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Hexose metabolism in discs excised from developing potato (*Solanum tuberosum* L.) tubers

II. Estimations of fluxes in vivo and evidence that fructokinase catalyses a near rate-limiting reaction

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Abstract. Metabolism of radiolabelled hexoses by discs excised from developing potato (*Solanum tuberosum* L.) tubers was been investigated in the presence of acid invertase to prevent accumulation of labelled sucrose in the bathing medium (Viola, 1996, Planta 198: 179–185). When the discs were incubated with either [U-^{14}C]glucose or [U-^{14}C]fructose without unlabelled hexoses, the unidirectional rate of sucrose synthesis was insignificant compared with that of sucrose breakdown. The inclusion of unlabelled fructose in the medium induced a dramatic increase in the unidirectional rate of sucroses synthesis in the tuber discs. Indeed, the decline in the sucrose content observed when discs were incubated without exogenous sugars could be completely prevented by including 300 mM fructose in the bathing medium. On the other hand, the inclusion of unlabelled glucose in the medium did not significantly affect the relative incorporation of [U-^{14}C]glucose to starch, sucrose or glycolytic products. Substantial differences in the intramolecular distribution of ^{13}C enrichment in the hexosyl moieties of sucrose were observed when the discs were incubated with either [2-^{13}C]fructose or [2-^{13}C]glucose. The pattern of ^{13}C enrichment distribution in sucrose suggested that incoming glucose was converted into sucrose via the sucrose-phosphate synthase pathway whilst fructose was incorporated directly into sucrose via sucrose synthase. Quantitative estimations of metabolic fluxes in vivo in the discs were also provided. The apparent maximal rate of glucose phosphorylation was close to the extractable maximum catalytic activity of glucokinase. On the other hand, the apparent maximal rate of fructose phosphorylation was much lower than the maximum catalytic activity of fructokinase, suggesting that the activity of the enzyme (unlike that of glucokinase) was regulated in vivo. Although in the discs incubated with or without fructose the rates of starch synthesis or glycolysis were similar, the relative partitioning of metabolic intermediates into sucrose was much higher in discs incubated with fructose (0.6% and 32.6%, respectively). It is hypothesised that the equilibrium of the reaction catalysed by sucrose synthase in vivo is affected in discs incubated with fructose as a result of the accumulation of the sugar in the tissue. This results in the onset of sucrose cycling. Incubation with glucose enhanced *all* metabolic fluxes. In particular, the net rate of starch synthesis increased from 2.0 μmol·hexose·g FW^{-1}·h^{-1} in the absence of exogenous glucose to 3.7 μmol·hexose·g FW^{-1}·h^{-1} in the presence of 300 mM glucose. These data are taken as an indication that the regulation of fructokinase in vivo may represent a limiting factor in the utilisation of sucrose for biosynthetic processes in developing potato tubers.

Key words: Carbohydrate metabolism – Fructokinase – Fructose – *Solanum* (hexose metabolism) – Sucrose metabolism – Sucrose synthase

Introduction

In developing potato tubers the bulk of incoming sucrose is degraded by sucrose synthase rather than invertase (Morrell and ap Rees 1986). This implies that only two enzymes (starch synthase and fructokinase) apparently catalyse irreversible reactions during the conversion of sucrose into starch in this tissue. However, other enzymic reactions involved (which are readily reversible in vitro) are thought to be essentially irreversible in vivo. For example, the synthesis of adenosine 5′-diphosphoglucose (ADPGlc) catalysed by ADPGlc pyrophosphorylase in the amyloplast is thought to be made thermodynamically irreversible by the hydrolysis of the reaction product, pyrophosphate. Displacement from equilibrium through rapid removal of a reaction product (fructose) has also been suggested as the mechanism by which fructokinase activity could make the reaction catalysed by sucrose

Abbreviations: ADPGlc = adenosine 5′-diphosphoglucose; Glc6P = glucose-6-phosphate; hexose-P = hexose phosphate; NMR = nuclear magnetic resonance; UDPGlc = uridine 5′-diphosphoglucose

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synthase essentially irreversible in vivo. Indeed, a coupling of sucrose synthase and fructokinase activities in developing potato tubers has been suggested on the basis of (i) the substantial and concomitant increase in sucrose synthase and fructokinase activities at the onset of starch synthesis (Ross et al. 1994) and (ii) the rapid decline in fructose content as the stolons stop elongating and start to swell (Davies 1984; Ross et al. 1994). Although appealing, this hypothesis has never been properly tested. Indeed, there is still much controversy on the role of sucrose synthase in the control of sucrose metabolism in storage organs and its function as a determinant of sink strength. Attempts have been made to establish a direct correlation between sink strength of tissues importing sucrose and sucrose synthase activity (Sung et al. 1989). However, recent experiments have cast doubts on this hypothesis by showing that sucrose synthase can catalyse a readily reversible reaction in vivo in potato tubers and other plant tissues (Geigenberger and Stitt 1993). In general, such reversibility and the existence of "futile" cycles of synthesis and degradation of sucrose have been studied under specific experimental conditions. In particular, in most cases sucrose synthase activity in vivo has been investigated by supplying the tissue with exogenous fructose (Wendler et al. 1990; Geigenberger et al. 1993, Geigenberger and and Stitt 1993). However, Viola (1996) has reported that radiolabelled precursors, and [U-^{14}C]fructose in particular, can be converted into sucrose prior to uptake by tuber tissue. This finding has implications for the issue of sucrose metabolism in potato tubers given the fact that a substantial proportion of label in sucrose in tuber discs incubated with [U-^{14}C]fructose appeared to have originated directly in the medium via sucrose synthase (Viola 1996). In the present work the metabolism of labelled hexoses by developing tuber tissue has been thoroughly re-examined. Here the accumulation of label from labelled hexoses into sucrose prior to uptake has been prevented by including acid invertase in the incubation medium. Unidirectional rates of sucrose synthesis and breakdown in the absence or presence of exogenous hexoses have been obtained. The results obtained indicate that sucrose synthase catalyses the net breakdown of sucrose in vivo in developing tubers and that the equilibrium of the reaction can be markedly affected by exogenously supplied fructose. Estimations of rates of glucose and fructose phosphorylation in vivo indicate that fructokinase activity is strongly regulated and may be an important determinant in the conversion of sucrose into starch.

Materials and methods

Plant material. Potato (*Solanum tuberosum* L. cv Record) plants were grown from seed tubers in unheated glasshouses in pots (30 cm^2) containing UC compost (Thompson and Taylor 1979). Tubers approximately 3 cm in diameter were selected and used within 1 h after excision from the mother plant.

Labelling experiments. Preparation of the tuber discs was carried out exactly as described previously (Viola 1996) except that prior to use the discs were washed three times. After the final wash, the discs were blotted onto filter paper and transferred to glass vials (20 discs/vial) containing 2 mL of bathing medium. In experiments with radiotracers, the bathing medium comprised 25 mM 2-(N-morpholino)ethanesulfonic acid (Mes)-KOH buffer, 37 kBq·mL^{-1} of [U-^{14}C]glucose or [U-^{14}C]fructose (both at 9.17 GBq·mmol^{-1}), various concentrations of unlabelled glucose or fructose (as indicated in the text), mannitol (enough to make up 325 mM final osmotic strength) and 167 nkat, yeast acid invertase (lyophylised powder; Boehringer Mannheim UK, Lewes, UK), resuspended in distilled water and desalted on pre-packed PD-10 columns (Pharmacia, Uppsala, Sweden) before use. The vials were sealed with rubber caps fitted with paper filters impregnated with 200 µL KOH (10%, w/v) for trapping evolved CO_2. Vials were kept dark and gently shaken at room temperature (21 °C).

