Plant Physiol. (1992) 98, 287–293
0032-0889/92/98/0287/07/$01.00/0

Received for publication June 27, 1991
Accepted August 16, 1991

Sucrose Metabolism in Tubers of Potato (*Solanum tuberosum* L.)

Effects of Sink Removal and Sucrose Flux on Sucrose-Degrading Enzymes

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ABSTRACT

Excision of developing potato (*Solanum tuberosum* L.) tubers from the mother plant, followed by storage at 10°C, resulted in a rapid, substantial decrease in sucrose synthase activity and considerable increases in hexose content and acid invertase activity. A comparison of the response of three genotypes, known to accumulate different quantities of hexoses in storage, showed that both sucrose synthase activity and the extent to which activity declined following excision were similar in all cases. However, there was significant genotypic variation in the extent to which acid invertase activity developed, with tubers accumulating the highest hexose content also developing the highest extractable activity of invertase. Similar effects were found in nondetached tubers when growing plants were maintained in total darkness for a prolonged period. Furthermore, supplying sucrose to detached tubers through the cut stolon surface prevented the decline in sucrose synthase activity. Maltose proved to be ineffective. Western blots using antibodies raised against maize sucrose synthase showed that the decline in sucrose synthase activity was associated with the loss of protein rather than the effect of endogenous inhibitors. Although there were indications that maintaining a flux of sucrose into isolated tubers could prevent the increase in acid invertase activity, the results were not conclusive.

Sucrose cleavage is catalyzed either by sucrose synthase (UDP-glucose: D-fructose-2-glucosyl transferase, EC 2.4.1.13) or by invertase (β-D-fructofuranoside fructohydrolase, EC 3.2.1.26). Sucrose synthase is a cytosolic enzyme (8), whereas invertase can be located in the cell wall, vacuole, or cytosol (9, 11). Neutral or alkaline invertases are generally believed to be cytosolic enzymes (12), although there are reports of association with the cell wall (3).

In starch-storing sinks such as potato tubers, in-coming sucrose is degraded predominantly via the sucrose synthase pathway (15, 24). An important feature of the sucrose synthase reaction is the conservation of the energy in the glycosidic bond of sucrose, thus making it more efficient, energetically, than the invertase pathway (5, 27). Sucrose synthase activity declines as tubers mature on the plant (19) and in mature, stored tubers, acid invertase activity predominates (20). In a previous article, it was reported that the decline in sucrose synthase can be accelerated by excising the tuber from the mother plant (18), although the effects on sugar balance and of tuber maturity in response to excision were not addressed.

In the present study, we examine in detail the consequence of excising tubers at various stages of development on the change in sugar balance and the activities of sucrose-cleaving enzymes during a subsequent storage period at 10°C. The effect of genotype on the response is also reported. This complements work published previously on the influence of genotype on sugar accumulation in tubers stored at low temperature (21) and on sugar metabolism in developing tubers (15, 23). Furthermore, whereas Claussen *et al.* (2) suggested that sucrose regulates sucrose synthase activity, this hypothesis has not been adequately tested for starch-storing sink tissues such as potato tubers. The present study addresses the question of regulation of enzyme levels by sucrose (assimilate) flux.

MATERIALS AND METHODS

Tuber Excision and Postharvest Storage Experiments

Potatoes (*Solanum tuberosum* L.) cvs Cara, Record, and Brodick, were grown in the field at the Scottish Crop Research Institute as described previously (21). Four sequential harvests were taken between August 1 and September 26 to provide tubers of varying maturity. Mean individual tuber weights were about 70 g fresh weight at the first harvest and 125 g fresh weight at the end of September. After tuber excision, five tubers of each genotype were selected at random for immediate analysis (within 2 h). The remainder were stored at 10 ± 1°C and analyzed at regular intervals over a period of up to 18 d.

Exogenous Application of Sugars to Intact, Detached Tubers

Developing tubers with a mean fresh weight of 40 g (±10%) were excised at the point of attachment of the stolon with the stem. The stolons were then immersed in distilled water or in solutions of either sucrose or maltose (10 individual tubers per treatment). To determine the rate of carbohydrate flux into the tubers, solutions were spiked with either [*U*-^{14}C]-D-

maltose (specific activity of stock isotope, 15.5 GBq $mmol^{-1}$) or [U-^{14}C]sucrose (specific activity of stock, 13.4 GBq $mmol^{-1}$). The concentration of isotope for all solutions was 37 KBq ml^{-1}.

