Starch Biosynthesis and Degradation in Plants

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Based in part on the previous version of this eLS article 'Starch Biosynthesis and Degradation in Plants' (2007) by Alison M Smith.

Advanced article

Article Contents

Online posting date: 15th July 2016

Starch is the main form in which plants store carbon. Its presence and turnover are important for proper plant growth and productivity. The glucose polymers that constitute the semi-crystalline starch granule are synthesised by the concerted actions of well-conserved classes of isoforms of starch synthase and starch-branching enzyme, via a process that also requires the debranching enzyme isoamylase. The degradation of the granule proceeds via different pathways in different types of starch-storing tissues. The pathway of starch degradation differs between different plant tissues, but has been elucidated in most detail in leaves. The polymer is first phosphorylated to allow access to the insoluble granule by enzymes that cleave bonds between glucose residues. The main product of this degradation is maltose, which is exported into the cytosol before a series of enzymatic steps convert it to sucrose.

Introduction

Starch is the main form in which plants store carbon. Its role in plants can be regarded as physiologically equivalent to that of glycogen in animals. Starch is the major component of many plant storage organs – for example, 50–80% of the dry weight of cereal, pea and bean seeds, cassava roots and potato tubers consists of starch. In these organs, starch synthesis and starch degradation occur during distinct developmental periods, which may be separated by months or even years. In starch-storing seeds, starch synthesis occurs during most of the period of growth and maturation. Starch degradation occurs after the onset of germination, providing carbon for the initial growth of the seedling. In vegetative storage organs, starch is synthesised during growth periods favourable for photosynthesis. It persists during unfavourable periods, when the photosynthetic parts of the plant may die. It is then degraded after the onset of regrowth, to provide carbon for initial growth until photosynthetic organs are reestablished. In addition to its role in storage organs, starch also serves as a temporary store of carbon in many other cells and organs of the plant. In leaves of many species, it is synthesised in parallel with sucrose as a product of photosynthesis during the day and accumulates in chloroplasts. It is then degraded at night to provide a continuing supply of carbon to nonphotosynthetic organs. This process is almost certainly regulated on a number of levels including control of transcription, post-translational mechanisms such as allosteric and redox control, and the circadian clock. Starch is also found in nonphotosynthetic parts of the plant such as stem parenchyma, cells peripheral to meristems, young fruits and seed coats and stomatal guard cells. In these cells synthesis and degradation may occur simultaneously, so that starch provides a 'carbon buffer' that allows for temporary imbalances between the rate of supply of sucrose from leaves and the rate of its utilisation in cellular metabolism. Starch is not only important as a carbon store in plants but is also used in many industries. As such much research has been performed examining the enzymes involved in its metabolism to either increase the amount produced in storage organs or alter its structure in a way that would make it more amenable to industrial use. Because of its industrial importance, starch synthesis has been intensively studied in major storage organs, particularly cereal seeds. Since the publication of the *Arabidopsis* genome sequence in 2000, however, there has been a rapid increase in the understanding of starch synthesis and degradation in leaves. This has demonstrated that although the pathway present in leaves and storage organs is catalysed by the same enzymatic activities, the isoforms involved are often different in different organs. The reason for this is most likely that starch needs to be synthesised with different structures in these organs in order to fulfil the variety of roles it plays, as outlined above.

eLS subject area: Plant Science

How to cite:
Lloyd, James R and Kötting, Oliver (July 2016) Starch Biosynthesis and Degradation in Plants. In: eLS. John Wiley & Sons, Ltd: Chichester.
DOI: 10.1002/9780470015902.a0020124.pub2

Starch Synthesis

The starch granule

Starch granules are semi-crystalline structures, typically ovoid or spherical in shape and ranging in size from less than a micron to over 100 µm depending on the organs and developmental stage examined. Granules consist almost entirely of two different sorts of glucose polymers. Amylopectin, which makes up 70–80% of most granules, is a highly branched α1,4, α1,6-linked polymer. Chains of about 12–20 glucoses are linked together at branch points that occur at intervals of 9 nm along the axis of the molecule, resulting in a regular clustering of chains along the molecule's axis (**Figure 1**). Within the clusters, adjacent chains form double helices, which become organised into regular arrays called crystalline lamellae. These sandwiches of crystalline lamellae and branch-point zones (amorphous lamellae) make up part of the matrix of the granule (**Figure 1**). Various levels of larger scale organisation also exist in the matrix. At intervals of several hundreds of nanometres (called growth rings), the 'sandwich' structure appears to break down, giving rise to concentric rings that can be revealed by various chemical and enzymic etching processes (**Figure 1**). Advanced microscopic techniques suggest that the matrix is also organised into 'blocklets' with dimensions between 20 and 500 nm (Kossmann and Lloyd, 2000).

The second glucose polymer, amylose, does not appear to contribute to the structural organisation of the matrix. Mutant plants lacking this polymer can still form granules of normal structure. Amylose consists of long chains of glucoses with very few branch points. It probably exists in an unorganised form inside the granule, perhaps in the less organised regions of the growth rings.

