Biochem. J. (1995) 307, 521–526 (Printed in Great Britain)

Analysis of carbohydrate transport across the envelope of isolated cauliflower-bud amyloplasts

Torsten MÖHLMANN, Olaf BATZ, Uwe MAAß and H.-Ekkehard NEUHAUS*
Pflanzenphysiologie, Universität Osnabrück, D-49069 Osnabrück, Germany

Using isolated amyloplasts from cauliflower buds, we have characterized the interaction and transport of various carbohydrates across the envelope membrane of a heterotrophic plastid. According to our results, glucose 6-phosphate (Glc6*P*) and glucose 1-phosphate (Glc1*P*) do not share the same transport protein for uptake into cauliflower-bud amyloplasts. Glc6*P*-dependent starch synthesis is strongly inhibited in the presence of dihydroxyacetone phosphate (DHAP) or 4,4′-di-isothiocyano-2,2′-stilbenedisulphonic acid (DIDS), whereas Glc1*P*-dependent starch synthesis is hardly affected by these compounds. Analysis of the Glc6*P* uptake into proteoliposomes reconstituted from the envelope proteins of cauliflower-bud amyloplasts indicate that Glc6*P* is taken up in a counter-exchange mode with P_i, DHAP or Glc6*P*, whereas Glc1*P* does not act as a counter-exchange substrate. P_i is a strong competitive inhibitor of Glc6*P* uptake (K_i 0.8 mM) into proteoliposomes, whereas Glc1*P* does not significantly inhibit Glc6*P* transport. Beside a hexose-phosphate translocator, these amyloplasts possess an envelope protein mediating the transport of glucose across the membrane. This translocator exhibits an apparent K_m for glucose of 2.2 mM and is inhibited by low concentrations of phloretin, known to be a specific inhibitor of glucose-transport proteins. Maltose inhibits the uptake of glucose (K_i 2.3 mM), indicating that both carbohydrates share the same translocator.

INTRODUCTION

Starch synthesis and degradation represent some of the most important biochemical features of plant metabolism. In heterotrophic tissues, starch synthesis is preceded by the uptake of soluble carbohydrates from the phloem and subsequent transport of carbon units into the amyloplasts. For several years there has been a contradictory debate on the chemical structure of the carbon compounds transported across the envelope membrane of amyloplasts.

Randomization experiments using specifically labelled glucose demonstrated that uptake of carbon into amyloplasts occurs via the import of C_6 units (Keeling et al., 1988; Hatzfeld and Stitt, 1990). Pozueta-Romero et al. (1991) claimed to have identified ADP-glucose (ADPGlc) as a cytosolic precursor for starch synthesis in isolated amyloplasts from a cell-suspension culture of *Acer pseudoplatanus*. We have demonstrated recently that this observation does not hold true for other types of amyloplasts. Amyloplasts purified from cauliflower buds are also able to import ADPGlc, but under physiological conditions, which favour hexose-phosphate-dependent starch synthesis, the rate of ADPGlc-dependent starch synthesis is negligible (Batz et al., 1994). Tyson and ap Rees (1988) and Tetlow et al. (1993) identified glucose 1-phosphate (Glc1*P*) to be the most efficient precursor for starch synthesis in isolated amyloplasts from wheat endosperm. This result has recently been confirmed for starch synthesis in amyloplasts isolated from soybean or potato cell-suspension cultures respectively (Coates and ap Rees, 1994; Kosegarten and Mengel, 1994). In contrast, Hill and Smith (1991) and Neuhaus et al. (1993a,b) demonstrated that glucose 6-phosphate (Glc6*P*) acts as the most efficient precursor for starch biosynthesis in isolated amyloplasts from pea roots, cauliflower buds, or maize endosperm, respectively. The last two types of amyloplasts are also able to use Glc1*P* as a source for starch synthesis (Neuhaus et al., 1993a,b), but the rate of incorporation into newly synthesized starch is lower than with Glc6*P*.

Glc6*P* is taken up into plastids isolated from pea roots by carrier-mediated counter-exchange with P_i, whereas Glc1*P* is not transported by this protein (Borchert et al., 1989, 1993). Recently, this group demonstrated that proteoliposomes reconstituted from envelope membranes of chloroplasts from green-tomato fruits are able to import both Glc6*P* and Glc1*P*, in counter-exchange with P_i (Schünemann and Borchert, 1994). This result indicates that plants may contain a further hexose-phosphate translocator exhibiting transport capacities for both Glc6*P* and Glc1*P*. However, this result is in contrast with observations made by our group. As we have demonstrated by analysing the precursor dependency of starch synthesis in isolated chloroplasts from green-pepper fruits, these chloroplasts solely take up Glc6*P* (Batz et al., 1995).

