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Starch Synthesis and Carbohydrate Oxidation In Developing Potato Tuber Amyloplasts

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Abstract: Starch, the major source of calorific intake in the human diet, is synthesised in the amyloplasts of non-photosynthetic tissues. To understand its synthesis, to determine which substrate is taken up by the organelle and which metabolite is broken down for the release of energy, the present studies were started from potato tuber (cv. Record). Glucose 1-phosphate (Glc1P) was the most effective substrate for starch synthesis in amyloplasts. The rate of incorporation of hexose (Glc1P) into starch was dependent on the presence of exogenous ATP and on the intactness of the preparation. Rates of starch synthesis from Glc1P plus ATP were linear for up to 1h and was saturated by 5-10 mM Glc1P. The uptake of Glc1P was inhibited by the addition of exogenous glucose 6-phosphate (Glc6P) and was not effected by the addition of 3-phosphoglyceric acid (3PGA). Other than Glc1P, ADPglucose (ADPG) also supported similar rates of starch synthesis, however, rate was saturated by 1mM ADPG. The import of ADPG and ATP into the amyloplast indirectly indicate the presence of an adenylate translocator on the organelle membrane. Carbohydrate oxidation in the amyloplast was stimulated by the addition of 2-oxoglutarate and glutamine. Glucose 1-phosphate also proved to be a better substrate for oxidative pathways than glucose 6-phosphate. The implications of these results for our understanding of the pathway of starch synthesis and carbohydrate oxidation are discussed.

Keywords: Carbohydrate oxidation, amyloplast, 2-oxoglutarate, glutamine and Potato

Introduction

Atmospheric free carbon is stored in plants in the form of starch and makes up 50% or more of the dry weight of many storage organs (Burton *et al.* 1995). Amyloplasts are the plastid that accumulate starch. They are usually found in storage tissues i.e endosperm, roots and tubers of plants. They lie in tissues that under normal conditions receive no light, which clearly shows that the enzymatic machinery of starch formation in these cells is independent of photosynthesis. Synthesis of starch in amyloplasts takes place after the translocation of sucrose from photosynthetic tissues. The amyloplast membrane is impermeable to sucrose and therefore it must be degraded into form which can across the amyloplast membrane. Starch synthesis in the amyloplasts of non-photosynthetic tissues depends upon the import of carbon compound(s) from the cytosol. Work with a variety of tissues has indicated that a six carbon compound enters the amyloplast for starch synthesis (Hatzfeld and Stitt, 1990; Viola *et al*,. 1991). This is supported by work with amyloplasts isolated from wheat endosperm (Tyson and ap Rees, 1988) which preferentially incorporate ^{14}C from glucose 1-phosphate into starch. However, the preferred substrate for starch synthesis and carbohydrate oxidation in amyloplasts isolated from pea embryos (Hill and Smith, 1991) and pea roots (Bowsher *et al.*, 1989) appears to be glucose 6-phosphate. It has also been suggested that some amyloplasts may take up ADP-glucose directly from the cytosol for starch synthesis (Pozueta-Romero *et al.*, 1991).

Using a rapid method, we have isolated amyloplasts from the developing potato tuber (Naeem *et al.*, 1997), allowing us to determine the substrates which are most likely to enter the amyloplast and support starch synthesis *in vivo*. An assessment of the impact of carbohydrate oxidation on starch synthesis within purified organelles has been carried out by supplying the substrate for glutamate synthase and stimulating the OPPP (Bowsher et al, 1992).

Materials and Methods

Chemicals: Enzymes, Substrates and Co-factors were from Sigma (UK) or Boehringer Corp. (UK). Nycodenz was brought from Nycomed Co., UK.