For nuclear magnetic resonance (NMR) experiments bathing medium comprised 25 mM Mes-KOH buffer (pH 6.5) containing [2-^{13}C]glucose or [2-^{13}C]fructose (both 99.9% ^{13}C) at 50, 100 or 300 µmol·mL^{-1}. The ^{13}C-NMR analyses of HPLC-purified sucrose (see below) were carried out as described earlier (Viola et al. 1991).

Analysis of ^{14}C distribution. At the end of the incubation time, the medium containing labelled substrates was removed and the discs were washed five times with 3 mL of Mes-KOH (pH 6.5) containing 300 mM mannitol (3 min/wash) to remove free-space solutes. The discs were blotted onto filter paper and then transferred to beakers containing 50 mL of boiling 80% (v/v) ethanol. Tissue was exhaustively extracted at 65 °C on a hotplate (the procedure was repeated twice) and extracts centrifuged (10 000·*g*) for 20 min. Extracted tissue was then ground in a mortar with 10 mL cold 80% (v/v) ethanol and centrifuged. Pellets were washed twice more. All supernatants were added to the original soluble extracts. All insoluble fraction were combined, resuspended in 20 mL distilled water and incubated in a boiling waterbath for 2 h. Aliquots of the gelatinised starch (1 mL) were incubated with 1 mL of 200 mM acetate buffer, pH 4.5 containing 33.4 nkat amyloglucosidase (*Aspergillus niger*, Sigma, Poole, Dorset, UK) for 12 h at 37 °C. The treatment resulted in the total dissolution of starch as determined by staining with iodine of material precipitated following centrifugation of samples (10 000·*g*) for 30 min. Radioactivity in starch was determined by liquid scintillation counting of aliquots of the supernatant (HPLC of supernatant showed that > 99% of ^{14}C co-eluted with glucose). Radioactivity in the insoluble fraction after treatment with amyloglucosidase was determined as described in Viola and Davies (1994).

Soluble fractions were gently reduced to the aqueous phase on a hotplate, freeze-dried and then resuspended in 2 mL distilled water. Aliquots (1 mL) were then passed in succession through pre-packed ion-exchange columns for solid-phase extraction (SAX [anion exchange], and SCX [cation exchange,] both from HPLC Technology, Macclesfield, UK). Columns were washed with distilled water (1 mL × 3) which was added to the unbound fractions. Charged compounds were eluted from the column with 1 N HCl (1 mL × 3). Radioactivity in bound and unbound fractions was determined by liquid scintillation counting. Quantification of $^{14}CO_2$ was carried out as described previously (Viola and Davies 1991).

Analysis by HPLC. The HPLC of soluble components was carried out as described in Viola (1996). For determination of ^{14}C specific activities, peaks of interests were collected, pooled and metabolite content quantified spectrophotometrically (see below). Quantification of ^{14}C in hexosyl moieties of sucrose (or purification of sucrose for NMR analyses) required repeated injections of individual samples and collection of the sucrose peak. Pooled fractions were then freeze-dried and resuspended in 0.5 mL distilled water (or 0.5 mL D_2O for ^{13}C samples). For hydrolysis of purified sucrose, 0.1 mL of 100 mM acetate buffer containing 167 nkat yeast acid invertase was added and samples incubated 3 h at 21 °C. Samples were then incubated in a boiling waterbath for 15 min, centrifuged (14 000·*g*) for 10 min and radioactivity in glucose or fructose determined by HPLC.

Metabolite and enzyme analysis. Soluble sugars or hexose monophosphates were quantified spectrophotometrically as previously described (Viola and Davies 1991; 1992). Optimised methods for

sucrose synthase, fructokinase and glucokinase assays are given elsewhere (Ross et al. 1994).

Results

Sugar balance in the tuber tissue and metabolism of exogenous hexoses

When discs excised from developing potato tubers were incubated with Mes-KOH (25 mM, pH 6.5) containing 300 mM mannitol, sucrose content declined at a linear rate of 1.5 $\mu mol \cdot g FW^{-1} \cdot h^{-1}$ (Fig. 1). No net changes in the other carbohydrate pools were observed. Incubation of the discs for 1.5 h with either glucose or fructose resulted in an increase in the content of the respective hexose in the tissue compared with discs incubated without exogenous sugars (Fig. 2). The possibility that such an increase was attributable to exogenous sugar in the apoplasm was tested by assessing the presence of radioactivity in extracts of discs incubated with the non-permeant [U-^{14}C]mannitol. After the discs had been taken through the washing procedure, less than 0.3% of the initial radioactivity was recovered in the extract (not shown). Assuming that this percentage represented the degree of contamination of the free space (from exogenous sugars) across all the hexose concentrations used, it could be estimated that exogenous sugars should account for no more than 19.1% and 16.0% of the observed accumulation of fructose or glucose, respectively. Thus, it is concluded that the observed increase in hexose content when discs are incubated with either glucose or fructose largely occurs within the tuber cells. Such an accumulation indicates that the hexose-phosphorylating capacity of the tissue becomes gradually saturated as the net uptake of sugars from the medium increases. Incubation of the discs with 50 or 100 mM fructose reduced, and at 300 mM completely prevented, the decline in sucrose content observed in discs incubated in buffer alone. Incubation of discs with glucose had no effect on the sucrose content.

Metabolism of [U-^{14}C] glucose and [U-^{14}C] fructose

Figure 3 shows the distribution of ^{14}C incorporated when discs were incubated for 3 h with [U-^{14}C]fructose (Fig. 3A) or [U-^{14}C]glucose (Fig. 3B) in the presence of 167 nkat acid invertase. No sucrose was detected in the bathing media at the end of the incubation, independently of the concentration of unlabelled hexose used (not shown). In discs incubated with glucose, the net rate of glucose metabolisation (calculated from the total label incorporated minus that still present as the original substrate) and the net incorporation of glucose into starch and sucrose saturated at approximately 100 mM external concentration. Fructose incorporation into starch also saturated at 100 mM external fructose concentration. On the other hand, the net rate of fructose metabolisation and incorporation into sucrose increased linearly at all external fructose concentrations. The incorporation of glucose into sucrose was substantially lower than that into starch at all exogenous glucose concentrations. The incorporation of fructose into sucrose was lower than that into starch at 10 and 50 mM exogenous fructose

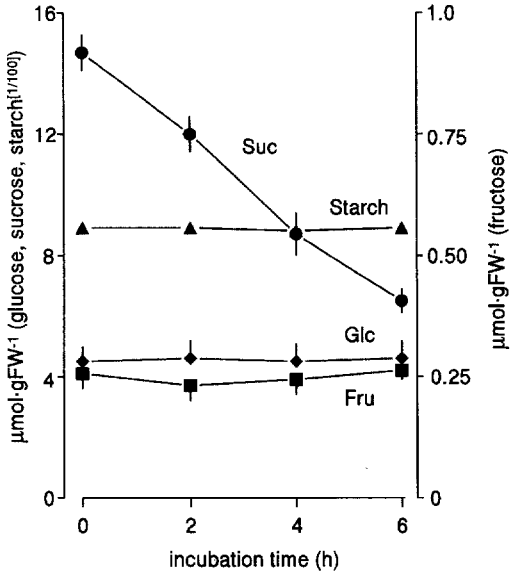

Fig. 1. Changes in sucrose (*circles*), starch (*triangles*), glucose (*diamond*) and fructose (*squares*) contents of developing potato tuber discs incubated with 25 mM Mes-KOH (pH 6.5) containing 300 mM mannitol. The results are given as means ± SE ($n = 4$ sets of discs)

