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Structure, Physical, and Digestive Properties of Starch from *wx ae* Double-Mutant Rice

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Amylopectin is the principal component of starch. The *amylose extender* (*ae*) gene encodes the starch-branching enzyme IIb, which is critical in determining the fine structure of endosperm starch. To determine the relationship between the fine structure of amylopectin and its physical properties, rice mutant lines defective in the *ae* function with altered fine structure of amylopectin and in combination with the *waxy* (*wx*) background were selected for comparative studies with primary wild-type and *ae* starches. The *ae* mutant endosperms accumulated a high amylose content starch with long amylopectin chains. The *ae* and *wx ae* starches showed no significant difference in the unit chain-length distribution of amylopectin and starch granule morphology. The *wx ae* starch displayed a higher pasting temperature and higher peak viscosity. The gelatinization peak temperatures of the *wx*, *ae*, and *wx ae* starches were 2.2, 13.1, and 17.1 °C higher, respectively, than that of the wild-type starch, and the *wx ae* starch showed a retrogradation peak with a shorter cooling period than that of *ae* starch. The raw *ae* and *wx ae* starches were almost indigestible by α-amylase *in vitro*. Rats fed the *wx ae* starch showed slowly increasing blood glucose at a lower level than the rats fed the *wx* or wild-type starch. These results indicate that the primary structure of the rice *wx ae* amylopectin with enriched long chains changes the granular structure of the starch, including its crystal structure, and results in resistance to *in vitro* or *in vivo* degradation.

KEYWORDS: Amylopectin fine structure; rice; mutant; starch; *amylose extender*; *waxy*

INTRODUCTION

Starch serves a fundamental role in the life cycle of plants as the carbohydrate storage substance and the most important energy reserve in the human diet. Starch consists of two major components: amylose and amylopectin. Amylose has mainly linear molecules with α-1,4-linked D-glucosyl units and a few branches of α-1,6 linkages (*1*), whereas amylopectin is a highly branched molecule consisting of short amylose chains connected with α-1,6 linkages (*1*, *2*).

One maize mutant, the *amylose extender* (*ae*) mutant, produces starch with amylose and amylopectin branch chains significantly longer than those of normal maize starch (*3*−*10*), as a result of a defective branching enzyme IIb function in starch synthesis (*11*, *12*). The maize *ae* mutant starch shows a higher gelatinization temperature and B-type crystallinity similar to potato starch, whereas cereal starch generally shows A-type crystallinity. The amylose/amylopectin ratio and amylopectin fine structure have a significant impact on the physical properties of starch. As with the maize *ae* mutation, biochemical and genetic analyses of the rice *ae* mutation showed that the *ae* starch has a higher gelatinization temperature and contains a larger amount of amylose and amylopectin with longer chains than wild-type starch, as a result of branching enzyme IIb deficiency (*13*−*15*). To eliminate the effects of amylose on the physical and structual properties of starch granules in the *ae* mutant, we tested starch from the amylose-free *ae* mutant line *wx ae*, which was produced by crossing the *ae* and *waxy* (*wx*) mutants (*15*, *16*). This starch needs a higher urea concentration to gelatinize because of the enriched long chains in its amylopectin fine structure. These observations suggest that the *ae* and *wx ae* mutants might be useful in understanding not only the function of branching enzymes in starch biosynthesis but also the properties of starch as a novel material for food and industrial applications.

Resistant starch is defined as the part of starch that cannot be digested in the small intestine (*17*) and is classified into four types: (I) tissue entrapped starch, (II) native raw starch granules having the B-type polymorphism and resistant to enzyme hydrolysis, (III) retrograded amylose, and (IV) chemically modified starch. Maize high-amylose starch is classified as type-II-resistant starch, and rice *ae* starch is likely to be classified similarly. The glycemic index (GI), which characterizes the carbohydrate in foods, is ranked on the basis of the postprandial increase in blood glucose (*18*). By virtue of the slow digestion and absorption of their carbohydrate, low GI foods produce a more gradual rise in

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blood glucose and insulin levels and are increasingly associated with health benefits. The health benefits of resistant starch include hypoglycemic and hypocholesterolemic effects, prevention of colon cancer, and inhibition of fat accumulation (*19*). Thus, it would be important to develop rice with resistant starch. The amylopectin structure has been shown to correlate with properties of slowly digestible starch (*20*), so that rice *wx ae* starch is a good material to provide a fundamental basis for amylopectin structures modified by mutation breeding.

