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SUGAR METABOLISM IN DEVELOPING TUBERS OF *SOLANUM TUBEROSUM*

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Key Word Index—*Solanum tuberosum*; Solanaceae; potato; tuber; sugar content; enzymes; fructose-2,6-bisphosphate; UDP-glucose.

Abstract—Regulation of the sugar content of the developing tubers of three varieties (King Edward, Maris Bard, Pentland Javelin) of *Solanum tuberosum* was investigated. Sucrose, glucose, fructose, UDP-glucose and fructose-2,6-bisphosphate were measured during tuber development as were the maximum catalytic activities of acid invertase, alkaline invertase, sucrose synthase, α-glucan phosphorylase, hexokinase, phosphofructokinase and pyrophosphate: fructose 6-phosphate 1-phosphotransferase [PFK(PPi)]. Sucrose was the dominant sugar and there was less fructose than glucose; the amounts of all three per gram fresh weight fell during tuber development. The activity of hexokinase per gram fresh weight declined during development but those of the other enzymes listed did not alter significantly. No change in the amounts of fructose-2,6-bisphosphate or UDP-glucose per gram fresh weight were found. The above measurements suggest that much of the sucrose translocated to the developing tuber is metabolized via sucrose synthase to UDP-glucose that is converted to glucose 1-phosphate by UDP-glucose pyrophosphorylase using pyrophosphate generated by PFK (PPi).

INTRODUCTION

The aim of this work was to investigate the mechanisms responsible for the immediate metabolism of the sucrose that is delivered to the developing potato tuber. Particular attention was paid to steps that might influence sugar content. As the translocated sucrose is the starting point of the tuber's metabolism, the manner in which it is partitioned beteween respiration, biosynthesis and storage is central to the growth and metabolism of the tuber. Knowledge of the control of the sugar content is important in respect of both the economy and the economic use of the tuber [1].

Perhaps the most important initial question to be resolved is the relative roles of invertase (EC 3.2.1.26) and sucrose synthase (EC 2.4.1.13) in metabolizing the sucrose delivered to the tuber [2]. We investigated this question by determining the relationship between the maximum catalytic activities of enzymes likely to be involved in sugar metabolism, and the contents of sugar, UDP-glucose and fructose-2,6-bisphosphate (Fru-2,6-P_2) throughout the development of three varieties of tuber. UDP-glucose was measured because it would be formed directly from incoming sucrose if the latter were broken down by sucrose synthase [2]. Although sucrose synthase can function with ADP it does not do so effectively in the presence of UDP. Sucrose synthase is cytosolic as is UDP-glucose pyrophosphorylase, a major enzyme using UDP. Thus it is highly likely that there will be sufficient UDP in the cytosol to ensure that UDP-glucose is the product of sucrose synthase *in vivo*. This view is supported by the demonstration that the enzymes for ADP-glucose metabolism are confined to the plastid [2]. Fru-2,6-P_2 was measured because of its known role in regulating sucrose metabolism [3]. Apart from the ones mentioned above the enzymes measured were: hexokinase (EC 2.7.1.1), α-glucan phosphorylase (EC 2.4.1.1), phosphofructokinase [PFK (ATP); EC 2.7.1.11], and pyrophosphate: fructose 6-phosphate 1-phosphotransferase [PFK (PPi); EC 2.7.1.90]. Estimates of some of these enzymes have been published [4–6]. However, we have no developmental picture, and the published estimates have not been shown to reflect the maximum catalytic activities of the tubers. Such authentication is essential before comparisons may be made between tubers or enzymes. The need is particularly pressing for potato tubers because of the clear demonstration that extensive and differential inhibition of enzymes can occur during extraction and analysis [7].

RESULTS AND DISCUSSION

Carbohydrate content

We relate our measurements of substrates and enzymes to fresh weight, and have used tuber weight as an indicator of development. Figure 1 provides fair evidence of a linear relationship between fresh weight and age for each variety studied. Linear regressions were 0.91, 0.80 and 0.74 for King Edward, Maris Bard and Pentland Javelin, respectively. The corresponding rates of growth were 0.18, 0.16 and 0.12 g fresh weight per day.

