Involvement of soluble sugars in reactive oxygen species balance and responses to oxidative stress in plants

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Received 16 September 2005; Accepted 24 October 2005

Abstract

Soluble sugars, especially sucrose, glucose, and fructose, play an obviously central role in plant structure and metabolism at the cellular and whole-organism levels. They are involved in the responses to a number of stresses, and they act as nutrient and metabolite signalling molecules that activate specific or hormone-crosstalk transduction pathways, thus resulting in important modifications of gene expression and proteomic patterns. Various metabolic reactions and regulations directly link soluble sugars with the production rates of reactive oxygen species, such as mitochondrial respiration or photosynthesis regulation, and, conversely, with anti-oxidative processes, such as the oxidative pentose-phosphate pathway and carotenoid biosynthesis. Moreover, stress situations where soluble sugars are involved, such as chilling, herbicide injury, or pathogen attack, are related to important changes in reactive oxygen species balance. These converging or antagonistic relationships between soluble sugars, reactive oxygen species production, and anti-oxidant processes are generally confirmed by current transcriptome analyses, and suggest that sugar signalling and sugar-modulated gene expression are related to the control of oxidative stress. All these links place soluble carbohydrates in a pivotal role in the pro-oxidant and antioxidant balance, and must have constrained the selection of adaptive mechanisms involving soluble sugars and preventing de-regulation of reactive oxygen species production. Finally, in line with the specific role of sucrose in oxygenic photosynthetic organisms, this role of soluble sugars in oxidative stress regulation seems to entail differential effects of glucose and sucrose, which emphasizes the unresolved issue of characterizing sucrose-specific signalling pathways.

Key words: Antioxidant, gene expression, oxidative stress, pro-oxidant, reactive oxygen species, signalling pathways, soluble sugars.

Introduction

Soluble sugars, which are commonly defined as mono- and disaccharides, play a major role in the structure and function of all living cells. Their origin appears to be tightly linked with prebiotic and early biotic evolution (Hirabayashi, 1996). Thus, glucose and fructose are ubiquitous sources of carbon and energy for eukaryotic cells. In oxygenic photosynthetic organisms, especially higher plants, sucrose and all the array of enzymes and proteins related to its processing developed into a central role between photosynthesis, transport, and heterotrophic utilization (Salerno and Curatti, 2003).

In an environment of molecular oxygen (O2), all living cells are confronted with the reactivity and toxicity of active and partially reduced forms of oxygen: singlet oxygen (1O2), superoxide anion (O2−), hydroxyl radical (HO·), and hydrogen peroxide (H2O2), which can lead to the complete destruction of cells (Mittler et al., 2004). These reactive oxygen species (ROS) can show acute production under conditions such as ultraviolet light, environmental stress, or anthropic action through xenobiotics such as herbicides. However, their production is also directly and constantly linked with fundamental metabolic activities in different cell compartments, especially peroxisomes, mitochondria, and chloroplasts. In plants, the links between ROS production and photosynthetic metabolism are particularly...
important (Rossel et al., 2002). All of this has led to the selection of antioxidative defence mechanisms, which place all aerobic cells in a state of pro-oxidant/antioxidant balance that prevents cellular damage. Moreover, control of the levels of endogenous ROS has opened the evolutionary possibility of using ROS as signalling and effector molecules in growth, development, stress responses, and biotic interactions (Ryter and Tyrrell, 1998; Mittler et al., 2004). This seems to be the case for most eukaryotic organisms.

Soluble sugars seem to assume a dual role with respect to ROS. Soluble sugars can be involved in, or related to, ROS-producing metabolic pathways. In reverse, soluble sugars can also feed NADPH-producing metabolic pathways, such as the oxidative pentose-phosphate (OPP) pathway, which can contribute to ROS scavenging. Thus, in yeasts and in mammalian cells, high glucose is associated with toxicity and pathogenesis through increased production of ROS by glucose auto-oxidation and glucose metabolism (Russell et al., 2002; Barros et al., 2004). Correlatively, low glucose and calorie restriction strongly decrease mitochondrial ROS production and increase chronological life span in Saccharomyces cerevisiae (Barros et al., 2004). By contrast, glucose feeding of the OPP pathway can enhance NADPH production, which is a major cofactor of ROS scavenging pathways such as ascorbate-glutathione cycles (Gaetani et al., 1989; May et al., 1998); indeed, glucose has been shown to enhance cellular defences against cytotoxicity of hydrogen peroxide in certain mammalian cell types (Averill-Bates and Przybytkowski, 1994).

