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# Pathways of starch and sucrose biosynthesis in developing tubers of potato (*Solanum tuberosum* L.) and seeds of faba bean (*Vicia faba* L.)

## Elucidation by $^{13}$C-nuclear-magnetic-resonance spectroscopy

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**Abstract.** Tissue slices from developing potato tubers (*Solanum tuberosum* L.) and developing cotyledons of faba bean (*Vicia faba* L.) were incubated with specifically labelled [$^{13}$C]glucose and [$^{13}$C]ribose. Enriched [$^{13}$C]glucose released from starch granules was analysed by nuclear magnetic resonance (NMR). Spectral analyses were also performed on sucrose purified by high-performance liquid chromatography. In both tissues a low degree of randomisation (< 11% in potato and < 14% in *Vicia*) was observed between carbon positions 1 and 6 in glucose released from starch when material was incubated with [$^{13}$C]glucose labelled in positions 6 and 1, respectively. Similarly, with [2-$^{13}$C]glucose a low degree of randomisation was observed in position 5. These findings indicate that extensive transport of three-carbon compounds across the amyloplast membrane does not occur in storage organs of either species. This is in agreement with previously published data which indicates that six-carbon compounds are transported into the plastids during active starch synthesis. When [1-$^{13}$C]ribose was used as a substrate, $^{13}$C-NMR spectra of starch indicated the operation of a classical pentose-phosphate pathway. However, with [2-$^{13}$C]glucose there was no preferential enrichment in either carbon positions 1 or 3 relative to 4 or 6 of sucrose and starch (glucose). This provides evidence that entry of glucose in this pathway may be restricted in vivo. In both faba bean and potato the distribution of isotope between glucosyl and fructosyl moieties of sucrose approximated 50%. The degree of randomisation within glucosyl and fructosyl moieties ranged between 11 and 19.5%, indicating extensive recycling of triose phosphates.

**Key words:** *Solanum* (starch biosynthesis) – Starch (biosynthesis) – Sucrose – Triose phosphate recycling – *Vicia* (starch biosynthesis)

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*Abbreviation:* NMR = nuclear magnetic resonance

### Introduction

The complete pathway involved in the conversion of sucrose into starch in cells of non-green storage organs is not known. In these cells, sucrose breakdown commonly occurs in the cytosol but starch synthesis takes place in another subcellular compartment, the amyloplast. Thus, to sustain starch biosynthesis, one or more products derived from sucrose breakdown must cross the amyloplast membrane. The exact nature of the intermediates transported has still not been identified.

Investigations with isolated amyloplasts from suspension cultures of soybean *(Glycine max)* (MacDonald and ap Rees 1983a) and starch-storing plastids of cauliflower *(Brassica oleracea)* (Journet and Douce 1985) have shown that the enzymic capacities for the synthesis of starch from triose phosphates are present. Similar evidence has been provided for rice (Nakamura et al. 1989) and maize amyloplasts (Echeverria et al. 1988). However, using antibody to plastidic fructose-1,6-bisphosphatase from spinach leaves, Entwistle and ap Rees (1988) could not detect any fructose-1,6-bisphosphatase in wheat endosperm. Furthermore, appreciable labelling of starch in purified wheat plastids has been observed with glucose-1-phosphate but not with triose phosphates (Tyson and ap Rees 1988). Recent experiments with the use of specifically labelled [$^{14}$C]glucose (Hatzfeld and Stitt 1990) have shown that in potato tubers, maize kernels and cell suspensions of *Chenopodium rubrum*, carbon is not transported into the amyloplast principally as three-carbon compounds. However, pea root plastids appear to possess the capacity to transport glucose-6-phosphate together with triose phosphate and 3-phosphoglycerate (Borchert et al 1989). Although the recent consensus of opinion is that six-carbon compounds rather than three-carbon compounds are transported into amyloplasts of non-green starch-storing tissues, it is clear that some controversy still remains.

Keeling et al. (1988) have provided independent evidence against the transport of three-carbon compounds in developing wheat endosperm with non-destructive $^{13}$C-NMR techniques. This technique obviates the need for lengthy and difficult procedures for isolation of fragile amyloplasts and offers the possibility of analysing the transport properties from a range of non-photosynthetically active sink tissues in vivo. This is important if general conclusions are to be made on the control of starch biosynthesis in such tissues. The ability to allocate isotopic enrichment in specific carbon positions within a molecule is also extremely useful in the analysis of the contributions of specific pathways to carbon fluxes.

