices [12]. Presumably, Al^{3+} reacts directly with Tris bound to the slices [13]. The ready reversibility by Al^{3+} of the Tris inhibition of glucose release is additional evidence for the presence of a maltase on or outside the plasmalemma.

In Fig. 3 the rates of sucrose and maltose uptake as a function of disaccharide concentration are compared. Sugar uptake refers to total sugar disappearance from the bathing solution calculated as disaccharide. In the sucrose bathing solutions the glucose concentration did not exceed 0·5 mM; and, therefore, the rate of sugar disappearance and the rate of sucrose uptake were essentially the same. However, at the higher maltose concentrations (50 and 100 mM) considerable glucose was released from the Tris-treated slices but not from the HCl-treated slices (see Fig. 2). Nevertheless, maltose uptake into the two kinds of slices was almost identical (Fig. 3). Evidently, glucose had little effect on the total sugar uptake, and it is concluded that the maximum rates of sucrose and maltose uptake are about the same. Tris treatment inhibited sucrose uptake (20–25%), and probably there also was some inhibition of maltose uptake as a result of Tris treatment. Maltose uptake was inhibited 85% when Tris-treated slices were incubated in a maltose–Tris solution (50 mM maltose, 80 mM Tris, pH 7·5).

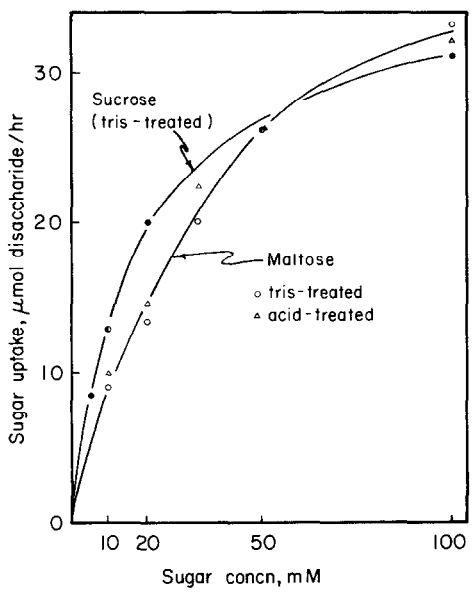

Fig. 3. Rates of sucrose and maltose uptake in Tris- and HCl-treated scutellum slices as a function of disaccharide concentration. Each point on the graph represents an average rate calculated from the results of three to five experiments. See footnote, Fig. 1 for preparation of Tris- and HCl-treated slices.

Neither the maltose curve nor the sucrose curve in Fig. 3 follows the typical hyperbolic substrate concentration curve. In contrast, the sucrose uptake vs concentration curve obtained with untreated slices closely fits the hyperbolic Michaelis–Menten curve [5]. However, even with untreated slices the time-course of sucrose uptake indicates that the kinetics are more complicated than the pseudo first order kinetics of the Michaelis–Menten type [5].

In a previous paper [14] the maximum sucrose flux through the plasmalemma of the scutellum cell was calculated (from data obtained with untreated slices) to be 12·3 pmol/cm^2 sec; the maximum maltose flux must be close to this figure.

Endogenous maltose and sucrose

One g of freshly prepared scutellum slices contained 60–70 μmol of sucrose, less than 2 μmol of glucose and less than 1 μmol of maltose. It took about 1 hr to prepare "freshly prepared" slices; and, therefore, the above sugar data were obtained from cells that had been removed from their external glucose and maltose supply (the endosperm)

Table 2. Tissue sugar contents after incubation of scutellum slices in water or sugar solutions

Treatment*	Tissue sugars (μmol/g) Sucrose	Maltose	Glucose
Zero time control	68·1	0·6	1·7
Water	44·1	0·5	2·4
Maltose	91·7	12·0	4·9
Sucrose	125·0	0·4	5·6
Glucose	123·6	0·4	6·7

* 0·2 M solutions of the sugars; measurements were made after 4 hr.

for 1 hr. The effects of incubating slices in water or sugar solution on the tissue sugar levels are shown in Table 2. Incubation in maltose, sucrose or glucose substantially increased the endogenous sucrose level and also increased the glucose level. An increase in endogenous maltose was obtained only after incubation in maltose. These results are not surprising since in higher plants the synthesis of maltose from hexose has not been demonstrated, and maltose is thought to be produced only from starch degradation [15]. The starch content of the slices (6·0–7·7 mg starch/g fr. wt) did not change during a 4-hr incubation in water, glucose, sucrose or maltose. Although an occasional intracellular starch grain was seen in electron micrographs of scutellum cells, most of the starch was probably endosperm starch that adhered to the slices. Starch metabolism does not appear to be a factor in these experiments.