The amount of starch per tuber increased linearly at least until the tubers reached 25 g. For plots of starch per tuber against tuber weight r was 0.99, 0.84 and 0.96 for King Edward, Maris Bard and Pentland Javelin, respectively. The average starch contents over the period of

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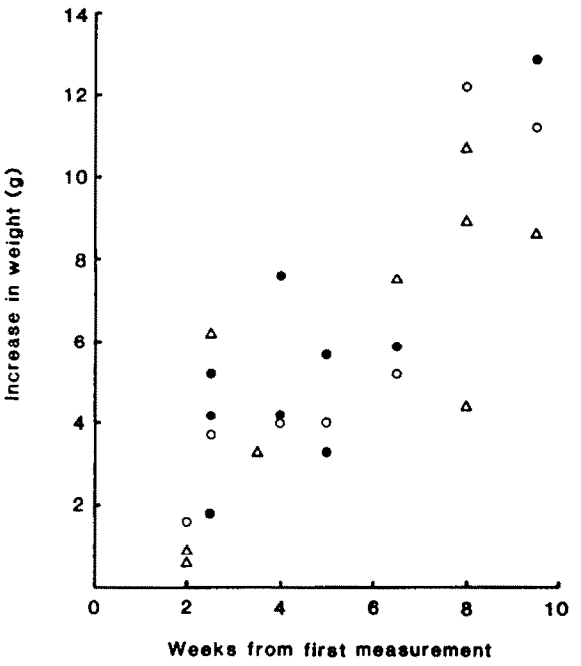

Fig. 1. Relationship between age and fresh weight of developing tubers of variety King Edward (○), Maris Bard (●) and Pentland Javelin (△). Tubers of initial fr. wt 0.5–1.5 g were tagged, harvested after varying times and weighed to give increase in tuber weight.

development shown in Fig. 1 were (means ± s.e.m. from 11 tubers of each variety): King Edward, 73 ± 5; Maris Bard, 53 ± 6; Pentland Javelin, 83 ± 6 mg starch per g fresh weight. From these values and the data in Fig. 1 we calculate the following rates of starch accumulation (nmol anhydrohexose/min per g fresh weight): King Edward, 47; Maris Bard, 32; Pentland Javelin, 47.

The methods that we used to measure sugar content (Fig. 2) have been shown to be reliable [1]. We draw the following conclusions from our measurements of sugars, and from comparable data published for other tubers [8, 9 and references therein]. First, our results in particular show that there may be very considerable variation in the sugar content of tubers of a given variety at the same stage of development. Thus analyses should be based on a sufficient number of samples to reflect this variation. Second, sucrose is the major sugar, and there is more glucose than fructose. Third, the sugar content of the tubers, unlike that of starch, does not increase throughout the period of development. In our experiments the amounts of all three sugars per g fresh weight declined as the tubers grew larger. Finally, although the above conclusions seem to be generally applicable, the precise relationship between the three sugars varies widely between different varieties. For example, we found that Pentland Javelin contained half as much sucrose and twice as much glucose as the other two varieties. Further, we found no convincing evidence that the relative amounts of sucrose and reducing sugar altered during development, nor did we find the very high ratios of glucose to fructose recently reported for Maris Piper tubers [9]. Collectively, the above strongly suggests that the sugar contents of the tubers are regulated during development.

Enzyme activities

We investigated the reliability of our assays as follows. For each enzyme we optimized the concentration of each component, and the pH, of the assay mixture, and we showed that activity was linearly related to time and amount of extract. For invertase we homogenized tissue at pH 8, centrifuged the homogenate at 32 000 *g* for 30 min, and then measured activity in the supernatant over the pH range 3.5–8.0. We found peaks of activity at pH 5.0 and 7.5, which we attribute to acid and alkaline invertase, respectively. There was no detectable acid invertase in the sediment obtained at 32 000 *g*. We also showed that no measurable hexokinase was discarded in the sediment formed when homogenates were centrifuged at 1000 *g*. The assay for hexokinase was optimized for fructose and for glucose; similar rates were obtained for the two sugars.