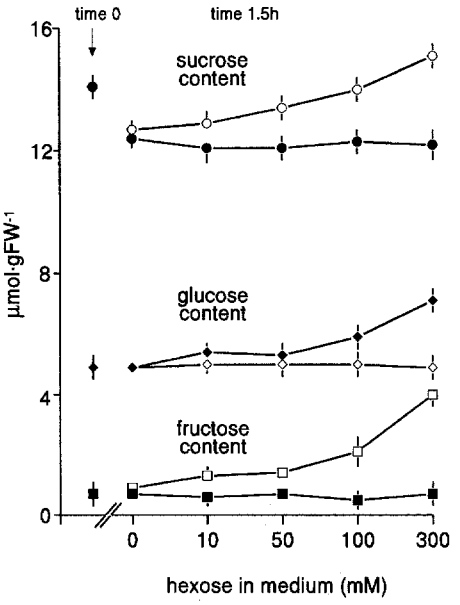

Fig. 2. Changes in sucrose, glucose and fructose contents (symbols as in Fig. 1) of developing potato tuber discs after 1.5 h incubation with 25 mM Mes-KOH (pH 6.5) containing 300 mM mannitol or various concentrations of fructose (*open symbols*) or glucose (*closed symbols*) adjusted to 325 mM total osmoticum with mannitol. Sugar content of discs at time 0 is also shown. The results are means ± SE ($n = 4$ sets of discs)

concentrations. However, at higher concentrations there was a progressive increase of fructose incorporation into sucrose and at 300 mM, the incorporation of fructose into sucrose was nearly fourfold higher than that into starch.

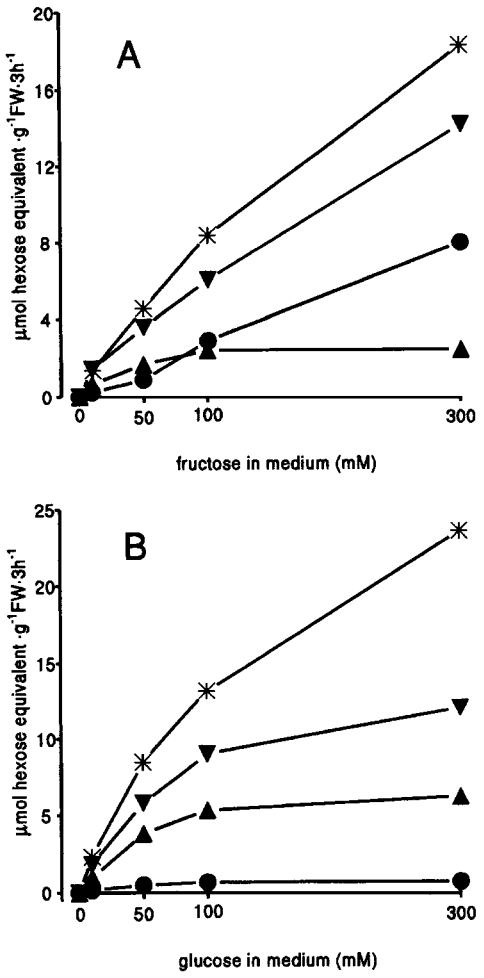

Fig. 3A, B. Net total uptake (*asterisks*) of (**A**) fructose and (**B**) glucose from solutions containing 25 mM Mes-KOH (pH 6.5) adjusted to 325 mM total osmoticum with mannitol (3 h incubation). Radiolabel was recovered in sucrose (*circles*) and starch (*triangles*). Net total label metabolised (label incorporated minus label present as the substrate supplied) is also shown (inverted *triangles*). The results are means of four replicates. Standard errors are smaller than the symbols

Metabolism of [2-^{13}C] glucose and [2-^{13}C] fructose

The pathways of sucrose biosynthesis in discs incubated with glucose or fructose were investigated by the use of specifically labelled ^{13}C-hexoses. Discs were incubated for 3 h with various concentrations of [2-^{13}C]fructose or [2-^{13}C]glucose and subsequently sucrose was extracted from the tissue, HPLC-purified and the ^{13}C-enrichment distribution assessed by ^{13}C-NMR spectroscopy. Table 1 shows the distribution of ^{13}C enrichment between and within the hexosyl moieties of sucrose when discs were incubated with [2-^{13}C]glucose or [2-^{13}C]fructose. With [2-^{13}C]glucose the ^{13}C enrichment was always equally distributed between the two hexosyl moieties. With [2-^{13}C]fructose the ^{13}C enrichment was always higher in the fructosyl moiety than in the glucosyl moiety. Very similar results were obtained when discs were incubated with ^{14}C-labelled hexoses (data not shown). The intramolecular distribution of ^{13}C enrichment in the hexosyl moieties of sucrose also differed substantially. With both substrates used, ^{13}C enrichment within the moieties was apparent only in the C_2 or C_5 position. With [2-^{13}C]glucose, approximately 20% of the total enrichment in both hexosyl moieties was recovered in the C_5 position. With [2-^{13}C]fructose, distribution of ^{13}C enrichment in the C_5 position was detected *only* in the glucosyl moiety of sucrose whilst the fructosyl moiety of sucrose appeared enriched uniquely in the C_2 position. These results are taken as an indication that the incorporation of exogenous fructose or glucose into sucrose in potato tuber discs occurs via independent pathways. In particular, the pattern of ^{13}C-enrichment distribution in sucrose in discs incubated with [2-^{13}C]fructose indicates that this precursor was directly incorporated into sucrose via sucrose synthase.

Estimation of metabolic fluxes in vivo

The rates expressed in Fig. 3 are estimations of fluxes based on the assumption that no dilution of incoming labelled substrates with internal unlabelled pools occurred prior to incorporation. However, realistic assessments of fluxes in vivo can only be derived by taking into account the dilution of incoming ^{14}C-hexoses by internal pools. Firstly, the isotopic dilution in the endogenous pools of hexoses will be considered and used to derive the

Table 1. Distribution of ^{13}C enrichment in hexosyl moieties of sucrose isolated from discs of developing potato tubers incubated for 3 h with [2-^{13}C]glucose or [2-^{13}C]fructose at the concentration stated. ^{13}C-Enrichment in individual carbon atoms was deduced from comparison of NMR spectra of enriched sucrose and spectra of natural abundance (in all cases enrichment was quantifiable only in C_2 and C_5 positions of hexosyl moieties). Data given as means ± SD of three replicates

Substrate		^{13}C enrichment in hexosyl moieties		
		Fructosyl/glucosyl enrichment ratio	Fructosyl C5/C2	Glucosyl C5/C2
[2-^{13}C]fructose	50 mM	1.6 ± 0.2	n.d.[a]	0.19 ± 0.01
	100 mM	1.5 ± 0.2	n.d.	0.18 ± 0.03
	300 mM	1.5 ± 0.1	n.d.	0.19 ± 0.02
[2-^{13}C]glucose	50 mM	0.9 ± 0.1	0.19 ± 0.01	0.18 ± 0.02
	100 mM	1.1 ± 0.1	0.21 ± 0.02	0.19 ± 0.03
	300 mM	1.0 ± 0.2	0.19 ± 0.03	0.20 ± 0.01

[a] n.d. = no enrichment detected in C_5

rate of hexose phosphorylation in vivo. Subsequently, the dilution of incoming ^{14}C-hexoses by internal unlabelled hexose-monophosphates and uridine 5'-diphosphoglucose (UDPGlc) pools will be estimated and used to derive the rates of glycolysis, starch and sucrose synthesis in vivo. The concentration of radioactivity in the various internal pools has been expressed as "isotopic dilution factor", i.e. the ^{14}C specific radioactivity in the pool relative to that of the radiolabelled substrate used.