In addition to a transporter catalysing the flux of phosphorylated intermediates, spinach chloroplasts possess a translocator protein which mediates the flux of glucose and maltose (Schäfer et al., 1977; Herold et al., 1981). Obviously, the same holds true for amyloplasts purified from cauliflower buds. We have demonstrated that isolated amyloplasts from cauliflower buds are able to use exogenous glucose for the synthesis of starch (Batz et al., 1994). In addition, this glucose translocator is involved in the release of carbohydrates during starch mobilization in isolated cauliflower-bud amyloplasts (Neuhaus et al., 1995). Up to now, the biochemical features of glucose transport across the envelope of heterotrophic plastids have not been analysed.

The aim for this work was to characterize the transport processes of carbohydrates across the membrane of isolated

Abbreviations used: ADPGlc, ADPglucose; DHAP, dihydroxyacetone phosphate; DIDS, 4,4′-di-isothiocyano-2,2′-stilbenedisulphonic acid; Glc1*P*, glucose 1-phosphate; Glc6*P*, glucose 6-phosphate; PGA, 3-phosphoglyceric acid.

* To whom correspondence should be addressed.

cauliflower-bud amyloplasts in more detail. In particular we were interested in answering the following questions: (1) what are the biochemical features of the hexose-phosphate transport into isolated cauliflower-bud amyloplasts and into proteoliposomes reconstituted from envelopes of these plastids?; (2) what are the biochemical features of glucose transport across the membrane of cauliflower-bud amyloplasts?

MATERIALS AND METHODS

Isolation of cauliflower-bud amyloplasts and hexose-phosphate-dependent starch synthesis

Cauliflower (*Brassica oleracea* L.) bud amyloplasts were isolated by the method of Journet and Douce (1985) with the modifications given by Neuhaus et al. (1993b). Amyloplasts purified by this method are characterized by high degrees of intactness and purity (Neuhaus et al., 1993b). Hexose-phosphate-dependent starch synthesis in isolated amyloplasts was performed as described in Batz et al. (1994).

Estimation of short-term uptake of carbohydrates across the envelope membrane of isolated cauliflower-bud amyloplasts

For short-term uptake experiments the silicone-oil filtration technique by the method of Heldt and Sauer (1971), with the modifications given in Batz et al. (1992), was applied. Eppendorf reaction vessels (400 μl) were filled with three layers. The lower one consisted of 50 μl of 700 mM sucrose, the middle one of 100 μl of silicone oil AR 200 (gift from Wacker-Chemie, Munich, Germany), and the upper layer consisted of 100 μl of incubation medium as given in Batz et al. (1994), containing in addition [U-^{14}C]glucose (30 MBq/mmol) or [U-^{14}C]Glc1*P* (4 MBq/mmol); both radioactively labelled substrates were purchased from Amersham-Buchler, Braunschweig, Germany. The uptake was initiated by addition of 50 μl of plastid solution containing 100 μg of plastid protein. The uptake of Glc1*P* was measured at room temperature; the uptake of glucose was measured at 16 °C. All solutions were equilibrated to this temperature before the experiment. Transport rates were corrected for the radioactivity trapped in the sorbitol-permeable space as described by Heldt and Sauer (1971). In control experiments, we checked that uptake was linear with time and increasing amounts of protein (results not shown).

Uptake of Glc6*P* into proteoliposomes prepared from membranes of cauliflower-bud amyloplasts

Proteoliposomes containing membrane proteins of isolated cauliflower-bud amyloplasts were essentially prepared as given in Flügge and Weber (1994). Proteoliposomes were prepared by using 100 mg/ml acetone-washed phosphatidylcholine (type IV-S from soybean; Sigma, Deisenhofen, Germany) in 100 mM Tricine/NaOH, pH 7.5, containing 20 mM P_i or phosphorylated intermediates (if not stated otherwise), and 30 mM potassium gluconate. Purified amyloplasts were lysed by sonication and the membranes were sedimented at 100000 ***g*** for 30 min in a precooled ultracentrifuge. The membrane sediment was resuspended in a small volume of an extraction medium consisting of 200 mM Tricine/KOH, pH 8, 10 mM EDTA, 4 mM dithiothreitol, 6 mM sodium ascorbate, 0.1 % (w/v) BSA and 0.1 % (w/v) polyvinylpyrrolidone. The protein content was measured by using a detergent-insensitive assay containing a bicinchoninic acid/$CuSO_4$ solution (Sigma) according to the supplier's instructions. A 100 μg portion of membrane proteins was reconstituted per ml of the proteoliposome suspension. For the solubilization of membrane protein, 0.8 % Triton X-100 was added for less than 10 s. The solubilized protein solution was combined with the liposome suspension (with a resulting lipid/detergent ratio of more than 40). The resulting suspension was immediately transferred into liquid nitrogen. Incorporation of the membrane proteins was achieved by the freeze–thaw technique. After thawing at room temperature, the proteoliposomes were sonicated for 20 s (40 % line voltage, 20 % duty cycle) under a continuous stream of nitrogen gas. Unincorporated solution was removed from the liposomes by passing the preparation over a NAP-5 gel filtration column (Pharmacia, Freiburg, Germany) equilibrated with 10 mM Tricine/NaOH (pH 7.5)/150 mM potassium gluconate. The eluted proteoliposomes were stored in an ice bath and used for the transport measurements within the next 60 min. Uptake experiments were performed in 1 ml reaction vessels, and the incubation was initiated by addition of 100 μl of proteoliposome solution to 100 μl of extraction medium containing [1-^{14}C]Glc6*P* (4–10 MBq/mmol; NEN, Dreieich, Germany). The latter medium was equilibrated to 25 °C by incubation in a thermostatically controlled Eppendorf shaker. The uptake was terminated by addition of 200 μl of inhibitor mixture [3 mM 4,4′-di-isothiocyano-2,2′-stilbenedisulphonic acid (DIDS), 10 mM pyridoxal 5-phosphate final concentrations], and the unincorporated radioactivity was removed by passing the proteoliposomes over a 1 ml Dowex AG-1X8 column (Cl^- form, 100–200 mesh) equilibrated with 200 mM Tricine/NaOH, pH 7.5. The proteoliposomes were eluted from the column by washing with 1 ml of the equilibration medium, and the radioactivity passing through this column was quantified in a Tri-Carb 2500 scintillation counter (Packard, Frankfurt, Germany). Linearity of the uptake with time and increasing amounts of proteoliposomes was checked in previous experiments (results not shown).

