Journal of Experimental Botany, Vol. 54, No. 382,
Regulation of Carbon Metabolism Special Issue, pp. 457–465, January 2003
DOI: 10.1093/jxb/erg074

Journal of Experimental Botany

Regulation of sucrose to starch conversion in growing potato tubers

Peter Geigenberger[1]

Max-Planck-Institut für Molekulare Pflanzenphysiologie, Am Mühlenberg 1, D-14476 Golm, Germany

Received 29 August 2002; Accepted 27 September 2002

Abstract

Growing potato tubers have been used as a model system to investigate the regulation of starch synthesis. Results indicate that sucrose degradation and starch synthesis are controlled via regulatory signals in response to sucrose and oxygen availability. (i) Sucrose leads to a co-ordinated up-regulation of sucrose synthase and ADP-glucose phosphorylase at the transcriptional and post-transcriptional level. Transcriptional regulation of ADP-glucose phosphorylase leads to rapid changes in transcript levels, but relatively slow changes in protein levels. The rapid regulation of this enzyme in response to sucrose is mediated by a novel mechansism, involving redox-activation of ADPGlc pyrophosphorylase. Sucrose synthase is regulated via transcriptional regulation, but again the resulting changes in enzyme activity occur relatively slowly. More rapid changes in the flux of this enzyme follow due to rapid changes in the levels of uridine nucleotides. (ii) Internal oxygen concentrations fall to low levels in growing tubers, triggering a restriction of respiration, a decrease in the adenylate energy status, and a widespread decrease in metabolic and biosynthetic activity. These metabolic adaptations will allow oxygen consumption to be decreased and prevent the tissue from becoming anoxic. It will be discussed how these factors interact at different levels and different time-scales of control to regulate tuber metabolism in response to physiological and environmental inputs.

Key words: Potato, regulation, starch synthesis, sucrose degradation, oxygen, sucrose, nucleotides.

Introduction

Starch is the most widespread carbon reserve stored in plants and is of considerable industrial significance for food and non-food uses. Despite recent advances in the genetic analysis of starch biosynthesis, the regulation of this important process is still poorly understood. Attempts to improve starch yield were mainly hampered by the lack of a complete understanding of the factors and mechanisms regulating this pathway (Nelson and Pan, 1995; Fernie *et al*., 2002*a*).

To investigate the regulation of sucrose to starch conversion in more detail, growing potato tubers have been used as a model system. The reasons are that (i) unlike many other tissues the entry of sucrose into metabolism is relatively simple, in that it is unloaded symplastically from the phloem (Oparka and Prior, 1988; Viola *et al*., 2001) and degraded via sucrose synthase (Susy) (Morell and ap Rees, 1986; Zrenner *et al*., 1995), (ii) the pathway for the conversion of sucrose to starch is known (Fig. 1), the enzymes mediating it are well characterized and most of the genes that encode them have been cloned (Kruger, 1997), and (iii) transgenic plants can be readily produced (Fernie *et al*., 2002*a*). In the following it will be discussed how conversion of sucrose to starch is regulated in potato tubers, and what are the major physiological and environmental factors affecting this process.

Transcriptional regulation of starch synthesis

Various transgenic lines have been generated in which the activities of most of the individual enzymes involved in the pathway of sucrose to starch have been independently modulated. A 2–3-fold decrease in AGP-glucose pyrophosphorylase (AGPase) (Müller-Röber *et al*., 1992) or SuSy activity (Zrenner *et al*., 1995) in antisense transformants

