Distribution and Characterization of Enzymes Causing Starch Degradation in Rice (*Oryza sativa* Cv. Koshihikari)

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The thermal dependency and stability of enzymes producing reducing sugar (RS) were examined in bran, the exterior 13% part (outer endosperm), and the remaining inner endosperm of rice grains. RS-producing enzymes in the inner endosperm showed a higher optimum temperature than those in other parts of the rice grain. Diethylaminoethyl-Sephacel chromatography of crude extracts revealed two peaks of RS-producing activity with different optimum temperatures (60 and 37 °C) in all three parts. α-Glucosidase (EC 3.2.1.20) and α-amylase (EC 3.2.1.1) isoform G were thought to be major components of the RS-producing activities with high and low optimum temperatures, respectively. The peak with a high optimum temperature was a more abundant component in the inner endosperm, compared with other parts of the rice grain. Thus, different parts of rice were found to have distinct enzyme sets having different thermal dependency and to be involved in starch degradation to various sugars.

Keywords: *Oryza sativa; reducing sugar; α-amylase; α-glucosidase*

INTRODUCTION

Rice (*Oryza sativa*) has been the major food and crop in most eastern and southeastern Asian countries, including Japan, since the beginning of agriculture. Attempts to assess the preference for cooked rice have been made, and recently taste analyzers, which are based on correlations between near-infrared reflectance (NIR) measurements of key constituents (e.g., amylose, protein, moisture, fat acidity) and preference sensory scores, have been developed (Champagne et al., 1996). In addition to texture, the taste of rice, especially its slight sweetness, is thought to be an important factor in consumers' preference, and the sweetness of cooked rice depends on the concentrations of reducing sugars (RS).

RS is mainly made from starch occupying 75% of the dry weight of rice grain. Starch is degraded by the combined actions of enzymes, such as α-amylase, *β*-amylase, and α-glucosidase, during cooking and processing, as well as germination (Dunn, 1974; Sun and Henson, 1991; Guglielminetti et al., 1995). α-Amylase has a major role in degrading native starch granules (Dunn, 1974; Sun and Henson, 1991). Several isoforms of α-amylases exist in rice. They are expressed depending on the tissue types and developmental stages (Karrer et al., 1991; Mitsui et al., 1996; Yu et al., 1996; Yamaguchi, 1998). Several heat unstable isoforms of *β*-amylase also exist in rice seeds, some of which are an inactivated form in ungerminated rice grain (Matsui et al., 1975, 1977; Shinke et al., 1973). Although amylase activities are highly induced during germination (Tanaka et al., 1970; Choi et al., 1996; Panabieres et al., 1989), the activity begins during seed maturation (Baun et al., 1970; Chrastil, 1993) and continues in dry seed to affect the quality of storage rice (Shin et al., 1985; Chrastil, 1990; Nandi et al., 1995).

Two isoforms of α-glucosidase have been isolated and purified from ungerminated rice seeds. These two isoforms have different actions on maltose and starch (Takahashi et al., 1971; Takahashi and Shimomura, 1973). α-Glucosidases have an important role in degrading native starch granules in some plants (Konishi et al., 1994; Sun et al., 1995; Sun and Henson, 1990).

Almost all studies have been concerned with isolated and/or purified enzymes in germinated cereal seeds. How these enzyme activities are coordinated as a whole to effectively degrade starch in rice grains during cooking is not clear.

Enzyme activities change during the soaking in parboiling, and they contribute to the increase in RS contents that may contribute to the sweetness of parboiled rice (Xavier and Raj, 1996). Therefore, the activities of these enzymes are thought to affect the quality of boiled rice.

Several components, such as amylose, starch, protein, and minerals, differ among sites in rice grains (Tanaka et al., 1974; Kennedy et al., 1974, 1975; Kennedy and Schelstraete, 1974, 1975). Moreover, the α- and *β*-amylase distributions in rice endosperm are mainly in the

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outer 20% of the endosperm (Barber, 1972). These components and enzymes are thought to be responsible for the taste of cooked rice.

We focused on RS production, and we called the enzymes catalyzing starch degradation to RS "RS-producing enzymes". Here, we examined the thermal dependency of RS-producing enzymes and α-glucosidase in three different parts of rice grains to determine their roles in starch degradation, which may be responsible for the tastiness of rice.

MATERIALS AND METHODS

Materials. Rice (*O. sativa* cv. Koshihikari) produced in Uonuma, Niigata Prefecture, in 1992, was purchased just after harvest and was stored at 4 °C as brown rice until use. The brown rice was polished to 90% using a National KG-1000 rice polisher (Matsushita Electric Industrial Co., Ltd., Osaka, Japan) and was separated into polished rice and bran (including the aleurone layer with a small amount of polish) immediately before use.

Thirteen percent (w/w) of the external part of the polished rice grain was scraped off using a Grain testing mill (Satake Engineering Co., Ltd., Tokyo, Japan). This material was used as the "outer endosperm". The remaining endosperm of the grains was milled using a Cyclone sample mill (Udy Corp., Fort Collins, CO) and was used as the "inner endosperm". Each material was filtered through a 3 mm mesh.