This review will assess this dual position of soluble sugars with respect to ROS in plants, where it can also be expected to occur. Given the importance of photo-oxidative stress in oxygenic photosynthetic organisms, examples will be drawn not only from higher plants, but also, to some extent, from cyanobacterial cells. Moreover, the ongoing characterization of sugar signalling (Jang et al., 1997; Pego et al., 2000; Smeekens, 2000; Rolland et al., 2001, 2002; Gibson, 2005) and of its involvement in stress responses will allow the question of sugar and ROS interactions to be addressed in terms of possible interweaving between sugar (Conte et al., 2004; Price et al., 2004; Loreti et al., 2005) and ROS (Desikan et al., 2001; Mittler et al., 2004) gene networks, which may play an important role in adapting the carbohydrate and ROS balances. Finally, the possibly different roles of glucose and sucrose will be discussed.

Oxidative stress and carbon nutritional status

Accumulation of ROS in plants is generally ascribed to several possible sources (Klessig and Malamy, 1994; Corpas et al., 2001; Desikan et al., 2001; Blokhina et al., 2003): cell-wall-bound peroxidases, membrane-located NADPH oxidases, amine oxidases, xanthine oxidase, chloroplastic electron transport chains, mitochondrial electron transport chains, the peroxisomal glycolate-oxidase step of photorespiration, and peroxisomal fatty acid β-oxidation, which includes the H$_2$O$_2$-generating acyl-CoenzymeA oxidase steps. ROS are continuously produced by normal mitochondrial respiration (Møller, 2001; Doudican et al., 2005) and by normal chloroplast functioning (Asada, 1999). The mitochondrial source of ROS production is as important in non-photosynthesizing plant cells as it is in mammalian cells (Møller, 2001), but the relative production of ROS between mitochondria and chloroplasts in the light is not known.

In animal cells, high glucose concentrations can lead to activation of NADPH oxidase, which is one of the reasons for glucotoxicity (Bonnefont-Rousselot, 2002). Such a direct effect has not been characterized in plants to date. By contrast, intuitive thought would rather link glucose availability for plant cells with the possibility of heterotrophic growth and of avoiding phototrophic functioning and, therefore, its damaging photo-oxidative effects (Ashton and Ziegler, 1987). However, an important ROS-producing situation such as high photosynthetic activity is associated with accumulation of soluble sugars. Moreover, in reverse, accumulation of soluble sugars negatively regulates photosynthesis gene expression (Koch, 1996; Pego et al., 2000; Rolland et al., 2002), including expression of Calvin cycle genes. This may thus cause, at least transiently, poor recycling of NADP$^+$ and excessive electron transfer that may lead to ROS production, even under normal conditions of illumination. These relationships between excess light and sugar accumulation may have been the basis for the selection of parallel induction of gene expression by light and sugar in plant cells. Moreover, these relationships between light and sugar are strongly compounded in situations of abiotic stress, such as chilling, where chilling promotes both photo-oxidative damage (Harvaux and Kloppstech, 2001) and the accumulation of sugars, that are supposed to act as cold-stress protectants (Ciereszko et al., 2001). Indeed, this parallel induction by both excess light and excess sugar is verified for genes involved in excess photon removal, such as chalcone synthase, the pivotal step of flavonoid synthesis, or in ROS defence, such as superoxide dismutase (Feinbaum et al., 1991; Koch, 1996; Rossel et al., 2002).

Similarly, fluctuations of carbohydrate levels by carbohydrate starvation and reverse feeding lead to variations in respiration rates (Brouquisse et al., 1991). These experimental fluctuations may correspond to certain environmental or developmental situations where sugar limitation or sugar excess may occur (Koch, 1996). Under the extreme situation of experimental sugar starvation applied to plant cells or excised organs, where regulations by sugar limitation can be intensified and thus better characterized (Brouquisse et al., 1991; Dieuaide et al., 1993), the rate...
of ADP regeneration is found to decrease significantly (Brouquisse et al., 1991). Mitochondria consequently function in a situation of 'state 4' respiration, where reduced electron transfer through the cytochrome c oxidase step can result in higher production of ROS at the level of mitochondria (Braidot et al., 1999). All of this is particularly important in relation to the involvement of the mitochondrial respiratory chain functioning in antioxidant crosstalk with other organelles and in the overall cell redox homeostasis of plant cells (Dutilleul et al., 2003).