Plants. All experiments were performed using developing potato tubers (*Solanum tuberosum* L. c.v Record). Plant stock was maintained by culturing tissue in a regular order. The tissue was grown in 500 ml jars (50mm x 95mm) containing 25 ml nutrient medium at 20 °C and 16 h photoperiod. Sub-culturing was carried out in an air flow chamber at 15 °C. During culturing, aseptic technique was practised (Naeem *et al.*, 1997). The plants were placed in pots containing mixture of Levington M2 compost and Silvaperl in a 1:1 ratio. Tubers developed after three months from initial culturing. Uniform tubers approximately 3-4 cm across were used as the material for amyloplast isolation. Fresh tubers were harvested on the day of the experiment. The tubers were peeled to remove the skin / pigment. The washed and peeled tubers were sliced into small discs. During preparation sliced discs were placed on ice in wash buffer until all the tubers were sliced. The wash buffer was then removed and replaced with 100 ml extraction buffer (Tetlow *et al.*, 1993). Sliced potato discs were kept on ice in extraction buffer for atleast 30 minutes. The extraction buffer was removed and the tissues were rapidly chopped within 5 minutes with a sharp razor blade until pieces were less than 1 mm square size in 2 ml extraction buffer. The homogenate was filtered through 6 layers of muslin, previously dampened with the extraction buffer. The crude extract produced contains a mixture of intact amyloplasts, starch grains, cytosolic and mitochondrial enzymes and cellular debris. All enzyme assays were performed on uv spectrophotometer (Cecil 5000 series Uk.) at 25 °C. For determination of organelle distribution after passing through Nycodenz the amyloplasts were freeze-thawed three times with liquid nitrogen and the contents were centrifuged at 10,000g for 5 minutes. The supernatant was used as a source for enzyme assays. (Tetlow *et al.*, 1993). The purified amyloplasts were used to study the incorporation of ^{14}C-labelled substrate into starch. Metabolism of 14C-labelled substrate. Using purified amyloplasts incorporation of radioactivity from ^{14}C-labelled substrate into starch was assayed in the following medium: 50 mM Hepes (pH 7.5), I M Sucrose or Sorbitol, 1 mM KCl, 2 mM $MgCl_2$, 1 mM EDTA, 0.1% (w/v) bovine serum albumin (BSA), 5 mM ^{14}C-labelled substrate. In some experiments 1 mM ATP was added. Assays contained 1 ml plastid cuvette in a final volume of 500 ml. Reactions were initiated by the addition of 14C-labelled substrate (3.7- 7.4 kBq per sample), incubated at 25 °C for 60 minutes and mixed by gentle inversion after every 20 minutes. Controls contained boiled plastid and or were stopped within 15s of starting the incubation by the addition of 1 ml 75% (v/v) aqueous methanol-1% (w/v) KCl (methanol-KCl). Values for incorporation into starch were corrected by the values obtained in the zero-time boiled controls. Controls using lysed plastid were prepared by subjecting samples to three cycles of freezing in liquid nitrogen and thawing at 25 °C. For each assay triplicate samples of intact and ruptured amyloplasts were used. Reactions were stopped by the addition of 1 ml methanol- KCl and left to stand for 15 minutes. samples were then centrifuged at 10000g for 5 minutes. The supernatant was discarded and the starch containing pellet was washed a total of three times by re-suspension in 1 ml water. This was then taken up in 500 mM 50 ml sodium acetate (pH

4.8), heated at 100 °C for 30 minutes, cooled and mixed with 0.6 units α-amylase, 29 units amyloglucosidase and incubated for 16h at 37 °C. The digest was then centrifuged (10000.g for 5 min) and 400 ml assayed for ^{14}C by liquid scintillation counting with 3.6 ml Ecoscint.

