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Characterization of Glucose-6-Phosphate Incorporation into Starch by Isolated Intact Cauliflower-Bud Plastids[1]

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Intact plastids from cauliflower (*Brassica oleracea* var Prince de Bretagne) buds were isolated according to the method described by Journet and Douce (E.P. Journet and R. Douce [1985] Plant Physiol 79: 458–467). Incubation of these plastids with various ^{14}C-labeled compounds revealed that glucose-6-phosphate can act as a precursor for starch synthesis. However, significant rates (incorporation of 120 nmol glucose mg^{-1} protein h^{-1}) could only be observed when both 3-phosphoglyceric acid and ATP were present as well. Starch synthesis in isolated plastids was strongly dependent upon the intactness of the organelle. The presence of a high-affinity ATP/ADP translocator with a K_m for ATP of 12 μM was demonstrated by uptake experiments with [^{14}C]ATP. ADP inhibited both ATP uptake and effector-stimulated starch synthesis. Effector-stimulated glucose-6-phosphate-dependent starch synthesis was not significantly influenced by fructose-6-phosphate or 2-deoxyglucose-6-phosphate but was strongly inhibited by triose phosphate and inorganic phosphate. Starch synthesis was also inhibited by 4,4′-diisothio-cyanostilbene-2,2′-disulfonate, which is known to be a potent inhibitor of the chloroplast phosphate translocator. The data presented here support the view that starch biosynthesis in heterotrophic tissues is powered by increasing levels of cytosolic 3-phosphoglyceric acid and ATP when glucose-6-phosphate is available.

To date it is not clear which metabolite(s) enter(s) heterotrophic plastids as a precursor for starch biosynthesis. In contrast to well-documented transport processes across the envelope membranes of chloroplasts, transport across the membranes of heterotrophic plastids is poorly characterized. The reason for this fragmentary knowledge is that there have been problems in isolating intact and metabolically active plastids (Journet and Douce, 1985; Entwistle and ap Rees, 1988).

Generally, it is assumed that the imported precursors for starch synthesis in plastids are hexoses, hexose phosphates, or triose phosphates. Analysis of the enzymic equipment of isolated amyloplasts from maize endosperm (Echeverria et al., 1988) and of the incorporation of DHAP into newly synthesized starch in isolated amyloplasts from potato tubers (Mohabir and John, 1988) lead to the assumption that in these cases triose phosphates are the most likely candidates. These findings are in contrast with results obtained with "randomization" experiments that indicate the in vivo uptake of hexoses or hexose phosphates for wheat endosperm (Keeling et al., 1988), maize endosperm (Hatzfeld and Stitt, 1990), and potato tubers (Viola et al., 1991). Using those experimental approaches, however, it was not possible to differentiate between the uptake of carbon as hexose and as hexose phosphate. For amyloplasts purified from wheat endosperm, it has been shown that Glc-1-P is the specific precursor for starch biosynthesis (Tyson and ap Rees, 1988). On the other hand, Borchert et al. (1989) clearly demonstrated that amyloplasts from pea roots possess a translocator with high affinity for Glc-6-P. Moreover, Glc-6-P-dependent starch synthesis has been demonstrated for isolated amyloplasts from developing pea embryos (Hill and Smith, 1991).

Other results reported by Pozueta-Romero et al. (1991b) provide evidence that exogenously supplied ADPGlc can act as a substrate for starch synthesis in isolated, intact amyloplasts from *Acer pseudoplatanus* suspension culture cells. The general capability of cytosolic sucrose synthase to synthesize ADPGlc was demonstrated by assaying the enzyme in vitro (Pozueta-Romero et al., 1991c).

On the basis of these contradictory results concerning the nature of the precursor for plastidic starch synthesis in non-green tissues, we asked the following questions: (a) Is starch biosynthesis in purified cauliflower-bud (*Brassica oleracea*) plastids driven by the uptake of hexose phosphates or triose phosphates? (b) What are the effectors of starch synthesis in these plastids? (c) Is the uptake of one precursor inhibitable by other metabolites? Using this approach, we hoped to gain insight into the regulatory events affecting the rate of starch biosynthesis in a tissue of heterotrophic origin.