Loss of activity during preparation of the extracts was checked by either recovery or recombination experiments [1]. Each type of experiment was carried out with samples that were mixtures of material from all three varieties of tuber. Estimates of the recovery of pure enzymes from such samples were: α-glucan phosphorylase, 81%; PFK (ATP), 103%; PFK (PPi), 81%; hexokinase, 142%. In the recombination experiments we prepared three samples, one of potato, one of material from a club of *Arum maculatum* L., and one that was a mixture of the two tissues. The activity in the mixture is expressed as a percentage of the value predicted from measurements made on the separate components of the mixture. The results were: acid invertase, 96%; alkaline invertase, 111%; sucrose synthase, 90%; PFK (PPi), 110%; hexokinase, 97%. We argue that our estimates of enzyme activities reflect the maximum catalytic activities of the tubers.

We measured enzyme activities in tubers that weighed 1, 5, 10, 15 and 20 g, except that the last stage was omitted for hexokinase and α-glucan phosphorylase. Table 1 presents data for the first, third and final weights of tuber; values from the intermediate stages did not differ significantly from the preceding stage shown in Table 1. In nearly all instances enzyme activity increased linearly with tuber weight so that activity per g fresh weight did not alter during development. There were two exceptions. In all three varieties hexokinase activity did not increase as much as tuber weight so that activity per g fresh weight fell by as much as 57%. In Maris Bard the activity of sucrose synthase per g fresh weight fell once the tubers reached 15 g. Neither of these exceptions was related to any obvious change in sugar content. The enzyme activities showed some variation between the three varieties but the variation is not great and does not appear to be related to differences in sugar contents (Fig. 2). The lack of any discernible correlation between sugar content and the maximum catalytic activities of the enzymes lead us to suggest that coarse control by these enzymes is not primarily responsible for regulating the sugar content of the developing tuber.

Our results indicate how the tubers metabolize the sucrose delivered by the translocation stream. Such sucrose must supply the demands for the synthesis of structural and storage polysaccharide plus the demands of the respiratory pathways. The latter will include a significant need for carbon for the biosynthesis of amino acids and organic acids. Sucrose could be converted to structural polysaccharides directly via UDP-glucose formed by sucrose synthase. To meet the remainder of the above demands it is probable that the sucrose is converted to

