Two Forms of α -Glucosidase from Rice Seeds at the Milky Stage

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Received April 18, 1979

Two forms of α -glucosidase were isolated from rice seeds at the milky stage by a procedure that included fractionation with ammonium sulfate, DEAE-cellulose column chromatography, CM-cellulose column chromatography, SP-Sephadex column chromatography and preparative disc gel electrophoresis. The isolated α -glucosidases were designated as α -glucosidase I and α -glucosidase II, and were glycoproteins containing 5.5% and 3.4% carbohydrate, respectively. The molecular weight and isoelectric point were: 78,900 and pH 9.3 for α -glucosidase I, and 47,300 and pH 9.3 for α -glucosidase II. They were found to be homogeneous on polyacrylamide disc gel electrophoresis. The two enzymes hydrolyzed maltose, maltotriose, phenyl α -maltoside, amylose and soluble starch to liberate glucose, but did not act on sucrose. Their enzymes produced maltotriose and 4- α -nigerosyl-glucose from maltose as main α -glucosyltransfer products. They hydrolyzed amylose to liberate α -glucose.

In previous works,^{1~7)} α -glucosidases and glucoamylases from molds belonging to *Mucor*, *Penicillium* and *Basidiomycetes*, on which fewer reports have been published to date than on the enzymes from *Aspergillus* and *Rhizopus*, were isolated in crystalline and highly purified forms, and comparative studies on these enzymes have been undertaken. A crystalline α -glucosidase from *M. javanicus* could hydrolyze soluble starch at the same rate as maltose, that is to say, the α -glucosidase was the intermediate one between α -glucosidase and glucoamylase. All α -glucosidases from the molds except *Asp. sp.* readily hydrolyzed soluble starch. A conventional view that α -glucosidases from molds were oligo-glucosidases having no hydrolytic activity toward amylaceous polysaccharides held true only with *Asp. sp.* Moreover, all the α -glucosidases tested hydrolyzed amylose liberating α -glucose, whereas all the glucoamylases tested liberated

β -glucose from amylose without exception. We think that this is a great difference between α -glucosidase and glucoamylase.

Recently α -glucosidases which seemed to possess starch-degrading activity were also reported in several plants.^{8~12)} Takahashi *et al.*⁹⁾ reported two forms of α -glucosidase from matured seeds of rice, but nothing on their transglucosylating action and anomeric configuration of fission products. No attempt has yet been made to investigate whether or not multiple forms of α -glucosidase are obtained from rice seeds at the milky stage, or whether or not the properties of each enzyme are different from those of the α -glucosidases from matured seeds of rice. Igaue *et al.*¹³⁾ reported that a partially purified amylase preparation from rice seeds at the milky stage had a strong maltose-hydrolytic activity. However, the enzyme preparation has not yet been isolated in a highly purified form. It has not been made clear whether or not a single enzyme is responsible for hydrolytic activities on both maltose and starch. As starch is actively synthesized at the milky stage, the α -glucosidase at the milky stage may be different from the enzyme from matured seeds.

In the present study, two forms of α -gluco-

Abbreviations: buffer A, 0.01 M acetate buffer, pH 5.3, containing 10% sodium chloride and 5 mM calcium acetate; buffer P, 0.01 M phosphate buffer, pH 7.7, containing 10% sodium chloride and 5 mM calcium acetate; solution A, 0.01 M sodium acetate containing 500 mM urea and 2 mM 2-mercaptoethanol; AB, acetate buffer.

sidase were obtained from rice seeds at the milky stage. They were separable by polyacrylamide disc gel electrophoresis. This paper deals with the purification and properties of these enzymes.