MATERIALS AND METHODS

Isolation of Cauliflower-Bud Plastids

Cauliflower buds (*Brassica oleracea*) were purchased at the local farmers' market. The isolation of cauliflower-bud plastids was performed essentially as described by Journet and Douce (1985). The final plastid pellet was resuspended in medium C, consisting of 300 mM sorbitol, 15 mM Hepes-KOH, pH 7.2, 1 mM EDTA, and 2 mM $MgCl_2$. After removal of an aliquot for protein determination, BSA was added to

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Abbreviations: ADPGlc, ADPglucose; ADPGlc-PPase, ADPglucose pyrophosphorylase; DHAP, dihydroxyacetone phosphate; DIDS, 4,4′-diisothio-cyanostilbene-2,2′-disulfonate; FBPase, fructose-1,6-bisphosphatase; 3PGA, 3-phosphoglyceric acid; UDPGlc-PPase, UDPglucose pyrophosphorylase.

0.1% final concentration. The indicated rates of starch synthesis and ATP uptake could be slightly underestimated by a small amount of BSA carried over from the extraction medium B to medium C.

Protein Determination

Total protein was determined using a detergent-insensitive colorimetric assay. A bicinchoninic acid-copper II sulfate mixture (Sigma, Deisenhofen, FRG) was used according to the instructions of the supplier with BSA as a standard.

Estimation of Contaminating Cell Compartments

All enzyme activities were measured at 25°C using an Eppendorf spectrophotometer (M1101). ADPGlc-PPase measurements were carried out as described by Sowokinos (1976). UDPGlc-PPase as a cytosolic marker enzyme was measured according to the method of Bergmeyer (1974). Citrate synthase and α-mannosidase as marker enzymes for mitochondria and vacuoles, respectively, were measured according to the method of Stitt et al. (1989). Crude extracts were prepared as described by Batz et al. (1992). Based on the relative enzyme activities in the plastid preparations and in the crude extracts, contamination by mitochondria was calculated to be 6.0% (SE 0.4%), by cytosol below 1.0%, and by vacuolar contents below the limit of detection (0.5%). All measurements were performed with four individual preparations.

Estimation of Intactness

To estimate the intactness of isolated plastids, we performed latency experiments according to the method of Entwistle and ap Rees (1988). Initially, ADPGlc-PPase activity was measured as a plastidic marker; later, phosphoglucose isomerase (measured according to the method of Bergmeyer, 1974) was used because cytosolic contamination was below 1%. Enzyme activities were subsequently determined for a sample of intact plastids (in the presence of 330 mM sorbitol in the assay buffer) and for a sonicated aliquot. The ratio of the enzyme activities before and after lysis was used to calculate intactness (Batz et al., 1992). Plastids with an intactness of 94% (SE 1.2%, $n = 8$) were obtained routinely (and used in our experiments). Upon storage for more than 1 h (under experimental conditions), intactness declined only slightly (to about 90%).

ATP Uptake Experiments

For short-term transport experiments using isolated amyloplasts, the silicon oil filtration technique was applied according to Heldt and Sauer (1971), including the modifications as described by Batz et al. (1992). [^{14}C]ATP (43 MBq/mmol, concentrations up to 20 μM ATP; 8.6 MBq/mmol, concentrations above 20 μM ATP) was used at different concentrations as indicated. The incubations were carried out at room temperature for 10 s (the ATP uptake was linear for 30 s, data not shown) and was terminated by centrifugation for 20 s in a Beckman Microfuge E equipped with a horizontal rotor. The transport rates were corrected for the radioactivity trapped in the sorbitol-permeable (intermembrane) space. The sorbitol-permeable and the sorbitol-impermeable space were determined according to Heldt (1980). We determined a sorbitol-permeable space of 2.6 μL mg^{-1} protein (SE 0.4 μL, $n = 5$) and a sorbitol-impermeable space of 1.6 μL mg^{-1} protein (SE 0.1 μL, $n = 5$), respectively.

