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Osmotic stress-induced changes of sucrose metabolism in cultured sweet potato cells

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Abstract

The intra- and extracellular sugar contents, the activities of sucrose-metabolizing enzymes, and the metabolism of [U-^{14}C] glucose in a pulse-chase experiment were compared between the normal and osmotically stressed (by 0.6 M sorbitol) sweet potato (*Ipomoea batatas*) suspension cells. The stress enhanced the levels of sucrose and sucrose phosphate synthase (SPS) activity. Northern blot analysis also showed that prolonged osmotic stress enhanced the SPS gene expression at the transcriptional level. Stressed cells also had higher activities of sucrose cleaving enzymes, such as alkaline invertase and sucrose synthase. The ^{14}C-sucrose isolated from normal and stressed cells had ^{14}C-fructose and ^{14}C-glucose ratios of 0.68 and 1, respectively. These data suggest the continual cycling of degradation and synthesis of sucrose in both types of cells. Among the enzymes used in constructing such futile cycling, besides invertase and SPS, sucrose synthase (SS) should be involved in normal cells, but not in stressed ones. It is apparent that the osmotic stress caused a significant change in the pattern of sucrose metabolism.

Key words: Invertase, *Ipomoea batatas*, osmotic stress, radiotracer study, sucrose-phosphate synthase, sucrose synthase.

Introduction

Sucrose is the common carbon source in most plant tissue cultures. Sucrose is also accumulated in many plant tissues in response to environmental stress, including water deficit (Morgan, 1984; Quick *et al*., 1989; Zrenner and Stitt, 1991; Castrillo, 1992; Pelah *et al*., 1997; Ramos *et al*., 1999), salinity (Balibrea *et al*., 1997) and low temperature (Guy, 1990) for playing a role in osmoregulation and cryoprotection. Sweet potato suspension cells exposed to osmotic stress induced by 0.6 M sorbitol resulted in the accumulation of a large amount of sucrose without significant change in cell volume (Wang *et al*., 1999). Thus this system was used for studying the changes in sucrose turnover induced by osmotic stress.

In many plant systems, one of the common features of sucrose metabolism is the continual cycling of its degradation and synthesis (Su, 1982; Hargreaves and ap Rees, 1988; Wendler *et al*., 1990; Geigenberger and Stitt, 1991, 1993; Hill and ap Rees, 1995; Geigenberger *et al*., 1997). This biochemical process, if constructed according to the known enzymatic reactions, is at the expense of ATP hydrolysis and thus recognized as a futile cycle. Although known about for many years, the physiological significance of the cycle is still illusive. So, whether the futile cycle is still kept, or modified under a high osmotic stress, is an interesting problem.

There are three enzyme systems directly involved in sucrose metabolism. The first one is sucrose phosphate synthase (SPS, EC 2.4.1.14), which is a soluble enzyme located in the cytoplasm that catalyses the reaction UDPGlc + fructose 6-phosphate = sucrose phosphate + UDP. SPS is thought to play a major role in sucrose biosynthesis (Huber and Huber, 1996) because the hydrolysis of sucrose phosphate by an accompanying specific phosphatase renders the synthetic reaction irreversible in favour of sucrose accumulation. The second is sucrose synthase (SS, EC 2.4.1.13), which

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Abbreviations: IT, invertase; SPS, sucrose-phosphate synthase; SS, sucrose synthase; SSC, 0.15 M sodium chloride/0.015 M trisodium citrate, pH 7.0; UDPGlc, uridine 5-diphosphoglucose.

catalyses a reversible reaction UDPGlc + fructose = sucrose + UDP. SS is thought to catalyse sucrose degradation for synthesizing UDPGlc (Chourey and Nelson, 1979; Cobb and Hannah, 1988; Heim *et al.*, 1993). In growing potato tubers and some plant tissues, SS was also considered to make a major contribution to sucrose synthesis (Geigenberger and Stitt, 1993; Déjardin *et al.*, 1997*a*). The third is invertase (IT, EC 3.2.1.26), which is a hydrolytic enzyme catalysing an irreversible sucrose-cleaving reaction. In higher plants, there are IT isozymes which can be categorized as acidic and alkaline based on the pH optima of reaction (Avigad, 1982). The soluble alkaline IT is thought to reside in the cytoplasm. Two types of acid IT have been observed: a soluble form located in the vacuole and an extracellular form ionically bound to the cell wall.

The authors wanted to study how the sucrose metabolism is changed and what physiological significance the futile cycle of sucrose has upon cells under osmotic stress. To achieve this, the contents of intra- and extracellular sugars of sweet potato suspension cells was assayed under normal and stress-shocked conditions. The activities of enzymes involved in sucrose metabolism were assayed. Then a pulse-and-chase type study was done on the normal and osmotically stressed cells by feeding a [U-^{14}C]-glucose.