The present study exploits $^{13}$C-NMR to investigate the form in which carbon is transported into amyloplasts of potato tubers and faba bean cotyledons during starch biosynthesis. Amyloplasts of both potato tubers and wheat grains are derived from relatively undifferentiated plastids i.e. leucoplasts, whilst starch-storing plastids of legume embryos are apparently derived from chloroplasts (Smith et al. 1990). It is for this reason that we have compared the pathways of starch synthesis in potato tubers and *Vicia* cotyledons. The study also provides information on the involvement of the pentose-phosphate pathway in starch biosynthesis and on the extent of triose-phosphate cycling in these plant tissues.

## Material and methods

*Plant material.* Plants of *Solanum tuberosum* L. cv. Record were grown in compost in an unheated glasshouse. Tubers approximately 4 cm in diameter were selected. Developing seeds of *Vicia faba* L. cv. Maris Bead, were taken from a field-grown crop 40 d after anthesis.

*Incubation conditions.* Potato tubers were cut transversely into 3 mm-thick slices and discs 5 mm in diameter excised with a cork borer from the phloem-rich perimedulla. Discs were washed four times in distilled water to remove debris. Twenty discs, obtained by randomly combining slices from five tubers, were incubated in scintillation vials containing 650 µl of medium. The volume of the medium was kept to a minimum to avoid the possibilty of anaerobiosis. The basic medium comprised 50 mM 2(N-morpholino) ethanesulfonic acid (Mes) buffer, pH 6.5, and the labelled sugars. [U-$^{14}$C]glucose (Amersham International, Amersham, Bucks., UK) was used at a specific activity of 2.26 GBq · mmol$^{-1}$ and [$^{13}$C]glucose (Aldrich Chemical Co., Gillingham, Dorset, UK) at final concentrations of 50, 100 or 300 mM. [1-$^{13}$C]ribose was used at a final concentration of 40 mM. Vials were incubated with gentle shaking for 5 h at 20° C. Discs were subsequently washed five times with Mes buffer and frozen in liquid $N_2$. *Vicia* cotyledons were hand-sliced transversely to a thickness of approx. 2 mm and 1 g FW (randomly selected from 30 seeds) incubated as above.

*Tissue-handling procedures.* Frozen tissue was homogenised with liquid $N_2$ in a pestle and mortar. Prior to total evaporation, 5 ml of ice-cold 1 M perchloric acid (PCA) was added. After centrifuging at 3000 · *g* for 10 min at 3° C the supernatant was washed with a further 2 ml PCA and supernatants combined. Residual isotope was removed by thoroughly washing pellets (8–10 ml) with distilled water. The pellet was resuspended in 2 ml of 100 mM sodium-acetate buffer, pH 4.5, containing 10 U · ml$^{-1}$ amyloglucosidase. The amyloglucosidase (Sigma Poole, Dorset, UK; *Aspergillus niger*) was purified prior to use first by gel filtration on Superose 12 (Pharmacia, Milton Keynes, UK) in 20 mM 2-amino-2-(hydroxymethyl)-1,3-propanediol (Tris) buffer, pH 7, and subsequently by ion exchange on Mono Q (Pharmacia). Two peaks of activity eluted in a 0 to 1 M NaCl gradient, and the most active form used for starch degradation. No β-glucanase activity (laminarin substrate) could be detected in this preparation. Incubation of starch pellets with amyloglucosidase was carried out in a shaking water bath at 37° C. For potato tubers, routine incubations lasted 17 h and for bean cotyledons 1.5 h. Samples were then centrifuged at 12 000 · *g* for 20 min and the supernatant boiled for 5 min to precipitate protein. When establishing the time course of $^{14}$C-release from starch, radioactivity was determined at this stage by liquid scintillation counting. For $^{13}$C-NMR analysis the protein-free supernatant was reduced to 0.75 ml under reduced pressure at 45° C and $D_2O$ added (final concentration 10% [v/v]) to provide a lock signal.

The PCA-soluble fraction was neutralised (pH 7–8) with ice-cold 5 M KOH. After removing insoluble perchlorate by centrifugation the extract was de-ionised with Amberlite-MB1 (Keeling and Tames 1986) and concentrated by rotary evaporation. Soluble sugars were then separated by high-performance liquid chromatography (HPLC) on a 15-cm amino-column using a mobile phase of acetonitrile:water (80:20, v/v) at a flow rate of 2 ml · min$^{-1}$. Detection was by refractive index. Samples were concentrated to 0.75 ml and $D_2O$ added.

*Operating conditions for NMR.* The $^{13}$C-NMR spectra were obtained on a Bruker AM 300/WB FT NMR spectrometer (Bruker Analytische Messtechnik Rheinstetten, FRG) at a frequency of 75.47 MHz. Spectra were accumulated into 16K of memory using a 6-s pulse and an acquisition time of 0.36 s. Transformation was performed after exponential multiplication (line-broadening 3 Hz). The number of transients varied between 500 and 5000, depending on solution strength. Chemical shifts are expressed in ppm and are referred to the external tetramethylsilane resonance at 0 ppm.

