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Starch synthesis by isolated amyloplasts from wheat endosperm

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Abstract. The aim of this work was to discover which compound(s) cross the amyloplast envelope to supply the carbon for starch synthesis in grains of *Triticum aestivum* L. Amyloplasts were isolated, on a continuous gradient of Nycodenz, from lysates of protoplasts of endosperm of developing grains, and then incubated in solutions of ^{14}C-labelled: glucose, glucose 1-phosphate, glucose 6-phosphate, fructose 6-phosphate, fructose-1,6-bisphosphate, dihydroxyacetone phosphate and glycerol 3-phosphate. Only glucose 1-phosphate gave appreciable labelling of starch that was dependent upon the integrity of the amyloplasts. Incorporation into starch was linear with respect to time for 2 h. At the end of the incubations, 98% of the ^{14}C in the soluble fraction of the incubation mixture was recovered as [^{14}C]glucose 1-phosphate. Thus it is unlikely that the added [^{14}C]glucose 1-phosphate was extensively metabolized prior to uptake by the amyloplasts. It is argued that the behaviour of the isolated amyloplasts, and previously published data on the labelling of starch by [^{13}C]glucose, are consistent with the view that in wheat grains it is a C-6, not a C-3, compound that enters the amyloplast to provide the carbon for starch synthesis.

Key words: Amyloplast – Glucose 1-phosphate – Triose phosphate – *Triticum* (starch synthesis) – Starch synthesis.

Introduction

Much of the starch in higher plants is made in amyloplasts from carbon derived from translocated sucrose. We do not know what compound(s) crosses the amyloplast envelope to support this starch synthesis. Amyloplasts from soybean cells lacked sucrose synthase, acid and alkaline invertases, and uridine 5′-diphosphoglucose (UDPglucose) pyrophosphorylase, but contained all the enzymes needed to convert triose phosphate to starch (Macdonald and ap Rees 1983). Plastids, which contain both starch and lipid, from cauliflower florets have also been reported to contain the enzymes for conversion of triose phosphate to starch (Journet and Douce 1985). These observations indicate that in these instances carbon for starch synthesis may enter the plastids as triose phosphate, a hypothesis recently regarded as being applicable to storage tissues in general (Boyer 1985). Although the enzymic capacities of the soybean and cauliflower plastids permit carbon for starch synthesis to enter as triose phosphate, they do not prove that it does. The observations allow any one or more of the glycolytic intermediates from glucose 1-phosphate to dihydroxyacetone phosphate to cross the amyloplast envelope for conversion to starch.

A more direct approach to discovering which compounds enter the amyloplast for conversion to starch is to determine the relative abilities of the different compounds to support starch synthesis by intact isolated amyloplasts. There is now available a procedure for isolating amyloplasts from the endosperm of developing wheat grains that does not expose the amyloplasts to severe osmotic stress that is likely to damage the transport mechanisms (Entwistle et al. 1988). We now report on the ability of such amyloplast preparations to synthesize starch from labelled intermediates.

Our basic experiment was to incubate freshly prepared amyloplasts in ^{14}C substrates, and determine the extent to which material insoluble in methanol-KCl became labelled. To distinguish incorporation by intact plastids from any due to bro-

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Abbreviations: PPase = alkaline inorganic pyrophosphatase; UDPglucose = uridine 5′-diphosphoglucose

ken plastids we always took replicate samples of amyloplasts that had been ruptured and measured the ability of the preparation to catalyse incorporation into insoluble material. For each experiment we also checked whether there was any co-precipitation of the labelled substrate with the material insoluble in methanol-KCl. We did this by adding methanol-KCl to a sample of amyloplasts immediately after the labelled substrate was added. In order to determine whether incorporation into the material insoluble in methanol-KCl did represent incorporation into starch we digested the insoluble material with amyloglucosidase and measured the label present in glucose in the hydrolysate.