Extraction of Enzymes. RS-producing enzymes, including α-glucosidase, were extracted from each material (9 g for the inner endosperm, 6 g for the outer endosperm, or 4 g for bran) using an extraction buffer (0.05 M acetate buffer, pH 6.0) by shaking for 10 h at 4 °C. The suspension was centrifuged at 18800*g* at 0 °C for 30 min. The supernatant was dialyzed with the extraction buffer at 4 °C for 24 h to remove low molecular weight components such as sugars and was used in the assays of thermal dependency and stability of the enzyme activities, as well as high-performance liquid chromatography (HPLC) analysis of the degradation products.

Analysis of the Thermal Dependency and Stability of the Enzymes. The thermal dependency and stability of the RS-producing enzymes were examined in the inner and outer endosperms and bran of rice. α-Glucosidase activities were assayed using extracts from the inner and outer endosperms.

To assay the thermal dependency of RS-producing enzymes, 500 μL of reaction mixtures [100 μL of 10% boiled soluble potato starch (Wako Pure Chemical Industries, Ltd., Osaka, Japan), 170 μL of 0.2 M acetate buffer at pH 6.0, and a suitable amount of the crude extract were made up to 500 μL with distilled water] were incubated in glass tubes for 60 min in a water bath at 20, 30, 40, 50, 60, 70, and 80 °C. After 60 min of incubation, 0.5 mL of Somogyi reagent was added to stop the reaction, followed by incubation in a boiling water bath for 20 min. The amounts of RS produced in the mixture were assayed using the Somogyi−Nelson method (Nelson, 1944; Somogyi, 1945, 1952).

To assay the thermal dependency of α-glucosidase, 100 μL of reaction mixtures (20 μL of 10% maltose, 20 μL of 0.2 M acetate buffer at pH 5.0, 50 μL of crude extract, and 10 μL of distilled water) were incubated in glass tubes in a water bath at the same temperatures as for the assay for the RS-producing enzymes for 30 min. To stop the reaction, the labeling reagent used in the mutarotase−GOD method (Glucose CII. test WAKO, Wako Pure Chemical Industries, Ltd.) was added immediately after 30 min of incubation. The amounts of glucose in the mixture were assayed using the mutarotase−GOD method according to the manufacturer's instruction.

To calculate the enzyme activities, the contents of RS or glucose were measured after reaction without enzyme extract. These values, which represent RS or glucose contents in enzyme extracts, were subtracted from the values obtained with the assays described above.

Standard curves were drawn using maltose and glucose solutions (from 0 to 100 μg/100 and 500 μL, respectively) for the assays of RS and glucose, respectively. The activities of the RS-producing enzymes and α-glucosidase were described as milligrams of RS and glucose produced per microgram of protein per 60 and 30 min, respectively.

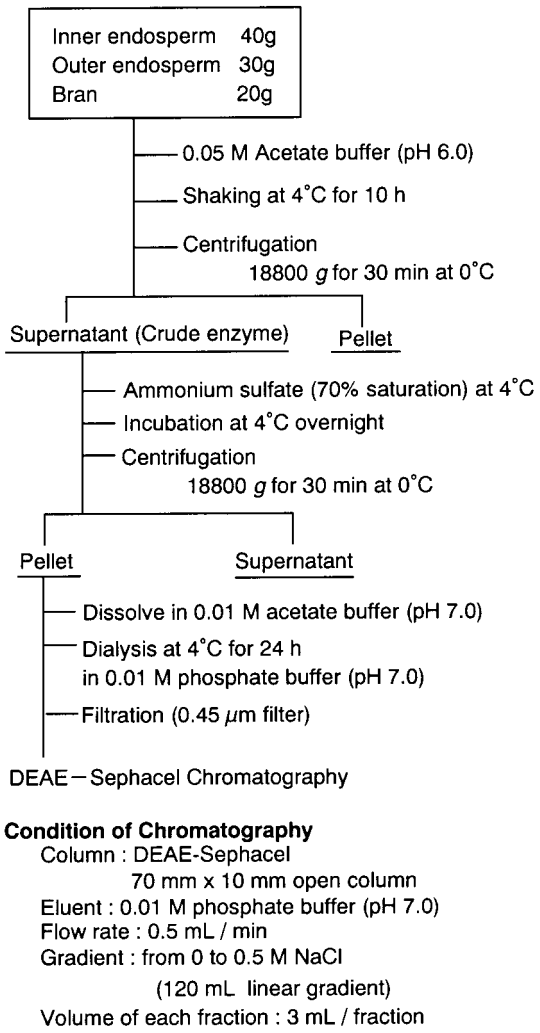

Figure 1. Methods of sample preparation and ion exchange chromatography using DEAE-Sephacel.

For the thermal stability test, 1 mL of crude extracts was treated in a water bath at 30, 40, 50, 60, 70, 80, and 100 °C for 20 min, and then the activities of the RS-producing enzymes and α-glucosidase were measured according to the two methods described above. The reactions were made at 37 °C.

