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Review

Starch metabolism in leaves

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Starch is the most abundant storage carbohydrate produced in plants. The initiation of transitory starch synthesis and degradation in plastids depends mainly on diurnal cycle, post-translational regulation of enzyme activity and starch phosphorylation. For the proper structure of starch granule the activities of all starch synthase isoenzymes, branching enzymes and debranching enzymes are needed. The intensity of starch biosynthesis depends mainly on the activity of AGPase (adenosine 5′-diphosphate glucose pyrophosphorylase). The key enzymes in starch degradation are β-amylase, isoamylase 3 and disproportionating enzyme. However, it should be underlined that there are some crucial differences in starch metabolism between heterotrophic and autotrophic tissues, e.g. is the ability to build multiprotein complexes responsible for biosynthesis and degradation of starch granules in chloroplasts. The observed huge progress in understanding of starch metabolism was possible mainly due to analyses of the complete *Arabidopsis* and rice genomes and of numerous mutants with altered starch metabolism in leaves. The aim of this paper is to review current knowledge on transient starch metabolism in higher plants.

Keywords: starch biosynhesis, starch degradation, chloroplast

INTRODUCTION

Starch is the major and the most abundant storage polysaccharide in plants. As a natural product it is obtained mainly from cereals and potatoes. Starch apart from sucrose is also a primary product of photosynthesis deposited transiently in the chloroplast in the form of insoluble granules. It is composed of glucose polymers, namely amylose and amylopectin. Starch is synthesized inside plastids, but its function depends upon the particular type of plastid and the plant tissue from which it is derived. All transient starch granules synthesised during the day undergo nocturnal breakdown, supplying sugars needed for metabolism in the whole plant. The observed periodical starch degradation could be divided into two steps: initiation of degradation and digestion, of amylopectin and amylose into maltose, glucose and maltotriose. Key role in the digestion is attributed to β-amylase, the product of its activity — β-maltose is transported to the cytosol where it is subjected to further conversions. Storage starch pro-

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Abbreviations: ADP-Glc, adenosine diphosphate glucose; AGPase, adenosine 5′-diphosphate glucose pyrophosphorylase; ApL, large subunit of AGPase; ApS, small subunit of AGPase; BAM/BMY, β-amylase; BE, starch branching enzyme; CBM, carbohydrate-binding modules; DBE, starch debranching enzyme; DP, degree of polymerisation; DPE, transglucosidase; DSP4/SEX4/PTPK1S2, dual-specifity protein kinase; Fru6P, fructose 6-phosphate; GBSS, granule bound starch synthase; Glc1P, glucose 1-phosphate; Glc6P, glucose 6-phosphate; GWD/R1/SEX1, glucan, water dikinase; ISA3, debranching enzyme/isoamylase 3; MEX1, maltose transporter; 3-PGA, 3-phosphoglycerate; PGI, phosphoglucoisomerase; PGM, phosphoglucomutase; PHS2, cytosolic glucan phosphorylase; PWD, phosphoglucan water dikinase; SnRK1, sucrose-non-fermenting-1-related protein kinase; SP, starch phosphorylase; SS, starch synthase; Tre6P, trehalose 6-phosphate.

duced in tuberous tissues or developing seeds accumulates carbon skeletons and energy used thereafter during germination or in other physiological processes.

There are same important factors affecting the rate of starch decomposition: the diurnal cycle, starch phosphorylation and post-translational regulation of enzyme activities. The post-translational control proceeds through redox potential, pH changes and the phosphorylation of proteins involved in starch degradation by specific kinases/phosphatases. Recently, a huge progress in our understanding of starch metabolism in leaves has been achived, chiefly due to characterization of natural mutants and by successive identification and isolation of genes encoding the enzymes involved in the starch biosynthesis and degradation pathways. The aim of this article is to review the recent progress in the understanding of transient starch metabolism and its regulation in higher plants.