Material and methods

Materials. Isotopes were from Amersham International, Amersham, Bucks., UK: all other chemicals, enzymes and co-factors were obtained as described by Entwistle et al. (1988). [^{14}C]Dihydroxyacetone phosphate was made in three ways. In method I, 0.058 μmol [U-^{14}C]glycerol (6.33 GBq·mmol^{-1}), 2 units glycerol kinase (EC 2.7.1.30), 2 units glycerol-3-phosphate oxidase (EC 1.1.3.21) and 130 units catalase (EC 1.11.1.6) were incubated for 10 min at 37° C in a total volume of 1 ml 60 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (Hepes), pH 8.0, 5 mM ATP, 5 mM $MgCl_2$. The reaction was stopped by heating in a boiling-water bath for 30 s and the [^{14}C]dihydroxyacetone phosphate was isolated by chromatography on Dowex-1 resin (Bartlett 1959) followed by concentration in vacuo. In method II, 47 nmol [U-^{14}C]glycerol 3-phosphate (6.29 GBq·mmol^{-1}), 10 units glycerol 3-phosphate oxidase and 130 units catalase were incubated for 10 min at 37° C in a total volume of 80 μl 50 mM Hepes, pH 8.0. The reaction was stopped as in method I and the product used without further purification. In method III, 57 nmol [U-^{14}C]fructose 6-phosphate (10.8 GBq·mmol^{-1}), 0.2 unit each of phosphofructokinase (EC 2.7.1.11), aldolase (EC 4.1.2.13), glyceraldehyde-phosphate dehydrogenase (EC 1.2.1.12) and phosphoglycerate kinase (EC 2.7.2.3), were incubated at 37° C for 15 min in a total volume of 1.0 ml 6 mM Hepes, pH 7.5, 5 mM $MgCl_2$, 0.5 mM ATP, 2 mM ADP, 2 mM KH_2PO_4, 1 mM NAD. The reaction was stopped as in the other methods and the products used without further purification. This method yielded a mixture of dihydroxyacetone phosphate and 3-phosphoglycerate. The same procedure was used to make [^{14}C]fructose-1,6-bisphosphate except that the aldolases glyceraldehyde-phosphate dehydrogenase and phosphoglycerate kinase were omitted.

Amyloplasts. Protoplasts were made from the endosperm of grains taken from the mid-ear region of wheat, *Triticum aestivum* L. cv. Mardler, 8–12 d after anthesis. The procedures used, and the methods for the fractionation of the protoplasts to yield the amyloplast preparations, were as described by Entwistle et al. (1988) except that: the grains were slightly heavier, 35–45 mg fresh weight; prior to digestion the freshly dissected endosperm halves were kept in 0.5 M sucrose, 10 mM KCl, 1 mM $CaCl_2$, 25 mM 2-(N-morpholino)ethanesulphonic acid (Mes), pH 5.5 (sucrose-Mes), and the layer of 20% Nycodenz was omitted from the gradient used to purify the protoplasts.