The objective of this study is to describe the structure, physical, and digestive properties of starch from *wx ae* double-mutant rice and compare them to those of wild-type, *wx*, and *ae* lines. In the present study, we focus on the fine structure of amylopectin and its relationship to the gelatinization, retrogradation, and the digestibility of rice starches *in vitro* and *in vivo*. To avoid the influence of amylose on physical and digestive properties of *ae* starch granules, starches from the amylose-free *ae* mutant line *wx ae* were used because they contain virtually 100% *ae* amylopectin.

MATERIALS AND METHODS

Materials. *wx* mutant line EM21 and *ae* mutant line EM16, genetically defective in granule-bound starch synthase I and starch-branching enzyme IIb, respectively, were generated by treating fertilized egg cells of wild-type *japonica* rice (*Oryza sativa*) cv Kinmaze with *N*-methyl-*N*-nitrosourea (*21*). We also used an amylose-free *ae* mutant line AMF18 (*wx ae*), which is a double-recessive mutant line for *ae* and *wx*. It was derived from a cross between EM21 and EM16. The parental cv Kinmaze (wild type) and *wx* were used for comparison. Mutant and wild-type rice plants were grown in the summer of 2007 in an experimental field at Osaka Prefecture University.

Starch Granule Isolation. A total of 100 g of 10% polished kernels was soaked in 0.05% (w/w) sodium hydroxide for 16 h. Kernels were washed with distilled water several times until the supernatant showed neutral pH. Wet starch was homogenized using a mortar and pestle. Slurry was filtered, and starch granules passing through a 0.3 mm mesh sieve were sedimented by centrifugation at 500*g* for 10 min. The isolated starch was washed with 70% (v/v) ethanol and then dried on filter paper. Dried starch was homogenized by mortar and pestle and filtered through a 0.3 mm mesh sieve before use.

Gel Permeation Chromatography (GPC). Three rice kernels were dehulled and ground in a mortar and pestle, and 10 mg of powder was suspended in 0.05 mL of 1 mol/L sodium hydroxide. After incubation for 30 min at room temperature, 0.95 mL of distilled water was added to the sample suspension, which was then centrifuged at 500*g* for 5 min. A 0.5 mL aliquot of supernatant was added to a Sepharose CL-2B (GE Healthcare, Buckinghamshire, U.K.) column (diameter of 1.5 cm and length of 45 cm) that had been equilibrated with the 50 mmol/L sodium hydroxide and 0.02% sodium azide solution at a flow rate of about 10 mL h^{-1} at room temperature. Fractions were taken at 60 drop intervals. The total carbohydrate content was measured using the phenolic sulfuric method of Dubois et al. (*22*). To determine λ_{max}, the absorbance of the starch fraction−iodine complex was obtained in the 500−700 nm range.

Chain-Length Distribution of Amylopectin. A sample preparation for determining chain-length distribution was obtained according to the methods of Fujita et al. (*23*). Briefly, boiled starch was debranched by treatment with *Pseudomonas* isoamylase, and reducing ends of linear polyglucans were fluorescently labeled with 8-amino-1,3,6-pyrenetrisulfonic acid. The samples were separated by capillary electrophoresis with a P/ACE MDQ carbohydrate system (Beckman Coulters, Brea, CA). The frequency of individual chain lengths in each population was normalized to the total peak area between degrees of polymerization (DPs) 3 and 70.

X-ray Diffraction Patterns. X-ray diffraction patterns were taken with an X-ray diffractometer (Rigakudenki RINT Ultima+) using the following conditions: X-ray (Cu Kα) wavelength, 0.15 nm; scanning step, 0.020°; time constant, 1 s.

Scanning Electron Microscope (SEM) Observation. Purified starch granules were sputter-coated with gold and examined with a JSM-5600LV SEM (JEOL, Japan) at 10 kV. Grains cut in a longitudinal plane with a cryostat Microm HM500-OM (Carl Zeiss, Oberkochen, Germany) were also examined.

Size Distribution of Starch Granules. Granule-size distribution in samples of endosperm starches from each genotype was determined in duplicate using a laser-scattering particle-size distribution analyzer LA-950 V2 (Horiba, Ltd., Kyoto, Japan). Approximately 10 mg of starch was transferred into distilled water in the dispersion tank of the instrument with circulation via sonication for 1 min. The granule size was taken to be the number-average diameter automatically calculated by the instrument software.

Pasting Properties of Starch. A total of 3 g of purified starch filtered through a 0.1 mm mesh were mixed with 30 mL of distilled water, and the viscosity development of the starch was measured using a rapid-visco analyzer (RVA) (RVA-4, Newport Scientific, Warriewood, Australia) by the method of Toyoshima et al. (*24*): the sample was installed on the rotor of the RVA, kept at 50 °C for 1 min, and then heated to 95 °C in 4 min. It was held at 95 °C for 7 min, cooled from 95 to 50 °C in 4 min, and kept at 50 °C for 3 min. The rotating speed of the paddle was 160 rpm.