Analysis by HPLC of the Degradation Products of the Starch. The reaction mixtures (275 μL in total) containing 50 μL of 5% boiled soluble potato starch (Wako Pure Chemical Industries, Ltd.), 75 μL of 0.2 M acetate buffer at pH 6.0, and 150 μL of crude extracts were incubated in glass tubes at 20, 37, and 60 °C. To produce enough sugars to be detected by HPLC, the reaction time was extended to 6 h. To stop the reaction, 150 μL of 10% trichloroacetic acid (final concentration = 4.6%) was added and the mixture was boiled for 1 min. The excess starch that was not used for the reaction was removed by centrifugation (18800*g*, 5 °C, and 5 min). These samples were then filtered through 0.45 μm nylon filters (Nihon Millipore Kogyo K.K., Tokyo, Japan), and the sugars were analyzed using an HPLC system (Hitachi, Ltd., Tokyo, Japan) with a GL-C611 column (300 mm × 10.7 mm) fused by a GL-C614 column (300 mm × 10.7 mm) (Hitachi Instruments Service Co., Ltd., Tokyo, Japan) at 60 °C equipped with deionized water (HPLC grade). Deionized water was used as the eluent, and the flow rate was 1.0 mL/min. Sugars were detected using a Hitachi 655A-30-type refraction index monitor (Hitachi, Ltd.)

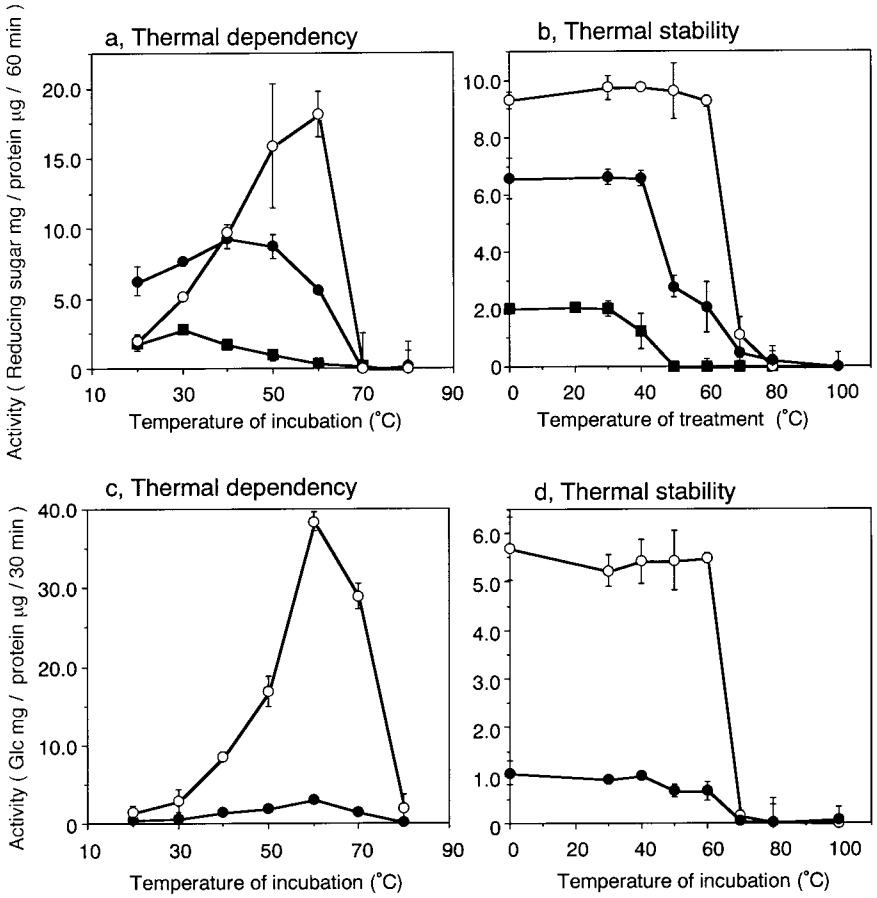

Figure 2. Thermal dependency and stability of RS-producing enzymes activity (a, b) and α-glucosidase activity (c, d) of the extracts. Crude enzymes were extracted three times with 0.05 M acetate buffer (pH 6.0) from the inner endosperm (○), the outer endosperm (●), and bran (■). The activity was assayed in triplicate for each extract. Averages and SD are shown ($n = 3$).

Protein Assay. The total protein was assayed using a Bio-Rad protein assay kit (Bio-Rad Co., Richmond, CA) with bovine serum γ-globulin solution (from 0 to 20.1 μg/200 μL) as a standard.

Anion Exchange Chromatography Using Diethylaminoethyl (DEAE)-Sephacel. The crude extracts in each endosperm of rice were fractionated by anion exchange chromatography (Figure 1).