Moreover, sugar starvation has been shown to activate lipid mobilization, fatty acid transfer, and peroxisomal β-oxidation, which involves activation of acyl-CoenzymeA oxidase steps, at the mRNA, protein, and activity levels (Hooks et al., 1995; Contenko et al., 2004). This activation of ROS production by sugar starvation is also suggested by transcriptome profiling analysis where sucrose starvation results in activation of oxidative stress genes, such as catalase (Contenko et al., 2004). Moreover, in yeast, growth in low glucose appears to induce long-term adaptive modifications that re-establish higher respiratory activity with decreased mitochondrial ROS release (Barros et al., 2004). In the reverse situation, sugar feeding results in repression of fatty acid mobilization and peroxisomal β-oxidation (Brouquisse et al., 1991; Dieuaide et al., 1992), thus acting negatively on a major source of ROS. Thus, through their relationships with photosynthesis, mitochondrial respiration, and regulation of fatty acid metabolism, variations in sugar levels can be expected to result in changes in ROS production, which thus place sugars in a central position relative to ROS balance, as summarized in Fig. 1.

**Carbon feeding of anti-oxidative metabolism**

Sugar metabolism and carbon skeletons are essential to the synthesis of numerous compounds that are involved in anti-oxidative protection. Glucose feeding of primary metabolism can result in enhanced reducing power in the form of NADH or NADPH through glycolysis and the OPP pathway, respectively (Averill-Bates and Przybytkowski, 1994; Zhang et al., 2000; Ryu et al., 2004). Thus, glucose has been shown to enhance cellular defences against cytotoxicity of hydrogen peroxide in animal cells (Averill-Bates and Przybytkowski, 1994). Glucose is also the main carbon initial precursor for carotenoid (Pallett and Young, 1993) and ascorbate (Foyer, 1993; Smirnoff et al., 2001) synthesis and for carbon skeletons of amino acids, including Cys, Glu, and Gly which are the building blocks of glutathione (Noctor and Foyer, 1998). All of these compounds have been involved in defences against oxidative stress, through implication in ascorbate–glutathione cycles, in redox homeostasis, and peroxide detoxification, or singlet oxygen protection. Thus, exogenous glucose treatment has been used in cyanobacterial cells in order to enhance cellular reducing power through the maintenance of higher NADPH concentrations (Alfonso et al., 2000; Li and Sherman, 2000; Ryu et al., 2004). In plants, the decrease in ascorbate levels in dark-treated leaves has been shown to be partially reversed by glucose or sucrose feeding (Smirnoff and Pallanca, 1996). Similarly, the loss of ascorbate content in harvested broccoli florets can be reversed by sucrose feeding (Nishikawa et al., 2005). In bacteria, sugar-based tolerance to abiotic stress leading to ROS accumulation has been shown to be hampered by mutation or dysfunctioning of glucose-6-phosphate dehydrogenase, thus strongly suggesting that the protective effects of sugars were dependent on OPP activity (Barra et al., 2003). On the other hand, glucose can also be expected to feed conjugation-based detoxification pathways, such as that of UDPG-glucosyltransferase, which is involved in the detoxification of some ROS-stress-mediated herbicides (Hatzios, 2000).

**Soluble sugars and protection against oxidative stress**

Different stress situations which cause, directly or indirectly, accumulation of ROS, such as pathogen challenge, drought, salt stress, abscisic acid (ABA) treatment, low temperature, or excess excitation energy, are also associated with soluble sugar accumulation, which has generally been considered to be an adaptive response to the stress condition (Roitsch, 1999). Although this relationship between stress and sugar accumulation is of great interest, it may be difficult to interpret because these situations of stress and the corresponding responses are clearly pleiotropic in terms of targets and therefore of protection mechanisms.

