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In situ observation of crystallinity disruption patterns during starch gelatinization

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ABSTRACT

Twelve starches were isolated from the tuberous root of sweet potato, the rhizomes of lotus and yam, the tuber of potato, the corm of water chestnut, and the seeds of pea, bean, barley, wheat, lotus, water caltrop, and ginkgo. Their gelatinization processes were in situ viewed using a polarizing microscope in combination with a hot stage. Four patterns of crystallinity disruption during heating were proposed. The crystallinity disruption initially occurred on the proximal surface of the eccentric hilum, on the distal surface of the eccentric hilum, from the central hilum, or on the surface of the central hilum starch granule. The patterns of initial disruption on the distal surface of the eccentric hilum and on the surface of the central hilum starch were reported for the first time. The heterogeneous distribution of amylose in starch granule might partly explain the different patterns of crystallinity disruption and swelling during gelatinization.

1. Introduction

Starch is produced by plants where it is stored as discrete semicrystalline granules, and consists of two main components: mainly linear amylose and highly branched amylopectin (Gallant, Bouchet, & Baldwin, 1997). Previous researches from electron microscopy show that the alternating semicrystalline and amorphous growth rings and the central amorphous starch region represent three distinct inner structural features of starch granules (Gallant et al., 1997; Li, Vasanthan, Hoover, & Rossnagel, 2003). Recently, Parker, Kirby, and Morris (2008) view the internal structure of starch granules using atomic force microscopy. Their results suggest that the granules contain alternating rings with different levels of crystallinity and different amylose/amylopectin ratios. Starches isolated from different botanical sources display characteristic granule morphologies. Starch granules vary in shape, including spherical, oval, polygonal, disk (lenticular), elongated and kidney shapes, and in size from <1 μm to 100 μm in diameter (Jane, 2009). The hilum, which is the core of the granule and the starting point from which the granule grows, is usually less organized than the rest of the granule. Most commonly it is situated near the middle of the granule, but it can be eccentric, i.e. towards one end of the granule (Gott, Barton, Samuel, & Torrence, 2006). According to the position of the hilum, starch granules have two types of central hilum granule and eccentric hilum granule.

Starch granules are insoluble in cold water. When starch is heated in the presence of enough water, granules absorb water and swell. The absorption of water by amorphous regions within the granules destabilizes their crystalline structure, results in the loss of birefringence, which is one definition of gelatinization (Parker & Ring, 2001). Gelatinization is an important factor contributing to starch functionality and is widely exploited in the food industry (Ratnayake & Jackson, 2007). Starch gelatinization and associated properties can be determined by various methods, including optical microscopy, electron microscopy, differential scanning calorimetry, X-ray diffraction, nuclear magnetic resonance spectroscopy, Fourier transform infrared spectroscopy, viscosity measurement, enzymatic digestibility, light extinction, and solubility or sedimentation of swollen granule (Liu, Charlet, Yelle, & Arul, 2002; Ratnayake & Jackson, 2006). All these methods measure slightly different physicochemical properties and have unique and inherent advantages and disadvantages.

The gelatinization behavior of individual starch granule can be examined using polarizing microscope with a hot stage. With the procedure, the disruption of crystalline structure and the swelling of disrupted areas can be clearly seen. For example, the sizes and distributions of potato and rice starch granules are determined using this procedure during gelatinization (Liu et al., 2002; Yeh & Li, 1996). The effect of heating rate on the morphology and size of wheat starch granule is also investigated using this procedure (Patel & Seetharaman, 2006). The disruption of crystalline structure during pea and potato starch gelatinization begins from the

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hilum area, is propagated along the granule and accompanied by swelling of disrupted areas (Bogracheva, Meares, & Hedley, 2006; Bogracheva, Morris, Ring, & Hedley, 1998; Tahir, Ellis, Bogracheva, Meares-Taylor, & Butterworth, 2011).

In this paper, 12 starches were isolated from a wide variety of plant sources, consisting of tuberous root, tuber, corm, rhizome and seeds. Their morphologies, amylose contents and crystalline properties were investigated. The gelatinization processes of granules were in situ viewed using a polarizing microscope in combination with a hot stage. Four patterns of crystallinity initial disruption and swelling during heating were proposed. The amylose distributions in native starch granules were also investigated using a confocal laser scanning microscope (CLSM) to explain the different gelatinization processes of starch granules. This study would be very useful for the understanding of the gelatinization processes of different starches and for further utilization of these starches.