The activities of RS-producing enzymes and α-glucosidase in each fraction were assayed at 20, 37, and 60 °C to determine the thermal dependency. The total protein contents were also assayed. The activities of the RS-producing enzymes and α-glucosidase were expressed as milligrams of RS and glucose produced per 50 μL fractions per 60 and 30 min, respectively.

Zymography. Crude enzymes extracted from the inner and outer endosperms were subjected to isoelectric focusing, and the gel was stained by iodine−starch reaction to detect starch degradation activities according to the methods of Perata et al. (1992).

To standardize rice α- and β-amylases, surface-sterilized embryoless half-seeds of rice (*O. sativa* cv. Nipponbare) were incubated in 0.5 mL of culture medium (10 mM sodium acetate, pH 5.3, containing 2 mM $CaCl_2$ and 1 μM GA_3) and were germinated at 30 °C in darkness. After a 4-day incubation, the embryoless half-seeds were homogenized with the culture medium with an additional 0.5 mL of homogenizing buffer (100 mM Hepes-HCl, pH 7.6, containing 10 mM $CaCl_2$ and 0.1% Triton X-100), and then the homogenate was centrifuged. The supernatant was used to determine the standard activity (Yamaguchi, 1998).

RESULTS

Thermal Dependency and Stability of the RS-Producing Enzymes and α-Glucosidase. Thermal dependency of RS-producing enzymes varied with the part of the grain (Figure 2a). The optimum temperature was higher in the inner endosperm, reaching a maximum at 60 °C. The activities at 40 and 20 °C were half and one-eighth of the maximum activity, respectively. However, in the outer endosperm, the activity was highest at ∼40 °C, and the activities at 20 and 60 °C were one-third and half of the activity at 40 °C, respectively. In bran, the optimum temperature was 30 °C. In every part of the grain, the RS-producing activity was not detected above 70 °C. The thermal stability of RS-producing enzymes also varied with the part of the rice grain. In the inner endosperm, the enzymes were stable up to 60 °C, whereas in the outer endosperm and bran, they were stable up to 40 and 30 °C, respectively (Figure 2b).

The activity of α-glucosidase in the inner endosperm was much higher than in the outer endosperm, but the optimum temperatures were the same, 60 °C (Figure 2c). The thermal stabilities of the enzymes in each portion of endosperm were also similar. The enzymes showed stable activity up to 60 °C and became inactive above 70 °C (Figure 2d).

Composition of the Degradation Products Using HPLC Analysis. The degradation products of starch produced by the enzymes from different parts of the rice grain at several temperatures were determined using HPLC analysis (Figure 3). The patterns of degradation products varied with the part of the rice grains.

In the inner endosperm, the major product was glucose. The amount of glucose increased as the reaction

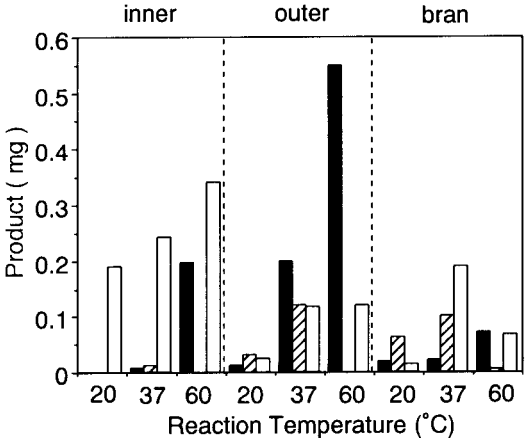

Figure 3. Reaction products of crude extracts obtained from the inner endosperm, the outer endosperm, and bran analyzed by HPLC: (black bar) maltotriose and maltotetraose; (slashed bar) maltose; (open bar) glucose. Enzymes were extracted once, and the reactions were made three times. The averages of two measurements are shown.

temperature increased. At 60 °C, in addition to glucose, a considerable amount of malto-oligosaccharides was detected, whereas maltose was hardly detected. The results of the analysis of these compounds suggested the presence of α-glucosidase and α-amylase with a high optimum temperature in the inner endosperm. In the outer endosperm, the major products were malto-oligosaccharides, which increased by increasing the reaction temperature, and a little production of maltose was detected at 37 and 20 °C. These results indicated the presence of α-amylase and a small amount of *β*-amylase in the outer endosperm. In bran, the production of malto-oligosaccharides was detected only slightly, and maltose and glucose production was more evident, indicating the presence of α-glucosidase and *β*-amylase in bran.

Elution Patterns and Thermal Dependency of RS-Producing Enzyme Activity from Anion Exchange Chromatography in Different Parts of the Rice Grain. The proteins were eluted as a single broad peak from the column loaded with the extract from each part of the rice grain (Figure 4), but the peak position varied among the samples. In the inner endosperm, the peak was near fraction 15, but in the outer endosperm and bran, the peak was near fraction 20. Although similar amounts of proteins were applied to the column, a larger amount of proteins was eluted in the outer endosperm than in other parts of the rice grain.