However, other striking cases of sugar protection of plants against abiotic stress can be related to more specific targets or mechanisms. Thus, in the case of anoxic injury, where exogenous sugar availability greatly enhances...
plantlet survival (Vartapetian and Andreeva, 1986; Loreti et al., 2005), it is seemingly straightforward to draw a link with fermentative metabolism which directly relies on glycolysis and therefore on the supply of glucose (Ricard et al., 1994). However, interestingly, this sugar treatment provides protection not only to the strictly anoxic phase of stress treatment, but also to the post-anoxic phase. This strongly suggests a significant level of protection against oxidative stress and induction of antioxidant defences, since both hypoxia and post-anoxic reoxygenation generate ROS, especially hydrogen peroxide and superoxide, which are a major cause of post-anoxic injury (Blokhina et al., 2003). Surprisingly, in the case of Arabidopsis seedlings, where tolerance to anoxia is strongly enhanced by sucrose, glucose has little effect (Loreti et al., 2005). An initial possible explanation for these differential effects of glucose and sucrose has been ascribed to differential metabolic efficiency of glucose and sucrose utilization, with glucose mobilization being more wasteful of ATP than sucrose mobilization (Bologa et al., 2003; Loreti et al., 2005). However, such differential metabolic effects are not a passive mechanism of sucrose versus glucose feeding, but depend on the activity of the sucrose synthase-mediated pathway and therefore of differential expression of genes encoding sucrose synthase (Loreti et al., 2005). Similarly, the positive effects of sucrose feeding on ascorbate levels in harvested broccoli have been interpreted to be a result of sucrose-dependent modifications of ascorbate biosynthesis gene expression (Nishikawa et al., 2005). Finally, the effects of glucose on the OPP pathway could also be mediated by specific activation of key enzymes. Thus, glucose treatment of potato leaf discs activates the activity of cytosolic glucose-6-phosphate dehydrogenase, but not that of plastidic glucose-6-phosphate dehydrogenase (Hauschild and von Schaewen, 2003). Moreover, in this case, sugar treatment was hypothesized to act both transcriptionally and post-transcriptionally on the induction of cytosolic glucose-6-phosphate dehydrogenase (Hauschild and von Schaewen, 2003). In mammalian and plant cells, increased glucose-6-phosphate dehydrogenase expression has been related to resistance to oxidative stress (Salvemini et al., 1999; Boada et al., 2000; Debnam et al., 2004). All of this thus implies effects on signal transduction and gene regulation mechanisms, rather than mere metabolic effects of carbon feeding.

Different classes of herbicides act on plants through direct induction of oxidative injury, thus providing useful tools to study more precisely oxidative stress injury and recovery. Methylviologen (paraquat) is thus largely used as a superoxide-producing treatment. Herbicides such as atrazine, which binds to the D1 protein, inhibits photosystem II (PSII), and blocks electron transfer to the plastoquinone pool (Zheleva et al., 1994; Rutherford and Krieger-Liszka, 2001), can be used to cause the production of triplet chlorophyll and singlet oxygen.

In cyanobacterial cells, singlet oxygen has been shown to cause direct photodamage to PSII and D1 protein (Nogushi, 2002; Lupinkova and Komenda, 2004) and to prevent PSII repair by suppressing elongation of D1 protein (Nishiyama et al., 2004). Furthermore, the fact that singlet oxygen can generate other ROS such as hydroxyl radicals (Rinalducci et al., 2004) and, probably, superoxide (Ryter and Tyrrell, 1998), cannot be ruled out. Singlet oxygen can also act as a signalling molecule inducing stress and necrotic responses in Arabidopsis (op den Camp et al., 2003; Wagner et al., 2004). The lethal effects of atrazine and of other PSII inhibitors are thus not due to nutritional stress and carbon starvation (Rutherford and Krieger-Liszka, 2001).

However, exogenous treatment with sucrose and, to a lesser extent, with glucose, was found to confer a very high level of atrazine tolerance to Arabidopsis seedlings (Sulmon et al., 2004, 2005). This protection by soluble sugars is concentration-dependent and parallel mannitol treatment does not confer any protection towards atrazine, thus showing that the protection effect cannot be ascribed to osmotic effects. Sugar-treated plants are able to maintain PSII activity and phototrophic growth in the presence of atrazine concentrations, up to 40 μM, that are otherwise, in the absence of sugar treatment, totally destructive by bleaching emerging cotyledons and leaves. Ashton and Ziegler (1987) had ascribed a similar protection of Chenopodium rubrum cell suspension cultures by exogenous sucrose treatment to the absence of oxygen evolution and transition from photoautotrophic to photoheterotrophic growth, thus generating less photo-oxidative damage. However, sucrose- and atrazine-treated Chenopodium cells showed an earlier decline than control sucrose-treated cells (Ashton and Ziegler, 1987). By contrast, sucrose-protected atrazine-treated Arabidopsis plantlets maintained active growth and oxygen evolution (Sulmon et al., 2004, 2005), thus suggesting that mechanisms other than phototrophic–photoheterotrophic transitions may be involved in such sucrose-based protection of Arabidopsis plantlets against atrazine and singlet oxygen injury. The difficulty of interpreting sugar protection effects in terms of mere carbon feeding has therefore initiated other lines of investigation.