Metabolism of labelled substrates. The amyloplast band was removed from the Nycodenz gradient and portions of 60–450 μl were put in 1.5-ml microfuge tubes and carefully mixed with 50–100 μl ice-cold 50 mM Hepes, pH 7.5, 40% (w/v) Nycodenz (prepared as described by Entwistle et al. 1988) 2.5 mM KCl and 5 mM $MgCl_2$. The labelled substrates were added to give a final concentration of 5 mM and the following specific activities (GBq·mol^{-1}): ADP[U-^{14}C]glucose, 4.1–7.4; UDP[U-^{14}C]glucose, 8.1–8.9; [U-^{14}C]glucose 1-phosphate, 26.6–80.7; [U-^{14}C]glucose 6-phosphate, 35.5–82.1; [U-^{14}C]fructose 6-phosphate, 29.2–83.3; [U-^{14}C]fructose-1,6-bisphosphate, 69; [U-^{14}C]dihydroxyacetone phosphate, 36–70; [U-^{14}C]glycerol 3-phosphate, 42.6; [U-^{14}C]glucose, 28.5. These reaction mixtures were incubated without shaking at 25° C for 2 h, then 1 ml 75% (v/v) methanol-1% (w/v) KCl was added (Ghosh and Preiss 1965). The tubes were then centrifuged at 1660·*g* for 1 min. The supernatant was kept for further analysis; the starch-containing pellet was washed a total of four times by resuspending in 200 μl water followed by addition of 1 ml methanol-KCl and centrifuging as above. The washed pellet was resuspended in 1.0 ml 0.1 M Na-acetate buffer, pH 4.8, and kept in a boiling-water bath for 30 min. After cooling, 750 μl of the starch suspension was assayed for ^{14}C by liquid scintillation counting with 2 ml LKB Optiphase (FSA Laboratory Supplies, Loughborough, Leics., UK). The results are referred to as incorporation into material insoluble in methanol-KCl. Some of the starch suspension remaining was incubated for 16 h at 37° C with 2 units amyloglucosidase (EC 3.2.1.3). This digest was then centrifuged (11000·*g* for 1 min) and [^{14}C]glucose was isolated from the supernatant by paper chromatography in ethanol:1 M ammonium acetate:acetic acid (50:20:33, by vol.). This [^{14}C]glucose was counted and the ^{14}C content attributed to starch. Samples of lysed amyloplasts were obtained by taking replicate samples of the above amyloplast preparations and subjecting them to three cycles of freezing in liquid nitrogen and thawing at 25° C. For each experiment, triplicate samples of intact and ruptured amyloplasts were used. In addition, for each experiment we set up zero-time controls in which the methanol-KCl was added to the amyloplasts within 30 s of the labelled substrate. Values for incorporation into the methanol-KCl-insoluble material are corrected by the values obtained in the zero-time controls. For glucose 1-phosphate, the latter amounted to 0.5–7.6% of the value found for intact samples incubated for 2 h. Analysis of the distribution of ^{14}C in amyloplast preparations at the end of the incubations in labelled substrates was by paper chromatography of the supernatant obtained after removal of the material insoluble in methanol-KCl. The solvent was that already described. The methods used for the assay of alkaline pyrophosphatase (PPase; EC 3.6.1.1) and UDPglucose pyrophosphorylase (EC 2.7.7.9) have been described (Entwistle et al. 1988). For the studies of α-glucan phosphorylase (EC 2.4.1.1), 0.5 g endosperm was homogenized in 5–10 vol. 50 mM Hepes, pH 7.5, 0.5 M sucrose. The unfractionated homogenate was assayed as described by Downton and Hawker (1973) in a reaction mixture that contained in 100 μl: 5 mM [U-^{14}C]glucose 1-phosphate (37 GBq·mol^{-1}), 1 mg glycogen, 25 mM KCl, 5 mM $MgCl_2$, 50 mM Hepes, pH 7.5, 40% (w/v) Nycodenz, 0.17 M sucrose.

Results and discussion

For each of the amyloplast preparations that we used we determined the starch content, the extent of cytosolic contamination as indicated by the cytosolic marker UDPglucose pyrophosphorylase, and the yield of amyloplasts as indicated by the plastid marker alkaline inorganic pyrophosphatase

Table 1. Properties of amyloplast preparations. Lysates of endosperm protoplasts were fractionated on Nycodenz gradients to give the amyloplast preparations. Data are means ± SE for amyloplast preparations from the 18 different lysates used in the experiments reported in Tables 2 and 3

Property	Value	
	PPase	UDPglucose pyrophosphorylase
Activities of marker enzymes:		
nmol·min^{-1} (100 µl amyloplasts)$^{-1}$	6.7±0.8	26.6±4.9
Percentage activity in lysate	25 ±2	7 ±1
Activity recovered from gradient as percentage of that added	96 ±5	99 ±4
Starch content (µg·(100 µl amyloplasts)$^{-1}$)		0.54±0.09

(PPase). Evidence that alkaline PPase is a satisfactory marker for plastids has been obtained for both wheat endosperm (Entwistle et al. 1988) and suspension cultures of soybean (Gross and ap Rees 1986). Table 1 shows the properties of the amyloplast preparations that we used in the work reported in this paper. The fact that we used slightly larger grains than in our previous work (Entwistle et al. 1988) resulted in slightly greater cytosolic contamination of the amyloplasts, but did not affect yield. On removal from the gradient the intactness of the amyloplasts was 54% as determined from measurements of the latency of PPase. We considered how best to express any ability of amyloplasts to incorporate labelled substrates into starch. As chlorophyll is the usual basis for expression of chloroplast properties, it could be argued that starch content should be used for amyloplasts. However, amyloplast preparations are significantly contaminated by starch from broken amyloplasts. Thus we suggest it is best to use a unique amyloplast stromal enzyme as the basis for expression of amyloplast properties and have expressed incorporation into starch per unit (µmol Pi produced·min^{-1}) PPase.

