Changes in Sugars and Activities of Sucrose Phosphate Synthase, Sucrose Synthase, and Invertase during Potato Tuber (Russet Burbank) Reconditioning at 10 °C in Air and 2.53 kPa Oxygen after Storage for 28 Days at 1 °C

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ABSTRACT. Potato tubers (*Solanum tuberosum* 'Russet Burbank') were stored at 1 °C in air for 28 days and then transferred to 10 °C in either air or 2.53 kPa O₂. During cold storage there was an increase in sucrose, glucose, and fructose. The activities of extractable sucrose phosphate synthase (SPS) and invertase increased by 2.2- and 7.7-fold, respectively, during 28 days at 1 °C. The activity of sucrose synthase (SS) remained constant at 1 °C and was similar to that found in tubers kept continuously at 10 °C. With the transfer of tubers from 1 to 10 °C, there was an initial sharp rise in respiration which peaked at ≈7 days, followed by a gradual decline. Sucrose declined rapidly during reconditioning, while glucose and fructose declined more slowly. With the transfer of tubers from 1 to 10 °C, the activity of SS increased sharply after 7 days at 10 °C, to be followed by a decline to the levels found in control tubers. The activities of both extractable SPS and invertase decreased during reconditioning, reaching the values of the control tubers within ≈15 days. Low O₂ inhibited the decrease in sugars and suppressed the rise in SS activity, but it did not alter the decrease in SPS and invertase. Western blot analysis showed that the amount of SPS protein remained unchanged at 1 and 10 °C. These results indicate that the activity of SPS is regulated by factors other than the amount of its protein. The activities of the above three enzymes showed no changes in tubers kept at 10 °C continuously. In control tubers SPS showed the highest activity, followed by SS and invertase.

Storing potato tubers at low temperatures is highly desirable because it inhibits sprouting and reduces microbial spoilage and weight loss (Burton, 1982). Low temperature, however, induces an increase in sucrose, glucose, and fructose; the increase in hexoses is detrimental to the appearance of processed potato products (Burton, 1982; Sowokinos, 1990). As a result, most of the potato crop is stored at 8 to 10 °C, and sprouting is inhibited with chemicals such as chloroisopropyl carbamate (CIPC) and isopropyl-*N*-chlorophenyl carbamate (IPC). The use of these chemicals is under review and they may not be available in the near future. Breeding potato cultivars with diminished sweetening capacity has been only partially successful. Genetic engineering offers an alternative approach. An understanding of the regulation of sweetening at the biochemical and molecular levels will be useful in developing nonsweetening potato cultivars through genetic engineering or classical breeding.

The accumulation of sugars during potato storage at chilling temperatures has been investigated extensively (Barker, 1968; Coffin et al., 1987; Isherwood, 1973, 1976; Sowokinos, 1987, 1990). Yet, the regulation of sugar accumulation is still not well understood. An increase in sugar content in potatoes is also induced by a number of stresses besides cold, such as aging (Burton, 1982; Isherwood, 1976), ethylene and HCN treatments (Solomos and Laties, 1975), and a variety of cultural practices (Sowokinos, 1990). The available data indicate that, during sweetening at chilling temperatures, starch is the sole source for sugars and respiration (Isherwood, 1973) and the conversion of starch to

sugars is mediated via starch phosphorylase because a) sucrose is the first sugar to accumulate upon transfer of the tubers to chilling temperatures (Barker, 1968; Isherwood, 1973) and b) neither maltose nor polymers of glucose larger than maltose, the usual products of amylolytic degradation of starch (Preiss and Levi, 1979), show any increase during sweetening at low temperatures (Isherwood, 1973; Zhou, 1994). However, starch phosphorylase is present in large quantities during the development of potato tubers and there are no changes in its activity at chilling temperatures (Kennedy and Isherwood, 1975; Pollock and apRees, 1975). It is clear that factors other than starch phosphorylase regulate sucrose biosynthesis at low temperatures (Isherwood, 1976).

