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The relationship between enzyme hydrolysis and the components of rice starches with the same genetic background and amylopectin structure but different amylose contents

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ABSTRACT

The relationship between starch components and hydrolysis previously focused on starches with different amylose contents (ACs) and amylopectin structures. In this study, starches were isolated from six rice seeds with the same genetic background but different activities of granule-bound starch synthase I. The starch components and enzyme hydrolysis were measured and analysed to reveal their relationships. The results showed that the six rice starches had the same amylopectin structure but different ACs ranging from approximately 1%–15%. The starch component parameters measured by different methods were significantly correlated with each other. The hydrolysis parameters of starch by both α-amylase and amyloglucosidase were analysed using three methods including a non-linear fit to the degree of hydrolysis, a first-order kinetics fit to the degree of hydrolysis, and a first-order kinetics fit to the hydrolysis rate, and they had significant correlation each other. Waxy starch had a fast hydrolysis rate and showed a single hydrolysis phase during early hydrolysis, while starch with a high AC had a bi-phasic hydrolysis process. Starch component parameters including the maximum absorption wavelength, iodine blue, AC, and low molecular weight fraction were significantly negatively correlated to the hydrolysis rate of starch. The above results indicated that starches with different ratios of amylose to amylopectin but the same amylopectin structure had significantly different hydrolysis properties. This study could provide some information for rice quality breeding and starch applications through changing the activity of granule-bound starch synthase I.

1. Introduction

Starch mainly consists of linear amylose and highly branched amylopectin, and it is stored as semi-crystalline granules. Starch granules are insoluble in water. Therefore, the hydrolysis of starch is usually required to utilize starch in food and non-food industries (Tawil, Viksø-Nielsen, Rolland-Sabaté, Colonna, & Buléon, 2011). Enzyme hydrolysis is commonly used for starch hydrolysis. Enzymes hydrolyse starch in two ways: hydrolysing starch from the outer to the inner portions of the granule (exocorrosion) and creating channels leading to the granule centre and consequently hydrolysing starch from the inner to the outer portions (endocorrosion) (Dhital, Shrestha, & Gidley, 2010a, 2010b). The enzymes α-amylase and amyloglucosidase are an endoamylase and exoamylase, respectively, and they are widely used to hydrolyse starch (Li, Hasjim, Dhital, Godwin, & Gilbert, 2011; Li, Vasanthan, Hoover, & Rossnagel, 2004).

The enzyme hydrolysis of starch is a dynamic process. It is important to investigate the hydrolysis dynamics. The kinetics of enzyme hydrolysis of starch is studied using various methods. For example, the curve method of hydrolysis degree establishes the relationship between the degree of hydrolysis and time, which can directly compare the degrees of hydrolysis of different starches at the same time, but it does

Abbreviations: AAC, apparent amylose content; AAG, *Aspergillus niger* amyloglucosidase; AC, amylose content; GBSSI, granule-bound starch synthase I; GPC, gel-permeation chromatography; PPA, porcine pancreatic α-amylase

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not quantitatively analyse and compare the hydrolysis rate (Man et al., 2012). The two-parameter non-linear fit method is to fit the degree of hydrolysis using a two-parameter model f = ax/(b + x), where "a" can be used to estimate the maximum theoretical degree of hydrolysis, and "b" can reflect the time required to reach half of the maximum degree of hydrolysis (Carciofi et al., 2012). The hydrolysis properties of starch can be evaluated quantitatively based on two parameters, but there is no way to compare the hydrolysis rate and hydrolysis efficiency of starch. For the first-order kinetics fit to the degree of hydrolysis, the natural logarithm of the starch degree of hydrolysis and hydrolysis time have a linear relationship, and the slope of the fit line is the hydrolysis rate coefficient, which reflects the rate of starch hydrolysis (Zhang, Dhital, & Gidley, 2013). For the first-order kinetics fit to the hydrolysis rate, the natural logarithm of the starch hydrolysis rate (slope) (i.e., log of slope, LOS) and hydrolysis time have a linear relationship, and the slope of the fit line is the hydrolysis rate coefficient (Butterworth, Warren, Grassby, Patel, & Ellis, 2012). The LOS method can analyse hydrolysis curves to reveal and quantify the differences during the hydrolysis process and predict the product concentration at the end of hydrolysis (Butterworth et al., 2012; Edwards, Warren, Milligan, Butterworth, & Ellis, 2014; Kim, Choi, Park, & Moon, 2017).

The enzyme hydrolysis of starch is influenced by many factors including the starch morphology, granule size, porosity, amylose content (AC), and crystalline structure (Blazek & Gilbert, 2010). A small granule with a larger relative surface area is hydrolysed faster than a large granule (Dhital, Butardo, Jobling, & Gidley, 2015; Dhital, Shrestha, & Gidley, 2010b; Lin, Cai, et al., 2016, 2015). The AC, as an important starch component, is negatively correlated with the hydrolysis of some rice starches and high-amylose maize starches (Cai et al., 2015; Chung, Liu, Lee, & Wei, 2011; Lin, Guo, Huang, et al., 2016; Lin, Zhang, Zhang, & Wei, 2017). The amylopectin structure, which determines the crystallinity, has significant effects on starch hydrolysis. The long branch-chains of amylopectin have higher resistance to hydrolysis than the short branch-chains (Dhital et al., 2015; Lin, Guo, Huang, et al., 2016). The previous studies on the enzyme hydrolysis of starch are mainly focused on starches with different granule sizes (Dhital et al., 2015, 2010b; Lin, Cai, et al., 2016, 2015) as well as starches with different ACs with different genetic backgrounds, thus leading to different crystallinity (Cai et al., 2015; Chung et al., 2011; Lin, Guo, Huang, et al., 2016, 2017). To our knowledge, there is no report about the hydrolysis of starches with a similar granule size, same amylopectin structure, and same genetic background but different ACs. Such starches, which exclude the influence of other factors including the granule size, amylopectin structure and crystallinity, are very important to investigate the effects of the AC on enzyme hydrolysis.