In other experiments the amount of ^{14}C-label recovered as evolved CO_2 was measured. Incubation mixtures were placed in 2.5 cm diameter glass vials, with a central well into which 200 ml 10% (w/v) KOH was placed to collect evolved CO_2 . Upon initiation of the reaction with labelled, substrate, the vial was sealed with a rubber stopper. The incubation medium was identical to that used for other experiments, but also included a mixture of 2-oxoglutarate and glutamine (each 5 ml) in order to provide substrate for plastidial reactions requiring reducing equivalents. Reactions were incubated as above and terminated by the addition of 0.1 ml 10 % (w/v) trichloroacetic acid (TCA). The KOH containing the evolved CO2 was removed and the well washed twice in 0.2 ml water. The water and KOH were combined and assayed for ^{14}C by liquid scintillation counting with 3.4 ml Ecoscint. The ^{14}C recovered in starch in the incubation mixture was washed and treated in the same way as previous incubations (Naeem *et al.*, 1997).

Results & Discussion

To determine which metabolite (s) may act as substrates supporting starch synthesis, intact and ruptured (Freeze-thawed 3 times) plastid were incubated with ^{14}C-labelled metabolites in the presence and absence of ATP. Whereas, the net value is that which is dependent upon the integrity of the organelles.

Purified potato amyloplasts were supplied with ^{14}C-labelled precursors, with and with out ATP. The data expressed here are shown as net incorporation of labelled hexose into starch, after subtracting the ruptured values from the intact values into starch. The activity of the amyloplast marker APPase has been measured in each preparation and used to express the amount of label being incorporated into starch.

Of the metabolites tested [U-^{14}C]Glc1P and [U-^{14}C]ADPG supported significantly higher rates of starch synthesis than any of the other compounds tested and were dependent on amyloplast integrity (Fig. 1). The incorporation of [U-^{14}C]Glc1P into starch in potato tuber amyloplasts were strictly dependent on exogenous ATP as well, contrary to the studies of Kosegarten and Mengel (1994). Without ATP the net incorporation of [U-^{14}C]Glc1P into starch was less than a quarter of that with ATP and was not statistically significant. Of the other metabolites tested, glucose, fructose and dihydroxyacetone phosphate (DHAP) with and without ATP, showed little net incorporation into starch and there was no significant difference between intact and ruptured amyloplasts with most of the substrates tested. However, fructose with ATP gave a statistically significant rate of 116 ± 35 nmol hexose/unit APPase net incorporation into starch. Previously when Tetlow *et al.* (1994) tested various metabolites with wheat amyloplasts, they did not find any net incorporation from fructose.

Since starch synthesis was reasonably linear for 1h, all subsequent experiments were completed within this time period. If there is a carrier in the amyloplast envelope for Glc1P this may be reflected in the response of starch synthesis to the exogenous concentration supplies. This effect was measured by varying Glc1P concentration in the presence of 1 mM ATP. The concentration of Glc1P was varied from 0.1 to 10.0 mM. The rate was saturated between 5 and 10 mM, where the net incorporation was 942.2 ± 108.8 and 1051.2 ± 122.2 nmol hexose/unit APPase respectively.

Similarly, the rate of incorporation was saturated at 5 mM in amyloplasts isolated from wheat endosperm and potato suspension cultured cells (Tetlow *et al.*, 1994; Kosegarten and Mengel, 1994). The same is true for the uptake of Glc6P by leucoplasts isolated from pea roots (Borchert *et al.*, 1993). Because of the lower values obtained at 1 mM Glc1P or less, no attempt was made to calculate a km for this process since the lower values were at the limit of what was experimentally unassurable and therefore prone to greater error.

Glc6P is a substrate for starch synthesis in amyloplasts of many species e.g, pea, cauliflower buds and oilseed rape etc. Despite the fact that Glc6P did not apparently support starch synthesis, this does not rule out the possibility that it is still transported into amyloplasts. If it is, then one possibility is that both hexose phosphates are transported on the same carrier.

To test this, the effect of elevated concentrations of unlabelled Glc6P on [U-^{14}C]Glc1P incorporation into starch was tested. Higher concentrations of unlabelled Glc6P showed a significant effect on the rate of starch synthesis from Glc1P. When 10 mM Glc6P was used in the presence of labelled 5 mM Glc1P plus 1 mM ATP, the rate of incorporation was half that of the rate observed in the absence of Glc6P. Where 20 mM Glc6P was used the rate of starch synthesis was three times less than that observed when 5 mM Glc1P plus 1mM ATP were supplied alone

From these data it seems that in potato tuber amyloplasts Glc6P has a strong effect on the uptake of Glc1P suggesting a common carrier for the transport of hexose phosphate.