RESULTS

Characterization of hexose-phosphate-dependent starch synthesis in isolated cauliflower-bud amyloplasts

Recently, we have shown that starch synthesis in isolated cauliflower-bud amyloplasts is driven by Glc6*P* (Neuhaus et al., 1993a). Figure 1 reveals that Glc6*P*-dependent starch synthesis is saturated at a Glc6*P* concentration of 3–5 mM. In contrast,

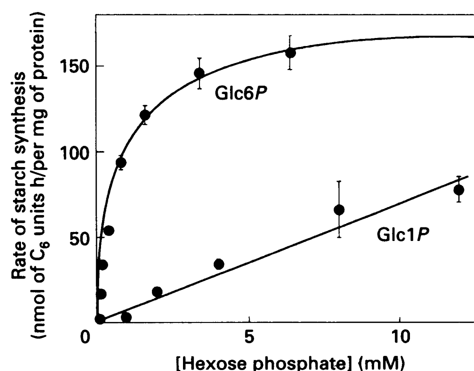

Figure 1 Starch synthesis from [^{14}C]Glc6*P* or [^{14}C]Glc1*P* in isolated amyloplasts from cauliflower buds

Plastids were incubated for 20 min in 330 mM sorbitol/15 mM Hepes/KOH (pH 7.2)/1 mM EDTA/2 mM $MgCl_2$ and substrates in the indicated concentrations. Data are means ± S.E.M. of three independent experiments.

Table 1 Effect of ATP and PGA on Glc1*P*-dependent starch synthesis in isolated amyloplasts from cauliflower buds

Data represent means ± S.E.M. of three indpendent experiments.

Glc1*P* (mM)	ATP (mM)	PGA (mM)	Rate of starch synthesis (nmol/h per mg of protein)
5	2	1	38.6 ± 2.8
5	–	1	8.8 ± 0.3
5	2	–	9.3 ± 0.4
5	2	1*	13.0 ± 0.2

* Plastids were lysed before incubation.

Table 2 Effector dependency of starch synthesis from Glc1*P* or Glc6*P* in isolated amyloplasts from cauliflower buds

Substrate concentrations were 3 mM. Data represent means of three independent experiments (S.E.M. < 8%). The rate of starch synthesis in the control samples was 123 nmol/h per mg of protein for Glc6*P* and 19.5 nmol/h per mg of protein for Glc1*P*.

Substrate	Effector/inhibitor	Rate of starch synthesis (% of control)
Glc1*P*	Glc6*P* (3 mM)	93.0
Glc6*P*	Glc1*P* (3 mM)	111.0
Glc6*P*	Glc1*P* (10 mM)	158.0
Glc6*P*	DHAP (5 mM)	5.7
Glc1*P*	DHAP (5 mM)	117.0
Glc6*P*	DIDS (30 μM)	11.6
Glc1*P*	DIDS (30 μM)	68.2

Glc1*P*-dependent starch synthesis is not saturable up to 12 mM. Increasing concentrations of Glc1*P* are linearly correlated with rising rates of starch synthesis (Figure 1).

Neuhaus et al. (1993a) demonstrated that Glc6*P*-dependent starch synthesis in isolated cauliflower-bud amyloplasts is strongly dependent on the presence of ATP and 3-phosphoglyceric acid (PGA) in the incubation medium and on the intactness of the plastid preparation. To increase our knowledge about the biochemical properties of Glc1*P*-dependent starch synthesis, we checked whether these requirements are necessary to drive starch synthesis from the precursor. Table 1 demonstrates that Glc1*P*-dependent starch synthesis is severely inhibited in the absence of ATP or PGA. Lysis of the plastids before the experiment decreases the rate of starch synthesis to around 33 % of the corresponding control value (Table 1).