STARCH STRUCTURE

The starch granule contains two distinct polysaccharide types, amylose and amylopectins. Amylose is essentially a linear polymer of glucose residues linked by α-(1-4) bonds, whereas amylopectin is a branched molecule consisting mainly of α-(1-4)-linked glucose residues with α-(1-6)-branch points every 24 to 30 glucose residues on average (Hizukuri, 1986). The regular distribution of the branch points within amylopectin allows formation of organized arrays of closely packed left-handed double helices in the semicrystalline zones of the starch granule. The essentially linear amylose is probably present in the so-called amorphous matrix of the granule that also contains amylopectin in a less ordered structure (Jenkins & Donald, 1995; Zeeman *et al.,* 2002). The variations in starch structure from different biological sources are the result of differences in a number of parameters such as the chain length distribution, degree of branching and granule size. For more detailed reviews of starch structure, see Buleon *et al.* (1998), Thompson (2000) and Vermeylen *et al.* (2004).

STARCH SYNTHESIS IN LEAVES

Starch is the most important storage polysaccharide in plants. All starch synthesised during the light period is then degraded during the night. In some species, e.g. *Beta vulgaris* and *Arabidopsis thaliana* (Fondy *et al.,* 1989; Zeeman & Rees, 1999) starch is the main storage form, whereas in others, e.g. *Pisum sativum* and *Spinacia olearacea* sucrose is the end product of photosynthesis, and only its surplus is directed to the starch biosynthesis path (Stitt *et al.,* 1983). The formation of the starch granule in the chloroplast is achieved through coordinated action of starch biosynthetic enzymes, including some which were associated previously with starch degradation. The amino-acid sequences of the various enzymes involved in starch metabolism are highly conserved and most isoforms of the many starch metabolic enzymes can be found in both chloroplasts and amyloplasts (Smith *et al.,* 1997; Ball & Morell, 2003). Several models of starch biosynthesis have been proposed to explain how the starch granule formation occurs in the plastid, based on results obtained in experiments *in vitro* as well as on the knowledge of the key enzymes of the pathway and analyses of mutants (Ball *et al.,* 1996; Waigh *et al.,* 1998; Zeeman *et al.,* 1998; Nakamura, 2002). In general, starch biosynthesis starts with the formation of ADP-glucose and then the glucose moiety is transferred on to an acceptor, usually a short chain of maltooligosaccharides and at the end of whole processes in which participate numerous enzymes they builds up final structure of starch granule (Fig. 1).

Formation of ADP-glucose

AGPase (adenosine 5′-diphosphate glucose pyrophosphorylase, EC 2.7.7.27) is the main enzyme responsible for the synthesis of ADP-glucose. The reaction catalysed by AGPase is considered as the first and key step in starch biosynthesis (Kleczkowski 2000; 2001). This enzyme comprises two large (ApL) and two small (ApS) catalytic subunits encoded by different genes. In *A. thaliana*, three genes encode the large (*ApL1, ApL2, ApL3*) and only one gene for the small subunit (*ApS*) of AGPase are known (Villand *et al.,* 1993; Kleczkowski *et al.,* 1999). Mutants in the *ApS* gene (Caspar, 1994) show starchless phenotype, and starch-deficient plants were obtained after mutation of the *ApL1* gene (Wang *et al.,* 1997). It is believed that the ApL1 subunit together with ApS is crucial for ADP-glucose formation and determines the rate of starch biosynthesis in leaves (Wang *et al.,* 1997). The *ApL2* and *ApL3* genes, encoding large subunits of AGPase are induced due to sucrose or glucose feeding to the detached leaves in the dark (Sokolow *et al.,* 1998).

In mature *Arabidopsis*, the ApS subunit was localised using GUS staining in almost all parts of plant, usually together with starch granules: in the leaf, both palisade and spongy mesophyll tissues and vascular bundles, in inflorescence stems, leaves and hypocotyl-root axis, as well as in flowers (Siedlecka *et al.,* 2003). In contrast to *Arabidop-*