[1] Fax: +49 331 5678408. E-mail: geigenberger@mpimp-golm.mpg.de

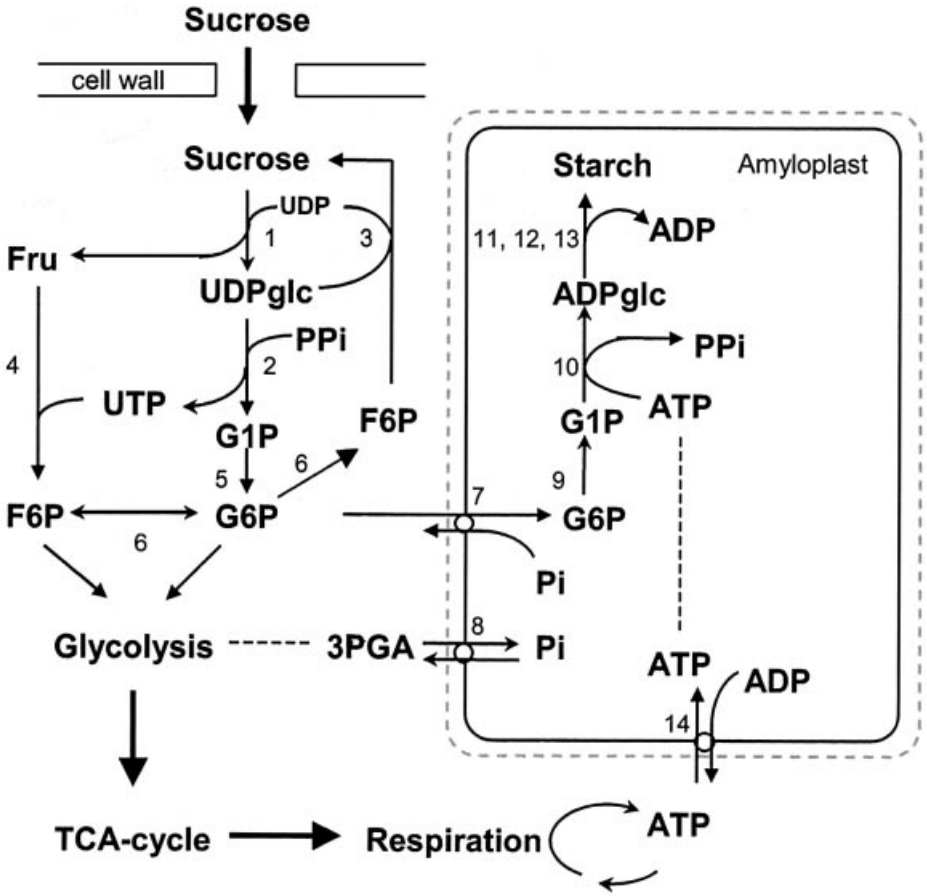

Fig. 1. Pathway of sucrose to starch conversion and its subcellular compartmentation in potato tubers. (1) Sucrose synthase, (2) UDP-glucose pyrophosphorylase, (3) sucrose-phosphate synthase, (4) fructokinase, (5) cytosolic phosphoglucomutase, (6) phosphoglucoisomerase, (7) hexose-phosphate translocator, (8) triose-phosphate translocator, (9) plastidial phosphoglucomutase, (10) ADP-glucose pyrophosphorylase, (11) soluble starch synthase, (12) granule-bound starch synthase, (13) branching enzyme, (14) adenylate translocator.

had no significant effect on tuber yield or starch synthesis. In analogous studies, a 30% decrease in plastidial phosphoglucomutase (Fernie *et al*., 2001*a*), or a more than 80% decrease of UDP-glucose pyrophosphorylase (Zrenner *et al*., 1993), sucrose-phosphate synthase (SPS) (Geigenberger *et al*., 1999), hexokinase (Veramendi *et al*., 1999, 2002), starch synthase (Marshall *et al*., 1996; Fulton *et al*., 2002), or branching enzyme (Safford *et al*., 1998) had no significant effect on starch accumulation in growing tubers. These studies show that the expression levels of individual enzymes of the pathway largely do not limit the flux from sucrose to starch, and demonstrate the need for co-ordinated mechanisms regulating starch synthesis at the transcriptional and post-transcriptional level.

Two key enzymes involved in sucrose to starch conversion are known to be subject to transcriptional regulation, SuSy and AGPase. SuSy, the first step in the pathway from sucrose to starch, catalyses the conversion of sucrose and UDP to UDPGlc and fructose via a readily reversible reaction (Geigenberger and Stitt, 1993), its expression being increased by sucrose, anaerobiosis and wounding (Salanoubat and Belliard, 1989; Zeng *et al*., 1998). AGPase catalyses the first committed step of starch biosynthesis in the plastid by converting Glc1P and ATP to ADPGlc and PPi. Its expression is increased by sucrose (Müller-Röber *et al*., 1990; Sokolov *et al*., 1998) and decreased by nitrate (Scheible *et al*., 1997) and phosphate (Nielsen *et al*., 1998). The levels of transcripts for *AGPB*, *AGPS* and *SUS1* are high in growing tubers, and both decrease rapidly after an interruption of assimilate supply, i.e. after detaching tubers (Ross and Davis, 1992; Tiessen *et al*., 2002). There are also parallel changes in AGPase and SuSy expression in response to diurnal changes of sucrose in tubers, the levels of both transcripts being high at the end of the light period and decrease at the end of the night (Geigenberger and Stitt, 2000). These results indicate a co-ordinated up-regulation of SuSy and AGPase in potato tubers in response to sucrose supply. The sensing and signalling mechanisms mediating this process are unknown. The finding that sucrose-induction of sucrose

synthase expression is reduced in SnRK1 antisense potato plants indicates that SNF1-related protein kinases are involved (Purcell *et al*., 1998).

Diurnal changes in AGPase and SuSy expression were, however, not accompanied by changes in the maximal activities of the encoded enzymes (Geigenberger and Stitt, 2000). Both activities also remained high for several days after detaching tubers, even though transcripts fell to low levels within 24 h (Geigenberger *et al*., 1994). This indicates that transcriptional regulation in response to sucrose allows only gradual changes in enzyme activity, which require up to days to develop. Further, large changes in *AGPB* and *SUS1* transcripts were required to produce a significant decrease in AGPase or SuSy activity in antisense potato transformants (Müller-Röber *et al*., 1992; Zrenner *et al*., 1995) indicating that on its own, transcriptional regulation is not an efficient method of altering pathway enzyme activities and fluxes.

Regulation of starch synthesis by changes in the levels of phosphorylated intermediates

A premium in the regulation of carbon metabolism in leaves is to maintain metabolite concentrations, which allow the fixation of carbon in the chloroplast. This requires rapid metabolic regulation in response to changes in light, CO_2 supply or sucrose export. Regulation is therefore occurring via regulatory circuits acting on key enzymes of photosynthetic metabolism in response to changes in metabolite levels (Stitt *et al*., 1987). In this context, a rising 3PGA/Pi ratio acts as a signal that fixed carbon is available beyond that required for sucrose synthesis. Starch synthesis is then stimulated, because AGPase is exquisitely sensitive to allosteric regulation, being activated by 3PGA and inhibited by Pi (Preiss, 1988).

AGPase from potato tubers resembles the leaf enzyme in being allosterically activated by 3PGA and inhibited by Pi (Sowokinos and Preiss, 1982). In contrast to leaves, however, the levels of phosphorylated intermediates in potato tubers are remarkably constant. There are only marginal changes in phosphorylated intermediates in the diurnal time-frame (Geigenberger and Stitt, 2000) or during tuber development (Merlo *et al*., 1993). The remarkable constancy of metabolite levels in tubers is mainly due to the operation of metabolic cycles in which the reversible reactions catalysed by SuSy and PFP play an important role (Hatzfeld and Stitt, 1990; Geigenberger and Stitt, 1993; Fernie *et al*., 2001*b*). These metabolic cycles allow large and rapid changes in the net rate of sucrose breakdown in response to the demand in the cell, even though the steady-state concentrations of metabolites hardly change.