Chromatograms of extracts of slices that had been incubated in water, sucrose or glucose contained strong sucrose and faint glucose and fructose spots, whereas those prepared from slices incubated in maltose contained light maltose spots in addition.

The amounts of endogenous maltose and sucrose in untreated slices as functions of time of incubation in maltose and of the maltose concentration of the bathing solution are shown in Fig. 4. Since untreated slices were used in these experiments considerable quantities of glucose appeared in the bathing solutions during the incubation (see Fig. 2).

During incubation of slices in 0·2 M maltose the endogenous sucrose level increased linearly during the first 2·5 hr, and during the last 1·5 hr of the incubation the rate of increase fell about 30%. In contrast, the endogenous maltose level increased linearly only during the first hr, and by the end of 4 hr had nearly reached a plateau (Fig. 4a). Figure 4(b) shows that the maltose content of the slices at the end of the 4-hr incubation was directly proportional to the maltose concentration of the bathing solution. The sucrose content, on the other hand, was increased to about the same level after incubation in each of the three maltose concentrations tested.

When the maltose solution bathing the slices was replaced with water, the endogenous maltose level declined with time (Fig. 5). Tris-treated slices

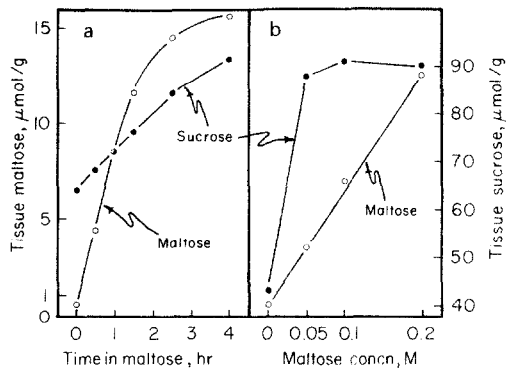

Fig. 4. Tissue contents of maltose and sucrose as functions of time of incubation in maltose and of maltose concentration. Following each maltose incubation the slices, before being killed, were given a 30 min H_2O incubation (see Experimental). (a) Untreated slices were incubated in 0·2 M maltose. (b) Untreated slices were incubated in maltose solutions for 4 hr.

contained less maltose than untreated slices. Tris causes scutellum slices to become leaky towards sucrose [12] and during the first 30 min water incubation period Tris-treated slices leaked 2 μmol more maltose than untreated slices. Presumably, however, most of the maltose that leaked initially was from intercellular spaces. Furthermore, when the initial wash water was replaced after 30 min with fresh water, less than 1·0 μmol of maltose or glucose leaked from the slices (Tris-treated or un-

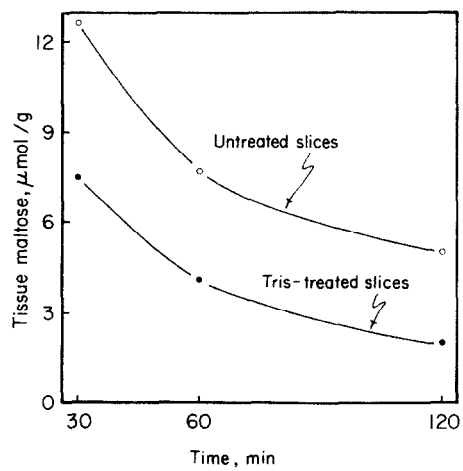

Fig. 5. Decline in maltose content of scutellum slices with time of incubation in water. Slices were incubated in 0·2 M maltose for 4 hr. At the end of the maltose incubation the slices were washed 2× with 10 ml H_2O, and then incubated in water for 30 min (see Experimental). The first two groups of slices were killed at this time. The other four groups were placed in fresh water and incubated for an additional 30 or 90 min.