Thermal Properties of Starch. Differential scanning calorimetry (DSC) analysis was performed using a Micro DSC VII (Setaram Instrumentation, Caluire, France) according to methods reported previously (*25*). A total of 40 mg of starch and 0.16 mL of distilled water were placed in a sample pan and stored at 20 °C for 30 min. Samples were heated to 105 °C and cooled to 5 °C at a scan rate of 0.5 K/min. For retrogradation analysis, gelatinized starch was rescanned after storing at 20 °C for 3 days, or at 5 °C for 5 s, 1 h, 6 h, and 3 days.

***In Vitro* Digestion with Porcine Pancreatic α-Amylase.** Digestibility was analyzed using a modification of the method of Ao et al. (*26*). Starch (10 mg) with 1 mL of sodium glycerophosphate-HCl buffer (1 mmol/L, pH 6.9) containing 25 mmol/L sodium chloride and 5 mmol/L calcium chloride was heated in a boiling water bath for 30 min. The solution was equilibrated at 37 °C for 5 min, and 5 units of porcine pancreatic α-amylase was added. Enzyme digestion was carried out at 37 °C, and 0.01 mL aliquots of hydrolyzed solution were withdrawn at different time intervals. The aliquots were immediately heated at 100 °C for 10 min to deactivate the enzyme. The equivalent reducing sugar value of maltose was determined using the Somogyi−Nelson method (*27*). Each sample was analyzed in duplicate.

Postprandial Blood Glucose Response in Rats. A 7% (w/w) starch sample was suspended in water. At 7 weeks of age, male Wistar rats ($n = 6$; body weight = 250−280 g) were orally administered starch slurry in dosages of 0.5 or 1 g/kg of body weight, and blood samples were taken from the tip of the tail after 0, 30, 60, 90, and 120 min. Blood glucose levels were determined by the glucose oxidase method using a Glucose Pilot meter (Aventir Biotech, LLC, Carlsbad, CA). Statistical analyses were carried out with the Statmate 3.18 software program (Brainpower, Calabasas, CA). Results are given as means ± standard error of the mean (SEM). Differences were considered significant when $p < 0.01$. The positive incremental blood glucose area under the curve (AUC), ignoring any areas below the baseline, for the blood glucose values from 0 to 120 min after gavage was calculated according to the method of Wolever et al. (*28*). The relative glycemic responses (RGRs) of mutant starches to wild-type starch were calculated according to the method of Wolf et al. (*29*): [(glucose AUC for mutant starch)/(glucose AUC for wild-type starch)] × 100.

RESULTS AND DISCUSSION

Effect of *ae* and *wx* Mutations on the α-Polyglucan Structure. In a previous report by Yano et al. (*13*), the higher amylose content of *ae* starch (EM16, 30.8%) compared to wild type (Kinmaze, 17.4%) was calorimetrically determined on the basis of the blue value of the starch−iodine complex. This value included the affinities of both amylose and amylopectin for iodine. In this study, we investigate compositional and structural changes in the endosperm starch that resulted from *ae* and *wx* mutations. To assess the relative amounts of component glucans, starches purified from wild-type and mutant grains were separated according to molecular mass by GPC on a Sepharose CL-2B column. As shown in **Figure 1**, a broad peak around fraction numbers 15−30 for wild type and *ae* is due to the presence of amylose. The major peak of amylopectin eluted from the column