During environmental perturbations like wounding (Hajirezaei and Stitt, 1991), water stress (Geigenberger *et al*., 1997), high temperature (Geigenberger *et al*., 1998), and hypoxia (Dixon and ap Rees, 1980; Geigenberger *et al*., 2000) this balance is disturbed and, consequently, large changes in metabolite levels occur in the tubers. During water stress, SPS is activated via protein phosphorylation and the subsequent stimulation of sucrose resynthesis leads to a decrease in metabolite levels including 3PGA (Geigenberger *et al*., 1997, 1999). A similar decrease in 3PGA levels takes place when respiration is stimulated in response to elevated temperatures (Geigenberger *et al*., 1998). In both cases the drop in 3PGA levels is accompanied by an inhibition of starch synthesis. The strong correlation between the levels of 3PGA and the levels of ADPGlc under these conditions provide evidence for the importance of the allosteric properties of potato AGPase for the regulation of starch synthesis *in vivo*. They operate to link the rate of starch synthesis to short-term changes in the balance between sucrose breakdown and respiration in potato tubers.

Redox-regulation of starch synthesis

Several situations have been reported in which changes in the rate of starch synthesis could not be explained by allosteric regulation or changes in expression of AGPase, including tuber detachment from the mother plant (Geigenberger *et al*., 1994), feeding sucrose to tuber discs (Geiger *et al*., 1998), diurnal changes in sucrose supply (Geigenberger and Stitt, 2000), and ectopic expression of sucrose phosphorylase in the cytosol of transgenic tubers (Trethewey *et al*., 2001). In all of these cases starch synthesis changed independently of overall AGPase activity, and reciprocally to the levels of phosphorylated intermediates, especially 3PGA.

Recent studies clarified that, under these conditions, starch synthesis is regulated by a novel mechanism, which involves post-translational redox-modification of AGPase (Tiessen *et al*., 2002). The site at which regulation occurs was clarified by analysis of the subcellular level of each metabolite between sucrose and starch using a non-aqueous fractionation technique (Farré *et al*., 2001), indicating AGPase as the only step whose substrates rise and mass-action ratios falls after detaching tubers. This was confirmed by substitution of higher plant AGPase with a heterologous bacterial AGPase in transgenic tubers, providing genetic evidence that the inhibitory mechanism requires the presence of a native AGPase.

The missing mechanism was subsequently clarified by the separation of extracts in non-reducing SDS gels revealing that the small subunit of the heterotetrameric AGPase (AGPB) was present as a mixture of monomers and dimers in growing tubers, and becomes completely dimerized in detached tubers (Tiessen *et al*., 2002). When activity was measured using a modified protocol omitting dithiothreitol in buffer preparations and allowing rapid

extraction and analysis, dimerization was accompanied by a decrease in enzyme activity due to changes in the kinetic properties of the enzyme, including a decrease in substrate affinities and sensitivity to allosteric effectors. Dimerization could be reversed and AGPase reactivated by incubating extracts (*in vitro*) or tuber slices (*in vivo*) with dithiothreitol. A similar activation of AGPase in response to dithiothreitol has been observed *in vitro* using heterologously over-expressed potato AGPase (Fu *et al*., 1998). In this case it could be shown that the enzyme is activated by the reduction and opening of an intermolecular disulphide bridge between the Cys12 of the two small subunits.

Redox-modulation of AGPase provides a novel mechanism that combines with allosteric and transcriptional control to co-ordinate AGPase activity in a network that allows starch synthesis to respond across a range of time-scales to a variety of physiological and environmental inputs (Fig. 2). Allosteric control by 3PGA and Pi operates in a time-frame of seconds to adjust the rate of starch synthesis to the balance between sucrose breakdown and respiration. Post-translational redox-modulation leads to changes in AGPase activity in a time-frame of about 30–60 min. Activation occurs in response to factors directly or indirectly related to an increased sucrose availability, and leads to stimulation of starch synthesis and a decrease in glycolytic metabolite levels (Tiessen *et al*., 2002). The signalling components leading to redox-modulation of AGPase are still unknown and may involve thioredoxins (Ballicora *et al*., 2000) as well as putative sugar sensors (Smeekens, 2000). Transcriptional regulation in response to changes of sucrose allows more gradual changes in AGPase activity, which requires several days to develop.

Relevance of nucleotide cofactors for sucrose to starch conversion and respiration

Attempts to understand the regulation of metabolism and to engineer metabolic fluxes have been dominated by investigations of the expression levels of enzymes and the role of their kinetic properties. Less attention has been paid to the possibility that metabolic fluxes may also be restricted by the levels of nucleotide cofactors. Sucrose degradation via the reversible reaction of SuSy requires uridine nucleotides (Geigenberger and Stitt, 1993). The *in vivo* concentration of UDP determined in the cytosol of potato tubers using non-aqueous fractionation (52–58 μM; Farré *et al*., 2001; Tiessen *et al*., 2002) is well below the

