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L-GLUTAMINE AND D-GLUCOSE UPTAKE BY DEVELOPING ENDOSPERMS OF MAIZE

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Key Word Index—*Zea mays*; Gramineae; maize; endosperm; uptake; amino acids; sugars.

Abstract—Developing maize (*Zea mays*) kernels were incubated with L-[G-^{3}H]glutamine and D-[U-^{14}C]glucose to characterize mechanisms of amino acid and sugar uptake by the endosperm tissue. The results indicate that mechanisms of L-glutamine uptake are different from those of D-glucose. In contrast to D-glucose uptake, L-glutamine uptake showed substrate-saturable kinetics and was inhibited by metabolic inhibitors and protein reagents. The pH optimum for L-glutamine uptake (pH 5.0–5.5) was lower than the optimum of D-glucose uptake (pH 7). EDTA (10 mM) stimulated L-glutamine uptake but inhibited D-glucose uptake. The uptake of D-glucose is not a simple diffusion process. L-Glucose, a stereoisomer of D-glucose, was taken up by endosperms at a much slower rate than was D-glucose, indicating a high degree of specificity of the sugar uptake system. The involvement of basal endosperm transfer cells in transport of assimilates into developing maize endosperms is discussed.

INTRODUCTION

The unloading of nutrients from phloem termini into pedicel and the subsequent uptake by the endosperm tissue are important processes which may affect kernel development. The unloading processes have been intensively studied, in part, due to the development of an empty seed coat technique [1–3]. In contrast, the mechanisms of amino acid uptake by developing maize endosperms are unknown, and the mechanisms of sugar uptake have only recently been partially characterized [4].

The unloaded nutrients in the pedicel have to pass through a layer of transfer cells before uptake by the endosperm. These cells are usually located in strategically important places where efficient transport of metabolites is required [5]. Transformation of the outermost layer of endosperm cells in the pedicel region into transfer cells suggests that developing endosperm tissue is confronted with a limitation of assimilate availability. The concentrations of sugar and amino acid are higher in the pedicel region than in the endosperm tissue [4, 6]. This may indicate that the rate of assimilate uptake by endosperms is slower than the rate of accumulation in the pedicel. The build-up of radioactivity in the pedicel region after $^{14}CO_2$-feeding of the ear leaf supports such an assumption [7].

Among organic nitrogen compounds, glutamine is the main amino acid transported into developing maize endosperms [8, 2, 6, 8]. It makes up about 20% of the total amino acid pool in the vascular sap, 30–50% in the pedicel region, and 30% in the endosperm tissue [2, 9]. The pedicel region, while containing the highest proportion of glutamine in the free amino acid pool among the major maize tissues tested [10], has a high activity of enzymes involved in amino acid metabolism [9]. These enzymes are probably responsible for modifications of free amino acid pools in this region relative to the vascular sap and the endosperm tissue. The conversion of amino acids between the seed coat and the embryo has been observed in soybean seeds [11]. However, the importance of these amino acid interconversions on uptake processes remains unknown.

The mechanisms of unloading and uptake differ among crop species and sink tissues [12, 13]. In many legume seeds, the process of amino acid unloading from phloem termini in the sink region requires energy and carriers [14, 15]. Similarly, the uptake of amino acids by developing seeds such as soybean, barley, oat, consists of selective transport systems with different degrees of specificity toward selected groups of amino acids [13, 14, 16, 17]. However, amino acid unloading in maize kernels is not inhibited by a short-term treatment with uncouplers or sulphhydryl-blocking reagents suggesting a diffusion-like process [2]. Sugar unloading in the pedicel region and its subsequent uptake by endosperm tissue show non-saturable kinetics in maize kernels as well. Furthermore, these processes are not affected by metabolic inhibitors [1, 2, 4, 9]. In this respect, the mechanisms of sugar transport in maize kernels appear to differ from those in many legumes [15, 18–20].

The purpose of these investigations was to characterize mechanisms of glutamine uptake by developing maize endosperms and to compare them with the uptake of D-glucose.