Tuber Analysis

The majority of analyses were carried out on material frozen in liquid N_2 and stored at −80°C. Frozen samples were lyophilized and finely ground before carbohydrate extractions and ^{14}C analyses. Enzyme extracts were prepared from fresh tissue. Three adjacent longitudinal slices (0.5-cm-thick) were taken from each tuber to provide sufficient material for analyses. This allowed a direct comparison of data on carbohydrate balance, enzyme activities, etc.

Determination of Sucrose, Glucose, and Fructose

Lyophilized and finely ground samples were extracted in 80% ethanol at 70°C and the concentration of glucose, fructose, and sucrose determined using enzyme-coupled reaction systems (21). In ^{14}C experiments, ethanol extracts were prepared as above and total ^{14}C in the soluble fraction determined. The remaining tissue pellet was gelatinized, digested with amyloglucosidase (25), the entire sample shaken thoroughly, and several aliquots removed for scintillation spectrometry (insoluble fraction). The ethanol extracts were freeze-dried and the distribution of label between glucose, fructose, sucrose, and maltose determined by HPLC. Sugars were separated on a 15-cm reverse-phase amino column using acetonitrile:H_2O (85:15 v/v) as the mobile phase (flow rate, 2 mL min^{-1}). Radioactivity in sugar peaks (refractive index detection) was measured by liquid scintillation counting. Preliminary studies with ^{14}C sugars showed that less than 2% of maltose or sucrose added to tuber tissue before extraction was degraded as a result of the procedures used.

Enzyme Determinations

Samples of fresh potato tissue were extracted with insoluble PVP (1% w/v) and acid-washed sand in 3 volumes of extraction solution. For sucrose synthase, the extraction solution contained 100 mM Tris-HCl (pH 7.5), 5 mM DTT, 3 mM magnesium acetate, and 2% (w/v) glycerol. For invertase determination, tissue was extracted in 100 mM acetate buffer (pH 5.0) containing 10 mM sodium sulfite. After centrifugation at 20,000g for 20 min at 5°C, extracts were dialyzed for 18 h at 3°C against 10 mM extraction buffer. Recoveries of sucrose synthase and invertase activities were unaffected by choice of de-salting method (dialysis or rapid de-salting with Sephadex G-25M [PD10; Pharmacia, United Kingdom]). Due to the number of samples processed, dialysis was chosen for convenience.

Invertase activity before (basal) and after (total) destroying the endogenous invertase inhibitor by extensive foaming was determined as described previously (21). Essentially, total invertase was measured after foaming de-salted extracts by vortexing for 90 min in test tubes fixed to a flask shaker operating at maximum speed. The recovery of yeast invertase added to tuber extracts and taken through the entire process was 82% for foamed extracts and 90% for nonfoamed extracts. The activity of yeast invertase added was approximately equal to the activity of tuber invertase. Both developing tubers detached from the mother plant for 12 d (high invertase samples) and tubers detached for only 5 min (low invertase samples) were used for recovery experiments. Results indicated no effect of tuber treatment on the recoveries of invertase activity. Invertase activity was assayed under optimum pH and substrate conditions in 0.1 M acetate buffer, pH 5, containing 250 mM sucrose. These conditions were optimal for both developing and stored tubers. Numerous pH curves were produced from the various treatments imposed. These revealed no specific alkaline invertase. Throughout the text, any reference to tuber invertase therefore refers to acid invertase only. Sucrose synthase was assayed in the cleavage direction under optimized pH (7.2) and sucrose (200 mM) and UDP (1 mM) concentrations. The method used was essentially that of Xu *et al.* (27). When developing cotyledons of bean (*Vicia faba L.*) were extracted together with tuber tissue, the recovery of sucrose synthase was 95% of that expected from independent extractions of tuber and bean material. It is concluded that no major losses of tuber sucrose synthase activity occurred during the extraction procedure.