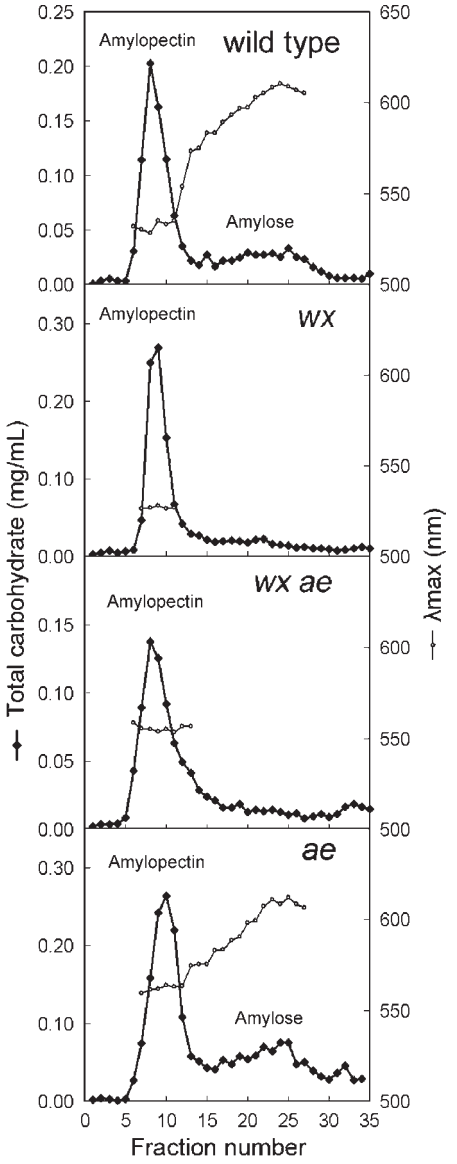

Figure 1. Size fractionation of glucan polymers from wild-type starch and *wx*, *wx ae*, and *ae* mutant starches. Starches were separated by GPC on a Sepharose CL-2B column, and the total carbohydrates (◆) in each column fraction and λ_{max} (○) of the polysaccharide−iodine complex were determined.

in the 6−11 range of fractions was detected in four starches, but the λ_{max} values of *ae* and *wx ae* amylopectin were around 560 nm, which is about 30 nm higher than those of the wild type or *wx*. In addition, **Figure 1** shows that the total carbohydrate content of *wx ae* is lower than that of the others. This could be partly attributed to the lower carbohydrate content of the *wx ae* kernel. The total carbohydrate content of *wx ae* kernels was determined to be 58.9%, which is about 10% lower than the others.

To confirm whether the higher λ_{max} values were due to the change in the fine structure of amylopectin, the frequency distribution of the linear glucan chain in starch from wild type and mutants was determined for each DP by fluorophore-assisted capillary electrophoresis. The amylopectins from *ae* and *wx ae* were identical or very similar to each other and were markedly depleted in chains $6 \leq DP \leq 14$ and enriched in chains of $15 \leq DP$ compared to those from the wild type (**Figure 2**).

On the basis of these observations, we confirmed that the rice *ae* mutation increased amylose content and also produced fewer short amylopectin chains as a result of the defective branching

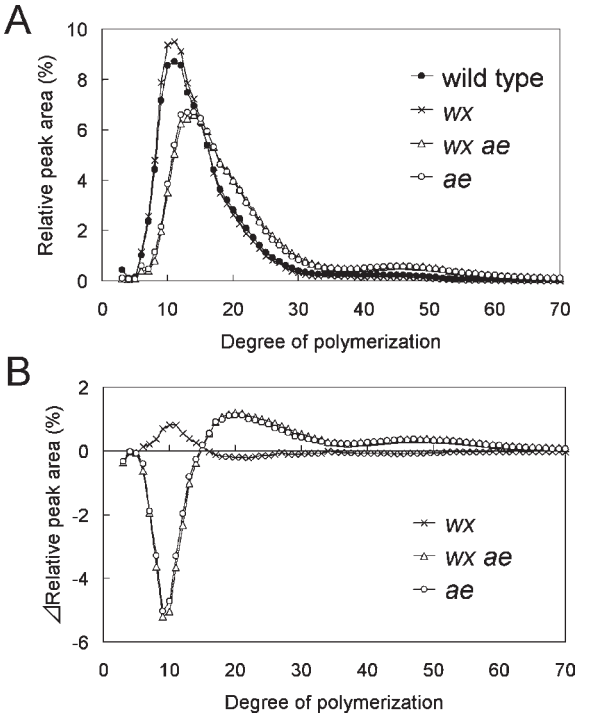

Figure 2. Chain-length distribution of polyglucans of wild-type, *wx*, *wx ae*, and *ae* starches. (A) Comparison of chain-length distribution of total polyglucans in wild type and mutants. (B) Differences in chain-length distribution of total polyglucans in mutant starches relative to wild-type starch.