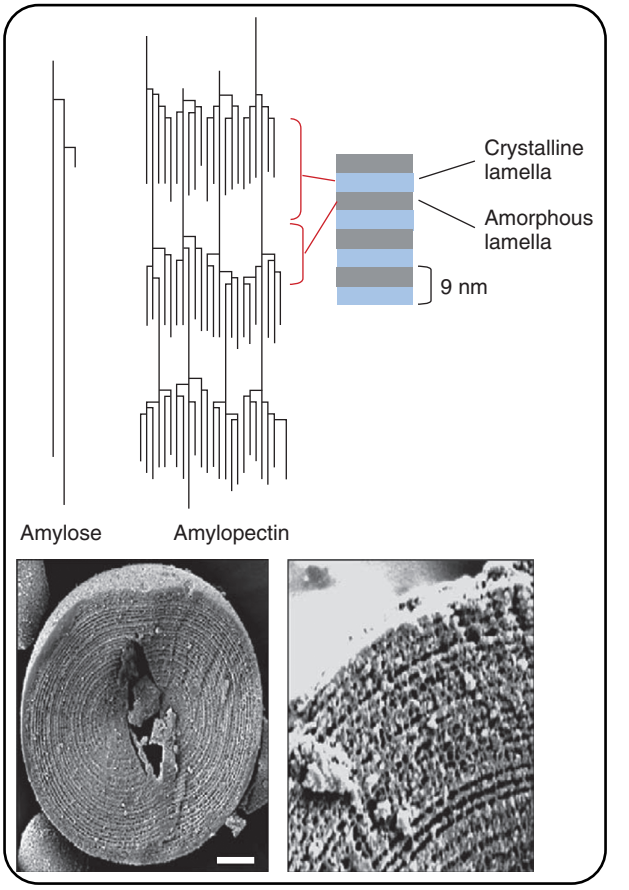

Figure 1 The structure of the starch polymers and the starch granule. Top left: representations of the structures of amylose and amylopectin. The chains in the amylose molecule are 1000 or more glucosyl residues in length. The short chains within the clusters of the amylopectin molecule are typically 12–20 glucosyl residues in length. Top right: adjacent chains within the clusters of the amylopectin molecule form double helices, and these associate together to form crystalline lamellae. The regions between the clusters that contain the branch points do not crystallise, giving rise to alternating crystalline and amorphous lamellae with a periodicity of 9 nm. The layers of the sandwich are parallel with the surface of the granule; in other words, the lamellae form concentric shells within the granule matrix. Bottom: scanning electron micrographs of the inner face of a starch granule from a potato tuber, cracked open and treated with a starch-degrading enzyme to reveal the growth rings. Each ring consists of tens of the 9 nm repeats shown above. The bar represents 5 µm; the picture on the right is a closer image of part of the picture on the left.

Amylopectin and amylose synthesis

Synthesis of ADPglucose

The substrate for synthesis of amylopectin and amylose is the sugar nucleotide adenosine diphosphate (ADP) glucose. This is synthesised from glucose 1-phosphate via the action of the enzyme ADPglucose pyrophosphorylase (**Figure 2**). In chloroplasts, glucose 1-phosphate for ADPglucose synthesis is derived from the Calvin cycle intermediate fructose 6-phosphate, via reactions catalysed by phosphoglucose isomerase and phosphoglucomutase. In nonphotosynthetic organs, ADPglucose is derived from sucrose imported from the leaves. In most nonphotosynthetic cells, sucrose is converted to glucose 6-phosphate in the cytosol. Glucose 6-phosphate is then imported into the plastid via a transporter that exchanges it for phosphate, and converted to ADPglucose via phosphoglucomutase and ADPglucose pyrophosphorylase inside the plastid. In cells of the developing endosperm of cereal and grass (Poaceae) seeds, a second pathway of ADPglucose synthesis also operates. These cells uniquely possess a cytosolic as well as a plastidial isoform of ADPglucose pyrophosphorylase. Glucose 1-phosphate derived from sucrose can thus be converted to ADPglucose in the cytosol. ADPglucose then enters the plastid via a transporter that exchanges it for ADP. Mutant cereals that lack either the cytosolic form of ADPglucose pyrophosphorylase or the ADPglucose transporter have strongly reduced rates of starch synthesis in the endosperm, suggesting that this pathway predominates under normal conditions in endosperm cells (Kossmann and Lloyd, 2000). **See also**: **Photosynthesis: The Calvin Cycle**; **Sucrose Metabolism**

ADPglucose pyrophosphorylase appears to exert significant control over the rate of starch synthesis in many starch-synthesising organs, and its catalytic and regulatory properties have been the subject of intensive research. It is a tetrameric enzyme consisting of two types of subunits known