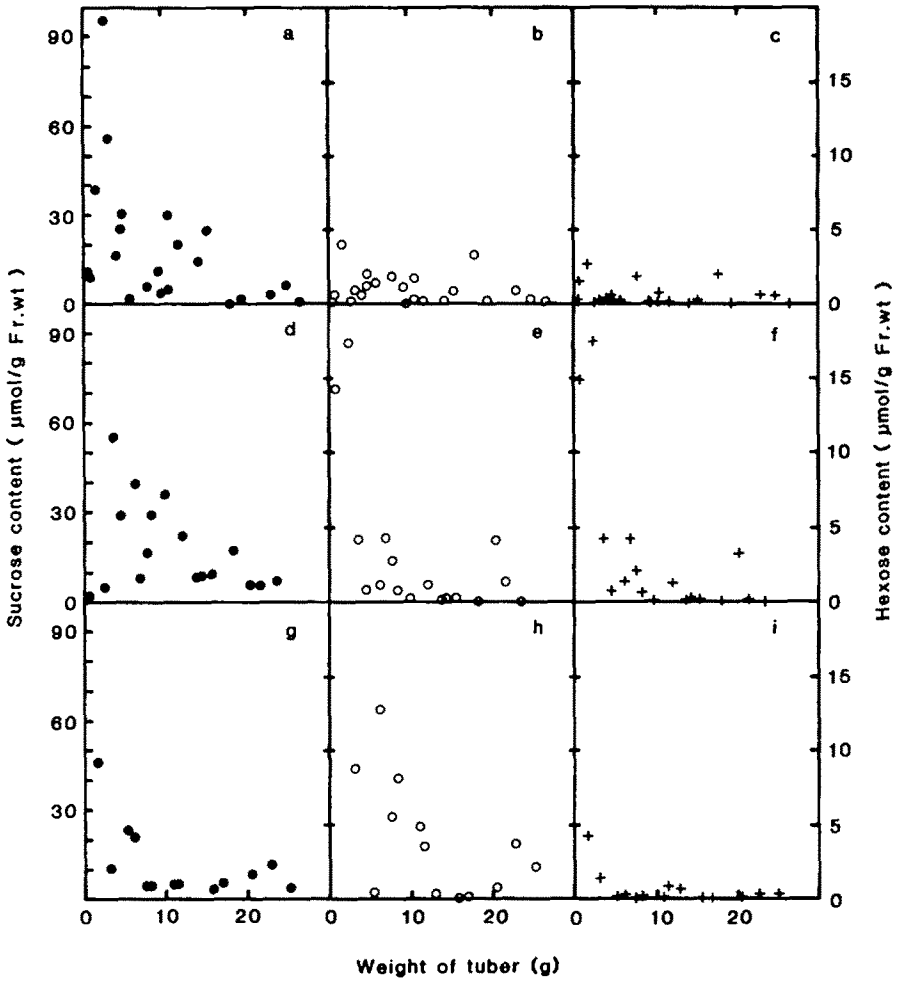

Fig. 2. Content of sucrose (●), glucose (○) and fructose (+) in developing tubers of variety King Edward (a, b, c), Maris bard (d, e, f) and Pentland Javelin (g, h, i). Each point represents measurements made on a single tuber.

hexose phosphate. This is likely to be true even for the conversion of sucrose to starch. The enzymes known to be capable of metabolizing sucrose and of synthesizing UDP-glucose appear to be confined to the cytosol, and those responsible for the synthesis of starch and its precursor ADP-glucose to the amyloplast [10]. We make minimal estimates of the rate at which sucrose is converted to hexose phosphate in the developing tubers by using our values for starch accumulation and Burton's [11] observation that young tubers consume oxygen at 50 ml per kg fresh weight/hr at 10°. Assuming a Q_{10} of 2, the latter corresponds to a consumption of hexose in respiration at 20°, the temperature at which the tubers were grown, of 6 nmol/min per g fresh weight. The distribution of label after feeding [^{14}C]sucrose [1] or [^{14}C]glucose [12] to tubers suggests that at least half of the hexose that enters the respiratory pathways is converted to organic acids, amino acids and protein. Thus hexose consumption in respiration is likely to have been at least 12 nmol hexose per g fresh weight/min. If this figure is added to the rates of starch accumulation then we argue that the following indicate the minimal rate at which sucrose is converted to hexose phosphate for the varieties King Edward, Maris Bard and Pentland Javelin, respectively, 59, 44 and 59 nmol hexose units per g fresh weight/min.

Neither of the invertases could be detected in sufficient activity to sustain the above rates of hexose phosphate formation in any of the varieties. In a rapidly growing tissue that is storing starch it is unlikely that much of the incoming sucrose will enter the vacuole where the acid invertase is located [13]. Thus any major contribution of invertase to the breakdown of the incoming sucrose is likely to be via the cytosolically located [10] alkaline invertase. The maximum catalytic activity of this enzyme (Table 1) could account for, at the most, 30% of the required flux from sucrose to hexose phosphate. Thus we suggest that invertases are not the major route from sucrose to hexose phosphates in developing potato tubers. A similar situation has been reported for the developing endosperm of maize [14].

In all three varieties the activity of sucrose synthase was well in excess of our estimates of the rates of sucrose breakdown. This, and the above arguments, strongly suggest a dominant role for sucrose synthase in sucrose breakdown. Such a suggestion has been made previously for potatoes [6] and for maize endosperm [14]. The central difficulty with this hypothesis is the conversion of the UDP-glucose, formed by sucrose synthase, to hexose phosphate.

In 1969 Turner [15] suggested that UDP-glucose,

Table 1. Maximum catalytic activities of enzymes of sugar metabolism during the development of tubers of three varieties of potato