Protein Determination, Electrophoresis, and Immunoblotting

Protein in enzyme extracts was quantified using the Bio-Rad protein assay with BSA as a standard. Approximately 10 μg of soluble protein extracted with the sucrose synthase extraction medium was subjected to SDS-PAGE (7.5% acrylamide) as described by Laemmeli (10) and polypeptides stained with Coomassie blue. In parallel gels, polypeptides were transferred to nitrocellulose and, after immunoblotting with polyclonal antibodies raised to maize sucrose synthase, cross-reacting bands were identified using anti-rabbit immunoglobulin conjugate labeled with alkaline phosphatase. The sucrose synthase antibody was raised against protein extracted from whole kernels of wild type maize and kindly supplied by Dr. Karen Koch, University of Florida.

RESULTS AND DISCUSSION

Sugar Balance and Enzyme Activities in Tubers after Detachment

Tubers from field-grown plants of cvs Cara, Record, and Brodick were excised on four occasions during the growing period to provide samples differing in physiological status and chronological age. Genotypic variation in tuber sugar balance, particularly during the postharvest period, is well known. The cvs used were chosen because of their differential accumulation of hexoses in storage (21). The hexose (glucose + fructose) and sucrose content of tubers after detachment and during a subsequent storage period at 10°C is shown in Figure 1. Data for harvests 1 and 4 only are presented. Similar patterns were observed with harvests 2 and 3, but the data were omitted for clarity. At each harvest date and for each genotype, the tuber hexose content was low at excision but increased substantially within the first few days in storage. As predicted, the three genotypes showed consistent differences in the rate and extent

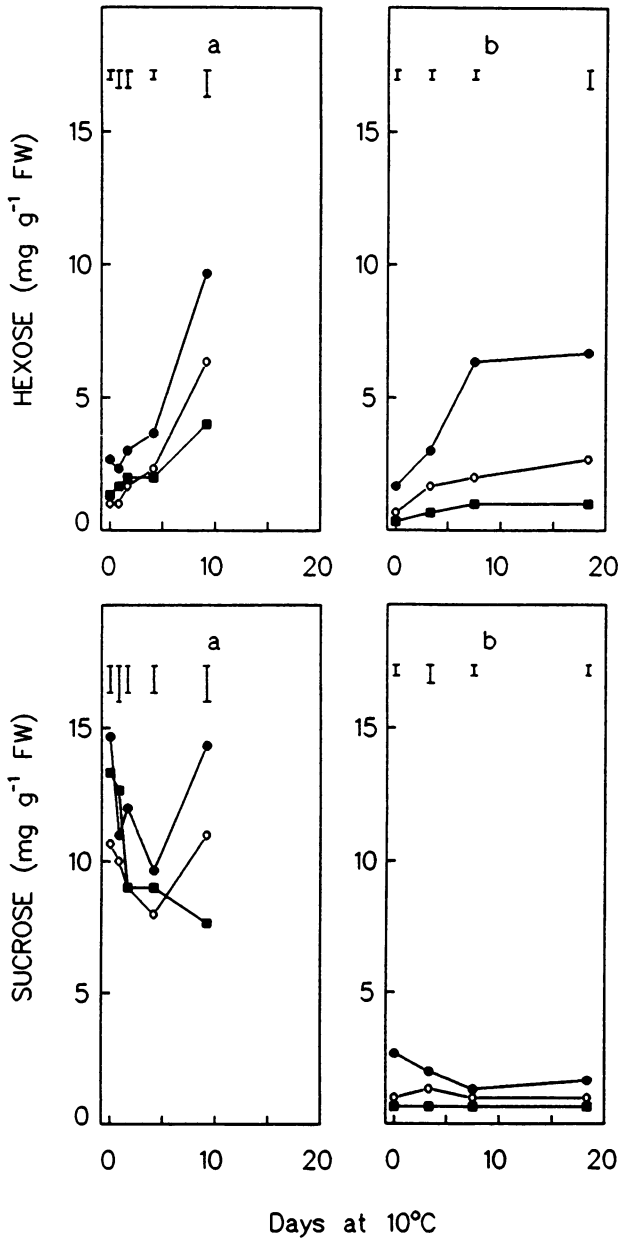

Figure 1. Effect of excising developing tubers from the mother plant on tuber hexose and sucrose content in storage at 10°C. Tubers were excised from growing plants either on August 1 (a) or September 26 (b). The cv used were Cara (●), Record (○), and Brodick (■). Bars indicate SE of the mean.