3PGA stimulates the rate of starch synthesis in chloroplast by activating AGPase and has been shown to have positive effect on starch synthesis in amyloplasts (Neuhaus *et al.*, 1993). The effect of 3PGA on starch synthesis from Glc1P in the amyloplasts of potato tuber was therefore tested. As indicated in Fig.2, there was nearly no effect of 5 mM 3PGA although this is more than sufficient to activate AGPase and was found to be saturating for cauliflower bud amyloplasts (Neuhaus *et al.*, 1993). The net rate of incorporation of Glc1P in the medium where 3PGA was present, was 1261.9 ± 42.9 nmol hexose / unit APPase, whereas the value without 3PGA was 1113.3 ± 82.5. There is no significant difference between these values, suggesting that the enzyme may already be fully activated in these preparations.

Carbohydrate oxidation in potato tuber amyloplasts was investigated by incubating plastid preparations with 5mM [U-14C]Glc1P plus 1 mM ATP or 5 mM [U-^{14}C]Glc6P plus 1 mM ATP. Since previous studies (Bowsher *et al.*, 1992; Tetlow *et al.*, 1994) had shown that the OPPP could be stimulated in plastid through the demand for reductant, the substrates for the glutamine were also supplied.

Table 1 shows the amount of label appearing as released carbon dioxide from plastid supplied with varied combinations of substrates for 1h at 25°C. Significant net release of labelled carbon dioxide was only observed when plastid were incubated with Glc1P, ATP, glutamine and oxoglutarate. There was some evolution of CO_2 from Glc1P plus ATP observed in intact and ruptured plastid but the values in ruptured plastid were higher than those from intact plastid. When [U-^{14}C]Glc6P was used as a substrate with ATP and with or without glutamine and 2-oxoglutarate, no CO_2 evolution was detected above the background value for the time zero control.

The pathway of carbon from translocated sucrose to storage starch in non-photosynthetic cells of potato tuber is controversial. One of the most important unanswered questions is which compounds cross the amyloplast envelope to support starch synthesis therein? There is no doubt about the sucrose of carbon and energy for starch synthesis in chloroplast, but starch synthesis in amyloplasts depends on the uptake of both carbon precursor and energy from the cytosol.

Historically, different views have been put forward about the precursors which enter the amyloplast stroma for the synthesis of starch. Proposed precursors have included triose-phosphate, hexose-phosphate and ADPglucose.

Of the metabolites tested, it appears that Glc1P is transported into the amyloplasts where it acts as a substrate for the synthesis of starch, a process which also depends on exogenous ATP. Thus the present investigations reveal one possible route for the synthesis of starch. Sucrose is degraded into Glc1P, which is transported via a phosphate-translocator type protein into the amyloplast. In the amyloplast, Glc1P is converted into ADPG and then into starch, and PPi is formed as a by product.

If Glc1P does enter the amyloplast for starch synthesis via 1:1 counter exchange with Pi then the concentration of Pi inside the organelle will still increase because pyrophosphate, a by product