Starch Synthesis with ^{14}C-Labeled Substrates

The specific radioactivities of the precursors tested for incorporation into starch were 8.0 MBq mmol^{-1} to 1.0 MBq mmol^{-1} for [^{14}C]Glc-6-P; 1 MBq mmol^{-1} for [^{14}C]Glc-1-P; 1.8 MBq mmol^{-1} for [^{14}C]DHAP; 1.8 MBq mmol^{-1} for [^{14}C]-glycerol-3-P; and 3.6 MBq mmol^{-1} for [^{14}C]glycerol. All radioactively labeled substrates were purchased from Amersham-Buchler (Braunschweig, FRG). [^{14}C]DHAP was synthesized from [^{14}C]glycerol-3-P according to the method of Tyson and ap Rees (1988).

Incorporation of ^{14}C-labeled substrates into starch was carried out in medium C. Starch synthesis was started by addition of 150 μL of the plastid suspension (equivalent to 130–180 μg of protein) to an equal volume of medium C containing ^{14}C-labeled substrates and effectors at double concentration. Incubation was carried out at room temperature for 20 min in 1.5-mL microfuge tubes using an overhead tumbler. The reaction was terminated by heating the samples to 95°C for 3 to 5 min in a thermostated shaker (model 5348, Eppendorf, FRG). After centrifugation (15 min, 15,000g, Beckman Microfuge E), the pellet was washed twice with double-distilled water and once with 90% (v/v) ethanol to remove soluble contaminants as detected by determination of radioactivity in the supernatants.

Assay for ^{14}C-Labeled Starch

To identify the insoluble product as starch, the final pellet was dissolved in 150 μL of 100 mM sodium acetate (pH 4.7) and autoclaved for 3 h (135°C). After cooling, the solution was incubated for 2 to 3 h (constant temperature 37°C) with 3 units each of α-amylase and amyloglucosidase in a shaker. After boiling in a water bath for 3 min, the solution was clarified by short centrifugation and the supernatant was loaded onto an anion-exchange column (0.8 mL Dowex 1, Cl^- form, for details see Kruckeberg et al. [1989]). The radioactivity in the neutral compounds (labeled Glc released upon hydrolytic digestion of starch) was eluted by adding five portions of 500 μL of double-distilled water.

To check whether the eluted radioactivity was indeed due to labeled Glc, we phosphorylated sample aliquots of 100 μL by incubation with 400 μL of assay medium containing 50 mM Hepes-KOH, pH 7.8, 5 mM $MgCl_2$, 2 mM ATP, and 5 units/mL of hexokinase. The phosphorylation reaction was stopped after 30 min by heating to 95°C for 3 min. The samples were subsequently applied to anion-exchange columns and washed with five portions of 500 μL of double-distilled water as above. The bound radioactivity (phosphorylated Glc) was eluted with five portions of 500 μL of 1 N HCl. Quantitation of the radioactivity in the phosphorylated and the unphosphorylated fractions indicated that more than 98% (SE 2.2%, $n = 10$) of the radioactivity was derived from [^{14}C]Glc incorporated into starch.

Table I. *Effect of 3PGA and ATP on Glc-6-P-dependent starch synthesis in isolated plastids*

Isolated plastids were incubated for 20 min in medium C containing Glc-6-P, 3PGA, and ATP in concentrations as indicated. Data are the mean (±SE) of three independent experiments.

Precursor	Effector	Rate of Starch Synthesis
		nmol mg^{-1} *protein h*$^{-1}$
Glc-6-P, 5.0 mM	3PGA, 10 mM ATP, 2.0 mM	117.5 ± 5.1
Glc-6-P, 5.0 mM	3PGA, 10 mM	3.1 ± 1.9
Glc-6-P, 5.0 mM	ATP, 2.0 mM	1.7 ± 0.9
Glc-6-P, 5.0 mM	3PGA, 10 mM ATP, 2.0 mM	7.4 ± 0.5[a]
Glc-6-P, 0.5 mM	3PGA, 10 mM ATP, 2.0 mM	35.3 ± 1.6
Glc-6-P, 0.5 mM	3PGA, 10 mM ATP, 0.5 mM	36.7 ± 2.4

[a] Amyloplasts were disintegrated by sonication prior to incubation.

