S24-004

Transcriptional regulation of genes of UDP-glucose synthesis in *Arabidopsis*

LA Kleczkowski[1], I Ciereszko[1,2], H Johansson[1], A Déjardin[1]

[1]*Umeå Plant Science Centre, Department of Plant Physiology, UmU, 901 87 Umeå, Sweden, fax. 46 90 786 6676, e-mail: Leszek.Kleczkowski@plantphys.umu.se*

[2]*Institute of Biology, University of Bialystok, 15-950 Bialystok, Poland*

Keywords: hexokinase, okadaic acid, sucrose synthase, UDP-glucose pyrophosphorylase

Introduction

Changes in carbohydrate concentration have frequently been implicated in plant responses to a variety of stresses, e.g. cold-stress, drought or Pi-deprivation. Little is known about exact mechanisms of these responses, but the upregulation of several genes coding for enzymes of sucrose synthesis/ metabolism has been proposed as part of acclimation mechanisms (Roitsch 1999). The key to synthesis of carbohydrates is the formation of UDPG, which serves as a direct or indirect precursor to sucrose and all polysaccharides (except starch) (Kleczkowski 1994). In plant tissues, UDPG is formed mainly by two cytosolic enzymes: UDPG pyrophosphorylase (UGPase) and sucrose synthase (Sus). In leaves, UGPase is normally involved in UDPG formation, whereas Sus has supposedly only a minor role; in young leaves it may break down sucrose imported from mature leaves. During stress conditions, however, expression of *Sus* genes (there are at least two *Sus* genes in *Arabidopsis*) is frequently stimulated in leaves and other organs (Koch 1996).

In the present study, we summarize our recent results concerned with studies on expression of genes for Sus and UGPase in *Arabidopsis*. Mutants impaired in sugar, starch and Pi content were used to directly assess relationship between carbohydrate and Pi content, expression of relevant genes and stress responses. Also, the roles of hexokinase (HXK) and protein phosphatase(s) in transducing sugar signals were studied using relevant transgenics/ mutants, as well as an inhibitor of protein phosphatases.

Sugars/ Osmoticum/ Pi differentially regulate Sus1 *and* Ugp *genes*

Possible effects of sugars and osmotica on *Ugp* expression were tested using excised leaves (Ciereszko et al. 2001b). The gene was strongly upregulated by sucrose and, to some extent, by PEG 6000. Glucose, mannitol, sorbitol and KCl were ineffective in inducing any appreciable change in *Ugp* expression. In contrast to *Ugp*, all the feeding compounds used in the present study were found to stimulate the expression of *A. thaliana Sus1*, an osmoticum-regulated gene, but not *Sus2* (Table 1). Light appeared to mimic the effect of sucrose both for *Ugp* and *Sus1* expression, although in most cases the light plus sucrose conditions resulted in a more pronounced effect on the transcript levels than individual sucrose or light treatments, possibly due to higher sugar levels in leaves exposed to both sucrose and light (Ciereszko et al. 2001b). Effects of light through factors other than sugar(s) can not be ruled out entirely, however, given close interactions between sugar and light signalling pathways in *A. thaliana* (Jang et al. 1997, Roitsch 1999).

Table 1. Regulation of *Arabidopsis Sus1*, *Sus2* and *Ugp* genes by different sugars/ osmotica (fed to the excised leaves) and abiotic factors. The data are from Déjardin et al. (1999) and Ciereszko et al. (2001a,b), except wounding and effects of Pi efficiency on *Sus2* (not published).

	Feeding conditions			Abiotic factor					
Gene	Glu	Suc	PEG-8000	Cold	Light	Drought	Anoxia	Pi-deficit	Wounding
Sus1	+	+	+	+	+	-	-	-	+
Sus2	-	-	-	-	-	-	+	-	+/-
Ugp	-	+	+	+	+	+/-	-	+	+