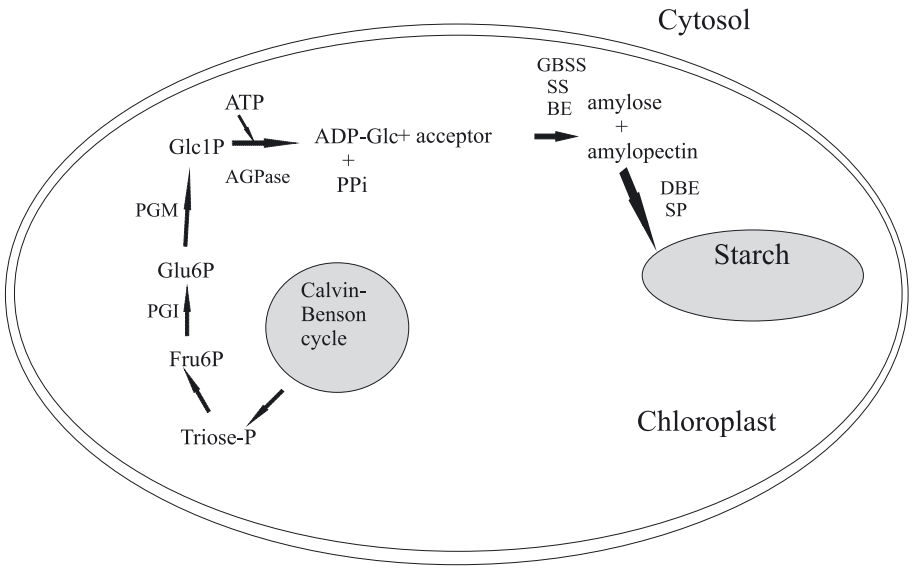

Figure 1. Starch synthesis in chloroplast.
Abbreviations: ADP-Glc, adenosine diphosphate glucose; AGPase, adenosine 5′-diphosphate glucose pyrophosphorylase; BE, starch branching enzyme; DBE, starch debranching enzyme; Fru6P, fructose 6-phosphate; GBSS, granule bound starch synthase; Glc1P, glucose 1-phosphate; Glc6P, glucose 6-phosphate; PGI, phosphoglucoisomerase; PGM, phosphoglucomutase; SP, starch phosphorylase; SS, starch synthase.

sis, in some cereals two genes encode small subunits of AGPase (Thorbjørnsen *et al.*, 1996; Johnson *et al.*, 2003; Cossegal *et al.*, 2008). The expression of at least one of them in barley (*Hv.AGP.S1*) is regulated by alternative splicing. One transcript of this gene is localised in the cytosol in endosperm, and the second, responsible for more than 90% of the total activity of AGPase, in chloroplasts. Expression of the *Hv.AGP.S2* gene takes place in embryo and in endosperm (Rösti *et al.*, 2006).

The plastidial isoform of AGPase is an allosteric enzyme activated by low 3-phosphoglycerate (3-PGA) concentration and inhibited as 3-PGA concentration increases and also by inorganic orthophosphate (P_i) and pyrophosphate (PP_i) (Kleczkowski, 2000). The ratio of 3-PGA and P_i concentration in chloroplast stoma is crucial for the speed of starch synthesis in leaves (Kleczkowski, 1999). The activity of AGPase is also controlled in various plant species by redox modulation in response to light and sugar levels (Fu *et al.*, 1998; Tiessen *et al.*, 2002; Hendriks *et al.*, 2003). Due to the nocturnal formation of disulfide bonds between the AGP-S subunits, an inactive dimer is formed (Hendriks *et al.*, 2003). The redox activation of AGPase in potato tubers is connected with the high level of sucrose or glucose, involves SnRK1 (sucrose-non-fermenting-1-related protein kinase) and hexokinase-mediated sugar signalling pathways, respectively (Tiessen *et al.*, 2003). An other signalling compound, Tre6P (trehalose 6-phosphate), is involved together with the sugar level in cytosol in the reductive activation of AGPase (Kolbe *et al.*, 2005; Geigenberger *et al.*, 2005). It is suggested that Tre6P plays a central role in linking the rate of starch synthesis to the demand for carbon and to plant growth (Smith & Stitt, 2007).

Starch granule formation

The starch granule formation is a very complicated process in which numerous enzymes and regulatory proteins participate. The most important ones are obviously starch synthases (SS, EC 2.4.1.21). SS catalyze the formation of new glycosidic linkages by transferring the glucosyl moiety of the soluble precursor ADP-glucose to the reducing end of an existing α-1,4-linked glucan chain (acceptor) to synthesise the insoluble glucan polymers, amylose and amylopectin. The regular branch point distribution that allows crystallization of amylopectin in the plastid is the effect of a concerted action of branching enzymes (BE, EC 2.4.1.18) and debranching enzymes (DBE, EC 3.2.1.68 or EC 3.2.1.41).