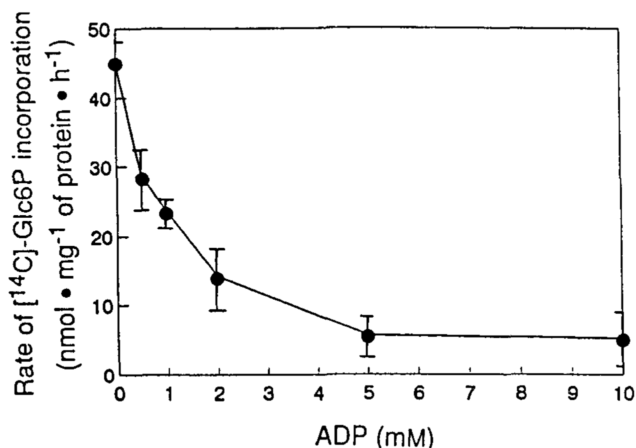

Figure 2. Effect of ADP on Glc-6-P-dependent starch synthesis in isolated intact plastids. Isolated plastids were incubated for 20 min in medium C containing 0.5 mM Glc-6-P, 10 mM 3PGA, 2 mM ATP, and various ADP concentrations. Data are the mean (±SE) of four independent experiments.

RESULTS

Effectors of Glc-6-P-Dependent Starch Synthesis

Attempts to demonstrate Glc-6-P-dependent starch synthesis with isolated intact cauliflower-bud plastids initially resulted in extremely low rates of starch synthesis. Therefore, various nonlabeled metabolites were added as effectors. Table I summarizes the results of these experiments. The highest rates of Glc incorporation, 110 to 120 nmol mg^{-1} protein h^{-1}, were obtained when both ATP and 3PGA were present. The stimulatory effect of ATP (2 mM) and 3PGA (10 mM) on Glc-6-P-dependent starch synthesis was, however, not very pronounced when these compounds were added separately. At a lower Glc-6-P concentration (0.5 mM), a decrease of the ATP concentration to 0.5 mM did not result in any change in the rate of starch synthesis (Table I). Under the given conditions, the rate of starch synthesis was shown to be completely dependent upon the intactness of the plastids. Disruption of the plastids by sonication prior to the start of the incubation decreased the rate of starch synthesis to 6.3% (SE 0.5%, $n = 8$) compared with the control (Table I).

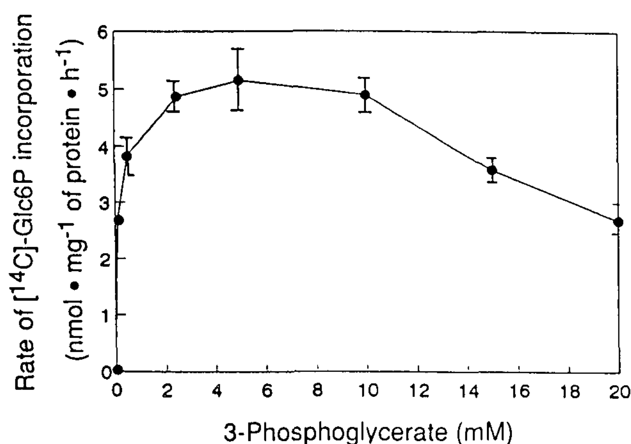

Figure 1. Effect of 3PGA upon Glc-6-P-dependent starch synthesis in isolated intact plastids. Isolated plastids were incubated for 20 min in medium C containing 0.1 mM Glc-6-P, 2 mM ATP, and 3PGA at various concentrations. Data are the mean (±SE) of four independent experiments.