Two peaks of RS-producing activity were detected. The enzymes in the first peak (fraction 10) and the second peak (fraction 20) were named RS-producing enzymes I and II, respectively. The activities of these two enzymes varied with the part of the rice grain. The activity of RS-producing enzyme I was markedly high in the inner endosperm, very low in the outer endosperm, and not detected in bran. However, the activity of RS-producing enzyme II was highest in bran. In the inner and outer endosperms, the activity of RS-producing enzyme II was less than half that in bran.

The thermal dependency (Figure 5) was also markedly different between RS-producing enzymes I and II. In all parts of the rice grain, the activity of RS-producing enzyme I was highest at 60 °C and was lower at 37 and 20 °C. However, the activity of RS-producing enzyme II was highest at 37 °C in all parts of the rice grains.

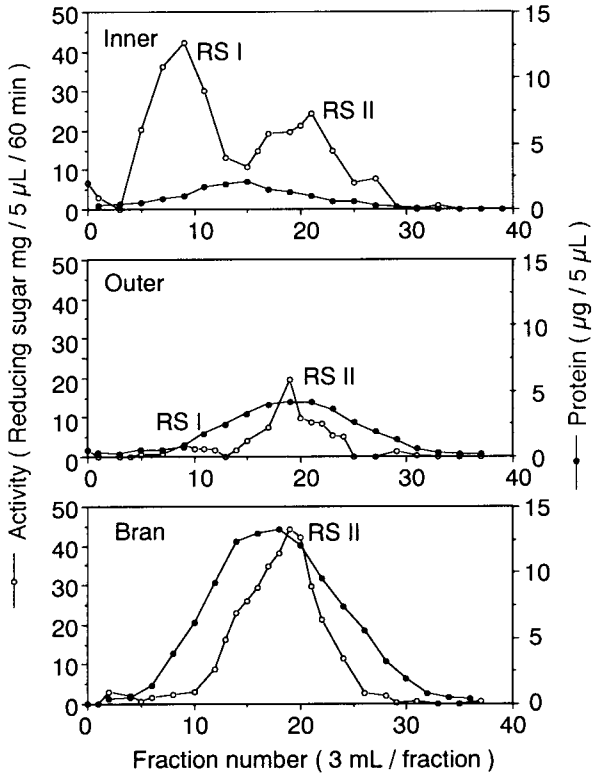

Figure 4. Elution patterns of RS-producing activity (○) and total proteins (●) assayed in each fraction that was separated by ion exchange chromatography using DEAE-Sephacel. RS I and II in the chromatograms indicate RS-producing enzymes I and II, respectively. The averages of two measurements are shown. Experiments were repeated three times. This figure shows a typical result.

However, the ratios of the activity at 20 °C to that at 37 °C varied with the part of the rice grain. In the inner endosperm, the activity at 20 °C was about one-third that at 37 °C, whereas in the outer endosperm, it was half that at 37 °C. However, in bran, the activity at 20 °C was at the same level as at 37 °C.

Elution Patterns and Thermal Dependency of α-Glucosidase Activity from Anion Exchange Chromatography in Different Parts of the Rice Grain. α-Glucosidase, α-amylase, and *β*-amylase are among the major RS-producing enzymes. As glucose was the major product of starch degradation in extracts from the inner endosperm, especially at high temperature (Figure 3), α-glucosidase was the most likely candidate for the activity at the higher optimum temperature.

Three peaks of α-glucosidase activities were found around fractions 10, 15, and 23 and were named α-glucosidase I, II, and III, respectively (Figure 6a). The position of α-glucosidase I was consistent with the position of RS-producing enzyme I. The level of this activity was highest in the inner endosperm and was only slightly detected in the outer endosperm and bran. α-Glucosidases II and III were eluted earlier and later, respectively, compared with the peak of RS-producing enzyme II. The peak of α-glucosidase II was found only in the inner endosperm extract. The activity of α-glucosidase III was highest in the bran extract, followed by that in the outer endosperm extract, and it was not detected in the inner endosperm extracts, in contrast to α-glucosidase II.

The α-glucosidase activities for each fraction at 20 and 60 °C showed that the thermal dependencies of these peaks differed in each parts of the rice grain (Figure