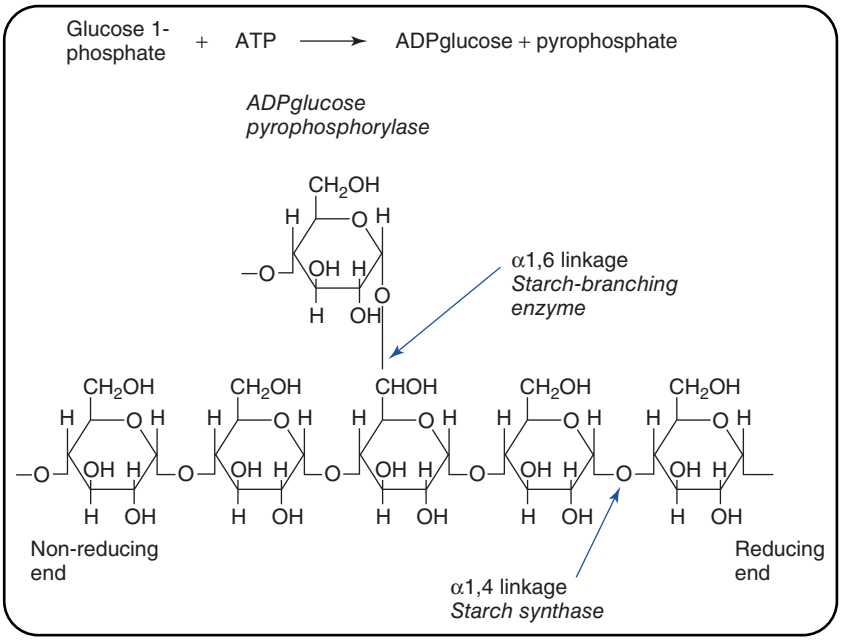

Figure 2 The actions of ADPglucose pyrophosphorylase, starch synthase and starch-branching enzyme. Starch synthase catalyses the addition of the glucosyl moiety of ADPglucose on to the nonreducing end of a chain via an α1,4 linkage. Starch-branching enzyme cleaves sections of chains from the nonreducing end and adds them to the side of the same or an adjacent chain via an α1,6 linkage.

as the small and large subunits. These proteins are related in sequence, but play distinct roles in determining the properties of the enzyme. Plant genomes contain several large subunit genes and may contain more than one small subunit gene. The large subunit genes have different patterns of expression in the plant and each is thought to confer distinct properties on the enzyme. As a result, the subunit composition and the properties of the enzyme vary from one organ and developmental stage to another.

The activity of ADPglucose pyrophosphorylase is regulated in a complex manner by the redox state and by allosteric interaction with the metabolites 3-phosphoglycerate and inorganic phosphate. In leaves, activation of the enzyme (by reduction of sulfydryl groups on the protein and by a high ratio of 3-phosphoglycerate to inorganic phosphate) occurs in conditions in which the rate of sucrose synthesis exceeds demand for sucrose by nonphotosynthetic parts of the plant. This activation diverts carbon from the Calvin cycle into starch rather than sucrose synthesis (Hendriks *et al*., 2003). The high level of regulation has been demonstrated to be important for increasing flux into starch synthesis. Expression of an unregulated *Escherichia coli* form of the enzyme leads to increased starch accumulation in both leaves (Gibson *et al*., 2011) and storage organs (Sonnewald and Kossmann, 2013), while expression of a mutated subunit of the plant protein that is unaffected by redox also leads to increased rates of starch synthesis in leaves (Hädrich *et al*., 2012).

Synthesis of glucose polymers

Two types of enzymes are required to synthesise α1,4, α1,6-linked glucose polymers: starch synthase and starch-branching enzyme. Starch synthase adds glucosyl moieties from ADPglucose to the nonreducing ends of chains. The branching enzyme cleaves linear chains and reattaches the cleaved fragment via an α1,6 linkage, creating a branch point (**Figure 2**).

The synthesis of amylopectin in the plant involves the actions of several different isoforms of starch synthase and starch-branching enzyme. All plants so far examined have at least five starch synthase genes and either two or three branching enzyme genes. The isoforms encoded by these genes have different structures and properties. Based on their primary amino acid sequences they fall into distinct classes, which are highly conserved across the plant kingdom (Kossmann and Lloyd, 2000). In maize endosperm, for example, starch synthase activity is mainly contributed by four different isoforms, SSI, SSIIa, SSIII and granule-bound starch synthase (GBSS). The starch-branching enzyme activity is contributed by two different isoforms, BEI and BEIIb. Different starch-synthesising organs on the same plant contain different complements of these isoforms. In maize leaves, for example, the predominant isoform of starch-branching enzyme is isoform IIa. Isoform IIb, which predominates in endosperm, is unimportant in leaves.

Different isoforms of starch synthase and starch-branching enzyme play distinct roles in the synthesis of amylopectin. In mutant and transgenic plants in which activity of one or more isoforms has been reduced or eliminated, amylopectin structure and/or the ratio of amylose to amylopectin is altered. For example, when SSII is eliminated from potato tubers (by the expression of antisense RNA (ribonucleic acid)) there is a large increase in chains of 8–11 glucoses in amylopectin molecules,

and when SSIII is eliminated there is a large increase in chains of six glucoses. When GBSS is eliminated, the structure of amylopectin is barely affected but the amylose content is drastically reduced (Kossmann and Lloyd, 2000). Generally speaking, elimination of a particular class of isoform has similar effects across many species of plants, suggesting that each class has a distinct and conserved function in the synthesis of the starch polymers. The most striking example is GBSS, which is specifically and exclusively responsible for the synthesis of amylose in all species examined thus far.