RESULTS

Kinetics of L-*glutamine uptake*

The uptake of L-glutamine by endosperms containing the pedicel region was dependent on the L-glutamine concentration in the medium (Fig. 1). The slope of the uptake–response curve at L-glutamine concentration range of 0–20 mM was greater than that above 20 mM L-glutamine. It indicates a saturation system at higher L-glutamine concentrations. Although, it is not clear whether the metabolism of amino acids in the pedicel

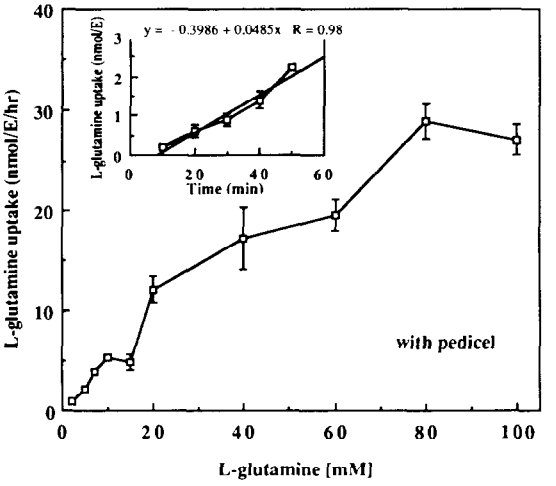

Fig. 1. Concentration dependence of L-glutamine uptake by maize endosperms with the intact pedicel region. Insert; time course of L-glutamine uptake at 10 mM after 15 min preincubation. Each data point represents the mean ± s.e. The uptake rates were determined as depicted in the insert and expressed as nmol of L-glutamine taken up per endosperm (E) per hr.

region may regulate uptake, a short term incubation of 80 min with L-glutamic acid indicated that no significant conversion into L-glutamine occurred despite a very high activity of glutamine synthetase in this region (data not shown).

Similar uptake kinetics were observed when kernels with the pedicel tissues removed were incubated (Fig. 2); however, the uptake was several times higher than in kernels with intact pedicel (Figs 1 and 2). At low L-glutamine concentrations in the range 0.05–10 mM, the uptake rate was a linear function of concentrations (Fig. 2; insert).

Treatment of kernels without pedicel tissue with 2,4-dinitrophenol (DNP), *p*-chloromercuribenzene sulphuric acid (*p*CMBS), diethylstilbestrol (Des), and carbonyl-cyanide *m*-chlorophenyl-hydrazone (CCCP) markedly inhibited L-glutamine uptake, whereas EDTA at 10 mM increased the rate of L-glutamine uptake. Sucrose at 100 mM showed a slightly inhibitory effect on L-glutamine uptake; however, this difference was not statistically significant (Table 1). The uptake of L-glutamine was dependent on the pH of the incubation mixture (Fig. 3). The pH optimum of L-glutamine uptake was *ca* 5.0–5.5 when assayed at 30°.

Kinetics of sugar uptake

Similar experiments were performed using D-glucose as a substrate. Metabolic inhibitors and protein reagent showed only a slight inhibition on D-glucose uptake

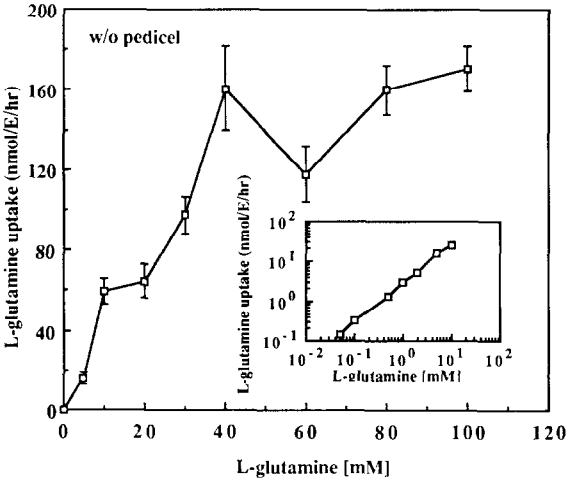

Fig. 2. Concentration dependence of L-glutamine uptake by maize endosperms without the pedicel region. Each data point represents the mean ± s.e. Insert; kinetics of L-glutamine uptake at low concentrations of L-glutamine. The uptake rates were calculated and expressed as in Fig. 1.