Plants possess multiple isoforms of SS. Based on amino-acid sequences, five classes of starch synthase may be distinguished: granule-bound starch synthase (GBSS) involved in amylose biosynthesis, and starch synthase (SSI, SSII, SSIII, SSIV) involved solely in amylopectin synthesis. The exact function of the SSIV isoform is not determinated. The SSI, SSII and SSIII isoenzymes are involved in elongation of amylopectin chains of a defined length (Zeeman *et al.*, 2007b). SSI preferentially elongates the shortest chains with a DP (degree of polymerisation) of 4–10 glucosyl units, SSII — medium-length chains (DP of 12–24 glucosyl units), and SSIII — the longest chains and is able to influence the rate of starch accumulation in leaves (Morell *et al.*, 2003; Zhang *et al.*, 2005). Among the tree SSII genes that were found in monocots, SSIIb predominates in photosynthetic tissues, and also one form of *SSIII* gene — SSIIIb is exclusively expressed in the leaf (Ohdan *et al.*, 2005). Analysis of starches produced as a result of the combined reduction of SSII and SSIII activities in potato

by Edwards *et al.* (1999) and Lloyd *et al.* (1999) reveals that different SS isoforms make distinct contributions to amylopectin biosynthesis, and that they act synergistically, rather than independently, during amylopectin synthesis (Tetlow, 2006).

Amylose is synthesised by the granule-bound starch synthase (GBSS). There are two genes encoding isoforms of GBSS (I and II). Expression of GBSSI appears to be mostly confined to storage tissues and that of the gene encoding GBSSII is mainly localised in the chloroplast. For that reason it is supposed that GBSSII is responsible for amylase synthesis in chloroplasts (Fujita & Taira, 1998; Vrinten & Nakamura, 2000). Mutations in the *Waxy* locus leading to a loss of GBSS activity result in amylose-free (*Waxy*) starch (Nelson & Rines, 1962). Analyses of transcript levels of GBSS in *Arabidopsis* leaves showed strong diurnal changes that follow the pattern of leaf starch accumulation in the light and degradation at night (Smith *et al.*, 2004).

Branching of the glucan chain by starch branching enzymes

Starch branching enzymes (SBE/BE, EC 2.4.1.18) introduce α-(1,6)-bonds by cleaving internal α-(1,4) linkages and transferring glucan segment of six or more glucose residues to the same or neighbouring chain of the amylopectin molecule (Borovsky *et al.*, 1975). Also BE exists in multiple isoforms. Two classes of BEs have been distinguished: I or B and II or A (Burton *et al.*, 1995; Rahman *et al.*, 2001). BEI preferentially transfers longer chains than BEII (Guan & Preiss, 1993). Down-regulation or removal of BEI activity in photosynthetic and nonphotosynthetic tissues appears to have minimal effects on starch quantity and composition (Safford *et al.*, 1998; Blauth *et al.*, 2002; Satoh *et al.*, 2003). In maize, mutation of the gene encoding BEIIb (also known as *amylose extender* (*ae*) or *amo* in barley) produces a high-amylose starch phenotype (Yun & Matheson, 1993). A mutation causing inhibition of BEII activity in potato resulted in increase of amylose content and modification of amylopectin structure due to the higher content of average chain length, i.e. DP 23–60 (Jobling *et al.*, 1999). In wheat, high-amylose starch can only be produced by suppression of both genes encoding the SBEIIa and SBEIIb forms (Regina *et al.*, 2006). Taken together, results obtained with different BE mutants suggest that BSI is unable alone to produce branching points in amylopectin molecule, and that both classes of BE together participate in the formation of the proper structure of the starch granule.