The precise contribution made by a particular isoform depends on what other isoforms are present, and probably also on other background factors. This is seen when two or more isoforms are eliminated together: the effects on amylopectin are usually different from, and bigger than, those predicted from examining the effects of elimination of one or the other isoform. For example, elimination of both isoforms of starch-branching enzyme from potato tubers has a catastrophic effect on amylopectin structure. Most of the chains of 12–20 glucoses disappear, leaving only very long chains. However, elimination of BEII by itself has a much less severe effect and elimination of BEI by itself has almost no effect (Schwall *et al*., 2000). Thus isoforms of starch synthase and starch-branching enzyme act cooperatively rather than independently in amylopectin synthesis. Evidence suggests that some of the isoforms of these two enzymes may exist in loose complexes, formation of which is controlled in part by protein phosphorylation (Tetlow *et al*., 2004).

A requirement for debranching

Although starch synthase and starch-branching enzyme can together create α1,4, α1,6-linked glucose polymers, in most plant organs a third type of enzyme is required for the production of normal starch granules. The requirement for isoamylase – a debranching enzyme that cleaves α1,6 linkages – was discovered by studying mutant plants in which a proportion of the starch is replaced by soluble α1,4, α1,6-linked glucose polymers called phytoglycogen (James *et al*., 1995). Phytoglycogen has more α1,6 linkages than amylopectin, and these are not spaced to give the cluster pattern of chain distribution seen in amylopectin. The link between phytoglycogen accumulation and loss of isoamylase activity was first established in the *sugary1* mutant of maize (the original sweet corn). Subsequent work has shown that loss of isoamylase causes phytoglycogen production in many plant organs including other cereal seeds, *Arabidopsis* leaves and potato tubers (Streb and Zeeman, 2012).

Both the nature of the enzyme and the consequences of its loss vary from one plant organ to another. In developing cereal grains, most of the isoamylase activity is due to a tetramer of a single protein, ISA1. In potato tubers and *Arabidopsis* leaves, activity is due to a tetramer of two different proteins, ISA1 and ISA2, both of which are necessary for activity (Hussain *et al*., 2003). In some plant cells, loss of isoamylase activity results in the almost complete replacement of starch with phytoglycogen, whereas in others loss of activity results in accumulation of both phytoglycogen and abnormally large numbers of small starch granules.

Although isoamylase is clearly very important for starch synthesis, its exact contribution remains unclear. One proposal is that isoamylase contributes directly to amylopectin synthesis by removing some of the branches introduced by the starch-branching enzyme, creating a structure capable of spontaneous crystallisation to form the granule matrix. Other proposals include a role in controlling the production of glucose polymers in the soluble fraction of the amyloplast, preventing the elaboration of these polymers at the expense of polymers at the starch granule surface (Myers *et al*., 2000).

Granule formation

Starch synthesis occurs exclusively within plastids and starch granules grow by the addition of new material on to the surface. The enzymes of amylopectin synthesis (see above) produce nascent amylopectin molecules at the surface, probably primarily by elongating and then branching chains projecting from the matrix. Amylopectin molecules are thought to have the properties of a side-chain liquid crystalline polymer: a type of polymer that can self-organise into regular arrays (Waigh *et al*., 2000). Thus it seems likely that as amylopectin is elaborated at the granule surface it spontaneously crystallises to form new matrix material. In contrast, amylose is probably synthesised inside the matrix formed by amylopectin crystallisation by an enzyme that is tightly bound within the matrix (see above).

The number and shape of granules per plastid varies considerably between organs and between species. For example, the starch-storing plastids (amyloplasts) of potato tubers and maize seeds typically contain only one granule, whereas those of rice seeds and cassava roots contain multiple granules. Potato starch granules are smooth and ovoid, granules of unripe bananas are extremely elongated, the so-called 'A' granules of wheat and barley seeds are bun-shaped with deep central grooves and leaf starch granules are typically flattened plates. This indicates that there are genetic factors controlling the number and shape of granules within plant tissues, and recent evidence has started to identify some of these. For example, in Arabidopsis leaves elimination of starch synthase IV leads to plants that synthesise one large starch granule per chloroplast rather than many small ones (Roldán *et al*., 2007), while plants mutated in both starch synthase IV and starch synthase III accumulate almost no starch (Szylowdski *et al*., 2009). Interestingly, mutations in isoforms of similar starch synthases in rice endosperm alter the shape of the starch granule (Toyosawa *et al*., 2016), while mutations in two others eliminate starch accumulation (Fujita *et al*., 2011). It is likely that other proteins are also involved in this process. Decreased activities of some debranching enzymes have been shown to influence starch granule size (Hussain *et al*., 2003; Kubo *et al*., 2010), while recent studies in rice have identified three proteins that affect starch granule shape or size, but for which there is no known catalytic activity (Matsushima *et al*., 2014, 2016; Peng *et al*., 2014). This indicates that all of these proteins are involved in starch granule initiation and determining the size and shape of granules, although the precise mechanism of their action in these processes is unknown.