To analyze in more detail the mechanism of sucrose responsiveness of *Ugp*, we used transgenic plants with modified expression of a HXK gene (*AtHXK1*) that is thought to be involved in transmitting sugar signal for expression of a number of plant genes (Jang et al. 1997). In these studies (Ciereszko et al. 2001b, and data not published), neither the excess of *HXK1* transcript nor its reduction in leaves had any marked effect on *Ugp* expression. On the other hand, sucrose-dependent increase of *Sus1* transcript was dependent on *HXK* status (Fig. 1A). It seems important to emphasize that, for wt plants, concentration of sucrose (50 mM) that was used in this experiment was sufficient for marked upregulation of *Ugp*, but had only a small effect on *Sus1* expression. The latter, however, was strongly upregulated in *HXK* overexpressing plants (Fig. 1A). The involvement of HXK in sugar-dependent upregulation of *Sus1* is quite unexpected, given that *Sus1* is generally regulated by osmotic pressure (Déjardin et al. 1999). We assume that the signal recognized by HXK comes from glucose, a product of sucrose breakdown, rather than sucrose itself, since *Arabidopsis* has the ability to rapidly metabolize the exogenously provided sucrose (Déjardin et al. 1999). It seems possible that the regulation of *Sus1* involves several transduction pathways, depending on the nature and strength of the signal, e.g. HXK involvement at low sugar concentration, and an osmoticum pathway at a higher [sugar].

Abiotic stresses trigger differential expression of Sus *and* Ugp *genes*

Both *Ugp* and *Sus* genes were profoundly and differentially regulated in leaves exposed to environmental stresses (cold stress, Pi-deficiency, O_2 deficiency, drought, wounding) (Déjardin et al. 1999; Ciereszko et al. 2001a,b). Most notably, transcript levels of both *Ugp* and *Sus1* increased upon exposure to cold, while *Sus2* mRNA was induced specifically by O_2 deficiency (Table 1). *Sus1* was also upregulated by drought, and *Ugp* – by Pi-deficiency. An increase in *Ugp* expression was accompanied by increase in UGPase protein, whereas Sus protein level increased profoundly only upon O_2 deficiency. Both cold and drought exposures induced accumulation of soluble sugars and caused a decrease in leaf osmotic potential, whereas O_2 deficiency was characterised by a near complete depletion in sugars. Feeding abscisic acid (ABA) to detached leaves or submitting *Arabidopsis* ABA-deficient mutants to cold stress conditions had no effect on both *Ugp* and *Sus* genes expression profiles.

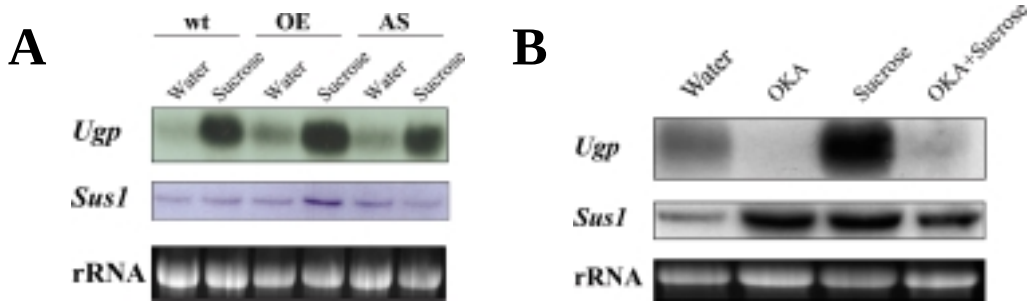

Fig. 1. (A) The HXK-independent and HXK-dependent sucrose upregulation of *Ugp* and *Sus1* in *Arabidopsis*. Excised rosette leaves were fed with water or 50 mM sucrose for 12 h in the dark. Wt, wild-type; OE, HXK-overexpressing plants; AS, HXK-"antisense" plants. **(B)** The role of OKA-dependent protein phosphatases in regulation of *Ugp* and *Sus1* in *Arabidopsis*. Excised rosette leaves were fed with water or 150 mM sucrose in the absence or presence of 2 µM OKA for 10 h in the dark.

Based on this, both *Ugp* and *Sus1* are probably regulated *via* ABA-independent signal transduction pathways that are related to perception of an increase in a specific sugar content (*Ugp* and *Sus1*, with the latter responding in this way only at low sugar concentration) and/or a decrease in leaf osmotic potential (*Sus1,* for high sugar concentration) during stresses. On the other hand, the expression of *Sus2* was independent of sugar/ osmoticum effects, suggesting the involvement of a signal transduction mechanism that is distinct from that regulating *Sus1* expression.

It is unknown whether the cold signal effects for both *Ugp* and *Sus1* are transmitted *via* the same pathways as the sucrose (osmoticum)/ light–mediated regulation, but sucrose content increases markedly during cold exposure of *A. thaliana* (with a concomitant decrease in osmotic potential) (Déjardin et al. 1999) and, thus, sucrose-signalling (*Ugp*) or osmoticum signalling (*Sus1*) must be seriously considered. Sugars/ osmoticum are probably not the main culprit in Pi-deprivation-induced upregulation of *Ugp*, pointing out to different signal sensing/ transduction mechanism (Ciereszko et al. 2001a).