The role of debranching enzymes and starch phosphorylase in biosynthesis of starch granules

Other enzymes traditionally connected with starch decomposition have been implicated in the pathway of starch biosynthesis, but their role in granule synthesis has yet to be elucidated. One of the important enzyme groups are debranching enzymes that are able to cleave the α-(1,6)-linkages in α-glucan. Two types of DBE exist in plants; isoamylase-type (EC 3.2.1.68; three isoamylase-type DBE genes: *isa-1*, *isa-2*, and *isa-3* have been found in the *Arabidopsis* genome) and one pullulanase-type (EC 3.2.1.41, also known as limit-dextrinase — *lda*), efficiently hydrolyze α-(1,6)-linkages not only in amylopectin, but also in pullulan (a fungal polymer of maltotriose residues). Studies on barley mutants and transgenic rice suggest that isoamylases play a crucial role in starch granule synthesis initiation (Burton *et al.*, 2002; Kawagoe *et al.*, 2005). DBEs are involved in starch biosynthesis in conjunction with the SSs and SBEs, and two models have been proposed for their regulative roles. The decrease or loss of either the ISA-1 or ISA-2 isoamylase-type DBE activity is thought to be responsible for the accumulation of phytoglycogen (disordered water-soluble polysaccharide) rather than starch in mutant or transgenic plants (Bustos *et al.*, 2004; Zeeman *et al.*, 1998) and algae (Mouille *et al.*, 1996). One possibility described by Zeeman and co-workers (1998) is that DBEs play a ''clearing'' role, removing soluble glucan that is not attached to the granule from the stroma. Such activity of DBEs prevents the accumulation of phytoglycogen due to single activity of SSs and SBEs and reduces the rate of starch synthesis. The second model of DBEs role in starch synthesis is based on so-colled ''glucan-trimming'' (pre-amylopectin trimming) model. In this model, it is supposed that glucan trimming is required for amylopectin aggregation into an insoluble granular structure (Ball *et al.*, 1996; Myers *et al.*, 2000). The activity of DBEs should remove branches appearing at wrong position which could disturb the crystallisation of starch granule.

The second enzyme traditionally connected with starch degradation is starch phosphorylase (SP). This enzyme (EC 2.4.1.1) catalyzes the reversible transfer of glucosyl units from glucose 1-phosphate to the non-reducing end of α-1,4-linked glucan chains. The role of glycogen phosphorylase in mammalian metabolism is well known but the function of SP in the plant kingdom remains unclear. Results of experiments suggest a role of plastidic isoform of SP (Pho1 or L-form) in starch synthesis rather than starch degradation (Yu *et al.*, 2001b; Zeeman *et al.*, 2004b). It is supposed that Pho1 fulfils a similar

function as this of DBEs, that is the "clearing" i.e. soluble glucan not connected to starch granules.

The role of multi-enzyme complex formation

Starch granule synthesis in the chloroplast seems to involve protein–protein interactions. The formation of complexes of starch metabolic enzymes *via* protein–protein interactions may enhance the efficiency of biosynthesis of amylopectin and amylose with proper structure, due to conformational changes of the enzymes, substrate channelling and also prevention of phytoglycogen formation. Analysis of mutants (Nishi *et al.,* 2001; Dinges *et al.,* 2001; 2003) warrant the formation such a thesis. However, most experiments were performed using heterotrophic tissue, mainly endosperm of cereals. It has been proposed that the synchronization of DBEs, BEs, and SSs activities required for starch synthesis is completed by physical association of the enzymes in a complex or complexes within the amyloplast (Ball & Morell, 2003). Formation of such a complex could explain the reduction of starch biosynthesis rate and numerous pleiotropic effects associated with well-characterized mutants in cereal endosperms (Hennen-Bierwagen *et al.,* 2008). Tetlow and co-workers (2004a; 2004b; 2008) asserted that formation of such complexes is dependent upon the enzymes' phosphorylation status. In this case, phosphorylated SBEs and SSs are able to build the complex but non-phosphorylated enzymes are not. Reversible enzyme phosphorylation, a well known mechanism of enzyme activity modulation (Klimecka & Muszynska, 2007), is now very intensively studied with respect to starch metabolism (see below).