Table 3. Disaccharidase activity of insoluble residues prepared from scutellum slices*

Residue from slices pretreated with	Substrate	Disaccharidase activity† pH 7·0	pH 4·0	K_m (mM) pH 4·0
H_2O	Maltose	8·2	46·8	4·6
HCl		3·6	6·5	1·4
H_2O	Sucrose	4·8	7·1	2·1
HCl		4·0	6·4	1·3

* Slices (1·0 g. for acid treatment; 0·5 g for H_2O treatment) were treated in 0·01 N HCl or in H_2O (footnote, Fig. 1) and then were ground in citrate–phosphate buffer at pH 5·0 or 7·4. The insoluble residue (see Experimental) from the pH 5·0 preparation was assayed at pH 4·0; the insoluble residue from the pH 7·4 preparation was assayed at pH 7·0.

† Activity expressed as μmol 1 hr disaccharide hydrolyzed per g of fresh slices when the preparations were incubated at 30° in buffered disaccharide solutions. Disaccharide concentration: 50 mM for residues from H_2O-treated slices and 25 mM for residues from HCl-treated slices.

treated) during the next 1·5 hr during which the decline in tissue maltose was measured (Fig. 5). Therefore, the results of Fig. 5 represent utilization of endogenous maltose, not leakage.

In higher plants maltose utilization requires maltose hydrolysis as a first step. The low rate of endogenous maltose utilization (Fig. 5) perhaps results from a low level of maltase activity in the cytoplasm. For this reason maltase and invertase activities and distributions were determined in extracts of scutellum slices. Slices were ground in sand with citrate–phosphate buffer at pH 5·0 or 7·4. A soluble invertase was found in the pH 7·4 extract (activity: 5·8 μmol sucrose hydrolyzed per hr per g of fresh slices). The rest of the invertase and maltase activities were found in the insoluble residue. The insoluble disaccharidases were not made soluble by changing the pH (5·0–7·4 or vice versa) or by a 30-min incubation in 0·5% Triton X100. The insoluble disaccharidases had pH optima near 4 irrespective of whether the slices were ground at pH 5·0 or 7·4. Some groups of slices were treated with HCl (footnote, Fig. 1) before being ground in order to destroy the surface maltase. The activities of the insoluble disaccharidases are shown in Table 3.

The following points can be made from the results of Table 3. (a) Most of the maltase was sensitive to HCl treatment; and, therefore, was either on or outside the plasmalemma. (b) The maltase remaining after HCl treatment differs from the HCl-sensitive maltase since it has a different K_m. (c) Most of the insoluble invertase activity remained after HCl treatment. (d) In the insoluble residue from acid-treated slices, essentially the same disaccharidase activity and the same K_m were found when maltose was the substrate as when sucrose was the substrate.

The amounts of maltase activity, both the total amount and the amount remaining after acid treatment, found in preparations of frozen and thawed slices (see Experimental) were essentially the same as those found in the insoluble residues of ground slices. Furthermore, the amount of activity lost as a result of acid treatment was very nearly the same as the "maltase activity" (i.e. the rate of glucose release in the presence of maltose) found with fresh slices (see Fig. 2). These results (Table 4) are additional evidence that the slices contain two kinds of maltase: one situated on or outside the plasmalemma; the other situated in or interior to the plasmalemma.

Table 4. Estimates of extracellular maltase activity in three scutellum-slice preparations*

Kind of slice used	Maltase activity† Insoluble residue	Frozen–thawed slices	Fresh slices
H_2O-treated	44·4	46·4	39·4
HCl-treated	6·5	6·0	<1
Difference = extracellular	37·9	40·4	39·4

* The preparations of the insoluble residue and the frozen–thawed slices are given in the Experimental.

† See footnote Table 3. The maltase activities in this table were obtained at pH 3·5 (65 mM citrate–phosphate buffer) since this was optimum for the fresh-slice assay.