of hexose accumulation, with cv Cara accumulating the highest concentration and cv Brodick the lowest. Changes in sucrose content after excision were far more variable with no consistent differences between genotypes. The fact that a decline in sucrose content did not always accompany hexose accumulation implies that the sucrose pool may be replenished as a result of starch breakdown (6). The loss of starch required to deliver the observed increase in hexoses is not measurable with any accuracy (70% of tuber dry matter is starch, but less than 1–2% is soluble sugar).

Tuber excision also resulted in a rapid and substantial increase in acid invertase activity at each stage of tuber development examined. This was evident when both total and basal activities were plotted (Fig. 2). Again, data for harvests 2 and 3 are omitted for clarity. In some cases, a 10-fold increase in activity occurred within 3 d after excision. In general, invertase activity in the high sugar accumulating genotype Cara was significantly and consistently higher than in the lowest sugar accumulator, Brodick. Often, the most substantial difference in invertase activity between genotypes

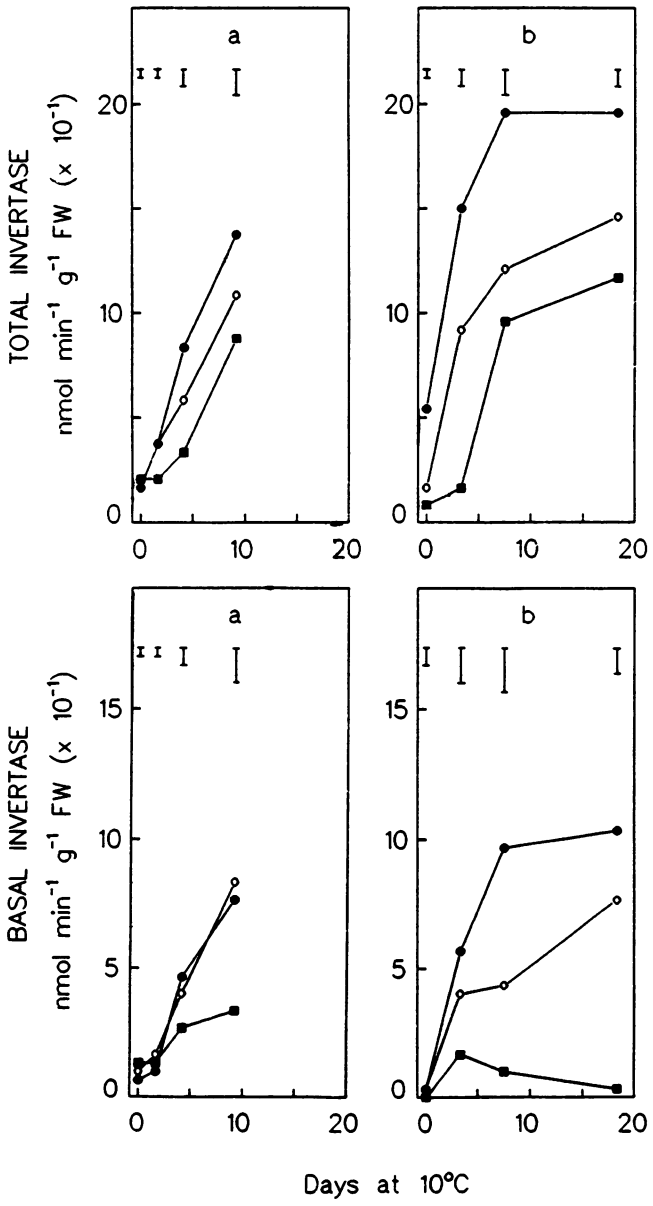

Figure 2. Effect of tuber excision on the development of total and basal acid invertase activity in storage at 10°C. Basal activity was determined in the presence of invertase inhibitor protein. Total activity, in the same extract, was determined after the destruction of inhibitor by rapid vortexing. Letters and symbols used to identify excision dates and potato genotypes are as in Figure 1.