## Results

The successful use of NMR for studies on amyloplast transport depends upon the isolation of starch with a high specific activity of $^{13}$C. The technique of Keeling et al. (1988) was used to extract glucose highly enriched with $^{13}$C from the outer layers of the starch granule. Clear $^{13}$C-NMR spectra of glucose were obtained following incubation of starch granules for 17 h (potato) and 1.5 h (*Vicia*) with purified amyloglucosidase. Spectra obtained with *Vicia* were closely similar to those for potato. For this reason only the spectra for potato are presented. Fig. 1a shows the $^{13}$C-NMR spectrum of glucose released from potato starch (natural abundance). Resonances have been assigned on the basis of the chemical-shift information for glucose (Pfeffer et al. 1979). The spectra of glucose released from starch following incubation of tuber discs with [1-$^{13}$C]glucose, [6-$^{13}$C]glucose, and [2-$^{13}$C]glucose are shown in Fig. 1b–d.

The $^{13}$C-NMR spectrum of native sucrose (natural abundance) with carbon assignment is shown in Fig. 2a. Resonances have been assigned as in Pfeffer et al. (1979). Different peak assignments for the fructosyl moiety of sucrose have been recently published (Keeling et al. 1988); this was the result of a misrepresentation of the molecular structure of sucrose. The NMR spectra of sucrose isolated from potato tuber discs supplied with [1-$^{13}$C], [6-$^{13}$C] or [2-$^{13}$C]glucose are shown in Fig. 2b–d.

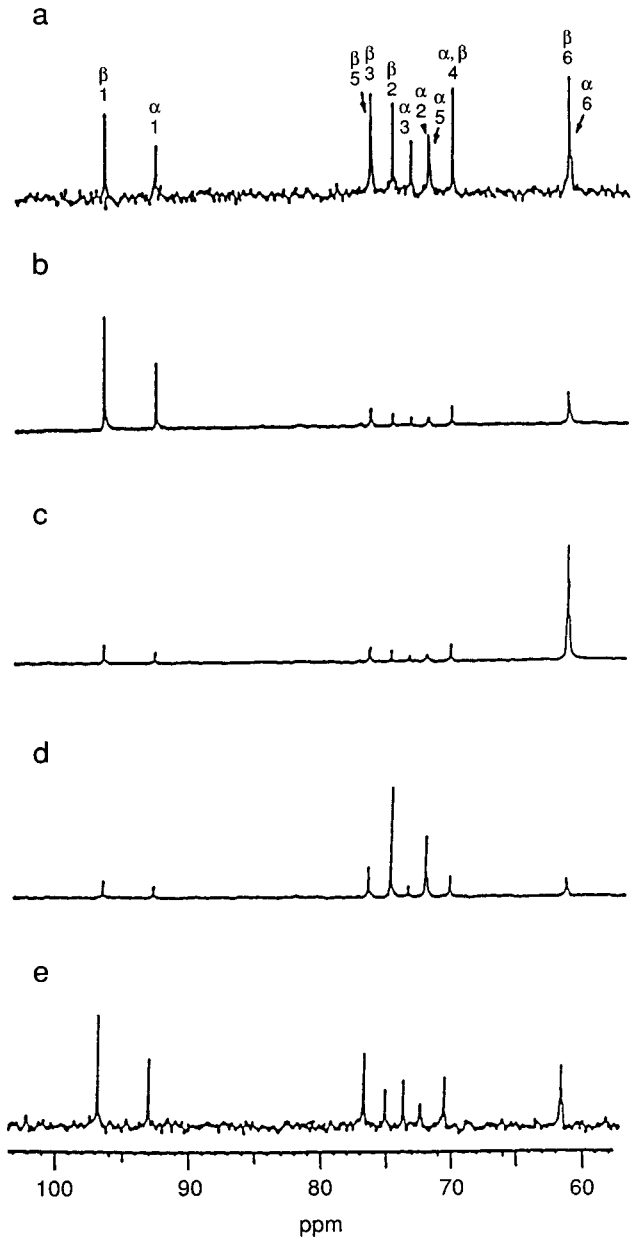


**Fig. 1a–e.** The $^{13}$C-NMR spectra of glucose released from partially digested starch granules of potato. The spectrum of natural $^{13}$C abundance is shown in **a.** Spectra obtained following the incubation of tuber discs with [1-$^{13}$C]glucose, [6-$^{13}$C]glucose, [2-$^{13}$C]glucose (all at 100 mM) and [1-$^{13}$C]ribose (40 mM) are shown, in the order given, in **b–e**