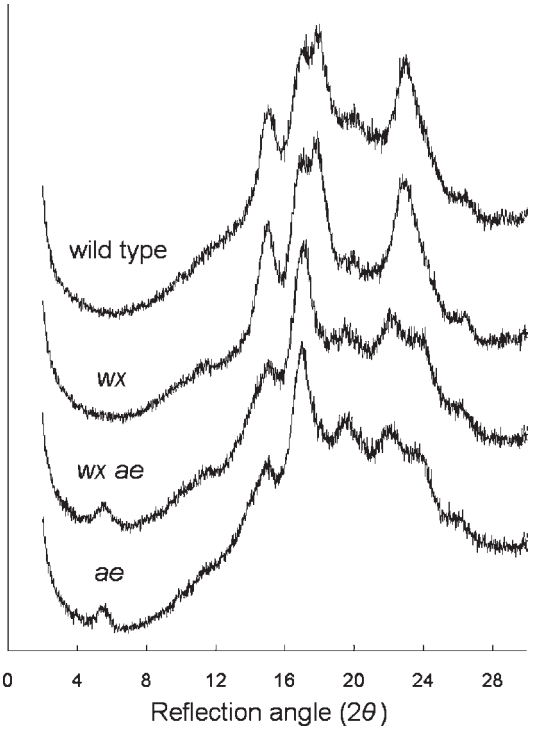

Figure 3. X-ray diffraction patterns of rice starches of wild type and mutants.

enzyme IIb function, as previously reported by Nishi et al. (*15*). The *wx* mutation was predominant in determining amylose content but did not change the fine structure of amylopectin, which was modified by the *ae* mutation.

X-ray Diffraction Pattern. Figure 3 shows X-ray diffraction patterns of the four samples of starches. The wild type and *wx*

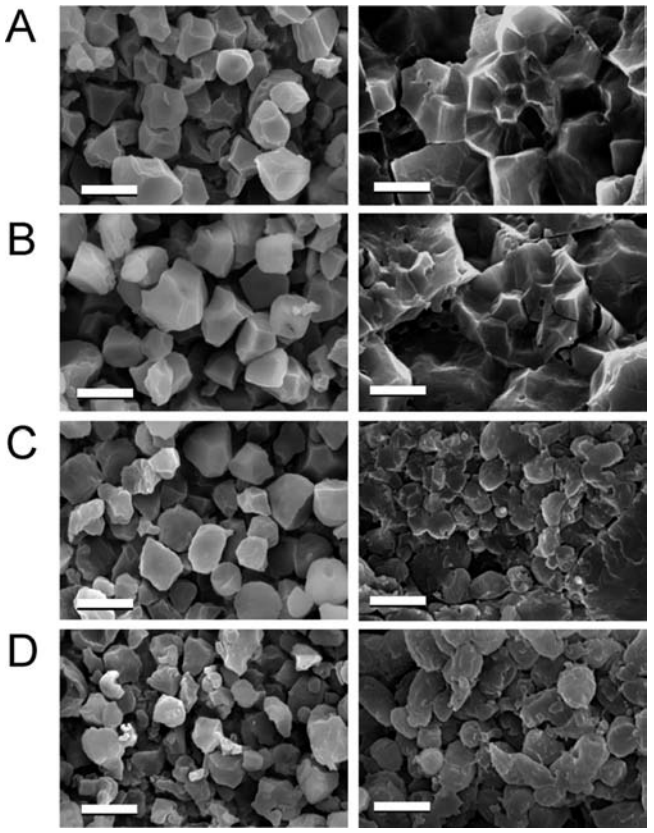

Figure 4. Morphology of starch granules from (A) wild type, (B) *wx*, (C) *wx ae*, and (D) *ae* as observed by scanning electron microscopy. (Left panels) Purified starch granules. (Right panels) Starch granules in the endosperm. The scale bars are 5 μm.

starches had four strong reflections at 2θ values of 15°, 17°, 18°, and 23°, which are typical of the A-polymorphic form. In comparison, the *ae* and *wx ae* starches showed peaks of reduced intensity at 15°, 18°, and 23°, and additional peaks appeared at 5°, 22°, and 24°. These characteristics indicate that the *ae* and *wx ae* starches are typical of the B-polymorphic form. The B pattern was similar to that of the original rice *ae* mutant starch (*13*), and *ae* and *wx ae* showed an almost identical B pattern, except for the relatively high intensity of the twin peaks at 22° and 24° of *wx ae*.

Starch Granule Morphology. Scanning electron microscopy was used to reveal whether there were significant changes in granule morphology in the absence of amylose or with the modification of the amylopectin structure. The starch granules from the wild type and *wx* were very similar in size and morphology (**Figure 4**). The *ae* starch consisted of spheroidal granules with some space in the tissue compared to polygonal granules from the wild type and *wx* starches. The shape of the *wx ae* granules was similar to that of the *ae* and many small granules were also observed. The wild-type and *wx* starch granules had an average mean diameter of 5.37 ± 1.17 and 4.68 ± 1.22 μm, while those of *wx ae* were smaller, with values of 3.96 ± 1.22 μm. These measurements were made using a laser-diffraction particle-size analyzer.