**Interactions between sugar- and oxidative-stress-regulated gene expression**

The demonstration that sugars acted as regulators of gene expression in plants (Graham et al., 1992; Koch, 1996; Rolland et al., 2002) has rapidly led to the characterization of a growing number of sugar-regulated genes. Thus, initial candidate-gene approaches showed that sugar variations, whether by limitation or by excess, could significantly modify the expression of genes involved in the response to abiotic stress. For instance, chalcone synthase, which allows the synthesis of photoprotective anthocyanins, and
superoxide dismutase are among ‘feast’ genes induced by glucose (Koch, 1996). Thus, in the particular case of chalcone synthase, there is a parallel induction by ROS-producing high-light stress (Feinbaum et al., 1991), by ROS and redox modifications (Long and Jenkins, 1998), and by soluble sugars (Koch, 1996; Hellmann et al., 2000; Martin et al., 2002). In cyanobacterial cells, Ryu et al. (2004) showed that glucose induced expression of carotenoid synthesis genes in the dark, thus mimicking the effects of high light on carotenoid synthesis genes and opening the possibility that glucose plays a role in the regulation of carotenoid synthesis in response to high light. Transcription profiling in Arabidopsis seedlings not only confirmed that glucose induced abiotic stress response genes, but also revealed that this induction by glucose affected scores of stress response genes (Price et al., 2004), including oxidative-stress-related genes, such as chalcone synthase, glucose-6-phosphate dehydrogenase, glutathione-S-transferases, and glutathione conjugate transporters (supplemental data in Price et al., 2004). However, these approaches using exogenous sugar treatments cannot distinguish between induction of stress defence mechanisms by sugars and induction of mechanisms against sugar-induced stress, such as sugar-modulated oxidative stress as discussed above.

Moreover, approaches combining controls, sugar treatment, stress treatment, and sugar plus stress treatment have shown that situations of sucrose and stress caused specific interactions and patterns of gene expression which were different from those of sugar-treated plants in the absence of stress treatments. Thus, in the case of the protective effects of sugars on anoxic and post-anoxic stress in Arabidopsis seedlings, genome-wide transcriptome analysis showed the induction of large sets of genes, including heat-shock (Loreti et al., 2005) and oxidative-stress (supplementary data in Loreti et al., 2005) response genes, and that this gene induction appeared to depend on sucrose and anoxia interactions, rather than on the additive effects of sucrose and anoxia (Loreti et al., 2005).

Although some genes are induced by sugar treatment as well as by oxidative stress and by high light, it is therefore not sufficient to compare the sugar-regulated transcriptome (Price et al., 2004) and the oxidative-stress transcriptome (Desikan et al., 2001; Rossel et al., 2002) in order to conclude that sugar treatment and sugar-regulated gene expression per se will automatically result in protection against oxidative stress. It is necessary to investigate negative or positive interactions between metabolic and stress signals, through combinations of varying levels of sugar treatment and of stress condition (Loreti et al., 2005).

Photosynthetic gene regulation clearly shows the importance of interactions between sugar and oxidative cues. Glucose or sucrose treatments in the absence of abiotic stress usually repress photosynthesis-related genes in plants (Pego et al., 2000) and in cyanobacterial cells (Li and Sherman, 2000). This is thus the case for psbA mRNA and D1 protein accumulation in higher plants (Pego et al., 2000; Sulmon et al., 2004). In the cyanobacterium Synechocystis, glucose feeding depresses the steady-state mRNA levels of PSII genes (Li and Sherman, 2000) and, under dark conditions, induces the destabilization of psbA transcripts (Alfonso et al., 2000). Surprisingly, sugar treatment of Arabidopsis seedlings in the presence of atrazine results in a markedly enhanced accumulation of psbA mRNA and D1 protein, which can thus be interpreted as derepression of sugar-induced repression of photosynthesis-related genes (Sulmon et al., 2004). Moreover, application of ROS, especially H2O2, or changes of the glutathione redox state in the dark enhances psbA gene expression, which may thus help replenish D1 protein under conditions of oxidative stress (Pfannschmidt et al., 2001). Given that atrazine treatment itself has negative effects on D1 protein levels, the observed derepression in the presence of sugar and atrazine (Sulmon et al., 2004) is therefore likely to result from interactions between sugar and oxidative signalling cues. Similarly, concomitant changes of photosynthetic electron transport and sugar levels can interact and affect photosynthesis gene expression in Arabidopsis (Oswald et al., 2001). Since modifications of photosynthetic electron transport were obtained by DCMU [3-(3′,4′-dichlorophenyl)-1,1′-dimethyleurea] treatment, which generates ROS, especially singlet oxygen (Rutherford and Krieger-Liszkay, 2001), the observed interaction was also likely to involve ROS and soluble sugars. The major effect of this PSII–herbicide treatment was the inhibition of low-sugar induction of photosynthetic gene expression (Oswald et al., 2001), which thus showed the same trend as the lifting of psbA high-sugar repression by atrazine treatment (Sulmon et al., 2004).