If Glc6*P* and Glc1*P* are taken up into isolated amyloplasts by the same translocator protein, competition between Glc6*P* and Glc1*P* has to be expected if both hexose phosphates are present in the incubation medium. However, the data in Table 2 show that this is not the case. Glc1*P*-dependent starch synthesis is only slightly decreased by the simultaneous presence of Glc6*P* in the incubation medium. Moreover, the rate of Glc6*P*-dependent starch synthesis is stimulated by more than 58 % when Glc1*P* is added at 10 mM concentration. These results are strong evidence that these metabolites do not share the same translocator protein for import into isolated cauliflower-bud amyloplasts.

This interpretation is further documented by analysing the effect of dihydroxyacetone phosphate (DHAP) or DIDS on Glc6*P*- or Glc1*P*-dependent starch synthesis, respectively. Glc6*P*-dependent starch synthesis is strongly inhibited by millimolar concentrations of DHAP, whereas Glc1*P*-dependent starch synthesis is not decreased by the simultaneous addition of this triose phosphate (Table 2). Similar observations hold true for the effect of micromolar concentrations of DIDS on hexose-phosphate-dependent starch synthesis. DIDS applied at a concentration of 30 μM decreases the rate of Glc6*P*-dependent starch synthesis to 12 % of the control value, whereas Glc1*P*-dependent starch synthesis is only decreased to 70 % of the corresponding control value (Table 2).

Table 3 Uptake of Glc6*P* into proteoliposomes reconstituted from envelopes of cauliflower-bud amyloplasts

Liposomes were preloaded with counter-exchange substrate (20 mM) and then incubated for 2 min in buffer medium containing in addition 1 mM [^{14}C]Glc6*P*. Inhibitor-stop with DIDS and pyridoxal phosphate was carried out as described in the Materials and methods section. Data are calculated on the basis of 25 μg of reconstituted protein per sample. Data represent means ± S.E.M. of three independent experiments.

Counter-exchange substrate	Rate of Glc6*P* uptake (nmol/h per mg of protein)
None	16.0 ± 3.6
P_i	117.5 ± 7.8
DHAP	115.0 ± 2.8
Glc6*P*	142.5 ± 13
Glc1*P*	8.0 ± 1.9

Uptake of Glc6*P* into proteoliposomes reconstituted from cauliflower-bud amyloplast envelope protein

To analyse the biochemical characteristics of the transport processes which are involved in the uptake of hexose phosphates into isolated amyloplasts from cauliflower buds, we used a proteoliposome system similar to that described by Flügge and Weber (1994). This system has the advantage of preloading the liposomes with different potential counter-exchange substrates before incubation with the radioactively labelled substrate. We have verified in previous experiments that the rate of uptake was linear with time and increasing amounts of liposomes added (results not shown).

It has been demonstrated that isolated plastids from pea roots import Glc6*P* in counter-exchange with P_i (Borchert et al., 1989). In order to analyse whether this holds true for the uptake of Glc6*P* into proteoliposomes containing the membrane proteins from isolated cauliflower-bud amyloplasts, we preloaded these liposomes with different potential counter-exchange substrates. Table 3 shows that proteoliposomes preloaded with P_i, DHAP or Glc6*P* are able to import [^{14}C]Glc6*P*. Proteoliposomes which contain only incubation medium or incubation medium plus Glc1*P* do not import Glc6*P* at significant rates.

In contrast with the uptake of Glc6*P*, we could not induce uptake of external Glc1*P* into proteoliposomes under any of the conditions mentioned above (results not shown).

Figures 2(a) and 2(b) show that increasing concentrations of P_i inside the proteoliposomes lead to a linear increase in the rate of Glc6*P* uptake, and that Glc6*P* uptake is saturable with increasing concentrations of external substrate, exhibiting an apparent K_m of 1.1 mM (insert of Figure 2b). To analyse the effect of P_i or Glc1*P* on Glc6*P* transport in more detail, we tried to estimate the K_i values of both P_i or Glc1*P* respectively. As demonstrated in the insert of Figure 2(b), the addition of Glc1*P* does not lead to a significant decrease in Glc6*P* uptake, whereas P_i decreases the rate of Glc6*P* transport severely, exhibiting an apparent K_i value of 0.8 mM.

