STARCH DEGRADATION

I. PLANT AMYLASES AND PHOSPHORYLASES

Many starch-degrading enzymes have been isolated from fungi, yeasts, and bacteria, and their mode of action varies greatly. Glucoamylase occurs almost exclusively in fungi, pullulanase in bacteria, and α-D-glucosidase and isoamylase are produced by both fungi and bacteria. Although these enzymes are used by the biochemist as research tools and their mode of action is clearly relevant to the subject of this chapter, only the starch-degrading enzymes found in plants are described here. The reader is referred to the excellent review of Galliard (1987) for information on the fungal and bacterial amylases.

Plants have a number of enzymes that can contribute to starch breakdown and they have been studied in some detail. Endoamylases such as α-amylase (EC 3.2.1.1) can cleave hydrolytically 1,4-α-glucosidic bonds, producing a mixture of linear and branched oligosaccharides, and eventually maltotriose, maltose, glucose, and a range of branched α-limit dextrins. Starch and other α-glucans can be hydrolyzed by β-amylase (EC 3.2.1.2), which catalyzes the removal of successive maltose units from the nonreducing end of α-glucan chains. The maltooligosaccharides produced by these amylolytic enzymes can be further hydrolyzed to glucose by α-glucosidase (EC 3.2.1.20). Manners (1985) presents a good discussion of the need for extensive purification before characterization of starch degradative enzymes (see also the chapter, "Branching Enzymes," for similar points on the characterization of branching enzymes) and the artifacts encountered by workers who did not follow this rule.

For a glucan to be a substrate for starch phosphorylase, it must be longer than maltotetraose. Shorter oligosaccharides, however, can be used by glucosyltransferases such as D-enzyme (EC 2.4.1.25) in the reaction

$$\alpha\text{-glucan} + \alpha\text{-glucan} \Leftrightarrow \alpha\text{-glucan}_{(m+n-1)} + \text{glucose}$$

These enzymes increase the degree of polymerization of short oligosaccharides, converting them into suitable substrates for starch phosphorylase.

None of the enzymes discussed so far can hydrolyze the α-1,6 bonds present in starch. These branch points are cleaved hydrolytically by the debranching enzyme (EC 3.2.1.41), which releases linear oligosaccharides for further metabolism by amylases or phosphorylase.

II. DEBRANCHING ENZYMES

The enzymes that hydrolyze (1 → 6)-α-D-glucosidic linkages in starch and glycogen and in related α- and β-dextrins are called debranching enzymes. The nomenclature for this type of enzyme was confusing, but has been clarified.

The latest International Union Biochemicals report includes an enzyme 3.2.1.41 α-dextrin endo-1,6-α-glucosidase, other names limit dextrinase, amylopectin 6-glucanohydrolase, pullulanase.

Dohlert and Knutson (1991) and D. J. Manners (personal communication) reported that extracts of sugary maize contain a mixture of limit dextrinase and isoamylase. However, James *et al.* (1995) reported that *su* 1 codes for the isoamylase.

The plant and bacterial enzymes capable of hydrolyzing pullulan do not have identical specificities. In particular, the plant enzymes have little or no action on glycogen and phytoglycogen under conditions in which they readily hydrolyze amylopectin and its β-dextrin. To stress this difference (the bacterial enzymes are capable of degrading both glycogen and phytoglycogen), Manners (1997) recommended different nomenclature for bacterial enzymes, to be called *pullulanase,* and the plant enzymes, to be called *limit dextrinases.*

Manners' (1997) classification is based on the specificity for the substrate [e.g., how readily they hydrolyze glycogen or pullulan—a glucan synthesized by *Pullularia pullulans* (*Aureobasidium pullulans*)—consisting essentially of a linear chain of (1 → 6)-linked α-maltotriose residues]. According to this classification, higher plant debranching enzymes are called either *limit dextrinases,* which hydrolyze amylopectin, pullulan, and α-dextrins, but do not hydrolyze glycogen, or *isoamylases,* which act on amylopectin, glycogen, and some α-dextrins, but not on pullulan. A third debranching activity, R-enzyme, was discovered independently of limit dextrinases but was later found to be the same enzyme. Hizukuri (1995) classifies them (according to their mode of action) direct and indirect debranching enzymes. The enzymes belong to the first-group if they hydrolyze (1 → 6)α-branch linkages in one step (e.g., isoamylase and pullulanase). The second group has an indirect action (e.g., an enzyme from rabbit muscle, the amylo 1,6-glucosidase 4-α-D-glucanotransferase, hydrolyzes only a single glucosyl side-

chain residue after the other residues of the chain have been transferred to other chains); it also has activity as a 1,4-α-glucanotransferase.