The involvement of 14-3-3 proteins in the regulation of starch metabolism has been considered as a second controlling mechanism. It has been shown by Sehnke and co-workers (2001) that one form of 14-3-3 protein is starch granule-associated in *Arabidopsis*. Antisense inhibition of the granule-bound 14-3-3 protein resulted in leaf starch accumulation (Sehnke *et al.,* 2001). These results together with those obtained by Alexander and Morris (2006) suggest formation of protein complexes between 14-3-3 proteins and starch synthases (SS) or starch branching enzymes (BE) in *Arabidopsis* leaf and in developing barley endosperm. Apart from those preliminary results, the exact role of 14-3-3 proteins in protein complex formation with enzymes involved in starch metabolism needs to be elucidated. Taken together, the phosphorylation status of enzymes involved in starch metabolism as well as the facilitatory role of 14-3-3 proteins may influence the rate of starch synthesis/degradation and the structure of the starch granule.

STARCH DEGRADATION IN CHLOROPLAST

Starch degradation in chloroplasts differs from that in amyloplasts of germinating cereal grains (Ritchie *et al.,* 2000; Lovegrove & Hooley, 2000; Zeeman *et al.,* 2004a). The observed periodical starch degradation at night could be divided into two phases: initiation of degradation, and digestion of amylopectin and amylose into maltose, glucose and maltotriose. Native starch granules are a poor substrate for most amylolytic enzymes (Tester *et al.,* 2004). For many years it was thought that α-amylases initiate starch granule degradation in the chloroplast, but recent studies have questioned this assumption (Yu *et al.,* 2005; Stanley *et al.,* 2005).

In the initiation of starch breakdown, the phosphorylation of starch caused by starch dikinases (GWD/PWD) becomes the crucial step (Lloyd *et al.,* 2005; Fettke *et al.,* 2006b; Weise *et al.,* 2006; Zeeman *et al.,* 2007a). Both dikinases do not themselves catalyse starch degradation. One possibility is that β-amylase and isoamylase 3 cannot readily attack the ordered, semi-crystalline surface of the granule in the chloroplast. The addition of phosphate groups to starch polymers may disrupt the organization of the granule matrix and make it a better substrate for exoamylolytic attack (Hejazi *et al.,* 2008; Edner *et al.,* 2007; Ritte *et al.,* 2006; Kötting *et al.,* 2005; Engelsen *et al.,* 2003).

Currently it is considered that the hydrolysis of the starch granule is catalysed mainly by β-amylase and a debranching enzyme (isoamylase 3) (Fig. 2). These two types of enzymes hydrolyse the 1,4- and 1,6-glycosidic bonds, respectively. The principal product of their combined activity is maltose, which is exported from the chloroplast to the cytosol *via* MEX1 transporter. The second product of starch degradation is glucose, which is also exported to the cytoplasm (Delatte *et al.,* 2006; Zeeman *et al.,* 2007a). β-Amylase is an exoamylase. In *Arabidopsis thaliana*, there are nine genes encoding β-amylases expressed in leaves. At least four of them are localised in the chloroplast (Lao *et al.,* 1999; Scheidig *et al.,* 2002; Kaplan & Guy, 2005). On the basis of recombinant analysis of protein activities, it has been shown that three forms (BAM1, -2, -3) have a typical β-amylase activity and BAM4 remains inactive. Surprisingly, *bam4* mutants have a starch excess phenotype and less maltose at night compared to the control (Fulton *et al.,* 2008). To sum up, Fulton and co-workers (2008) have proposed that β-amylase isoenzymes are the key enzymes in starch breakdown. BAM3 and BAM1 catalyse mainly glucan hydrolysis, and BAM4 probably fulfils a regulatory or facilitatory role in starch degradation. BAM2 does not play a significant role in the breakdown of starch in

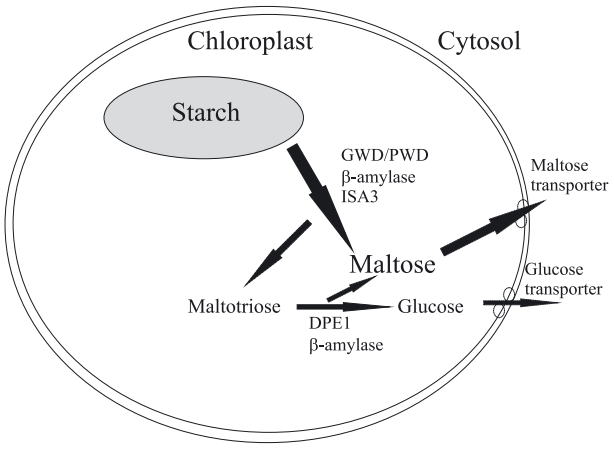

Figure 2. Pathway of starch degradation in leaves. Abbreviations: DPE1, transglucosidase; GWD, glucan water dikinase; ISA3, debranching enzyme/isoamylase 3; PWD, phosphoglucan water dikinase.