Starch Degradation

During starch degradation, the glucose polymers are converted to sugars that can either enter the metabolism of the cell in which the starch is stored or be exported for metabolism in some other part of the plant. In most cases starch degradation occurs in living cells. The initial stages occur in the plastid; then, a product is exported to the cytosol for metabolism to hexose phosphate and then to sucrose. In germinating cereal seeds the process is different: cell integrity is lost during seed maturation so starch degradation occurs in a nonliving tissue. The product in this case is glucose, which is taken up into living cells in the scutellum for transfer to the developing shoot and root axis. Starch degradation is well understood in cereal seeds, because of its importance to the brewing and distilling industries. Glucose and oligosaccharides (short chains of glucoses) produced from starch during germination in these seeds are the substrates for the synthesis of alcohol by yeast during beer and whisky production.

The pathway in cereal seeds

The pathway by which starch is converted to glucose in the endosperm of germinating cereal seeds is relatively simple (**Figure 3**). The starch granule is attacked by α-amylase, which is an endoamylase. α-Amylase thus releases both linear and branched oligosaccharides from the starch granule. These soluble products are then acted on by two further enzymes. A debranching enzyme called limit dextrinase (LDA) generates linear chains by cleaving the α1,6 linkages. β-Amylase is an exoamylase that releases the disaccharide maltose. Maltose is hydrolysed to two glucoses by an α-glucosidase (also known as maltase). All of these enzymes are classified as glycoside hydrolases (see www.cazy.org for information on the structures of the starch synthesising and degrading enzymes mentioned in this review).

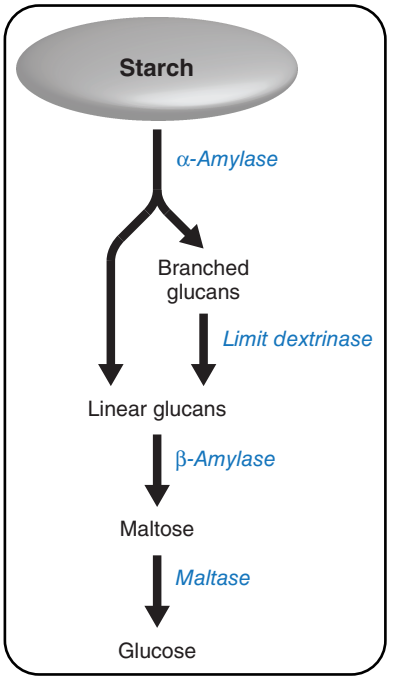

Figure 3 The pathway of starch degradation in the endosperm of a germinating cereal seed. The starch granule is attacked by the endoamylase α-amylase, which releases soluble linear and branched glucans. These are acted on by the debranching enzyme limit dextrinase and the exoamylase β-amylase to produce maltose. Maltose is then hydrolysed to glucose by an α-glucosidase (maltase). The glucose is taken up into the growing embryo.

The pathway in *Arabidopsis* leaves

The pathway of degradation of chloroplastic starch to sucrose in leaf cells was elucidated in *Arabidopsis* (Smith *et al*., 2005; **Figure 4**). It differs markedly from the pathway in cereal endosperm. First, leaf starch degradation is dependent on reversible glucan phosphorylation, a process that facilitates the access of starch degrading enzymes to the starch granule by partially disrupting the granule surface. Second, although some starch-degrading enzymes are common to both pathways, α-amylase activity plays at most a minor role in leaf starch degradation. Third, the pathway in leaves is characterised by a network of reactions rather than a linear pathway (Streb and Zeeman, 2012).

The process of reversible glucan phosphorylation is initiated by enzymes called glucan, water dikinases (GWD). GWD transfers phosphate from adenosine triphosphate (ATP) to C6-positions of glucosyl residues within amylopectin (Ritte *et al*., 2002, 2006). A second GWD enzyme, *phospho*glucan, water dikinase (PWD), catalyses essentially the same reaction, but is specific for C3-positions (Baunsgaard *et al*., 2005; Kötting *et al*., 2005; Ritte *et al*., 2006). Furthermore, PWD cannot phosphorylate unphosphorylated starch and hence requires the previous action of GWD. The presence of active GWD and PWD is essential for normal starch degradation. Loss-of-function mutations in the GWD gene completely prevent starch phosphorylation and drastically reduce the rate of starch degradation. Consequently, leaves of these mutants (*gwd* or *starch excess 1*/*sex1*) accumulate up to seven times more starch than wild-type leaves (Yu *et al*., 2001). Since PWD acts downstream of GWD, mutations in the PWD gene prevent phosphorylation of C3-positions, but not of C6-positions (Ritte *et al*., 2006). The reduction in the rate of starch degradation and the accumulation of starch in leaves of these mutants (*pwd*) is thus not as severe as in *gwd* mutants. Phosphate groups occur at a low frequency in *Arabidopsis* leaf starch (about 1 in every 2000 glucosyl residues is phosphorylated) and phosphate at C6-positions is about five times more abundant than at C3 (Ritte *et al*., 2006). This low level reflects the phosphate incorporated into starch during starch synthesis. However, starch phosphorylation occurs at considerably higher rates during times of active starch degradation (Ritte *et al*., 2004). The reduced starch degradation rates and the excess starch in leaves of *gwd* and *pwd* mutants demonstrate the importance of glucan phosphorylation for starch degradation. How glucan phosphorylation promotes starch degradation is not entirely clear. It has been proposed that phosphate groups may influence the organisation of the glucose polymers in the granule and hence the susceptibility of the granule surface to attack by degrading enzymes. This idea was