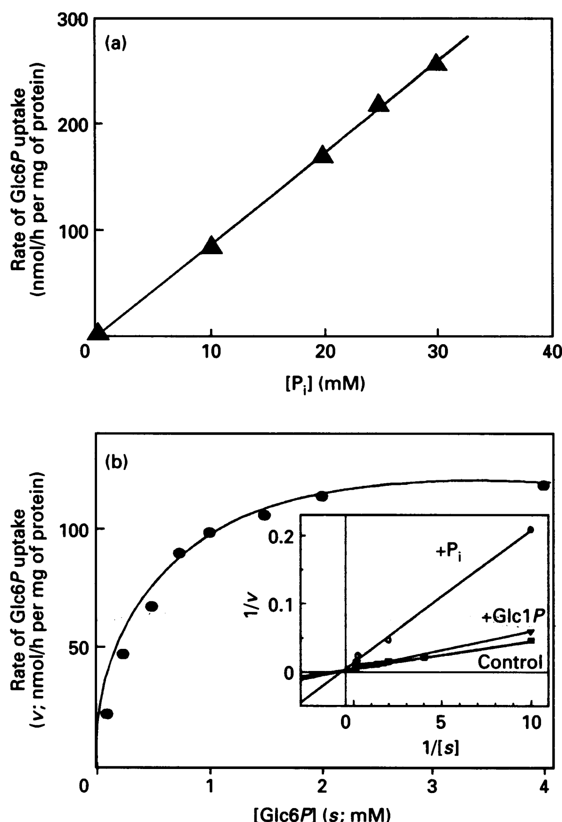

Figure 2 Uptake of [^{14}C]Glc6*P* into proteoliposomes reconstituted from cauliflower-bud amyloplast envelope protein

(**a**) Dependency of Glc6*P* uptake on increasing concentrations of internal P_i. Data were corrected for background without internal P_i (S.E.M. < 8%). (**b**) Substrate saturation curve for Glc6*P* uptake at an internal P_i concentration of 20 mM. Insert: Lineweaver–Burk plot of Glc6*P* uptake in the absence or presence of P_i (1 mM) or Glc1*P* (1 mM).

Glc1*P* uptake into isolated cauliflower-bud amyloplasts

As mentioned above, it was not possible to induce uptake of Glc1*P* into proteoliposomes containing the membrane proteins of isolated cauliflower-bud amyloplasts. However, the observation that the Glc1*P*-driven starch synthesis is dependent on the intactness of the plastid suspension (Table 1) and that Glc1*P* acts to stimulate the rate of Glc6*P*-dependent starch synthesis (Table 2) indicates that Glc1*P* must be taken up into the intact amyloplast. Therefore, we tried to characterize the uptake of this hexose phosphate into isolated cauliflower-bud amyloplasts.

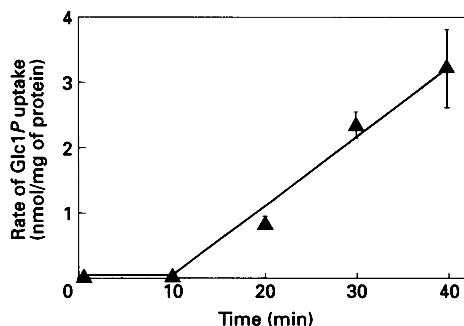

Figure 3 Time course of [^{14}C]Glc1*P* uptake into isolated amyloplasts from cauliflower buds

Uptake was measured by using the silicone-oil filtration technique as described in the Materials and methods section. Data are corrected for uptake into the sorbitol-permeable space by measuring uptake of [^{14}C]sorbitol. Data are means ± S.E.M. of three independent experiments.

Table 4 Uptake of Glc1*P* into isolated amyloplasts from cauliflower buds in the presence or absence of DIDS or glucose

Plastids were incubated for 30 min and separated by the silicone-oil filtration technique as given in the Materials and methods section. Data represent means ± S.E.M. of three independent experiments.

Substrate (mM)	Inhibitor (mM)	Rate of Glc1*P* uptake (nmol/h per mg of protein)
Glc1*P* (1)	None	4.68 ± 0.4
Glc1*P* (1)	DIDS (1)	0.08 ± 0.08
Glc1*P* (1)	Glucose (10)	4.04 ± 0.28

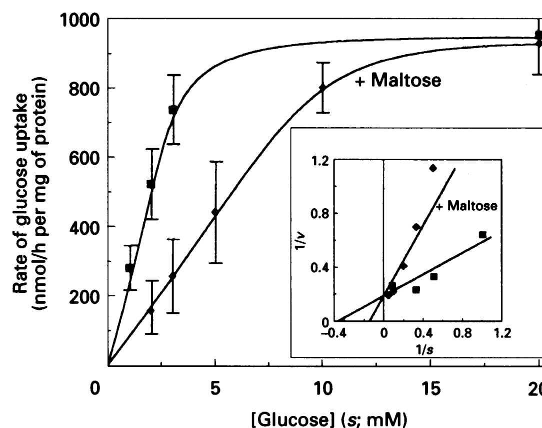

Figure 4 Uptake of [^{14}C]glucose into isolated cauliflower-bud amyloplasts

The substrate-saturation curve of glucose uptake was performed in the absence or presence of maltose (5 mM). Data are corrected for uptake into the sorbitol-permeable space by measuring uptake of [^{14}C]sorbitol, and are means ± S.E.M. of three independent experiments. Insert: Lineweaver–Burk plot of glucose uptake in the absence or presence of maltose (5 mM).