plants growing at either 12-h or 16-h photoperiod (Fulton *et al.*, 2008). Instead it may play a role in plant adaptation to stress conditions but it is only a speculation at present.

Regulation of starch degradation in leaf tissues

Diurnal cycle

Under standard conditions (day no longer than 16 h) starch is synthesised during the light period and all transient starch granules are degraded at night. During the first 2 h in the darkness, the process is accelerated and after that proceeds at a constant rate. The frequency of starch degradation depends on the length of day and intensity of starch synthesis, i.e. the lower the rate of starch synthesis the lower the rate of its breakdown (Zeeman & Rees, 1999; Gibon *et al.*, 2004; Smith *et al.*, 2004; Lu *et al.*, 2005; Smith & Stitt, 2007). The diurnal starch synthesis and products of its degradation supply plants continually with carbohydrates necessary for metabolism.

Starch phosphorylation

The importance of different enzymes in starch degradation was elucidated in studies with *starch excess* (*sex*) *Arabidopsis* mutants, isolated earlier by Caspar *et al.* (1991). Mutant *sex1* accumulated over a 7-fold more starch than the wild type after darkness. This mutation was attributed to the inactivation of GWD1 (SEX1) in *A. thaliana* leaves (Yu *et al.*, 2001a). Repression of GWD (R1) in potato resulted in starch accumulation in leaves and slowdown of the cold-sweetening process in tubers (Lorberth *et al.*, 1998). Both effects indicate a disturbance of starch degradation in potato. Starch phosphorylation takes place during both the synthesis and breakdown of starch (Ritte *et al.*, 2000). Normal rates of starch granule degradation require the presence of two glucan water dikinases (GWDs: GWD1 and PWD/GWD3), enzymes that add phosphate groups from ATP onto either the 3-position (PWD/GWD3) or the 6-position (GWD) of glucose residues within starch polymers (Ritte *et al.*, 2006). Recently, a third isoform (AtGWD2) of water dikinase localised outside plastids in *Arabidopsis* leaves was described and characterised (Glaring *et al.*, 2007). It was shown that AtGWD2 is not directly involved in transient starch degradation. It has been supposed that this isoform of GWD acts on starch granules released from degraded plastids in ageing plants (Glaring *et al.*, 2007). It is worth mentioning that phosphorylation of starch is important as the initiation step of starch degradation for some plant species, e.g. potato and *A. thaliana*, but not as much for rice or others cereals. In these plants, starch phosphorylation level is very low (less than 0.01% glucose moieties are phosphorylated) (Yu *et al.*, 2001a).

Transcriptional control

In general, the transcript levels of enzymes involved in starch degradation show diurnal changes, they increase during the day and decrease during the night (Smith *et al.*, 2004; Lu *et al.*, 2005; Bläsing *et al.*, 2005; Smith & Stitt, 2007).

A bioinformatics studies applied after analysis of gene expression using Affymetrix *Arabidopsis* ATH1 chips have been published recently (Li *et al.*, 2007; Usadel *et al.*, 2008). A profile of expression of 12 genes whose functions are well elucidated and attributed to starch degradation (e.g. genes of sugar transporters, starch dikinases, starch phosphorylases, starch dikinases, transglucosidases) was shown (Usadel *et al.*, 2008). Besides those known genes, there are others that are similarly co-expressed, but their physiological functions are still not fully understood. It clearly demonstrates that starch degradation is a highly regulated process involving the action of multiple enzymes and regulatory genes (Li *et al.*, 2007).