MATERIALS AND METHODS

Materials. Rice seeds (*Oryza sativa* L. cultivar, *Yashiromochi*) at the milky stage (15 days after the beginning of heading) were harvested in the experimental field of the Institute for Agricultural and Biological Sciences, Okayama University, Kurashiki, in 1977. Amylose (EX-I, $\overline{\text{Dpn.}}^* \text{ ca. } 18$), maltose (HHH), maltotriose and isomaltose (Hayashibara Biochemical Laboratories, Inc., Okayama, Japan), amylopectin from waxy maize (Nippon Food Industry Co., Ltd., Tokyo, Japan), glycogen from shellfish (Nakarai Chemicals, Co., Ltd., Kyoto, Japan), soluble starch (E. Merck, Darmstadt, Germany), and glucoamylase from *Rhizopus niveus* (pure grade) and saccharifying α -amylase from *Bacillus subtilis* (crystalline grade) (Seikagaku Kogyo Co., Ltd., Tokyo, Japan) were obtained from commercial sources. Panose was kindly supplied by Prof. K. Matsuda, Faculty of Agriculture, Tohoku University. 4- α -Nigerosyl-glucose was kindly supplied by Associate Prof. S. Chiba, Faculty of Agriculture, Hokkaido University.

Analytical techniques

Enzyme assays. α -Glucosidase activity was determined by measuring the amount of glucose liberated from the substrate in accordance with the method of Papadopoulos *et al.*⁴⁾, as modified by Dahlqvist.⁵⁾ One unit of α -glucosidase activity is defined as the amount of enzyme which forms 1 μmol of glucose from maltose per 15 min under the standard conditions.¹⁾

Determination of protein. Protein was determined by the method of Warburg *et al.*¹⁶⁾ The protein profiles in column chromatography were followed by measuring the absorbance of eluates at 280 nm.

Disc gel electrophoresis. Electrophoresis of the enzyme was carried out in a polyacrylamide gel, pH 4.0, by the method of Reisfeld *et al.*¹⁷⁾ After the run, the gel was stained for proteins with 1% amido black 10 B in 7% acetic acid.

Determination of optical rotation. Optical rotation studies were performed with a Yanaco model OR-50 automatic polarimeter.

Paper chromatography. The sugar solution was spotted on Toyo No. 50 filter paper, and the paper was

developed three times at room temperature by the ascending method with a solvent system of *n*-butyl alcohol-pyridine-water (6:4:3, v/v). After drying, the products were detected by the silver nitrate dip method.¹⁵⁾

Liquid column chromatography. For determination of transglucosylation products, the reaction mixture of α -glucosidase with maltose was subjected to a Nihon Denshi model JLC 6-AUH liquid chromatograph. Neutral sugars in intact enzymes were also identified by the liquid chromatograph. Orcinol dissolved in conc. sulphuric acid (1.5 g/l) was used as assay reagent.

Determination of molecular weight. Molecular weight was determined by the low speed sedimentation equilibrium method with a Hitachi ultracentrifuge model 282 equipped with a light absorption scanning recorder.

Determination of isoelectric point. Analytical gel electrofocusing was performed with 7.5% polyacrylamide gels containing the carrier ampholyte (pH range 3.5 to 10) in accordance with the method of Wrigley.¹⁰⁾ Electrofocusing was run at a constant voltage of 80 V for 48 hr with a conventional disc electrophoretic apparatus having $0.5\phi \times 8$ cm tubes.

RESULTS

I. Purification of α -glucosidases

Unless otherwise indicated, the operations were carried out at 4°C. Rice seeds (20 kg) were homogenized for 3 min in a National model MX-120 S mixer with buffer P (2.5 liter per kg of the rice seeds). After being kept at 4°C for 24 hr, the homogenate was filtered with a centrifugal dehydrater.

Step 1. Fractionation with ammonium sulfate. To the filtrate was added solid ammonium sulfate to give 80% saturation under stirring. After the mixture was allowed to stand overnight, the resulting precipitate was collected by vacuum filtration through a thick bed of Celite No. 535 over a Toyo No. 2 filter paper on a Büchner funnel, and dissolved in buffer A. Insoluble material was removed by filtration. The enzyme solution was dialyzed for 48 hr against solution A.