Pasting Property. Figure 5 shows RVA viscograms obtained for the wild-type, *wx*, *wx ae*, and *ae* starches. The higher gelatinization temperature of the *wx ae* and *ae* starches could be attributed to the amylopectin structure, with relatively long unit chains. The peak viscosity of wild type and mutant starches, on the other hand, correlated not with the ratio of short amylopectin chains but with the amylose content, as shown by the high peak viscosities of the *wx* and *wx ae* starches and the low

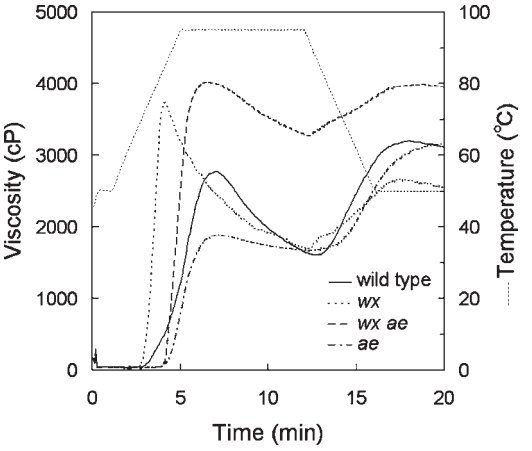

Figure 5. Pasting curves of rice starches (10%) of wild type and mutants measured by RVA.

peak viscosity of the *ae* starch. The negative correlation of proportion of long chains in amylopectin and paste breakdown, such as the small paste breakdown of the *ae* and *wx ae* starch, was similar to that reported by Han and Hamaker (*30*). The higher paste setback shown by the wild-type and *ae* starches correlated with the amylose content and was consistent with a previous report (*31*), but the viscosity did not. *ae* and *wx ae* have significantly different RVA profiles, indicating a difference in swelling and pasting behavior as a function of the temperature for *ae* and *wx ae* starch granules. The major factor affecting these behaviors is not clear at this stage, but the key is to know how the amylose fraction is organized in starch granules and behaves with changing temperature. This is a subject for further investigation.

Gelatinization and Retrogradation Properties. Thermal properties of the wild-type and mutant starches are summarized in **Table 1**. The gelatinization peak temperatures of the *ae* (78.9 °C) and *wx ae* (82.9 °C) starches were markedly higher than those of wild type (65.8 °C) and *wx* (68.0 °C) because of their many long-branch chains of *ae* amylopectin. The gelatinization enthalpy of the *ae* and *wx ae* starches was greater than that of wild type and *wx*. This result was in agreement with a previous report (*32*), showing that the gelatinization peak temperature and gelatinization enthalpy are indicators of molecular (double-helical) order. The relatively higher gelatinization peak temperature of the *wx* and *wx ae* starches compared to wild type and *ae*, respectively, may be affected by the amylose defection. However, the change in amylopectin fine structure affected the gelatinization endotherm property more than the amylose defection. The gelatinization ranges of the rice *ae* and *wx ae* starches were narrow, whereas the commercial high-amylose maize starch showed a broad peak. Similar broad gelatinization peaks have been observed in previous studies using maize *ae* mutants (*6*, *8*). Although we only focus on the endothermic peaks for gelatinization in this paper, the melting of amylose lipids for these samples is also of interest and is a topic for further research.

Retrogradation of these four starches after heating in water to 105 °C and storing under various conditions was analyzed by DSC (**Table 1**). The retrograded gels of *ae* and *wx ae* starches gave higher peak temperatures in the same order as the order of gelatinization temperatures of the four native starches: wild type < *wx* < *ae* < *wx ae*. This result was similar to the data on retrogradation properties of maize *wx* and *wx ae* starches (*7*). Apparently, from the ΔH values, the rate of nucleation at 5 °C was slow for *wx* starch compared to wild-type starch containing amylose. However, the starches of *ae* and *wx ae* showed a remarkably larger retrogradation extent (ΔH) than wild-type