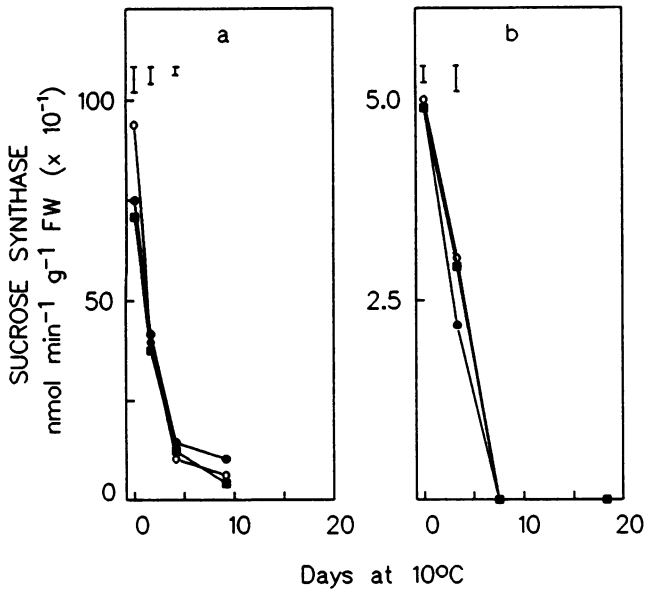

Figure 3. Sucrose synthase activity in tubers immediately detached from the mother plant and during a subsequent storage period at 10°C. Activity was determined in the cleavage direction. Letters and symbols are identified as in Figures 1 and 2.

was observed when basal rather than total activity was measured. This could imply that the proteinaceous invertase inhibitor present in tubers plays a role in regulating invertase activity *in vivo* (20). It must be pointed out, however, that the use of techniques such as rapid vortexing and foaming of extracts, developed to destroy the inhibitor, are severe, and the possibility that losses of invertase as well as inhibitor protein occur cannot be ruled out. It should also be noted that no alkaline invertase activity was detected in tubers.

In contrast to acid invertase, sucrose synthase activity declined rapidly after tuber detachment and by as much as 84% in 2 d (Fig. 3). Experiments in which extracts from attached and detached were mixed showed that this was not due to the presence of an inhibitor in excised tubers. Similar activities were found in all three genotypes. As with sucrose content, enzyme activity declined between the first and final harvests (Fig. 4) and in many cases there was no measurable activity in stored tubers. The results suggest that acid invertase rather than sucrose synthase regulates sucrose hydrolysis in stored tubers and that sucrose concentration or sucrose flux modifies sucrose synthase activity. Sucrose is known to regulate the expression of a number of genes in plants (4, 7). Of particular relevance is the positive effect of sucrose on the expression of potato genes including those encoding patatin (26), sucrose synthase (22), and ADP-glucose pyrophosphorylase (16). The latter experiments were carried out with leaf and stem tissue.

Effect of Modifying Assimilate (Sucrose) Supply to Tubers

As with tuber detachment, excluding light completely from growing plants (cv Record), by covering them with two layers of black polythene produced increases in tuber hexose content and invertase activity and decreases in sucrose content and sucrose synthase activity (Fig. 5). The magnitude of the effect on basal and total invertase activities was similar, although basal activity was about threefold lower than the total (data not shown). Compared with the excision experiment, the effects were not as rapid, with substantial differences between covered and uncovered plants occurring more than 10 d after eliminating light interception. The result again implies that the rate of assimilate supply to developing tubers modifies sucrose-hydrolyzing potential via both sucrose synthase and invertase pathways. The delayed response referred to above may be attributed to the mobilization of starch and sucrose supplies in leaf, stem, or root tissues.

