Characterization of cell wall hydrolases induced by sugar starvation

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Abstract. **In our previous work in transcriptional regulation of sugar, expression of genes encoding putative glycosyl hydrolases in Arabidopsis was induced by sugar starvation. They were annotated as b-galactosidase (At5g56870), β-xylosidase (At5g49360) and β-glucosidase (At3g60140), which belong to glycosyl hydrolase family that has a catalytic domain of polysaccharides. From the primary structure of deduced amino acid sequence, they were predicted to localize to cell wall. Further investigation of these cell wall hydrolases implicated that cell wall polysaccharides provide metabolizable sugars to nutrient allocation under sugar starvation.**

From solar energy, plants produce sugars that are used as an energy source and as building blocks for complex carbohydrates such as starch and cell wall polysaccharides. Sugars have been recognized to act as a signaling molecule. Gene expression underlying plant metabolism and development are regulated by signalsthat derive from sugar. We previously identified many genes regulated by sugars in Arabidopsis leaves using a cDNA macroarray. Interestingly, many genes encoding glycosyl hydrolase and carbohydrate metabolism enzymes are remarkable induced by sugar starvation. Glycosyl hydrolases are broadly distributed enzymes that hydrolyze the glycosidic bonds, these enzymes have been classified into 95 families. In this work, we focus on enzymes whose function is considered to be in the cell wall breakdown.

Materials and Methods

Plant material. Arabidopsis Col-0 cell suspension derived from roots of Arabidopsis ecotype Columbia (Mathur *et al.* 1998) and whole plants (ecotype Columbia) were used.

RNA gel-blot analysis. Isolation of total RNA from suspension-culturedcells and RNA gel-blot analysis were performed as described (Lee *et al.* 2004).

Enzyme assay. Total soluble protein was isolated from medium of suspension cultured-cells by a method of ammonium sulfate precipitation. The pellet was resuspended in 0.5M sodium phosphate (pH 5.0) and desalted by NAP-5 columns to obtain 1 ml of active pool in 10 mM sodium phosphate buffer (pH 5.0). The substrates of β-galactosidase, β-xylosidase and β-glucosidase were used the corresponding 4-methylumbelliferyl

glycopyranoside. The activity was measured employing a spectrofluorometer with the wavelength fixed at 365 and 460 nm for excitation and emission, respectively. The experiment was repeated three times on the independent active pools and expressed as rmol MU per µl medium.

Production of recombinant b-galactosidase and its antibody. Purification of recombinant β -galactosidase (At5g56870) protein produced in *E. coli* and antibody production was conducted in Medical & Biological Laboratories MBL Co., LTD (Nagano, Japan).

Immunoblot analysis. Soluble proteins were separated on a 10% SDS-polyacrylamide gel. Proteins were further electro-transferred to a polyvinylidene difluoride membrane. The membrane was probed with anti-β-galactosidase (At5g56870) or anti-phosphoenolpyruvate carboxylase (PEPC)antibodies as the primary antibodies. Signals were detected with the HRP-conjugating secondary antibodies and ECL western blotting detection reagent.

Determination of cell wall polysaccharides contents. Cell wall polysaccharides were fractioned by the method of Hoson *et al.* (1995). The sugar content of each fraction was determined with the gas chromatography, and expressed as glucose equivalents by the phenol-sulfuric acid method (Dubois *et al.* 1956).

Gus staining. Seedlings and excised organs were placed directly in buffer containing 1.5 mM $K_3Fe(CN)_6$, *1.5 mM* $K_4Fe(CN)_6$, *0.9% Triton X-100, and 5.7 mM X-gluc. After successive clearing in ethanol, samples were incubated in 99% ethanol overnight and photographed through a dissecting microscope.*

Results and Discussion

Transcripts of genes encoding putative glycosyl hydrolases were induced by sugar depletion. They were annotated as β-galactosidase (At5g56870), β-xylosidase (At5g49360) and β -glucosidase (At3g60140). Their deduced polypeptides contained signal peptides for secretion. The β-galctosidase sequence is very similar to that of an induced β-galactosidase associated with fruitsoftening in pear, and β-xylosidase is highly similar to β-glucan exohydrolases detected in germinated barley grain. We therefore assumed that these enzymes function in utilization of cell wall polysaccharides as alternate carbon sources under sugar depletion. Based on this hypothesis, we conducted further experiments. The activities of β -galactosidase, β-xylosidase and b-glucosidase increased in the culture medium of suspension cells when sugar was depleted from the medium. Transcription was repressed in suspension cells grown with galactose, xylose and glucose, as well as with sucrose, suggesting that plant cells release these sugars from the cell wall and metabolize them. Using a specific antibody, secretion of the β-galactosidase protein into culture medium and cell wall fraction of leaves under sugar-depleted condition was confirmed. Determination of composition and

quantity of monosaccharides in cell wall polysaccharides fraction prepared from detached leaves showed that the amount of galactose and xylose in pectin and hemicellulose decreased with sugar depletion. In plants grown under normal condition, transcript and protein accumulation of the β-galactosidase were detected when photosynthesis was inhibited by shading. Dark induction of β-galactosidase transcripts was also shown using a GUS reporter gene driven by the β-galactosidase gene promoter. These findings suggested that the cell wall functions as a reserve carbon source, as well as determining cell shape and mechanically supporting the plant body.

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Publications

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