Table 1. Effects of metabolic inhibitors and other compounds on the uptake of 10 mM L-glutamine and 50 mM D-glucose by maize endosperms

	Uptake rate			
	L-Glutamine		D-Glucose	
Treatment	(nmol/E/hr)	(%control)	(nmol/E/hr)	(%control)
Control	63 ± 4	100	171 ± 13	100
Des (100 μM)	42 ± 6	67*	137 ± 15	80
*p*CMBS (0.48 mM)	31 ± 5	49**	145 ± 46	85
CCCP (50 μM)	43 ± 4	69**	136 ± 16	80
DNP (1 mM)	31 ± 2	50**	ND	ND
EDTA (10 mM)	143 ± 6	227**	109 ± 14	64**
L-glucose (100 mM)	ND	ND	173 ± 31	101
Sucrose (100 mM)	54 ± 3	86	76 ± 37	44

*Significant difference from control at $p < 0.1$.

**Significant difference from control at $p < 0.05$.

ND not determined

Kernels were incubated without the pedicel region as described in Experiments after 30 min pre-incubation. Values represent means ± s.e. of the uptake rate expressed as nmol of substrate taken up per endosperm per hr of incubation.

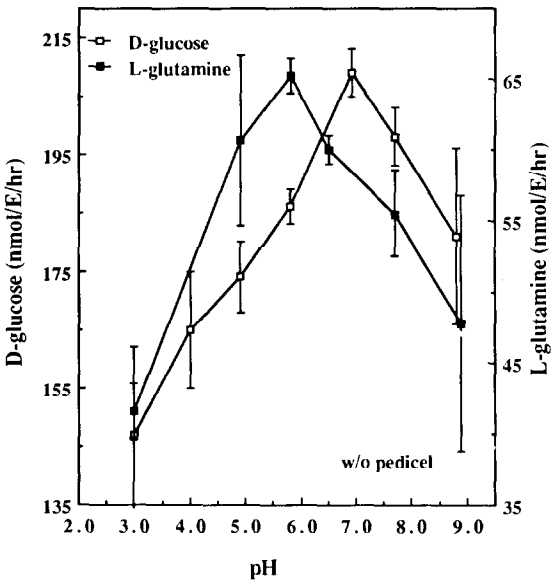

Fig. 3. pH dependence of L-glutamine and D-glucose uptake by maize endosperms. Kernels were pre-incubated for 15 min at a given pH and then incubated in the same pH for 30 min at 30° with radioactive L-glutamine (20 mM) or D-glucose (50 mM). Each data point represents the mean ± s.e.

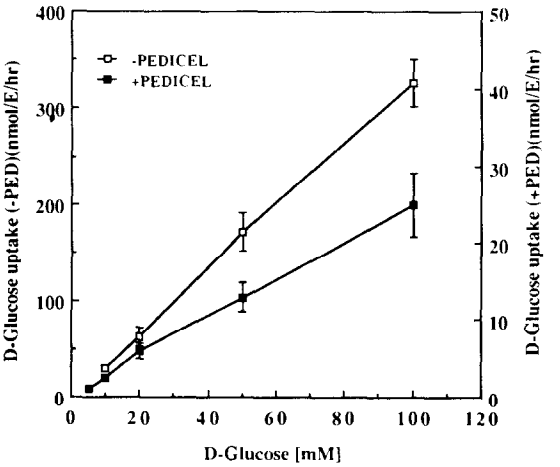

Fig. 4. Concentration dependance of D-glucose uptake by endosperms with(+Pedicel) or without (−Pedicel) the pedicel region. The incubation conditions were the same as for L-glutamine uptake described in Fig. 1. Each data point represents the mean ± s.e.

(Table 1). The same was true for the uptake of sucrose (data not shown). However, inhibition of D-glucose uptake by EDTA and sucrose was evident (Table 1). The uptake of D-glucose was also pH-dependent (Fig. 3) with optimum of about 7.0.