Effect of Supplying Sucrose Exogenously to Intact Tubers

Detached tubers were supplied with sucrose at a range of concentrations through the cut stolon surface, and enzyme activities were determined after 12 d (Table I). The loss of sucrose synthase activity after tuber detachment was prevented by sucrose. A concentration of 750 mM was most effective. The effect of sucrose concentration on invertase activity was more variable. The large SEs obtained with certain treatments probably reflect variation in the rate of sucrose uptake between individual tubers. As Figure 6 clearly shows, there was a good correlation ($r = 0.75$) between sucrose uptake by, and sucrose synthase activity in, individual tubers. Clearly, a proportion of incoming [^{14}C]sucrose will either be compartmentalized in the vacuole or converted into starch. It was beyond the scope of this study to determine the concentration

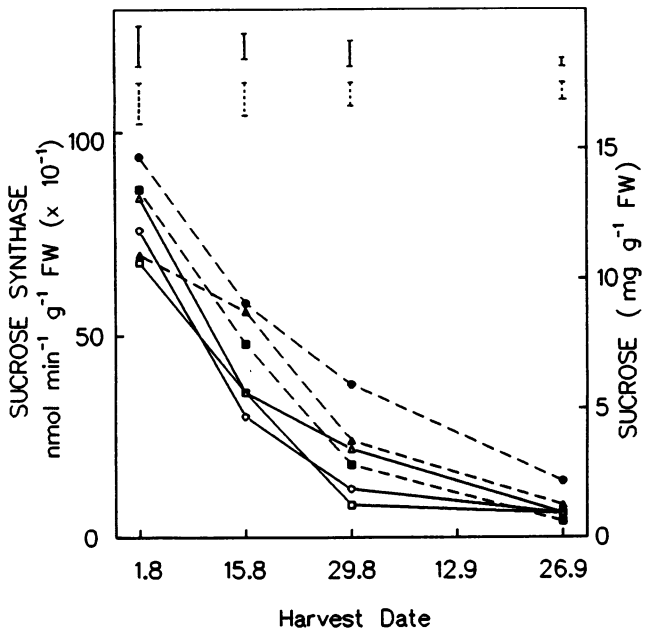

Figure 4. Sucrose synthase activity in (——), and sucrose content of (- - - -), tubers collected at various stages of the growing season. Analyses were carried out immediately after tubers were collected. Key to genotypes: Cara (○, ●), Record (△, ▲), and Brodick (□, ■). Bars represent SE of the mean.

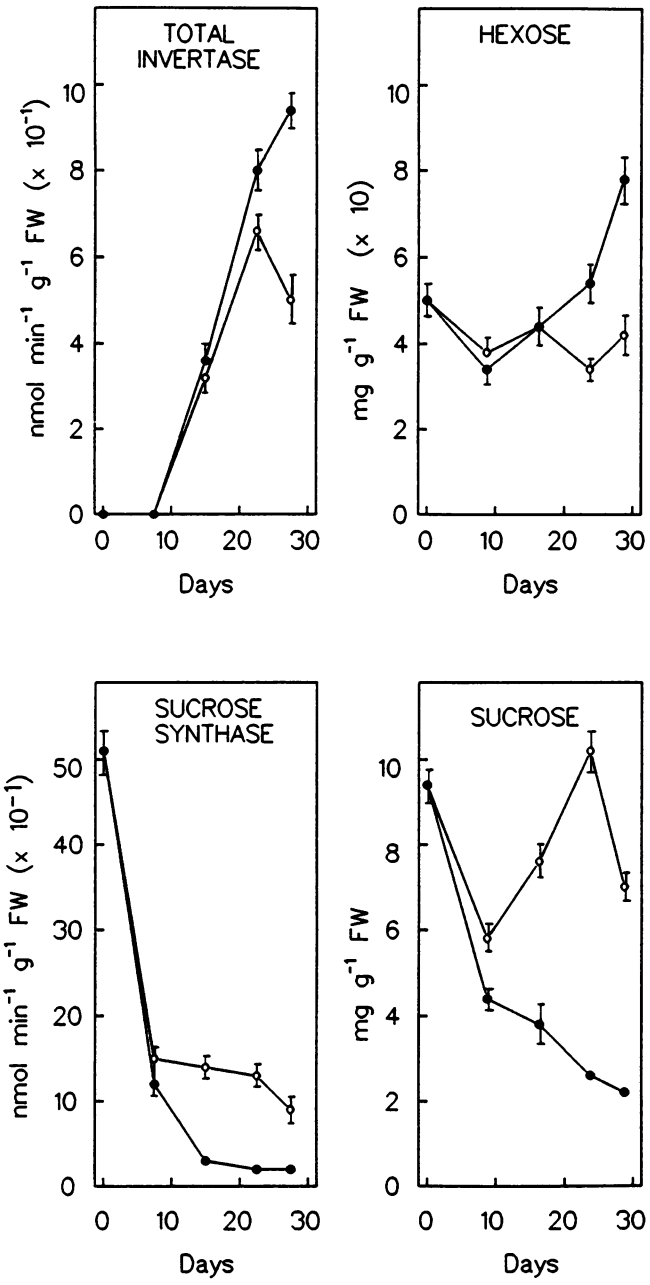

Figure 5. Effects of eliminating light interception for up to 30 d on tuber invertase (total) and sucrose synthase activities and sugar content. ●, Light interception prevented; ○, control.

