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Starch modification with microbial alpha-glucanotransferase enzymes

Marc J.E.C. van der Maarel[a,b,*], Hans Leemhuis[b]

[a] Food Innovation Center, AVEBE U.A., Veendam, The Netherlands
[b] Microbial Physiology, Groningen Biomolecular Sciences and Biotechnology Institute (GBB), University of Groningen, Groningen, The Netherlands

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ABSTRACT

Starch is an agricultural raw material used in many food and industrial products. It is present in granules that vary in shape in the form of amylose and amylopectin. Starch-degrading enzymes are used on a large scale in the production of sweeteners (high fructose corn syrup) and concentrated glucose syrups as substrate for the fermentative production of bioethanol and basic chemicals. Over the last two decades α-glucanotransferases (EC 2.4.1.xx), such as branching enzyme (EC 2.4.1.18) and 4-α-glucanotransferase (EC 2.4.1.25), have received considerable attention. These enzymes do not hydrolyze the starch as amylases do. Instead, α-glucanotransferases remodel parts of the amylose and amylopectin molecules by cleaving and reforming α-1,4- and α-1,6-glycosidic bond. Here we review the properties of α-glucanotransferases and discuss the emerging use of these enzymes in the generation of novel starch derivatives.

1. Enzymatic starch conversion

Starch is the major dietary carbohydrate for humans, as rice, corn, tapioca, wheat and potato form a major part of our diet. It is an abundant storage carbohydrate found in the leaves, seeds, roots and tubers of many plants (Zeeman, Kossmann, & Smith, 2010). Green plants use sunlight to transform carbon dioxide and water into sucrose which is then polymerized into starch and stored as semi-crystalline granules inside amyloplast organelles. Plants have two types of starch, one is found in leaves and the other in seeds, roots and tubers. Transitory leaf starch is made during the day to store excess energy. During the night most of the transitory leaf starch is consumed again while some of it is converted into sucrose that is transported to seeds, roots or tubers where it is converted back into starch. This type of starch is meant for long term energy storage that can be consumed by the young seedling during the first stage of germination. Over thousands of years man has cultivated starch containing crops for maximum starch yield. Commonly used starch-containing crops are corn, wheat, potato, rice, rye, and barley.

Normal starches are composed of about 20% of amylose, a virtually linear polymer of α-1,4 linked glucose residues, and about 80% of amylopectin, a branched polymer of α-1,4 linked glucose residues with about 5% α1,6-glycosidic bonds (Zeeman et al., 2010). There are also starches that contain almost exclusively amylopectin (the so-called waxy variants) and a few corn varieties that have elevated amounts of amylose (the Hylon varieties that have up to 50% amylose). The semi-crystalline starch granules are insoluble in cold water but swell and fall apart when heated. Subsequent cooling of the heated starch slurry leads to the formation of a firm, opaque gel due to hydrogen bonding between parallel oriented amylose and or long side chains of amylopectin. This process, termed retrogradation, is irreversible resulting in an insoluble gel.

Besides serving as food, starches are also industrially processed into a series of derivatives that are applied in various industries such as oil drilling fluids, adhesives, paper and cotton coatings, or gelling, emulsifying and viscosifying agents in food products. Starches are also converted into various syrups such as high fructose corn syrup on industrial scale (Buchholz & Seibel, 2008; Crabb & Shetty, 1999; van der Maarel, van der Veen, Uitdehaag, Leemhuis, & Dijkhuizen, 2002) to be used as filling agents and/or sweeteners in, *e.g.* soft drinks. Glucose syrups derived from mainly corn or wheat starch have become the basic raw material for the bioethanol industry (Lin & Tanaka, 2006). Glucose production starts with the liquefaction of 30–35% dry solid starch slurry by jet-cooking at temperatures above 100 °C and a first treatment with a heat stable α-amylase (EC 3.2.1.1) for a few minutes at 90 °C. The next step is saccharification using a thermostable glucoamylase (EC 3.2.1.3) and pullulanase (EC 3.2.1.41) at 60 °C, yielding over 97% of glucose

Abbreviations: AGTases, alpha-glucanotransferases; 4αGT, 4-α-glucanotransferase (amylomaltase); BE, branching enzyme; CGTase, cyclodextrin glucanotransferase; GI, Glycemic Index.