The ability of intact and ruptured amyloplasts to incorporate labelled substrates into starch is shown in Table 2. The highest incorporation was found when ADPglucose was supplied to ruptured amyloplasts. A lower, but still marked, incorporation was found with the preparations of intact amyloplasts and ADPglucose. We attribute this to starch synthase in broken or damaged amyloplasts. The data for ADPglucose could be taken as a measure of the latency of starch synthase in our amyloplast preparations. A value of 18% may be calculated, much less than the 54% we obtained for PPase. These two estimates of latency are not directly comparable. Two factors probably contribute to the low value for starch synthase. First, the

Table 2. Starch synthesis by isolated amyloplasts. In each experiment three samples of intact and three of ruptured amyloplasts were prepared from the same preparation of amyloplasts, and then incubated with labelled substrate for 2 h. Incorporation into material insoluble in 75% methanol-1% KCl was determined; then the insoluble material was digested with amyloglucosidase; label released as [^{14}C]glucose, isolated by paper chromatography, is attributed to starch. Each value is from a different preparation of amyloplasts; where there are sufficient samples, values are given as means ± SE for the number of different amyloplast preparations shown in parenthesis

Substrate	Incorporation into methanol insoluble material (nmol/unit PPase)		^{14}C in starch as % of the total incorporation into methanol-insoluble material	
	Intact	Ruptured	Intact	Ruptured
ADPglucose	4269±324 (3)	5245±505 (3)	80	91
Glucose	47	5		
Glucose 1-phosphate	248± 63 (13)	23± 12 (9)	94,95	
Glucose 6-phosphate	61	0		
	38	21		
Fructose 6-phosphate	35	0		
	54	34		
Fructose-1,6-bisphosphate	153	145	12	11
Dihydroxyacetone phosphate[a]	63± 38 (4)	44± 22 (4)	48,80	84,63
Glycerol 3-phosphate	7	3		
Glucose 1-phosphate+1 mM ATP	636±161 (5)	422±173 (5)	85,94	80
Glucose 1-phosphate+1 mM UTP	820±219 (3)	677±304 (3)	28	
UDPglucose	3033	4701	20	14
	4888	4168		

[a] Prepared by method II

latency of PPase was determined directly after the isolation of the amyloplasts, whereas that of starch synthase was determined over a 2-h incubation, during which it is likely that there was a progressive rupture of the amyloplasts. Second, the percentage of the starch-synthase activity in the unfractionated protoplast lysate that is recovered in our amyloplast preparations (26%) is higher than that for the two other amyloplast markers, alkaline PPase (18%) and ADPglucose pyrophosphorylase (18%) (Entwistle et al. 1988). This probably represents starch synthase bound to starch grains that have been lost from ruptured amyloplasts but have co-purified with the amyloplasts. Such activity would not be latent. Our results for ADPglucose, plus the evidence that both starch synthase and ADPglucose pyrophosphorylase are confined to the amyloplast (Entwistle et al. 1988), lead us to suggest that ADPglucose does not readily cross the amyloplast envelope and is synthesized in the amyloplast from compound(s) imported from the cytosol.

We found no evidence that any of the following were imported by the amyloplast to any significant extent: glucose, glucose 6-phosphate, fructose 6-phosphate, fructose-1,6-bisphosphate, glycerol 3-phosphate, dihydroxyacetone phosphate. None gave significant incorporation into material insoluble in methanol-KCl that was dependent upon amyloplast integrity. We stress that we obtained similarly negative results in experiments in which we used [^{14}C]dihydroxyacetone phosphate prepared by methods I and III. Thus our failure to label starch with triose phosphate does not appear to be an artifact arising from the method used to prepare [^{14}C]dihydroxyacetone phosphate.

When glucose 1-phosphate was supplied to intact amyloplasts there was appreciable incorporation into material insoluble in methanol-KCl. This incorporation was almost entirely dependent upon the intactness of the amyloplasts and could be demonstrated consistently. Almost all of the ^{14}C incorporated into the insoluble material was released as [^{14}C]glucose by treatment with amyloglucosidase. The possibility that ruptured amyloplasts within our intact preparations contributed significantly to the incorporation of glucose 1-phosphate into starch is made unlikely by the very low incorporation shown by the completely ruptured preparations (Table 2). The data in Table 3 illustrate the agreement between replicates in a single experiment and show that incorporation was roughly linear with respect to time for 2 h.