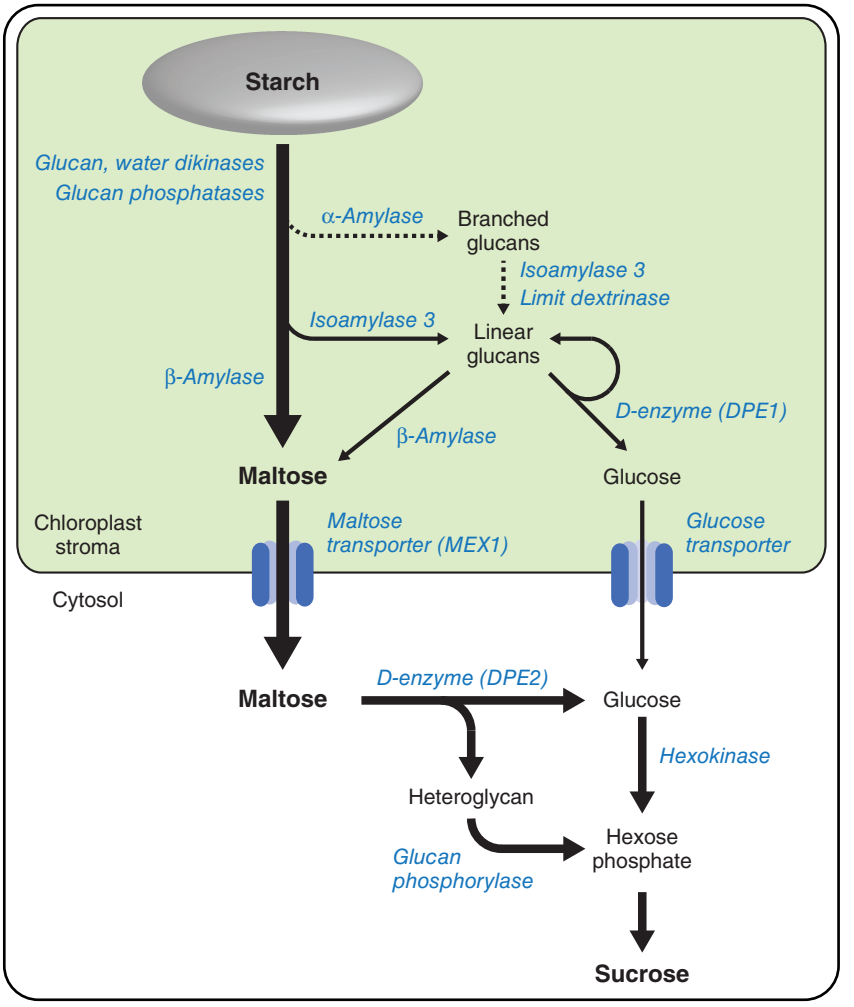

Figure 4 The pathway of starch degradation in an *Arabidopsis* leaf at night. Leaf starch degradation is initiated by phosphorylation of amylopectin via glucan, water dikinases (GWD and PWD). Dephosphorylation of the phosphoglucans by glucan phosphatases (SEX4 and LSF2) presumably occurs concurrently. Debranching of the starch polymers at the granule surface is mainly via isoamylase 3, and linear glucans are metabolised via β-amylase to yield maltose as the main product, with maltotriose as a more minor product. Possibly, α-amylase can release branched glucans from the granule surface, which are then debranched via the debranching enzymes isoamylase 3 and limit dextrinase. However, this is at most a minor pathway as indicated by the dashed arrows. Maltose is exported from the chloroplast to the cytosol via the maltose transporter MEX1, and then metabolised via the cytosolic disproportionating enzyme (D-enzyme; DPE2). DPE2 releases one of the glucosyl moieties of maltose as free glucose and transfers the other to a cytosolic heteroglycan, from which it is released via glucan phosphorylase as hexose phosphate. The maltotriose product of β-amylase is converted via chloroplastic D-enzyme (DPE1) to maltopentaose and free glucose. The maltopentaose is a substrate for the further action of β-amylase, and the glucose is assumed to be transported to the cytosol via a glucose transporter. For convenience, maltotriose and maltopentaose in this figure are represented under the generic term 'linear glucans'. Hexose phosphates produced in the cytosol from free glucose and the deglucosylation of the heteroglycan are converted to sucrose for export to the nonphotosynthetic parts of the plant.

substantiated by modelling studies of phosphorylated glucans, which suggest that phosphate groups at C6-positions locally disrupt the packing of neighbouring amylopectin double helices and that phosphate at C3-positions could destabilise the amylopectin helices (Engelsen *et al*., 2003; Kötting *et al*., 2009).