To analyze further the effect of 3PGA on Glc-6-P-dependent starch synthesis, we incubated isolated plastids in the presence of a limiting Glc-6-P concentration (0.1 mM) at 2 mM ATP in the presence of various concentrations of 3PGA (Fig. 1). A relatively low concentration of 3PGA (0.2 mM) led to a significant increase in starch synthesis (50% of the maximum rate) followed by a broad optimum between 2.5 and 10 mM 3PGA. Concentrations above 10 mM 3PGA decreased Glc-6-P-dependent starch synthesis. This inhibition was also observed at a higher Glc-6-P concentration (0.5 mM, data not shown).

For further characterization of the stimulatory effect of ATP, ADP was supplied at various concentrations in addition to 2 mM ATP and 10 mM 3PGA. Figure 2 demonstrates the inhibitory effect of ADP on the rate of starch synthesis at 0.5 mM Glc-6-P, with 1 mM ADP inhibiting by 50%.

ATP Import into Isolated Intact Cauliflower-Bud Plastids

It is generally accepted that autotrophic and heterotrophic plastids possess an ATP-translocator protein (Heldt, 1969; Pozueto-Romero et al., 1991b). In chloroplasts, the role of this protein is unknown; in amyloplasts or chromoplasts, it catalyzes the import of ATP (Kleinig and Liedvogel, 1980; Pozueto-Romero et al., 1991b).

The results presented in Table I clearly demonstrate that cauliflower-bud plastids possess a high-affinity ATP-translocator catalyzing the uptake of the exogenously added ATP. Consequently, we performed short-term uptake experiments with ^{14}C-labeled ATP. The uptake exhibited saturation kinetics with an apparent K_m of 12 μM ATP and a maximal velocity of 167 nmol mg^{-1} protein h^{-1} (Fig. 3). This rate of ATP uptake was decreased in the presence of ADP. At 18 μM ATP, the

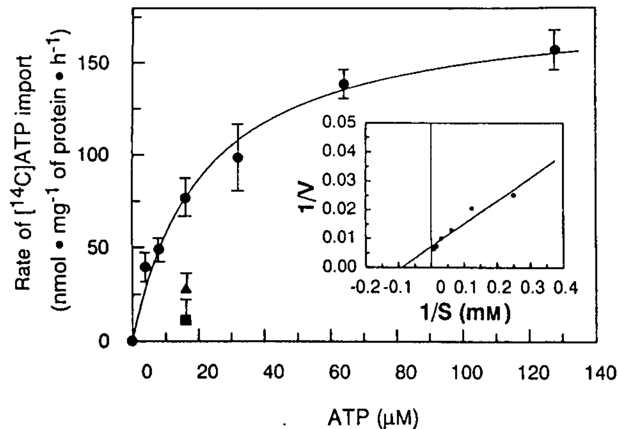

Figure 3. Uptake of [^{14}C]ATP by isolated plastids. Isolated plastids were incubated in medium C, with various concentrations of [^{14}C]ATP for 10 s as described in "Materials and Methods." The triangle represents the result of an experiment where 50 μM ADP was added to medium C. The square indicates the rate of [^{14}C]ATP uptake in the presence of 100 μM ADP. The inset is a double-reciprocal plot of the data; the x-axis intercept indicates an apparent K_m (ATP) of 12 μM; the y-axis intercept indicates a V_{max} of 167 nmol mg^{-1} protein h^{-1}. Data are the mean (±SE) of four independent experiments.

addition of 50 μM ADP resulted in 60% inhibition; 100 μM ADP inhibited by 80% (Fig. 3). This observed decrease in ATP uptake when ADP is applied simultaneously is in agreement with the strong inhibitory effect of rising ADP levels on Glc-6-P-dependent, ATP-stimulated starch synthesis (Fig. 2).

Characterization of Effector-Stimulated Glc-6-P-Dependent Starch Synthesis

Glc-6-P-dependent starch synthesis in the presence of ATP and 3PGA is saturated at concentrations around 5 mM Glc-6-P (Fig. 4A). After a short initial lag-period, the rate is nearly linear with time (Fig. 4B). For further characterization of Glc-6-P-dependent starch synthesis, various compounds known to be competitive and uncompetitive inhibitors of the chloroplastic phosphate translocator were added. As expected, DIDS (at 0.5 mM) inhibits starch biosynthesis almost completely (Table II). Even a 20-fold molar excess of Fru-6-P over Glc-6-P decreased Glc-6-P-dependent starch synthesis by only 30% (Table II). This result indicates that the translocator protein is highly specific for Glc-6-P. The weak inhibition obtained by the addition of the substrate analog 2-deoxyglucose-6-P is taken as further evidence for the high specificity of this translocator.