of sucrose in the cytosol and vacoule and to relate these to sucrose synthase activity. When tubers were supplied with maltose, the correlation between maltose uptake and sucrose synthase activity was poor ($r = -0.35$). Similarly, there was no significant correlation between invertase activity and sucrose uptake (total $r = -0.28$, basal $r = -0.061$). HPLC analysis showed that at the end of the incubation with [^{14}C] sucrose, more than 50% of radiolabel in the soluble sugar fraction was present as sucrose, 1 to 2% as maltose, and the remainder equally distributed between fructose and glucose.

Table I. *Effect of Sucrose Concentration Supplied to Detached Tubers on Sucrose Synthase and Acid (Total) Invertase Activities after Storage at 10°C*

Day 0, enzyme activities immediately after tuber detachment. Day 12, enzyme activities 12 d after supplying sucrose. Values are ± SE of the mean ($n = 5$).

Treatment	Sucrose Synthase	Total Invertase
	nmol min⁻¹ g⁻¹ fresh wt	
Day 0	154 ± 32	30 ± 17
Day 12 control (H_2O)	57 ± 7	143 ± 12
150 mM sucrose	85 ± 21	111 ± 44
300 mM sucrose	106 ± 10	66 ± 8
750 mM sucrose	260 ± 103	71 ± 18
1500 mM sucrose	93 ± 22	122 ± 29

With [^{14}C]maltose, 50% of the label was recovered as sucrose, 7% as maltose, and the remainder again equally distributed between glucose and fructose. Despite the substantial conversion of maltose into sucrose, the decline in sucrose synthase was not prevented. This result is difficult to interpret in the light of the sucrose effect. On a daily basis, the rate of maltose conversion may be low compared with the rate of sucrose influx directly from the external medium. This would not prevent an accumulation of significant amounts of sucrose by the end of the incubation period, but this is most likely to occur in the vacuole.

Immunoblotting of protein extracts from the above experiment with maize sucrose synthase antibody revealed a cross-reacting polypeptide (M_r 90,000) (Fig. 7). The molecular mass of potato tuber sucrose synthase has been estimated at 290 kD (19), but generally the enzyme is a tetramer consisting of subunits ranging from 87 to 100 kD (14, 17). In Figure 7, the respective activities of sucrose synthase in extracts electropho-

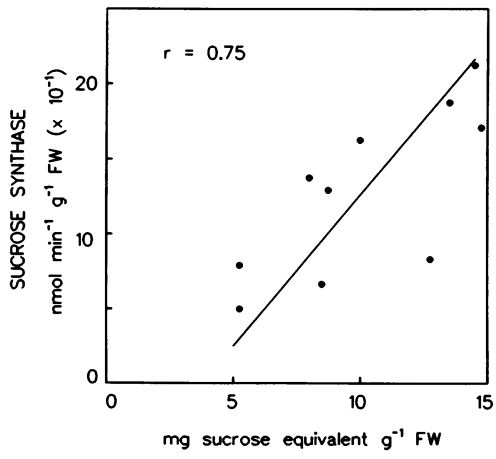

Figure 6. Correlation between sucrose synthase activity and sucrose flux into individual tubers. Tubers were excised from the mother plant and the cut stolon surface immersed in 750 mM sucrose containing a known specific activity of [*U*-^{14}C]sucrose. ^{14}C incorporated by tubers after 12-d incubation was used to determine the quantity of sucrose transported.

