Plant Physiology and Biochemistry 100 (2016) 141–149

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy

Short communication

Reduction of the plastidial phosphorylase in potato (*Solanum tuberosum* L.) reveals impact on storage starch structure during growth at low temperature

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ARTICLE INFO

Article history:
Received 2 November 2015
Received in revised form
15 January 2016
Accepted 18 January 2016
Available online 20 January 2016

Keywords:
Potato
Solanum tuberosum L.
Plastidial phosphorylase
Starch synthase
Starch metabolism
Starch granule

ABSTRACT

Tubers of potato (*Solanum tuberosum* L.), one of the most important crops, are a prominent example for an efficient production of storage starch. Nevertheless, the synthesis of this storage starch is not completely understood. The plastidial phosphorylase (Pho1; EC 2.4.1.1) catalyzes the reversible transfer of glucosyl residues from glucose-1-phosphate to the non-reducing end of α-glucans with the release of orthophosphate. Thus, the enzyme is in principle able to act during starch synthesis. However, so far under normal growth conditions no alterations in tuber starch metabolism were observed. Based on analyses of other species and also from *in vitro* experiments with potato tuber slices it was supposed, that Pho1 has a stronger impact on starch metabolism, when plants grow under low temperature conditions. Therefore, we analyzed the starch content, granule size, as well as the internal structure of starch granules isolated from potato plants grown under low temperatures. Besides wild type, transgenic potato plants with a strong reduction in the Pho1 activity were analyzed. No significant alterations in starch content and granule size were detected. In contrast, when plants were cultivated at low temperatures the chain length distributions of the starch granules were altered. Thus, the granules contained more short glucan chains. That was not observed in the transgenic plants, revealing that Pho1 in wild type is involved in the formation of the short glucan chains, at least at low temperatures.

1. Introduction

Starch is the almost ubiquitous storage polysaccharide of plants and algae. Two types of starch exist: transitory and storage starch. Transitory starch is formed in the photoautotrophic cells as product of photosynthesis. Therefore, transitory starch underlies a diurnal metabolism. In the light, when photosynthesis is functional, the transitory starch is formed. In contrast, in the dark, when photosynthesis is impossible, the transitory starch is degraded to overcome limitations of carbon and energy, and must be managed prudently to optimize growth and reproductive success (Andriotis et al., 2012; Stitt and Zeeman, 2012; Fettke and Fernie, 2015).

In contrast, storage starch is formed over a long time period in heterotrophic tissues and normally degraded only once.

Starch biosynthesis is a complex process that proceeds by a close collaboration of starch synthase isozymes and starch branching/debranching isozymes (Brust et al., 2013, 2014; for review see Keeling and Myers, 2010).

Discussion is ongoing over the involvement of another glucan transferase in starch synthesis, the plastidial phosphorylase (Pho1; EC 2.4.1.1). In potato two Pho1-type phosphorylases exist, Pho1a and Pho1b, which are highly homologous except a large insertion (Albrecht et al., 1998). In tubers the dominant Pho1 is the homodimeric Pho1a, whereas Pho1b is normally not detected (Albrecht et al., 1998).

In contrast, the cytosolic phosphorylase is reported to be involved in starch derived maltose metabolism during starch degradation (Fettke et al., 2012a; Fettke and Fernie, 2015).

For potato storage starch as well as for Arabidopsis transitory starch lack of plastidial phosphorylase has no impact on starch metabolism, when plants were grown under normal conditions (Sonnewald et al., 1995; Zeeman et al., 2004). Nevertheless, for Arabidopsis it was reported, that when in addition to the lack of

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http://dx.doi.org/10.1016/j.plaphy.2016.01.013
0981-9428/

plastidial phosphorylase, the maltose pathway of starch degradation is impeded, the plants reveal an obvious altered starch phenotype, including reduction of starch granule number per chloroplast (Malinova et al., 2014).

For various plant species increasing evidences were collected for an involvement of the plastidial phosphorylase in starch metabolism. Thus, *Chlamydomonas reinhardtii* strains carrying mutations in one of the two plastidial phosphorylase genes display reduced starch amounts during storage, abnormally shaped starch granules with modified amylopectin structure, and higher amylose content (Dauvillée et al., 2006). Furthermore, an interaction of plastidial phosphorylase and branching enzymes during synthesis of branched maltodextrins and initiation of starch synthesis were discussed for rice endosperm (Nakamura et al., 2012). In addition, for starch synthesis in maize a multi-subunit complex was described, consisting of starch synthases, branching enzymes and in some cases also the plastidial phosphorylase (Liu et al., 2009, 2012). Finally, rice mutants deficient in plastidial phosphorylase activity reveal smaller starch granules in the endosperm accompanied with modified amylopectin structure when grown at low temperature (Satoh et al., 2008).

In vitro data obtained by incubation of potato tuber slices with ^{14}C-labeled glucose-1-phosphate reveal a direct incorporation of glucosyl residues into starch via the plastidial phosphorylase (Fettke et al., 2010). As a consequence a transport of glucose-1-phosphate over the amyloplast and cytoplasm membrane has to be postulated, but so far transporters are unknown (Fettke et al., 2010). Similarly, for other species a transport of glucose-1-phosphate over the plastidial membrane was reported (Coates and ap Rees, 1994; Fettke et al., 2011; Tetlow et al., 1996).

