Purification and Properties of α -Glucosidase and Glucoamylase from *Lentinus edodes* (Berk.) Sing.

Yoshiki YAMASAKI and Yukio SUZUKI

Institute for Agricultural and Biological Sciences, Okayama University,
Kurashiki, Okayama

Received October 24, 1977

An α -glucosidase and a glucoamylase have been isolated from fruit bodies of *Lentinus edodes* (Berk.) Sing., by a procedure including fractionation with ammonium sulfate, DEAE-cellulose column chromatography, and preparative gel electrofocusing. Both of them were homogeneous on gel electrofocusing and ultracentrifugation. The molecular weight of α -glucosidase and glucoamylase was 51,000 and 55,000, respectively. The α -glucosidase hydrolyzed maltose, maltotriose, phenyl α -maltoside, amylose, and soluble starch, but did not act on sucrose. The glucoamylase hydrolyzed maltose, maltotriose, phenyl α -maltoside, soluble starch, amylose, amylopectin, and glycogen, glucose being the sole product formed in the digests of these substrates. Both enzymes hydrolyzed phenyl α -maltoside into glucose and phenyl α -glucoside. The glucoamylase hydrolyzed soluble starch, amylose, amylopectin, and glycogen, converting them almost completely into glucose. It was found that β -glucose was liberated from amylose by the action of glucoamylase, while α -glucose was produced by the α -glucosidase.

Maltotriose was the main α -glucosyltransfer product formed from maltose by the α -glucosidase.

In the previous papers,^{1~7)} we have reported the purification and properties of α -glucosidases from *Mucor javanicus*, *M. racemosus*, *Penicillium purpurogenum*, *Pen. oxalicum*, and *Aspergillus awamori*, and those of glucoamylases from *M. rouxianus*, *Pen. oxalicum*, and *Asp. awamori*. α -Glucosidases from *Asp. niger*,⁸⁾ *Asp. oryzae*,⁹⁾ and *M. javanicus*¹⁾ have so far been isolated in crystalline form. Glucoamylases from *Rhizopus delemar*,¹⁰⁾ *Asp. awamori*,¹¹⁾ *Asp. niger*,¹²⁾ *Endomyces* sp.¹³⁾ and *M. rouxianus*⁶⁾ have so far been isolated in crystalline form.

The enzymes from *Basidiomycetes* have not been studied in the same detail as those from the fungi belonging to *Phycomycetes* and *Ascomycetes*.

In the present study, α -glucosidase and glucoamylase from fruit bodies of *Lentinus edodes* (Berk.) Sing. ("shiitake") were isolated in highly purified forms, and their properties were investigated. The results obtained are given here.

MATERIALS AND METHODS

Materials. Fruit bodies of *Lentinus edodes* (Berk.) Sing. used were obtained from the local greengrocer's shop. A glucoamylase from *Rhizopus niveus* (pure grade) and a crystalline α -amylase from *Bacillus subtilis* (saccharifying type) were purchased from Seikagaku Kogyo Co., Ltd., Tokyo, Japan. Amylose (EX-I, $\overline{DP}_n$ approx. 18), isomaltose, maltose and maltotriose (Hayashibara Biochemical Laboratories, Inc., Okayama, Japan), amylopectin from waxy maize (Nippon Food Industries, Ltd., Tokyo, Japan), glycogen from shellfish (Nakarai Chemicals Co., Ltd., Kyoto, Japan), soluble starch (E. Merck, Darmstadt, Germany), corn starch and potato starch (Wako Pure Chemical Industries, Ltd., Osaka, Japan), rice starch (Shimada Chemical Industries, Ltd., Niigata, Japan) were obtained from commercial sources. Panose was a gift from Prof. K. Matsuda, Faculty of Agriculture, Tohoku University. Other chemicals and reagents used in this study were the same as described in the previous papers.^{1~7)}

Analytical techniques

Enzyme assays. α -Glucosidase activity was determined by measuring the amount of glucose liberated from the substrate according to the method of Papadopoulos *et al.*,¹⁴⁾ as modified by Dahlqvist.¹⁵⁾ One

unit of α -glucosidase activity is defined as the amount of enzyme which forms 1 μ mole of glucose from maltose per 15 min under the standard conditions.¹³ Glucoamylase activity was determined under the same conditions as employed in the assay for the α -glucosidase activity, except that soluble starch was used in place of maltose.⁷⁾

Determination of protein. Protein was determined by the method of Warburg *et al.*¹⁷⁾ The protein profiles in column chromatography were followed by measuring the absorbance of eluates at 280 nm.

Gel electrofocusing. Analytical gel electrofocusing was performed with 7.5% polyacrylamide gels containing the carrier ampholyte (pH range, 4 to 6) according to the method of Wrigley.¹³⁾ A sample gel containing the purified enzyme was photopolymerized on the top of separation gel, and electrofocusing was run at a constant voltage of 80 V for 48 hr at 4°C using a conventional disc electrophoretic apparatus. Gel was stained for 1 hr with 0.2% bromophenol blue in ethyl alcohol–water–acetic acid (50: 45: 5, v/v) and destained with ethyl alcohol–water–acetic acid (30: 65: 5, v/v).