The rate of D-glucose uptake by maize endosperms was a linear function of D-glucose concentrations in the range 5–100 mM (Fig. 4). As with the L-glutamine uptake, the rate of D-glucose uptake was higher, when the pedicel tissues were removed. The uptake of D-glucose into

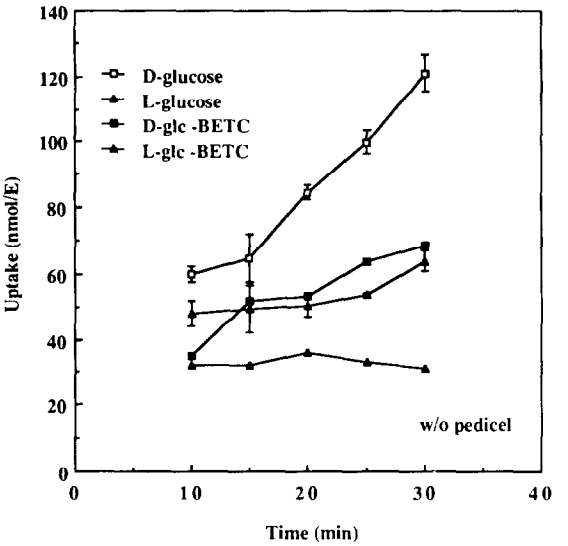

Fig. 5. Time-course of D-glucose and L-glucose uptake at 50 mM concentration. Endosperms without basal endosperm transfer cells (−BETC) were obtained by cutting off the lower portion of the endosperm tissue and were incubated in the presence of radioactive substrate in the same conditions as described in Experimental section. Each data point represents the mean ± s.e.

endosperms was linear for at least 30 min (Fig. 5). The time course of uptake did not extrapolate to zero because some of the labelled sugar in the incubation mixture probably adsorbs on the exterior surfaces of the endosperm. The rate of L-glucose uptake at 50 mM was low compared with D-glucose uptake rate (175 nmol/endosperm (E)/hr) at the same concentration (Fig. 5).

Because the presence of transfer cells adjacent to the pedicel region of the maize kernel suggests their function to facilitate the movement of nutrients into endosperm tissue, D-glucose was used to evaluate the role of basal endosperm transfer cells in the uptake process. The removal of the basal endosperm transfer cells reduced the rate of D-glucose uptake from 175 to 112 nmol/E/hr (Fig. 5).

DISCUSSION

The rates of D-glucose as well as sucrose uptake were linear over the concentration range tested, and the uptake rate was not significantly reduced by metabolic inhibitors. These results agree with previous studies [4]. It has been proposed that mechanisms of sugar uptake by maize endosperms are influenced by a diffusion-like process, a situation similar to the unloading processes within the pedicel region [4, 21]. On the other hand, L-glutamine uptake by maize endosperms was, at least partially, saturable and was inhibited by metabolic inhibitors such as CCCP (respiratory uncoupler), DNP (a mitochondrial uncoupler), *p*CMBS (non-penetrating sulphhydryl group reagent) and Des (ATPase inhibitor). Both L-glutamine and D-glucose uptake responded to alterations in pH with the pH optimum for L-glutamine uptake lower than for D-glucose. Another difference between L-glutamine and D-glucose uptake was that EDTA at 10 mM stimulated L-glutamine but inhibited D-glucose uptake. At this

concentration, EDTA probably causes significant changes in membrane structure affecting its physiological function by chelating divalent cations. Thus, the observed effects of EDTA on L-glutamine and D-glucose uptake might suggest that intact membranes of basal endosperm transfer cells are more restrictive for L-glutamine than for D-glucose uptake.

These data do not disclose an exact mechanism of L-glutamine transport across membranes in the transfer cell layer, but they suggest that energy and protein carriers are required. However, the kinetics of glutamine uptake cannot be described by a simple mechanism. It is likely that the uptake processes of L-glutamine into endosperms may consist of diffusion, facilitated-diffusion, and active transport acting in concert although the contribution of each process may be dependent on substrate concentrations in the pedicel region. Partial inactivation by metabolic inhibitors and relatively small response to pH changes suggest such a possibility. But even so, the contribution of active transport mechanisms for L-glutamine uptake seems to be very pronounced.

On the other hand, evidence does not indicate that energy and protein carriers are involved in D-glucose uptake, but it provides indications that this process may not be just a simple diffusion. Metabolic inhibitors could have some side effects on physical properties of membranes, resulting in an enhanced permeation of membranes [15]. Accordingly, they may result in increased diffusion and mask the inhibitory effects on sugar uptake. In certain systems, the non-saturable components of uptake are not affected by metabolic inhibitors but do show certain characteristics of an active uptake process. The linear component of sucrose uptake in *Beta vulgaris* leaf tissues operates against a concentration gradient, is pH-dependent, is inhibited by anoxia, and can be selectively promoted by changes in turgor [22]. These observations prompt authors to conclude that the linear component of uptake in *Beta vulgaris* is not due to simple diffusion. Similarly, the D-glucose uptake of maize endosperms may not be the result of a simple diffusion despite the fact that the system exhibits several properties which are characteristic of diffusion processes.