Dilution of incoming [U-^{14}C]fructose. Incoming [U-^{14}C]fructose has been assumed to rapidly equilibrate with the internal fructose pool. In developing tubers, fructose is produced during sucrose degradation catalysed mainly, if not entirely, via sucrose synthase (Morrell and ap Rees 1986). The enzyme is located exclusively in the cytosolic compartment of plant cells (Keller at al. 1988). Developing tubers also characteristically contain low amounts of fructose (Davies and Oparka 1985) and this has been attributed to the presence of high fructokinase activity (Gardner et al. 1992; Renz et al. 1993). Thus, it appears reasonable to assume that the fructose pool is located in the cytosolic compartment of the tuber cell where it undergoes a very rapid turnover (as also suggested by Geigenberger and Stitt 1993). Hence, it is proposed that any incoming [U-^{14}C]fructose would rapidly equilibrate with the internal fructose pool and that the ^{14}C specific radioactivity of tissue fructose rather than that of incoming [U-^{14}C]fructose should be used for quantitative assessments of its metabolism in vivo. Table 2 shows the ^{14}C specific radioactivity of tissue fructose relative to that of medium fructose (isotopic dilution factors) after 1.5 h incubation of tuber discs with [U-^{14}C]fructose and various concentrations of unlabelled fructose.

Dilution of incoming [U-^{14}C]glucose. When the rates of glucose metabolisation in vivo were calculated using the isotopic dilution factors in tissue glucose (i.e. assuming equilibration of incoming [U-^{14}C]glucose with internal glucose), the values ranged between 16.8 and 159 $\mu mol \cdot g FW^{-1} \cdot h^{-1}$ (not shown). Even the lower estimate was so much in excess of the maximum catalytic activity of glucokinase in the tubers (Table 3) that it was concluded that little or no equilibration between incoming [U-^{14}C]glucose and the internal glucose could take place under the conditions used. The possibility of glucose compartmentalisation in developing tubers has been previously suggested (Hardy and Norton 1968). Figure 4 shows the progression of ^{14}C incorporation in the glucose-6-phosphate (Glc6P) pool when the tuber discs were incubated with 50 mM glucose containing [U-^{14}C]glucose. The steady-state labelling of the pool was established

Table 2. Total fructose content and ^{14}C isotopic dilution in endogenous fructose in developing potato tuber discs incubated for 1.5 h with 25 mM Mes-KOH (pH 6.5), [U-^{14}C]fructose (37 $kBq \cdot mL^{-1}$), and various concentrations of unlabelled fructose adjusted to 325 mM final osmoticum with mannitol. The ^{14}C specific radioactivity (s.r.) in fructose is expressed as "isotopic dilution factor" (s.r. endogenous fructose/s.r. medium fructose). Data given as average ± SD of four determinations

Concentration of unlabelled fructose (mM)	Tissue fructose content ($\mu mol \cdot g FW^{-1}$)	Isotopic dilution factor in tissue fructose (s.r. tissue/s.r. medium)
0	0.27 ± 0.09	0.015
10	0.48 ± 0.06	0.19
50	0.90 ± 0.10	0.46
100	1.41 ± 0.15	0.65
300	3.33 ± 0.34	0.81

Table 3. Maximum catalytic activities of sucrose synthase and hexose-kinases in developing potato tubers ($n = 4$)

Enzyme	Activity ($\mu mol \cdot g FW^{-1} \cdot h^{-1}$)
Sucrose synthase	75.5 ± 10.1
Glucokinase	4.3 ± 0.5
Fructokinase	60.1 ± 5.5

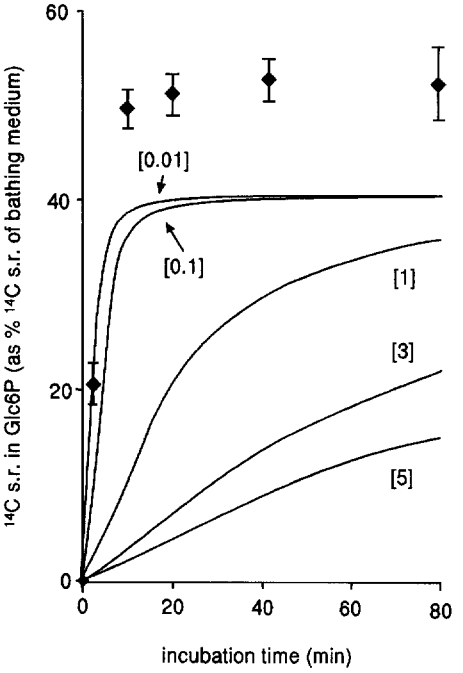

Fig. 4. Timecourse of labelling of intracellular Glc6P (*diamonds*; means ± SD) in developing potato tuber discs incubated with Mes-KOH (25 mM, pH 6.5) containing [U-^{14}C]glucose and 50 mM glucose. Theoretical curves (*solid lines*) were computed from the model given in the text by varying the endogenous glucose pool size (shown in brackets as $\mu mol \cdot g FW^{-1}$). The experimental points were obtained in the following way. Discs of developing tubers were incubated with Mes-KOH (25 mM, pH 6.5) containing [U-^{14}C]glucose (54 kBq), 50 mM glucose and 250 mM mannitol. At various intervals, discs were removed from the incubation medium, quickly blotted on filter paper and metabolism quenched by immersion in 80% (v/v) boiling aq. ethanol. The ^{14}C specific activity in Glc6P was obtained following purification of fractions containing hexose-monophosphates by HPLC and quantification of Glc6P in the fraction by spectrophotometric assay. Data are expressed as ^{14}C specific radioactivity (s.r.) in tissue Glc6P as a percentage of initial ^{14}C s.r. of the medium of incubation