Step 2. DEAE-cellulose column chromatography. The dialyzed solution obtained from Step 1 was applied to a DEAE-cellulose column

* Average degree of polymerization.

($4.5\phi \times 20$ cm) previously equilibrated with solution A. The active fractions which passed through the column were collected and brought to 90% saturation by adding solid ammonium sulfate. The resulting precipitate was collected by centrifugation and dissolved in solution A. The enzyme solution was dialyzed overnight against solution A without 2-mercaptoethanol.

Step 3. CM-cellulose column chromatography. The dialyzed solution obtained from Step 2 was applied to a CM-cellulose column ($4.5\phi \times 20$ cm) previously equilibrated with 0.01 M AB, pH 5.0. The column was washed with the same buffer and 0.01 M AB, pH 6.0, and eluted with a linear gradient of 0 to 0.5 M sodium chloride. The active fractions eluted with sodium chloride were collected and precipitated with ammonium sulfate as mentioned above. The resulting precipitate was collected by centrifugation and dissolved in 0.01 M AB, pH 5.0. The enzyme solution was dialyzed overnight against 0.01 M AB, pH 5.0.

Step 4. SP-Sephadex C-50 column chromatography. The dialyzed solution obtained from Step 3 was applied to an SP-Sephadex column ($2.8\phi \times 10$ cm) previously equilibrated with 0.01 M AB, pH 5.0. The column was washed with 0.02 M AB, pH 5.0, 0.02 M AB, pH 5.5 and 0.02 M AB, pH 6.0. Elution was

carried out with a linear gradient of 0 to 0.5 M sodium chloride in 0.02 M AB, pH 6.0 (Fig. 1). The active fractions (Nos. 235 to 257) were collected and precipitated with ammonium sulfate as mentioned above. The resulting precipitate was collected by centrifugation, dissolved in 0.01 M AB, pH 5.0, and dialyzed overnight against the same buffer.

Step 5. Preparative disc gel electrophoresis. The dialyzed solution was subjected to preparative disc gel electrophoresis, pH 4.0, by the procedure described in MATERIALS AND METHODS. The running column (diameter: 2.5 cm) was composed of 12 ml of a stacking gel and 64 ml of a separating gel. Each of the upper and lower chambers contained about 1,000 ml of buffer. To the enzyme solution was added sucrose to a final concentration of 20%, and the mixture was layered on the surface of the stacking gel. Electrophoresis was carried out at 60 mA for 8 hr with the cathode in the lower chamber. After electrophoresis, the gel was cut into 5 mm sections and crushed in 1 M AB, pH 5.3. After being allowed to stand overnight, the gel was removed by filtration. The active two filtrates (Nos. 1-3 and 5-9) were pooled (Fig. 2) and concentrated by ultrafiltration with a collodion bag. The two enzymes will be called α -glucosidase I and α -glucosidase II. Each concentrated solution was dialyzed against 0.01 M AB, pH 5.0. A summary of the purification procedure is given in Table I. Each of the two enzymes was found to be homogeneous on disc gel electrophoresis (Fig. 3).

FIG. 1. SP-Sephadex C-50 Column Chromatography.

Protein applied, 1,760 mg; flow rate, about 30 ml per hr; fraction volume, 15 ml; $\bullet$ — $\bullet$, absorbance at 280 nm; $\circ$ — $\circ$, α -glucosidase activity; — — —, concentration of sodium chloride.

FIG. 2. Preparative Disc Gel Electrophoresis.

TABLE I. PURIFICATION OF α -GLUCOSIDASES I AND II

	Protein		α -Glucosidase activity		
	Total	A_{290}/A_{280}	Total units	Specific activity	Recovery
	mg		units/mg protein		%
1. Ammonium sulfate fractionation	50,100	1.03	231,600	4.62	100
2. DEAE-cellulose column chromatography	4,630	1.29	149,700	32.33	64.6
3. CM-cellulose column chromatography	1,760	1.35	117,000	66.48	50.5
4. SP-Sephadex column chromatography	270	1.54	87,610	324.48	37.8
5. Preparative disc gel electrophoresis I	12	1.23	7,390	615.83	3.2
II	20	1.42	14,140	707.00	6.1

FIG. 3. Disc Gel Electrophoresis of α -Glucosidases I and II.