DISCUSSION

The hydrolysis of maltose by a maltase situated at the cell surface obscured the existence of maltose transport in the maize scutellum. However, the fact that maltose hydrolysis could be strongly inhibited by treatments that allowed appreciable rates of sugar uptake to occur (Fig. 1, Table 1) clearly indicates that maltose transport took place. The results suggest that there are at least two transport systems for maltose, one driven either by free or facilitated diffusion, and the other accepting maltose at the cell exterior but not releasing free maltose into the cytoplasm.

The results shown in Fig. 4 support the idea that maltose can enter the slices either by free or facilitated diffusion. The amount of tissue maltose was directly proportional to the exogenous maltose concentration (Fig. 4b), and it took *ca* 4 hr to equilibrate the maltose between tissue and bathing solution (Fig. 4a). The length of time required to reach equilibrium suggests free instead of facilitated diffusion as the mechanism of entry.

Garrard and Humphreys [16] found an intracellular glucose space in scutellum slices whose volume was about 0·1 ml/g fr. wt of tissue. Glucose space is defined as the volume of tissue water necessary to contain the glucose of the tissue at the concentration of the bathing solution [17]. Therefore, 1 g of scutellum slices incubated in 0·1 M or 0·2 M glucose contained 10 μmol or 20 μmol of glucose [16]. The maltose contents were lower than this (Fig. 4b), but there was undoubtedly some utilization of tissue maltose during the 30 min water incubation used to remove extracellular maltose (Fig. 5). Furthermore, tissue maltose probably had not reached equilibrium with the bathing solution even after 4 hr (Fig. 4a). Glucose, on the other hand, equilibrates between tissue and bathing solution in 30 min [16]. It is suggested, therefore, that the intracellular maltose space coincides with the glucose space. The size of the space and the fact that glucose rapidly leaks from the space until the tissue is nearly devoid of glucose [16] suggest that the glucose maltose space coincides with the cytoplasm. If this is correct, the cytoplasmic maltose concentrations in Fig. 5 would range from 120 to 50 mM in untreated slices and from 75 to 20 mM in Tris-treated slices. These endogenous maltose concentrations supported rates of maltose utilization (Fig. 5) that were much lower than rates of maltose uptake from the same concentrations of exogenous maltose (Fig. 3). Low rates of endogenous maltose utilization would be expected from the results of Tables 3 and 4.

The low rates of tissue maltose utilization (Fig. 5) rule out the idea that maltose is taken up *only* by free or facilitated diffusion and that curves supposedly showing maltose uptake (e.g. Figs. 1 and 3) actually show maltose utilization from a cytoplasmic pool in equilibrium with the bathing solution.

From the above considerations it is concluded that there is a second maltose transport system, that this system contains a carrier saturable with maltose and that this system does not release free maltose into the cytoplasm. The bulk of maltose transport is carried by the second system.

If the results shown in Fig. 4 indicate an equilibration between exogenous and endogenous maltose pools, why did not the endogenous maltose equilibrate with the bathing solution when the slices were removed from maltose and placed in water? In the experiment of Fig. 5 less than 0·1 μmol of maltose or glucose leaked from the slices during the 1·5 hr period following the initial 30 min wash. A possible explanation is that as maltose diffuses out of the cell across the plasmalemma it is picked up (from within the interstices of water-filled membrane pores, for instance) by the second maltose transport system and does not reach the bathing solution. This explanation places transport via the second maltose transport system as a first step in the utilization of both exogenous and endogenous maltose. The low level of maltase activity at pH 7 (Table 3) and the fact that all the maltase activity is insoluble and, therefore, perhaps membrane bound, are consistent with this interpretation.

The conclusion that free maltose is not released into the cytoplasm by the second transport system matches the conclusion of a previous paper [14] that sucrose transport does not release free sucrose into the cytoplasm. The conclusion about sucrose was based on the observation that scutellum slices could take up and utilize exogenous sucrose under conditions that completely inhibited the utilization of cytoplasmic and vacuolar sucrose. If not free maltose or free sucrose, what is released?