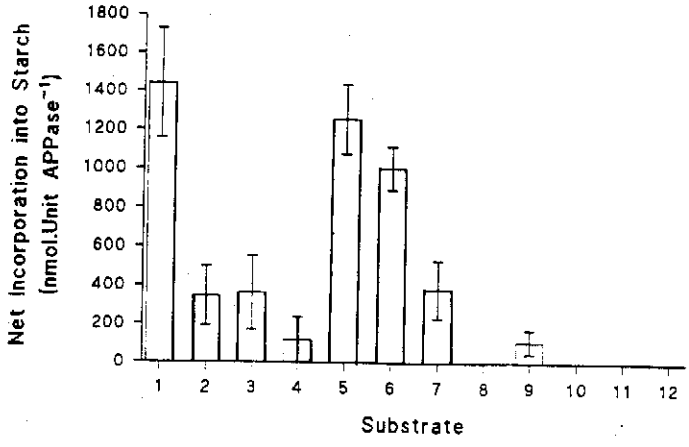

Fig. 1: Substrates for starch biosynthesis in purified amyloplasts from potato tuber. Each replicate consisted of three samples of intact and three ruptured (freeze-thawed three times) amyloplasts prepared from the same preparation. Amyloplasts were incubated for 1h at 25 °C with labelled sugars and sugar phosphates (5 mM) with or without 1 mM ATP. Incorporation of isotope into starch was determined. Bars shown are the net rate of incorporation (intact minus ruptured) of labelled substrate into starch. Each value is the mean ± S.E. of the number of independent amyloplast preparations.

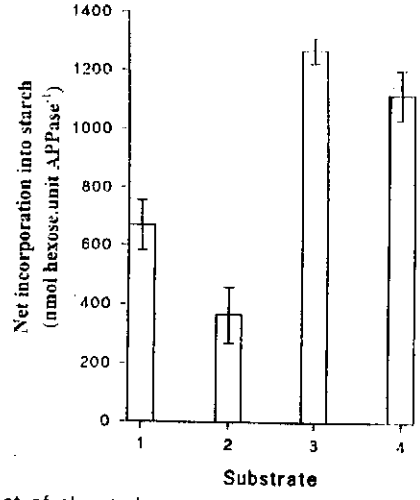

Fig. 2: Effect of elevated concentrations of Glc6P and 5 mM 3PGA on incorporation of [U-^{14}C]-Glc1P (5mM) into starch. Assays were performed in the presence of 1mM ATP. Bars shown are the difference between the values obtained with intact and ruptured preparations incubated at 25 °C. The reactions were terminated by the addition of 1 ml methanol/KCl. Results are the mean of 3 experiments ± S.E. 1, 10 mM Glc6P + 5 mM Glc1P + 1 mM ATP; 2, 20 mM Glc6P + 5 mM Glc1P + 1 mM ATP; 3, 5 mM 3PGA + 5 mM Glc1P + 1mM ATP; 4, 5 mM Glc1P + 1 mM ATP.

Table 1: Carbohydrate oxidation in amyloplasts isolated from developing potato tuber. Intact and ruptured (freeze-thawed 3 times) preparations of amyloplasts were incubated with either 5 mM Glc1P or Glc6P with 1 mM ATP and in the presence or absence of 5 mM 2-oxoglutarate and 5 mM glutamine (2OG + Gln). The data presented give the amount of ^{14}C from the labelled substrates recovered as evolved $^{14}CO_2$ after 1h incubation at 25 C. Each value is the mean ± S.E of three replicated experiments. The P-value indicates any significant statistical difference between intact and ruptured amyloplasts within different treatments.

Substrate	CO_2 (nmol CO_2/unit APPaase)			
	Intact	Ruptured	Net	P-value
Glc1P + ATP + 2OG + Gln	1333.9 ± 265.7	399.8 ± 126.1	934.2 ± 160.3	< 0.05
Glc6P + ATP + 2OG + Gln	N.D	N.D	-	-
Glc1P + ATP	200.0 ± 82.8	259.6 ± 167.1	-	-
Glc6P + ATP	N.D	N.D	-	-

N.D = Not detectable

of starch synthesis is cleaved into 2 Pi (Preiss, 1993).

Under such conditions, presumably another mechanism must operate to remove the additional phosphate. However, Ambudkar *et al.* (1986) demonstrated a variable stoichiometry of Pi/Glc6P in *Streptococcus lactis*. They observed 2:1 stoichiometry of Pi/Glc6P instead of 1:1 in the membrane vesicles of *S. lactis* and further revealed that the exchange stoichiometry was dependent on pH. On the other hand, pH had no effect on the stoichiometry of the exchange catalysed by the reconstituted wheat amyloplast translocator (Tetlow *et al.*, 1996).