Reconditioning previously chilled potatoes at high temperatures is associated with a reduced sugar content (see Isherwood, 1973). Nevertheless, reconditioning does not always lower the concentration of reducing sugars to acceptable levels (Burton 1982). The enzymatic changes associated with reconditioning have not yet been investigated as extensively as those occurring during sweetening at low temperatures. The conversion of sugars to starch at high temperatures may, on the one hand, suppress the enzymes involved in sugar accumulation and, on the other hand, induce those that convert sucrose to starch.

It has been observed previously that 2.53 and 1.53 kPa O₂ decrease the rate of sugar accumulation attending storage of potatoes at chilling temperature (Sherman and Ewing, 1983; Zhou, 1994). Further, the inhibition by low O₂ of sugar accumulation coincides with the suppression of the induction or enhancement of proteins associated with starch–sugar interconversion. In addition, hypoxia completely inhibits the induction by cold of the alternative oxidase (Zhou, 1994). In fruit, hypoxia suppresses the expression of genes associated with fruit ripening (Kanellis et al., 1991) and the synthesis of novel mRNA in potatoes in response to wounding (Butler et al., 1990). The effect of reduced O₂ pressures on desweetening has not been investigated. If low O₂ inhibits desweetening, as in the case of the inhibition of sugar accumulation in the cold, it might also be expected to inhibit the rise in the

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activities of the enzymes that convert sucrose to starch. The effects of hypoxia may help us understand the mechanism of the starch-sucrose interconversion in potato tubers.

Materials and Methods

Freshly harvested potato tubers (*Solanum tuberosum* 'Russet Burbank') were shipped from Idaho to our laboratory. The tubers were washed, and tubers free of blemishes were selected and cured for 3 weeks at 10 °C under high relative humidity. From 20 to 25 tubers were put in desiccators and stored either at 1 or 10 °C. A humidified CO₂-free air stream of 50 to 60 mL·min⁻¹ was passed through the desiccators. After 28 d at 1 °C, the desiccators were transferred to 10 °C and connected to gas lines supplying either air or 2.58 kPa O₂. The rate of CO₂ output at 10 °C was monitored hourly by using a Horiba infrared gas analyzer. The samples were positioned through a Scannivalve, which was timed with an intervalometer.

SUGAR EXTRACTION AND DETERMINATION. At each sampling time, three tubers were picked at random and peeled. Ten-gram slices were dropped in 100 mL of 70% boiling ethanol. The subsequent extraction and determination of individual sugars were carried out as described by Warman and Solomos (1988). The rest of the tubers were sliced individually into liquid N₂ and powdered therein. The powder was stored at -70 °C and used for enzyme extraction.

EXTRACTION AND ASSAY OF SUCROSE PHOSPHATE SYNTHASE. Ten grams of frozen powder was allowed to thaw on ice in 5 mL extraction buffer consisting of 200 mM MOPS (pH 7.5), 20 mM MgCl₂, 2 mM EDTA, 10 mM mercaptoethanol, 2 mM PMSF, 0.4% diethyldithiocarbamic acid (DMTC), 0.4% NaHSO₃, and 1 g PVPP and homogenized using a polytron homogenizer with a 3-cm grinding probe (model 28S; Brinkman Inst., Piscataway, N.J.). The resultant slurry was filtered through eight layers of cheesecloth moistened with extraction buffer. The filtrate was centrifuged at 30,000 g_n for 30 min at 4 °C. An aliquot of the supernatant (2.5 mL) was loaded onto a prepacked gel filtration column (PD-10 Sephadex G-25 M; Pharmacia-LKB, Westbury, N.Y.) that had been equilibrated with elution buffer consisting of 50 mM MOPS (pH 7.5), 10 mM MgCl₂, 1 mM EDTA, 10% ethylene glycol, and 5 mM DTT. The first 3.5 mL was collected and used to determine SPS activity. The latter was determined, following the method of Walker and Huber (1989), by measuring the F6P-dependent synthesis of sucrose. Three reaction tubes were used for each sample with varying amounts of extract (5 to 15 µL) in a final volume of 70 µL. The results of all enzymatic assays were subjected to statistical treatment and the values of LSD and level of significance are indicated in the figures.