The purified enzyme protein was subjected to polyacrylamide disc gel electrophoresis at pH 4.0. Electrophoresis was carried out at a constant current of 5 mA per tube for 120 min by the method described in MATERIALS AND METHODS.

I, α -glucosidase I (70 μ g); I+II, mixture of α -glucosidase I (20 μ g) and α -glucosidase II (20 μ g); II, α -glucosidase II (90 μ g).

II. Properties of enzymes

1) Molecular weight. Molecular weight

was determined by the low speed sedimentation equilibrium method as described in MATERIALS AND METHODS. Experimental conditions were as follows: temperature, 20°C; cell used, a multichannel cell; concentration of α -glucosidases I and II, about 0.03% (in 0.01 M acetate buffer, pH 5.3); speed, 9000 r.p.m.. The molecular weights of α -glucosidases I and II were 78,900 and 47,300, respectively.

2) *Isoelectric point.* Analytical gel electrophoresis was performed as described in MATERIALS AND METHODS. The isoelectric point of the two enzymes was determined to be 9.3.

3) *Neutral sugar constituent.* The neutral sugar content in terms of mannose was calculated to be 5.5% for α -glucosidase I and 3.4% for α -glucosidase II by the method of the Tillman reaction.²¹⁾ As shown in Fig. 4, the hydrolyzates of the two enzymes gave a peak of neutral sugar corresponding to mannose.

4) *Effect of pH on α -glucosidase activity.* The pH optimum was at 4.0 for the activity of each of the α -glucosidases. The two enzymes were stable in the pH range of 4.0 to 5.5 (Table II).

5) *Effect of temperature on α -glucosidase activity.* The temperature optimum was at 37°C to 55°C for the activity of α -glucosidase I and at 37°C to 50°C for α -glucosidase II. The two enzymes were stable at up to 30°C (Table II).

FIG. 4. Liquid Chromatogram of Neutral Sugar Constituents from α -Glucosidases I and II.

A Nihon Denshi model JLC 6-AUH carbohydrate analyzer was used. A column ($0.8\phi \times 15$ cm) was packed with LC-R-3 quarternary ammonium ion-exchange resin. α -Glucosidases I (0.580 mg) and II (0.555 mg) were separately hydrolyzed, each with 1 ml of 1 N HCl at 100°C for 6 hr in a sealed glass tube. The hydrolyzate was cooled and neutralized with 4 M NaOH. The solution was assayed by liquid chromatography.

TABLE II. pH OPTIMUM, pH STABILITY, TEMPERATURE OPTIMUM AND HEAT STABILITY

	α -Glucosidase	
	I	II
pH Optimum	4.0	4.0
pH Stability ^a	4.0–5.5	4.0–5.5
Temperature optimum	37°C – 55°C	37°C – 50°C
Heat stability ^b	up to 30°C	up to 30°C

^a Enzyme was preincubated for 20 hr at 30°C with 0.05 M McIlvaine's buffer at various pH.

^b Enzyme was preincubated for 15 min at various temperatures.

TABLE III. SUBSTRATE SPECIFICITY

	Relative rate of hydrolysis ^a (%)		K_m value ($\times 10^{-4}$ M)	
	I*	II**	I*	II**
Isomaltose	2.7	2.5		
Kojibiose	13.2	18.9		
Maltose	100	100	6.94	6.94
Sucrose	0	0		
Maltotriose	36.3	45.4	13.20	13.20
Panose	3.2	3.6		
Amylopectin	5.4	6.0		
Amylose EX-I	31.9	29.7		
Glycogen	1.4	1.9		
Soluble starch	18.7	19.0		

* α -Glucosidase I; ** α -Glucosidase II.

