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Sucrose synthase activity and carbohydrates content in relation to phosphorylation status of *Vicia faba* root meristems during reactivation from sugar depletion

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S U M M A R Y

Carbohydrate starvation of *Vicia faba* root meristems leads to readjustment of carbohydrate metabolism and blocks the cell cycle in two principal control points (PCP1/2). The cell cycle reactivation is possible after sucrose provision, although with a delay of about 12 h. During this period, the cells are sensitive to 6-dimethylaminopurine (6-DMAP) and okadaic acid (OA), inhibitors of protein kinases and phosphatases, respectively. The aim of the present study was to investigate whether those inhibitors are involved in inhibition of cell cycle revival through interference with the activities of two sucrose-cleaving enzymes: sucrose synthase (SuSy; EC 2.4.1.13) and invertase (INV; EC 3.2.1.26).

In sugar-starved cells, the *in situ* activity of both enzymes decreased significantly. Following supplementation of root meristems with sugar, INV remained inactive, but SuSy activity increased. Despite the lack of INV activity, glucose was present in meristem cells, but its content was low in cells treated with OA. In the latter case, the size of plastids was reduced, they had less starch, and Golgi structures were affected. In sugar-starved cells, SuSy activity was induced more by exogenous sucrose than by glucose. The sucrose-induced activity was strongly inhibited by OA (less by 6-DMAP) at early stages of regeneration, but not at the stages preceding DNA replication or mitotic activities. The results indicate that prolongation of regeneration and a marked decrease in the number of cells resuming proliferation (observed in previous studies) and resulting from the action of inhibitors, are correlated with the process of SuSy activation at the beginning of regeneration from sugar starvation.

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Introduction

Cell divisions in root meristems strongly rely on a constant supply of nutrients, including sucrose. Depending on physiological activities of heterotrophic root tips, sucrose is stored in vacuoles or utilized for respiration, changed into structural and storage polysaccharides or sugar derivatives in different subcellular compartments. Sucrose can also act as a signaling and regulatory molecule causing alterations in gene expression or enzymatic activities, and can consequently turn cell cycle progression on or off (Riou-Khamlichi et al., 2000; Ciereszko and Kleczkowski, 2002a; Koch, 2004; Rolland et al., 2006; Ciereszko, 2009). Plant cells are equipped with two principal control points (PCP1 and PCP2), which block cell cycle progression in response to carbohydrate starvation at G1 and G2 phases (Van't Hof, 1985; Polit et al., 2003). Since this blockade is reversible, application of sucrose switches the cell cycle on again, yet with a delay (about 12 h) defined as metabolic regeneration period (Polit and Maszewski, 2004; Polit et al., 2004), during which numerous phosphorylation and dephosphorylation processes occur, especially just after provision of sucrose. Meristematic cells are extremely sensitive to inhibitors of protein kinases (cyclin-dependent kinases, CDK) and protein phosphatases (PP1/PP2A), which give rise to the prolonged cell cycle block (Polit and Maszewski, 2004, 2005). However, the pathway(s) and messengers passing information about the abundance of nutrients to main regulators that trigger DNA replication and mitosis are still unknown.

In plants, sucrose cleavage into hexoses is catalyzed by invertases (INVs; EC 3.2.1.26; β-D-fructofuranoside fructohydrolases) and sucrose synthase (SuSy; EC 2.4.1.13; UDP-glucose: D-fructose-2-α-D-glycosyl transferase). INV catalyzes irreversible hydrolysis of sucrose to glucose and fructose. SuSy, in the presence of UDP,

Abbreviations: 6-DMAP, 6-dimethylaminopurine; INV, invertase; MPF, M-phase promoting factor; NBT, nitroblue tetrazolium salt; OA, okadaic acid; PCP1-2, principal control points; PP1/PP2A, specific protein phosphatases; SPF, S-phase promoting factor; SuSy, sucrose synthase; WM, White's nutrient medium.

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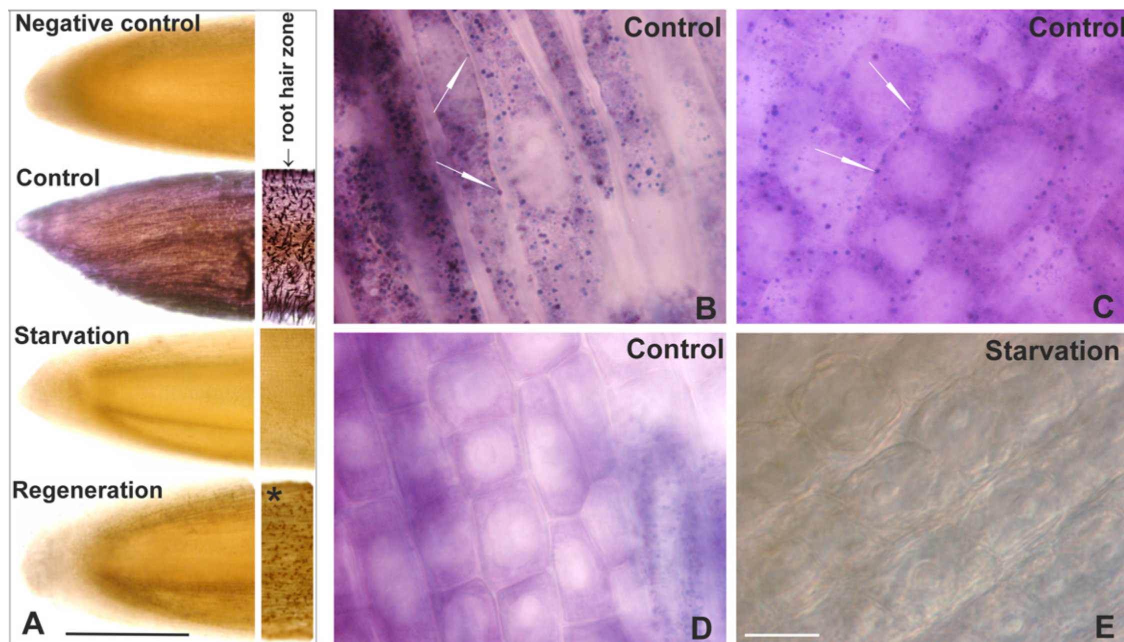