EXTRACTION AND ASSAY OF INVERTASE AND SS. Ten grams of frozen potato tissue powder was allowed to thaw on ice in 5 mL extraction buffer consisting of 100 mM Tris-maleate (pH 6.3) and 0.5% NaHSO₃. The subsequent procedures were identical to those described above for SPS. In desalting, the PD-10 column was equilibrated and eluted with 50 mM Tris-maleate buffer (pH 6.3).

Sucrose synthase cleavage activity was determined as described by Sowokinos et al. (1985) with slight modifications. Briefly, 1 mL reaction mixture contained 250 mM sucrose, 10 mM UDP, 40 mM Tris-phosphate buffer (pH 6.6), 10 mM

NaF, and extract containing ≤0.1 mg protein. Reaction blanks consisted of all reagents minus UDP and boiled extract. The reaction arrangements were similar to those of SPS. The reaction mixture was incubated at 30 °C for 20 min.

In determining invertase activity, the tubes containing the extract were vortexed for 30 min as a precaution to destroy the invertase inhibitor (Pressey, 1966). The reaction mixture consisted of 100 mM sodium acetate buffer (pH 5.0), 250 mM sucrose, and 10 mM NaF. The tubes were incubated at 37 °C for 1 h. The production of reducing sugars in SS and invertase was determined by the method of Nelson (1944). Blanks contained all reagents and boiled extract.

WESTERN BLOTTING. Tissue powder was extracted and desalted as described above for SPS. The proteins were separated on a nondenatured native gel consisting of 4% stacking and 7.5% resolving polyacrylamide. A volume of extract containing 30 µg protein from each sampling time was loaded onto the wells. Two gels were run simultaneously, and the proteins of one gel were electroblotted onto a nitrocellulose membrane. Vertical strips of the second gel, corresponding to individual lanes, were excised and incubated for 30 min in 7.5 mM MOPS buffer (pH 7.5). The strips were then cut into 1-mm-wide segments. The segments were inserted in small tubes and stirred with a glass rod to break them into smaller pieces. The activity of SPS present on the gel segments was assessed by incubating the strips at 30 °C for 1 h in a 70-µL solution containing 50 mM MOPS (pH 7.5), 10 mM MgCl₂, 25 mM UDP-G, and 10 mM F6P. In this way the location of SPS on the gels was identified. The nitrocellulose membranes were probed with an antibody raised against SPS, purified by successive column chromatography steps, and followed by gel electrophoresis on native-PAGE (Illeperuma, 1994). The antibody against the native protein (anti-NSPS) was further purified by binding it onto, and subsequently eluting it from, nitrocellulose strips containing a 120-kD polypeptide that was obtained from a SDS-PAGE of partially purified potato tuber protein (Illeperuma, 1994).

Results

RESPIRATION. A common feature of dormant potato tubers kept at 10 °C is that their rate of respiration remains constant until the growth of sprouts begins to contribute to CO₂ output (Barker,

Fig. 1. Rate of CO₂ output (µL CO₂/g/h) in potato tuber during reconditioning at 10 °C. Tubers were stored at 1 °C for 28 d, divided in two, and transferred to 10 °C either in air or 2.58 kPa O₂. One set of tubers was kept at 10 °C in air continuously.

1968; Isherwood, 1973; Sherman and Ewing, 1983; Zhou, 1994).

The conversion of sugars to starch is a sink for ATP use in dormant potato tubers (Isherwood, 1973). Substantial desweetening would, therefore, be expected to be accompanied by a rise in respiration. The results presented in Fig. 1 show that the rate of CO₂ output increases sharply with the transfer from 1 to 10 °C in air and then declines gradually to the level of tubers kept at 10 °C. Low O₂ suppresses the rise in respiration, which initially remains above that of tubers kept continuously at 10 °C (Fig. 1). A similar respiratory drift during desweetening has been reported previously (Isherwood, 1973).