Materials and methods

Plant materials

Suspended cells of sweet potato (*Ipomoea batatas* cv. Tainong 57) were cultured as reported (Wang *et al.*, 1999). Briefly, they were maintained by weekly transferring into a fresh Murashige-Skoog medium containing 2 μg ml^{-1} 2,4-D, 0.2 μg ml^{-1} kinetin and 30 g l^{-1} sucrose. Cells for experiment were harvested at 7 d after a routine transfer, washed with modified Gamborg's B5 salt solution in which a part of the KNO_3 was substituted by NH_4NO_3 (Wang *et al.*, 1999), then transferred into the same solution supplemented with 2.2 μg ml^{-1} 2,4-D and 30 g l^{-1} sucrose without (normal) or with (sorbitol-stressed) 0.6 M sorbitol added. NH_4NO_3 in the medium was needed to avoid cell aggregation. Cells were harvested after 1, 3 and 7 d of cultivation and then used as for analysis.

Determination of free sugars

The intracellular sugars were exhaustively extracted by a hot 80% EtOH as previously described (Wang *et al.*, 1999). The ethanolic extract and the extracellular medium were filtered through a Millipore Millex-GX nylon membrane. Sugars in these filtrates after concentration were separated on a high pH anion-exchange chromatographic column (CarboPA1, Dionex, USA) using a carbonate–bicarbonate-free sodium hydroxide eluant and quantified by a pulsed amperometric detector (Dionex, USA).

Enzyme extraction

To prepare enzyme extracts, cells were quickly washed under an aspirator-suction with the medium from which sucrose was omitted. The washed cells were homogenized in a pre-chilled mortar and pestle with 3–5 times in weight of an extraction buffer (50 mM HEPES-KOH [pH 7.0], 2 mM DTT, 1 mM EDTA, 5 mM $MgCl_2$, and 0.5 mM PMSF), 2% (w/w) insoluble PVP, and 20% (w/w) sea sand. The homogenate was centrifuged at 26000 *g* for 20 min. The supernatant was immediately desalted by passing through an Excellulose column (Pierce, No.20440), which had been equilibrated with the extraction buffer from which PMSF was omitted. All steps of extraction were done at 4 °C. The extract was used as the enzyme source immediately after the desalting treatment.

For extracting the cell-wall bound IT, the residue from the enzyme extraction was ground and washed three times with a large amount of the enzyme extraction buffer until the supernatant did not show any IT activity. The residue was stirred overnight with the extraction buffer with 1.0 M NaCl added (Hill *et al.*, 1996). The IT activity in the supernatant was accounted for the cell-wall-bound form.

Enzyme activity assay

Sucrose synthase was assayed in the direction of both sucrose degradation and formation. In the cleavage direction, the activity was estimated by a UDPGlc dehydrogenase coupling method (Su *et al.*, 1977). In the synthetic direction, the amount of synthesized sucrose was determined by a resorcinol reaction (Roe, 1934). Invertases were assayed in 50 mM Na-acetate (pH 5.0) for acid and cell wall-bound forms, and 50 mM Na-phosphate (pH 7.5) for alkaline IT (Singh and Asthir, 1988). The amount of reducing sugar produced from sucrose was determined (Nelson, 1944). SPS activity was assayed in 50 mM HEPES-NaOH (pH 7.5) containing 10 mM fructose 6-phosphate, 40 mM glucose 6-phosphate and 10 mM UDPGlc. Sucrose phosphate formed was quantified using an anthrone reagent (Walker and Huber, 1989). Assay conditions for linear correlation to the amount of enzyme and reaction time were ascertained in all tests. Enzyme activities are expressed as μmol of product generated min^{-1} g^{-1} FW of washed cells.

Pulse-and-chase experiment

After being cultured in either the normal or stressed medium for 8 d, cells were washed with the respective medium from which sucrose was omitted. A 5 ml cell suspension, containing about 4 ml of packed cells, was put in a 50 ml plastic tube, mixed with 0.1 ml of a high specific activity [U-^{14}C]-glucose (9.3 GBq mmol^{-1}, Amersham, UK), and shaken at 120 rpm. At various time intervals after the addition of radio-glucose, the residual radioactivity in the supernatant was measured. The time interval needed to reduce the applied radioactivity to 50% was taken as the optimal pulsing time.

Once the optimal pulsing time is reached, a non-radioactive medium, either the normal or the osmotically stressed, was added, and a chasing for 2 h was done in a 125 ml flask. Cells harvested immediately after adding the media were used as samples chased for 0 h.