James *et al.* (1995) cloned the *Sugary* 1 (*Su* 1) gene of maize; when this gene is disrupted, the endosperm has reduced amounts of starch and substantial amounts of a water-soluble polysaccharide reminiscent of glycogen (hence its name, *phytoglycogen*). The *Su* 1 cDNA was expressed in *Escherichia coli,* where it displayed debranching activity. The deduced amino-acid sequence of the product of *Su* 1 had strong homology with the bacterial isoamylases. Thus, preliminary evidence suggests that this enzyme could participate in the debranching of a amylopectin precursor, leading to the formation of an amylopectin capable of crystallizing and forming the water-insoluble starch granule (see the chapter, "Open Questions and Hypotheses in Starch").

It is likely that, as research on the structure and function of the plant debranching enzymes progresses, and as the enzymes are compared with their bacterial and fungal counterparts, classification will be based on both mode of action and structure.

III. THE PATHWAY OF STARCH DEGRADATION IN PLANTS

The enzymes capable of degrading starch have been discussed, but the steps required to convert the intact starch granule into soluble maltooligosaccharides have not been addressed yet. Which of the enzyme(s) named in the preceding text are actually involved in the initial degradation of the intact and insoluble starch granule? Is degradation initiated by a particular α-amylase or by one of the phosphorylases? Although some thought that only endoamylases were capable of catalyzing the initial steps of starch degradation, it is known that phosphorylase from pea chloroplasts can release labeled glucose 1-phosphate from ^{14}C-labeled starch granules (Kruger and ap Rees, 1983). It is worth noting that because starch is accumulated in many different tissues, some variation in the pathway of degradation should be expected, if only to accommodate the different metabolic demands of those tissues. For example, reserve starch is compartmentalized differently within legume cotyledon and cereal endosperm. Within the living cotyledon cells, the amyloplast membranes disintegrate during seed maturation and the granules are exposed to the cytosolic enzymes. In the endosperm of cereal seeds, the cells die early and, at maturity, the starch granules are embedded in a matrix of storage protein and are surrounded by a net of dead cell walls. During germination of cereal grains, starch breakdown is associated with the destruction of the endosperm, which softens and eventually liquifies. Starch degradation in these circum-

stances can be considered to be extracellular, and it seems to occur via a hydrolytic route rather than by phosphorolysis (Boyer, 1985b). The glucose released by the combined action of α-amylases, debranching enzymes, and α-glucosidases is absorbed by the scutellum, converted into sucrose, and transported to the embryo. In other seeds, germination occurs without the destruction of storage tissue, although it is likely that amyloplast membranes are broken down, making starch accessible to cytosolic enzymes. In one of these cases, which is similar to that in pea cotyledons, starch breakdown may be phosphorolytic, but in soybeans and lentils it seems that α-amylolysis may be the major route of starch breakdown. The role of β-amylase in the many seeds is uncertain and, although it may contribute to the degradation of oligosaccharides released by α-amylase, β-amylase does not seem to be essential for starch breakdown (ap Rees, 1988).

The onset of the dark period induces an amylolytic enzyme in the leaves of *Arabidopsis thaliana* plants grown under a light–dark cycle (Kakefuda and Preiss, 1997). Zymograms, obtained from samples harvested over a 24-hour period, indicated that the activity of this hydrolase is induced by the onset of darkness, is higher after 1 hour, and then rapidly disappears from the soluble protein fraction. The peak of activity of this amylase coincides with a decrease in starch content in the leaf. The dark-induced amylase was separated from other, noninducible hydrolases, using polyethylene glycol precipitation, DE-52 ion exchange, Bio-Gel A-0.5M gel filtration, and amylose affinity chromatography. The partially purified enzyme was characterized using the release of Remazol brilliant blue dye from starch azure, hydrolysis of beta-limit dextrin and periodated amylose, and the results obtained indicate that it is an endo-(α)amylase. Activity was inhibited by dialysis of the enzyme preparation against buffers containing EDTA or EGTA, and calcium protected the activity from this inhibition. This amylase differs from other amylolytic enzymes in that it has a relatively high pH optimum for activity (pH 7.0).

Although native starch granules from storage organs seem to be first eroded by amylases before other enzymes can further hydrolyze it, the chloroplastic phosphorylase can release labeled glucose 1-phosphate from ^{14}C-labeled starch granules, at least in pea leaves (Kruger and ap Rees, 1983).