SUGARS. In dormant potato tubers kept at nonchilling temperatures, the concentration of sugars is low until the tubers begin to senesce (Burton, 1982; Isherwood, 1973; Zhou, 1994). In the present study, in tubers kept at 10 °C continuously, the concentration of total simple sugars, i.e., sucrose, glucose, and fructose, was

≈0.32%. The concentration of individual sugars was 2, 7.2, and 6.8 μmol·g⁻¹·h⁻¹ for sucrose, glucose, and fructose, respectively. The value at -2 d in Fig. 2 corresponds to the samples taken 2 d before the transfer to 1 °C. Storage for 28 d at 1 °C resulted in about a 3-fold increase in the above three simple sugars (Fig. 2). Within 7 d after the transfer from 1 to 10 °C in air, sucrose declined sharply (Fig. 2C), while the decrease in hexoses was not as steep as in sucrose (Fig. 2 A and B). Low O₂ (2.53 kPa) inhibited the decrease in sucrose by 2.5-fold compared to air (Fig. 2C). In contrast the decrease in fructose and glucose was completely inhibited by 2.53 kPa O₂.

ENZYMES. In tubers kept for 30 d at 10 °C the activity of invertase remained low and showed no changes with time (Zhou, 1994). In the present study, the constant level of activities of SPS, SS, and acid invertase in tubers kept at 10 °C in air were 315, 114, and 33 nmol·h⁻¹·g⁻¹, respectively (Table 1).

The data presented in Table 1 and Fig. 3A show that storage for 28 d at 1 °C resulted in a 2.2-fold increase in SPS activity compared to control tubers kept at 10 °C. With transfer to 10 °C, the activity of SPS declined rapidly and within ≈15 d it dropped to its constant level at 10 °C in air (Table 1, Fig. 3A). The changes in the activity of SPS, however, were not reflected in the amount of protein, since western blot analysis did not indicate substantial changes in SPS protein content in all treatments (Fig. 4).

It has been mentioned that acid invertase activity was very low in tubers kept in air at 10 °C (Table 1). Storage for 28 d at 1 °C increased the extractable activity of acid invertase by 7-fold, from ≈33 to 210 nmol·h⁻¹·g⁻¹ (Table 1, Fig. 3B). During desweetening at 10 °C in air, the activity of acid invertase decreased in parallel with that of SPS to approach its constant level at 10 °C between 14 and 21 d (Fig. 3B). As in the case of SPS, low O₂ did not inhibit the decline in invertase activity (Fig. 3B).

In contrast to SPS and acid invertase, the activity of SS was not altered during storage at 1 °C for 28 d (Table 1, Fig. 3C). There was a sharp increase in SS activity upon transferring the tubers from 1 to 10 °C in air (Fig. 3C). The steep increase in SS activity was followed by a similar sharp decline, which reached its background activity at 10 °C in air within ≈15 d (Fig. 3C). Storage during desweetening under 2.53 kPa O₂ suppressed the rise in SS activity (Fig. 3C).

Discussion

Earlier work has shown that the increase in respiration following the transfer of potatoes from chilling to nonchilling temperatures is necessary for the conversion of sugars to starch (Isherwood, 1973). The results presented in Figs. 1 and 2 also show a close temporal relationship between the rise in CO₂ evolution and decline in sucrose content.

Sucrose synthase catalyzes a readily reversible reaction in vivo (Geigenberger and Stitt, 1993). The close association between SS activity and decrease in sucrose content (Figs. 2C and 3C) indicates that this enzyme may be important in the conversion of sucrose to starch. It should be noted that the activity of SS after 28 d at 1 °C, coincident with the peak values of the rates of sucrose synthesis

Table 1. Activities of sucrose phosphate synthase (SPS), sucrose synthase (SS), and invertase in tubers kept at 10 °C for 58 d.

Enzyme	Activity ^a (nmol·g ⁻¹ ·h ⁻¹)
SPS	318
SS	114
Invertase	33

^aLSD = 59, 18, and 7, respectively, for SPS, SS, and invertase at *P* = 0.05.