Analysis of radioactive extracts

The cells were harvested and washed three times with respective sucrose-free medium by centrifugation (1000 rpm, 5 min). The radiolabelled sugars were extracted from the washed cells with hot 80% EtOH as described earlier. The extract was vacuum dried and dissolved in deionized water. The neutral fraction of the extract was obtained by passing through a cation exchanger

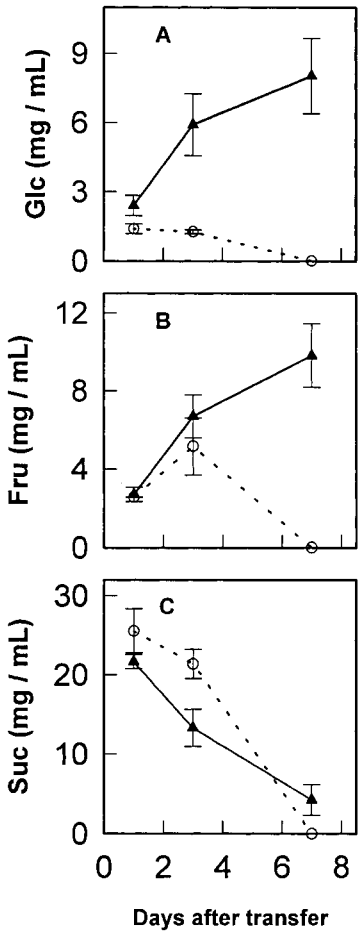

Fig. 1. Changes in the contents of Glc (A), Fru (B), and Suc (C) of cultured media of sweet potato cells under sorbitol-stressed (▲) and normal (○) conditions. All points are the average of duplicate determinations from two independent experiments.

and then an anion-exchanger (as in Quick *et al.*, 1989). The neutral compound was dried and further separated on thin-layer plates (cellulose, Merck 1.05574 or silica, Merck 1.05735), developed twice with ethyl acetate, pyridine and H_2O (20:7:5, by vol.). Authentic ^{14}C-labelled glucose, fructose and sucrose were applied as standards. Autoradiograms were obtained and quantitatively analysed by a Bio-imaging analyser (Fuji, BAS1000Mac, Japan).

The band migrated as sucrose was scraped out. The sugar was eluted with 50 mM sodium acetate (pH 5.0) and hydrolysed by 20 U ml^{-1} invertase at 37 °C for 1 h (Wendler *et al.*, 1990). Radioactive glucose and fructose in the hydrolysate were separated on a thin-layer plate and quantified.

RNA extraction and Northern blot analysis

Total RNA was extracted from sweet potato cells, which was ground to a fine powder in liquid N_2 with a handful of diethylpyrocarbonate-treated sea sands, according to a commercial phenol/guanidine isothiocyanate kit (Trizol, GIBCOBRL). The amount of total RNA was determined by A_{260}, and a sample of 20 μg was separated on a 1.0% SeaKem LE agarose (FMC)-formaldehyde gel. The RNA was transferred onto a nylon membrane (GeneScreen Plus, DuPont) according to the downward alkaline capillary transfer method (Chomczynski, 1992) with a minor modification. The paper towel was replaced with a pulp sponge (Spontex, France). After transferring for 1–2 h, the membrane was quickly washed with 2×SSC and RNA was fixed on the membrane by UV-cross-linking (Stratagene) before the membrane dried completely. An SPS cDNA was used as the template for a PCR reaction that amplified a 445 bp fragment (accession no. BankIt 259059, AF 135800) using oligonucleotide primers 5′-ATGGATCTTGCCAAGGCA-3′ and 5′-CAAGCTTGTTTCTACCCAGCG-3′. The PCR product was separated in an agarose gel, eluted from the gel and used as the template in a random-primed synthesis of a DNA probe (Promega) in the presence of [α-^{32}P] dCTP. Unincorporated nucleotides were removed using a Sephadex G-50 column (Amersham Pharmacia). The membrane was treated with 8 ml of a hybridization buffer (50% formamide, 0.1% SDS, 0.1% Ficoll, 0.1% PVP, 20 mM sodium phosphate [pH 6.5], 5×SSC, 2 mg denatured salmon sperm DNA) at 42 °C for 4–16 h. The denatured probe was added and allowed to hybridize at 42 °C for 16 h. The membrane was washed twice in 2×SSC and 0.1% SDS at room temperature for 10 min, then washed twice in 0.1×SSC and 0.1% SDS at 50 °C for 30 min. Autoradiograms were taken and quantitatively analysed by a Bio-imaging analyser as before.