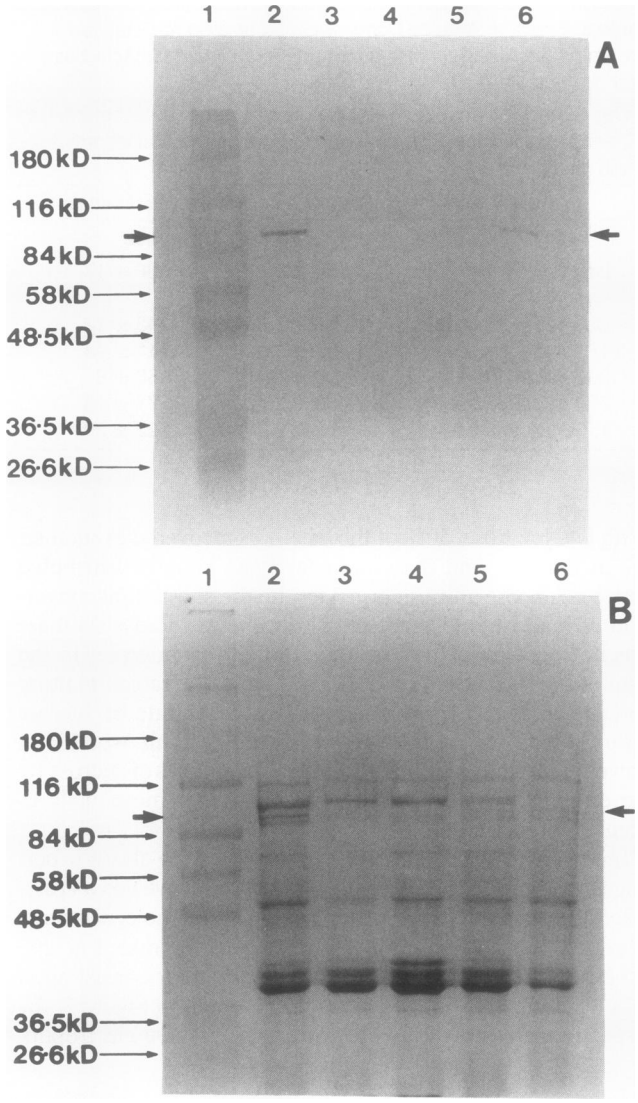

Figure 7. A, Western blot of crude potato extracts with antibodies raised against maize sucrose synthase. Lanes: 1, mol wt markers; 2, newly harvested tuber; 3, 4, 5, and 6, 12 d after supplying excised tubers with water, 750 mM maltose, 750 mM sucrose, and 1500 mM sucrose, respectively. B, Coomassie-stained gel of crude potato extracts (protein loading 10 μg). Lane numbers correspond with treatments described in panel A. Arrow indicates sucrose synthase polypeptide.

resed in lanes 2, 3, 4, 5, and 6 were 412, 45, 39, 281, and 445 nmol min^{-1} g^{-1} fresh weight. The relative abundance of sucrose synthase protein therefore follows sucrose synthase activity. It can be concluded that in potato tuber storage parenchyma, the level of sucrose synthase protein, and hence activity, is regulated by sucrose supply. The work of Salanoubat and Belliard (22) has shown that transcription of the gene in potato leaf and stem is modified by sucrose supply. One must conclude that an identical control mechanism is operating in the tuber. This is unlike the situation in maize protoplasts in which sucrose appears to affect, negatively, transcription of the NTP 11 reporter gene fused to the maize sucrose synthase promoter (13).

CONCLUSIONS

Treatments that interfered with the supply of photosynthate to developing potato tubers stimulated hexose accumulation, caused a rapid increase in acid invertase activity, but resulted in a substantial depletion of sucrose synthase activity and sucrose synthase protein. The data are taken as evidence that acid invertase rather than sucrose synthase regulates hexose accumulation in mature, stored tissues. Differences between genotypes in the rate of hexose accumulation were related to invertase activity. Maintaining a flux of sucrose into excised tubers prevented the decline in sucrose synthase, substantiating the view that sucrose has a positive effect on the expression of the gene in potato. The mechanism by which sucrose regulates gene expression is not known as yet. There is no convincing evidence that sucrose has a negative effect on the synthesis of invertase protein.

ACKNOWLEDGMENTS

The authors wish to thank E. Cuthbert and D. McRae for their careful technical assistance.

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