To test the effect of metabolites that are known to interact with the chloroplastic and amyloplastic phosphate translocator (Fliege et al., 1978; Borchert et al., 1989), we analyzed the influence of Pi and DHAP on Glc-6-P-dependent starch synthesis (Table II). Both metabolites decreased starch synthesis significantly, indicating a strong competition with Glc-6-P for the same transport protein. Alternatively, Pi could inhibit ADPGlc-PPase (Preiss, 1982).

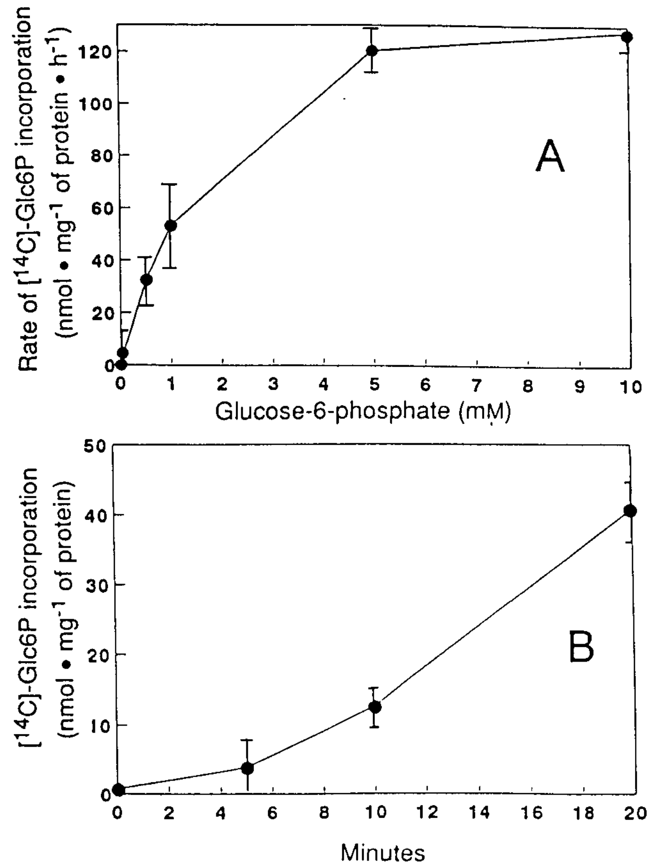

Figure 4. Effects of substrate concentration and incubation time upon starch synthesis in isolated intact plastids. A, Plastids were incubated for 20 min in medium C containing, in addition to various Glc-6-P concentrations, 2 mM ATP and 10 mM 3PGA. B, Plastids were incubated in the same medium as in A (including 5 mM Glc-6-P). The reactions were terminated at the indicated time points by transfer to a 95°C water bath for 3 min. Data are the mean (±SE) of four independent experiments.

Table II. *Inhibition of Glc-6-P-dependent starch synthesis in isolated plastids by competitive and uncompetitive inhibitors*

Isolated plastids were incubated for 20 min in medium C, containing 0.5 mM Glc-6-P, 10 mM 3PGA, and 2 mM ATP (control). All samples contained the same concentration of these effectors plus inhibitors in concentrations as indicated. Data are the mean (±SE) of four independent experiments.