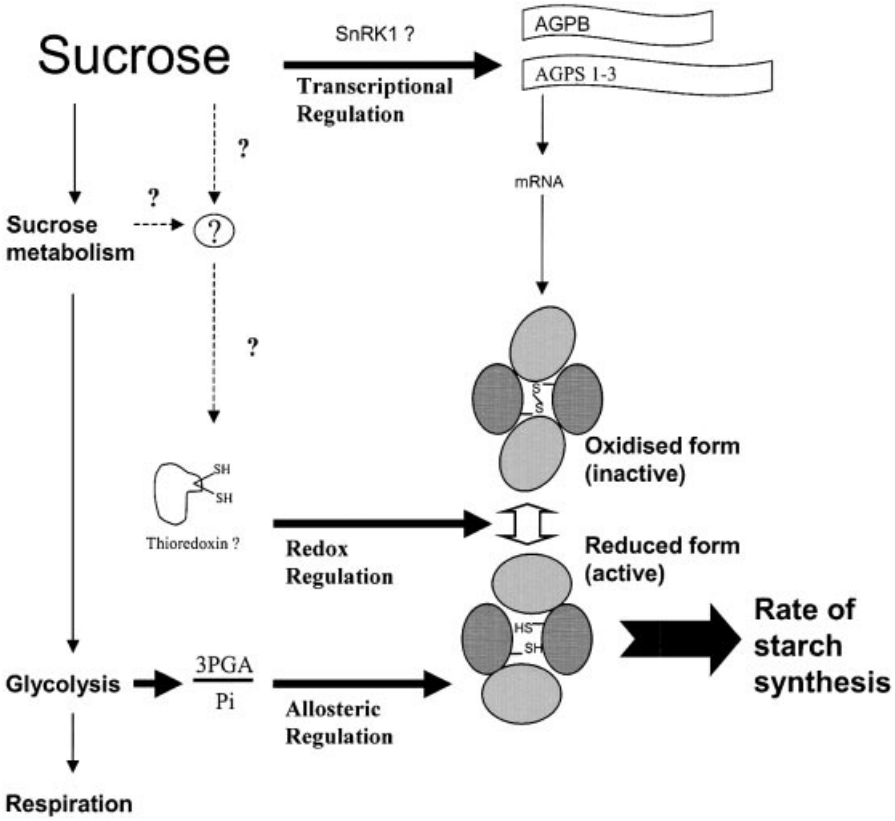

Fig. 2. ADP-glucose pyrophosphorylase regulates starch synthesis at different time scales and different levels of control in growing potato tubers (modified from Tiessen *et al*. (2002).

K_m of potato tuber SuSy for UDP (100–700 μM; Avigad, 1982), indicating that UDP levels might co-limit the rate of sucrose degradation. Recent experiments using potato tuber slices showed that sucrose degradation and starch synthesis are indeed stimulated when the overall uridine nucleotide pool is increased by feeding orotate, an intermediate of the *de novo* pathway of purine synthesis (Loef *et al*., 1999).

Conversion of sucrose to starch via fructokinase and AGPase requires ATP. The ATP required for the AGPase reaction is imported into the plastid via an ATP/ADP transport protein located on the inner-envelope membrane (Heldt, 1969; Kampfenkel *et al*., 1995). Recent studies with transgenic potato tubers showed that a relatively small decrease in ATP/ADP transporter activity leads to reduced levels of ADPGlc and total starch content and a lower amylose:amylopectin ratio, whereas increased transporter activity had the opposite effect (Tjaden *et al*., 1998; Geigenberger *et al*., 2001). These findings demonstrate that the rate of ATP import exerts considerable control on the rate of starch synthesis (control coefficient of *c*. 0.7) and also affects the molecular composition and morphology of starch granules in potato tubers. It also indicates that AGPase is ATP limited *in vivo*. This conclusion is further strengthened by subcellular analysis of the plastidial concentrations of ATP in growing wild-type potato tubers (80–179 μM; Farré *et al*., 2001; Tiessen *et al*., 2002), which are in the range of the K_m of potato tuber AGPase for ATP (120–190 μM; Sowokinos and Preiss, 1982; Ballicora *et al*., 1995). Recently, it was shown that when adenine is fed to tuber discs, there is an increase in ATP level and a stimulation of starch synthesis (Loef *et al*., 2001).

These studies show that sucrose breakdown and starch synthesis are restricted by the levels of adenine and uridine nucleotide cofactors. Increased adenine nucleotides, but not uridine nucleotides also lead to a stimulation of respiration, probably due to elevated levels of ADP (Loef *et al*., 2001). During the rapid starch accumulation phase in bulking tubers, increased expression of SuSy is accompanied by a marked increase of uridine nucleotides, but not of adenine nucleotides (Merlo *et al*., 1993; Loef *et al*., 1999). This allows sucrose mobilization to be increased, without a parallel and potentially counterproductive stimulation of respiration (see discussion below). Similar to the expression of SuSy, the increase in uridine nucleotide levels during tuber bulking could be linked to sucrose. There is a rapid decrease of uridine nucleotide levels in potato tuber slices incubated for 2 h in buffer, which can partially be prevented by adding sucrose (Loef *et al*., 2001; Geigenberger and Stitt, 2000). In intact tubers, diurnal changes in sucrose supply are accompanied by changes in uridine nucleotide levels, leading to increased levels of UDPGlc, UTP and UDP and an increased ratio of UDP/UTP in tubers when sucrose is high at the end of the light period (Geigenberger and Stitt, 2000). The rapidity of these changes in the uridine nucleotide levels indicate that post-transcriptional mechanisms are involved.

On the role of oxygen and sucrose as regulators of tuber metabolism

The results presented so far suggest a pivotal role of sucrose in regulating tuber metabolism at the transcriptional and post-transcriptional level to stimulate sucrose degradation and starch biosynthesis in response to sucrose supply (Fig. 3A). Recent studies, however, show that this concept has to be extended. By using oxygen microelectrodes it could be shown that oxygen falls to low levels (4–8% versus ambient levels of about 21%) inside bulking potato tubers (Geigenberger *et al*., 2000), which is in agreement with older studies summarized in Stiles (1960). The internal oxygen concentration decreases with increasing tuber size. There are marked gradients of oxygen inside growing tubers, with typical values being 8–10% in the periphery and 2–5% in the centre of the tuber. The decrease in oxygen tension from the periphery towards the centre of the tuber is accompanied by a decrease in the ATP/ADP ratio and the cellular adenylate energy charge indicating an inhibition of respiration (Geigenberger *et al*., 2000), as well as by a decrease in starch level indicating an inhibition of starch biosynthesis (P Geigenberger, AR Fernie, unpublished results). Crucially, this decrease in the adenylate status is not accompanied by lactate accumulation, and occurs at oxygen concentrations that are 100-fold above the K_m (O_2) of cytochrome oxidase.