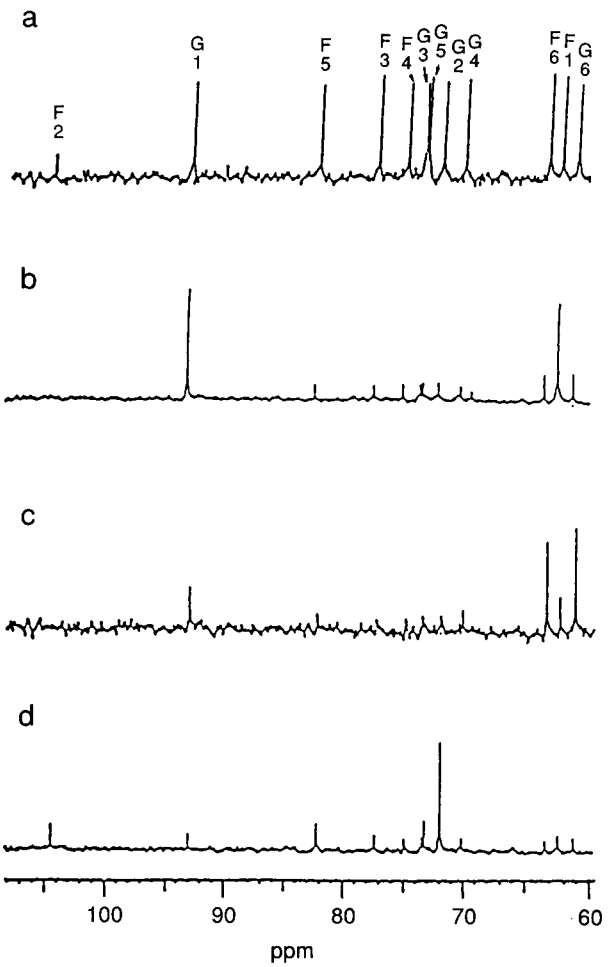


**Fig. 2a–d.** The $^{13}$C-NMR spectra of sucrose purified by HPLC from perchloric-acid extracts of tuber discs. The spectrum of natural $^{13}$C abundance together with peak assignments is shown in **a.** Spectra obtained following the incubation of potato tuber discs with [1-$^{13}$C]glucose, [6-$^{13}$C]glucose and [2-$^{13}$C]glucose (all at 100 mM) are shown, in the order given, in **b–d.** *F* = fructosyl moiety; *G* = glucosyl moiety

*Determination of enrichment.* When [1-$^{13}$C] or [6-$^{13}$C]glucose were supplied, peak intensities for both alpha and beta anomers were considered. Owing to poor separation of carbons 2 and 5 in the alpha anomer, peak intensities of carbon atoms of the beta anomer only were considered following incubation of tissue with [2-$^{13}$C]glucose. Enrichment of specific carbon positions following incubation with either [1-$^{13}$C] or [6-$^{13}$C]glucose was identified as follows: Spectra of enriched samples were superimposed on a spectrum obtained from glucose released from unlabelled starch (natural abundance spectrum) such that peak heights were identical for positions 2 to 5 (i.e. positions 2 to 5 were normalised). Twenty five replicate analyses of natural-abundance spectra showed less than 5% variation in the percentage contribution of each carbon position to total peak height. This technique allowed the enrichment of carbon position 1 relative to position 6 to be determined. There was no evidence of preferential enrichment in positions 2,3,4 or 5. When [2-$^{13}$C]glucose was supplied, enrichment was evident in positions 2 and 5. There was no specific enrichment in positions 1 or 3 relative to 4 or 6. Therefore, these four carbon positions were used to normalise spectra. With [1-$^{13}$C]ribose, positions 2,4 5 and 6 were used for normalisation. Sucrose spectra were were normalised in the same way as spectra of glucose released from starch, but using both hexosyl moieties. Once relative enrichment in specific carbon positions was identified by normalisation of spectra, the degree of enrichment was obtained by calculating increase in peak height over natural abundance. The percentage redistribution of isotope from the