Okadaic acid differentially affects expression of Sus1 *and* Ugp *genes*

More details of sucrose-signalling pathway for *Ugp* have emerged from using okadaic acid (OKA) in the feeding solution (Fig. 1B). This compound is a potent and specific inhibitor of protein phosphatases PP1 and PP2A (Bialojan and Takai 1988). OKA completely inhibited *Ugp* expression, blocking also any effect of sucrose (provided at 150 mM), whereas it upregulated *Sus1* (Ciereszko et al. 2001b). The OKA-stimulated upregulation was earlier observed for a *Sus* gene in sweet potato (Takeda et al. 1994). The activating effect of OKA on *Sus1* expression has indicated that a phosphoprotein (X-P) that serves as a substrate for PP1 and/or PP2A is mediating the upregulating signal for this gene, whereas upregulation of *Ugp* requires dephosphorylation of X-P or some other phosphoprotein that acts as a substrate for PP1 and/or PP2A. This is consistent with expression of *Sus1* responding to osmotic potential rather than to any specific sugar [with the exception of low (50 mM) sugar concentration, see Fig. 1A] (Déjardin et al. 1999) and that components of the osmoticum-regulated pathways in plants are distinct from those comprising the sugar-signalling pathways (Miyata et al 1998).

Summary

Differential effects of sugars, osmoticum and abiotic stresses on *Ugp* and *Sus* genes (Table 1) imply the presence of several signal transduction pathways that mediate activation of these genes at the transcriptional level. This is further supported by evidence for HXK-dependent and independent sugar regulation of *Sus1* and *Ugp*, respectively (Fig. 1A) and differential

effects of OKA (Fig. 1B). The biosynthesis of sucrose itself is regulated by changes in sucrose/ Pi content, either through metabolite feedback or signal transduction mechanisms (Koch 1996, Roitsch 1999, Ciereszko 2001a).

There is accumulating evidence for crosstalk, modulation and integration between signalling pathways responding to sugars, Pi, phytohormones, and biotic and abiotic stress-related stimuli. These interactions at the signal transduction levels and coordinated regulation of gene expression play a central role in source-sink regulation (Roitsch 1999). Both *Ugp* and *Sus* genes seem no exception, with their expression susceptible to an array of signals and, most probably, to different transduction pathways. Whereas exact details of regulation of *Ugp* and *Sus* genes are unknown, it is perhaps not surprising that they are subject to a complex transcriptional control, given the role of UDPG in anabolic pathways in plant cells. For instance, both UGPase and Sus were earlier proposed to provide UDPG for cellulose synthesis, the key process for cell growth in plants (Kleczkowski 1994, Amor et al. 1995), and the presence of distinct signalling pathways for both genes may represent a mechanism where UDPG will be assured to be produced even if one of the pathways is inactive or blocked. The data point toward both *Ugp* and *Sus1/ Sus2* as important regulatory entities that are closely involved in homeostatic readjustments of plant responses to environmental/ metabolic signals

Acknowledgements

We thank Drs. J.-C. Jang and J. Sheen for seeds of *AtHXK1* plants. This research was supported, in part, by the Swedish Natural Science Research Council, Swedish Foundation for Strategic Research, The Swedish Institute, and The Carl Trygger Foundation.

References

Amor Y, Haigler CH, Johnson S, Wainscott M, Delmer DP (1995) *Proceedings of the National Academy of Sciences USA* **92**, 9353-9357.

Bialojan C, Takai A (1988) *Biochemical Journal* **256**, 283-290.

Ciereszko I, Johansson H, Hurry V, Kleczkowski LA (2001a) *Planta* **212**, 598-605.

Ciereszko I, Johansson H, Kleczkowski LA (2001b) *Biochemical Journal* **354**, 67-72.

Déjardin A, Sokolov LN, Kleczkowski LA (1999) *Biochemical Journal* **344**, 503-509.

Jang J-C, León P, Sheen J (1997) *Plant Cell* **9**, 5-19.

Kleczkowski LA (1994) *Phytochemistry* **37**, 1507-1515.

Koch KE (1996) *Annual Review of Plant Physiology and Plant Molecular Biology* **47**, 509-540.

Miyata S, Urao T, Yamaguchi-Shinozaki K., Shinozaki K (1998) *FEBS Letters* **437**, 11-14.

Roitsch T (1999) *Current Opinion in Plant Biology* **2**, 198-206.

Takeda S, Mano S, Ohta M, Nakamura K (1994) *Plant Physiology* **106**, 567-574.