Results

The extra- and intracellular sugar levels

Although the changes of sucrose levels present in the media appeared rather similar between the normal and stressed conditions, the external hexose levels were significantly different (Fig. 1). Under the normal condition, the fructose level was always higher than that of glucose during the first 3 d (Fig. 1A, B). This result implied that a part of the sucrose in the medium was hydrolysed before being absorbed, and the rate of fructose uptake is lower than that of glucose (Fig. 1B). Similar observation was made for suspension cell cultures of *Phaseolus vulgaris* (Botha and O'Kennedy, 1998) and *Catharanthus roseus* (Sagishima *et al.*, 1989). It is noteworthy that the hexose contents in the stressed medium gradually increased with increase in culture time, and fructose-to-glucose ratio was maintained close to 1.

Glucose, fructose and sucrose were the major sugars in cells. After being transferred into a fresh normal medium, the changes in their levels were similar, first showed a decrease and then an increase (Fig. 2A–C). However, the stressed cells showed a decrease in hexoses, but a significant increase in sucrose from the start, and the ratio of sucrose to hexoses in the 7-d-stressed cells was 12-fold higher than that in the normal.

Enzyme activities

With the onset of sorbitol-stressed condition, the activity of the cell-wall-bound IT increased strikingly, and then declined rapidly at day 7 (Fig. 3A). Conversely, the apoplastic capacity remained fairly low in the normal cells, but increased slightly at the 7th day when the external

carbon source was almost depleted (Fig. 1C). Irrespective of the normal and stressed cells, the activity of alkaline invertase was much higher than that of soluble acidic IT (Fig. 3B, C). The activity of alkaline IT in stressed cells was significantly higher, and the level remained rather constant (Fig. 3C). Results in Fig. 3D also showed that the stressed cells had a higher activity of SS, assayed in the direction of sucrose hydrolysis, than that of the normal cells, even though they both showed a downward change in the time course.

The ability to synthesize sucrose was assessed by assaying SS and SPS. Both the normal and stressed cells showed a declining tendency for SS activity in the direction of sucrose synthesis, but the stressed cells always showed a higher activity (Fig. 4A). Unlike SS, SPS activity patterns were different between the two types of cells. Sorbitol stress resulted in a substantial increase in SPS activity (Fig. 4B), in good agreement with the very significant increase of sucrose content in stressed cells (Fig. 2C).

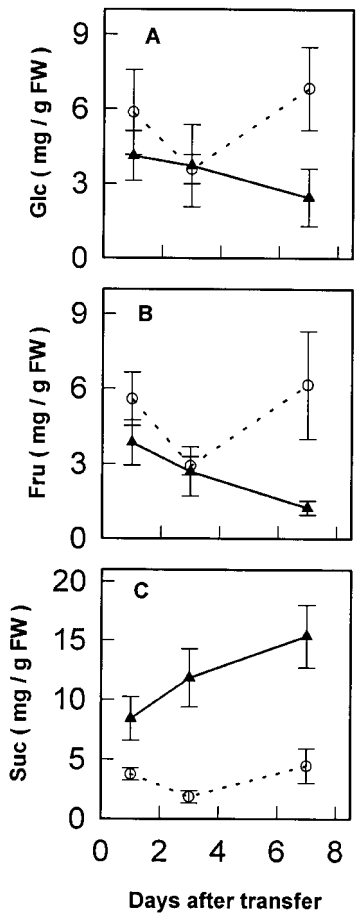

Fig. 2. Changes in the contents of Glc (A), Fru (B), and Suc (C) of sweet potato cells under sorbitol-stressed (▲) and normal (○) conditions. Each point is the mean ± SE of four independent experiments.

Expression of SPS gene

The levels of SPS mRNA in the normal and sorbitol-stressed cells were markedly different (Fig. 5). In the normal cells, the mRNA level rose during the 3 d period following the medium renewal, then declined rapidly. In contrast, the mRNA in the stressed cells continued to increase during the whole experimental period. A quantitative analysis showed that the SPS expression level in the stressed cells was six times higher than that of the normal on the 7th day after medium renewal.

Free sugars derived from absorbed ^{14}C-glucose

The rate of ^{14}C-glucose absorption by the sweet potato cells was greatly reduced under the osmotic-stressed condition. On the 8th day after medium renewal, normal cells took less than 30 min to absorb 50% of added ^{14}C-glucose, while the sorbitol-stressed cells took 180 min to absorb the same amount (Fig. 6). Consistent with the data in Fig. 1A and B, hexose levels in the stressed medium showed only a slow increase, suggesting that the hexose transport system in the stressed cells was partly inhibited.