Fig. 2. Changes in sugars during reconditioning at 10 °C: (A) fructose, (B) glucose, (C) sucrose. Tubers were treated as in Fig. 1. Tubers that were kept at 10 °C in air continuously were analyzed 2 d before the transfer of the cold-treated tubers from 1 to 10 °C and at the end of the experiment. Each point represents three replications.

(Zhou, 1994), is similar to that of tubers kept at 10 °C (Table 1, Fig. 3). It is known that sugar content affects the expression of genes involved in starch synthesis (Salehuzzamam et al., 1994) and sucrose metabolism (Koch et al, 1992). In addition, Solanoubat and Belliard (1989) found that, in potato leaves and petioles, elevated sucrose levels enhanced SS. It is not clear whether the rise in SS activity during reconditioning is induced by the elevated sucrose concentration or is a response to changes in temperature.

The effect of temperature on the activity of SPS has not been unequivocally resolved. Some experimental results showed no change in SPS activity at chilling temperatures (Pollock and apRees, 1975), while Sowokinos (1987, 1990) observed that sweetening was attended by a rise in SPS activity in six potato

breeding lines. The present data show a 2.2-fold increase in SPS activity after 28 d at 1 °C, indicating that it may play an important role in sucrose synthesis at chilling temperatures. Reconditioning results in a rapid decrease in SPS activity (Fig. 3A). The changes in activity, however, are not reflected in the amount of protein (Fig. 4). It appears that, *in vivo*, the activity of SPS may be regulated by factors other than the amount of protein, as in photosynthetic tissues and potato tubers where SPS is subjected to fine and covalent modification control (Huber et al., 1989; Reimholtz et al., 1994; Walker and Huber, 1989).

Acid invertase catalyzes the hydrolysis of sucrose to glucose and fructose. Previous experimental results have been presented for and against a role for invertase controlling the level of reducing sugars at chilling temperatures (Pressey, 1969; Richardson et al., 1990). More recently, a very close temporal relationship between activity of acid invertase and levels of reducing sugars has been observed during sweetening of 'Russet Burbank' tubers at 1 °C. Further, the increase in acid invertase activity is associated with an induction of its mRNA, indicating that its synthesis is regulated at the transcriptional level (Zhou, 1994). The results presented in Table 1 and Fig. 3B also show that storing tubers for 28 d at 1 °C results in a 7-fold increase in acid invertase activity and that it decreases rapidly during reconditioning at 10 °C. In short, changes in acid invertase activity coincide with similar changes in the levels of reducing sugars during sweetening at 1 °C as well as during desweetening at 10 °C. It has been shown recently that a suppression of the induction of acid invertase by reverse genetics results in a simultaneous decrease in invertase activity and in the hexose to sucrose ratio during storage of potato tubers at chilling temperatures (Zrenner et al., 1996). The crucial role of invertase in the regulation of the levels of glucose and fructose has been amply demonstrated in tomatoes (Klann et al., 1993; Miron and Shaffer, 1991; Ohyama et al., 1995; Yelle et al., 1988). Since acid invertase plays such an important role in the synthesis of reducing sugars, its suppression at chilling temperatures may prevent their accumulation.