IV. STARCH DEGRADATIVE ENZYMES LOCATED OUTSIDE THE CHLOROPLAST: POSSIBLE FUNCTION

In germinating seeds and tubers, starch degradation is essentially an irreversible process and continues until all the starch is metabolized. This

is not so in young and mature leaves, in which starch is a temporary reserve and cellular and organelle integrity is maintained. In the case of young and mature leaves, at least the initial stages of starch breakdown occur inside the chloroplast, and breakdown seems to be mobilized by the joint action of endoamylases and the chloroplastic phosphorylase (Stitt and Steup, 1984).

Incubation of spinach leaf starch granules with extrachloroplastic phosphorylase resulted in the formation of glucose 1-phosphate (Steup *et al.*, 1983). Hammond and Preiss (1983) reported a large increase in cytosolic phosphorylase from spinach leaf in a time course that approximates the time of leaf senescence (i.e., when starch chloroplast must be hydrolyzed and exported to active sinks).

The localization of β-amylase has been a matter of discussion for some time. Okamoto and Akazawa (1979) found β-amylase to be associated with starch granules, an obvious location for an enzyme capable of metabolizing starch, but later, cell fractionation (Beck and Ziegler, 1989) indicated that β-amylase was localized in the vacuole. Wang *et al.* (1995), using monoclonal antibodies selected for phloem-specificity, proposed that the β-amylase of *A. thaliana* is localized in the phloem. They attributed the putative localization of the enzyme in the vacuole and its association with starch granules to the similarity in epitopes between β-amylase and phosphorylase (i.e., it was the starch phosphorylase that had been located and had been described in previous publications). Regarding the possible role for the β-amylase, Wang *et al.* (1995) suggested that it might be there to prevent buildup of starch in the sieve elements during sugar translocation. In seeds, it has been suggested that the enzyme does not fulfill any significant metabolic role (mutants deficient in β-amylase can germinate and grow normally), but is rather a storage protein.

V. DIGESTION OF STARCH IN HUMANS

Starch is a major nutrient in human diet. Digestion of starch consists in the breaking up of the glycosidic bonds linking the glucose residues by glycosidases in order to liberate the reducing components; cooking hydrates starch, making its digestion more efficient.

The principal locations for digestion of starch in humans are the mouth, the lumen of the small intestine, and the brush border of the epithelial cells of the intestinal mucosa. Food is masticated in the mouth, forming a bolus ready for swallowing, while the salivary α-amylase attacks the hydrated starch. The enzyme breaks the starch at random intervals, hydrolyzing internal α-1,4 bonds (and not the α-1,6 bonds constituting the branching points). The α-amylase will not break the bonds nearest the nonreducing

ends, nor the bonds next to the α-1,6 branch points, and for this reason the product of its action is a mixture of glucose, maltose, and smaller units of the starch molecule called *starch dextrins,* which contain all of the original α-1,6 bonds.

When food that has been thoroughly chewed reaches the stomach, acidity inactivates the salivary α-amylase, but by then the large starch molecules have been reduced from several thousands to a few glucose units, provided the food has been chewed thoroughly. Little hydrolysis of the carbohydrate occurs in the stomach, although sucrose can be broken up to some extent because the linkage between glucose and fructose, a β-D-fructofuranoside bond, is sensitive to acidity ("acid-labile").

As the stomach empties, the hydrochloric acid in the material entering the small intestine is neutralized by secretions from the pancreatic ducts, bile, and pancreatic juice. The digestion of the starch dextrins is continued by the action of the pancreatic α-amylase.

The pancreatic amylase is very similar to the salivary amylase; indeed, there is only a 1% difference in amino acid composition between the two. Pancreatic amylase is secreted in excess relative to starch intake; it is more important that the salivary enzyme be from a digestive point of view, because food generally does not remain in the mouth long enough to be digested thoroughly by salivary α-amylase. The products of the digestion by α-amylase are mainly maltose and maltotriose, and α-limit dextrins containing about eight glucose units with one or more α-1,6 glucosidic bonds.

It is worth noting that ingested cellulose cannot be digested by humans. Although cellulose is also a polymer of glucose, the linkages between glucose residues are by means of $(1 \rightarrow 4)$-β-D-glycosidic linkages (rather than an α-glycosidic linkages as in starch), and there is no human enzyme capable of hydrolyzing them.

Final hydrolysis of di- and oligosaccharides is carried out by surface enzymes of the small intestinal epithelial cells, called the *brush border,* a term that comes from the appearance of the enterocytes, in which the luminal plasma membrane is enlarged by a regular array of projections called *microvilli.* The enzymes are not secreted into the lumen, but are embedded in the cell membrane, many of these enzymes can protrude into the intestinal lumen up to 10 μm, as they are attached to the plasma membrane by an anchoring polypeptide that has no role by itself in the hydrolysis.

