Review

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Phosphoglucan phosphatase function sheds light on starch degradation

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Phosphoglucan phosphatases are novel enzymes that remove phosphates from complex carbohydrates. In plants, these proteins are vital components in the remobilization of leaf starch at night. Breakdown of starch is initiated through reversible glucan phosphorylation to disrupt the semi-crystalline starch structure at the granule surface. The phosphoglucan phosphatases starch excess 4 (SEX4) and like-SEX4 2 (LSF2) dephosphorylate glucans to provide access for amylases that release maltose and glucose from starch. Another phosphatase, LSF1, is a putative inactive scaffold protein that may act as regulator of starch degradative enzymes at the granule surface. Absence of these phosphatases disrupts starch breakdown, resulting in plants accumulating excess starch. Here, we describe recent advances in understanding the biochemical and structural properties of each of these starch phosphatases.

Phosphoglucan phosphatases

Phosphatases are enzymes that remove phosphate groups (PO_4^{3-}) from a wide array of substrates that includes proteins, lipids, and glucans [1–3]. These proteins often operate in conjunction with kinases to control a multitude of cellular processes. Phosphoglucan phosphatases form a distinct clade within the dual-specificity phosphatase (DSP) family of the protein tyrosine phosphatase (PTP) superfamily [4] (Box 1), indicating that these proteins have evolved specific phosphatase domains for glucan dephosphorylation. Over the past decade, the phosphoglucan phosphatases have surfaced as fundamental proteins that regulate storage carbohydrate metabolism in plants (starch) and mammals (glycogen). For instance, the mammalian phosphoglucan phosphatase laforin controls the phosphorylation state of glycogen, and deregulation of human laforin causes patients to accumulate hyperphosphorylated polyglucosan bodies that result in the fatal neurodegenerative disorder Lafora disease (recently reviewed in [5,6]). In this review, we focus on the laforin-related plant phosphoglucan phosphatases SEX4, LSF1, and LSF2, which are vital components in the remobilization of leaf starch. Several recent physiological, biochemical, and structural studies have pinpointed each of these proteins as having discrete functions, revising the current model of leaf starch degradation in plants.

Plant phosphoglucan phosphatases contain not only the DSP domain, but also other structural features that make these proteins suitable for glucan dephosphorylation (Figure 1). Each of these phosphatases initially has a chloroplast transit peptide (cTP), which is cleaved off after translocation into the chloroplast (the site of starch metabolism), and a C-terminal (CT) domain for protein stability [7–12]. SEX4 and LSF1 are the only plant phosphatases that contain carbohydrate-binding modules (CBMs) [7,8]. These noncatalytic protein domains bind polysaccharides and orient the active site of the DSP domain near the phosphoglucan [13,14]. In addition, LSF1 has an N-terminal extension with a PDZ* domain, which in other eukaryotic proteins mediates protein–protein interactions [15–17]. Altogether, the protein architecture of these phosphoglucan phosphatases represents a unique combination of domains that aid in starch breakdown in plants.

Degradation of leaf starch

Starch is the major carbohydrate reserve in plants. During daytime photosynthesis, starch is synthesized from assimilated sugars and assembled into storage granules in specific tissues (e.g., seeds, tubers, and leaves) [18]. Granules are composed of two glucose polymers: linear α-1,4-glucosidic-linked amylose (20–30%) and branched α-1,6-glucosidic- and α-1,4-glucosidic-linked amylopectin (70–80%). Starch granules are highly ordered, semi-crystalline structures: adjacent glucan chains in unbranched regions of amylopectin can form double helices that cluster together into crystalline lamellae, which alternate with the less-ordered, amorphous lamellae, comprising mostly α-1,6-glucosidic-linked branch points [19,20] (Figure 2A).

Leaf chloroplasts store transient starch that is remobilized into sugars (i.e., maltose and glucose) for metabolism and growth at night, when carbon derived from photosynthesis is unavailable [18,21]. Maltose is cleaved from the linear sections of amylopectin by β-amylases (BAMs; EC 3.2.1.2). In addition, other starch hydrolases release

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*PDZ is an acronym of the first three proteins identified with this domain: postsynaptic density protein 95 (PSD95) [77], disc large tumor suppressor [78], and zonula occludens-1 protein (ZO-1) [79].

Box 1. The protein tyrosine phosphatase family

PTPs (EC 3.1.3.48) have the most diverse substrate groups and, therefore, function of phosphatases in eukaryotes [1]. PTPs were originally classified based on phosphotyrosine dephosphorylation, but other substrates of PTPs also include mRNA (e.g., RNA capping enzymes) [61], phosphoinositides (e.g., myotubularins) [62], and phosphoglucans (e.g., phosphoglucan phosphatases) [34]. These nonproteinaceous substrates are hydrolyzed by the DSP subfamily [63]. DSPs have a shallower and often wider active site compared with tyrosine-specific PTPs and, therefore, can accommodate larger substrates [64]. In humans, 112 PTPs are encoded, whereas fewer exist in *Arabidopsis* (31) and rice (25) [4,65], which is partially attributed to a lack of receptor PTPs in the Plantae. Outside of starch degradation, the cellular function of most plant PTPs is unknown, or is only inferred based on sequence relationship to human and yeast enzymes.

Catalytic mechanism

PTPs have the signature motif [I/V]HCXXGXXR[S/T] (or HCX_5R), which contains the invariant cysteine positioned at the base of the active site cleft [57]. This cysteine exists as an active thiolate anion during catalysis and is stabilized (i.e., relative pK_a lowered) by the electrostatic interactions of the neighboring histidine [51]. Dephosphorylation occurs in two steps [66–68]: (i) a cysteinyl-phosphate intermediate is formed after nucleophilic attack by the thiolate ion of the cysteine and coordinated by the arginine of the signature motif. Transfer of the phosphate is promoted through the protonation of the dephosphorylated substrate by the aspartic acid in the WPD loop (approximately 30 residues upstream of the signature motif); and (ii) phosphate is released after hydrolysis by the aforementioned aspartate (general base) and a nearby glutamine to restore the thiolate anion. Additional residues in the flexible variable region (V loop) mold the active site, which contributes to substrate recognition for PTPs.

