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Morphology, structure and gelatinization properties of heterogeneous starch granules from high-amylose maize

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ABSTRACT

High-amylose cereal endosperm is rich in heterogeneous starch granules. In this paper, we investigated the morphology, structure and gelatinization properties of high-amylose maize endosperm starch. Starch had individual, aggregate and elongated heterogeneous granules. Most of individual granules were round with small size and had one central hilum. Aggregate and elongated granules consisted of many subgranules with central hila, and had irregular and rod/filamentous shapes, respectively. Iodine stained starch granules showed five types of polarization colors: blue, purple, fuchsia, dark red, and interior dark blue and exterior brown. Most of individual and aggregate granules had the color of dark red, that of elongated granules the color of interior dark blue and exterior brown. Amylose was mainly distributed in the hilum region and the circumference of starch granules. Aggregate and elongated granules had higher amylose content than individual granules. Elongated and individual granules had the highest and the lowest gelatinization resistance among high-amylose maize heterogeneous starch granules, respectively.

1. Introduction

Starch is stored as discrete semicrystalline granules in higher plants, and consists of two main components: linear amylose and highly branched amylopectin. The amylose content has an important effect on physicochemical properties and applications of starch. For example, starch with high-amylose content has a high resistance to gelatinization and hydrolysis (Man et al., 2012; Wei et al., 2011). Most of the starch in the diets of humans is digested rapidly in the small intestine. However, a variable proportion, known as resistant starch (RS), cannot be hydrolyzed in the upper gastrointestinal tract and functions as a substrate for bacterial fermentation in the large intestine (Englyst, Kingman, & Cummings, 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. Many high-amylose cereal varieties have been developed via mutation or transgenic breeding approaches. Some have been shown to contain a high-level of RS and show potential health benefits (Bird et al., 2004; Li, Jiang, Campbell, Blanco, & Jane, 2008; Regina et al., 2006; Wei et al., 2010).

Cereal endosperm starch granules with high-amylose content always show markedly different morphology, structure and physicochemical properties compared with waxy and normal starches (Chen et al., 2009; Jiang, Campbell, Blanco, & Jane, 2010; Kim et al., 2005; Regina et al., 2006, 2010; Slade et al., 2012; Wei et al., 2010). For examples, normal maize starch has starch granules with spherical and angular shapes, whereas starches from high-amylose maize *ae* and GEMS-0067 mutants consist of about 7% and 32% elongated granules, respectively (Jiang, Campbell, et al., 2010). Normal wheat and barley endosperm starch granules consist of disk-shaped large granules and globular small ones. Starch granules from wheat and barley endosperms with inhibition of starch branching enzyme increase amylose contents, and display significant morphological alterations. These large granules appear to be sickle-shaped, and their hila are hollow (Regina et al., 2006, 2010; Slade et al., 2012). Starches from normal rice endosperm are homogeneous polygonal granules with sizes of 3–5 μm. Starch granules from high-amylose rice show significantly heterogeneous shapes, consist of aggregate granules with large size, hollow granules and elongated granules (Kim et al., 2005; Wei et al., 2010).

Starches from high-amylose cereal endosperms consist of highly heterogeneous granules. The mixture of heterogeneous granules

Abbreviations: APTS, 8-amino-1,3,6-pyrenetrisulfonic acid; CLSM, confocal laser scanning microscopy; SEM, scanning electron microscopy.

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shows significantly different physicochemical properties from normal starches (Bird et al., 2004; Jiang, Campbell, et al., 2010; Kim et al., 2005; Li et al., 2008; Man et al., 2012; Regina et al., 2006, 2010; Slade et al., 2012; Wei et al., 2010, 2011). For *ae* high-amylose mutant maize, the heterogeneity in chemical and physical structure is observed within individual granule, between granules within cells, and spatially within the kernel (Liu et al., 2013; Wellner, Georget, Parker, & Morris, 2011). However, the differences in morphology, structure and properties are not clear among heterogeneous starch granules. In this paper, morphology, structure and gelatinization properties of heterogeneous starch granules from high-amylose maize were investigated with several microscopy techniques, including normal light microscopy, polarized light microscopy, hot stage microscopy, confocal laser scanning microscopy (CLSM), and scanning electron microscopy (SEM). This research could add to our understanding of heterogeneous starch granules, and would be useful for various applications of high-amylose cereal starches in the food and nonfood industries.

2. Materials and methods

2.1. Starch

Normal and high-amylose (HYLON VII) maize starches were obtained from National Starch LLC (Bridgewater, NJ, USA), and HYLON VII is a corn hybrid. The amylose contents determined by the potentiometric iodine method were about 27% and 71% for normal and high-amylose maize starches, respectively (Brewer, Cai, & Shi, 2012).