These results were confirmed and extended in an independent approach in which tuber discs were incubated in various oxygen concentrations from 0–40% using premixed gases (Geigenberger *et al*., 2000). There was a continuous decrease in energy state and glycolytic flux when external oxygen was decreased from 40% to 1%. Also respiration rates were shown to decrease when external oxygen fell from 21% (ambient level) to 4% or 8%. Labelling studies demonstrated that starch synthesis and other biosynthetic fluxes were inhibited at 4–8% oxygen. This was, however, not due to fermentation, since there was no increase of lactate under these oxygen concentrations. Oxygen had to be decreased below 1% before glycolysis was stimulated and lactate accumulated due to the onset of fermentation. The responses to low oxygen and anoxia are, therefore, diametrically opposed. Similar oxygen gradients and changes in response to external oxygen have been reported to occur in seeds from *Arabidopsis* (Porterfield *et al*., 1999; Gibon *et al*., 2002) and pea (Rolletscheck *et al*., 2002).

It is an interesting question why plants, unlike multicellular animals, did not evolve specialized circulation systems to allow a more efficient transport of gases to internal tissues. However, the above studies suggest that

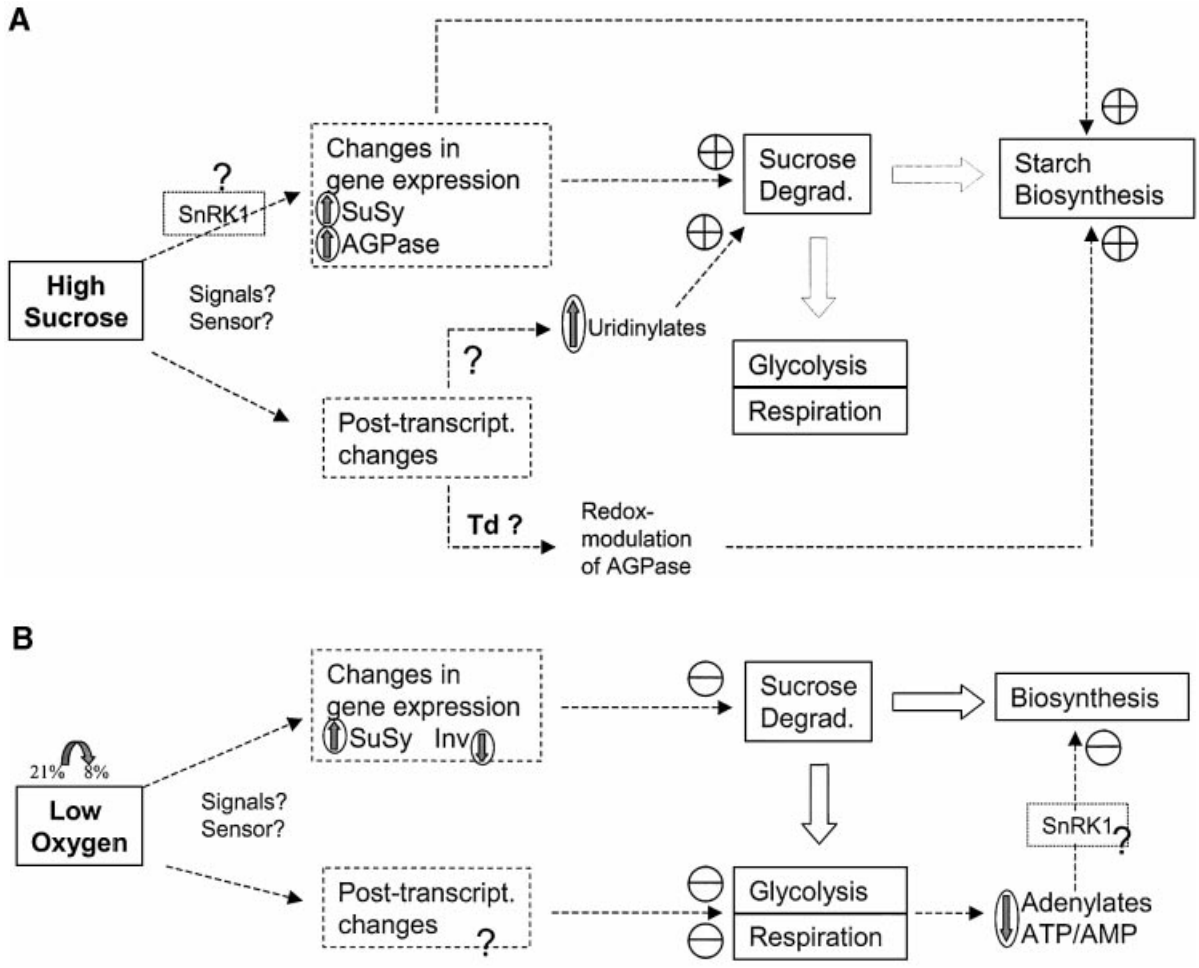

Fig. 3. Regulation of potato tuber metabolism in response to sucrose (A) and oxygen supply (B). Td, thioredoxin; SnRK1, sucrose-non-fermenting-1-related kinase.

falling internal oxygen tensions trigger metabolic adaptations, including a restriction of respiration, a decrease in adenylate levels and a widespread decrease in metabolic and biosynthetic activity, which will allow oxygen consumption to be decreased and prevent tissue from becoming anoxic (Fig. 3B). Less is known about the mechanisms involved in the scenario. It is highly unlikely that cytochrome oxidase (K_m for oxygen is 0.01%) is directly limited by oxygen in this concentration range (4–40%), which suggests that an oxygen sensor may be involved. More studies are needed to investigate how oxygen is sensed in plants, and what are the factors leading to the adaptive changes in metabolism.

This premium on biochemical adaptations to conserve energy and allow oxygen consumption to be decreased may explain the unusual use of PPi as an alternative energy donor in plants (Sonnewald, 1992; Stitt, 1998). Whereas breakdown of a molecule of sucrose via invertase requires 2 molecules of ATP, breakdown of sucrose via SuSy and UGPase requires only 1 molecule of PPi. In seeds and tubers, invertase is typically expressed during the early stages and SuSy during the later stages of development (Appeldoorn *et al*., 1997; Weber *et al*., 1997). This switch correlates with a transition from cell division to cell expansion and storage and has initially been interpreted in terms of regulation by sugar-related signals (Borisjuk *et al*., 1998). An alternative explanation would be that it is related to the decrease in oxygen tension that develops as seeds and tubers grow. As observed in maize roots (Zeng *et al*., 1999) and potato tubers (K Bologa, AR Fernie, P Geigenberger, unpublished results) low oxygen repress invertase and induce specific SuSy genes.