EFFECTS OF LOW O₂. Hypoxic conditions suppress the expression of genes associated with fruit ripening and wounding of potato tubers (Butler et al., 1990; Kanellis et al., 1991). Hypoxia also induces the synthesis of anoxic proteins in a number of plant tissues (Kanellis et al., 1991; Saglio et al., 1988). In 'Russet Burbank' tubers, 1.52 kPa O₂ inhibits sugar accumulation and induction of the alternative oxidase and invertase at chilling temperatures (Zhou, 1994). This suggests that holding potatoes under low O₂ during reconditioning may be a useful experimental approach in elucidating the mechanism of the regulation of sugar biosynthesis in potato tubers. It is well known that O₂ levels that induce partial anaerobiosis in fruit are highly detrimental (Kader, 1986; Solomos, 1994). To avoid the creation of partial anaerobiosis inside the tuber, we calculated the concentration of O₂ at the center of the tuber. We used the average values for O₂ diffusivity of 6.81×10^{-7} and 2.62×10^{-4} cm²·s⁻¹ through the skin and flesh, respectively, of 'Russet Burbank' tubers (Abdul-Baki and Solomos, 1994), a rate of respiration of 1.5 μL·g⁻¹·h⁻¹ (Fig. 1), and the following dimensions for an average tuber: 200 g mass, 2.75 cm radius, 12 cm length, and 0.012 cm skin thickness. By means of the method reported previously (Abdul-Baki and Solomos, 1994) we calculated that its partial pressure for a 2.5 kPa external O₂ at the center of the tuber is 2.02 kPa, which in turn results in a 42 μM O₂ aqueous solution (Hitchman, 1978), a concentration that is not expected to restrict plant mitochondrial terminal oxidases (Douce, 1985). Even at the peak of respiration (Fig. 1), the internal O₂ concentration is expected to be 1.65 kPa. No visible symptoms of

Fig. 3. Changes in the activities of (A) sucrose phosphate synthase, (B) acid invertase, and (C) sucrose synthase during reconditioning of potato tubers at 10 °C. Each point is the mean of three replications. Tubers were treated as in Fig. 2.

Fig. 4. Western blot of a 7.5% N-PAGE of crude potato extracts exposed to different storage conditions, visualized with anti-NSPS-120. Proteins (30 µg) were loaded into each lane, electrophoresed, transferred onto nitrocellulose membrane, and probed with anti-NSPS-120. Lane 1 = tubers stored continuously at 10 °C in air; lane 2 = tubers stored at 1 °C for 28 d in air; lanes 3 to 6 = tubers transferred from 1 °C to 10 °C and held in air for 5, 10, 20, and 30 d; lanes 7 to 10 = tubers transferred from 1 °C + 10 °C and held in 2% to 5% for 5, 12, 10, and 30 d.

anoxic injuries (Sherman and Ewing, 1983) were observed, and the rate of CO₂ output under 2.53 kPa O₂ was similar to that of the control tubers (Fig. 1), indicating that the tubers were not anaerobic. Thus, the inhibition by 2.53 kPa O₂ of the respiratory rise during reconditioning may not be ascribed to the restriction of cytochrome oxidase. We have also observed recently that there was no ethanol accumulation in tubers kept under 0.81 kPa O₂ at 4 °C for 60 d (unpublished observations).

It is clear from the preceding discussion that the suppression of the respiratory rise by low O₂ is not due to the curtailment of cytochrome oxidase but rather to its suppressive effects on tuber metabolism in general and inhibition of sugar conversion to starch, in particular. Low O₂ suppresses the rise in SS activity (Fig. 3C) but has no effect on the decline of either acid invertase or SPS activities (Fig. 3 A and B). It should be noted that the only difference between the effects of air and 2.53 kPa O₂ treatments on enzyme activities lies in the inhibition by the latter of the rise in SS (Fig. 3C), coincident with an inhibition of the decline in sucrose. This, in turn, supports a role for SS in the regulation of the conversion of sucrose to starch (Isherwood, 1973).

Low O₂ does not completely inhibit the initial decline in sucrose during the first 7 d at 10 °C (Fig. 3C). The lack of inhibition could be attributed to the high levels of invertase present at the time of transfer from 1 to 10 °C. It should also be pointed out that the low levels of sugars observed in tubers kept continuously at 10 °C in air coincide with a steady level of activity of the three enzymes (Table 1, Fig. 2).

In summary, the present data indicate that SS may play an important role in the regulation of the conversion of sugars to starch during reconditioning, because the prevention of its rise coincides with an inhibition of the decline in the sugar content. Further, in 'Russet Burbank' tubers, 2.53 kPa suppresses the induction or enhancement of SS but does not affect the decline of SPS and acid invertase, a response similar to that observed with the same tubers during sweetening at chilling temperatures where 1.53 kPa O₂ prevented the induction of acid invertase and alternative oxidase but did not affect the decrease in cytochrome oxidase that occurred in air (Zhou, 1994).

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