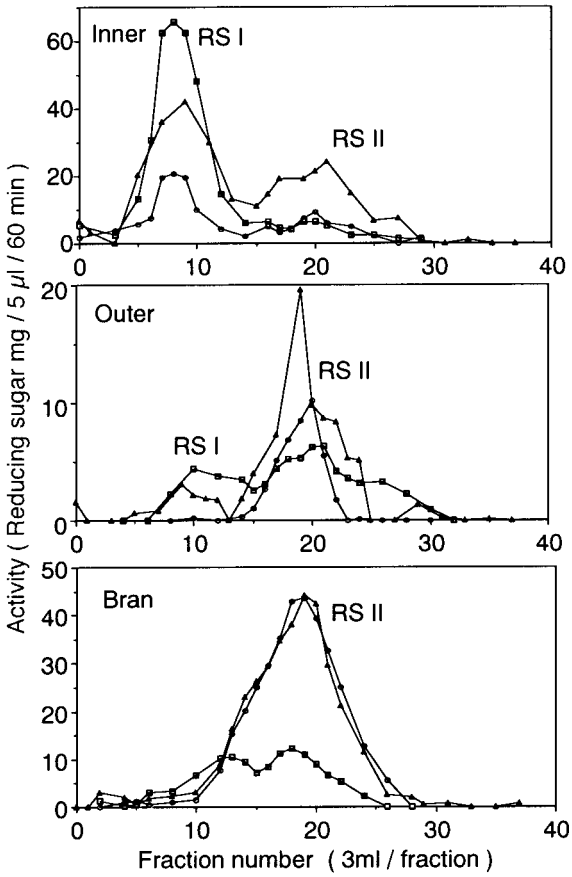

Figure 5. Activity of RS-producing enzymes of each fraction obtained by ion exchange chromatography using DEAE-Sephacel. The assays were at 20 °C (○), 60 °C (□), and 37 °C (△) to determine the thermal dependency of each peak. RS I and II in the chromatograms indicate RS-producing enzymes I and II, respectively. The averages of two measurements are shown. Experiments were repeated three times. This figure shows a typical result.

6b). The activities of α-glucosidase I and II at 60 °C were the highest, and they decreased as the reaction temperature decreased. At 60 °C, α-glucosidase II was detected in extracts of the outer endosperm and bran as in those of the inner endosperm. However, the activity of α-glucosidase III at 37 °C was the highest, and the activities at 20 and 60 °C were about half and less than one-third of the activity at 37 °C, respectively. The thermal dependencies of α-glucosidase I, II, and III were similar in all parts.

Activity Detection of Starch Degradation Enzymes by Zymography. As shown, for germinated embryoless half-seeds of rice (Figure 7, lane 6), α- and *β*-amylases were clearly detected, as reported by Perata et al. (1992), indicating that both enzymes are strongly induced during germination.

In contrast, the activity of the starch degradation enzymes was detected only in the extract from the outer endosperm at the position of near p*I* 5.2 (Figure 7, lanes 3 and 5).

DISCUSSION

Thermal Dependency and Stability of the RS-Producing Enzymes and α-Glucosidase in Rice Grains. The levels of activity, thermal dependency, and stability of enzymes causing starch degradation varied with the part of the rice grain. The RS-producing enzymes in the inner endosperm had a higher optimum temperature than those in other parts of the rice grain (Figure 2a,b). The inner endosperm contained higher α-glucosidase activity than the outer endosperm (Figure 2c,d). As far as we know, this is the first study suggesting that the properties of the enzymes causing starch degradation were different among the various parts of a rice grain. Their difference may be related to the localization of storage compounds such as starch, which is found predominantly in the inner side of the rice grain (Tanaka et al., 1974; Kennedy et al., 1974, 1975; Kennedy and Schelstraete, 1974, 1975).

α-Glucosidase I as RS-Producing Enzyme I. The thermal dependency of RS-producing enzymes in crude extracts varied with the part of the rice grain (Figure 2a). A possible cause of this difference is the presence of different enzymes with different optimum temperatures. To test this possibility, crude extracts were separated by anion exchange chromatography using DEAE-Sephacel, and the thermal dependency of each peak was determined.

By the separation, two peaks with RS-producing activities (RS-producing enzymes I and II, Figures 4 and 5) were detected. The optimum temperatures of the two peaks differed. Moreover, different parts of a rice grain had different ratios of these two peaks. In the inner endosperm, in which the optimum temperature of the RS-producing activity in crude extracts was high, the proportion of RS-producing enzyme I, with a high optimum temperature, was higher than in the other parts. Thus, the difference in the ratio of two peaks with different optimum temperatures may cause a difference in thermal dependency of the total RS-producing activity in crude extracts.

Assays of α-glucosidase activity of each fraction revealed one of the peaks of α-glucosidase activity (α-glucosidase I, Figure 6a,b) at the same elution time as the peak of RS-producing enzyme I. α-Glucosidase I had a higher optimum temperature (60 °C) and was localized mainly in the inner endosperm. These characteristics were consistent with those of RS-producing enzyme I. As the substrate specificity of α-glucosidase was not so high, and it can hydrolyze the α-1−4 linkage of not only maltose but also starch to produce glucose (Takahashi et al., 1971; Takahashi and Shimomura, 1973), the major component of RS-producing enzyme I was considered to be α-glucosidase I.

Other peaks were detected (α-glucosidase II and III, Figure 6a,b). α-Glucosidase II had the same optimum temperature (60 °C) and the same localization as α-glucosidase I, but its activity was at a lower level than that of α-glucosidase I. α-Glucosidase III had a lower optimum temperature (37 °C) and a different localization than α-glucosidase I and II. This peak was mostly in the outer endosperm. The elution times of these peaks were not consistent with those of RS-producing enzyme II. RS-producing activity was not found in the fraction corresponding to the peaks of α-glucosidase II or III, possibly because α-glucosidases II and III might be highly specific to maltose and cannot degrade starch. However, the starch degradation activities of α-glucosidase II and III were possibly masked by RS-producing enzyme II, because the amounts of α-glucosidase II and III were relatively small.