Intriguingly, ectopic expression of invertase (Trethewey *et al*., 1998) or sucrose phosphorylase (Trethewey *et al*., 2001) to bypass the endogenous sucrose synthase route in potato tubers leads to a decrease in tuber sucrose levels, but also to a large stimulation of respiration, a fall in the internal oxygen levels down to almost zero and a decrease in the cellular energy state (Fernie *et al*., 2002*b*; K Bologa, AR Fernie, P Geigenberger, unpublished data). These

results indicate that the inhibition of starch synthesis in invertase and sucrose phosphorylase expressing tubers is attributable to two different factors: (i) the decreased levels of sucrose (see above) and (ii) the increased energy consumption due to introduction of an alternative pathway of sucrose degradation. This also implies that sucrose degradation via sucrose synthase is important to maintain a relatively large sucrose pool and to minimize ATP consumption required for normal metabolic function in the wild type.

Acknowledgements

I wish to thank Mark Stitt for his support, stimulating discussions, and helpful comments on the manuscript. I am grateful to Axel Tiessen, Janneke HM Hendriks, Yves Gibon, Karin Bologa, Alisdair R Fernie, Eva M Farré, Richard N Trethewey, Ralph Reimholz, and Michael Geiger for their contributions to cooperative work. Work from the author's laboratory has been supported by grants from the Deutsche Forschungsgemeinschaft (Ge 878/1-1 & 1-3).

References

Appeldoorn NJG, de Bruijn SM, Koot-Gronsveld EAM, Visser RGF, Vreugdenhil D, van der Plas LHW. 1997. *Developmen*tal changes of enzymes involved in conversion of sucrose to hexose-phosphate during early tuberization of potato. *Planta* **202,** 220–226

Avigad G. 1982. Sucrose and other disaccharides. In: Loewus TA, Tanner W, eds. *Encyclopedia of plant physiology*. Heidelberg: Springer, 217–347.

Ballicora MA, Laughlin MJ, Fu Y, Okita TW, Barry GF, Preiss J. 1995. Adenosine 5′-diphosphate-glucose pyrophosphorylase from potato tuber. Significance of the N terminus of the small subunit for catalytic properties and heat stability. *Plant Physiology* **109,** 245–251.

Ballicora MA, Frueauf JB, Fu Y, Schürmann P, Preiss J. 2000. Activation of the potato tuber ADP glucose pyrophosphorylase by thioredoxin. *Journal of Biological Chemistry* **275,** 1315–1320.

Borisjuk L, Walenta S, Weber H, Mueller-Kliesig W, Wobus U. 1998. High resolution mapping of glucose concentrations in developing cotyledons of *Vicia faba* in relation to mitotic activity and storage processes: glucose as a possible developmental trigger. *The Plant Journal* **15,** 583–591.

Dixon WL, ap Rees T. 1980. Identification of the regulatory steps in glycolysis in potato tubers. *Phytochemistry* **19,** 1297–1301.

Farré EM, Tiessen A, Roessner U, Geigenberger P, Trethewey RN, Willmitzer L. 2001. Analysis of the compartmentation of glycolytic intermediates, nucleotides, sugars, amino acids, and sugar alcohols in potato tubers using a non-aqueous fractionation method. *Plant Physiology* **127,** 685–700.

Fernie AR, Roessner U, Trethewey RN, Willmitzer L. 2001*a*. The contribution of plastidial phosphoglucomutase to the control of starch synthesis within the potato tuber. *Planta* **213,** 418–426.

Fernie AR, Roscher A, Ratcliffe RG, Kruger NJ. 2001*b*. Fructose 2,6-bisphosphate activates pyrophosphate: fructose-6-phosphate:1-phosphotransferase and increases triose-phosphate to hexose-phosphate cycling in heterotrophic cells. *Planta* **212,** 250–263.

Fernie AR, Tiessen A, Stitt M, Willmitzer L, Geigenberger P. 2002*b*. Altered metabolic fluxes result from shifts in metabolite levels in sucrose phosphorylase expressing potato tubers. *Plant, Cell and Environment* **25,** 1219–1232.

Fernie AR, Willmitzer L, Trethewey RN. 2002*a*. Sucrose to starch: a transition in molecular plant physiology. *Trends in Plant Science* **7,** 35–41.

Fu Y, Ballicora MA, Leykam JF, Preiss J. 1998. Mechanism of reductive activation of potato tuber ADP-glucose pyrophosphorylase. *Journal of Biological Chemistry* **273,** 25045–25052.

Fulton DC, Edwards A, Pilling E, *et al*. 2002. Role of granule-bound starch synthase in determination of amylopectin structure and starch granule morphology in potato. *Journal of Biological Chemistry* **277,** 10834–10841.

Geigenberger P, Fernie AR, Gibon Y, Christ M, Stitt M. 2000. Metabolic activity decreases as an adaptive response to low internal oxygen in growing potato tubers. *Biological Chemistry* **381,** 723–740.

Geigenberger P, Geiger M, Stitt M. 1998. High-temperature inhibition of starch synthesis is due to inhibition of ADPGlc pyrophosphorylase by decreased levels of 3PGA in growing potato tubers. *Plant Physiology* **117,** 1307–1317.

Geigenberger P, Reimholz R, Deiting U, Sonnewald U, Stitt M. 1999. Decreased expression of sucrose phosphate synthase strongly inhibits the water stress-induced synthesis of sucrose in growing potato tubers. *The Plant Journal* **19,** 119–129.