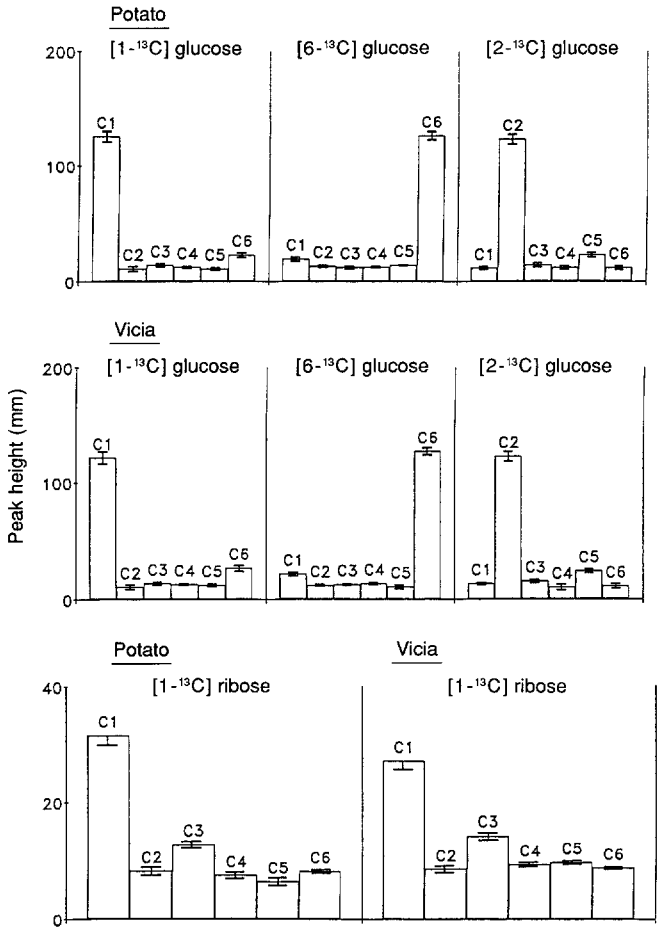


**Fig. 3.** Distribution of $^{13}$C in all carbon atoms of glucose released from starch isolated from potato tubers and *Vicia faba* cotyledons incubated with [1-$^{13}$C]glucose, [6-$^{13}$C]glucose, [2-$^{13}$C]glucose or [1-$^{13}$C]ribose. Final concentrations of [$^{13}$C]glucose and [$^{13}$C]ribose were 100 mM and 40 mM, respectively. Bars indicate SEs ($n=4$)

carbon position originally labelled (99% $^{13}$C) to other carbon positions was then calculated.

*Redistribution of specifically labelled [$^{13}$C]glucose in starch.* It is evident from the spectra shown in Fig. 1 that most of the $^{13}$C in glucose released from starch was located in the carbon position in which the substrate was originally supplied. A schematic representation of the relative heights of individual carbon positions was prepared from representative spectra and is shown in Fig. 3. Data for both potato and *Vicia* are presented. With [1-$^{13}$C]glucose as a substrate, some enrichment was found in the C6 of glucose released from starch. Conversely, after incubation with [6-$^{13}$C]glucose there was some redistribution to C1. Redistribution to C5 only was evident when [2-$^{13}$C]glucose was supplied. There was no apparent enrichment in C1 or C3 relative to C4 or C6.

Figure 1e shows the spectrum of glucose released from starch when [1-$^{13}$C]ribose was supplied to potato tuber discs. In both potato and *Vicia*, enrichment was apparent in carbons 1 and 3 (Fig. 3).

Table 1 shows the percentage redistribution of $^{13}$C between carbons 1 and 6 and between carbons 2 and 5 obtained with both species. The degree of redistribution varied between 3.8 and 10.8% in potato, depending on substrate concentration used and original carbon position labelled. The percentage varied between 8.6 and 13.5% with *Vicia*. With both tissues there was less randomisation with [6-$^{13}$C]glucose than with either [1-$^{13}$C]glucose or [2-$^{13}$C]glucose. Table 2 shows that following incubation of potato and Vicia with [1-$^{13}$C]ribose, approx. 25% of the enrichment was recovered in C3 in glucose released from starch and approx. 75% in C1.

**Table 1.** Percentage redistribution (± SE) of $^{13}$C in glucose released from starch after incubation of potato tuber discs or *Vicia faba* cotyledons with [1–$^{13}$C], [6–$^{13}$C] or [2–$^{13}$C] glucose ($n=4$)

| Tissue | Isotope | Isotope concn. (mM) | % redistribution | | |
|---|---|---|---|---|---|
| | | | C1 to C6 | C6 to C1 | C2 to C5 |
| Potato | [1–$^{13}$C]glucose | 300 | 6.3 ± 0.3 | | |
| | [6–$^{13}$C]glucose | 300 | | 3.8 ± 0.5 | |
| | [1–$^{13}$C]glucose | 100 | 9.5 ± 2.1 | | |
| | [6–$^{13}$C]glucose | 100 | | 5.0 ± 0.3 | |
| | [1–$^{13}$C]glucose | 50 | 10.8 ± 1.2 | | |
| | [2–$^{13}$C]glucose | 100 | | | 9.6 ± 1.1 |
| | [2–$^{13}$C]glucose | 50 | | | 10.7 ± 1.5 |
| *Vicia* | [1–$^{13}$C]glucose | 100 | 13.5 ± 0.6 | | |
| | [6–$^{13}$C]glucose | 100 | | 8.6 ± 1.1 | |
| | [2–$^{13}$C]glucose | 100 | | | 10.7 ± 0.7 |