Glucose 1-phosphate is the substrate for α-glucan phosphorylase. We thought it possible that the incorporation of ^{14}C from [^{14}C]glucose 1-phosphate could have been due to phosphorylase, possibly through some association of the enzyme with starch granules caused by the diethylaminoethyl (DEAE)-dextran and dextran sulphate, and that such activity could have been abolished by the freezing and thawing used to rupture the amyloplasts. The following is evidence against this view. First, unfractionated extracts of wheat endosperm showed no detectable activity of phosphorylase when assayed under conditions comparable to those used in the experiments described in Table 2. Activity was found if glycogen or amylopectin was added to the reaction mixture, but was very low in comparison with that of the enzymes involved in starch synthesis, 20 $nmol \cdot min^{-1} \cdot g^{-1}$ as opposed to 5.3 $\mu mol \cdot min^{-1} \cdot g^{-1}$ for PPase. Second, neither addition of DEAE-dextran plus dextran sulphate nor freezing and thawing affected such activity of phosphorylase that we found. Our values for ADPglucose in Table 2 show that there was Sample starch-synthase activity in preparations of ruptured amyloplasts. Similarly, measurements of the latency of ADPglucose pyrophosphorylase have revealed appreciable activities of this enzyme in frozen and thawed preparations of wheat endosperm. Thus we suggest that the low incorporation of glucose 1-phosphate by ruptured amyloplasts is the consequence of the breakage of the plastids.

Table 3. Labelling of starch by [^{14}C]glucose 1-phosphate supplied to isolated amyloplasts. Replicate samples of amyloplasts were incubated in 5 mM [U-^{14}C]glucose 1-phosphate: incorporation of ^{14}C into material insoluble in 75% methanol-1% KCl was determined at zero time and at intervals thereafter. Values are means ± SE for triplicate samples

Incubation period (h)	^{14}C incorporated (kBq/sample)
0	10.7 ± 0.4
1	104.8 ± 2.5
2	171.7 ± 12.4
3	149.3 ± 7.0

We investigated whether it was glucose 1-phosphate itself that entered the amyloplasts or some derivative formed by metabolism of the added glucose 1-phosphate by some contaminating enzyme(s) in the amyloplast preparations. The following observations make the latter possibility unlikely. Even when supplied exclusively, none of the obvious derivatives of glucose 1-phosphate did label starch to any appreciable extent (Table 2). In addition we chromatographed the soluble components of the incubation mixture after the methanol-KCl had been added. This fraction would have comprised the suspending medium, containing the

[^{14}C]glucose 1-phosphate plus the soluble fraction of the amyloplasts. We found that, of the label in this fraction, 98% was recovered as [^{14}C]glucose 1-phosphate and 2% as [^{14}C]glucose. Even when supplied at the same concentration, glucose was a poor substrate for starch synthesis in comparison with glucose 1-phosphate (Table 2).

We suggest that our data strongly indicate that amyloplasts from wheat endosperm can incorporate glucose 1-phosphate into starch. Our evidence of low activity of α-glucan phosphorylase, and of the confinement of appreciable activities of ADPglucose pyrophosphorylase, starch synthase, and PPase to the amyloplast (Entwistle et al. 1988), make it very probable that the incorporation of glucose 1-phosphate into starch proceeded via ADPglucose pyrophosphorylase and starch synthase. This pathway requires ATP for the formation of ADPglucose in the amyloplast. The ATP could be imported from the cytosol or made within the plastid by amyloplast glycolysis. The fact that ATP stimulated incorporation of glucose 1-phosphate into starch by intact plastids (Table 2) indicates that at least some of the ATP required for starch synthesis in the plastid may be imported. This possibility is weakened by our observation that ATP also stimulated incorporation of glucose 1-phosphate by ruptured amyloplasts. However, it is conceivable that ATP affected the intact and ruptured preparations in different ways. The stimulation in intact preparations may represent import of ATP and stimulation of ADPglucose pyrophosphorylase in intact amyloplasts. The effect in ruptured preparations may have been caused by rupture of the amyloplasts, allowing both ATP and glucose 1-phosphate unrestricted access to all of the ADPglucose pyrophosphorylase in the preparations.