Enzyme	Variety of tuber	Enzyme activity (nmol per min/g fr. wt) at the following stages*		
		1 g	10 g	20 g
Acid invertase	King Edward	11 ± 4	9 ± 2	11 ± 2
	Maris Bard	14 ± 6	3 ± 1	3 ± 0.4
	Pentland Javelin	2 ± 1	2 ± 0.3	3 ± 0.1
Alkaline invertase	King Edward	11 ± 4	8 ± 2	16 ± 1
	Maris Bard	14 ± 2	7 ± 1	7 ± 0.2
	Pentland Javelin	12 ± 4	7 ± 1	8 ± 0.2
Sucrose synthase	King Edward	160 ± 30	100 ± 20	100 ± 30
	Maris Bard	360 ± 80	210 ± 20	130 ± 10
	Pentland Javelin	340 ± 10	220 ± 30	220 ± 20
Hexokinase	King Edward	84 ± 14	56 ± 6	52 ± 4†
	Maris Bard	73 ± 2	60 ± 10	59 ± 8†
	Pentland Javelin	78 ± 10	43 ± 5	43 ± 5†
PFK (ATP)	King Edward	57 ± 10	63 ± 8	53 ± 5
	Maris Bard	96 ± 50	130 ± 15	87 ± 5
	Pentland Javelin	88 ± 11	78 ± 10	75 ± 7
PFK (PPi)	King Edward	790 ± 150	650 ± 150	520 ± 40
	Maris Bard	820 ± 320	1000 ± 70	840 ± 70
	Pentland Javelin	810 ± 40	710 ± 70	860 ± 70
α-Glucan phosphorylase	King Edward	460 ± 30	350 ± 50	330 ± 10†
	Maris Bard	440 ± 80	390 ± 60	400 ± 40†
	Pentland Javelin	600 ± 100	370 ± 50	380 ± 20†

*Each value is the mean ± s.e.m. of estimates from 3–5 tubers.
†These values are from tubers of 15 g fr. wt.

formed by sucrose synthase, could be converted to glucose 1-phosphate by UDP-glucose pyrophosphorylase, an enzyme known to catalyse a readily reversible reaction *in vitro* [16]. The conviction that the cytosol contained too little pyrophosphate (PPi) to permit this reversal may be challenged by the recent evidence that plant tissues contain appreciable amounts of PPi [17, 18], and that acid [19] and alkaline [20] pyrophosphatase (PPase) are located in the vacuole and the plastid, respectively. We investigated the above hypothesis further by determining whether the maximum catalytic activity of UDP-glucose pyrophosphorylase was high enough to mediate sucrose breakdown. The activity, 31.4 ± 1.3 μmol/min per g fresh weight (mean ± s.e.m. of values from six Pentland Javelin tubers at the 10 g stage), is well in excess of the estimates of sucrose breakdown. This very high activity, also found in other tissues [21], is consistent with UDP-glucose pyrophosphorylase being on the main pathway of sucrose breakdown, and is difficult to reconcile with a role for the enzyme that is confined to the formation of the relatively small amount of UDP-glucose needed for the synthesis of structural polysaccharides in potato tubers. No significant activity of UDP-glucose phosphorylase was found in potato tubers [22].

If the above view of sucrose catabolism is correct then the latter will be dependent upon a supply of cytosolic PPi. The very high activities of PFK (PPi) that we found in all tubers are consistent with this enzyme, known to be confined to the cytosol [23], providing this PPi.

Metabolite measurements

We investigated whether the amounts and behaviour of PPi, UDP-glucose and Fru-2,6-P_2 in developing and mature tubers were compatible with the above hypothesis. We did recovery experiments to investigate whether losses occurred during killing and extraction. For each experiment we prepared duplicate samples of tissue. One was freeze-clamped, killed and extracted in the usual way; the other was treated similarly except that a measured amount of the compound in question was added to the freeze-clamped tissue. Comparison of the two samples permitted calculation of the percentage of the added compound that had survived killing and extraction. The amounts of added compounds were comparable to those found in the tissue samples.

Our assay for PPi will detect as little as 2 nmol/g fresh weight [17]. For mature tubers (Maris Piper) recovery of added PPi was 86 ± 8% (mean ± s.e.m. of values from six tubers). We detected no PPi in any of these six tubers and conclude that they contain less than 2 nmol PPi/g fresh weight. As the mature tubers were detached and not receiving sucrose, a low content of PPi is consistent with our hypothesis. With developing tubers we were unable to either detect PPi in the extracts or to recover any of the added PPi. We attribute the latter to an inability to inhibit PPase by methods that did not lead to chemical hydrolysis of PPi. The question of whether developing tubers contain PPi thus remains open, though we stress that appreciable amounts of PPi have been demonstrated in other tissues in which there is massive conversion of translocated sucrose to starch [22, 24].