Analysis of potato cultivars reveals that high yielding cultivars like Kuras showed reduced level of plastidial phosphoglucomutase activity but higher level of glucose-1-phosphate suggesting a direct import of glucose-1-phosphate into the amyloplast for starch synthesis (Kaminski et al., 2012). Furthermore, in *in vitro* incubation experiments with ^{14}C-labeled sucrose and glucose-1-phosphate the incorporation of glucosyl residues into starch was compared. It was concluded that incorporation of glucosyl residues from glucose-1-phosphate is dominant under low temperature conditions (Fettke et al., 2012a,b). Moreover, the plastidial phosphorylase activity seems to be less affected by temperature than the total starch synthase activity. Thus, plastidial phosphorylase activity remained constant over a wide temperature range, whereas the starch synthase activity was gradually reduced with decreasing temperature (Fettke et al., 2012a,b).

The indications for a possible impact of the plastidial phosphorylase activity on potato tuber starch synthesis, especially under low temperature conditions, were starting point for the analyses presented in this communication. Potato plants were cultivated under different temperatures and the starch properties were analyzed. Transgenic potato plants, that reveal a strong reduction of plastidial phosphorylase activity (line A: 8.2%, B 8.8%, and C 12.4% compared to wild type set at 100%; see Fettke et al., 2005, 2010) were included.

2. Materials and methods

2.1. Plant materials and growth conditions

Wild type (cv. Desiree) and three independent plastidial phosphorylase transgenic potato plants (contain two antisense constructs each of which is directed against one of the plastidial phosphorylase isoforms, Pho1a and Pho1b; Fettke et al., 2005, 2010) were cultivated under controlled conditions (16 h light: 8 h dark; 200 μmol quanta m^{-2} s^{-1}; 50% relative humidity; 22 °C) for six weeks. Plants were not flower. Control plants were kept at 22 °C, while other plants were transferred to 15 °C and 5 °C for eight weeks. In an independent experiment the plants were transferred to 17 °C and 13 °C for eight weeks. Except the growth temperature all other growth conditions remain unchanged. Tubers were harvested, cut into small slices and the tuber slices were frozen immediately in liquid nitrogen and stored at −80 °C until use.

2.2. Extraction and quantification of buffer soluble proteins

For the quantification of enzyme activities by native PAGE tuber material (0.5 g) was homogenized in grinding medium [50 mM HEPES-NaOH pH 7.5, 1 mM EDTA, 5 mM dithioerythritol (DTE), 1 mM phenylmethylsulphonyl fluoride, 0.1% (w/v) sodium sulphite, 0.075% (w/v) sodium disulphite, and 10% (v/v) glycerol] using an Ultra Turrax (2 times, 30 s each). The homogenate was centrifuged (20 min at 14,000 *g*, 4 °C). The supernatant was either directly used for protein quantification and native PAGE or was frozen in liquid nitrogen and stored at −80 °C until use. Proteins were quantified using the micro version of the Bio-Rad protein assay kit according to Bradford (1976).

2.3. Native PAGE and staining for glucosyl transferase activities

In a discontinuous native PAGE (as described in Fettke et al., 2005) the separation gel contained 7.5% (w/v; T) acrylamide-bisacrylamide, and 0.04% or 0.02% (w/v) glycogen from oysters (type II; Sigma, Taufkirchen, Germany) for phosphorylase activity staining or 0.2% (w/v) glycogen for starch synthase activity staining. Proteins were loaded as indicated. During electrophoresis, the temperature was kept at 4 °C. For phosphorylase zymograms, separation gels were washed for 10 min with 100 mM citrate-NaOH buffer (pH 6.5). Subsequently, gels were incubated in a mixture containing 20 mM glucose-1-phosphate and 100 mM citrate-NaOH pH 6.5 for 2 h. For soluble starch synthase zymograms, gels were incubated in a mixture consisting of 50 mM Tricine-KOH (pH 8.0), 0.025% (w/v) bovine serum albumin, 5 mM dithioerythritol, 2 mM EDTA, and 25 mM potassium acetate. After 10 min, the incubation mixture was replaced by fresh solution containing in addition 1 mM ADP-glucose. Gels were incubated for 2 h at room temperature. For both phosphorylase activity and starch synthase activity, gels were stained with iodine and the intensity of the staining was quantified using the AIDA software. The analyzed activities in the gels were in linear range for all assays.

2.4. Preparation of native starch granules

1 g potato tuber material was cut into small pieces, subjected to starch isolation buffer [50 mM HEPES-KOH pH 8.0, 1 mM EDTA, 5% (w/v) glycerol, and 1% (v/v) Triton X-100] and disrupted using a Ultra Turrax (2 times, 30 s each). The homogenate was kept on ice for 20 min. The supernatant was decanted and the settled starch granules were washed three times with starch isolation buffer and five times with water.

2.5. Chain length distribution of starch

Isolated starch [0.5–1 mg] was solubilized by heating for 5 min at 95 °C. The solubilized starch was incubated with 12 units isoamylase in a total volume of 300 μl (10 mM ammonium-acetate, pH 5.5) overnight in a thermomixer at 37 °C and 1,400 rpm. Reaction was stopped by heating (95 °C for 3 min). Following filtration (MWCO 10 kDa) glucans (10 nmol reducing ends) were labeled and analyzed using CE-LIF as described in Malinova et al. (2014).