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Thermal and rheological characteristics of mutant rice starches with widespread variation of amylose content and amylopectin structure

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ABSTRACT

Of starch biosynthesis-related enzymes, starch synthases (SSs) and starch branching enzymes (BEs) play important roles in the control of starch structure. Measurements of the particle size distribution, swelling power, differential scanning calorimetry (DSC), X-ray diffraction (XRD) and dynamic viscoelastic measurements were used to investigate the physicochemical properties of rice endosperm starch from SSIIIa, granule-bound starch synthase (GBSSI) and/or BEIIb deficient mutants including double mutants having widespread variation of amylose content and amylopectin structure. The predicted relative starch crystallinity (RSC) of A-type starches and B-type starches of these mutants, as computed based on amylopectin contents, were almost equal. These results suggest that SSIIIa and BEIIb deficiency does not affect the degree of crystallinity of amylopectin. A newly developed double mutant line (*ss3a*/*be2b*) with high amylose content (ca. 45%) and a lower proportion of amylopectin short chains showed a higher temperature of gelatinization. Moreover, retrogradation of the gel was extremely rapid. Additionally, *be2b* with extremely low proportion of amylopectin short chains and lower amylose contents (28%) showed higher gelatinization temperature and more rapid retrogradation than *ss3a*, with a lower proportion of amylopectin long chains with DP $\leq$ 33 and higher amylose content (30%). Granules of *ss3a*/*be2b* and *be2b* in gels after viscoelastic measurement were mostly maintained, indicating that most starch granules were not ruptured during heating. These results clarify that not only amylose contents but also the fine structure in amylopectin strongly affected rice gel viscoelastic properties.

1. Introduction

Starch is not only a major resource of carbohydrates and materials for food processing. Its wider use as functional material for added value is also anticipated. Starch comprises amylose and amylopectin, which are α-D-glucose polymers of different structures. The amylose content influences starch physicochemical properties and processing characteristics (Singh, Inouchi, & Nishinari, 2006). Resistant starch is attracting attention as a new functional property of starch (Jiang, Campbell, Blanco, & Jane, 2010; Kubo et al., 2010; Zhu, Liua, Wilson, Gu, & Shi, 2011).

Starch biosynthetic enzymes of four types are well known: ADP-glucose pyrophosphorylase (AGPase), starch synthase (SS), starch branching enzyme (BE), and debranching enzyme (DBE). Multiple isozymes existing in these enzymes can control the chain length distribution of amylopectin and amylose contents (Ball & Morell, 2003; Myers, Morell, James, & Ball, 2000; Nakamura, 2002; Smith, Denyer, & Martin, 1997). Hizukuri (1986) reported that one amylopectin cluster consists of chains with DP $\leq$ 24. The chain length of amylopectin also affects the gelatinization, retrogradation, and pasting properties of starch (Han & Hamaker, 2001; Jane et al., 1999; Lu et al., 2009; Miles, Morris, Orford, & Ring, 1985; Yuan, Thompson, & Boyer, 1993). However, our understanding of these relations between physicochemical properties and the starch structure remains incomplete (Fujita, 2015).

In recent years, our research group has developed several rice mutant lines producing starch biosynthesis-related enzymes. We have investigated their characteristics intensively (Fujita, 2012, 2014; Fujita, 2015). SSIIIa is the second major soluble SS isozyme in rice developing endosperm. It elongates long B_{2-3} chains with the degree of polymerization (DP) $\geq$ 33 connecting amylopectin clusters (Fujita et al., 2007). Deficiency of SSIIIa led to the enhanced expression of *SSI* and granule-bound starch synthase I (*GBSSI*). The former decreases the distribution of chains with DP6–DP9 and

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DP16–DP19 while increasing chains with DP10–DP15 and DP20–DP25. The latter results in increased amylose contents by 1.5-times compared with wild-type (WT) (Fujita et al., 2007). Actually, GBSSI has been shown to be an amylose synthase. The GBSSI-deficient mutants of several crops have no amylose in the stored starches. The expression levels of *GBSSI* directly affect the amylose content (Sano, 1984). The *be2b* starch exhibits markedly lower amylopectin short chains with DP < 14. The average length of the amylopectin branch chains is very long, with high amylose contents compared to those of WT (Asaoka, Okuno, Sugimoto, Omura, & Fuwa, 1986; Ikawa, Glover, Sugimoto, & Fuwa, 1981; Nishi, Nakamura, Tanaka, & Satoh, 2001). The *be2b* starch in maize and rice endosperm exhibited a B-type diffraction pattern and a higher gelatinization temperature in those earlier studies (Fuwa et al., 1999; Jane et al., 1999; Kasemsuwan, Jane, Schnable, Stinard, & Robertson, 1995; Kubo et al., 2010; Tanaka et al., 2004; Yano, Okuno, Kawakami, Satoh, & Omura, 1985; Yuan et al., 1993).

Analysis of double mutant lines with mutations in genes encoding SS and BE isozymes is one strategy to elucidate these enzymes' interaction and their functions (Abe et al., 2014; Asai et al., 2014). A double mutant line (*ss3a*/*be2b*) was generated by crossing with *ss3a* and *be2b* mutant lines. Surprisingly, the apparent amylose contents of *ss3a*/*be2b* were 45%, as estimated using gel filtration of debranched starch. That value is the highest reported to date among non-genetically modified (GM) rice plants (Asai et al., 2014). In addition, amylopectin short chains DP < 14 of *ss3a*/*be2b* were reported as markedly fewer than in the WT. The concentrations of long chains (DP ≥ 25) connecting clusters of amylopectin in *ss3a*/*be2b* were higher than in the WT and lower than in the *be2b*. For *waxy* type of *ss3a*/*gbss1*, which is generated by crossing with *ss3a* and *gbss1* mutant lines, the endosperm has no amylose with similar traits to those of *ss3a* amylopectin structure (Ando et al., 2013).