very rapidly, again suggesting that incoming [U-^{14}C]glucose was phosphorylated without prior equilibration with a large internal pool. In order to estimate the size of the internal pool which diluted incoming [U-^{14}C]glucose in the experiment, the system was modelled with the use of the software SB-ModelMaker (Zeton Ltd, Nottingham UK). The model consisted of three pools (external and internal glucose, and phosphorylated intermediates) connected in series. A fourth pool, sucrose, was connected to that of phosphorylated intermediates. The only variable used was the size of the internal glucose pool whilst all the other parameters were assumed to be constant. The following parameters, derived from the labelling experiment, were used: 1) external glucose pool = 100 µmol containing 74 kBq ^{14}C glucose; 2) internal sucrose pool = 15 µmol; 3) internal hexose-monophosphates/UDPGlc pool = 0.4 µmol; 4) net rate of glucose uptake from the medium = 2.61 µmol · g FW^{-1} · h^{-1}; 5) rate of glucose phosphorylation, derived from the total label metabolised, = 1.91 µmol · g FW^{-1} · h^{-1}; 6) rate of hexose-monophosphates/UDPGlc production from sucrose degradation = 3.06 µmol · g FW^{-1} · h^{-1}, derived from the net glucose degradation plus the unidirectional rate of sucrose synthesis. Sucrose degradation was assumed to occur solely via sucrose synthase and all the fructose produced was assumed to be phosphorylated since no fructose accumulated during the experiment. For simplicity it was assumed that there was instantaneous isotopic equilibration between hexose-monophosphates (Glc6P, fructose-6-phosphate, glucose-1-phosphate) and UDPGlc and for this reason these metabolites were pooled together. When the model was run, the expected relationship between the time required for steady-state labelling of the hexose-monophosphates/UDPGlc pool and the size of the internal glucose pool diluting incoming [U-^{14}C]glucose was observed (Fig. 4). For all inputs of internal glucose concentrations the label dilution in the hexose-monophosphates/UDPGlc pool predicted by the model at the steady-state was somewhat higher than that observed experimentally (for Glc6P). When the input was 5.1 µmol · g FW^{-1}, i.e. equal to the actual glucose content of the tissue, the time required for steady-state labelling of the intermediates exceeded 750 min (not shown). On the other hand, when the input was only 10 nmol · g FW^{-1} there was a very close correlation between the experimental and theoretical observations in the time required for steady-state labelling of the pools. These results suggest that when tuber discs were incubated with [U-^{14}C]glucose, the incoming sugar was incorporated without any significant dilution. For this reason the rates of glucose metabolism in tuber discs in vivo will subsequently be calculated using the ^{14}C specific radioactivity of glucose in the medium.

Rates of hexose phosphorylation in vivo. Figure 5 shows the effect of external hexose concentration on the rate of glucose or fructose phosphorylation in vivo in tuber discs. As no dilution of incoming [U-^{14}C]glucose with internal glucose was assumed, the rates of glucose phosphorylation are identical to the net rates of [U-^{14}C]glucose metabolisation illustrated in Fig. 3B. On the other hand, incoming fructose was assumed to equilibrate with endogenous pools and the rates of fructose phosphorylation were obtained by multiplying the isotopic dilution factors given in Table 2 by the net rates of [U-^{14}C]fructose metabolisation shown in Fig. 3A. However, given the evidence from ^{13}C-NMR experiments that fructose was directly incorporated into sucrose via sucrose synthase the proportion of label which was recovered in the fructosyl moiety of sucrose (see Table 2) was excluded from the fraction representing [U-^{14}C]fructose metabolisation. For experiments with 10 mM fructose, where ^{13}C-NMR data were not available, the label recovered in the fructosyl moiety of sucrose was quantified by hydrolysing labelled sucrose isolated from discs incubated for 3 h with [U-^{14}C]fructose (which yielded a fructosyl/glucosyl labelling ratio of 1.7:1). In the absence of exogenous sugars, the apparent rate of fructose phosphorylation in vivo was 1.5 µmol · g FW^{-1} · h^{-1}, similar to the net rate of sucrose breakdown in the tissue. The apparent rates of fructose and glucose phosphorylation in vivo increased with the external concentrations of hexoses. However, the apparent maximal rates of phosphorylation in vivo were insufficient for complete metabolism of incoming hexoses (compare Fig. 5 with Fig. 3) and this would explain the accumulation of hexoses observed under these conditions (see Fig. 2). The apparent maximal rate of glucose phosphorylation in vivo approached the V_{max} of glucokinase in the tissue whilst the rate of fructose phosphorylation was always substantially lower than the maximum catalytic activtiy of fructokinase (Table 3).

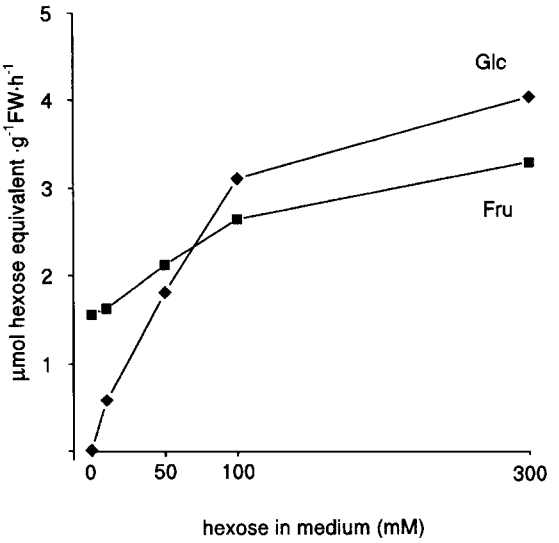

Fig. 5. Estimates for rates of glucose (*diamonds*) or fructose (*squares*) phosphorylation in vivo in potato tuber discs incubated with various concentrations of glucose or fructose, respectively. The rate of fructose phosphorylation was deduced from the net rate of total label metabolised shown in Fig. 3A, re-calculated using the isotopic dilution factors of endogenous fructose (see Table 2). However, as fructose incorporation into sucrose occurred via sucrose synthase, label recovered in the hexosyl moiety of sucrose was omitted from the net ^{14}C metabolised. The rate of glucose phosphorylation is identical to that shown in Fig. 3B as "net total label metabolised", since no dilution of incoming label was assumed

Dilution of label in the hexose-monophosphate/UDPGlc pools. This has been calculated using the following parameters. The rates shown in Fig. 5 were used to deduce the rates of labelled hexose-monophosphate generation from incoming [U-^{14}C]glucose or [U-^{14}C]fructose at the various exogenous sugar concentrations. The ^{14}C specific radioactivity of medium glucose or of tissue fructose was used to calculate the ^{14}C specific activity of labelled hexose phosphate (hexose-P) produced following the supply of [U-^{14}C]glucose or [U-^{14}C]fructose, respectively. The rate of fructose phosphorylation in experiments without exogenous fructose was assumed to be constant at 1.5 $\mu mol \cdot g FW^{-1} \cdot h^{-1}$, which was the "basal" rate of fructose phosphorylation in absence of exogenous fructose (see Fig. 5). This assumption was also justified by the finding that fructose content did not change when discs were incubated with glucose. Unlabelled hexose-monophosphates/UDPGlc were assumed to be generated solely from sucrose breakdown which was assumed constant at a rate of 1.5 $\mu mol \cdot g FW^{-1} \cdot h^{-1}$. As previously, it was assumed a complete equilibration between ^{14}C-hexose-P generated and the endogenous hexose-monophosphate pool and also the rapid isotopic equilibration between hexose-monophosphates and UDPGlc (combined pool size kept constant at 0.4 μmol). Table 4 shows the estimated steady-state isotopic dilution factors of the tissue hexose-monophosphate/UDPGlc pool following incubation of the discs with [U-^{14}C]glucose or [U-^{14}C]fructose and various concentrations of unlabelled hexoses. The more substantial dilution of incoming label with [U-^{14}C]fructose is expected given its equilibration with the internal fructose pool prior to phosphorylation. In Table 4 are also given the isotopic dilution factors with [U-^{14}C]fructose calculated by using a different approach. In this case, the fructosyl/glucosyl enrichment ratio in sucrose (see Table 2) was assumed to reflect the ^{14}C specific activity of UDPGlc and fructose, the substrates of sucrose synthase. Therefore, the isotopic dilution factor of the UDPGlc pool could be derived from that, known, of fructose (see Table 1). The results obtained with these independent methods were remarkably similar, providing an important validation for the assumptions used for the calculations.