Most uptake processes of carbohydrates into an organelle are characterized by saturation with time. However, this does not hold true for the uptake of Glc1*P* into isolated cauliflower-bud amyloplasts (Figure 3). Due to the low rate of uptake, it was not possible to estimate the influx of Glc1*P* within the first 10 min. In the time interval between 10 and 40 min of incubation the uptake proceeded linearly with time (Figure 3). Since this kinetic behaviour would also be expected for a diffusion process not facilitated by a protein, we analysed whether DIDS given in a high concentration non-specifically inhibits the rate of Glc1*P* uptake; indeed, 1 mM DIDS decreases the rate of Glc1*P* uptake almost completely (Table 4).

Kosegarten and Mengel (1994) claimed that Glc1*P* uptake into isolated amyloplasts from a potato tuber cell-suspension culture is decreased in the simultaneous presence of glucose in the incubation medium. However, this interaction does not occur in the case of Glc1*P* uptake into isolated cauliflower-bud amyloplasts. Glucose applied at a 10 mM concentration does not

Table 5 Effect of Glc1*P* or phloretin on the short-term glucose uptake into isolated cauliflower-bud amyloplsts

Uptake experiments were performed as described in the Materials and methods section. Radioactively labelled glucose was given at a 1 mM concentration. Data are means ± S.E.M. of three independent experiments

Condition	Rate of glucose uptake (nmol/h per mg of protein)
Control	1085 ± 23
+ Glc1*P* (5 mM)	1087 ± 132
+ phloretin (50 μM)	923 ± 45
+ phloretin (100 μM)	628 ± 111
+ phloretin (250 μM)	Not detectable

significantly decrease the rate of Glc1*P* uptake into the amyloplasts (Table 4).

Glucose uptake into isolated amyloplasts from cauliflower buds

Batz et al. (1994) and Neuhaus et al. (1995) have shown that isolated cauliflower-bud amyloplasts are able to transport glucose across the envelope membrane. Figure 4 demonstrates that glucose uptake into isolated cauliflower-bud amyloplasts is saturable at high concentrations of external substrate. Glucose uptake is half-saturated at a substrate concentration of 2.2 mM, reaching a V_{max} of 0.99 μmol/h per mg of protein.

Phloretin is known to act inhibitorily on the glucose transport into chloroplasts (Schäfer et al., 1977). The same holds true for glucose uptake into isolated amyloplasts from cauliflower buds. Phloretin applied at a concentration of 50 μM inhibits the rate of glucose uptake by 15%; 250 μM phloretin prevents glucose transport completely (Table 5).

It is known that both maltose and glucose are transported by the same protein into isolated chloroplasts (Herold et al., 1981; Beck, 1985). Obviously, the same holds true for the interaction of glucose and maltose transport across the envelope of isolated cauliflower-bud amyloplasts (insert of Figure 4). The degree of maltose-induced inhibition of glucose uptake into amyloplasts is decreased by rising concentrations of glucose, indicating a competitive mode of inhibition. From the data presented in the insert of Figure 4, an apparent K_i (maltose) of 2.3 mM can be calculated.

DISCUSSION

Both Glc6*P* and Glc1*P* have been identified as acting as potential precursors for starch biosynthesis in isolated amyloplasts of different origins (Tyson and ap Rees, 1988; Hill and Smith, 1991; Neuhaus et al., 1993a,b). As demonstrated in the Results section, both of these metabolites can induce starch synthesis in isolated cauliflower-bud amyloplasts (Figure 1, Table 2). However, Figure 1 also indicates that these two hexose phosphates do not exhibit the same substrate-saturation curve for starch biosynthesis. In contrast with the rate of Glc6*P*-dependent starch synthesis, the rate of Glc1*P*-dependent starch synthesis is not saturable in a physiological concentration range of this intermediate. Information about metabolic levels in heterotrophic tissues is scarce. However, Hill and Smith (1991) calculated that the concentration of Glc6*P* in pea endosperm tissue is around 5 mM. If we assume that the reaction catalysed by the cytosolic phosphoglucose mutase is close to the thermodynamic equilibrium, this Glc6*P* concentration would lead to a Glc1*P* concentration of 0.6–0.8 mM. In this concentration range, the rate of Glc1*P*-dependent starch synthesis is extremely low compared with the rate of Glc6*P*-dependent starch synthesis (Figure 1).

The observation that Glc6*P*-dependent starch synthesis is clearly saturable, whereas the Glc1*P*-dependent starch synthesis is not, further indicates that they are not taken up by the same transport protein. This assumption is reinforced by analysing the effect of DHAP or DIDS on Glc6*P*- or Glc1*P*-dependent starch synthesis, respectively. Glc6*P*-dependent starch synthesis is strongly inhibited in the presence of DHAP, whereas Glc1*P*-dependent starch synthesis is nearly unchanged. These results can be explained because DHAP and Glc6*P* are transported in a counter-exchange mode at the same translocator, whereas Glc1*P* does not act as a counter-exchange substrate (Table 3). A strong inhibitory effect of DHAP on the Glc6*P* uptake has also been demonstrated for isolated pea-root plastids (Borchert et al., 1989).