The metabolism of absorbed ^{14}C-glucose was chased with cold sucrose absorbed from the normal or stressed media. The thin layer chromatograms of neutral ethanol extracts were shown in Fig. 7. The R_F values for glucose and sorbitol were very close in the cellulose plate (Merck, 5574) when developed with ethyl acetate : pyridine : H_2O, 20 : 7 : 5 (by vol.). In the same solvent system, sucrose and sorbitol had an approximately equal mobility in the silica

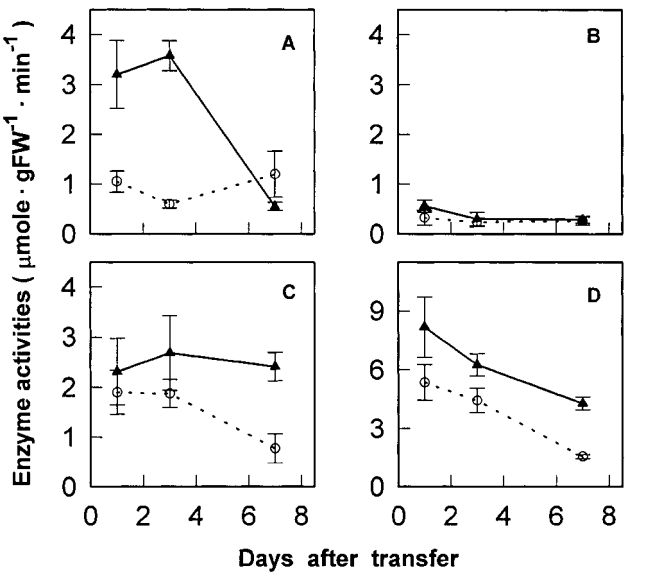

Fig. 3. Changes in the activities of enzymes involved in sucrose hydrolysis. The extracts were prepared from sweet potato cells under sorbitol-stressed (▲) and normal (○) conditions. (A) Cell wall-bound invertase; (B) acid invertase; (C) alkaline invertase; (D) sucrose synthase assayed in the direction of sucrose cleavage. Each point is the average of three independent experiments.

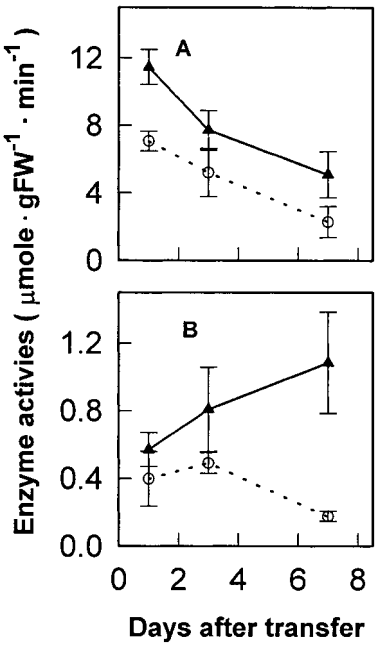

Fig. 4. Changes in the activities of enzymes involved in sucrose synthesis. The extracts were prepared from sweet potato cells grown under sorbitol-stressed (▲) and normal (○) conditions. (A) Sucrose synthase assayed in the direction of sucrose synthesis; (B) sucrose phosphate synthase. Each point is the average of three independent experiments.

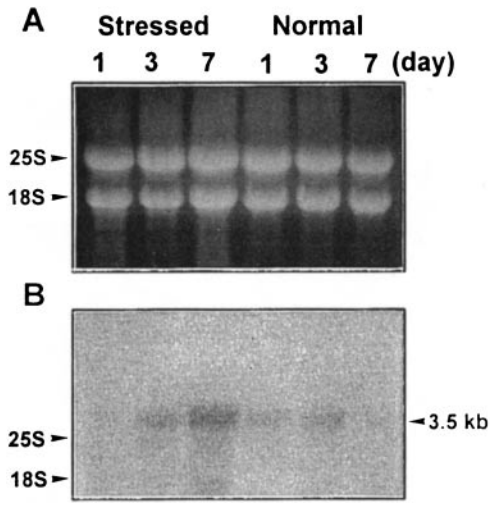

Fig. 5. RNA gel blot analysis for the expression of gene encoding SPS. RNA was isolated from sorbitol-stressed and normal sweet potato cells. Total RNA (20 μg) was separated by electrophoresis, blotted onto a nylon membrane, and hybridized with an SPS cDNA probe. (A) EtBr staining; (B) Northern-blot analysis.

plate (Merck, 5735), and their R_F values were significantly lower than those of glucose and fructose (data not shown). Hence, to separate glucose and fructose in the presence of a high concentration of sorbitol, such as the extract from sorbitol-stressed cells, the silica plate was the adsorbent of choice.