During sucrose transport the evidence suggests that sucrose is split into glucose and fructose but

that free hexoses are not released into the cytoplasm [18]. The argument against free hexose being released is that the presence of free hexose in the cytoplasm should result in hexose leakage into the bathing solution when, in fact, very little hexose is found in the bathing solution during disaccharide uptake. For example, during 4 hr in 0·1 M maltose the HCl-treated slices took up about 120 μmol of maltose (Fig. 3), but less than 4 μmol of glucose appeared in the bathing solution.

A speculative working model of disaccharide transport at the scutellum plasmalemma has been presented [18]. The model contains a disaccharidase sequestered in the interior of the membrane and connected to the outer surface by a specific disaccharide carrier and to the inner surface by a phosphotransferase. In this model, hexose phosphates are the transport products released into the cytoplasm. The presence of insoluble disaccharidase activity in acid-treated slices (Tables 3 and 4) and the fact that very nearly the same activity and the same K_m is found with maltose and sucrose are consistent with this model and with the similar rates of uptake obtained with the two disaccharides (Fig. 3). The low disaccharidase activity in comparison to rates of disaccharide uptake could be a result of an uncoupling between the carrier or the phosphotransferase and the disaccharidase in the preparations used to assay disaccharidase activity (Table 4).

Maltose transport in yeasts [19] and bacteria [20] releases free maltose into the cell. In contrast, there is good evidence that in the amphibian and mammalian intestine the disaccharide transport systems of the brush border accept their respective disaccharides but release free hexoses into the epithelial cell [21,22]. In fact, a disaccharidase may be the carrier in the membrane. Storelli *et al.* [23] prepared a lipid bilayer from the total lipids of hamster small intestinal brush borders that was essentially impermeable to sucrose, glucose and fructose. When a purified sucrase-isomaltase complex (from rabbit brush borders) was added to the lipid bilayer system, the resulting synthetic membrane released glucose and fructose on one side when sucrose was placed in the compartment facing the other side.

EXPERIMENTAL

Plant material. Maize grains (*Zea mays* L., cv. Funks G-4455) were soaked in running tap water for 24 hr and then placed on moist paper towels in the dark at 24–25° for 72 hr. The scutella were excised and cut transversely into slices 0·5 mm or less in thickness. The slices were washed in H_2O until the washings remained clear, blotted on filter paper and weighed in groups of 1·0 g.

Experimental procedure. Slices were incubated in 10 ml of appropriate soln at 30°. For sugar uptake experiments, slices were incubated in H_2O for 30 min and then given 2 washes, 1 with 10 ml H_2O and 1 with 3 ml of an appropriate sugar soln. The wash solns were removed by suction, 10 ml of an appropriate sugar soln were added to the flasks, and uptake measurements were begun. Uptake was measured by measuring the disappearance of sugar from the bathing solution.

Analysis of sugars. Glucose was determined by the glucose oxidase method. Samples were incubated with commercial (Sigma) amyloglucosidase (for maltose analysis) or invertase (for sucrose analysis) solns for 2 hr in 10 mM acetate buffer, pH 4·8, before determining glucose. Amyloglucosidase completely hydrolyzed maltose but had no effect on sucrose, whereas invertase completely hydrolyzed sucrose but had no effect on maltose. To extract sugars, scutellum slices (1·0 g) were "killed" with 20 ml boiling 80% EtOH for 30 sec. After 1 hr at room temp. the EtOH was decanted, and the tissue re-extracted with 20 ml and 3 × 5 ml 50% EtOH. The combined filtered extracts were evaporated to 2 ml and made up to 50 ml with H_2O. Prior to analysis, the extract was centrifuged (1000 *g* for 10 min) and refiltered. Portions were then chromatographed (EtOA–pyridine–H_2O; 8:2:1) and the chromatograms were sprayed with aniline–diphenylamine. Starch was determined in the residue of the slices remaining after EtOH extraction by the method of Dekker and Richards [24]. Before killing, slices that had been incubated in sugar solutions were washed 2 × 10 ml H_2O, and incubated in 10 ml H_2O at 30° for 30 min to remove extracellular sugars (less than 0·1 μmol of any sugar leaked during this additional 30 min incubation).