Table 1. Gelatinization and Retrogradation Properties of Wild-Type and Mutant Starches[a]

	gelatinization endotherm					retrogradation endotherm				
sample	n^b	T_o (°C)	T_p (°C)	T_c (°C)	ΔH (J/g)	storing condition	T_o (°C)	T_p (°C)	T_c (°C)	ΔH (J/g)
wild type	9	60.0 ± 0.1	65.8 ± 0.3	71.2 ± 0.1	9.6 ± 0.4	5 °C, 6 h	48.2	55.7	66.9	1.8
						5 °C, 3 days	42.9	57.2	68.9	1.9
						20 °C, 3 days	47.4	58.1	68.8	1.2
wx	9	60.8 ± 0.1	68.0 ± 0.1	74.2 ± 0.3	11.0 ± 0.6	5 °C, 6 h	45.0	55.5	61.2	1.2
						5 °C, 3 days	45.5	58.7	69.9	1.8
						20 °C, 3 days	48.4	61.0	67.2	1.0
ae	7	73.1 ± 0.0	78.9 ± 0.1	83.9 ± 0.2	12.3 ± 0.1	5 °C, 5 s	44.4	61.8	72.4	3.9
						5 °C, 1 h	47.0	62.6	72.8	4.8
						5 °C, 6 h	42.3	62.8	75.2	9.1
						5 °C, 3 days	43.1	60.8	73.2	9.9
						20 °C, 3 days	39.7	61.7	73.5	7.2
wx ae	11	78.1 ± 0.1	82.9 ± 0.1	86.4 ± 0.1	14.5 ± 0.9	5 °C, 5 s	48.7	67.8	74.6	3.5
						5 °C, 1 h	47.0	66.6	76.5	7.4
						5 °C, 6 h	44.9	64.8	75.2	8.2
						5 °C, 3 days	45.4	64.7	75.4	9.1
						20 °C, 3 days	44.3	69.1	76.9	7.4

[a] Samples (40.0 mg) and deionized water (160.0 mg) were used for the analysis. T_o, T_p, T_c, and ΔH are onset, peak, and conclusion temperatures and enthalpy change, respectively. [b] Number of measurements. The parameters T_o, T_p, T_c, and ΔH are given as the average value ± standard deviation.

and *wx* starches even stored at 20 °C, and *wx ae* starch retrograded most quickly. In general, amylose is responsible for instantaneous retrogradation, and amylopectin is responsible for slow retrogradation for normal starches. However, if the average amylopectin unit chain is longer in *ae* and *wx ae* starches, very fast retrogradation could occur because there is a high local concentration of longer unit chains participating in double-helix formation. The structural homogeneity of *wx ae* starch also speeds retrogradation.

***In Vitro* Digestion of Starches with Porcine Pancreatic α-Amylase.** The digestion profiles of raw starches using porcine pancreatic α-amylase are shown in **Figure 6A**. The digestion rates of *wx ae* and *ae* were markedly slower than those of wild-type and *wx* starches. In contrast to the rate of digestion of wild-type raw starch, the rates for *wx ae* and *ae* incubated for 8 h fell by 40 and 22%, respectively. Besides the increased amount of amylose in the *ae* starch, few-branched *ae* amylopectin is most likely to restrict hydrolysis of raw starch. When starches were gelatinized by boiling, all samples were mostly digested in 30 min by α-amylase (**Figure 6B**). However, the starch gel of not only *ae* but also *wx ae* was digested relatively slowly after cooling at 5 °C for 2 days (**Figure 6C**), indicating that hydrolysis of retrograded *ae* and *wx ae* starch was inhibited by the recrystallization of their *ae* amylopectin. This result suggests that *ae* amylopectin could play a functional role in starch retrogradation. Therefore, our results do not conflict with the result that amylopectin is the starch molecule associated with slowly digested starch (*20*).

Postprandial Blood Glucose Response to Ingestion of Starches in Rats. Figure 7 shows the postprandial incremental blood glucose response of rats improved when wild-type and mutant starches were administered orally by gavage at a dosage of 0.5 g/kg of rat. Blood glucose concentrations of fasting subjects did not differ before each treatment ($p > 0.01$). The incremental blood glucose level was higher when rats were fed *wx* starch and lower when rats were fed *ae* starch ($p < 0.001$; 30 and 60 min) compared to wild-type starch, indicating that the amylose ratio influenced glucose response as reported by Granfeldt et al. (*33*) and Denardin et al. (*34*). However, the incremental blood glucose excursions were also reduced ($p < 0.001$) when rats were fed *wx ae* starch at 30, 60, and 90 min. In comparison to wild-type starch, the blood glucose at 30 min was reduced 27% when *wx ae* starch was fed. When the rats were fed *ae* or *wx ae* starch, the positive incremental blood glucose AUC (*28*) was reduced in comparison to wild-type starch and the RGR (*29*) was 35 and 25%, respectively. A higher