Fig. 1. Comparison of INV activity staining in *Vicia faba* roots. (A) Whole root tips and fragments of root hair zone: (negative control) – no INV activity staining in the control reaction without sucrose (control) – INV activity staining in the control roots – *in planta* (starvation) – INV activity staining in excised carbohydrate-starved roots cultivated for 3 days in the WM (regeneration) – INV activity staining in carbohydrate-starved and then regenerated roots; roots from several regenerated series regardless of time and type of medium looked the same; vestigial activity of INV in root hair zone was visible only after prolongation of detection, enzymatic reaction from 1 to 3 h (*). (B) Control rhizodermis cells; (C) first layer of control meristem cells lying directly beneath rhizodermis; (D) cells from the central part of control root tips; (E) carbohydrate-starved root meristem cells. Arrows indicate INV activity connected with cytoplasmic organelles and cell wall. Black bar = 1.5 mm, white bar = 20 μm.

catalyzes a reversible sucrose conversion to fructose and UDP-glucose. Most plant species possess at least two isoforms of soluble vacuolar acid INV and several isoforms of insoluble acid INV, ionically bound to the cell wall (Sturm, 1999; Winter and Huber, 2000). Acid INVs localized in vacuoles or cell walls share several biochemical properties and are regulated by a small (below 20 kDa) proteinous inhibitor (Weil et al., 1994). They differ in structure from neutral/alkaline INVs, which are less characterized, and are located in the cytosol, mitochondria or plastids (Sturm, 1999; Barratt et al., 2009; Ciereszko, 2009 and papers cited therein). Vacuolar INVs are involved in cellular catabolism, cell osmoregulation and enlargement, and also in the control of soluble sugar composition and fluxes in sink organs, which guarantees normal elongation and development of plants (Sturm, 1999; Sergeeva et al., 2006). Cell wall INVs may play an important role in sucrose unloading and plant responses to wounding or infection (Sturm, 1999; Koch, 2004). Inactivation of acid INVs or loss of one of the isoforms of neutral INV affects plants development and reduces primary root extension (Jia et al., 2008; Barratt et al., 2009).

SuSy is encoded by several genes, with various expression patterns in different plant tissues (Baud et al., 2004; Duncan and Huber, 2007; Hirose et al., 2008; Goren et al., 2011). The expression of SuSy genes is regulated by sugars or okadaic acid (OA)-sensitive protein phosphatases, among other regulators (Ciereszko and Kleczkowski, 2002b; Kleczkowski et al., 2004, 2010). SuSy is found mainly in the cytosol, but is also located in the nucleus, mitochondria (Subbaiah et al., 2006), plastids (Núnez et al., 2008), and it also associates with Golgi membrane (Buckeridge et al., 1999), plasmalemma (Cai et al., 2011) or cell wall (Persia et al., 2008). The activity of SuSy may be regulated by oligomerization, phosphorylation, interactions with other proteins, rapid changes in sub-cellular localization, or modulated protein turnover (Winter and Huber, 2000; Koch, 2004). SuSy can exist either as a tetramer (promoted by sucrose) or dimer; oligomerization may affect its cellular localization (Duncan and Huber, 2007; Kleczkowski et al., 2010). Two isozymes of barley SuSy are able to interact with 14:3:3 proteins (Alexander and Morris, 2006), while a maize isozyme forms a complex with several proteins involved in starch biosynthesis (Hennen-Bierwagen et al., 2009). In tobacco, an increase of sucrose concentration abolishes binding of cytoplasmic SuSy to actin filaments, simultaneously increasing the affinity of membrane SuSy to filaments, which seems to indicate that sucrose can affect enzyme distribution (Cai et al., 2011). Dephosphorylation increases the surface hydrophobicity of the SuSy polypeptide and promotes its association with the membrane (Winter and Huber, 2000). SuSy exists in two phospho-isoforms: one phosphorylated at the N-terminal domain on seryl residues, abundant mostly in the cytoplasm and cell wall, and the other, not phosphorylated, specific to the plasma membrane (Persia et al., 2008). UDP-glucose, a product of SuSy activity, is utilized in various metabolic pathways, including synthesis of cellulose and callose, plastid starch biosynthesis or xyloglucan synthesis in the Golgi structure (Amor et al., 1995; Buckeridge et al., 1999; Subbaiah and Sachs, 2001; Kleczkowski et al., 2004).

The aim of the present study was: (i) to estimate modifications of INV and SuSy activities *in situ* at early and final stages of metabolic regeneration in carbohydrate-starved root meristems; (ii) to determine the influence of protein kinase and phosphatase inhibition (by 6-DMAP and OA) on the ability of both enzymes to hydrolyze sucrose and to prolong cell metabolic regeneration in the presence of sugar, and (iii) to analyze the effects of these inhibitors and sugar on carbohydrate content *in situ* and cell ultrastructure in *V. faba* root meristems.

Materials and methods

Seed germination and root culture

Seeds of *Vicia faba* var. *minor* cv. Nadwiślański (Center for Seed Production in Sobiejuchy, Poland) were germinated on wet filter paper (Petri dishes), at room temperature for 4 days in the dark.

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