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Linking Chloroplast Antioxidant Defense to Carbohydrate Availability: The Transcript Abundance of Stromal Ascorbate Peroxidase Is Sugar-Controlled via Ascorbate Biosynthesis

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ABSTRACT All genes encoding chloroplast antioxidant enzymes are nuclear-encoded and posttranscriptionally targeted to chloroplasts. The transcript levels of most of them decreased upon sucrose feeding like the transcript levels of many genes encoding components of the photosynthetic electron transport chain. However, the transcript abundance of stromal ascorbate peroxidase (*s-APX*; At4g08390) increased. Due to mild sugar application conditions, the plants kept the phosphorylation status of the ADP+ATP pool and the redox states of the NADPH+NADP$^+$ and the ascorbate pools under control, which excludes them as signals in *s-APX* regulation. Correlation with ascorbate pool size regulation and comparison of transcript abundance regulation in the starch-biosynthetic mutant *adg1*, the ascorbate biosynthesis mutant *vtc1*, and the abscisic acid (ABA) biosynthetic mutant *aba2* showed a link between sugar induction of *s-APX* and ascorbate biosynthesis.

Key words: ascorbate; antioxidant; carbohydrate; chloroplast; gene expression; stromal ascorbate peroxidase.

INTRODUCTION

Sugar availability regulates the expression of many genes for chloroplast proteins (Rook and Bevan, 2003; Rolland et al., 2006). While low sugar concentrations support seedling growth, excess carbohydrates antagonize greening and seedling development (Koch, 1999). Carbohydrate surplus inhibits the Calvin-Cycle (Macdonald and Buchanan, 1992) and promotes generation of reactive oxygen species (ROS) via feedback inhibition of photosynthetic electron transport (Rook et al., 2006a).

Antioxidant enzymes and low-molecular-weight antioxidants, such as ascorbate (Asc) and glutathione (GSH), form a ROS-protective network (Asada, 1999; Dat et al., 2001; Dietz et al., 2002; Chang et al., 2009). Stromal and thylakoid-bound ascorbate peroxidase (s-APX (At4g08390) and t-APX (At1g77490)) detoxify H_2O_2 on the expense of ascorbate (Asada, 1999). The co-substrate is regenerated by monodehydroascorbate and dehydroascorbate reductases (MDHAR and DHAR). In parallel, glutathione peroxidases (GPX) and peroxiredoxins (PRX) reduce peroxides via ascorbate-independent thiol-mediated pathways (Dietz et al., 2002). These enzymes are nuclear-encoded and posttranslationally targeted to the organelles by N-terminal transit peptides (Pitsch et al., 2010). Most proteins, like the four peroxiredoxins and t-APX, for example, are exclusively targeted to chloroplasts. s-APX and MDHAR (At1g63940) can be alternatively targeted to chloroplasts and (the intermembrane space of) mitochondria (Chew et al., 2003), yet they display strong preference towards chloroplasts. To acclimate the chloroplast antioxidant protection upon (photo-)oxidative stress, the genes for chloroplast antioxidant enzymes respond to organellar signals.

Organelle-to-nucleus signaling has been best studied for photosynthesis associated genes, like cab (encoding chlorophyll-a/b-binding proteins/light-harvesting complex proteins) and rbc-S (encoding the small subunits of ribulose-1,5-bisphosphate carboxylase/oxygenase). They are suppressed by carbohydrates (Arenas-Huertero et al., 2000), while apl3 (encoding a large subunit of ADP-glucose pyrophosphorylase; At4g39210) is induced by sugars (Rook et al., 2006b).

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Screens for sugar signaling mutants demonstrated a strong, but diverse, crosstalk of ABA, sugar, and ROS/redox signaling (Rook et al., 2001; Gonzalez-Guzman et al., 2002; Staneloni et al., 2008). So far, little is known about the transcriptional regulation of genes encoding chloroplast antioxidant enzymes. Array studies and RT–PCR demonstrate that transcript abundances often increase only by mild stress, but are decreased upon severe stress due to general inactivation of chloroplast function (summarized in Baier et al. (2010)).

Regulation of the 2-Cys peroxiredoxin-A gene (At3g11630) has been investigated in detail (Baier et al., 2004b): *cis*-acting motifs located within 200 bp upstream of the transcription initiation site mediate developmental responses. Photosynthetic redox signals and ABA-signals integrate antagonistically at a more distally located promoter region, designated as redox-box. There, the APETALA-2-type transcription factor RAP2.4a induces 2cpa transcription upon moderate oxidative stress (Shaikhali et al., 2008). Upon severe stress, RAP2.4a oligomerizes. As a consequence, 2cpa transcription activity decreases (Shaikhali et al., 2008).