^a The reaction mixture (0.5 ml) containing enzyme solution, 0.1 ml of 1% substrate and 0.05 M acetate buffer, pH 5.3, was incubated at 37°C for 15 min. The enzyme solution used was as follows: α -glucosidase I (0.543 units), α -glucosidase II (0.752 units).

6) *Substrate specificity.* With various substrates, the activities of the two enzymes were examined, and the relative rates of hydrolysis are listed in Table III. Maltose, phenyl α -malto-side, maltotriose, amylose and soluble starch were readily served, but sucrose was resistant to the two enzymes. As shown in Table III, there was a remarkable similarity between the two enzymes in substrate specificity. The Michaelis constants were also assessed (Table III).

7) *α -Glucosyltransfer products.* When the two α -glucosidases were incubated with 5% maltose, transfer products (temporarily named X_1 and X_2) were detected on a paper chromatogram (Fig. 5). The substance X_1 produced by α -glucosidase I was isolated by multiple one-dimensional paper chromatography with a

solvent system of *n*-butyl alcohol-pyridine-water (6:4:3, v/v), after the same reaction mixture (2 ml) as described in Fig. 4 was incubated at 37°C for 5 hr. The substance X_1 was presumed to be maltotriose from their similarity in paper chromatographic mobility (Fig. 5), and to be the mixture of maltotriose and 4- α -nigerosyl-glucose from their similarity in liquid chromatographic mobility (Fig. 6). As shown in Fig. 7, maltotriose was already detected in a hydrolysis degree of 13.2%.

FIG. 5. A Paper Chromatogram of Transglucosylation Products from Maltose.

The reaction mixture (2 ml) containing enzyme solution, 1.0 ml of 10% maltose and 0.96 ml of 0.05 M acetate buffer, pH 5.3, was incubated at 37°C. At various time intervals, 0.05 ml of the reaction mixture was chromatographed by the same procedure as described in MATERIALS AND METHODS. The enzyme solution used was as follows: α -glucosidase I (9.00 units), α -glucosidase II (11.88 units). I, glucose (60 μ g)+maltose (150 μ g)+maltotriose (300 μ g)+ isomaltose (150 μ g)+panose (300 μ g).

FIG. 6. Liquid Chromatogram of Transglucosylation Products from Maltose.

A Nihon Denshi model JLC 6-AUH carbohydrate analyzer equipped with a two-line system was used. Lines 1 and 2 were equipped with a long-type jacketed column ($0.8\phi \times 100$ cm) and a short-type jacketed column ($0.8\phi \times 15$ cm), respectively. The columns were packed with LC-R-3 quaternary ammonium ion-exchange resin. The reaction mixture containing α -glucosidase I in Fig. 5 was incubated at 37°C for 5 hr, and 1 ml of the mixture was assayed by liquid chromatography.

FIG. 7. Time-Course of Transglucosylation Products from Maltose.

The reaction mixture containing α -glucosidase I in Fig. 5 was incubated at 37°C. At various time intervals, 1 ml of the mixture was assayed by liquid chromatography as described in Fig. 6.

●—●, maltotriose; ○—○, nigerosyl glucose; Δ — Δ , glucose; ——, hydrolysis of maltose.

There was a little accumulation of maltotriose, as it was readily hydrolyzed by the enzymes.

8) *Anomeric configuration of fission products.* Time-course of the reaction of the two enzymes with amylose was investigated by

FIG. 8. Optical Rotation Study of the Action on Amylose by *B. subtilis* Amylase, *Rh. niveus* Amylase and the Two Forms of α -Glucosidase.

The mixture (25 ml) containing the enzyme solution, 5.0 ml of 1% amylose aqueous solution and deionized water was incubated at 40°C. The optical rotation was measured at various intervals with a Yanaco model OR-50 automatic polarimeter, a 10 cm-light pathway length cell being used. When the rotation became constant, 10 mg of anhydrous sodium carbonate was added to 25 ml of the digest, the pH being brought to 10. The arrow indicates the optical rotation after the addition of alkali to the digests.