Inhibitor	Concentration	Rate of Starch Synthesis
	mM	*nmol mg⁻¹ protein h⁻¹*
Control		40.0 ± 2.5
DIDS	0.5	0.7 ± 0.1
Fru-6-P	10.0	33.5 ± 1.8
2-Deoxyglucose-6-P	5.0	23.3 ± 1.2
Pi	10.0	1.0 ± 0.1
DHAP	10.0	0.5 ± 0.1

Substrate Specificity of Starch Synthesis in Plastids Isolated from Cauliflower Buds

To identify the most efficient precursor molecules leading to starch synthesis in heterotrophic plastids, we analyzed the rate of starch synthesis using various other ^{14}C-labeled substrates (Table III). In the presence of ATP and 3PGA, only Glc-6-P was able to maintain starch synthesis at significant rates. Glc-1-P at the same concentration as Glc-6-P drove starch synthesis only with 16% of the rate obtained using Glc-6-P. The efficiency of DHAP was only 14%, taking into account that two molecules of triose phosphates have to be taken up to provide one molecule of Glc for starch synthesis. The efficiency was even lower when using glycerol-3-P as a precursor, whereas glycerol supplied at a concentration of 5 mM was practically unable to act as a substrate.

DISCUSSION

Currently, there is an ongoing debate over the various metabolite(s) that could act as precursor(s) for starch synthesis in heterotrophic plastids. Using isolated intact cauliflower-bud plastids, we found significant incorporation of Glc-6-P into starch in the presence of 3PGA and ATP, whereas Glc-1-P was much less effective under these conditions. In accordance with the fact that the equilibrium of all hexose phosphates lies on the side of Glc-6-P (Bergmeyer, 1974), in our study this metabolite emerges as the most likely candidate to be the precursor of starch synthesis in vivo. This is in agreement with findings for amyloplasts purified from developing pea embryos (Hill and Smith, 1991). Amyloplasts from wheat endosperm seem to use Glc-1-P as a substrate for starch synthesis (Tyson and ap Rees, 1988). However, these authors did not test Glc-6-P-dependent starch synthesis in the presence of other effectors (e.g., ATP and 3PGA). We cannot rule out the possibility that amyloplasts from other sources use substrates other than Glc-6-P for starch synthesis. One possible reason for a difference could be the fact that wheat, as opposed to cauliflower and pea, is a monocotyledonous plant. Isolated amyloplasts from *Acer pseudoplatanus* use ADPGlc for starch synthesis (Pozueta-Romero et al., 1991b). These plastids are derived from a cell-suspension culture and could have adapted to these special conditions. More experiments are still needed to clarify this situation.

Table III. *Starch synthesis in isolated plastids is dependent on various precursors*

Isolated plastids were incubated for 20 min in medium C, containing different precursors at 5 mM concentration, in addition to 10 mM 3PGA and 2 mM ATP. Data are the mean (±SE) of four independent experiments.

Precursor	Rate of Starch Synthesis
	nmol mg^{-1} protein h^{-1}
Glc-6-P	127.5 ± 7.9
Glc-1-P	19.8 ± 1.1
DHAP	35.5 ± 1.7
Glycerol-3-P	3.3 ± 0.8
Glycerol	1.0 ± 0.2

In contrast with the chloroplastic phosphate translocator (Fliege et al., 1978), the translocator of cauliflower-bud plastids has the ability to transport Glc-6-P at significant rates because this compound was shown to drive starch synthesis when added exogenously (Fig. 4; Table III). The specificity for Glc-6-P is documented by the relatively moderate inhibition of Glc-6-P-dependent starch synthesis by the structural analog 2-deoxyglucose-6-P. The low inhibitory effect of an excess of Fru-6-P (Table II) and the low rate of Glc-1-P-dependent starch synthesis (Table III) further support our findings. Similarly, a phosphate translocator that is also competent for Glc-6-P transport has been described for amyloplasts isolated from pea roots (Borchert et al., 1989).

The pronounced inhibitory effect of Pi and DHAP is in good agreement with the possible physiological role of this translocator. During starch synthesis, Pi is liberated (enzyme sequence of ADPGlc-PPase and alkaline pyrophosphatase) and could be released from plastids in counter-exchange with newly imported Glc-6-P. In addition, Pi would inhibit starch synthesis by allosteric inhibition of ADPGlc-PPase inside the plastids (Preiss, 1982). Using our experimental attempt to study starch synthesis in cauliflower-bud plastids, it is not possible to distinguish between these two possible effects of Pi. DHAP seems to act competitively on the uptake of phosphorylated intermediates (e.g. Glc-6-P and 3PGA) into isolated cauliflower-bud plastids, as in the case of the chloroplastic phosphate translocator (Fliege et al., 1978) and the amyloplastic phosphate translocator from pea roots (Borchert et al., 1989). The general ability of cauliflower-bud plastids to take up phosphorylated C_3-molecules has already been shown for glycerol-3-P-dependent synthesis of fatty acids (Journet and Douce, 1985).