Recoveries of UDP-glucose from developing (Pentland Javelin) and mature (Maris Piper) tubers were satisfactory, 91 ± 2 and 84 ± 6% (means ± s.e.m. of estimates from six tubers), respectively. We detected no change in the amounts of UDP-glucose per g fresh weight throughout development (Fig. 3). The amount present in the

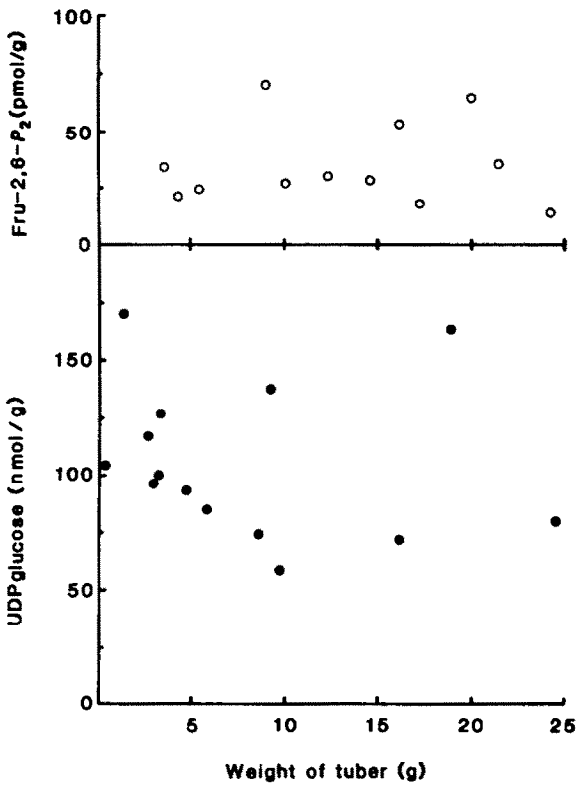

Fig. 3. Content of fructose-2,6-bisphosphate (○) and UDP-glucose (●) in developing tubers of variety Pentland Javelin. Each point represents measurements made on a single tuber.

mature tubers (Table 2) was appreciably less than that in the developing tubers. This is consistent with UDP-glucose acting as a major product of sucrose breakdown.

The recoveries of Fru-2,6-P_2 from developing and mature tubers were 68 ± 5 and 68 ± 2% (means ± s.e.m. of values from six tubers), respectively. Our values are thus underestimates but the degree of underestimation was constant. Again, we detected no change in the amounts per g fresh weight during development (Fig. 3). These amounts are well above those required for PFK (PPi) activity [25].

The role we suggest for PFK (PPi) contrasts with recent proposals that it acts glycolytically and synthesizes fructose-1,6-bisphosphate [26, 27]. In an attempt to discover the direction of PFK (PPi) *in vivo*, we determined the effects of anoxia and chilling on the amounts of UDP-glucose and Fru-2,6-P_2 in developing (Pentland Javelin) and mature (Maris Piper) tubers (Table 2). Anoxia stimulates glycolysis, at least in part, via the reaction that converts fructose 6-phosphate to fructose-1,6-bisphosphate [28]. Chilling preferentially inhibits the entry of hexose phosphate into the respiratory pathways and leads to an accumulation of sucrose [7, 12]. Removal of developing tubers from the plant was used as a means of rapidly reducing the supply of sucrose to the tuber.

Although we found striking changes in the metabolites that are consistent with our hypothesis, they do not demonstrate it (Table 2). The effect of anoxia on UDP-glucose, a fall by 50%, is almost exactly that demonstrated previously [28] for the hexose 6-phosphates. This suggests near equilibrium between UDP-glucose and the hexose phosphates, a condition expected on our hypothesis. The fall in hexose phosphates and rise in Fru-2,6-P_2 in anoxia is evidence of a glycolytic role for PFK (PPi). However, these observations are just as readily explained by the proposal that the increase in Fru-2,6-P_2 stimulates the formation of PPi by PFK (PPi) to provide the substrate required for the accelerated glycolysis. Transfer of the developing tubers to 4° involved severing them from the plant, so the appropriate controls are the tubers kept at 20° for the same length of time. The rise in UDP-glucose on cooling coincides with a comparable rise in hexose monophosphates [29] and adds further to the evidence of near equilibrium between UDP-glucose and hexose monophosphates in potatoes. The fall in Fru-2,6-P_2 could reflect either a decline in the need for PPi or the reduced rate of glycolysis. Finally we note that severance of the tubers from the plant led to a fall in UDP-glucose and Fru-2,6-P_2. Both falls would be expected if PFK (PPi) produced the PPi needed for the metabolism of UDP-glucose formed from sucrose.