**Table 2.** Percentage distribution or enrichment in glucose released from starch after incubation of potato tuber discs or *Vicia faba* cotyledons with 40 mM [1–$^{13}$C]ribose ($n=3$)

| Tissue | % total enrichment in C1 | % total enrichment in C3 | SE |
|---|---|---|---|
| Potato | 77.7 | 22.3 | 3.5 |
| *Vicia* | 70.7 | 29.3 | 4.1 |

*Redistribution of specifically labelled [$^{13}$C]glucose in sucrose.* With both potato and *Vicia* approx. 50% of the label was recovered in the fructosyl moiety of sucrose when [1-$^{13}$C], [6-$^{13}$C] and [2-$^{13}$C]glucose was supplied (Table 3). There was less redistribution from C6 to C1 than from C1 to C6 or from C2 to C5. In general, redistribution was more extensive within the fructosyl moiety compared with the glucosyl moiety. Redistribution within the hexosyl moieties ranged from approx. 11 to 19.5% with both species. Sucrose isolated from tissues incubated with [1-$^{13}$C]ribose was not sufficiently enriched to allow accurate determinations.

## Discussion

A high degree of isotopic randomisation in starch following incubation of tissues with specifically labelled [$^{13}$C]glucose is expected if glucose is converted to triose

**Table 3.** Redistribution (±SE) or $^{13}$C enrichment in sucrose isolated from potato tuber discs and *Vicia* cotyledons after supply of [1–$^{13}$C], [6–$^{13}$C] and [2–$^{13}$C] glucose ($n=3$)

| Tissuse | Isotope (100 mM) | % of label in sucrose in the fructosyl moiety | % redistribution of enrichment in the hexosyl moieties of sucrose | | | |
|---|---|---|---|---|---|---|
| | | | Glycosyl C1 to C6 | Fructosyl C1 to C6 | Glycosyl C2 to C5 | Fructosyl C2 to C5 |
| Potato | [1–$^{13}$C]glucose | 51±3 | 16.2±2.6 | 19.5±2.1 | | |
| | [6–$^{13}$C]glucose | 52±3 | 12.9±1.8 | 15.4±1.9 | | |
| | [2–$^{13}$C]glucose | 54±5 | | | 16.1±2.4 | 18.0±3.1 |
| *Vicia* | [1–$^{13}$C]glucose | 49±2 | 13.2±2.8 | 14.1±2.2 | | |
| | [6–$^{13}$C]glucose | 47±4 | 11.9±1.5 | 10.9±0.7 | | |
| | [2–$^{13}$C]glucose | 49±4 | | | 15.5±1.9 | 17.5±0.9 |

phosphates prior to entry into the amyloplast. The reaction catalysed by triose-phosphate isomerase, both in the cytosol and the amyloplast, would produce an extensive redistribution of label between carbons 1 and 6, 2 and 5 and between 3 and 4. The low percentage of randomisation in starch in both growing potato tubers and developing cotyledons of faba bean indicates that in both species carbon flux into the amyloplast does not occur principally as triose phosphates. It seems most likely that six-carbon compounds such as hexose and-or hexose-phosphates are transported. Although the starch-storing plastids of *Vicia* may be derived from chloroplasts, the evidence from $^{13}$C-NMR indicates that they share similar transport properties with starch-storing plastids from non-green storage organs. Our data could also be consistent with the hypothesis that sucrose is translocated into the amyloplast. Sucrose has been detected in starch-granule preparations from potato tubers (Isherwood 1976) and maize endosperm (Liu and Shannon 1981). Furthermore, early developmental stages of *Avena* plastids possess the capacity for sucrose uptake (Hampp and Schmidt 1976). However, there is increasing evidence that plastids in general lack enzymes capable of metabolizing sucrose (ap Rees 1988 and references therein). This makes it unlikely that sucrose is transported directly into amyloplasts during starch synthesis.

The fact that, in the tissues examined, starch glucosyl residues and sucrose hexosyl moieties both show redistribution of label indicates that sucrose and starch are synthesised largely from the same cytosolic pool of precursors, presumably hexose phosphates. This confirms $^{13}$C-NMR data on developing wheat grains (Keeling et al. 1988), and the $^{14}$C data of Haztfeld and Stitt (1990) obtained with maize kernels, cell suspension cultures of *Chenopodium rubrum* and potato tubers. With potato these authors observed 12.8% redistribution of carbon between positions 1 and 6 in starch ([1-$^{14}$C]glucose substrate). In the same study, redistribution within sucrose was 13.3%. In the present study the lower percentage of redistribution in starch compared to sucrose may relate to the high glucose concentration used in the experiments. These results could be explained if glucose is transported directly into the amyloplast together with hexose phosphates. With the assumption that redistribution of the label occurs solely in the cytosol, this would result in a lower degree of randomisation in starch as compared with the hexosyl moieties of sucrose. Although the flux of carbon through the chloroplast membrane has been attributed mainly to the transport of trioses via the phosphate translocator (for a review, see Heber and Heldt 1981) the capacity for hexose transport is also present (Schafer et al. 1977; Heber and Heldt 1981). Transport into the chloroplast of six-carbon molecules could explain the low degree of carbon randomisation in starch when tobacco leaf tissue is supplied with specifically labelled [$^{14}$C]glucose (Maclachlan and Porter 1961). Furthermore, isolated wheat amyloplasts are able to incorporate [$^{14}$C]glucose into starch (although at a lower rate than [$^{14}$C]glucose-1-phosphate) and this is dependent upon membrane integrity (Tyson and ap Rees 1988). However, the maximum capacity of such preparations to convert glucose into starch can only be assessed in the presence of optimum concentrations of ATP.