Geigenberger P, Merlo L, Reimholz R, Stitt M. 1994. When growing potato tubers are detached from their mother plant there is a rapid inhibition of starch synthesis, involving inhibition of ADP-glucose pyrophosphorylase. *Planta* **193,** 486–493.

Geigenberger P, Reimholz R, Geiger M, Merlo L, Canale V, Stitt M. 1997. Regulation of sucrose and starch metabolism in potato tubers in response to short-term water deficit. *Planta* **201,** 502–518.

Geigenberger P, Stamme C, Tjaden J, Schulz A, Quick PW, Betsche T, Kersting HJ, Neuhaus HE. 2001. Tuber physiology and properties of starch from tubers of transgenic potato plants with altered plastidic adenylate transporter activity. *Plant Physiology* **125,** 1667–1678.

Geigenberger P, Stitt M. 1993. Sucrose synthase catalyses a readily reversible reaction in developing potato tubers and other plant tissues. *Planta* **189,** 329–339.

Geigenberger P, Stitt M. 2000. Diurnal changes in sucrose, nucleotides, starch synthesis, and *AGPS* transcript in growing potato tubers that are suppressed by decreased expression of sucrose phosphate synthase. *The Plant Journal* **23,** 795–806.

Geiger M, Stitt M, Geigenberger P. 1998. Metabolism in potato tuber slices responds differently after addition of sucrose and glucose. *Planta* **206,** 245–252.

Gibon Y, Vigeolas H, Tiessen A, Geigenberger P, Stitt M. 2002. Sensitive and high throughput metabolite assays for inorganic pyrophosphate, ADPGlc, nucleotide phosphates, and glycolytic intermediates based on a novel enzymic cycling system. *The Plant Journal* **30,** 221–235.

Hajirezaei MR, Stitt M. 1991. Contrasting roles for pyrophosphate: fructose-6-phosphate phosphotransferase during aging of tissues from potato tubers and carrot storage tissues. *Plant Science* **77,** 177–183.

Hatzfeld WD, Stitt M. 1990. A study of the rate of recycling of triose phosphates in heterotrophic *Chenopodium rubrum* cells, potato tubers and maize endosperm. *Planta* **180,** 198–201.

Heldt HW. 1969. Adenine nucleotide translocation in spinach chloroplasts. *FEBS Letters* **5,** 11–14.

Kampfenkel K, Möhlmann T, Batz O, van Montagu M, Inzé D, Neuhaus HE. 1995. Molecular cloning of an *Arabidopsis thaliana* cDNA encoding a novel putative adenylate translocator of higher plants. *FEBS Letters* **374,** 351–355.

Kruger NJ. 1997. Carbohydrate synthesis and degradation. In: Dennis DT, Turpin DH, Lefebvre DD, Layzell DB, eds. *Plant metabolism*. Harlow, UK: Longman, 83–104.

Loef I, Stitt M, Geigenberger P. 1999. Feeding orotate leads to a specific increase in uridine nucleotide levels, resulting in a stimulation of sucrose degradation and starch synthesis in discs of growing potato tubers. *Planta* **209,** 314–323.

Loef I, Stitt M, Geigenberger P. 2001. Increased adenine nucleotide levels modify the interaction between respiration and starch synthesis when adenine is fed to discs of growing potato tubers. *Planta* **212,** 782–791.

Marshall J, Sidebottom C, Debet M, Martin C, Smith AM, Edwards A. 1996. Identification of the major starch synthase in the soluble fraction of potato tubers. *The Plant Cell* **8,** 1121–1135.

Merlo L, Geigenberger P, Hajirezaei M, Stitt M. 1993. Changes of carbohydrates, metabolites and enzyme activities in potato tubers during development, and within a single tuber along a stolon–apex gradient. *Journal of Plant Physiology* **142,** 392–402.

Morell S, ap Rees T. 1986. Sucrose metabolism in developing tubers of *Solanum tuberosum*. *Phytochemistry* **25,** 1579–1585.

Müller-Röber BT, Kossmann J, Hannah LC, Willmitzer L, Sonnewald U. 1990. One of two different ADP-glucose pyrophosphorylase genes from potato responds strongly to elevated levels of sucrose. *Molecular and General Genetics* **224,** 136–146.

Müller-Röber BT, Sonnewald U, Willmitzer L. 1992. Inhibition of ADP-glucose pyrophosphorylase leads to sugar storing tubers and influences tuber formation and expression of tuber storage protein genes. *EMBO Journal* **11,** 1229–1238.

Nelson O, Pan D. 1995. Starch synthesis in maize endosperms. *Annual Review of Plant Physiology and Plant Molecular Biology* **46,** 475–496.

Nielsen TH, Krapp A, Röper-Schwarz U, Stitt M. 1998. The sugar-mediated regulation of genes encoding the small subunit of Rubisco and the regulatory subunit of ADP glucose pyrophosphorylase is modified by nitrogen and phosphate. *Plant, Cell and Environment* **21,** 443–455.

Oparka KJ, Prior DAM. 1988. Movement of Lucifer Yellow CH in potato tuber storage tissues: a comparison of symplastic and apoplastic transport. *Planta* **176,** 533–540.

Porterfield DM, Kuang A, Smith PJS, Crispi ML, Musgrave ME. 1999. Oxygen-depleted zones inside reproductive structures of Brassicaceae: implications for oxygen control of seed development. *Canadian Journal of Botany* **77,** 1439–1446.

Preiss J. 1988. Biosynthesis of starch and its regulation. In: Preiss J, ed. *The biochemistry of plants*, Vol. 14. San Diego, California: Academic Press, 181–254.

Purcell PC, Smith AM, Halford NG. 1998. Antisense expression of a sucrose non-fermenting-1-related protein kinase sequence in potato results in decreased expression of sucrose synthase in tubers and loss of sucrose-inducibility of sucrose synthase transcripts in leaves. *The Plant Journal* **14,** 195–202.