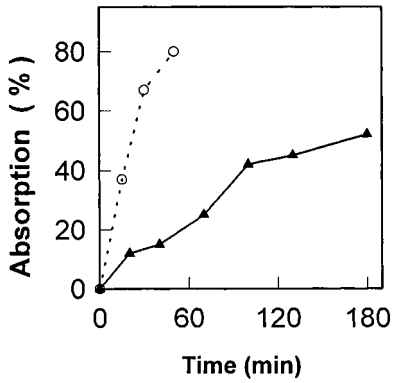

Fig. 6. [U-^{14}C]-glucose absorption efficiency of sweet potato cells. The cells were cultured under normal (▲) and sorbitol-stressed (○) conditions for 8 d, rapidly harvested and washed with respective sucrose-free medium by centrifugation for three times, then resuspended in [U-^{14}C]-glucose (about 10 μCi). At various time intervals, the residual radioactivity in the supernatant was measured.

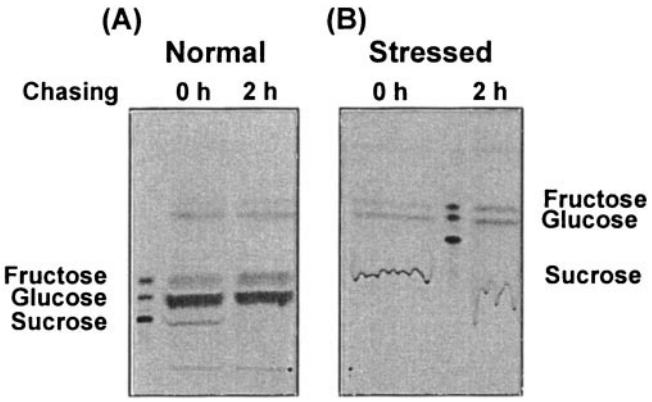

Fig. 7. Thin layer chromatograms of neutral fraction of ethanol extracts of sweet potato cells. Extracts were prepared from sweet potato cells cultured under normal (A) and sorbitol-stressed (B) conditions for 8 d, pulsed with [U-^{14}C]-glucose and chased with sucrose for different time. The TLC plates are cellulose (Merck 5574) for (A) and silica gel (Merck 5735) for (B), respectively.

Figure 7 showed that the distributions of ^{14}C in free glucose and fructose was significantly different between the normal and stressed cells. In normal cells, the radioactivity ratio of free-fructose to free-glucose was far below 1, or *c*. 0.3 (Fig. 7A), but close to 1, or from 0.8–0.9, in stressed cells (Fig. 7B). These results implied that the pathway of glucose converting into fructose was much facilitated under the stressed condition.

Due to the interference of a large amount of sorbitol, the chromatographic band of radio-labelled sucrose appeared irregular and had a tailing (Fig. 7B). In order to obtain pure sucrose from the ethanolic extract of stressed cells, the neutral extract must be developed on the cellulose thin layer plate, and the band migrating as sucrose scraped off, eluted, concentrated and chromatographed at least three times, thus rendering the quantitative recovery of sucrose impossible.

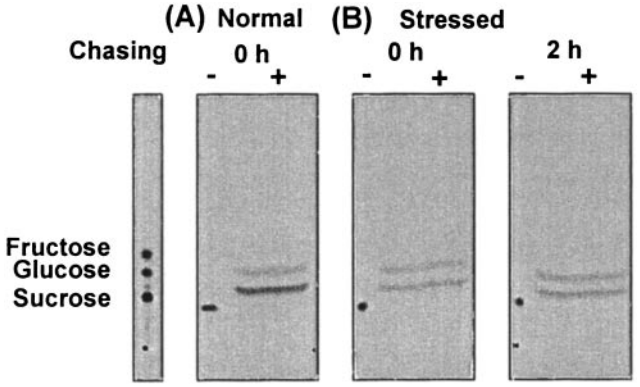

Fig. 8. Thin layer chromatograms of isolated sucrose incubated either without (−) or with (+) invertase. The labelled sucrose was extracted and isolated from sweet potato cells cultured under normal (A) and sorbitol-stressed (B) conditions for 8 d, pulsed with [U-^{14}C]-glucose and chased with sucrose for different times.