Moreover, typical markers of ROS response have been shown to respond to interacting sugar and oxidative cues. Thus, Sulmon et al. (2005) have shown that, during sugar-induced protection against atrazine treatment, FSD1 (encoding a chloroplastic Fe-superoxide dismutase) gene expression, which is slightly increased by sugar treatment per se and does not respond to atrazine treatment per se, is greatly enhanced in the presence of both sugar and atrazine. Similarly, induction of pathogenesis-related genes during plant–pathogen interactions, where ROS play an early signalling and transduction role, as well as a direct role in plant defence (Fellbrich et al., 2002), is enhanced by exogenous sugar treatment or an increase in endogenous sugar levels (Badur et al., 1994; Herbers and Sonnewald, 1998; Thibaud et al., 2004) in a signal-dependent manner (Thibaud et al., 2004).

Thus, on the one hand, the effects of soluble sugars on gene expression are mediated through sugar-specific signalling pathways and, on the other hand, these effects are also linked to regulations by redox, ROS, light, stress, and photosynthesis electron transfer signals. These interactions...
can be mediated through common target genes, such as those listed in Table 1, especially photosynthesis genes and ROS defence genes. They may also be mediated by interactions between signal transduction pathways, which, however, remain to be fully elucidated.

**Mechanisms of sugar signalling and oxidative stress responses**

Characterization of the mechanisms of sugar signalling in plants has mainly focused on the involvement of hexokinase, with the characterization of various *Arabidopsis* mutants, especially gin2 mutants and catalytically inactive *HEXOKINASE1* mutants, which retain some of its glucose signalling functions in spite of the lack of hexokinase enzymic activity (Moore *et al*., 2003). In the case of sugar-induced atrazine protection, interactions between sugar signalling and atrazine sensitivity were analysed in mutant backgrounds affected in the effects of sugars on photosynthesis and greening (Sulmon *et al*., 2004). The study of the sense and antisense *HEXOKINASE* transgenic lines described by Jang *et al.* (1997) showed that sugar-induced tolerance to atrazine was independent of the level of glucose-sensing *HEXOKINASE* (Sulmon *et al*., 2004). This was consistent with the negative effects of the hexokinase pathway on photosynthesis gene expression (Pego *et al*., 2000; Rolland *et al*., 2002), which may thus aggravate, rather than alleviate, the depression of photosynthesis gene expression by singlet oxygen (Nogushi, 2002; Nishiyama *et al*., 2004). Ryu *et al.* (2004) also showed that the induction of carotenoid biosynthesis genes by glucose in the cyanobacterium *Synechocystis* was not mimicked by glucose analogues that were substrates of glucokinase, such as mannose and 2-deoxyglucose.

Indeed, analysis of various sugar-regulated processes strongly suggests that sugar signalling involves not only the well-described glucose-sensitive hexokinase-dependent pathway, but also the hexokinase-independent glucose-sensitive and sucrose-specific pathways (Jang *et al*., 1997; Rook *et al*., 1998; Lalone *et al*., 1999; Xiao *et al*., 2000; Rolland *et al*., 2002). This complexity is compounded by the possible existence of metabolism-dependent signals, involving, for instance, metabolic signatures of sucrose degradation and allocation (Koch, 1996, 2004), cytosolic redox state, ATP/ADP ratio, or cytosolic pH (Rolland *et al*., 2001; Ryu *et al*., 2004). Moreover, analysis of ABA and ethylene signalling mutants has shown cross-talk between hormonal and metabolic regulation (Gazzarrini and McCourt, 2001; León and Sheen, 2003). Thus, glucose induction of abiotic stress response genes has been interpreted as a link with the stress response hormones ABA and ethylene (Price *et al*., 2004). Finally, soluble sugars can also interact with signalling by gibberellins (Moalem-Beno *et al*., 1997), auxins and cytokinins (Rolland *et al*., 2002), salicylate (Thibaud *et al*., 2004), and jasmonate (Berger *et al*., 1995).