Dodecyl sulfate-polyacrylamide gel electrophoresis. For the determination of the molecular weight, the enzyme and markers were separately subjected to electrophoresis by the method of Weber *et al.*,¹⁹⁾ with the use of polyacrylamide gel containing sodium dodecyl sulfate at a concentration of 0.1%. The markers used were lysozyme (mol. wt. 14,307), sperm whale myoglobin (mol. wt. 17,800), beef pancreas chymotrypsinogen A (mol. wt. 25,000), ovalbumin (mol. wt. 45,000), and bovine serum albumin (mol. wt. 67,000).

Paper chromatography. Samples were spotted on a Toyo No. 50 filter paper, and the paper was developed three times at 26°C by the ascending method with the 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.²⁰⁾

Analytical ultracentrifugation. Ultracentrifugal studies were performed with a Hitachi model 282 analytical ultracentrifuge.

Oxidation of amylose. Amylose EX-I (1 g) was oxidized according to the method of J. J. Marshall *et al.*,²¹⁾ except that sodium metaperiodate (0.15 g) was added to the amylose solution and the mixture was stored in the dark for 15 hr.

RESULTS

I. Purification of α -glucosidase

Unless otherwise indicated, the operations were carried out at 4°C.

Step 1. Preparation of crude extract. Fruit bodies of *L. edodes* (Berk.) Sing. (5 kg) were homogenized in a National model MX-120 S mixer with 10 liters of 0.02 M acetate buffer, pH 5.3, containing 10% sodium chloride. The mixture was left overnight at 4°C and the mycelial debris was removed by filtration.

Step 2. Fractionation with ammonium sulfate. To the filtrate (14 liters) was added solid ammonium sulfate to give 95% saturation under stirring. After allowing to stand overnight, the resulting precipitate was collected by filtration, and dissolved in 0.05 M acetate buffer, pH 5.3. Insoluble material was removed by centrifugation, and solid ammonium sulfate was added to the solution to give 55% saturation. The resultant precipitate (Fraction I) was collected by centrifugation. The ammonium sulfate concentration of the supernatant solution was then brought to 60% saturation, and the precipitate was discarded. To the supernatant solution was added solid ammonium sulfate to give 90% saturation, and the precipitate (Fraction II) was collected by centrifugation. Fractions I and II were dissolved in 0.05 M acetate buffer, pH 5.3, separately. Each fraction was dialyzed overnight against 0.01 M acetate buffer, pH 5.3.

Step 3. DEAE-cellulose column chromatography. Fraction II, after being dialyzed, was applied to a DEAE-cellulose column (1.8 ϕ $\times$ 10 cm) equilibrated previously with 0.025 M Tris-HCl buffer, pH 7.42. Elution was carried out with 0.02 M acetate buffer, pH 5.3 (Fig. 1). The active fractions (Nos. 16 to 38) were collected, and solid ammonium sulfate was added to the solution to give 95% saturation. Any other active fraction was not obtained from the eluate with a linear gradient with 0 to 0.5 M sodium chloride in the same buffer. The resulting precipitate was collected by centrifugation and dissolved in 0.05 M acetate buffer,

Fig. 1. DEAE-cellulose Column Chromatography.

Applied protein, 3000 mg; flow rate, about 30 ml per hr; fraction volume, 16.25 ml; ●—●, absorbance at 280 nm; ○—○, α -glucosidase activity; ---, NaCl (mole).

pH 5.3. The solution was dialyzed overnight against 0.01 M acetate buffer, pH 5.3.

Step 4. Preparative gel electrofocusing. The solution obtained from Step 3 was subjected to preparative gel electrofocusing with 7.5% polyacrylamide gel containing carrier ampholyte (pH range, 4 to 6). The equipment was made up of a running column ($0.5\phi \times 10$ cm) and two chambers. The running column was composed of 0.56 ml of a sample gel and 1.2 ml of a separating gel. The upper and lower chambers contained 300 ml of 1% ethylenediamine aqueous solution and 300 ml of 1% phosphoric acid aqueous solution, respectively. Electrofocusing was carried out at 80 V for 48 hr with the anode in the lower chamber. After electrofocusing, one of the gels was stained and destained by the same procedure as described in MATERIALS AND METHODS. Sections of the unstained gel corresponding to protein bands in the stained gel were excised and crushed in 1 ml of deionized water per a gel. The pH of the water suspension of each piece was measured with a Hitachi-Horiba model M-5 pH meter, and then the same volume of 1 M acetate buffer, pH 5.3, was added to the water suspension. After standing for 2 to 3 hr, the gel was removed by filtration. The active filtrates of α -glucosidase were pooled and dialyzed overnight against 0.02 M