Therefore, it is argued that another mechanism must operate for the removal of the additional Pi. Neuhaus and Maaß (1996) while they were working on cauliflower bud amyloplasts,, reported the unidirectional transport of Pi from the amyloplasts. But it is unclear in the case of potato tuber amyloplasts, whether this is a function of the bi-directional hexose-phosphate translocator or a separate protein, though Schwartz *et al.* (1994) have demonstrated that the triose-phosphate translocator of spinach chloroplast possesses this ability.

The plastid used in the present study were unable to support relevant rates of starch synthesis from Glc1P in the absence of exogenous ATP. Dependence of starch synthesis on exogenous ATP has been shown in plastid from many species (Hill and Smith, 1991; Neuhaus *et al.*, 1993; Tetlow *et al.*, 1994). Starch synthesis from Glc1P in the absence of ATP is likely to involve a reversal of the starch phosphorylase reaction, which is generally regarded as having a role in starch breakdown (Smith *et al.*, 1995).

An important question is whether the present rates of starch synthesis observed *in vitro* are relevant to the rates of starch synthesis *in vivo*. Comparison of these entities is somewhat difficult at this stage due to the lack of sufficient information in the literature. However ap Rees and Morrell (1990) reviewed that ADPglucose starch synthase, ADPglucose and alkaline are present in sufficient amounts to mediate the rates of starch synthesis *in vivo*, and are confined to the amyloplast. Other than Glc1P, the other substrate which seems to be able to support starch synthesis is ADPglucose (ADPG). In this way sucrose is degraded into ADPG outside the amyloplast membrane. ADPG thus formed from cytosolic AGPase crosses the organelle membrane. If this is the route of starch synthesis then it raises the question whether there is enough activity of AGPase present outside the amyloplast *in vivo*. It has been suggested by Villand and Kleczkowski (1994) that AGPase is extra-amyloplastic in barley seed endosperm. These workers proposed that ADPG formed by the extra-amyloplastic enzyme is transported to the amyloplasts via an ADPglucose carrier in the plastid membrane, and then utilized by the starch synthesising machinery of these organelles.

In addition to the extra-amyloplastic form of AGPase, barley endosperm contains also a second isozyme of AGPase, located in the amyloplasts. There is now substantive evidence in support of this, in which plastidic form and a distinct cytosolic isozyme have been demonstrated in barley endosperm (Thorbjornsen *et al.*, 1996).

Similarly, immunoblotting of gels of endosperm and plastid extracts from barley and maize also indicated that the enzyme is both inside and outside the plastid (Denyer *et al.*, 1996). Their results suggest that there are distinct plastidial and cytosolic forms of AGPase in these species which are composed of different subunits. The cytosolic AGPase is likely to be involved in the provision of ADPglucose for starch synthesis. Its activity is far in excess of the plastidial form is only slightly greater (barley) or less (maize) than the rate of starch synthesis. However, if AGPase is present outside the amyloplast then this will affect the turnover of PPi. Kleczkowski (1994) has suggested that activity of a cytosolic AGPase could be coupled to that of UDPglucose pyrophosphorylase (UGPase) through production and consumption of pyrophosphate and Glc1P, potentially allowing integration of the breakdown of sucrose and the synthesis of starch. Similarly, parallel to the formation of starch, the possibility that the extra-plastidial AGPase provides ADPG for some other process, for example the synthesis of the considerable amounts of ß-glucans found in barley endosperm (Bhatty *et al.*, 1991) can not be ruled out.