Transcriptional regulation of other genes for chloroplast antioxidant enzymes has not been studied so far. Mutants impaired in redox regulation of the 2cpa promoter (Heiber et al., 2007) and knockout lines of the 2cpa redox-regulating transcription factor RAP2.4a (Shaikhali et al., 2008) show links between *s-APX*, *t-APX*, and *2CPA* in redox regulation. To investigate the impact of the photosynthetically controlled parameters, carbohydrate availability, light, and ABA on the expression of nuclear genes for chloroplast antioxidant enzymes, here the transcript abundances were analyzed in *Arabidopsis* following light and sugar treatments. The responses were compared to those in the ascorbate-biosynthetic mutant *vtc1* (Conklin et al., 1997), the starch-biosynthetic mutant *adg1* (Lin et al., 1988), and the ABA-biosynthetic mutant *aba2* (Léon-Kloostersiel et al., 1996) after short- and long-term Suc treatment. In this comparison, a specific regulation is shown for *s-APX*. It is concluded that *s-APX* regulation is indirectly sugar-responsive via ascorbate biosynthesis.

RESULTS

Optimization of the Experimental Set-Up

In nature, the mucilage of the outer seed coat provides the first carbohydrate source upon germination. In experiments, most of the mucilage is washed off during seed sterilization. Here, *Arabidopsis* was screened for the optimal background sucrose (Suc) concentration prior to gene-expression analyses. In continuous moderate light (50 µmol quanta m^{-2} s^{-1}), the seedling biomass increased on Suc-supplemented MS-media up to 0.8% (w/v) Suc (data not shown). With Suc concentrations higher than 1.6% (w/v), anthocyanins accumulated in hypocotyls and cotyledons indicating excess carbohydrates (Solfanelli et al., 2006). Suc concentrations higher than 2.5% (w/v) resulted in growth inhibition. Based on these observations, 1% (w/v) Suc was chosen as optimal sugar concentration and 0.5% (w/v) Suc was used to establish moderate sugar starvation.

Sorbitol (Sor) was used as an osmotic control. Compared to Suc, it is taken up and metabolized much less efficiently (Shabala and Lew, 2002; Gibson, 2005). Chemically, the osmolarities of Suc and Sor are similar. *In planta*, hydrolysis of Suc into glucose and fructose increases the osmolarity and the heterotrophic metabolism decreases it. The Sor concentration best suited as an osmotic control for Suc treatments was determined empirically based on biomass comparison. At 10 d, the fresh weights of seedlings grown on 1% (w/v) Suc supplemented with 0.8–1.3% (w/v) Sor were in the range of seedlings grown on 2% (w/v) Suc (data not shown). Finally, 1% Sor was chosen as control for 2% Suc application.

Long-Term Carbohydrate Effect in *Arabidopsis* Seedlings

Long-Term Carbohydrate Effect on the Transcript Abundance of Nuclear-Encoded Chloroplast Proteins

Long-term effects of Suc and Sor on the expression of genes encoding chloroplast antioxidant enzymes were studied in *ACTIN-2*-standardized cDNA samples by RT–PCR (Figure 1). As secondary control, the transcript level of ubiquitin-11 (*UBQ11*; At4g05050) was quantified. Relative to *ACTIN-2* transcript levels, the *UBQ11* transcript levels were not significantly changed by any of the treatments (Figure 1). *APL3* (encoding a large subunit of ADP-glucose-pyrophosphorylase) served as a control for induction by Suc (Rook et al., 2001). *RBC-S* (encoding the small subunit of Ribulose-1,6-bisphosphat-carboylase/oxygenase; At5g38430) and *STP1* (encoding a high-affinity monosaccharide/proton symporter; At1g11260) were included to monitor suppression by surplus amounts of carbohydrates (Figure 1; Koch, 1999; Sherson et al., 2000).

This study focuses on regulation of chloroplast antioxidant enzymes. On 2% (w/v) Suc, the transcript levels of the four chloroplast peroxiredoxins, *2CPA* (At3g11630), *2CPB* (At5g06290), *Prx-Q* (At3g26060), and *Prx-IIE* (At3g52960), of CuZn-superoxide dismutase 2 (*CSD2*; At2g28190), *MDHAR* (At1g63940), *t-APX* (At1g77490), the Rieske protein *PET-C* (At4g03280), the photosystem-II antenna protein *LHCB2.2* (At2g05070), and of the cytochrome b_6f proteins *PET-M* (At2g26500) were decreased compared to 1% (w/v) Suc and the osmotic control containing 1% (w/v) Sor demonstrating a broad inactivation of genes encoding chloroplast proteins. On 3% (w/v) Suc, the transcript levels were below those observed on 1% (w/v) and 2% (w/v) Suc. On 1% (w/v) Suc supplemented with 2% (w/v) Sor, they were barely detectable (Figure 1).

PET-E1 (At1g76100) and *PET-E2* (At1g20340) levels, encoding plastocyanins, were unchanged irrespective of the treatment. The *s-APX* mRNA level was increased on 2% (w/v) Suc, but not on 1% (w/v) Suc supplemented with 1% (w/v) Sor demonstrating Suc-dependent induction.