α-Amylase as RS-Producing Enzyme II. We considered that the main component of RS-producing enzyme II is an α-amylase isoform for the following

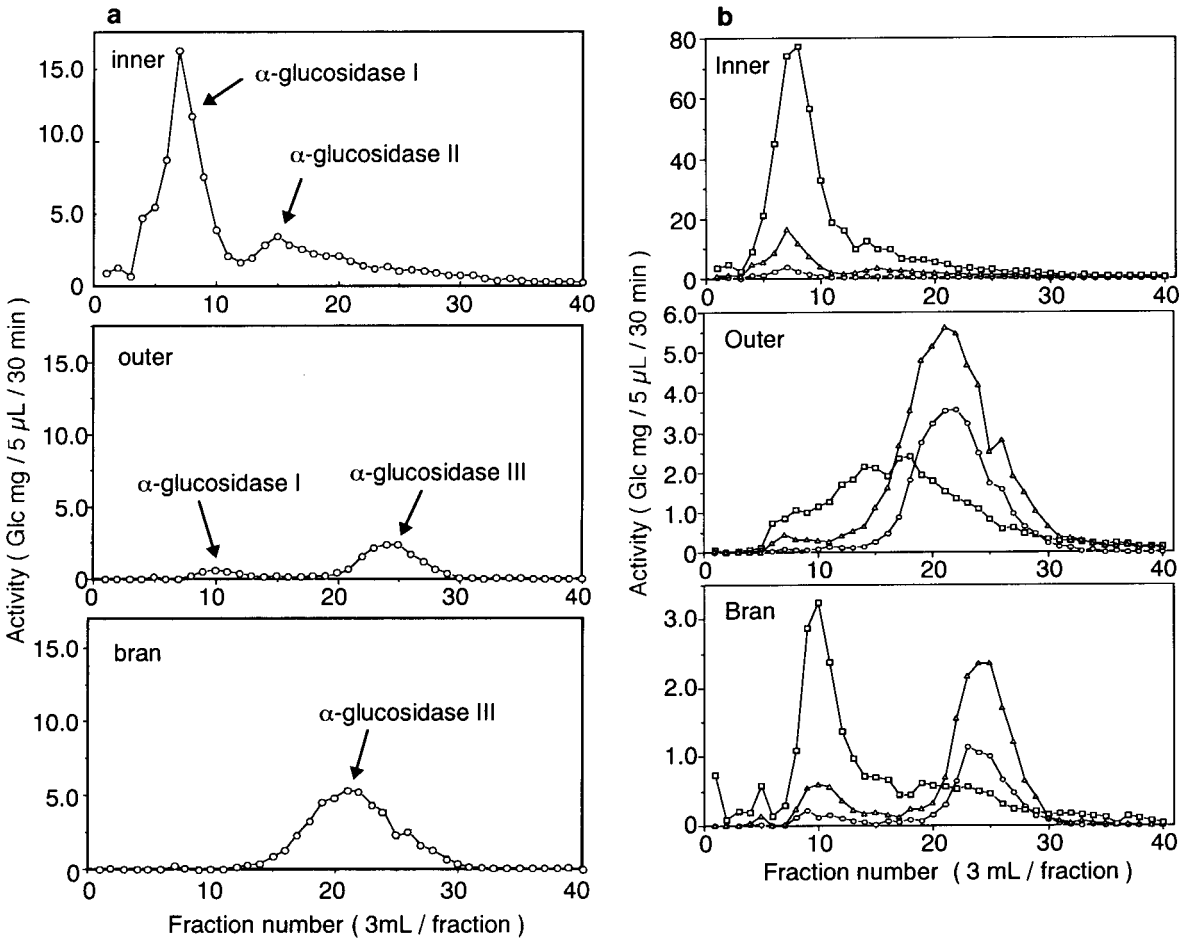

Figure 6. Elution pattern and thermal dependency of α-glucosidase separated by ion exchange chromatography using DEAE-Sephacel: (a) α-Glucosidase activities were assayed at 37 °C in each fraction obtained by ion exchange chromatography using DEAE-Sephacel and the elution patterns are shown. Each fraction was assayed twice, and the averages are shown. Experiments were repeated three times and a typical result is shown. (b) To determine the thermal dependency of each peak of α-glucosidase, the reactions were carried out at 20 °C (○), 37 °C (□), and 60 °C (△).