Table 4. Estimated ^{14}C isotopic dilution in hexose-P/UDPGlc pools following the uptake and metabolism of [U-^{14}C]glucose or [U-^{14}C]fructose by developing potato tuber discs. In columns 1,2 (glucose and fructose^{I}) are shown the isotopic dilution factors (s.r. hexose-P-UDPGlc/s.r. medium) calculated assuming a constant rate (1.53 $\mu mol \cdot g FW^{-1} \cdot h^{-1}$) of sucrose breakdown generating unlabelled hexose-P/UDPGlc. The rate of labelled hexose-P production was calculated from the estimated rates of hexose phosphorylation (see Fig. 5) taking into account dilution of incoming isotope in the endogenous hexose pool. In column 3 (fructose^{II}) are shown isotopic dilution factors estimated assuming that the labelling of the glucosyl moiety of sucrose relative to that of the fructosyl moiety (when labelled fructose is supplied) represents the dilution of label in the tissue hexose-P/UDPGlc pool relative to that (known) of endogenous fructose (Table 2). The calculations assume instantaneous isotopic equilibration between hexose-P and UDPGlc

Carrier concn. (mM)	Isotopic dilution factor in hexose-P/UDPGlc pool following application of labelled substrates:		
	[U-^{14}C]glucose	[U-^{14}C]fructose^{I}	[U-^{14}C]fructose^{II}
0	0.008	0.009	n.d.[a]
10	0.17	0.08	0.08
50	0.41	0.27	0.29
100	0.52	0.39	0.43
300	0.59	0.58	0.54

[a] n.d. = not determined

Rates of starch synthesis, sucrose synthesis and glycolysis in vivo. Figure 6 shows the effects of glucose or fructose on the main metabolic fluxes in vivo in discs as determined by multiplying the rates shown in Fig. 3 by the isotopic dilution factors in the hexose-P/UDPGlc pools given in Table 4. The net glycolytic rate was estimated as in Viola and Davies (1994) from the incorporation of radioactivity in CO_2, charged fractions and non-starch insoluble material. Unidirectional sucrose synthesis was estimated assuming that in the absence or presence of exogenous fructose it occurred via sucrose-phosphate synthase or sucrose synthase, respectively. When discs were supplied with undiluted [U-^{14}C]glucose or [U-^{14}C]fructose, the extremely low concentration of hexoses in the medium enabled the tracing of metabolic fluxes in the tissue without significant disturbance. These "basal metabolic fluxes" were very similar whether estimated from experiments with [U-^{14}C]glucose or with[U-^{14}C]fructose. This finding is very important because of the different parameters used for calculation of data. For example, [U-^{14}C]fructose but not [U-^{14}C]glucose was assumed to equilibrate with the internal hexose pool. The basal rate of glucose or fructose phosphorylation also differed quite substantially. The apparent unidirectional rate of sucrose biosynthesis increased from 0.04 to 0.05 μmol hexose $\cdot g FW^{-1} \cdot h^{-1}$ in the absence of exogenous hexoses to 3.2 μmol hexose $\cdot g FW^{-1} \cdot h^{-1}$ in the presence of 300 mM fructose in the incubation medium. This value is very close to the net rate of sucrose degradation in discs incubated in buffer alone (see Fig. 1) and would explain the finding that the decline of sucrose in tuber discs could be prevented by the inclusion of 300 mM fructose to the incubation media (see Fig. 2). This also implies that exogenous fructose induces substantial cycling of sucrose synthesis and breakdown in the tissue. With glucose, the unidirectional rate of sucrose synthesis apparently saturated at 0.4 $\mu mol \cdot$ hexose $\cdot g FW^{-1} \cdot h^{-1}$, a value which is too low to affect the decline in sucrose content in the tissue. Such a close similarity between the estimated rates of metabolism in vivo and the experimental observations represent a further validation of the criteria used for processing the data. Although the rates of starch biosynthesis and glycolysis appeared little affected by exogenous fructose, the relative *proportion* of intermediates which were partitioned to these pathways decreased from 65.1% to 40.6% and from 34.3 to 26.7%, respectively, between 0 and 300 mM exogenous fructose. Under the same conditions the partitioning of intermediates to sucrose increased from 0.6% (basal rate) to to 32.6%. Indeed, nearly 77% of the additional 1.5 $\mu mol \cdot g FW^{-1} \cdot h^{-1}$ hexose-P generated when discs were incubated with 300 mM exogenous fructose (relatively to the basal rate) were apparently incorporated into

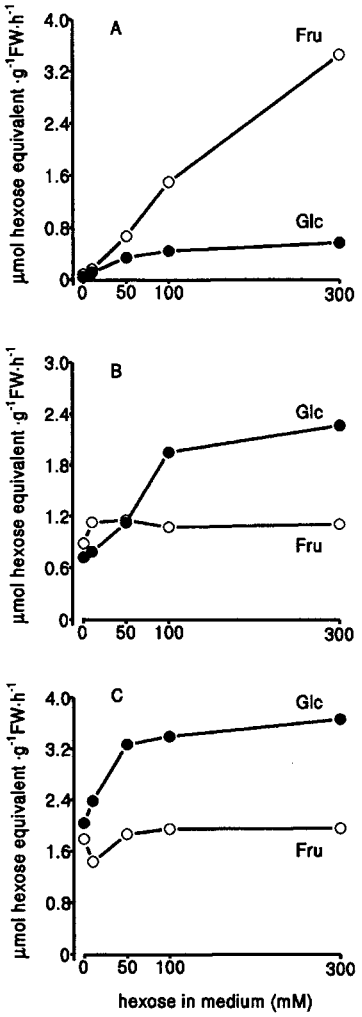

Fig. 6A–C. Estimation of metabolic fluxes in vivo in developing potato tuber discs incubated with various concentrations of glucose (*closed circles*) or fructose (*open circles*). Rates were obtained from data shown in Fig. 3, re-processed to take into account (i) isotopic dilution of incoming hexoses with the endogenous pools (for fructose only; see Fig. 5) and (ii) the isotopic dilution caused by equilibration between products of hexose phosphorylation (labelled) and those of unidirectional sucrose degradation (unlabelled). The rate of sucrose breakdown was assumed constant at 1.53 μmol hexose · $g FW^{-1} \cdot h^{-1}$. For estimation of sucrose synthesis (**A**) it was assumed that biosynthesis occurred through sucrose synthase or sucrose-P synthase for experiments with fructose and glucose, respectively. Estimates of glycolytic rates (**B**) were obtained from label recovered in the charged fraction (anions + cations), CO_2 and the residue of the fraction insoluble in ethanol following starch digestion. Note that rates are overestimated since, with the techniques used, glycolytic intermediates (such as phosphate esters etc.) were not separated from organic acids. Estimates of rates of starch synthesis are also shown (**C**)

sucrose. In the presence of glucose all the metabolic fluxes apparently increased and the relative partitioning of intermediates did not appear to be substantially affected. The saturation of all the fluxes considered, which became apparent above 100 mM exogenous glucose concentration, was probably attributable to the saturation of glucose phosphorylation in vivo which was also observed at similar concentrations (see Fig. 5).

