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Interactive effects of phosphate deficiency, sucrose and light/dark conditions on gene expression of UDP-glucose pyrophosphorylase in *Arabidopsis* ☆

Iwona Ciereszko[a,*], Henrik Johansson[b], Leszek A. Kleczkowski[b]

[a]*Institute of Biology, University of Bialystok, Swierkowa 20b, 15-950 Bialystok, Poland*
[b]*Department of Plant Physiology, Umeå Plant Science Centre, Umeå University, 901 87 Umeå, Sweden*

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Summary

The effects of inorganic phosphate (Pi) status, light/dark and sucrose on expression of UDP-glucose pyrophosphorylase (UGPase) gene (*Ugp*), which is involved in sucrose/polysaccharides metabolism, were investigated using *Arabidopsis* wild-type (wt) plants and mutants impaired in Pi and carbohydrate status. Generally, P-deficiency resulted in increased *Ugp* expression and enhanced UGPase activity and protein content, as found for wt plants grown on P-deficient and complete nutrient solution, as well as for *pho1* (P-deficient) mutants. *Ugp* was highly expressed in darkened leaves of *pho1*, but not wt plants; daily light exposure enhanced *Ugp* expression both in wt and *pho* mutants. The *pho1* and *pho2* (Pi-accumulating) mutations had little or no effect on leaf contents of glucose and fructose, regardless of light/dark conditions, whereas *pho1* plants had much higher levels of sucrose and starch in the dark than *pho2* and wt plants. The *Ugp* was up-regulated when leaves were fed with sucrose in wt plants, but the expression in *pho2* background was much less sensitive to sucrose supply than in wt and *pho1* plants. Expression of *Ugp* in *pgm1* and *sex1* mutants (impaired in starch/sugar content) was not dependent on starch content, and not tightly correlated with soluble sugar status. Okadaic acid (OKA) effectively blocked the P-starvation and sucrose-dependent expression of *Ugp* in excised leaves, whereas staurosporine (STA) had only a small effect on both processes (especially in -P leaves), suggesting that P-starvation and sucrose effects on *Ugp* are transmitted by pathways

Abbreviations: OKA, okadaic acid; *pgm1*, phosphoglucomutase-deficient mutant; *pho1*, Pi-deficient mutant; *pho2*, Pi-excess mutant; Pi, inorganic phosphate; –P, phosphorus-deficient plants, +P, control plants; PP, protein phosphatase; *sex1*, starch accumulating mutant; STA, staurosporine; UGPase, UDPG pyrophosphorylase; UDPG, UDP-glucose

☆ Article dedicated to the memory of Dr. Anna Siedlecka (1965–2004)

*Corresponding author. Tel.: +48 85 7457315; fax: +48 85 7457302.
E-mail address: icier@uwb.edu.pl (I. Ciereszko).

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that may share similar components with respect to their (in)sensitivity to OKA and STA. The results of this study suggest that *Ugp* expression is modulated by an interaction of signals derived from P-deficiency status, sucrose content and dark/ light conditions, and that light/ sucrose and P-deficiency may have additive effects on *Ugp* expression.

Introduction

UDP-glucose pyrophosphorylase (UTP α-D-glucose-1-P uridylyltransferase, EC 2.7.7.9, UGPase) is an important cytosolic enzyme producing/utilizing UDP-glucose (UDPG), a key precursor to numerous polysaccharides in plants. For example, UDPG is utilized in the synthesis of sucrose and cell wall polysaccharides, production of carbohydrate moiety of glycolipids, glycoproteins and proteoglycans, and as a precursor to UDP-glucuronic acid (Gibeaut, 2000; Johansson et al., 2002; Kleczkowski et al., 2004). In non-photosynthetic tissues, UGPase may be linked to sucrose degradation pathways providing carbon skeletons for starch synthesis (Sowokinos et al., 1993; Kleczkowski, 1994a; Borovkov, et al., 1996; Kleczkowski et al., 2004).

Despite the importance of UGPase in sucrose and polysaccharide metabolism in all plants, generally, little is known about regulatory events that control UGPase activity and the expression of the corresponding gene(s). It has been reported that the enzyme exhibits a negative non-linear cooperation for the substrates, and controls the flow of carbon to sucrose, especially under stress conditions (Sowokinos et al., 1993). It has been suggested that a barley UGPase is regulated by (de)oligomerization processes, as found using site-directed mutants (Martz et al., 2002) and by homology modeling (Geisler et al., 2004). In previous studies, UGPase gene expression was up-regulated by sucrose and low temperature stress, as found for potato tubers (Spychalla et al., 1994) and *Arabidopsis* leaves (Ciereszko et al., 2001b). The sucrose effect was independent of hexokinase-sensing mechanism (Ciereszko et al., 2001b). Further, our earlier studies (Ciereszko et al., 2001a) have suggested that enzymatic activity of UGPase increases upon inorganic phosphate (Pi) deficiency stress via regulation at the gene expression level. One study has shown that in pea roots, the UGPase protein increased upon cadmium stress, perhaps reflecting cadmium-dependent reduction in Pi uptake (Repetto et al., 2003).

Plant Pi-deficiency is a common stress condition experienced in many different environments (Vance et al., 2003). Plants adapt to such stress conditions by developing a number of mechanisms for increasing Pi uptake from soil and Pi mobilization/recycling from intercellular pools; most of these changes are preceded by activation or repression of specific genes (Raghothama, 2000; Rausch and Bucher, 2002; Hammond et al., 2003; Ciereszko and Kleczkowski, 2005). Under Pi-deficiency, accumulation of soluble carbohydrates and/or starch in different plant tissues has often been observed (Ciereszko et al., 1996; Kondracka and Rychter, 1997; Ciereszko and Barbachowska, 2000), whereas adenylates content decreased (Rao et al., 1989). Sugar accumulation appears to be the early plant response to Pi-deficiency and may be involved in plant acclimation to low-nutrient conditions. Plants often respond to changes in sugar content by regulating the expression of various genes via a variety of signal transduction pathways (Koch, 1996; Smeekens, 2000; Loreti et al., 2001). In leaves, photosynthesis is a potent generator of sugars and osmotica, and the light/dark effects may have direct relevance to the signal transduction pathways, both via generating sugars and independently, as in light-dependent signaling (Sheen, 1993; Sugiyama et al., 2001; Jiao et al., 2003). Little is known about the effects of phosphorus and light on gene expression, although effects of these two factors on the physiological and morphological components of growth or carbohydrate content/metabolism are relatively well established (Rao et al., 1989; Qiu and Israel, 1992; De Groot et al., 2001).

In the present study, we used mutants affected in Pi status to dissect the effects of Pi-deficiency and Pi-excess from those of sugar and light/dark conditions on *Ugp* expression in *Arabidopsis thaliana* leaves. The approaches in this study included feeding the leaves of both wt plants and *pho* mutants with sugar, Pi, growth of wt plants on liquid media lacking Pi, and the effects of photoperiod for both wt and mutants. Details of sucrose/Pi-starvation regulation were further analyzed using mutants with modified starch/soluble sugar content, and using inhibitors of protein phosphatases/kinases that might be