The isolated-labelled sucrose was hydrolysed with an IT. The glucose and fructose in the hydrolysate were separated by thin layer chromatography and quantitatively analysed in an image analyser. These data were compared with the distribution of radiocarbon in free glucose and fructose isolated from the same sample. Based on the rationale that the synthesis of sucrose via SPS will result in a symmetrical labelling of glucose and fructose moieties of sucrose, while in the SS-catalysed reaction the labelling will not be symmetrical, the contribution of SPS and SS on sucrose synthesis may be distinguished (Wendler *et al*., 1990; Geigenberger and Stitt, 1993). In the labelled sucrose isolated from 8-d-stressed cells, the distribution of ^{14}C in the two hexose moieties was quite similar, namely the ratio of ^{14}C in fructose to glucose was very close to 1 (Fig. 8B). This indicated that the irreversible SPS pathway was solely responsible for the sucrose synthesis in stressed cells. On the contrary, the ratio was 0.68 in the 8-d-normal cells (Fig. 8A). These data supported the view that the reversible SS pathway contributed partly to the sucrose synthesis under the normal condition. The relative contribution of SS to the unidirectional sucrose synthesis was estimated to be 32% according to the equation proposed earlier (Geigenberger and Stitt, 1993) which was based on the prediction that the fructosyl moiety in sucrose synthesized via SS could not be labelled with the radiocarbon.

Discussion

Sucrose serves as the carbon source in most heterotrophic cell suspension cultures, and the cells used here were no exception. Although sucrose disappears from the normal and sorbitol-stressed media at a similar rate (Fig. 1C), the cell growth was severely retarded under the stressed condition (Wang *et al*., 1999), showing that the fate of sucrose in the medium was altered by the osmotic stress.

Osmotic stress provoked a sharp increase of the cell-wall-bound IT activity (Fig. 3C). This result could explain the findings that the ratio of free fructose to glucose in the stressed medium was maintained close to 1, and that the cell growth was greatly retarded in the high osmotic stress medium. This explanation is offered by accepting the view that the extracellular IT facilitates an apoplastic cleavage of sucrose and the hydrolysis products are not utilized efficiently by the cells. Besides, the doubling in sugar molarity by hydrolysing a disaccharide into two monosaccharides also intensified the osmotic stress.

In rice calli, a high concentration of mannitol (0.6 M) also enhanced the activity of cell wall IT (Huang, 1998). An immunohistochemical study localized the bound IT in peripheral cells, especially on the side of cells attached to the solid culture medium. Except the osmotic stress, mechanical wounding (Sturm and Chrispeels, 1990; Zhang *et al*., 1996) and pathogen infection (Sturm and Chrispeels, 1990; Krishnan and Pueppke, 1988; Ehness *et al*., 1997) also induced the expression of cell-wall IT at the transcriptional and translational levels. In the pea phloem, an *in situ* hybridization study also showed that the activation of the cell wall IT gene was limited to the wounded area (Zhang *et al*., 1996). These observations seemed to indicate that the expression of an extracellular IT is likely to be a universal phenomenon in plant tissues or cells under adverse environmental conditions.

Extracellular IT was considered as a sink-specific enzyme which was important for determining the sink strength (Ho, 1988; Zhang *et al*., 1996; Ehneß and Roitsch, 1997; Ehness *et al*., 1997). This view agreed with previous observations that the stress-shocked cells accumulated a large amount of starch, typical energy storage in the sink (Wang *et al*., 1999).

A temporary starch accumulation took place within 3 d after renewing medium in normal cultures, but it disappeared rapidly as the sucrose level in the medium was depleted (Wang *et al*., 1999). This starch mobilization probably could account for the increase in sugar level at the 7th day of normal culture (Fig. 2). Therefore, even though the carbon source in normal medium was consumed almost entirely, cells at the 7th day after medium renewal were not in the starved condition. Cell wall-bound IT is typically present in plant cell suspension cultures (Dancer *et al*., 1990). Figure 3A showed that the level of bound IT activity slightly increased for cells transferred into normal condition at the 7th day. It is not clear whether the cell wall-bound IT contributed to this increase in hexose contents.

The accumulation of sucrose is one of the pronounced features for sweet potato cells exposed to a high osmotic condition (Fig. 2C). It has been reported that SPS is activated by an osmotic stress in spinach leaves (Zrenner and Stitt, 1991; Quick *et al*., 1989) and potato tubers (Reimhole *et al*., 1994; Geigenberger *et al*., 1997), and

caused an enhancement of sucrose synthesis. Moreover, it was shown the water stress-induced synthesis of sucrose was strongly inhibited in transgenic potato with reduced expression of SPS (Geigenberger *et al*., 1999). These data provided direct evidence that SPS regulates sucrose synthesis under a stress condition. Indeed, there are three pieces of evidence that strongly indicate that SPS has a predominant role of sucrose *de novo* synthesis for sorbitol-shocked sweet potato cells. First, the pattern of the increase of sucrose level (Fig. 2C) is consistent with the pattern of SPS activity changes (Fig. 4B). Second, in the sorbitol-stressed cells, the intra-molecular labelling of sucrose derived from ^{14}C-glucose was symmetric (Fig. 8B). Third, the Northern blot data also showed that SPS mRNA was more significantly expressed in cells continually exposed to osmotic stress (Fig. 5).