The *Waxy* (*Wx*) gene encodes granule-bound starch synthase I (GBSSI) in rice (*Oryza sativa* L.), which is directly responsible for amylose synthesis in the rice endosperm. In the study of Liu, Wang, and Cai (2014), a series of transgenic rice lines with different activities of OsGBSSI and ACs was developed by introducing site-directed mutated *Wx* gene constructs into the *wx* mutant glutinous rice Guang-ling-xiang-nuo (GLXN). The site-directed mutagenesis of OsGBSSI has no effect on the protein dosage of other enzymes involved in starch biosynthesis as well as the granule size and crystalline structure of the starch; however, the grain appearance and starch physicochemical properties show gradient changes with an increasing AC (Liu et al., 2014; Zhang et al., 2017). In this study, starches were isolated from the six rice seeds with the same genetic background but different ACs, and their components as well as enzyme hydrolysis were measured. Our objective was to reveal the relationship between enzyme hydrolysis and the components of starches with the same genetic background, amylopectin structure, granule size and crystalline structure but different ACs.

2. Materials and methods

2.1. Materials

Five transgenic rice lines (P1300, E410D, Y268F, R408G, and CDS) and their wild-type, the glutinous *Japonica* rice cultivar Guang-ling-xiang-nuo (GLXN), were used in this study. The P1300 is a negative control, and it carries an empty pCAMBIA1300 vector, thus showing the same glutinous phenotype as GLXN. The CDS is a positive control and represents the wild-type OsGBSSI, whereas the R408G, Y268F, and E410D represent amino acid substitutions at residues 408, 268, and 410 in the wild-type OsGBSSI protein, respectively. The activity of OsGBSSI gradually decreases from CDS to R408G, Y268F, and E410D (Liu et al., 2014). The six rice lines with the same genetic background were grown simultaneously in the same transgenic close experimental field of Yangzhou University and were subjected to identical cultural practices as well as environmental conditions. Mature seeds were harvested and used to isolate the starches.

Dimethyl sulphoxide (DMSO) was purchased from Merck KGaA (Darmstadt, Germany). Porcine pancreatic α-amylase (PPA, A3176), protease from *Streptomyces griseus* (P5147), and Sepharose CL-2B (CL2B300) were purchased from the Sigma-Aldrich Co. Protease K was purchased from Amresco (Solon, OH). Amyloglucosidase from *Aspergillus niger* (AAG, E-AMGDF) and isoamylase (E-ISAMY) were obtained from Megazyme Ltds (Bray, Ireland). All other reagents were of analytical grade.

2.2. Isolation of starch

The starch was isolated from rice seeds following the methods of Zhang et al. (2017). Briefly, the polished rice (10 g) was soaked in 30 mL of 50 mM Tris-HCl buffer (pH = 7.0) including 10 mM $CaCl_2$ at 4 °C overnight and ground in a home blender. Five milligrams of protease K was added. The starch slurry was stirred with a magnetic stir bar at 37 °C for 24 h, filtered with a 75 μm sieve, and centrifuged (3000 g, 5 min). The dirty layer on top of the white starch precipitate was scraped off. The starch was washed five times with deionized water and dehydrated two times with anhydrous ethanol. Finally, the starch was dried at 40 °C and passed through a 100-mesh sieve.

2.3. Measurements of the iodine absorption spectrum and AC of starch

The iodine absorption spectrum of starch was measured according to the methods of Lin, Cai, et al. (2016) and Man et al. (2014) with some modifications. Briefly, 10 mg of starch was incubated in 1 mL of protease solution (0.25 M tricine buffer, pH = 7.5, 2 U protease) in a ThermoMixer with shaking at 350 rpm and 37 °C for 30 min, and then, it was centrifuged (4000 g, 10 min). The starch precipitate was incubated in 1 mL of 0.45% sodium bisulphite solution (w/v) and centrifuged again as in the above method. The starch precipitate was incubated in 5 mL of 85% (v/v) methanol at 65 °C for 1 h and centrifuged. Finally, the starch sample was incubated in 5 mL of DMSO containing 10% 6.0 M urea at 95 °C for 1 h with intermittent vortexing. The 1 mL of starch-DMSO solution and 1 mL of iodine solution (0.2% I_2 and 2% KI, w/v) were made up to 50 mL with deionized water in a 50 mL volumetric flask. The sample was mixed, settled in darkness for 20 min, and scanned from 400 to 900 nm using an Ultrospec 6300 pro spectrophotometer. The apparent AC (AAC) was measured from the absorbance at OD 620 nm. In order to exclude the influence of amylopectin long branch-chains on the AAC, the AC in starch was also measured using the Megazyme Amylose/Amylopectin Assay kit (K-AMYL) following the instructions supplied with the Kit.

2.4. Molecular weight distribution of fully branched starch

The molecular weight distribution of fully branched starch was