Echeverria et al. (1985) reported that uridine 5′-triphosphate (UTP) was more effective than ATP in promoting incorporation of label into starch from hexose phosphates supplied to ruptured amyloplasts from maize endosperm. We found that UTP was particularly effective in promoting incorporation of label from [^{14}C]glucose 1-phosphate into material insoluble in methanol-KCl (Table 2). However, analysis of the incorporation in the presence of UTP strongly indicated that very little of it represented the synthesis of starch. In contrast to the incorporation from glucose 1-phosphate, either alone or in the presence of ATP, incorporation in the presence of UTP was not solubilized by treatment with amyloglucosidase. A very similar situation was found when labelled UDPglucose was supplied to the wheat amyloplasts. Since UDPglucose pyrophosphorylase has a very high activity in lysates of wheat endosperm protoplasts (Entwistle et al. 1988), even slight cytosolic contamination of the amyloplasts will amount to significant activities of UDPglucose pyrophosphorylase. We suggest that the addition of UTP and glucose 1-phosphate resulted in the synthesis of UDPglucose. Most of the incorporation from the latter appeared to be into some polymer other than starch. The incorporation that was into starch is likely to have been caused by granule-bound starch synthase from broken amyloplasts working with UDPglucose (Macdonald and Preiss 1985).

Given the enormous amount of work that was needed to devise a means of isolating intact functional chloroplasts, it is to be expected that our present method of isolating amyloplasts will be found to be far from perfect. Two points in particular require attention. First, the rate at which the isolated amyloplasts incorporated glucose 1-phosphate into starch, 0.12 μmol hexose·h^{-1}·(unit PPase)$^{-1}$, is less than that observed when wheat endosperm halves were incubated in 20 mM glucose, 0.7–1.0 μmol hexose·h^{-1}·(unit PPase)$^{-1}$ (Entwistle et al. 1988). However, in the presence of ATP the incorporation by the amyloplasts was increased to a rate roughly half that observed in the endosperm halves (Table 2). The second question is whether the concentration of glucose 1-phosphate in the cytosol is high enough to sustain an adequate rate of transport into the amyloplast. A definitive answer to this question requires the development of a method for measuring metabolite concentrations in the cytosol of the non-photosynthetic cells of plants. However, measurement of the total amount of glucose 1-phosphate in wheat endosperm gave a value of 112 ± 15 nmol·(g fresh weight)$^{-1}$, mean ± SD for six samples (S. Baird, personal communication). This indicates that there is an appreciable concentration of glucose 1-phosphate in the endosperm. Similarly high values for glucose 1-phosphate content have been found for two other tissues in which massive synthesis of storage starch is occurring; the developing cotyledons of pea, 70 nmol·(g fresh weight)$^{-1}$ (Edwards and ap Rees 1986); maize endosperm, 120 nmol·(g fresh weight)$^{-1}$ (Dancer 1987). Each of the above values is high when compared with the likely content of glucose 1-phosphate in tissue in which starch is made during photosynthesis. Gerhardt et al. (1987) showed that spinach leaves contained up to 135 nmol glucose 6-phosphate·(mg chlorophyll)$^{-1}$. If we assume that phosphoglucomutase catalyses an equilibrium reaction with an apparent equilibrium constant of 19 (ap Rees 1977) then the amount of glucose 1-phosphate in

spinach leaves is likely to be in the region of 10 nmol·(g fresh weight)$^{-1}$.

We suggest that amyloplasts from developing wheat grains can import glucose 1-phosphate and convert it to starch. It is not clear whether this is the sole source of carbon for starch synthesis in wheat amyloplasts. The negative results with other substrates may have been the consequence of differential loss of their transport systems during the isolation of the amyloplasts. The labelling of the glucosyl units of starch from wheat grains supplied with [1-^{13}C]- and [6-^{13}C]glucose shows that equilibration between these two glucose carbons was slight during the conversion of glucose to starch (Keeling et al. 1983). This is entirely consistent with the movement into the amyloplast of carbon for starch synthesis as glucose 1-phosphate. The above labelling pattern is difficult to explain if the route from sucrose to starch involves conversion to triose phosphate for entry into the amyloplast. Thus both the labelling patterns and the behaviour of the isolated amyloplasts are consistent with the view that in wheat grains, at least, it is hexose not triose that enters the amyloplast to provide the carbon for starch synthesis.

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