DIDS has been identified to inhibit the Glc6*P*-dependent starch synthesis in isolated cauliflower-bud amyloplasts in micromolar concentrations (Batz et al., 1994). The affinity of the hexose-phosphate translocator for this inhibitor is sufficient to allow the use of radioactively labelled DIDS for the identification of the transport protein in the amyloplastic envelope (Batz et al., 1993). The strong inhibitory effect of DIDS on Glc6*P*-dependent starch synthesis is documented in Table 2. In contrast, Glc1*P*-dependent starch synthesis is less sensitive to DIDS, indicating in addition that Glc1*P* is not taken up into isolated cauliflower-bud amyloplasts by the action of the hexose-phosphate translocator. This result is in agreement with the low inhibitory effect of Glc1*P* on the Glc6*P* uptake into proteoliposomes (insert of Figure 2b). Borchert et al. (1989, 1993) have shown that the same holds true for the hexose-phosphate translocator from plastids purified from pea roots.

As we have shown recently, chloroplasts isolated from green-pepper fruit also possess a hexose-phosphate translocator (Batz et al., 1995). Since these fruit chloroplasts use solely Glc6*P* as an external precursor for starch biosynthesis, we predict that this translocator has similar transport characteristics to the hexose-phosphate translocator from pea-root plastids (Borchert et al., 1989) or cauliflower buds (Table 3, Figure 2). In contrast, the group of Borchert demonstrated that proteoliposomes reconstituted from the envelope membrane of green-tomato fruit chloroplasts transport both Glc6*P* and Glc1*P*, in a counter-exchange with P_i (Schünemann and Borchert, 1994). Since these transport processes occur with similar rates, we have to assume that at least some plastids contain a hexose-phosphate translocator which exhibits a high affinity towards both Glc6*P* and Glc1*P*.

However, as we have demonstrated above, the uptake of Glc1*P* into isolated cauliflower-bud amyloplasts is mediated by a protein different from the hexose-phosphate translocator responsible for the import of Glc6*P* (Tables 2 and 3; Figures 1 and 2b). Kosegarten and Mengel (1994) proposed that the uptake of Glc1*P* into amyloplasts prepared from a potato cell-suspension culture is competitively inhibited by glucose. However, the uptake of Glc1*P* into isolated cauliflower-bud amyloplasts is not affected by the simultaneous presence of glucose (Table 4), nor is the uptake of glucose inhibited by the presence of Glc1*P* (Table 5). At this point, we cannot rule out the possibility that these two types of amyloplasts possess a different kind of membrane proteins responsible for the uptake of Glc1*P* and/or glucose.

The observation that Glc1*P* acts to stimulate Glc6*P*-dependent starch synthesis (Table 2) is surprising, but might be explained as follows: Glc1*P* enters the amyloplastic stroma by a slow protein-mediated transport mechanism and is subsequently used for starch biosynthesis. This import is not dependent on the sim-

ultaneous export of P_i, as described for Glc6*P* import (Table 3). Upon conversion of Glc1*P* into starch, the phosphate moiety is liberated and leads to an increase in the concentration of P_i inside the plastids. The rising concentration of phosphate energizes the Glc6*P* translocator and induces a more rapid uptake of Glc6*P* into the amyloplasts. This assumption is reinforced by the observation that rising concentrations of internal P_i lead to a linear increase in the rate of Glc6*P* uptake into proteoliposomes (Figure 2a). This model depends on the premise that under the chosen conditions the velocity of Glc6*P* uptake limits the rate of starch synthesis. Indeed, the activity of the stromal enzymes involved in the conversion of Glc6*P* into starch in isolated amyloplasts from cauliflower buds is several times higher than required (Journet and Douce, 1985). The assumption that the rate of Glc6*P* uptake into amyloplasts is limiting the rate of starch synthesis is further strengthened by the observation that the K_m of the Glc6*P* uptake into proteoliposomes (around 1 mM; Figure 2b) is similar to the K_m of Glc6*P*-dependent starch synthesis in intact plastids under conditions allowing high rates of starch synthesis (PGA and ATP present in the incubation medium; Figure 1).

Figure 4 demonstrates that glucose is taken up into isolated cauliflower-bud amyloplasts by carrier-mediated transport. The short-term uptake exhibits a $V_{max.}$ of 0.99 μmol of glucose/h per mg of protein and an apparent K_m of 2.2 mM. According to these data, this translocator exhibits a higher affinity towards the substrate glucose than does the corresponding translocator protein in spinach chloroplasts. Schäfer et al. (1977) demonstrated that the glucose translocator from spinach chloroplasts exhibits a K_m for glucose of 20 mM.