Incubation with [1-$^{13}$C]glucose resulted in a higher degree of randomisation in starch and in the hexosyl moieties of sucrose compared with [6-$^{13}$C]glucose. This phenomenon has also been observed elsewhere (Hatzfeld and Stitt 1990) and attributed to the operation of the pentose-phosphate pathway (PPP) in the cytosol during which C1 atoms are lost as $CO_2$. In developing wheat grain, Keeling et al. (1988) observed less redistribution of $^{13}$C in starch with [6-$^{13}$C]glucose as a subtrate compared with [1-$^{13}$C]glucose. However, this was not apparent in the hexosyl moieties of sucrose. They concluded that the PPP was operating in the amyloplast. To investigate the involvement of the PPP in more detail we supplied potato tubers and *Vicia* cotyledons with [1-$^{13}$C]ribose. Enrichment in starch was distributed between carbons 1 and 3. The ratio approximated 7:3, the ratio expected if ribose were incorporated into starch via a single cycle of the PPP (for a review, see Axelrod and Beevers 1956). Therefore, our data indicate the presence of PPP enzymes in the tissues examined. However, the presence of high activities of enzymes of a particular pathway does not necessarily imply an operational pathway in vivo (Earl and Gibbs 1955). Consequently, [2-$^{13}$C]glucose was used to determine the extent to which the PPP contributed to hexose phosphates metabolism in vivo. Preferential enrichment in both starch and sucrose of carbons 1 and 3 relative to 4 and 6 is expected if glu-