SPS is an enzyme under a complex regulation involving a fine control by allosteric effectors and a coarse control by protein phosphorylation (Huber and Huber, 1996). It has been postulated that, in spinach leaves, SPS is phosphorylated at Ser-424 during osmotic stress, resulting in antagonizing the inhibitory effect of phosphorylation of Ser-158, which is also a regulatory site (Toroser and Huber, 1997). In sweet potato cells, there is a close correspondence between the levels of gene transcript and activity of SPS. Whether the transcription or post-transcription is the major regulatory step for SPS expression remains to be investigated. However, since the enzyme activities extracted from both normal and stressed cells were not allosterically regulated by either inorganic phosphate or glucose 6-phosphate (data not shown), it may be said that the increase in the SPS activity was not due to the additional expression of an isozyme gene.

Sucrose can be degraded via SS or IT. Vacuole acid IT was considered to be important in regulating sucrose content in leaves (Huber, 1989; Scholes *et al*., 1996), or non-photosynthetic tissues (Miron and Schaffer, 1991; Ross and Davies, 1992; Zrenner *et al*., 1996). Figure 3B and C illustrated that the activity of alkaline IT was significantly higher than that of acid IT in both normal and stressed cells. Similar results have been obtained in cell suspension cultures of *Chenopodium rubrum* (Dancer *et al*., 1990) and *Saccharum sp*. (Wendler *et al*., 1990; Ebrahim *et al*., 1999) where the activity of soluble acid IT was barely detectable. Alkaline IT and SS are both located in the cytoplasm. For cells under the osmotic stress, the level of alkaline IT seemed to remain fairly constant (Fig. 3C) while that of SS showed a gradual decrease (Fig. 3D). These data suggested that alkaline IT rather than SS dominated the sucrose breakdown in cells exposed to an extended stress treatment. The declining of SS activity assayed in the direction of sucrose cleavage (Fig. 3D) probably contributed to a net increase of sucrose in stressed cells.

The sorbitol-stressed cells contain higher levels of sucrose-degrading and sucrose-synthesizing enzyme activities than that of the normal cells. These results implicate that the futile cycle of sucrose is an active process in the cytoplasm of stressed cells. In general, the cardinal feature of the futile cycle is to amplify the metabolic signal; a small change in either of the unidirectional fluxes will result in a much larger change in the net rate of carbon storage (Wendler *et al*., 1990; Geigenberger and Stitt, 1991; Geigenberger *et al*., 1997). Hence, it may be postulated that the physiological significance of a rapid cycling of sucrose for stressed-cells is an enhanced sensitivity to respond to the metabolic flux, thus enhancing the carbon partitioning in favour of sucrose accumulation for counteracting the osmotic stress condition.

In fact, the futile cycle of sucrose is also in operation in normal cells. The direct evidence is the rapid appearance of labelled sucrose under the condition of no net increase in sucrose, and the labelling of fructose in normal cells supplied with ^{14}C-glucose (Fig. 7A). The ^{14}C distribution in the sucrose isolated from 8 d normal cells is that the majority of radioactivity is present in the glucosyl moiety, resulting in an asymmetric labelling of sucrose (Fig. 8A). These data revealed that SS was partly involved in the sucrose synthesis and could explain why the radioactivity in free-fructose was very much less than in free-glucose (Fig. 7A). Compared with the symmetric labelling of sucrose in stressed cells, it is concluded that the osmotic stress markedly altered the pattern of sucrose metabolism by shutting down the reversible bypass of carbon flow through the SS catalysed system and enhanced the unidirectional flow through the irreversible SPS and IT catalysed pathway.

In rice grains, a tracer feeding study using a double labelled sucrose ([U-^{14}C]-D-glucosyl-[6-^{3}H]-D-fructoside) showed that SS participated in starch synthesis by supplying a sugar nucleotide precursor (Lee and Su, 1982). Many authors have found that the SS activity and/or the expression of SS gene had a positive correlation with the amount of starch in various plant systems. So, the activity of SS was considered as a biochemical marker for sink strength (Chourey, 1981; Heim *et al*., 1993; Wang *et al*., 1993; Zrenner *et al*., 1995; Déjardin *et al*., 1997*b*). However, a negative correlation was observed between SS activity and starch level in stressed sweet potato cells. This disparity may reflect that carbohydrate metabolism in osmotically stressed sweet potato cells is different from other plant systems. The fact that the deposition of starch and sucrose occurred simultaneously in stressed cells strongly implicated that not only the sucrose pools but the starch pool also turned over rapidly. What the biochemical mechanism is linking the sucrose and starch pools in sweet potato suspension cells under osmotic stress would be an interesting problem to study.

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