2. Materials and methods

2.1. Plant material

The tuberous root of sweet potato (*Ipomoea batatas* L.), the rhizomes of lotus (*Nelumbo nucifera* Gaertn.) and yam (*Dioscorea opposita* Thunb.), the tuber of potato (*Solanum tuberosum* L.), the corm of water chestnut (*Eleocharis dulcis* (Burm. F.) Trin. ex Hensch.), and the seeds of pea (*Pisum sativum* L.), bean (*Vicia faba* L.), barley (*Hordeum valgare* L.), wheat (*Triticum aestivum* L.), lotus (*N. nucifera* Gaertn.), water caltrop (*Trapa bispinosa* Roxb.), and ginkgo (*Ginkgo biloba* L.) were used to isolate native starch. Freshly harvested sweet potato tuberous root, lotus and yam rhizomes, potato tuber, water chestnut corm, water caltrop seed, and mature dry seeds of pea, bean, lotus and ginkgo were obtained from a local natural food market (Yangzhou City, China). Barley and wheat mature seeds were harvested in the experiment field of Yangzhou University, Yangzhou, China.

2.2. Isolation of native starches

Native starches were isolated following a method described by Man et al. (2012). Briefly, the seeds of pea, bean, barley, wheat and lotus were steeped overnight in double-distilled water at 4 °C. Sweet potato tuberous root, lotus and yam rhizomes, potato tuber and water chestnut corm were peeled and sliced into small pieces. The hard seed coats of ginkgo and water caltrop were removed with the help of a sharp and clean stainless steel knife and the edible portions were used to isolate starches. These above materials were homogenized with ice-cold water in a home blender. The homogenate was filtered with 100-, 200-, and 300-mesh sieves, successively. The starch suspension was centrifuged at 3000 × *g* for 10 min. The yellow gel-like layer on top of the packed white starch granule pellet was carefully scraped off and discarded. The process of centrifugation separation was repeated several times until no dirty material existed. The precipitated starch was further washed two times with anhydrous ethanol, dried at 40 °C for 2 days, ground into powders, and passed through a 100-mesh sieve. The starch samples were stored at −20 °C for analysis.

2.3. Morphology observation of starches

A starch suspension (1%) was prepared with 50% glycerol. A small drop of starch suspension was placed on the microscope slide and covered with a coverslip. The granule shape and Maltese cross were viewed under the Olympus BX53 polarizing light microscope equipped with a CCD camera.

2.4. Light microscope with hot stage

Starch suspensions were prepared by suspending about 10 mg starch in 1.0 ml of 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 (50× magnification) using the Olympus polarizing microscope equipped with cross polarizers and λ-plate during heating. The hot stage was heated from 25 to 50 °C at a heating rate of 5 °C/min and from 50 to 90 °C at a heating rate of 1 °C/min. The behavior of individual starch granule during heating was viewed under normal and polarizing light and photographed using an Olympus DP72 CCD camera.

2.5. Confocal laser scanning microscope

Starch granules were prepared for CLSM essentially as previously described (Blennow et al., 2003). Briefly, starch granules (about 2 mg) were stained in 3 μl of APTS solution (20 mM 8-amino-1,3,6-pyrenetrisulfonic acid (molecular probes, Sigma–Aldrich) dissolved in 15% acetic acid) and 3 μl of 1 M sodium cyanoborohydride. Samples were incubated at 30 °C for 15 h, washed five times in double-distilled water and suspended in 20 μl of 50% glycerol. 2 μl of the starch granule suspension was added to 8 μl of a highly viscous mixture containing 2% agar and 85% glycerol in water. The sample thoroughly mixed using a plastic pipette tip. The sample was immediately mounted on a glass plate for microscopy. 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 to 535 nm. Image analysis was performed using the Carl Zeiss ZEN 2010 software and granules were viewed using the channels from red to white.

2.6. Amylose content determination of starches

Amylose content was determined following a modified method (Man et al., 2012) according to the iodine adsorption method of Konik-Rose et al. (2007). The experiments were performed thrice.

2.7. Crystalline property of starches

Crystalline property of starches was analyzed on an X-ray powder diffraction (XRD) (D8, Bruker, Germany) following the method described by Wei et al. (2010).

3. Results and discussion

3.1. Morphology of starches

Photomicrographs of starch granules taken from polarizing light microscope under normal light and polarizing light are presented in Fig. 1. Potato starch had large and small granules, they showed the oval and spherical shapes, respectively (Fig. 1A). The hilum positions of potato oval and spherical starches were at one end of granules (Fig. 1a). Lotus rhizome starch showed significantly heterogeneous shapes, including elongated, oval, spherical and irregular shapes. The elongated and oval granules were larger than the spherical and irregular ones (Fig. 1B). The hilum positions were at one end of granules (Fig. 1b). Yam starch was slightly oval with the hilum at one end of granules (Fig. 1C and c). Pea and bean starches were oval with the hilum in the center of granules (Fig. 1D, d, E and e). Barley and wheat starches had bimodal size distributions, the large granules had disk shapes, whereas the small granules had spherical shapes. Their hilum positions were all in the