Reversible oxidation

PTPs are susceptible to oxidation because the cysteine exists as a thiolate anion at physiological pH (pK_a 4.7–5.7) [69]. Initial oxidation rapidly converts the thiolate anion ($-S^-$) to sulfenic acid (-SOH), rendering the enzyme inactive [70]. PTPs can protect this cysteine through reversible oxidation by first forming a sulfenyl-amide bond (with the protein backbone) or a disulfide bond (with a nearby cysteine), and then reducing the cysteine back to the thiolate ion via glutathione or thioredoxins [71,72]. These structural rearrangements prevent the cysteine from becoming irreversibly oxidized to sulfinic acid ($-SO_2H$) and sulfonic acid ($-SO_3H$) [73]. Reversible oxidation of PTPs can serve as a regulatory mechanism for phosphatase activity. AtPTP1 [74] and SEX4 [41] are the only plant PTPs known to undergo reversible oxidation, the latter recently shown to form disulfides to allow reactivation.

linear and branched malto-oligosaccharides that are further hydrolyzed into sugars in the chloroplast stroma.

Phosphoglucan phosphatases in the reversible glucan phosphorylation of starch

Numerous studies have revealed that reversible glucan phosphorylation at the granule surface is required to initiate and achieve complete remobilization of leaf starch. Glucan phosphorylation is the only known *in vivo* modification of starch [22], yet its biological role in starch metabolism has only recently been deduced [23]. Phosphorylation occurs at C6 and C3 positions of amylopectin glucosyl units [24] by the dikinases α-glucan, water dikinase (GWD; EC 2.7.9.4) and phosphoglucan, water dikinase (PWD; EC 2.7.9.5), respectively [25]. Steady-state starch phosphate levels vary between species and tissues. Reported levels of *Arabidopsis thaliana* (*Arabidopsis*) leaf

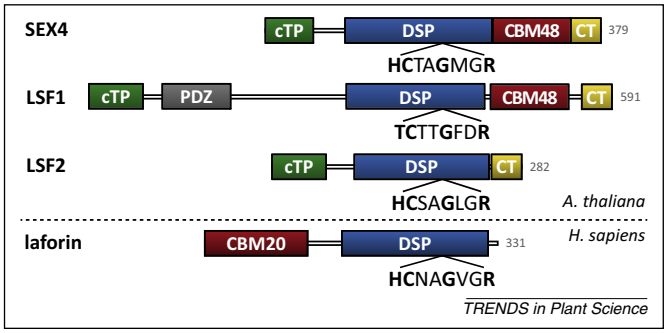

Figure 1. Domain architecture of phosphoglucan phosphatases. Phosphoglucan phosphatases include starch excess 4 (SEX4), like-SEX4 1 (LSF1), and LSF2 in plants (shown from *Arabidopsis thaliana*) and laforin in mammals (shown from *Homo sapiens*). Protein domains are the chloroplast transit peptide (cTP), dual-specificity phosphatase domain (DSP), carbohydrate-binding module (CBM), C-terminal domain (CT), and protein–protein interaction domain (PDZ). Residues pertaining to the signature motif (HCX_5R) are displayed for each phosphatase. Domain boundaries for SEX4 and LSF2 were defined by solved crystal structures [12,48], whereas LSF1 and laforin were annotated using PFAM [75] and SMART [76].

starch phosphate range from 0.8 to 1.4 nmol phosphate per μmol glucose [11,26,27], whereas potato tuber starch is generally more highly phosphorylated (3.4–5.8 nmol phosphate per μmol glucose) [22,26]. The addition of phosphate induces granule surface hydration that disrupts the semi-crystalline matrix of amylopectin and exposes linear chains for subsequent hydrolysis by BAMs (Figure 2A). Recent structural data indicate that these modifications by GWD and PWD have different effects on the semi-crystalline layer of starch [28]. GWD marks sections for glucan hydrolysis via C6 phosphorylation to initiation granule surface hydration [29]. PWD then recognizes these partially solubilized sections [30] and catalyzes C3 phosphorylation of nascent glucans to induce steric strain that breaks the helical structure and prevents recrystallization. Altogether, GWD and PWD operate in a concerted relay of phosphorylation to initiate glucan hydrolysis. Notably, Nitschke and colleagues [31] showed that mammalian glycogen is phosphorylated at the C6 position and that this modification is a carefully controlled event, rather than a side-reaction of glycogen synthase, as previously thought.

Just as glucan phosphorylation triggers leaf starch breakdown, the removal of these phosphate groups is also necessary for complete glucan hydrolysis. Starch-bound phosphate can impede the movement of starch degradative enzymes along the glucan chain [32], limiting the release of maltose and malto-oligosaccharides from the starch granule. The enzymes responsible for removing these phosphates were eventually identified as the phosphoglucan phosphatases SEX4 (EC 3.1.3.48) [9,27], and later LSF2 [11]. SEX4 releases phosphate from both the C3 and C6 position of amylopectin [33], whereas LSF2 is specific for the C3 position [11] (Figure 2B). The discovery of this novel function of glucan dephosphorylation signified that reversible phosphorylation, not just phosphorylation, was required for the breakdown of leaf starch (Figure 2). The recent evidence of LSF1 as a putative inactive, scaffold protein also suggests another mode of regulation for these phosphatases [10,34].