In contrast to sugar unloading from phloem termini into the pedicel region [2], uptake of D-glucose by endosperm responded to changes in pH. Furthermore, uptake of L-glucose, a stereoisomer of D-glucose, was substantially slower, indicating that D-glucose may be taken up by selective processes or that substrate metabolism within the endosperm tissue is the main driving force of D-glucose uptake. However, a relatively short time of incubation should partially eliminate the effects of metabolism on uptake rates. In fact, about 90% of radioactivity taken up by the endosperm tissue still remained in the D-glucose form with 20 min of incubation (data not shown).

The outermost layer of endosperm cells in the region of pedicel is transformed into transfer cells. The characteristic wall ingrowths of these cells increase the plasma membrane area [5]. The efficiency of transport through this cell layer should be increased regardless of the mechanisms involved. Removal of the transport cell layer before incubation significantly reduced the rate of D-glucose uptake. This is important evidence of an active role of the basal endosperm transfer cells in assimilate transport processes.

Based on presented results, L-glutamine is taken up by the endosperm tissue in processes which require energy and the presence of carrier proteins. Evidence does not indicate that energy and carriers are involved in D-glucose uptake. However, because of significant differences between L-glucose and D-glucose uptake rates and pH-dependence of this process, D-glucose uptake by endosperms may not proceed by a simple diffusion mechanism.

EXPERIMENTAL

Plant materials. Maize (*Zea mays* L.) inbred W64A was grown at the Purdue University Agronomy Farm in 1986 and 1987. All plants were self-pollinated. Ears were harvested 20 days after pollination and immediately brought to the laboratory. Whole kernels from the middle section of the cob were used for the study. At this stage of development, dry matter accumulation is most active in kernels and the separation of the pedicel tissue from endosperm is relatively easy.

The uptake system. The investigation of assimilate uptake mechanisms by maize endosperms is obstructed because of the complicated morphology of kernel tissues involved in transport processes. Two experimental designs were applied to investigate L-glutamine and sugar uptake. Kernels with or without the pedicel region were used for incubations. The former represents an intact system with the pedicel cells, placento-chalazal region, basal endosperm transfer cells, and endosperm cells unaffected by preparation procedure. When kernels with the pedicel tissue were used, only the tip of the pedicel region was removed before incubation. In the latter, the pedicel region was removed. At this stage of kernel development, the pedicel region can be removed leaving the endosperm tissue with basal endosperm transfer cells intact.

Incubation conditions. Five kernels were incubated at 30° in 2 ml of incubation mixture containing: 5 mM Na-Pi buffer, pH 6.5, specified amounts of cold and radioactive substrates (2 μCi; 44 Ci/mmol and 271 mCi/mmol for L-[G-^{3}H]glutamine and D-[U-^{14}C]glucose, respectively) and other compounds where indicated. Kernels were pre-incubated for 30 min (or 15 min where indicated) in the same solns as the incubation mixture except that the radioactive substrates were omitted. During incubation, kernels were sampled at 10 min and 5 min intervals for kernels with and without pedicel tissue, respectively.

Determination of radioactivity and uptake rates. After incubation, each kernel was washed in 200 ml of H_2O and the endosperm tissue was separated. Intact endosperms were rinsed again in 100 ml of H_2O for 5–10 sec and placed in scintillation vials containing 1 ml of 70% EtOH and 100 μl mixture of $HClO_4$–H_2O_2 (1:1). Individual endosperms were crushed in the scintillation vial using a glass rod. Radioactivity was measured using Budget Solve scintillation cocktail (10 ml). The least-squares fitting of experimental points to the straight line was used to calculate the uptake rates. In most cases, the values of coefficient of correlation were higher than 0.9. For testing the effect of other factors on uptake rate, each treatment contained its own control. This rate determination method was more accurate than direct measurements after a defined time of incubation because the initial rate was not a linear function to time.

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