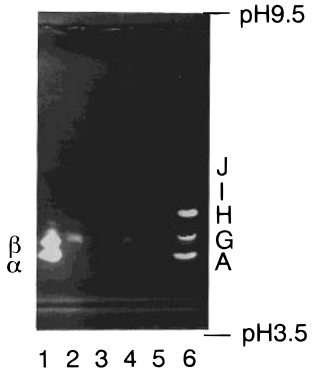

Figure 7. Zymogram of crude extracts obtained from the inner and outer endosperm: (lane 1) extract from germinated embryoless half-seed of rice, α-amylase isoform A (p*I* 4.5), *β*-amylase (near p*I* 5.0); (lane 2) outer endosperm (30 *μ*L); (lane 3) inner endosperm (30 *μ*L); (lane 4) outer endosperm (10 *μ*L); (lane 5) inner endosperm (10 *μ*L); (lane 6) marker α-amylase, isoform A (p*I* 4.5), isoform G (p*I* 5.1), isoform H (p*I* 5.6), isoform I (p*I* 6.3), isoform J (p*I* 6.7). See Materials and Methods for preparation of embryoless half-seeds.

reasons: (1) RS-producing enzyme II was a major component of the total RS-producing enzymes in the outer endosperm (Figure 4). (2) Production of malto-oligosaccharide was greater than by glucose and maltose in the crude extract from the outer endosperm (Figure 3). (3) RS-producing enzyme II has an optimum temperature at 37 °C. Isoform A of rice α-amylase is heat stable, whereas isoforms G, H, and I have low optimum temperatures (Terashima et al., 1994; Mitsui et al., 1996), indicating an agreement with RS-producing enzyme II. (4) The activity band of RS-producing enzyme II was near 5.2 as a p*I*, identical to that of isoform G (Figure 7; Mitsui et al., 1996). The overall results indicate that the main component of RS-producing enzyme II is α-amylase isoform G.

RS-Producing Enzymes in Rice Grains. This indicated that α-glucosidase and α-amylase isoform G might be the main enzymes causing starch degradation in the inner and outer endosperm of raw rice grains, respectively. A high proportion of maltose was detected in the reaction products of crude extract from rice bran (Figure 3), suggesting the presence of *β*-amylase in bran. Indeed *β*-amylase mRNA is in the aleurone layer of germinating rice grain (Yamaguchi et al., 1999).

These results indicate that different sets of starch degradation enzymes are in ungerminated rice grain.

We found that rice grains contain, especially in the inner endosperm, high levels of α-glucosidase with a high optimum temperature that may be responsible for starch degradation to RS. In raw sweet potato, the presence of a large amount of *β*-amylase has been suggested to cause production of sugars from starch during cooking (Damir, 1989). During germination, amylases are synthesized to degrade starch into sugars

in rice seeds (Tanaka et al., 1970; Panabieres et al., 1989; Choi et al., 1996; Yamaguchi et al., 1998). However, dry rice seeds have small amounts of amylases and large amounts of α-glucosidase, which are capable of starch degradation, especially in the inner endosperm that has abundant starch.

Rice Cooking and RS-Producing Enzymes. We propose that α-glucosidase may be more active than amylases in RS production in rice during cooking, and therefore α-glucosidase may be more advantageous in rice cooking than amylases. Texture, especially stickiness, is an important factor for Japanese preference of rice, which is not so for sweet potato. The stickiness of gelatinized starch is less, as the molecular weight of the starch molecule is lower (Cowie and Greenwood, 1957). Thus, α-glucosidase, which hydrolyzes starch at the ends of an α-1−4-glucosyl chain, may be able to produce sugars effectively without spoiling the texture of cooked rice compared with amylases. In this study, although the activity of RS production from starch was high in the crude extract from the inner endosperm (Figure 2a,b), starch degradation activity was not detected by the iodine−starch reaction (Figure 7). This indicated that α-glucosidase can produce sugars from starch without markedly influencing the molecular size of the starch molecule. Production of sugars with a smaller influence on starch texture would be an advantage for preference of rice when both texture and sweetness are considered.

During cooking of sweet potato, especially when heating is slow, large quantities of RS are produced, presumably because of the extended action period of β-amylase (Damir, 1989). Therefore, slowly cooked sweet potato is thought to be sweeter and preferred, compared with rapidly cooked sweet potato. The sweetness of cooked rice may also be determined by the activity of the enzymes that catalyze degradation of starch into RS during cooking, as in sweet potato. Rice enzymes that maintain activity above 60 °C may be able to act for a long time during heating. Furthermore, gelatinization of rice starch occurs from 66 to 77 °C (Guilbot and Mercier, 1985). Considering this result, at around 60 °C starch granules may break and the starch may be loosened. Therefore, starch may be degraded more easily at this temperature than raw starch due to easy access. If the enzymes causing starch degradation can act above this temperature (60 °C), such enzymes should be able to degrade starch effectively. Consequently, rice having a higher level of these enzymes would produce more sugar during cooking, making the rice sweeter and tastier. In nature, enzymes having a high optimum temperature, such as 60 °C, could not be advantageous because such high temperatures are rarely reached. Rice containing α-glucosidase with a high optimum temperature is considered to have been selected as good quality rice by the Japanese people. Further evidence to support this view may be obtained by comparing the α-glucosidase activities between poor quality rice and good quality rice.

ABBREVIATIONS USED

RS, reducing sugar; GA, gibberellic acid; HPLC, high-performance liquid chromatography; DEAE, diethylaminoethyl.

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