DHAP displayed a lower efficiency in acting as a precursor for starch synthesis than Glc-6-P. This is in contrast with its action as a source for carbon *and* energy (i.e., ATP) during starch synthesis in barley etioplasts (Batz et al., 1992). Mohabir and John (1988) postulated DHAP-dependent starch synthesis in amyloplasts isolated from potato tubers. A high degree of contamination by cytosolic marker enzymes and the comparably low intactness of the organelles used in their experiments, however, cast some doubts on the results. "Randomization" studies using position-labeled Glc applied to potato tubers and maize endosperm (and subsequent analysis of the position of the ^{14}C-label within the Glc recovered from starch) clearly demonstrated that hexoses are directly taken up into the plastids of these tissues (Hatzfeld and Stitt, 1990; Viola et al., 1991).

Our results indicate that Glc-6-P-dependent starch synthesis in cauliflower-bud plastids strongly depends on 3PGA as an activator (Table I). This effect could be due to an allosteric activation of the ADPGlc-PPase by a rising 3PGA/Pi ratio. Such allosteric effects have been established for ADPGlc-PPase of green as well as heterotrophic tissues (Preiss and Walsh, 1981; Preiss, 1982; Batz et al., 1992). 3PGA at higher concentrations (above 10 mM) competes with Glc-6-P, indicating that both metabolites are transported by the same translocator. If Glc-6-P and 3PGA would be transported by separate translocators, one for 3PGA and one with a higher specificity for hexose phosphates, the curve should exhibit

strict saturation characteristics with no inhibition at higher 3PGA concentration (see Fig. 1).

Starch synthesis in isolated cauliflower-bud plastids is strictly dependent upon external ATP (Table I). A similar effect has already been demonstrated for starch synthesis in amyloplasts from developing pea embryos (Hill and Smith, 1991). The ability of isolated cauliflower-bud plastids to use exogenously applied ATP is evidence for the presence of a functional ATP-translocator protein. A protein cross-reacting with antibodies against the mitochondrial ATP/ADP translocator from *Neurospora crassa* has already been detected in envelope preparations of amyloplasts from *A. pseudoplatanus* cell-suspension cultures (Ngernprasirtiri et al., 1988). We have demonstrated now that isolated cauliflower-bud plastids possess an ATP-transport protein with a relatively high affinity for ATP. A high-affinity ATP translocator with a K_m of 10 μM has been described for chloroplasts purified from spinach leaves (Pozueto-Romero et al., 1991a).

To gain insight into the regulatory events affecting starch synthesis in isolated plastids, we have carried out experiments with changing effector concentrations. Figure 2 shows that starch synthesis is strongly modulated by the external ATP/ADP ratio, thus linking the rate of starch synthesis with the energy supply of the cell. The information about metabolite changes during the transition from carbohydrate starvation to storage in heterotrophic tissues is scarce. Using *Chenopodium rubrum* suspension-culture cells, Hatzfeld et al. (1990) analyzed the metabolic events after initiating carbohydrate storage by feeding Glc. In their experiments, an increase in respiration can be correlated with increased levels of hexose phosphates, 3PGA, and ATP. According to our results, increasing concentrations of metabolites, especially of 3PGA and ATP, can act as a feed-forward signal for plastidic starch synthesis when Glc-6-P is present as a precursor. Here we postulate that heterotrophic plastids respond to metabolic signals imposed by the cytosol. The same seems to be true for plastids from green tissues, where chloroplastic starch synthesis is controlled mainly by regulatory events in the cytosol (Neuhaus and Stitt, 1989; Stitt, 1990).

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