Figure 3 shows that maltose causes a significant inhibition of the glucose uptake into isolated amyloplasts from cauliflower buds (K_i 2.3 mM). As we have shown recently, both glucose and maltose are exported from isolated cauliflower-bud amyloplasts during the process of starch mobilization (Neuhaus et al., 1995). Obviously, both carbohydrates share the same transport protein. This observation is in agreement with the finding that glucose and maltose are transported by the same translocator protein across the envelope of spinach chloroplasts (Herold et al, 1981; Beck, 1985).

Since the glucose translocator from cauliflower-bud amyloplasts exhibits a significantly higher affinity for glucose than does that of chloroplasts, we have performed further experiments to increase our knowledge about this transport protein. As demonstrated in Table 5, phloretin acts inhibitorily on the rate of glucose uptake into isolated cauliflower-bud amyloplasts. This inhibition occurs in a similar concentration range to that for the glucose uptake into both erythrocytes (Baldwin, 1992) or spinach chloroplasts (Schäfer et al., 1977), indicating some structural similarities between these translocator proteins.

As mentioned in the Introduction, Glc1*P* is identified as inducing starch synthesis in amyloplasts isolated from developing wheat endosperm (Tyson and ap Rees, 1988; Tetlow et al., 1993) or soybean cell-suspension culture (Coates and ap Rees, 1994). It would be worth comparing the rate of Glc1*P*-dependent starch synthesis with the endogenous starch contents of the isolated plastid preparations and analysing the kinetics of the uptake mechanism leading to the transport of Glc1*P* across the envelope membrane. Obviously, more studies are needed to answer the question as to which translocator catalyses the uptake of Glc1*P*, and how important this metabolite is in driving starch synthesis in specific types of amyloplasts.

We thank Professor Dr. Renate Scheibe (University of Osnabrück) for stimulating and helpful discussions. We thank Joachim Tjaden for help with the Glc6*P*-uptake experiments using proteoliposomes. T.M. is recipient of a 'Graduierten' fellowship of the Land Niedersachsen. This work was supported financially by the Deutsche Forschungsgemeinschaft, SFB 171-C16.

Received 10 November 1994; accepted 14 December 1994

REFERENCES

Baldwin, S. A. (1992) in Molecular Aspects of Transport Proteins (De Pont, J. J. H. H. M, ed), pp. 163–211, Elsevier, Amsterdam
Batz, O., Scheibe, R. and Neuhaus, H. E. (1992) Plant Physiol. **100**, 184–90
Batz, O., Scheibe, R. and Neuhaus, H. E. (1993) Biochem. J. **294**, 15–17
Batz, O., Scheibe, R. and Neuhaus, H. E. (1994) Biochim. Biophys. Acta **1200**, 148–154
Batz, O., Scheibe, R. and Neuhaus, H. E. (1995) Planta, in the press
Beck, E. (1985) Proc. Annu. Symp. Plant Physiol. 8th, pp. 27–44
Borchert, S., Grosse, H. and Heldt, H. W. (1989) FEBS Lett. **253**, 183–186
Borchert, S., Harborth, J., Schünemann, D., Hoferichter, P. and Heldt, H. W. (1993) Plant Physiol. **101**, 303–312
Coates, S. A. and ap Rees, T. (1994) Phytochemistry **35**, 881–883
Flügge, U. I. and Weber, A. (1994) Planta **194**, 181–185
Hatzfeld, W.-D. and Stitt, M. (1990) Planta **180**, 198–204
Heldt, H. W. and Sauer, F. (1971) Biochim. Biophys. Acta **234**, 83–91
Herold, A., Leegood, R. C., McNeil, P. H. and Robinson, S. P. (1981) Plant Physiol. **67**, 85–88
Hill, L. M. and Smith, A. M. (1991) Planta **185**, 91–96
Journet, E.-P. and Douce, R. (1985) Plant Physiol. **79**, 458–467
Keeling, P. L., Wood, J. R., Tyson, R. H. and Briggs, I. G. (1988) Plant Physiol. **87**, 311–319
Kosegarten, H. and Mengel, K. (1994) Physiol. Plant. **91**, 111–120
Neuhaus, H. E., Henrichs, G. and Scheibe, R. (1993a) Plant Physiol. **101**, 573–578
Neuhaus, H. E., Thom, E., Batz, O. and Scheibe, R. (1993b) Biochem. J. **296**, 496–501
Neuhaus, H. E., Henrichs, G. and Scheibe, R. (1995) Planta, in the press
Pozueta-Romero, J., Frehner, M., Viale, A. M. and Akazawa, T. (1991) Proc. Natl. Acad. Sci. U.S.A. **88**, 5769–5773
Schäfer, G., Heber, U. and Heldt, H. W. (1977) Plant Physiol. **60**, 286–289
Schünemann, D. and Borchert, S. (1994) Bot. Acta **107**, 461–467
Tetlow, I. J., Blisset, K. J. and Emes, M. J. (1993) J. Exp. Bot. **247** (Suppl.), 69
Tyson, R. H. and ap Rees, T. (1988) Planta **175**, 33–38