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Morphology and structural properties of high-amylose rice starch residues hydrolysed by amyloglucosidase

Jianmin Man [a,b], Yang Yang [a,b], Jun Huang [a,b], Changquan Zhang [a], Fengmin Zhang [c], Youping Wang [a,b], Minghong Gu [a], Qiaoquan Liu [a,*], Cunxu Wei [a,b,*]

[a] Key Laboratories of Crop Genetics and Physiology of the Jiangsu Province and Plant Functional Genomics of the Ministry of Education, Yangzhou University, Yangzhou 225009, China
[b] College of Bioscience and Biotechnology, Yangzhou University, Yangzhou 225009, China
[c] Testing Center, Yangzhou University, Yangzhou 225009, China

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ABSTRACT

High-amylose starches are attracting considerable attention because of their potential health benefits and industrial uses. Enzyme hydrolysis of starch is involved in many biological and industrial processes. In this paper, starches were isolated from high-amylose transgenic rice (TRS) and its wild type rice, Te-qing (TQ). The morphological and structural changes of starch residues following *Aspergillus niger* amyloglucosidase (AAG) hydrolysis were investigated. AAG hydrolysed TQ starch from the granule surface, and TRS starch from the granule interior. During AAG hydrolysis, the content of amorphous structure increased, the contents of ordered structure and single helix decreased, and gelatinisation enthalpy decreased in TQ and TRS starch residues. The A-type polymorph of TRS C-type starch was hydrolysed faster than the B-type polymorph. The short-range ordered structure and B-type polymorph in the peripheral region of the subgranule and the surrounding band of TRS starch increased the resistance of TRS starch to AAG hydrolysis.

1. Introduction

Starch is an abundant natural polysaccharide that is renewable and fully biodegradable, and consists of two main components: linear amylose and highly branched amylopectin. There are three reported types of starch crystallinity known as A-, B- and C-type according to their X-ray powder diffraction (XRD) patterns. The C-type starch is a mixture of both the A- and B-type (Cheetham & Tao, 1998). For nutritional purposes, starch is classified into three types: rapidly digestible starch, slowly digestible starch and resistant starch (RS) (Englyst, Kingman, & Cummings, 1992). RS is a portion of starch that cannot be hydrolysed in the upper gastrointestinal tract and functions as a substrate for bacterial fermentation in the large intestine (Englyst et al., 1992). RS has been reported to provide many health benefits for humans, as RS-enriched food can lower the glycaemic and insulin responses and reduce the risk for developing type II diabetes, obesity and cardiovascular disease (Nugent, 2005).

In general, the RS content of granular starch is positively correlated with the level of amylose (Sang, Bean, Seib, Pedersen, & Shi, 2008). Therefore, high-amylose starches are of interest because of their potential health benefits and industrial uses (Butardo et al., 2011). Many high-amylose cereal varieties have been developed via mutation or transgenic breeding approaches (Bird et al., 2004; Regina et al., 2006). Some have been shown to contain a high-level of RS and show potential health benefits. For example, high amylose barley and wheat grains have a significant potential to improve health by reduction of plasma cholesterol and production of increased large-bowel short-chain fat acids (Bird et al., 2004; Regina et al., 2006). A high-amylose transgenic rice line (TRS) containing a starch with an amylose content of about 60% was developed by antisense RNA inhibition of both starch branching enzyme I (SBE I) and SBE IIb in our lab (Wei, Xu et al., 2010; Zhu et al., 2012). TRS grains are rich in RS, are as safe as the conventional non-transgenic rice when tested for rat consumption, and have shown significant potential to improve the health of the large bowel in rats (Zhou et al., 2011, 2012). TRS starch has C-type crystallinity, and a high resistance to HCl hydrolysis and *Bacillus licheniformis* α-amylase hydrolysis (Wei, Xu et al., 2010).

Most applications of starch in foods and nonfoods (pharmaceutics, papers, adhesives, packaging, and biofuels) require the disruption of starch granules through acid, alkaline, enzyme, or hydrothermal treatments (gelatinisation/melting) (Tawil, Viksø-Nielsen, Rolland-Sabaté, Colonna, & Buléon, 2011). Enzyme hydrolysis of starch occurs in many biological and industrial processes

* Corresponding authors: Addresses: Agricultural College, Yangzhou University, Yangzhou 225009, China. Tel.: +86 514 87996648 (Q. Liu). College of Bioscience and Biotechnology, Yangzhou University, Yangzhou 225009, China. Tel.: +86 514 87997217 (C. Wei).
E-mail addresses: qqliu@yzu.edu.cn (Q. Liu), cxwei@yzu.edu.cn (C. Wei).