2.2. Light microscopy

A starch suspension (1%, w/v) was prepared with 50% glycerol. 10 μL of starch suspension was placed on the microscope slide and covered with coverslip. The starch granule shape and Maltese cross were viewed with an Olympus BX53 polarized light microscope equipped with a CCD camera. Iodine-stained starches were prepared and observed according to the method of Evans, McNish, and Thompson (2003) with some modifications. 5 mg starches were stained in 0.5 mL of iodine solution (0.5% NaAc buffer, pH4.5, 25% glycerol, 0.04% I_2, 0.06% KI) for 30 min in darkness. 10 μL of iodine-stained starch suspension was firstly viewed under normal light, and then the same field was viewed under polarized light. All the iodine-stained starch granules were photographed with the same condition in order to compare the polarization colors. Iodine stained starches had five kinds of polarization colors: blue, purple, fuchsia, dark red, and interior dark blue and exterior brown. For one type of heterogeneous starch granules, over fifty granules were randomly chosen to be analyzed for polarization color for one experiment. The experiments were performed in triplicate.

2.3. Scanning electron microscopy

Starches were suspended in anhydrous ethanol. One drop of the starch-ethanol suspension was applied to an aluminum stub using double-sided adhesive tape. The starch was coated with gold before viewing with an environmental SEM (Philips XL-30).

2.4. Confocal laser scanning microscopy

Starch granules were stained with the fluorophore APTS (8-amino-1,3,6-pyrenetrisulfonic acid) and prepared for CLSM essentially as previously described by Blennow et al. (2003). Images were recorded on a CLSM (LSM 710, Carl Zeiss MicroImaging GmbH, Jena, Germany) using a 488 nm laser line for excitation and light was detected in the interval from 500 nm to 535 nm. For morphology observation, laser power capacity and master gain were adjusted to maximum saturation. To compare the fluorescence intensity in all images of starch granules, the laser power was kept constant at 1%, and images were recorded at the master gain of 570 for all the starch granules. The condition allowed all granules to have no saturation of the detector for every dot in all granules. For every type of heterogeneous starch granules, over thirty granules were randomly chosen to be recorded. Image analysis was performed using the Carl Zeiss ZEN 2010 software.

2.5. Hot stage microscopy

Starch suspensions were prepared by suspending about 10 mg starch in 1.0 mL of double distilled water by using a vortex mixer. The suspension was transferred onto a slide, covered with a coverslip, and sealed with nail polish to prevent moisture loss during heating. The sealed specimen was then mounted on a Kitazato hot stage apparatus and observed under a long focus M Plan Semi Apochromat objective (20×, 50× magnification) using an Olympus polarized microscope equipped with cross polarizers during heating. The hot stage was heated from 25 °C to 50 °C at a heating rate of 5 °C/min and from 50 °C to 100 °C at a heating rate of 1 °C/min.

The gelatinization temperatures were measured according to the method of Konik-Rose et al. (2001) with some modifications. The starch granules were photographed under polarized light using an Olympus DP72 CCD camera during heating from 50 °C to 100 °C at 1 °C intervals. More than one hundred starch granules of every type of heterogeneous starch granules were analyzed for one experiment. The initial, middle and end gelatinization temperatures recorded, that was, from the point when 5%, 50% and 95% of starch granules had lost their birefringence. The experiments were performed in triplicate.

The swelling of individual starch granule during heating was viewed under normal light and photographed using an Olympus DP72 CCD camera from 40 °C to 95 °C at 5 °C intervals. The area of starch granule was analyzed from the micrograph with JEDA 801D morphological image analysis system (Jiangsu JEDA Science-Technology Development Co., Ltd, Nanjing, China). Over fifty starch granules were measured for every type of heterogeneous starch granules. The starch granule swelling was presented as area swelling percentage (ASP) from ungelatinized granule at 40 °C, and calculated by the equation: $ASP\ (\%) = A_t/A_i \times 100$, where A_i and A_t represented the area of starch granule at initial (40 °C) and specific testing temperature, respectively.

2.6. Statistical analysis

Analysis of variance (ANOVA) by Tukey's test ($p < 0.05$) was evaluated using the SPSS 16.0 Statistical Software Program.

3. Results and discussion

3.1. Morphology of heterogeneous starch granules

Isolated starch granules were viewed under normal and polarized light (Fig. 1). Most of normal maize starch granules were polygonal with large size. Some granules were spherical with small size (Fig. 1A and C). They all showed central hila with typical Maltese-crosses. One granule had only one hilum though starch granules had different sizes (Fig. 1a and c). Therefore, normal maize starch granules were called as individual granules and showed homogeneity. High-amylose maize starch granules showed significantly heterogeneity under normal and polarized light (Fig. 1B and b). These heterogeneous granules could be divided into three types: individual, aggregate, and elongated granules according to morphology and Maltese-cross (Fig. 1D–F and d–f). High-amylose