The synthesis of starch from Glc6P was not measurable and supports the previous observations of Coates and ap Rees (1994) and Tetlow *et al.*, (1994) etc. that Glc1P and not Glc6P is the preferred hexose phosphate entering the organelle for starch synthesis. The fact that Glc6P was not able to act as a substrate for starch synthesis also reveals that there is little interconversion between Glc6P and Glc1P outside the organelle by contaminating phosphoglucomutase, either released from broken organelles or present as a cytosolic contaminant (Borchert *et al.*, 1993).

In addition to the enzymic capacities of starch synthesis in potato tuber amyloplasts, the first two enzymes of the oxidative pentose phosphate pathway (OPPP), glucose 6-phosphate and 6-phosphogluconate dehydrogenase are present in potato tuber amyloplast (Naeem *et al.*, 1997). This is consistent with other studies which indicate that amyloplasts contain significant capacity for carbohydrate oxidation as well as starch synthesis (Tetlow *et al.*, 1994; Coates and ap Rees, 1994).

The stimulation of CO_2 evolution from Glc1P brought about by the combined presence of glutamine and 2-oxoglutarate is evidence that the oppp is responding to a demand for reductant from glutamate synthase (Bowsher *et al.*, 1992). It has previously been observed by Tetlow *et al.* (1994) that when Glc1P plus ATP were supplied to amyloplasts of wheat endosperm, rates of starch synthesis were reduced by 75% when carbohydrate oxidation was driven by the glutamate synthase reaction, which implies that the need for hexose phosphates to sustain carbohydrate oxidation in wheat endosperm amyloplasts had the potential to act as a considerable drain on carbon for starch synthesis may involve a reduction in starch synthesis in potato. Besides being involved in the synthesis of starch in potato tuber amyloplasts, Glc1P also acts as a substrate for the OPPP. This contrasts with other studies which have demonstrated that Glc6P is the best substrate for oxidation in amyloplasts of plant species (Bowsher *et al.*, 1992; Tetlow *et al.*, 1994; Coates and ap Rees, 1994). Though Glc6P was unable to act as a substrate for oxidation, the pathway could not proceed without synthesis of Glc6P. Therefore, it must be concluded that in amyloplasts there is enough activity of phosphoglucomutase present, able to isomerise Glc1P to Glc6P and hence complete the OPPP.

References

Ambudkar, S.V., L.A.Sonna and P.C. Maloney, 1986. Proc. Natl. Acad. Sci. USA. (83), 280-284.

Ap Rees, T. and S. Morrell, 1990. Carbohydrate metabolism in developing potatoes. American Potato J., 69: 835-847.

Bhatty, R.S., A.W.MacGregor and B.G. Rossnagel, 1991. Total and acid-soluble ß-glucan content of hulless barley and its relationship to acid-extract viscosity. Cereal Chem., 68: 221-227.

Borchert, S., J. Harborth, D. Schunemann, P. Hoferichter and H.W. Heldt, 1993. Studies of the enzymic capacities and transport properties of pea root plastid. Plant Physiol., 101: 303-312.

Bowsher, C.G., D.P. Hucklesby and M.J. Emes, 1989. Nitrite reduction and carbohydrate metabolism in plastid purified from pea roots. Planta, 177: 359-366.

Bowsher, C.G., E.L. Boulton, J. Rose, S. Nayagam and M.J.Emes, 1992. Reductant for glutamate synthase is generated by the oxidative pentose phosphate pathway in non- photosynthetic root plastid. Plant, J., 2: 893-898.

Burton, R.A., J.D. Bewley, A.M. Smith, M.K. Bhattacharyya, H.Tatge, S. Ring, V. Bull, W.D.O.Hamilton and C. Martin, 1995. Starch branching enzymes belonging to distinct enzyme families are differentially expressed during pea embryo development. The Plant J., 7: 3-15.

Coates, S.A. and T. Ap-Rees, 1994. Metabolism of glucose monophosphates by leucoplasts and amyloplasts from soybean cultures. Phytochem, 35: 881-883.