●—●, *B. subtilis* α -amylase (99.1 units); ●—●, *Rh. niveus* glucoamylase (124.2 units); ○—○, α -glucosidase I (900.0 units); ○—○, α -glucosidase II (1188.0 units).

following the changes in optical rotation.²⁰⁾ Two other amylases, from *B. subtilis* and *Rh. niveus*, were also studied for comparison. Figure 8 shows the changes in optical rotation with the duration of incubation for the two amylases and the two α -glucosidases from rice seeds. The downward rotation of the digests upon the addition of alkali indicates that the product from amylose by the two α -glucosidases from rice seeds has the α -configuration.

9) *Inhibitor.* Among the metal ions

TABLE IV. EFFECT OF INHIBITORS

For the assay of the effect of metal ions, 0.03 ml of the enzyme solution, 0.02 ml of 0.1 M metal ion solution and 0.35 ml of 0.05 M acetate buffer, pH 5.3, were preincubated at 37°C for 30 min. After preincubation, 0.1 ml of 1% maltose was added and the resulting mixture was kept at 37°C for 15 min.

For the assay of the effect of the other inhibitors, 0.03 ml of the enzyme solution, each of the reagents dissolved in deionized water and deionized water in a final volume of 0.4 ml were preincubated at 37°C for 5 min. To the mixture was added 0.1 ml of 1% maltose and the resulting mixture was kept at 37°C for 15 min.

The enzyme solution used was as follows: α -glucosidase I (0.326 units), α -glucosidase II (0.451 units).

Inhibitor	Concentration mm	Hydrolysis (residual activity %)	
		I*	II**
None		100	100
$\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$	5	61.9	67.5
$\text{BaCl}_2 \cdot 2\text{H}_2\text{O}$	5	72.7	86.6
$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	5	83.0	93.5
$\text{Cd}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$	5	72.2	76.6
CoCl_2	5	90.7	90.9
$\text{Cu}(\text{CH}_3\text{COO})_2$	5	64.9	64.9
HgCl_2	5	0.0	0.0
KCl	5	92.8	97.8
MgCl_2	5	82.7	82.7
MnCl_2	5	101.5	110.4
NaCl	5	100.5	103.9
NiCl_2	5	84.8	84.8
$\text{Pb}(\text{CH}_3\text{COO})_2$	5	8.2	10.8
$\text{SnCl}_2 \cdot 2\text{H}_2\text{O}$	5	5.7	4.3
ZnCl_2	5	56.7	80.1
EDTA	5	59.8	71.4
Monoiodoacetic acid	0.05	49.5	64.9
PCMB	0.05	32.5	45.0
Turanose	14.6	20.6	25.1

* α -Glucosidase I; ** α -Glucosidase II.

tested, Hg^{2+} , Pb^{2+} and Sn^{2+} proved potent inhibitors of the two α -glucosidases (Table IV). They were also inhibited by turanose.

DISCUSSION

The two α -glucosidases were isolated from rice seeds at the milky stage in highly purified forms. The two enzymes were separated by preparative disc gel electrophoresis. The molecular weight and isoelectric point of α -glucosidase I were 78,900 and at pH 9.3, and those of α -glucosidase II were 47,300 and at pH 9.3, respectively. α -Glucosidases I and II were glycoproteins containing 5.5% and 3.4% carbohydrate as mannose, respectively. The two enzymes were different in both molecular weight and sugar content, but possessed the same isoelectric point and similar properties. The two enzymes will be two forms of glycoprotein, which are different in sugar content.