Post-translational control of enzyme activity

Due to a weak correlation between the transcript level of known enzymes and their activity observed in the cell, it is thought that the control is mainly through post-translational modifications or by regulatory proteins (Smith *et al.*, 2004; 2005; Lu *et al.*, 2005). The post-translational regulation of enzyme activity may involve several phenomena:

— *redox potential*, potato GWD (Mikkelsen *et al.*, 2005) and one isoenzyme of β-amylase (BMY7, also called BAM1 or TR-BMY; Sparla *et al.*, 2006) were reported to be activated directly by thioredoxin-mediated reduction of an -SH group essential for the activity of the enzymes (Mikkelsen *et al.*, 2005; Fulton *et al.*, 2008). Some enzymes connected with CO_2 assimilation are also directly or indirectly activated by SH-reduction. It occurs by using electrons from Photosystem I, which are transferred *via* the ferredoxin–thioredoxin system (Buchanan *et al.*, 2002).

— *metabolite concentration*, in mutants with decelerated starch decomposition maltooligosacharide accumulation was observed (Critchley *et al.*, 2001; Niittylä *et al.*, 2004; Chia *et al.*, 2004; Messerli *et al.*, 2007).

— *reversible protein phosphorylation* involved in starch degradation due to specific kinases/phosphatases. Mutation of a dual-specifity protein phosphatase (SEX4, described also as DSP4 or PTPKIS2) in *A. thaliana* caused accumulation of starch granules and storing down of starch degradation (Fordham-Skelton *et al.*, 2002; Kerk *et al.*, 2006; Niittylä *et al.*, 2006; Sokolov *et al.*, 2006). SEX4 is able to bind to different polysaccharides through carbohydrate-binding modules (CBM), found also in PWD (Kötting *et al.*, 2005; Niittylä *et al.*, 2006). Interestingly, SEX4 has a similar structure to the human laforin. Laforin removes phosphate groups from both proteins (e.g., kinase 3, a part of the glycogen phosphorylase regulation system) and carbohydrates such as glycogen or amylopectin (Niittylä *et al.*, 2006; Worby *et al.*, 2006). By combining the activity of GWD/PWD and SEX4 starch degradation can be regulated by starch phosphorylation and dephosphorylation (Zeeman *et al.*, 2007a). However, it is also possible that specific phosphatases are part of a regulatory pathway that modulates the activities of enzyme(s) of starch degradation.

Control of starch degradation in cytosol

Maltose produced by β-amylase is exported from the plastid by the maltose transporter (Niittylä *et al.*, 2004). In the cytosol, maltose is converted to hexose phosphates *via* a pathway that requires a transglucosidase, DPE2 (EC 2.4.1.25) (Chia *et al.*, 2004; Lu *et al.*, 2006). The glucose moiety is transferred to an acceptor molecule in the cytosol — a heteroglycan containing glucose, mannose other saccharides (Fettke *et al.*, 2005; 2006a). The glucose residues of the heteroglycan are also a substrate for cytosolic glucan phosphorylase (PHS2), which converts them to glucose 1-phosphate (Fettke *et al.*, 2006a). This DPE2–PHS2 pathway is potentially reversible and for that reason the heteroglycan may act as a 'glucose buffer' between starch degradation and sucrose synthesis (Fettke *et al.*, 2006b). Free glucose is also produced in the cytosol due to DPE2 activity and further metabolised to hexose phosphate *via* hexokinase, a key enzyme in sugar sensing and signalling. The conversion of maltose to hexose phosphates in the cytosol seems to be a part of the mechanisms controlling the rate of starch degradation and depends on the plant's demand for carbohydrates.

CONCLUSIONS

Our understanding of starch metabolism in leaves is nowadays more comprehensive because of the full annotation of both *Arabidopsis* and rice genomes. In addition, using genetic approaches and various natural mutants, we have improved our knowledge on starch biosynthesis and degradation in the chloroplast. The key role in starch digestion is attributed to β-amylase. Other enzymes from the starch biosynthetic path, probably in the form of multi-protein complexes, affect the semi-crystalline structure of the starch granule. With respect to differences in starch metabolism in heterotrophic, e.g. cereal embryos, and autotrophic tissues, the new information could contribute to rapid progress in plant breeding. As a final result we should be able to obtain starches with novel technological features.

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