Disaccharidase assay. Slices (0·5 or 1·0 g) were ground with sand (1 g) and citrate–phosphate buffer (2 ml; 75 mM, pH 5·0 or 93 mM, pH 7·4), and the slurry, transferred to a centrifuge tube with an additional 8 ml of buffer, was centrifuged at 20000 *g* for 20 min. The supernatant fraction was dialyzed overnight against 1 liter 30 mM buffer (pH 5·0 or 7·4), the sediment was suspended in 10 ml fresh buffer and centrifuged again. The entire washed sediment (insoluble scutellum residue plus sand) and portions of the dialyzed supernatant fraction were assayed for disaccharidase activity at the appropriate pH and maltose or sucrose concentration. Another preparation for disaccharidase assay was obtained by freezing and thawing the slices. Slices (0·5 or 1·0 g) were washed with 5 ml of citrate–phosphate buffer (75 mM, pH 5·0), the buffer removed, and the slices then frozen at −20°. After 3–5 hr the slices were washed with 10 ml of buffer (75 mM, pH 5·0) at 30° for 20 min. The buffer was removed, and the slices were given a second 20-min wash with 30 mM buffer, pH 5·0. The maltase activity of the washed, frozen and thawed slices was then assayed at the appropriate pH and maltose concentration.

Acknowledgement—I thank Dr. L. A. Garrard for helpful discussions.

REFERENCES

1. Dure, L. S. (1960) *Plant Physiol.* **35,** 925.
2. Murata, T., Akazawa, T. and Fukuchi, S. (1968) *Plant Physiol.* **43,** 1899.
3. Nomura, T., Kono, Y. and Akazawa, T. (1969) *Plant Physiol.* **44,** 765.
4. Dure, L. S. (1960) *Plant Physiol.* **35,** 919.

5. Whitesell, J. H. and Humphreys, T. E. (1972) *Phytochemistry* **11,** 2139.
6. Chen, S. S. C. and Varner, J. E. (1969) *Plant Physiol.* **44,** 770.
7. Gottschalk, A. (1950) in *The Enzymes* (Sumner, J. and Myrback, K., eds.), p. 551. Academic Press, New York.
8. Humphreys, T. E. and Garrard, L. A. (1968) *Phytochemistry* **7,** 701.
9. Larner, J. and Gillespie, R. E. (1956) *J. Biol. Chem.* **223,** 709.
10. Garrard, L. A. and Humphreys, T. E. (1971) *Phytochemistry* **10,** 243.
11. Humphreys, T. E. and Garrard, L. A. (1971) *Phytochemistry* **10,** 981.
12. Humphreys, T. E. and Garrard, L. A. (1969) *Phytochemistry* **8,** 1055.
13. Mahler, H. R. (1961) *Ann. N.Y. Acad. Sci.* **92,** 426.
14. Humphreys, T. E. (1973) *Phytochemistry* **12,** 1211.
15. Whelan, W. J. (1971) *Biochem. J.* **122,** 609.
16. Garrard, L. A. and Humphreys, T. E. (1965) *Nature* **207,** 1095.
17. Morgan, H. E., Henderson, M. J., Regen, D. M. and Park, C. R. (1961) *J. Biol. Chem.* **236,** 253.
18. Humphreys, T. E. (1974) *Phytochemistry* In press.
19. Sols, A. and de la Fuente, G. (1961) in *Membrane Transport and Metabolism* (Klenizeller, A. and Kotyk, A., eds.), p. 361. Academic Press, New York.
20. Wiesmeyer, H. and Cohn, M. (1960) *Biochem. Biophys. Acta* **39,** 440.
21. Crane, R. K., Malathi, P., Caspary, W. F. and Ramaswamy, K. (1970) *Fed. Proc.* **29,** 595.
22. Parsons, D. S. and Prichard, J. S. (1968) *J. Physiol.* **199,** 137.
23. Storelli, C., Vogeli, H. and Semenza, G. (1972) *FEBS Lett.* **24,** 287.
24. Dekker, R. F. H. and Richards, G. N. (1971) *J. Sci. Food Agric.* **22,** 441.