The two enzymes readily hydrolyzed maltose, phenyl α -maltoside, maltotriose, amylose, soluble starch, and very weakly isomaltose, panose, amylopectin and glycogen. α -Glucosidases from plants,^{8~12)} *Mucor javanicus*,²¹⁾ *M. racemosus*,²²⁾ *Penicillium purpurogenum*,²⁾ *Lentinus edodes* (Berk.) Sing.⁷⁾ and pig liver²³⁾ are known to hydrolyze soluble starch. Like α -glucosidases from *M. javanicus*, *M. racemosus*, *Pen. purpurogenum*, pig liver, *Bacillus cereus*²⁴⁾ and green gram,¹²⁾ the two enzymes from rice seeds formed maltotriose as the main α -glucosyltransfer product from maltose. α -Glucosidases from molds may be classified into at least four groups according to their starch-degrading activity and mode of transglucosylation.¹²⁾ The enzymes of one group readily hydrolyze soluble starch and produce maltotriose as the main α -glucosyltransfer product from maltose, as exemplified by α -glucosidases from *M. javanicus*, *M. racemosus*, *Pen. purpurogenum* and *L. edodes* (Berk.) Sing. The two enzymes from rice seeds at the milky stage and the three α -glucosidases from green gram¹²⁾ belong to this group. This result suggests that the classification of α -glucosidases from molds may be applicable to the enzymes

from plants, too.

The changes in the optical rotation of the amylase digests upon addition of alkali indicated that the α -anomer of glucose was produced when the two α -glucosidases hydrolyzed amylose liberating glucose. α -Glucosidases from *M. javanicus*,^{22,25)} *M. racemosus*,²²⁾ *Pen. oxalicum*,³⁾ *L. edodes* (Berk.) Sing.⁷⁾ and green gram¹²⁾ hydrolyzed amylose to liberate α -glucose, whereas glucoamylases from *M. rouxianus*,²⁶⁾ *Pen. oxalicum*,²⁷⁾ *Aspergillus awamori*²⁸⁾ and *L. edodes* (Berk.) Sing.⁷⁾ did to liberate β -glucose. The two enzymes from rice seeds at the milky stage hydrolyzed amylose to liberate α -glucose. From these results, we can distinguish α -glucosidase from glucoamylase according to the anomeric configuration of fission products.

The two α -glucosidases existed at the milky stage of rice seed where starch was actively synthesized. It is suggested that the role of α -glucosidase in plant tissues is to hydrolyze the oligosaccharides produced from starch by α - and β -amylases in the germination stage and therefore to take part in a hydrolytic pathway with α - and β -amylases.^{29,20)} However, the role of the enzyme at the milky stage in plant tissues has not yet been made clear. The two enzymes readily hydrolyzed soluble starch and exhibited strong α -glucosyl transfer activity. If they take part only in the breakdown of starch, their existence is inexplicable. The enzymes may play a part in the biosynthesis of polysaccharides.

Takahashi *et al.*⁹⁾ reported the isolation of two forms of α -glucosidase from matured seeds of rice. The two α -glucosidases from matured seeds of rice were similar to the two enzymes from rice seeds at the milky stage in temperature optimum and pH optimum. The two enzymes from matured seeds of rice were different from each other in substrate specificity. α -Glucosidase I hydrolyzed maltotriose and soluble starch at a faster rate than α -glucosidase II, and the relationship between the two enzymes is reversed in kojibiose-hydrolyzing activity. However, the two enzymes from rice seeds at the milky stage exhibited similar sub-

strate specificities with each other, and were similar to α -glucosidase II from matured seeds of rice in various properties tested, although the former hydrolyzed soluble starch at a faster rate than the latter. The two α -glucosidases from matured seeds of rice will take part in the hydrolytic pathway with α - and β -amylases in the germination stage.

Acknowledgments. The authors wish to express their thanks to Professor J. Ozawa, Institute for Agricultural and Biological Sciences, Okayama University, for his encouragement and Associate Professor K. Kawazu, Faculty of Agriculture, Okayama University, for allowing them to use a Yanaco model OR-50 automatic polarimeter.

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