* Corresponding author. Present address: Aquatic Biotechnology and Bioproduct Engineering Research Group, Institute for Technology and Management, University of Groningen, Nijenborgh 4, 9747 AG Groningen, The Netherlands.
E-mail address: m.j.e.c.van.der.maarel@rug.nl (M.J.E.C. van der Maarel).

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Table 1
Starch products made by α-glucanotransferase enzymes.

Product	Applications	Enzyme
Cycloamylose	Protein folding	4αGT
Cyclic cluster dextrin (CCD)	Sport drink	BE
Cyclodextrins	Cholesterol removal	CGTase
Thermoreversible starch gel (Etenia™)	Gelatin/fat replacer	4αGT
Slowly digestible starch	Slow glucose release	BE
Resistant starch	Food fiber	4αGT followed by debranching
Highly branched starch	*E.g.* paper coating	BE
Highly branched amylopectin cluster	Slow glucose release	BE followed by maltogenic amylase

in about 72 h. This glucose syrup can then be converted into a glucose/fructose mixture using immobilized glucose isomerase (EC 5.3.1.5). The enzymatic production of glucose and glucose/fructose syrups from starch is a bulk process consuming large amounts of enzyme (Crabb & Mitchinson, 1997; Crabb & Shetty, 1999). The common feature of these enzymes is the hydrolysis of the α-1,4- or α-1,6-glycosidic linkages leading to the degradation of the amylose and/or amylopectin. Over the last 10 years the use of alpha-glucanotransferases (AGTases), *i.e.* non-hydrolyzing starch-active enzymes, has received considerable attention leading to a number of new commercial products (Table 1) (Biwer, Antranikian, & Heinzle, 2002; Dermaux, Peptitjean, & Wills, 2007; Kaper, van der Maarel, Euverink, & Dijkhuizen, 2004; Le et al., 2009; Norman, Pedersen, Stanley, Stanley, & Richmond, 2007; Richmond et al., 2008; Takaha & Smith, 1999; van der Maarel et al., 2005).

The widespread use of starch and starch derivatives, especially the easily digestible forms in all kinds of food products contributes to overweight/obesity, thereby being an important risk factor of several well-fare diseases (Brennan, 2005; Govindji, 2006; Swinburn, Caterson, Seidell, & James, 2004). Starch is a staple food present in many products we eat daily (*e.g.* bread, pasta, tortillas). Also starch hydrolysis products such as maltodextrins, glucose syrups and high fructose syrups are used in many food products (*e.g.* tomato ketchup, soft drinks, candy). Starch used in food and feed products is mostly processed by heating, mixing, or homogenization destroying the granules and thereby making the amylose and amylopectin easily accessible for digestive enzymes. After consumption, starch is converted along our gastrointestinal tract all the way to glucose, the actual energy source of our body. Degradation is initiated by salivary α-amylase, an endo-acting enzyme that randomly hydrolyzes α-1,4-glycosidic linkages in the amylose and amylopectin chains. The degradation continues in the small intestine where glucose is formed that is then taken up in the blood. The Glycemic Index (GI) expresses the rate at which glucose appears in the blood after the consumption of a starch containing foods. Food products with a high GI are white bread, cooked pasta and boiled potatoes, while green unripe bananas or kidney beans represent low GI foods (Atkinson, Foster-Powell, & Brand-Miller, 2008).

Starches can be categorized by the rate at which glucose is formed and appears in the blood. Rapidly degradable starches are converted in the small intestine within the first 20 min of digestion, whereas slowly digestible starches take more time to degrade. Resistant starch is mostly not degraded in the small intestine and enters the large intestine where it is degraded by gut bacteria *via* fermentation. Three classes of digestible starches are distinguished (Englyst, Kingman, & Cummings, 1992; Zhang & Hamaker, 2009):

- Rapidly digestible starch (RDS): starch that is completely converted within the first 20 min of the digestion test.
- Slowly digestible starch (SDS): starch that is degraded within a period of 120 min.
- Resistant starch (RS): typically defined as the sum of the unprocessed starch and its oligosaccharide degradation products that enter the colon.