Discussion

Exogenous fructose and glucose are converted into sucrose via different mechanisms. Analyses of ^{14}C incorporation in discs incubated with [U-^{14}C]glucose or [U-^{14}C]fructose showed that incoming glucose and fructose were metabolised differently. In particular, the vast majority of incoming glucose was always incorporated into starch whilst fructose was preferentially incorporated into sucrose at high exogenous fructose concentrations. Evidence for the existence of different mechanisms of hexose utilisation by tuber cells was provided by analysing the distribution of ^{13}C enrichment in sucrose after supplying the discs with [2-^{13}C]glucose or [2-^{13}C]fructose. With [2-^{13}C]glucose the proportion of label redistributed to the C_5 position was broadly similar in the two moieties. The appearance of ^{13}C enrichment in the C_5 position of hexosyl moieties of sucrose following the supply of [2-^{13}C]hexose is, per se, an indication that the direct precursors used for sucrose synthesis undergo isotopic exchange with the pool of hexose-P. This is because isotopic exchange between hexose-P and triose-P, accompanied by rapid isomerisation of triose-P results in isotopic exchange between the top and bottom carbon positions in the hexose-P molecules (Keeling et al. 1988; Viola et al. 1991). The symmetric intramolecular distribution of ^{13}C enrichment in the fructosyl and glucosyl moieties of sucrose observed following the supply of [2-^{13}C]glucose indicates that the direct precursors of sucrose synthesis were UDPGlc and fructose-6-phosphate and that these pools underwent rapid isotopic equilibration. Such an equilibration is expected since the two pools are connected via a series of highly active enzymes catalysing reversible reactions. Thus, the results obtained with [2-^{13}C]glucose are consistent with the hypothesis that this precursor was incorporated into sucrose via sucrose-P synthase. On the other hand, when discs were supplied with [2-^{13}C]fructose much more enrichment was present in the fructosyl moiety than in the glucosyl moiety of sucrose. Moreover, redistribution of ^{13}C enrichment to the C_5 position was evident only in the glucosyl moiety of sucrose. Such unsymmetrical distribution of ^{13}C enrichment in the hexosyl moieties of sucrose can be better explained if one assumes that in discs incubated with [2-^{13}C]fructose sucrose synthesis occurred via sucrose synthase. Firstly, the lack of any ^{13}C enrichment in the C_5 position of the fructosyl moiety of sucrose can be taken as indication of direct incorporation of incoming [2-^{13}C]fructose. Secondly, the presence of enrichment in the C_5 position of the glucosyl moiety and the lower degree of enrichment in the glucosyl moiety compared with the fructosyl moiety of sucrose would be expected to occur as a result of [2-^{13}C]fructose phosphorylation and subsequent convertion into UDPGlc prior to incorporation. Cumulatively, the results obtained with ^{14}C- and ^{13}C-hexoses suggest that: (i) sucrose synthesis in tuber discs is stimulated by fructose much more substantially than by glucose; (ii) the pathways of sucrose biosynthesis in the presence of exogenous fructose or glucose appear different. An hypothesis explaining these perplexing results can be put forward. The unidirectional rate of sucrose synthesis estimated with either [U-^{14}C]glucose or

[U-^{14}C]fructose without unlabelled carrier hexoses was similar (0.04 and 0.05 $\mu mol \cdot g FW^{-1} \cdot h^{-1}$) and insignificant compared with the net rate of sucrose breakdown (3 $\mu mol \cdot hexose \cdot g FW^{-1} \cdot h^{-1}$). Thus, it can be assumed that under these conditions very little cycling of sucrose synthesis and breakdown occurs in the tuber tissue and sucrose breakdown occurs as an essentially irreversible process. When exogenous [2-^{13}C]glucose was supplied to the discs, the pattern of ^{13}C enrichment distribution in sucrose indicated that the enhanced flux into sucrose occurred via sucrose-P synthase. Similar results have been obtained for a number of plant tissues supplied with specifically labelled glucose (Keeling et al. 1988; Hatzfeld and Stitt 1990; Viola et al. 1991). The conversion of glucose into sucrose in the discs was moderately stimulated by exogenous glucose and was proportional to the increased conversion into starch. This again would be expected if sucrose synthesis occurred via sucrose-P synthase which, as in starch synthesis, derives substrates from the cytosolic pool of phosphorylated intermediates in potato tubers (Hatzfeld and Stitt 1990; Viola et al. 1991). On the other hand, when the discs were incubated with fructose the specific increase in the unidirectional rate of sucrose synthesis and the pattern of ^{13}C-enrichment distribution in sucrose strongly indicated that the flux into sucrose was mediated by sucrose synthase. An explanation for this effect can be found in the accumulation of fructose in the tissue, which is expected to affect the equilibrium of the reaction catalysed by sucrose synthase in vivo (Viola 1996) given that the enzyme catalyses a readily reversible reaction in vitro (Cardini et al. 1954). The effect on the equilibrium of the reaction appears proportional to the accumulation of fructose and induces the onset of a cycle of sucrose synthesis and breakdown in the tissue.

On the reversibility of sucrose synthase in vivo. These findings can explain the results presented by Geigenberger and Stitt (1993) which implied that sucrose synthase catalysed a reversible reaction in vivo in potato tubers. The authors deduced this hypothesis from experiments in which tuber tissue was incubated with [U-^{14}C]glucose and [U-^{14}C]fructose together with carrier sugar, or [U-^{14}C]glucose together with cold glucose and fructose. The results presented here and in a previous report (Viola 1996) suggest that these experimental conditions may lead to overestimation of the rate of sucrose synthesis in the tissue. Firstly, labelled sucrose can be generated in the bathing medium of tuber tissue incubated with ^{14}C-hexoses (particularly [U-^{14}C]fructose) and then taken up by the tissue (Viola 1996). Secondly, the inclusion of cold fructose in the incubation medium stimulates the incorporation of [U-^{14}C]fructose and UDPGlc into sucrose in the medium itself (Viola 1996). Thirdly, the rate of unidirectional sucrose synthesis in the tuber tissue was clearly stimulated even in the presence of low exogenous fructose concentrations (Fig. 3A). In the present study, precautions were taken to prevent the accumulation of labelled sucrose in the medium when the discs were supplied with ^{14}C-hexoses. It was possible to demonstrate that sucrose breakdown in the tuber cells occurred almost irreversibly in the absence of exogenous hexoses whilst, in the presence of fructose, cycling of sucrose synthesis and breakdown via sucrose synthase was induced. Thus, the main conclusion from the data presented here is that one should be careful when concluding that sucrose synthase catalyses a reversible reaction in vivo when the system investigated has been supplied with fructose, even in low concentrations. Mass-action determinations of metabolites in intact potato tubers also suggested that sucrose synthase catalysed a readily reversible reaction in vivo (Geigenberger and Stitt 1993). However, it is arguable that compartmentation of substrates can complicate the interpretation of metabolite measurements. Indeed, on the basis of estimations of fluxes in intact growing tubers, the same authors concluded that sucrose synthase catalysed a reaction which was displaced from equilibrium in favour of sucrose breakdown (Geigenberger and Stitt 1993). The results presented here show that the latter conclusion can be drawn also for excised tuber discs, provided that no exogenous sugars are supplied.