The signalling pathways of sugar-induced responses to stress thus remain largely to be characterized, since the few studies that exist merely confirm the hexokinase-independent mechanism of sugar-induced stress responses and its relationships with hormones and growth regulators. The effects of low-to-moderate sugar concentrations, up to 100 mM, on sugar-induced tolerance to atrazine were found to be modified in the *sis1* mutant (Sulmon *et al*., 2004), which is allelic to *ctr1* mutations and is therefore affected in ethylene signal transduction (Gibson *et al*., 2001). Further analysis of sugar-induced atrazine protection showed that several ethylene-insensitive mutants, *etr1-1*, *ein4*, and *ein2-1*, were significantly less protected by sugars than wild-type in terms of chlorophyll accumulation, with *ein2-1* being the most affected (C Sulmon, unpublished results). The induction by sugars of PR-2 expression in *Arabidopsis* during plant–pathogen interaction, where ROS are also involved, has been shown to be hexokinase-independent and ethylene-independent, and dependent on a specific salicylate pathway (Thibaud *et al*., 2004), that is distinct from the NPR1-mediated salicylate pathway (Eulgem, 2005). Gibberellin-induced chalcone synthase gene expression in *Petunia* has been shown to be sugar-dependent (Moalem-Beno *et al*., 1997). Finally, Ryu *et al.* (2004) found evidence that glucose-induced enhancement of carotenoid biosynthesis gene expression in *Synechocystis* may be mediated by changes of cytosolic pH.

**Differential effects of sucrose and glucose in oxidative stress responses**

In some of the mechanisms of carbon feeding mentioned above, glucose should be as efficient as sucrose as the

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<th>Function</th>
<th>Gene</th>
<th>Regulating cues</th>
<th>References</th>
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<tr>
<td>Photosynthesis</td>
<td><em>psbA</em></td>
<td>Light, redox, ROS, sugars</td>
<td>Pfannschmidt <em>et al</em>., 2001; Sulmon <em>et al</em>., 2004</td>
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<td>OPP pathway</td>
<td>Cytosolic G6PDH</td>
<td>Light, sugars</td>
<td>Hausschild and von Schaewen, 2003</td>
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<td>ROS defence</td>
<td><em>CHS</em></td>
<td>Light, redox, ROS, sugars</td>
<td>Hellmann <em>et al</em>., 2000; Koch, 1996; Long and Jenkins, 1998</td>
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<td><em>GST</em></td>
<td>Light, ROS, sugars</td>
<td>Price <em>et al</em>., 2004; Rossel <em>et al</em>., 2002; Wagner <em>et al</em>., 2002</td>
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<td>Ascorbate synthesis genes</td>
<td>High light, sugars</td>
<td>Nishikawa <em>et al</em>., 2005; Rossel <em>et al</em>., 2002</td>
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<td>Carotenoid synthesis genes</td>
<td>High light, sugars</td>
<td>Ryu <em>et al</em>., 2004</td>
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<td>Stress defence</td>
<td><em>HSP</em></td>
<td>Anoxia, sugars</td>
<td>Loreti <em>et al</em>., 2005</td>
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<td>Pathogen defence</td>
<td><em>PR-2</em></td>
<td>Pathogen, sugars</td>
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metabolic precursor. Thus, in the hypothesis of sugars feeding the OPP pathway, no major difference should be expected between glucose and sucrose, inasmuch as OPP pathway activity in plant cells has been shown to be equivalent for glucose and sucrose feeding (Schwender et al., 2003). This is also why the similar effects of glucose and sucrose on ascorbate synthesis in dark-treated leaves have elicited interpretations linked to carbon feeding (Smirnoff et al., 2001). By contrast, in harvested broccoli florets, where sugar-induced ascorbate synthesis is thought to depend on gene expression modifications, sucrose feeding increases ascorbate levels, whereas glucose has no effect (Nishikawa et al., 2005). Significantly different effects of glucose and sucrose have also been reported for anoxic stress (Loreti et al., 2005) and herbicide-mediated photo-oxidative stress (Sulmon et al., 2004, 2005). Moreover, as discussed above, differences in ATP utilization between the sucrose synthase route of sucrose degradation and the invertase route, which yields glucose and fructose, depend on differential activity of the corresponding enzymes and differential expression of the corresponding genes rather than on differential feeding of pre-existing metabolic pathways (Bologna et al., 2003; Loreti et al., 2005). The differential effects of glucose and sucrose cannot therefore be interpreted as protection by mere metabolic carbon feeding of antioxidant processes.

In the hypothesis of action through sugar signalling and differential gene expression, these differential effects of glucose and sucrose greatly contrast with the similar effects of metabolizable sugars on the hexokinase-mediated signalling pathway (Graham et al., 1992; Rolland et al., 2002). However, as discussed above, all the mechanisms of sugar-signalled defence against oxidative stress that have been investigated to date point to hexokinase-independent pathways (Moalem-Beno et al., 1997; Ryu et al., 2004; Sulmon et al., 2004; Thibaud et al., 2004). The differential effects of glucose and sucrose would therefore agree with the existence of hexokinase-independent sucrose-specific signalling pathways, which have been postulated (Lalonde et al., 1999), but have remained elusive up to now.