Food scientist and technologists are continuously searching for starch processing methodologies that yield slower digestible starches. The view is emerging that AGTases enzymes can contribute to the generation of healthy starch based foods with respect to a controlled release of the glucose stored in the starch polymers.

2. The alpha-glucanotransferase enzymes

AGTases act on substrates with a number of consecutive α-1,4-glycosidic linkages such as amylose, amylopectin, maltodextrins and glycogen. The crucial feature of AGTases is that they catalyze a disproportion reaction, transferring the cleaved-off glucan to a glucan acceptor forming a new α-glycosidic bond (Fig. 1). AGTases belong to the superfamily of glycoside hydrolases (GHs; for a comprehensive overview see www.cazy.org). GHs cleave glycosidic bonds and transfer the cleaved off fragment to an acceptor molecule, which is usually water resulting in a hydrolysis reaction, although some GHs transfer the cleaved off fragment to another sugar forming a new glycosidic bond in the so-called disproportionation (or transfer) reaction (Fig. 1). The AGTases discussed in this paper are found in three GH families, 13, 57 and 77 (Cantarel et al., 2009; Kuriki & Imanaka, 1999; Murakami, Kanai, Takata, Kuriki, & Imanaka, 2006; Stam, Danchin, Rancurel, Coutinho, & Henrissat, 2006; Zona, Chang-Pi-Hin, O'Donohue, & Janecek, 2004). Of these, the GH13 family is by far the largest and contains numerous hydrolases such as α-amylase which plays a pivotal role in starch degradation, but also glycogen and starch branching enzymes and cyclodextrin glucanotransferases. The GH13, 57 and 77 enzymes are α-retaining enzymes meaning that the newly formed glycosidic bond has the same anomeric configuration as the bond cleaved in the substrate. In these enzymes the chemistry is carried out by two acidic amino acids, one acting as nucleophile and one acting as acid/base (Vocadlo & Davies, 2008). Reactions start with the cleavage of an α-1,4-glycosidic bond in the substrate resulting in a β-linked covalent glycosyl-enzyme intermediate. Following the departure of the glucan fragment from the acceptor subsites the non-reducing end of another glucan can enter the acceptor subsite +1 and attack the covalent intermediate with its 4-hydroxyl (4-α-glucanotransferases [4αGTs] (EC 2.4.1.15) and cyclodextrin glucanotransferases [CGTases] (EC 2.4.1.19)) or 6-hydroxyl (starch and glycogen branching enzymes [BEs] (EC 2.4.1.18)), resulting in the formation of an α-1,4- or α-1,6-glycosidic bond, respectively.

Three types of AGTase enzymes are distinguished (i) 4-α-glucanotransferase (4αGT; EC 2.4.1.25) sometimes indicated as amylomaltase, disproportionating or D-enzyme (Kaper et al., 2004), (ii) cyclodextrin glucanotransferase (CGTase; EC 2.4.1.19) (Biwer et al., 2002; Leemhuis, Kelly, & Dijkhuizen, 2010) and (iii) branching enzyme (BE; EC 2.4.1.18), also known as Q-enzyme (Boyer & Preiss, 1977). Because AGTases use polymeric substrates, the hydroxyl acceptor can also be located downstream on the glycosyl-enzyme intermediate leading to an intra-molecular transglycosylation generating a cyclic product. For CGTases intra-molecular transglycosylation is the dominant reaction producing α-, β- and γ-cyclodextrins with 6, 7 or 8 α-1,4 linked anhydroglucose residues (Fig. 1). CGTases can also transfer the glucan intermediate to a second glucan chain, *i.e.* inter molecular transglycosylation, forming a linear product. The later reaction is only dominant at very high concentrations of glucan acceptors with a free 4-hydroxyl group. In contrast to CGTases, 4αGTs preferably catalyze inter molecular