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such as starch metabolism in plants, digestion by mammals, malting, fermentation, glucose syrup, or bioethanol production. Enzymatic modification can change the physicochemical nature of starch including its morphological and crystalline properties (Mitsuiki et al., 2005; Zhou, Hoover, & Liu, 2004). Enzyme molecules affect the granules in two ways (Li, Gao, Wang, Jiang, & Huang, 2011). First, enzymes erode the outer surface of the granule and cause the occurrence of characteristic fissures and pits (exocorrosion). Second, enzymes create channels leading to the granule centre, which weakens granule integrity and consequently leads to its breakdown (endocorrosion) (Li, Vasanthan, Hoover, & Rossnagel, 2004; Oates, 1997). Starch is usually hydrolysed by three important amylolytic enzymes, namely, α-amylase, β-amylase, and amyloglucosidase. The α-amylase is an endoamylase that cleaves the α-1,4 glycosidic bonds of the amylose or amylopectin chain at internal positions (endo) to yield products (oligosaccharides with varying lengths and branched oligosaccharides called limit dextrins) with an α-configuration. The β-amylase and amyloglucosidase belong to the exoamylase family. The β-amylase hydrolyses maltosyl units from the nonreducing end of amylose or amylopectin to yield maltose in the β-configuration and β-limit dextrin. The amyloglucosidase catalyses the hydrolysis of both α-1,4 and α-1,6 glycosidic bonds at the branching point to release β-D-glucose residues of the polymer substrate (van der Maarel, van der Veen, Uitdehaag, Leemhuis, & Dijkhuizen, 2002). Many researchers have reported structures and physical properties of A-type and B-type starches after exposure to α-amylase hydrolysis, with the B-type crystals being more resistant to enzyme hydrolysis than the A-type (Mitsuiki et al., 2005; Tawil et al., 2011; Zhou et al., 2004). However, there is little information on amyloglucosidase hydrolysis of starches, especially for C-type starch.

In this paper, high-amylose C-type starch was isolated from rice TRS mature grains, and hydrolysed by amyloglucosidase. The granule morphology and structural characterisations of the hydrolysed starch residues were investigated by scanning electron microscope (SEM), XRD, solid-state ^{13}C cross-polarisation magic-angle spinning nuclear magnetic resonance (^{13}C CP/MAS NMR) spectroscopy, and attenuated total reflectance-Fourier transform infrared (ATR-FTIR) spectroscopy. The objective of this study was to obtain a better understanding of the resistance of high-amylose C-type TRS starch to amyloglucosidase hydrolysis. This research should be very helpful in the application of high-amylose rice TRS in food and nonfood industries.

2. Materials and methods

2.1. Plant materials

An *indica* rice cultivar Te-qing (TQ) and its transgenic line (TRS) with high amylose and RS contents were used in this study. TRS was generated from TQ after transgenic inhibition of both SBE I and SBE IIb through an antisense RNA technique, and was homozygous for the transgene. The expressions of SBE I and SBE IIb were completely inhibited in TRS grains (Zhu et al., 2012). TQ and TRS were cultivated in the transgenic close experiment field of Yangzhou University, Yangzhou, China, in 2011, and mature grains were used to isolate starches.

2.2. Native starch isolation

Starches were isolated from mature grains as previously described (Wei, Xu et al., 2010). In order to avoid the effect of enzyme or alkaline treatment on crystalline structure, samples were only treated with water during starch isolation. The isolated starch, which contained granule-associated protein and lipid, was referred to as native starch.

2.3. Hydrolysis of starch by amyloglucosidase

Starches were hydrolysed by *Aspergillus niger* amyloglucosidase (AAG) (EC 3.2.1.3) (Sigma–Aldrich A-7095) according to the method of Li et al. (2004) with some modifications. One gram of starch was suspended in 40 ml of enzyme solution (0.05 M acetate buffer, pH 4.5, 500 U AAG). The amylolysis was carried out in a constant temperature shaking water bath with continuous shaking (100 rpm) at 55 °C for 1, 2, 4, 8, and 16 h. After hydrolysis, undissolved residues were quickly obtained by centrifugation (3000*g*, 10 min) at 4 °C, and the supernatant was used for measurement of the solubilised carbohydrates to quantify the degree of hydrolysis by the anthrone-H_2SO_4 method (Viles & Silverman, 1949). The residues were subsequently washed three times with ddH_2O to remove residual enzyme and two times with anhydrous ethanol to dehydrate the residues, then dried at 40 °C for 2 days. The dried starches were ground into powders in a mortar with pestle, and passed through a 100-mesh sieve for further structural analysis.

2.4. SEM observation

The starch residues in ethanol were used for sample preparation of SEM. One drop of the starch–ethanol suspension was applied to an aluminium stub using double-sided adhesive tape, and the starch was coated with gold before viewing with an environmental SEM (Philips XL-30).

2.5. Apparent amylose content measurement

The apparent amylose contents of starch residues were determined using the iodine adsorption method of Konik-Rose et al. (2007) with some modifications. Ten milligram of starch was weighed (accurate to 0.1 mg) into a 10 ml screw-capped tube. For defatting, 5 ml of 85% (v/v) methanol was added and incubated at 65 °C for 1 h with occasional vortexing. After centrifugation at 13,000*g* for 5 min, the supernatant was removed. The defatting step was then repeated. The starch was dried at 37 °C overnight, then dissolved in 5 ml of urea dimethyl sulphoxide (UDMSO) solution (nine parts DMSO and one part of 6 M urea). Dissolution was obtained by incubating the mixture at 95 °C for 1 h with intermittent vortexing. A 1 ml aliquot of the starch-UDMSO solution was treated with 1 ml of iodine solution (0.2% I_2 and 2% KI, w/v) and made up to 50 ml with ddH_2O. The solution was immediately mixed and placed in darkness for 20 min. Apparent amylose content was evaluated from absorbance at 620 nm. The recorded values were converted to percent of amylose by reference to a standard curve prepared with amylose from potato (Sigma–Aldrich A-0512) and amylopectin from corn (Sigma–Aldrich 10120).

2.6. DSC analysis

A DSC (200-F3, NETZSCH, Germany) was used to examine the thermal properties of starch residues as described previously (Wei et al., 2011).

2.7. Swelling power measurement

Swelling powers of starch residues were determined with a small-scale swelling test method by heating starch–water slurries (2%, w/v) in a water bath at 95 °C for 30 min according to the procedure of Konik-Rose et al. (2001).