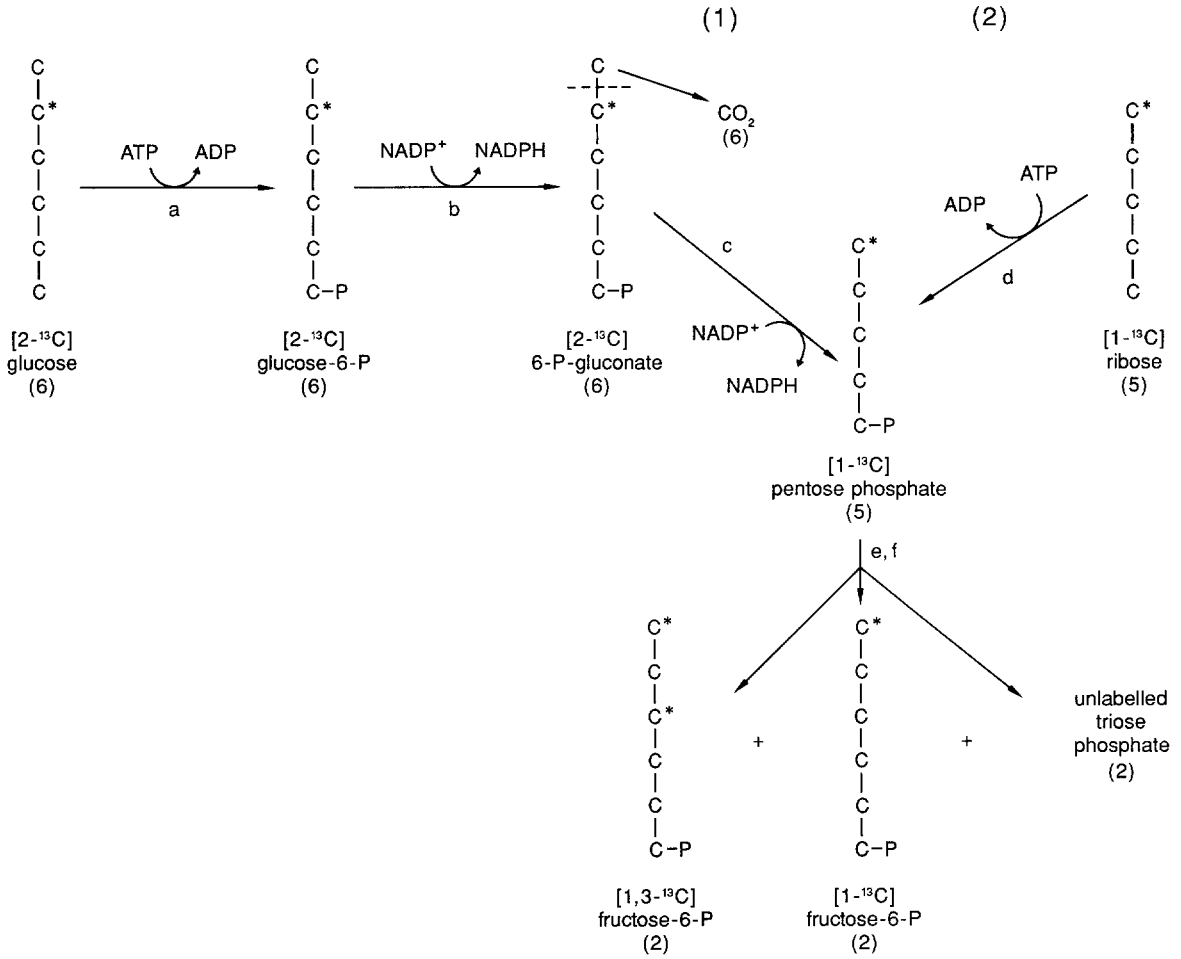


**Fig. 4.** Schematic representation of the redistribution of $^{13}$C from [2-$^{13}$C]glucose *(1)* and [1-$^{13}$C]ribose *(2)* following a cycle through the pentose-phosphate pathway. Letters denote the enzymes: *a* hexokinase; *b* glucose-6-phosphate dehydrogenase; *c* b-phosphogluconate dehydrogenase; *d* ribokinase; *e* transketolase; *f* transaldolase. *Asterisks* show the carbon positions specifically labelled. Numbers in ( ) denote number of molecules. *P* = phosphate

cose is cycled in the PPP prior to incorporation (Fig. 4). Shibko and Edelman (1957) reported redistribution of carbon from [2-$^{14}$C]glucose to carbons 1 and 3 relative to carbons 4 and 6 in sucrose and cellulose in barley seedlings. However, under our conditions, no enrichment was detected in either position 1 or 3 relative to position 4 or 6 in starch nor in either glucosyl or fructosyl moieties of sucrose. This was the case for both potato tubers and *Vicia* cotyledons. Although we cannot exclude the possibilty that the sensitivity of $^{13}$C-NMR is insufficient to detect a very low percentage of isotopic redistribution from carbon 2 either to carbon 1 or to carbon 3, our data indicate that in both tissues glucose does not enter the PPP to any great extent prior to starch or sucrose biosynthesis. The lower degree of redistribution of label in starch and sucrose with [6-$^{13}$C]glucose compared to [2-$^{13}$C]glucose provides further evidence against extensive operation of the PPP in the tissues examined. This difference in redistribution cannot be accounted for by specific losses of isotope as $CO_2$ in the PPP.

Nevertheless, incorporation of [1-$^{13}$C]ribose into starch does indicate the potential for PPP activity. A tight control of the PPP has been proposed (Turner and Turner 1980), with entrance into the pathway regulated by the activity of glucose-6-phosphate dehydrogenase. This enzyme is allosterically modulated by the NADPH:NADP ratio (ap Rees 1988).

In both potato and *Vicia,* carbon redistribution was observed in sucrose hexosyl moieties, indicating that a proportion of the hexose phosphates utilised for biosynthesis had been formed from randomised triose phosphates. Redistribution of label was higher in the fructosyl moiety compared with the glucosyl moiety, confirming the hypothesis that the hexose phosphates may not be completely in equilibrium (Hatzfeld and Stitt 1990). A pathway for the recycling of trioses in plants was reported as early as 1955 (Edelman et al. 1955) but has received more detailed attention only recently (Keeling et al. 1988; Hatzfeld et al. 1990). The incorporation, in non-gluconeogenic tissues, of radiolabel from glycerol into sucrose and starch (MacDonald and ap Rees 1983b; Sasaki and Kainuma 1984; Viola and Davies 1990) could also be explained by such a pathway. Our data do not provide insights as to how this pathway is regulated.

However, if we assume that in the tissues examined the triose phosphates are in equilibrium, the lower degree of redistribution observed in starch and sucrose with [6-$^{13}$C]glucose compared to [1-$^{13}$C] or [2-$^{13}$C]glucose could be tentatively explained by the involvement, in the recycling pathway, of a transaldolase exchange mechanism, similar to the one described by Ljungdahl et al. (1961). Whilst such an exchange has not yet been shown to occur in higher plants, the preferential recycling of glyceraldehyde-3-phosphate in this way could explain the lower degree of randomisation in starch and sucrose with [6-$^{13}$C]glucose.

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