Conclusion

Enhancement of anti-oxidant defence mechanisms by sugars may have evolved from the relationship between fluctuations of light intensity and the possibility of correlated photodamage (Asada, 1999) on one hand, and photosynthesis-dependent sugar accumulation (Brouquisse et al., 1998; Hendriks et al., 2003) on the other hand, and from the central position of sugars relatively to major sources of ROS production (Fig. 1). Thus, soluble sugars could be part of an array of defences and signals that are useful to the plant, not only to sense and control photosynthetic activity but also to sense and control the ROS balance. Consequently, soluble sugars may be expected to regulate defence against various ROS-producing stresses and, in particular, against ROS-producing xenobiotics. Moreover, the possibility that singlet oxygen may act as an apoptogenic signal in plant cells (op den Camp et al., 2003; Wagner et al., 2004) strongly suggests that the potential anti-apoptotic activity of sugars in plants should be studied further.

This protection by soluble sugars may occur in planta during the normal circadian cycle of sugar accumulation and utilization (Brouquisse et al., 1998). It may also occur through mechanisms of differential carbon allocation, if sugar fluxes can be maintained or targeted towards cellular zones of ROS stress with less carbon being invested in development, export, or storage, in the same way that experimental control of fluxes towards storage can result in greater investment in development of sink tissues such as roots (Fritzius et al., 2001; Freixes et al., 2002) or in the response to abiotic stress (Fukushima et al., 2003; Wagner et al., 2004) strongly suggests that the potential anti-apoptotic activity of sugars in plants should be studied further.

The possibility of preferential effects of sucrose may be strongly related to the central qualitative and quantitative importance of sucrose in higher plants as a major carbon structure and a major form of transport throughout the plant. In the hypothesis of sugar signalling, it is likely to be of the utmost importance to plants to sense and act differently on differential dynamics of glucose and sucrose. Glucose, fructose, and sucrose have often been considered to undergo parallel changes as a result of the use of biological models, such as excised organs (Brouquisse et al., 1991) or cell cultures (Graham et al., 1992), where carbohydrate depletion resulted in the concomitant decrease of these three soluble sugars. However, in planta, modifications of glucose, fructose, and sucrose levels do not follow the same patterns in a number of cases (El Amrani et al., 1992; Borisjuk et al., 1998, 2002; Weber et al., 2005) and do not have the same signalling and developmental impact (Borisjuk et al., 1998, 2002; El Amrani et al., 1998; Weber et al., 2005). Moreover, since sucrose is also a signalling molecule for assimilate partitioning through a hexokinase-independent sucrose-specific pathway (Chiou and Bush, 1998), sucrose-specific signalling pathways may be important for the allocation of sugars towards protection against ROS stress. The development of specific sucrose signalling mechanisms involving specific transduction proteins may be cognate to the origin of sucrose biosynthesis-related proteins in cyanobacterial and plant lineages (Salerno and Curatti, 2003), in the same way that, in yeast and in plants, glucose sensors are related to glucose
transporters and metabolizing enzymes, such as hexokinase. However, as pointed out by several authors (Koch, 1996, 2004; Rolland et al., 2002; Gibson, 2005), regulation by sucrose-specific metabolic signatures or fluxes must also be taken into account.

The relationship between soluble sugars and ROS production or between soluble sugars and ROS responses is not a straightforward positive correlation, since, as discussed above, high sugar levels can correspond to activation of some ROS-producing pathways and decrease of other ROS-producing pathways, and both high sugar level and low sugar level can result in the enhancement of ROS responses. Moreover, soluble sugars other than fructose, glucose, and sucrose are detected at significant levels in plants. Hexoses, such as mannose, and disaccharides, such as trehalose, may play important roles in relation to the main three soluble sugars. Thus, the positive effects of trehalose on stress responses in plants, including decrease of photo-oxidative damage, have been ascribed to interactions with sucrose metabolism, which is significantly modified by trehalose treatment (Bae et al., 2005), and with sugar signalling (Garg et al., 2002; Avonce et al., 2004). Mannose and galactose, which are directly involved in ascorbate synthesis (Smirnoff et al., 2001), may also have been advantageous targets for the selection of sensing mechanisms. Indeed, mannose appears to be sensed by plant cells, probably through the hexokinase-dependent pathway (Pego et al., 1999). Therefore, even though a major immediate challenge consists in fully understanding glucose and sucrose signalling pathways and the wiring between glucose, sucrose, light, redox balance, and ROS signalling pathways, much more complex relationships may lay ahead.

References