The saccharidases present in the surface of the small intestine relevant to the digestion of starch and its components are:

1. exo-1,4-α-glucosidase (glucoamylase), specific for α-(1,4)glucose bonds

2. oligo-1,6-glucosidase (isomaltase), which acts on α-(1-6)glucose bonds and breaks down isomaltose and α-dextrins
3. α-glucosidase (maltase), which acts specifically on α(1-4)glucose bonds, breaking down maltose and maltotriose

Most of the surface oligosaccharidases are exoenzymes, which clip off one monosaccharide at a time from the nonreducing end. The capacity of the α-glucosidases normally is much greater than that needed for completion of starch digestion. Absorption of the glucose resulting from digestion in the intestinal lumen into the cell is mediated by substrate-specific carriers in the plasma membrane. Glucose transport is the rate-limiting step of carbohydrate at the brush border membrane. Conversely, the duodenal amylase content of an adult is capable of hydrolyzing the starch content of a whole meal in a matter of minutes. Eventually, glucose passes into the portal blood system, through which it is transported first to the liver and then to the remainder of the body.

Because amylase activity is not rate limiting, blood glucose and insulin levels in a healthy adult will be similar if he is fed soluble starch, hydrolyzed starch, low molecular weight maltooligosaccharides, or glucose. Different types of starchy foods can, however, differ in the glycemic responses they elicit because of differences in factors such as particle size (determined in part by milling), amylase inhibitors, amylose–amylopectin ratio, starch–lipid interactions, and starch–protein interactions. Processing may have a large effect on starch availability. For example, raw wheat flour gives a very low sugar and insulin blood levels as compared with the same flour after gelatinization of starch by boiling, and roasting of beans increases the systemic response as compared with boiled beans. In other studies, extrusion cooking of whole grain crisp bread increased starch availability more than conventional baking.

Di-, oligo-, and polysaccharides that are not hydrolized by α-amylase and/or intestinal surface enzymes cannot be absorbed, and they reach the lower tract of the intestine, which, from the lower ileum onward, contains bacteria. Because bacteria contain a larger variety of saccharidases than humans, they can use many of the remaining carbohydrates.

VI. MECHANISM OF ACTION OF AMYLASES AND PHOSPHORYLASES

The nomenclature α- and β- for amylases had its origin in the optical properties of the hydrolysis products of starch. Another nomenclature, proposed in the 1950s, had its basis in the mode of action: endo- and

exoamylases. Since that time, research on amylases has moved toward an understanding of the detailed mechanism of action and the crystal structure of the enzymes involved (Yamamoto, 1995). This scientific field is active, partly because of the commercial importance of enzymatic starch degradation for the food and brewing industries.

Polysaccharides, such as starch, phytoglycogen, glycogen, and maltodextrin, are degraded by glucosylhydrolases. Despite large variations in the composition of their substrates, all polysaccharide hydrolases are thought to act by a general acid catalysis mechanism, in which two acidic amino-acid residues participate in either a single or double displacement reaction, resulting in the inversion or retention of configuration at the anomeric carbon atom of the glucosidic bond (Henrissat and Bairoch, 1993). As exemplified by glucoamylase, the nature of the polysaccharide binding site is generally well characterized and consists of several subsites. It is assumed that the total affinity of the oligomeric substrate corresponds to the sum of the individual subsite affinities. Cleavage occurs between subsites 1 and 2, where subsite 2 has the highest affinity of the oligomeric substrate.

In contrast to polysaccharide hydrolases, starch and glycogen phosphorylases (E.C. 2.4.1.1) transfer a glucosyl residue to a phosphate group rather than to water, resulting in the phosphorolytic cleavage of an α-1,4-linked glucose unit and the formation of α-D-glucose 1-phosphate (Glc-1-P) according to the equation

$$[\text{glucose}]_n + P_i \leftrightarrow [\text{glucose}]_{n-1} + \text{glucose 1-phosphate}$$

Another major difference between the phosphorylase-catalyzed reaction and the reaction of hydrolases is the free reversibility of the phosphorolytic cleavage. However, under certain conditions, the reaction of the hydrolysis is reversible, and may result in the synthesis of higher oligosaccharides from maltose and related di- and trioligosaccharides (as studied by French and Hehre in the 1960s and 1970s).

The polysaccharide phosphorylases and the other depolymerizing enzymes differ in both mechanism of action and structure, although it is generally accepted that phosphorylases share the general acid catalysis.

FURTHER READINGS

These sources provide additional in-depth coverage of this topic. For complete reference, please see the Reference section at the end of the book.

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