The key enzymes that differentiate the starch structure of the mutants from the WT in this study are SSIIIa, GBSSI, and BEIIb. We treated five rice mutants, *ss3a*, *be2b*, *gbss1*, *ss3a*/*be2b*, *ss3a*/*gbss1*, and Japonica WT rice lines showing widespread variation of the amylose content and amylopectin structure. To elucidate the starch structure of the rice lines used for this study, we present (Fig. 1) a three-dimensional plot of the relations among rates of the amount of short chains with DP ≤ 14 within one cluster of amylopectin chains (DP ≤ 24), the rate of the amount of long chains with 24 < DP ≤ 60 in all amylopectin chains (DP ≤ 60), and apparent amylose contents of starches. The ranges of the rates of the short chains (DP ≤ 14) and long chains (24 < DP ≤ 60) were, respectively, 36.8–56.0% and 10.3–21.5%. The range of the apparent amylose content was 0–45.1%.

This study examines the particle size distribution, swelling power, gelatinization temperature and heat of gelatinization as measured using differential scanning calorimetry (DSC), X-ray diffraction (XRD), and dynamic viscoelastic measurements using widespread variation of the endosperm starch in the rice mutants described above. The study objectives are clarification of the relations between physicochemical property characteristics and the starch structure.

2. Materials and methods

2.1. Plant materials

Six rice lines were grown in the summer of 2008 at an experimental field at Akita Prefectural University. This study examined double mutant lines *ss3a*/*be2b* (Asai et al., 2014) and *ss3a*/*gbss1* (Ando et al., 2013), the parental mutant lines *ss3a* (Fujita et al., 2007), *gbss1* (Ando et al., 2013) and *be2b* (Yano et al., 1985), and WT (*Oryza sativa* L. Japonica, cv. Nipponbare).

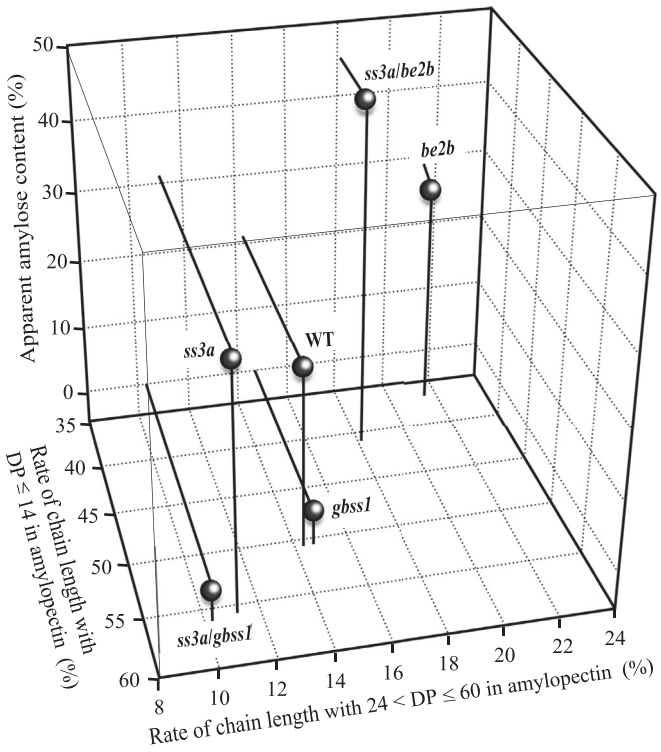

Fig. 1. Three-dimensional plot of relations between the rate of chain length of amylopectin and apparent amylose content in the double mutant lines (*ss3a*/*be2b* and *ss3a*/*gbss1*), the parental mutant lines (*be2b* and *ss3a*), and the wild type (WT) line.

2.2. Starch granule isolation

Starch samples from five mutant lines (*ss3a*/*be2b*, *ss3a*/*gbss1*, *ss3a*, *gbss1*, and *be2b*) and a WT examined in this study were isolated using cold alkaline steeping method (Yamamoto, Sawada, & Onogaki, 1973; Yamamoto, Sawada, & Onogaki, 1981). These starches were assumed to contain 10% moisture. The protein and lipid contents were, respectively, < 0.4% and <0.3%.

2.3. Particle size distribution

Particle size distributions of rice starches were measured using a laser diffraction particle size analyzer (MT3300EXII; Microtrac Inc., FL, USA). Starch samples were suspended in 1% (w/w) deionized water. The suspension was introduced with a sample delivery controller and was sonicated for 180 s to avoid the agglomerated particles. At least three replicates were analyzed for each sample.

2.4. Swelling power and water solubility index

Starch (30 mg, dry base) was weighed in a 2 mL centrifuging tube. Then 1470 mg of distilled water was added. The sealed tubes were allowed to stand for 30 min at room temperature of approximately 23 °C. The starch suspensions (2%, w/w) were heated to 60, 70, 80, 90, and 95 °C for 60 min in a water bath several times with gently inverted closed tubes. Then the tubes were cooled rapidly in an ice bath. After centrifugation at 12,500 × *g* for 10 min, the sediments were weighed to ascertain the swelling power (g/g; SP). The water solubility index (%; WSI) was measured using supernatant (Leach, McCowen, & Schoch, 1959). SP and WSI were measured in triplicate, at least.