Table 2. Effects of different physiological treatments on amounts of UDP-glucose and Fru-2,6-P_2 in developing and mature potato tubers

	UDP-glucose(nmol/g fr.wt)		Fru-2,6-P_2 (pmol/g fr.wt)	
	Developing	Mature	Developing	Mature
Prior to start of treatment I	105 ± 9	29 ± 3	34.9 ± 5.1	36.8 ± 7.1
Storage in N_2 for 90 min at 20° II	49 ± 10	16 ± 1	78.2 ± 7.2	83.7 ± 7.0
Storage in air for 12 hr at 4° III	83 ± 14	24 ± 2	3.9 ± 1.2	—
Storage in air for 12 hr at 20° IV	45 ± 3	—	18.2 ± 2.1	—
Fishers *P* values				
I vs. II	< 0.002	< 0.001	< 0.001	< 0.001
I vs. III	N.S.	N.S.	< 0.001	—
I vs. IV	< 0.001		< 0.02	—
III vs. IV	< 0.05	—	< 0.001	—

Mature tubers were taken from store and developing tubers were removed from growing plants, and immediately subjected to the treatments. Values are means ± s.e.m. of estimates from six separate tubers except those for UDP-glucose and Fru-2,6-P_2 for developing tubers prior to treatment, which are from 14 and 12 tubers, respectively.

Conclusions

Our failure to find any evidence of coarse control of sugar content of the developing tubers suggests that fine control is responsible for determining the relative amounts of sucrose, glucose and fructose. The central position of these sugars in the tuber's metabolism means that a wide variety of factors could affect sugar content and is likely to explain the marked variation seen between different varieties and different stages of development.

The available evidence strongly suggests that sucrose synthase, rather than either of the invertases, plays the major role in metabolizing the sucrose delivered to the developing tuber. As a tentative working hypothesis as to how this may occur we suggest the pathway shown in Scheme 1. Each of the enzymes shown in Scheme 1 is present in the cytosol and we envisage the complete sequence occurring in the cytosol. For each molecule of sucrose metabolized through the pathway one molecule of fructose 6-phosphate would be converted by PFK (ATP) to fructose-1,6-bisphosphate that would act as a substrate for PFK (PPi) in the generation of PPi. The fructose 6-phosphate formed by PFK (PPi) is envisaged as re-entering glycolysis via PFK (ATP). Thus for each molecule of sucrose broken down one molecule of fructose 6-phosphate would have to cycle through PFK (ATP) and PFK (PPi) before being broken down in glycolysis. Our hypothesis would allow Fru-2,6-P_2 to play a major role in the partitioning of sucrose delivered to the tuber.

EXPERIMENTAL

Material. Enzymes, cofactors and substrates were from Boehringer except that peroxidase, PFK (PPi), fructose 6-phosphate, fructose-2,6-bisphosphate, glucose-1,6-bisphosphate and UDP-glucose were from Sigma. Mature tubers, *Solanum tuberosum* L. cv. Maris Piper, were bought locally and stored at 10° for no more than 8 weeks before use. For studies of developing tubers we bought AI grade seed tubers (cv. King Edward, Maris Bard and Pentland Javelin), allowed them to sprout for 3 weeks, covered them with moist compost (Fison's Levington) and grew them for a further 4 weeks. Then we excised at the tuber surface single shoots (6–7 cm long) that had only one stem and planted them, one per pot (11 × 13 cm), in compost, which we moistened every other day, alternately, with water and with Hoagland's soln. Tubers began to appear after 10 weeks from planting the sprouts and were harvested as required. All the above growth procedures were carried out at 20°, 90% relative humidity, and 10.5-hr day length with light of minimum intensity of 130 μmol m^{-2} sec^{-1}. The developmental stage of the tuber was designated according to fresh weight; for most experiments the weights chosen were 1, 5, 10, 15 and 20 g. To establish the relationship between tuber age and weight, plants were dug up carefully at the start of tuberization and individual tubers were tagged. The initial fresh weight of these tubers was estimated by comparison with tubers of similar dimensions that were removed and weighed. The plants were carefully re-potted and the tagged tubers were harvested and weighed after known intervals of time.