Although starch phosphorylation stimulates starch degradation, it has been known for a long time that phosphate groups can also impede the action of some starch-degrading enzymes. For instance, β-amylase releases maltose from the nonreducing end of glucan chains, but cannot do so past phosphate groups and hence cannot fully degrade phosphorylated glucans (Takeda and Hizukuri, 1981). The mechanism by which plants metabolise phosphorylated glucans was only discovered recently. Two glucan phosphatases [SEX4 (for *S*tarch *Ex*cess *4*) and LSF2 (for *L*ike *S*ex *F*our *2*)] were shown to dephosphorylate amylopectin in Arabidopsis (Gentry *et al*., 2007; Kötting *et al*., 2009; Santelia *et al*., 2011). SEX4 releases phosphate from C3- and C6-positions of glucosyl units while LSF2 specifically releases C3-bound phosphate (Hejazi *et al*., 2010; Santelia *et al*., 2011). SEX4 is crucial for starch degradation as demonstrated by Arabidopsis

sex4 mutants, which accumulate excess starch in their leaves. *sex4* mutant plants also accumulate high levels of soluble phosphorylated glucans (phospho-oligosaccharides), intermediate products of starch degradation released by α-amylase and/or the debranching enzyme isoamylase 3 (ISA3). Total glucan-bound phosphate (i.e. phosphate on amylopectin and phosphate on phospho-oligosaccharides) is more than six times higher in *sex4* mutants than in the wild type (Kötting *et al*., 2009). The amount of phosphate groups on *sex4* amylopectin, however, is unchanged as compared to the wild type, indicating that SEX4 is not involved in starch biosynthesis. LSF2 activity is not crucial for starch degradation as mutant plants lacking LSF2 activity (*lsf2*) degrade starch normally and do not accumulate phospho-oligosaccharides (Santelia *et al*., 2011). When LSF2 activity is missing in addition to SEX4 (*sex4 lsf2* double mutants), however, starch degradation is even more impaired than in *sex4* mutants indicating a (minor) role of LSF2 in this process.

Upon phosphorylation of the starch granule surface, the accessible glucans are degraded by different enzymes. α1,4-linked glucose chains can potentially be degraded via two alternative pathways. Chloroplastic glucan phosphorylase converts linear chains to glucose 1-phosphate, whereas β-amylase converts them to maltose. Study of mutant *Arabidopsis* plants lacking the chloroplastic glucan phosphorylase shows that starch degradation can proceed at normal rates in the absence of this enzyme (Zeeman *et al*., 2004); however, work on both *Arabidopsis* and potato leaves shows that β-amylase (BAM) is important in starch degradation. Four of the nine β-amylases encoded in the Arabidopsis genome are localised to plastids, but only BAM1 and BAM3 are involved in actively degrading starch (Fulton *et al*., 2008; Streb and Zeeman, 2012). β-Amylase cannot be the only enzyme that degrades the starch polymer as it is only able to hydrolyse α-1,4 bonds, not α1,6 branchpoints. Hydrolysis of α1,6 linkages proceeds via two debranching enzymes, ISA3 and LDA. It seems that ISA3 is more important than LDA for starch degradation in Arabidopsis (Streb and Zeeman, 2012). The main product of the action of β-amylase on linear glucose chains is maltose: its fate is discussed below. A second, more minor product is maltotriose, an oligosaccharide consisting of three glucose residues. β-Amylase is unable to attack chains of less than four glucose residues, so one maltotriose molecule is produced when it acts on a linear chain with an odd number of residues. The maltotriose produced in this way in the chloroplast at night is acted on by disproportionating enzyme (DPE1), which uses two maltotriose molecules to create maltopentaose (a linear chain of five glucose residues) and a free glucose. The free glucose can be exported from the chloroplast via a glucose transporter in the chloroplast envelope, and the maltopentaose can be acted on by β-amylase (Smith *et al*., 2005).

The maltose produced by β-amylase in the chloroplast during starch degradation in *Arabidopsis* leaves is the major starch degradation product in chloroplasts and can be exported to the cytosol via a specific transporter in the chloroplast envelope, MEX1 (Niittylä *et al*., 2004). Mutant plants that lack MEX1 accumulate very large amounts of maltose in the chloroplast at night (40 times the normal amount). The metabolism of maltose after export to the cytosol is not fully understood. In the cytosol, maltose is not hydrolysed via a maltase as in cereal endosperm, but is instead metabolised via a glucosyl transfer reaction catalysed by the cytosolic disproportionating enzyme, DPE2. Like mutants lacking MEX1, mutant plants lacking DPE2 accumulate very high levels of maltose at night, consistent with the idea that metabolism of maltose exported from the chloroplast is via DPE2. The enzyme releases one of the glucosyl moieties of maltose as free glucose and transfers the other on to an acceptor molecule, called heteroglycan (Fettke *et al*., 2006). The heteroglycan is a complex cytosolic polysaccharide consisting of various sugars that are linked by more than 20 different glycosidic linkages (Fettke *et al*., 2005). Its main constituents are arabinose and galactose, but to a lesser extent it also contains glucose, rhamnose, fucose, xylose and mannose. Since the amount of glucose in heteroglycan is relatively low compared to the substantial amount of exported maltose, it was proposed that heteroglycan serves as a short-term buffer rather than an actual glucose store (Chia *et al*., 2004). Glucose can be released from the heteroglycan by the cytosolic isoform of glucan phosphorylase, an enzyme that generates glucose 1-phosphate (Fettke *et al*., 2004). This scheme implies the generation of one molecule each of glucose and glucose 1-phosphate for each molecule of maltose. After phosphorylation of the free glucose via hexokinase to form hexose phosphate, the two hexose phosphates could be used for conversion to sucrose (Smith *et al*., 2005). However, evidence for this pathway from maltose to sucrose in the plant is still fragmentary, and other pathways cannot be ruled out at present.