Denyer, K., T. Thorbjornsen and A.M. Smith. 1996. The major form of AGPase in maize and barley endosperm is extra-plastidial. Biochemistry of metabolic regulation in plants (BOMRIP) meeting, University of Manchester, UK.

Hatzfeld, W.D. and M.Stitt, 1990. A study of the rate of recycling of triose phosphates in heterotrophic *Chenopodium rubrum* L. cells, potato tubers and maize endosperm. Planta, 180: 198-204.

Hill, L. and A.M. Smith, 1991. Evidence that glucose 6-phosphate is imported as the substrate for starch synthesis by the plastid of developing pea embryos. Planta, 185: 91-96.

Kleczkowski, L.A., 1994. Glucose activation and metabolism through UDPglucose in plants. Phytochem., 37: 1507-1515.

Kosegarten, H. and K. Mengel, 1994. Evidence for a glucose1-phosphate translocator in storage tissue amyloplasts of potato (*Solanum tuberosum* L.) suspension-cultured cells. Physiologia Plantarum, 91: 111-120.

Naeem, M., T.J. Tetlow and M.J.Emes, 1997. Starch synthesis in amyloplasts purified from developing potato tubers. The Plant J. 11: 1095-1103.

Neuhaus, H.E., O. Batz, E. Thom and R. Scheibe, 1993. Purification of highly intact plastid from various heterotrophic plant tissue: analysis of enzymic equipment and precursor dependency for starch biosynthesis. Biochem. J., 296: 496-501.

Neuhaus, H.E. and U. Maa, 1996. Unidirectional transport of orthophosphate across the envelope of isolated cauliflower bud amyloplasts. Planta, 198: 542-548.

Pozueta-Romero, J., M. Frehner, A.M. Viale and T. Akazawa, 1991. Direct transport of ADP-glucose by an adenylate translocator is linked to starch biosynthesis in amyloplasts. Proc.Ntl. Acad. Sci. US. 88: 5769-5773.

Preiss, J., 1993. Biosynthesis of starch: ADP-glucose pyrophosphorylase. the regulatory enzyme of starch synthesis: structure-function relationships. Denpun Kagaku, 40: 117-131.

Schwartz, M., A. Gross, T. Steinkamp, U. I. Flugge and R. Wagner, 1994. Ion channel properties of the reconstituted chloroplast triose phosphate / phosphate translocator. J. Biol. Chem., 269: 29481-29489.

Smith, A.M., K. Denyer and C.R. Martin, 1995. What controls the amount and structure of starch in storage organs? Plant Physiol., 107: 673-677

Tetlow, I.J., K.J. Blissett and M.J. Emes, 1993. A rapid method for the isolation of purified amyloplasts from wheat endosperm. Planta, 189: 597-600.

Tetlow, I.J., K.J. Blissett and M.J. Emes, 1994. Starch synthesis and carbohydrate oxidation in amyloplasts from developing wheat endosperm. Planta, 194: 454-460.

Tetlow, I.J., C.G. Bowsher and M.J. Emes, 1996. Reconstitution of the hexose phosphate translocator from the envelope membranes of wheat endosperm amyloplasts. The Biochemical J., (in press).

Thorbjornsen, T., P. Villand, K. Denyer, O.A. Olsen and A.M. Smith, 1996. Distinct isoforms of ADPglucose pyrophosphorylase occur inside and outside the amyloplasts in barley endosperm. The Plant J., (in press).

Tyson, R.H. and T. ap Rees. 1988. Starch synthesis by isolated amyloplasts from wheat endosperm. Planta, 175: 33-38.

Villand, P. and L.A. Kleczkowski. 1994. Is there an alternative pathway for starch biosynthesis in cereal seeds. Z. Naturforsch., 49c: 215-219.

Viola, R., H.V. Davies and A.R. Chudeck, 1991. Pathways of starch and sucrose biosynthesis in developing tubers of potato (*Solanum tuberosum* L.) and seeds of faba bean (*Vici faba* L.). Planta, 183: 202-208.