Enzyme assays. Samples, 0.5–1.0 g fr. wt, were homogenized, first in a pestle and mortar, and then in an all-glass homogenizer. The procedures for acid invertase, alkaline invertase, sucrose synthase and α-glucan phosphorylase are in ref. [1]. Hexokinase was extracted as described for α-glucan phosphorylase, the extract was centrifuged at 1000 *g* for 5 min and the supernatant assayed according to ref. [30] in a 3-ml reaction mixture that contained: 80 mM glycylglycine, pH 7.9, 0.43 mM NADP, 6.7 mM $MgCl_2$, 20 mM glucose, 0.7 unit glucose-6-phosphate dehydrogenase, 0.024 unit 6-phosphogluconate dehydrogenase and 2.75 mM ATP. The remaining enzymes were extracted in 100 mM Tris–HCl, 20 mM EDTA, 20 mM cysteine-HCl, 20 mM diethyldithiocarbamate, pH 7.6. The homogenates were centrifuged at 100 000 *g* for 30 min and the supernatants were assayed according to the following refs and reaction mixtures: PFK (ATP) [31], 50 mM glycylglycine, pH 7.9, 0.1 mM NADH, 1.67 mM $MgCl_2$, 0.25 mM ATP, 1 unit fructose-1,6-bisphosphate aldolase, 1 unit triosephosphate isomerase, 1 unit glyceraldehyde-3-phosphate dehydrogenase, 5 mM fructose 6-phosphate in 3 ml; PFK (PPi) [25], 75 mM Hepes, pH 7.6, 2 mM $MgCl_2$, 7.5 mM fructose 6-phosphate, 2 μM Fru-2,6-P_2, 0.15 mM NADH, 0.6 mM $Na_4P_2O_7$, coupling enzymes as for PFK (ATP) in 1 ml; UDP-glucose pyrophosphorylase [16], 80 mM glycylglycine, pH 8.0, 1 mM $Na_4P_2O_7$, 1 mM $MgCl_2$, 10 μM glucose-1,6-bisphosphate, 0.4 mM NAD, 4 units phosphoglucomutase, 1.4 unit glucose-6-phosphate dehydrogenase (NAD-dependent from *Leuconostoc*), 0.8 mM UDP-glucose in 1.0 ml. Enzymes were extracted and kept at 4° and assayed at 25°.

Substrate assays. Measurements were made as in the accompanying refs: sugars [1], Fru-2,6-P_2 [24], starch [32]. For PPi and UDP-glucose, samples, 0.5–1.0 g fr. wt, were freeze-clamped, killed and extracted as in ref. [17] except that the time between adding the $HClO_4$ to the tissue and its removal by centrifugation at 25 000 *g* was 30 min for UDP-glucose and 60 min for PPi. UDP-glucose was assayed as in ref. [21], PPi ref. [17] and ^{14}C ref. [21].

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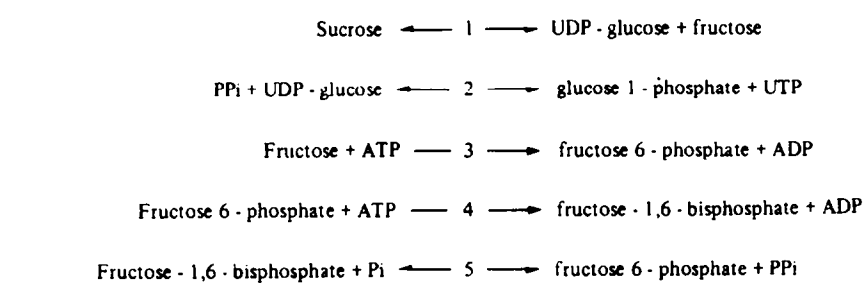

Scheme 1. Possible pathway for sucrose breakdown: 1, sucrose synthase; 2, UDP-glucose pyrophosphorylase; 3, fructokinase; 4, PFK (ATP); 5, PFK (PPi).

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