Starch degradation in leaves is very tightly controlled. In *Arabidopsis*, little or no starch degradation occurs during the day. The process is switched on at the onset of darkness by post-translational mechanisms: amounts of the enzymes of starch degradation change very little over the course of the day (Smith *et al*., 2004). The rate of degradation at night is closely coordinated with the rate of synthesis during the day, allowing a constant rate of sucrose synthesis through the dark period. Although the control mechanisms are not understood, it seems likely that they include modulation of enzyme activities by protein phosphorylation, by forming complexes with other proteins, and by reduction of sulfhydryl groups.

It is important to note that the pathway of starch degradation in *Arabidopsis* has thus far been studied under a very limited set of environmental conditions. It is possible that the pathway is different in plants grown under other conditions, or challenged with specific stresses. The *Arabidopsis* genome encodes several enzymes that are predicted to be capable of the metabolism of α1,4, α1,6-linked glucose polymers, but which appear from mutant analysis to be unnecessary for starch degradation in leaves at night. These enzymes may prove to have roles in other parts of the plant, or under stress conditions.

The pathway in other organs

Organs other than leaves and cereal seeds may well prove to have pathways of starch degradation that differ significantly from either of those described above. In the cotyledons of starch-storing legume seeds, for example, the plastid membrane breaks down as the seed reaches maturity, so that during germination the starch granule is degraded in the cytosol rather than the plastid. Circumstantial evidence indicates that the major enzymes involved are α-amylase, limit dextrinase and glucan

phosphorylase, producing glucose 1-phosphate for conversion to sucrose. It is surprising that such an important pathway is so poorly understood, particularly in crops such as tubers, storage roots and ripening fruits in which the rate of conversion of starch to sugar determines the quality of the harvest. This is an important area for future research.

Importance of Starch

Starch and plant productivity

During a day/night cycle plant leaves fix carbon when it is light and store parts of it as starch within chloroplasts, which is then degraded at night. There is an exceptional degree of control over this process, with starch becoming depleted from leaves just before dawn. This means that the amount of starch that is synthesised during the day must change depending on day length to ensure that there is a constant supply of sugar production through the dark period. More starch would, therefore, be present at the end of a short day than a long day to avoid night time sugar starvation (Graf and Smith, 2010). Circadian rhythms have been demonstrated to play a role in this as plants grown in either 17- or 28-h day lengths eliminate starch from their leaves approximately 24 h after the last dawn, irrespective of the actual dawn (Graf *et al*., 2010). This control over starch turnover is advantageous as demonstrated by plants that synthesise little starch, or are unable to degrade starch efficiently at night, which grow poorly and demonstrate a starvation response in the dark (Graf and Smith, 2010). On the other hand, Arabidopsis plants that synthesise more starch than usual during the day grow bigger (Gibson *et al*., 2011; Liu *et al*., 2016). Indeed starch is considered to be one of the most important metabolites involved in regulating plant growth (Sulpice *et al*., 2009). These alterations in growth have been linked to changes in levels of gibberellin biosynthesis (Paparelli *et al*., 2013). **See also**: **Gibberellin – Mechanism of Action**; **Plant Circadian Rhythms**

Industrial uses of starch

Many industries use starch as a raw material; for example, it can be degraded to glucose and fermented to manufacture biofuels or used as a thickener in the food industry. Industrial starch is harvested from storage organs, with most starch used by industry being obtained from maize seeds. The total annual market for starch is currently approximately $50 billion. Before it is used, starch is often modified using chemicals or physical processes that incur a cost onto industry. Research has generally examined how altering genes involved in starch metabolism could increase the amounts of starch that accumulate, or change the structure of amylose and amylopectin to make them more amenable for industrial use. Several studies have led to increased starch synthesis and these have tended to concentrate on increasing flux through ADP-glucose pyrophosphorylase, either by directly increasing its activity, or by increasing supply of substrates to it (Sonnewald and Kossmann, 2013). Manipulation of starch structure often leads to decreased amounts of starch that are synthesised by plants in storage organs, meaning that although the starch may have improved properties, the decreased yield makes it uneconomic. For example, it was mentioned above that repression of both BEI and BEII in potato led to starch with increased amylose; however, the amount of starch stored in the tubers from these plants was decreased by approximately a quarter (Hofvander *et al*., 2004). Other alterations that could be advantageous for industrial starch production are changes in the average chain lengths within amylopectin that have been produced by alterations in starch branching enzyme and soluble starch synthase activities, or changes in starch-bound phosphate that have been produced by manipulation of GWD (Carciofi *et al*., 2011). The main starch used by industry that has been altered through manipulation of the starch biosynthetic pathway is from plants that lack GBSS and, therefore, produce starch containing only amylopectin. Such plants have only minor yield decreases and the starch from them has properties that are particularly useful in the food industry. Plants synthesising altered starches have the potential to lead to more sustainable industries with feedstocks that are less environmentally damaging to produce. **See also**: **Starch and Starch Granules**

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