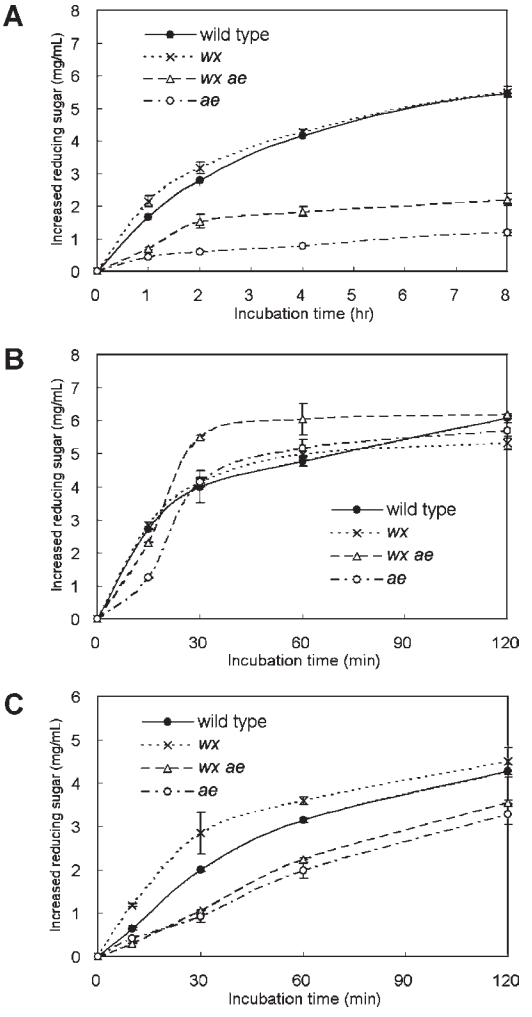

Figure 6. Digestion profiles of the wild-type and mutant starches incubated with porcine pancreatic α-amylase at different times. (A) Raw starch granules. (B) Gelatinized starches by boiling. (C) Retrograded starch gels by storing at 5 °C for 2 days.

dosage (1 mg/kg of body weight) yielded similar results. This study demonstrates that the change in amylopectin fine structure caused by inhibiting the branching enzyme IIb function by *ae*

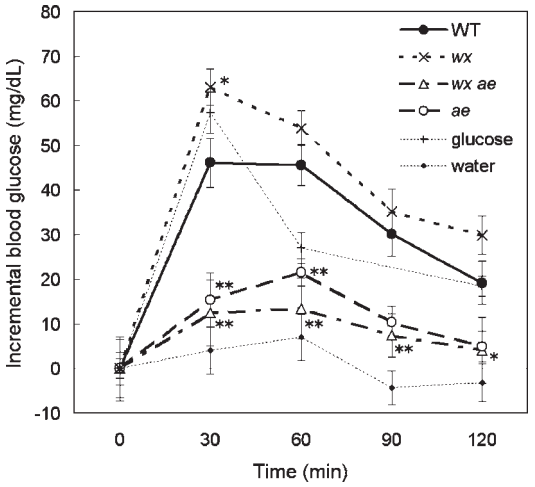

Figure 7. Postprandial blood glucose in rats after an oral gavage of starches at a dosage of 0.5 g/kg of rat. Values are means ± standard error (SE) ($n = 6$). (*) $p < 0.01$ and (**) $p < 0.001$, with wild-type starch versus mutants.

mutation dramatically decreased the digestibility of starch granules *in vivo* with or without amylose. When *in vivo* digestion behavior of *indica* rice *ae* mutant starch was examined, no significant difference between wild-type and *ae* mutant starches was observed (*35*). In this study, *japonica* rice containing relatively low amounts of amylose and short-chain enriched amylopectin, which are genetically regulated by lower expression levels of *GBSSI* (*36*) and *SSIIa* (*37*), respectively, was used, so that the difference between wild-type and mutant starches could be observed clearly.

Overall, rice *wx ae* accumulated long-chain enriched amylopectin similar to that of *ae*. The *ae* and *wx ae* starches had a higher gelatinization temperature, and the *wx ae* starch had increased peak viscosity. Raw *ae* and *wx ae* starches showed remarkable indigestibility, and retrograded starch gels of *ae* and *wx ae* were partially indigestible, whereas their boiled starches were gelatinized in a narrow range of temperatures and digested as easily as the wild-type or *wx* starch. The partially recrystallized starch of *wx ae* might be desirable when included in processed foods with reduced glycemic response.

In conclusion, *ae* and *wx ae* mutant rice starches contain similar amylopectin with relatively long unit chains, but *wx ae* starch amylopectin is more homogeneous because of the lack of amylose. This leads to the homogeneous structure of the starch granule at the molecular and higher ordered structural levels, higher gelatinization temperature, quick retrogradation, and lower digestion *in vivo*. We are now examining the effects of long-term administration of the starches used in this study on blood glucose and fat accumulation using model mice.

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