The phosphorylation of fructose (but not of glucose) is regulated in vivo. Incubation of tuber discs with fructose induced an accumulation of the hexose in the tissue at all external concentrations used. This finding was unexpected given the high fructokinase activity detected in the tissue (Table 3) and generally associated with developing tubers (Renz et al. 1993; Ross et al. 1994). For this reason the actual rates of hexose phosphorylation in vivo in the tissue were investigated. In the absence of exogenous sugars the apparent rate of fructose phosphorylation in vivo (1.5 $\mu mol \cdot g FW^{-1} \cdot h^{-1}$) approached that of sucrose breakdown, explaining the lack of any fructose accumulation under these conditions. It would appear that fructokinase acted as an efficient fructose-scavenger under these conditions, enabling sucrose breakdown via sucrose synthase to proceed as an essentially irreversible process. When exogenous fructose was included in the incubation medium, the apparent rate of fructose phosphorylation increased only moderately and saturated at a value which represented only 5.5% of the maximum catalytic activity of fructokinase in the tissue. It should be enphasised that the rates of fructose phosphorylation were calculated assuming complete dilution of incoming [U-^{14}C]fructose within the pool of internal fructose. As such, the estimates of the rates of fructose phosphorylation in vivo should be considered *minimal* estimates given the possibility of some compartmentalisation of fructose in the tuber cell. Significant product inhibition of potato tuber fructokinase activity has been reported for two out of three isoforms studied (Gardner et al. 1992; Renz and Stitt 1993). Thus one could argue that when discs were incubated with fructose, fructokinase activity in vivo could have been affected by the incoming fructose. However, this hypothesis does not fully explain the results obtained. For example, the highest estimated input of fructose in the system (7.5 $\mu mol \cdot g FW^{-1} \cdot h^{-1}$), derived from the combined rates of fructose uptake at 300 mM external concentration and of fructose production from sucrose breakdown was well below the maximal fructose phosphorylative potential in the tissue (60.1 $\mu mol \cdot g FW^{-1} \cdot h^{-1}$). Moreover, fructose accumulation in the discs occurred at all exogenous

concentrations used, even at 10 mM when the net rate of uptake was 0.4 $\mu mol \cdot g FW^{-1} \cdot h^{-1}$, only a fraction of the total estimated fructose input of 1.9 $\mu mol \cdot g FW^{-1} \cdot h^{-1}$. Nevertheless, the small increase in fructose content observed in these conditions was sufficient to increase the unidirectional rate of sucrose synthesis from 0.03 $\mu mol \cdot g FW^{-1} \cdot h^{-1}$ (in the absence of exogenous hexoses) to 0.18 $\mu mol \cdot g FW^{-1} \cdot h^{-1}$. For a comparison, the unidirectional sucrose synthesis with 10 mM exogenous glucose was 0.06 $\mu mol \cdot g FW^{-1} \cdot h^{-1}$. These findings suggest that mechanisms other than incoming fructose were involved in the regulation of fructokinase activity in vivo in the tissue. These mechanisms could play a very important role in the control of sucrose mobilisation in the tuber tissue. For example, the net rate of sucrose breakdown observed here was similar to that reported for intact developing tubers (Morrell and ap Rees 1986). Thus, it is possible to speculate that even small increases in the rates of sucrose unloading in the tuber may lead to (transient) accumulation of fructose in the tissue which in turn would activate sucrose recycling via sucrose synthase. The implication and relevance of such a mechanism in the modulation of sucrose mobilisation in storage organs have been discussed (Geigenberger and Stitt 1993). Unlike fructose, the rates of glucose phosphorylation in vivo have been deduced assuming no dilution of incoming [U-^{14}C]glucose. Indirect evidence in support of this hypothesis was provided by establishing that steady-state labelling radioactivity in the direct product of glucose phosphorylation (Glc6P) was very rapidly established. Mathematical processing of the data suggested that equilibrating of the internal glucose pool with incoming [U-^{14}C]glucose was insignificant (Fig. 4). More crucially, the maximal rate of glucose phosphorylation in vivo, calculated assuming no dilution of incoming [U-^{14}C]glucose by internal pools, coincided well with the maximum catalytic activity of glucokinase in the tissue. Hexokinases with very high affinity for glucose have been detected in potato tubers (Renz and Stitt 1993). The results indicate that unlike fructokinase, glucokinase activity is not regulated in vivo in developing tuber cells. It also indicates that in the same tissue the internal glucose pool is well segregated from the site of enzyme activity. This confirms speculations that the high glucose content of developing tubers derives from starch turnover or is a residual of acid invertase activity in the elongating stolon (Ross et al. 1994), implying compartmentalisation of the sugar in the amyloplast or vacuole, respectively.

The effects of exogenous hexoses on metabolic fluxes in vivo. The quantitative estimation of metabolic fluxes in vivo required assessments of the dilution of incoming ^{14}C hexose label by internal unlabelled pools. For these calculations the production of labelled hexose-P was derived from the rates of uptake and phosphorylation of [U-^{14}C]glucose or [U-^{14}C]fructose at the various concentration of unlabelled sugar used. Sucrose breakdown was assumed to be the sole sorce of unlabelled intermediates in the tissue. This assumption was justified, in discs incubated with glucose, by the finding that the net rate of sucrose breakdown was clearly not affected in these conditions. On the other hand, a decline in the net rate of sucrose breakdown was observed in discs incubated with fructose and it could be argued that this was due to the inhibition of sucrose synthase by fructose (Doehlert 1987; Dancer et al. 1990). However, it must be enphasised that the decline in the *net* rate of sucrose breakdown observed when discs were incubated with fructose was accompanied by a marked stimulation of the unidirectional rate of sucrose synthesis via sucrose synthase. It is also worth pointing out that when the unidirectional rate of sucrose synthesis in discs incubated with 300 mM fructose was calculated assuming no effect of fructose on unidirectional sucrose breakdown, the two rates balanced out. This was confirmed experimentally by the finding that the rapid decline of sucrose content in discs incubated without hexoses could be completely prevented when discs were incubated with 300 mM fructose. These results are entirely consistent with the hypothesis put forward that the equilibrium of the reaction catalysed by sucrose synthase becomes shifted in the direction of sucrose biosynthesis in discs incubated with fructose. This can also explain why the additional phosphorylated intermediates generated from incoming fructose were not partitioned to starch or glycolysis as was the case for those generated from incoming glucose (Fig. 5). The capacity of developing tuber tissue to convert exogenous glucose into starch much more efficiently than fructose has been repeatedly demonstrated (Oparka et al. 1990; Wright and Oparka 1990; Geigenberger and Stitt 1993). The substantial increase in the apparent rate of starch synthesis in tuber discs incubated with glucose is of particular interest. The maximal net rate of starch synthesis observed here in the absence of hexoses was 2 $\mu mol \cdot g FW^{-1} \cdot h^{-1}$, within the average rate of starch deposition in field-grown tubers (1.9–2.8 $\mu mol \cdot g FW^{-1} \cdot h^{-1}$; Morrell and ap Rees 1986). This indicates that excised discs continue to convert sucrose into starch at a rate which is representative of intact tubers. The incubation of excised discs with exogenous glucose increased the apparent rate of starch synthesis to 3.7 $\mu mol \cdot g FW^{-1} \cdot h^{-1}$. This suggests that availability of substrates may be a limiting factor for starch biosynthesis under normal growing conditions. Stark et al. (1992) have shown that ectopic overexpression of deregulated bacterial ADPGlc pyrophosphorylase in transgenic potato tubers does not lead to a proportional increase in starch content. The authors suggested that factors other than the step catalysed by ADPGlc pyrophosphorylase are likely to be involved in the control of starch deposition in potato tubers. The contribution of other enzymes directly involved in starch synthesis in the amyloplast (i.e. starch synthase) on the overall control of the flux is still unresolved. However, the data presented here suggest that the processing of sucrose through sucrose synthase and fructokinase may represent an important control point for starch synthesis in potato tubers.

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