Rolletscheck H, Borisjuk L, Koschorreck M, Wobus U, Weber H. 2002. Legume embryos develop in a hypoxic environment. *Journal of Experimental Botany* **53,** 1–9.

Ross HA, Davies HV. 1992. Sucrose metabolism in tubers of potato (*Solanum tuberosum* L.). *Plant Physiology* **98,** 297–293.

Safford R, Jobling SA, Sidebottom CM, Westcott RJ, Cooke D, Tober KJ, Strongitharm BH, Russell A, Gidley MJ. 1998. Consequences of antisense RNA inhibition of starch branching enzyme activity on properties of potato starch. *Carbohydrate Polymer* **35,** 155–168.

Salanoubat M, Belliard G. 1989. The steady-state level of potato sucrose synthase mRNA is dependent on wounding, anaerobiosis and sucrose. *Gene* **84,** 181–185.

Scheible WR, González-Fontes A, Lauerer M, Müller-Röber B, Caboche M, Stitt M. 1997. Nitrate acts as a signal to induce organic acid metabolism and repress starch metabolism in tobacco. *The Plant Cell* **9,** 783–798.

Smeekens S. 2000. Sugar-induced signal transduction in plants. *Plant Molecular Biology* **51,** 49–81.

Sokolov LN, Dejardin A, Kleczkowski LA. 1998. Sugars and light/dark exposure trigger differential regulation of ADP-glucose pyrophosphorylase genes in *Arabidopsis thaliana* (thale cress). *Biochemical Journal* **336,** 681–687.

Sonnewald U. 1992. Expression of *E. coli* inorganic pyrophosphatase in transgenic plants alters photoasimilate partitioning. *The Plant Journal* **2,** 571–581.

Sowokinos JR, Preiss J. 1982. Phosphorylases in *Solanum tuberosum*. III. Purification, physical and catalytical properties of ADP-glucose pyrophosphorylase in potatoes. *Plant Physiology* **69,** 1459–1466.

Stiles W. 1960. The composition of the atmosphere (oxygen content of air, soil, intercellular spaces, diffusion, carbon dioxide and oxygen tension). In: Ruhland W, ed. *Encyclopedia of plant physiology, plant respiration inclusive fermentations and acid metabolism*, Vol. XII (Part 2). Heidelberg: Springer Verlag, 114–148.

Stitt M, Huber S, Kerr P. 1987. Control of photosynthetic sucrose synthesis. In: Hatch MD, Boardman NK, eds. *The biochemistry of plants,* Vol. 10. Academic Press, 327–409.

Stitt M. 1998. Pyrophosphate as an energy donor in the cytosol of plant cells: an enigmatic alternative to ATP. *Botanica Acta* **111,** 167–175.

Tiessen A, Hendriks JHM, Stitt M, Branscheid A, Gibon Y, Farré EM, Geigenberger P. 2002. Starch synthesis in potato tubers is regulated by post-translational redox-modification of ADP-glucose pyrophosphorylase: a novel regulatory mechanism linking starch synthesis to the sucrose supply. *The Plant Cell* **14,** 2191–2213.

Tjaden J, Möhlmann T, Kampfenkel K, Henrichs G, Neuhaus HE. 1998. Altered plastidic ATP/ADP-transporter activity influences potato (*Solanum tuberosum* L.) tuber morphology, yield and composition of starch. *The Plant Journal* **16,** 531–540.

Trethewey RN, Geigenberger P, Hajirezaei M, Sonnewald U, Stitt M, Riesmeier J, Willmitzer L. 1998. Combined expression of glucokinase and invertase in potato tubers leads to a dramatic reduction in starch accumulation and a stimulation of glycolysis. *The Plant Journal* **15,** 109–118.

Trethewey RN, Fernie AR, Bachmann A, Fleischer-Notter H, Geigenberger P, Willmitzer L. 2001. Expression of a bacterial sucrose phosphorylase in potato tubers results in a glucose-independent induction of glycolysis. *Plant, Cell and Environment* **24,** 357–365.

Veramendi J, Fernie AR, Leisse A, Willmitzer L, Trethewey RN. 2002. Potato hexokinase 2 complements transgenic *Arabidopsis* plants deficient in hexokinase 1 but does not play a key role in tuber carbohydrate metabolism. *Plant Molecular Biology* **49,** 491–501.

Veramendi J, Roessner U, Renz A, Willmitzer L, Trethewey RN. 1999. Antisense repression of hexokinase 1 leads to an overaccumulation of starch in leaves of transgenic potato plants but not to significant changes in tuber carbohydrate metabolism. *Plant Physiology* **121,** 1–11.

Viola R, Roberts AG, Haupt S, Gazzani S, Hancock RD, Marmiroli N, Machray GC, Oparka KJ. 2001. Tuberization in potato involves a switch from apoplatic to symplastic phloem unloading. *The Plant Cell* **13,** 385–398.

Weber H, Borisjuk L, Wobus U. 1997. Sugar import and metabolism during seed development. *Trends Plant in Science* **2,** 169–174.

Zeng Y, Wu Y, Avigne WT, Koch KE. 1998. Differential regulation of sugar-sensitive sucrose synthases by hypoxia and anoxia indicate complementary transcriptional and post-transcriptional regulation. *Plant Physiology* **116,** 1573–1583.

Zeng Y, Wu Y, Avigne WT, Koch KE. 1999. Rapid repression of maize invertases by low oxygen. Invertase/sucrose synthase balance, sugar signaling potential, and seedling survival. *Plant Physiology* **121,** 599–608.

Zrenner R, Willlmitzer L, Sonnewald U. 1993. Analysis of the expression of potato uridinediphosphoglucose pyrophospharylase and its inhibition by antisense RNA. *Planta* **190,** 247–252.

Zrenner R, Salanoubat M, Willmitzer L, Sonnewald U. 1995. Evidence of the crucial role of sucrose synthase for sink strength using transgenic potato plants. *The Plant Journal* **7,** 97–107.