TABLE I. PURIFICATION OF α -GLUCOSIDASE AND GLUCOAMYLASE

	Protein			α -Glucosidase activity			Glucoamylase activity		
	Total mg	A_{280}/A_{290}	Total units	Specific activity units/mg protein	Recovery %	Total units	Specific activity units/mg protein	Recovery %	Total units
1 Crude extract	19600	0.70	8250	0.42	100	9240	0.47	100	
2 Ammonium sulfate fractionation saturation 0~55 (Fraction I)	9240	0.91	4730	0.51	57.3	7040	0.76	76.2	
saturation 60~90 (Fraction II)	3000	0.71	3600	1.20	43.6	2920	0.97	31.6	
I. Purification of α -glucosidase (Fraction II)									
3 DEAE-cellulose column chromatography	1200	0.66	1500	1.25	18.2	850	0.71	9.2	
4 Preparative gel electrofocusing	5	1.37	410	82.00	5.0	90	18.00	1.0	
II. Purification of glucoamylase (Fraction I)									
3' DEAE-cellulose column chromatography	2440	1.16	1370	0.56	16.6	4050	1.66	43.8	
4' Preparative electrofocusing	80	1.45	190	2.38	2.3	560	7.00	6.1	
5' Preparative gel electrofocusing	20	1.45	60	3.00	0.7	360	28.00	3.9	

acetate buffer, pH 5.3. The solution was concentrated by ultrafiltration, using a collodion bag. The concentrate was dialyzed overnight against 0.02 M acetate buffer, pH 5.3. A summary of the purification procedure is given in Table I.

II. Purification of glucoamylase

Step 3'. DEAE-cellulose column chromatography. After being dialyzed, Fraction I was chromatographed on a DEAE-cellulose column ($1.8\phi \times 20$ cm) under the same conditions as described in Step 3, and ammonium sulfate was added to the active fractions (Nos. 52 to 110) to give 60% saturation (Fig. 2). The resulting precipitate was collected by centrifugation and dissolved in 0.05 M acetate buffer, pH 5.3. The solution was dialyzed overnight against 0.01 M acetate buffer, pH 5.3.

FIG. 2. DEAE-cellulose Column Chromatography.

Applied protein, 9240 mg; flow rate, about 30 ml per hr; fraction volume, 16.25 ml; ●—●, absorbance at 280 nm; ○---○, glucoamylase activity; ---, NaCl (mole).

Step 4'. Preparative electrofocusing. The solution obtained from Step 3' was applied to a column of 110 ml capacity of an LKB 8100 Ampholine electrofocusing equipment with a density gradient of sucrose and a pH gradient of 4 to 6. After electrofocusing for 24 hr, 2 ml fractions were collected from the bottom of the column by means of a peristaltic pump. The active fractions were combined, and concentrated by ultrafiltration using a Diafilter G-05 T. The concentrate was dialyzed for 48 hr against two changes of 2 liters of 0.01 M acetate buffer, pH 5.3.

Step 5'. Preparative gel electrofocusing. The solution obtained from Step 4' was subjected to preparative gel electrofocusing by the same procedure as described in Step 4 except that pH range of carrier ampholyte used was 3 to 5. The active filtrates of glucoamylase were pooled and dialyzed overnight against 0.02 M acetate buffer, pH 5.3. The solution was concentrated by ultrafiltration, using a collodion bag. The concentrate was dialyzed overnight against 0.02 M acetate buffer, pH 5.3. A summary of the purification procedure is given in Table I.

III. Criteria of purity

1) *Gel electrofocusing.* The α -glucosidase and glucoamylase preparations were homogeneous on gel electrofocusing (Fig. 3). The isoelectric point of the α -glucosidase preparation was at pH 4.6, and that of the glucoamylase, at pH 4.1.

α -Glucosidase (300 μ g) Glucoamylase (200 μ g)

FIG. 3. Gel Electrofocusing of α -Glucosidase and Glucoamylase.

Gel electrofocusing was performed by the procedure as described in MATERIALS AND METHODS. Electrofocusing was run at a constant voltage of 80 V for 48 hr at 4°C using a conventional disc electrophoretic apparatus with $0.5\phi \times 8$ cm tubes. Migration was towards anode.

2) *Ultracentrifugation.* The sedimentation patterns of the α -glucosidase and the

FIG. 4. Sedimentation Pattern of α -Glucosidase and Glucoamylase.

Experimental conditions were as follows: temperature, 20°C; speed, 58,000 rpm; bar angle, 70°; cell used, a standard 12 mm double sector cell. Pictures were taken at indicated times after maximum speed had been reached. The protein concentration was 1.87 mg per ml of 0.01 M acetate buffer, pH 5.3, for α -glucosidase, and 7.28 mg per ml of 0.01 M acetate buffer, pH 5.3, for glucoamylase.

glucoamylase are illustrated in Fig. 4. The value of the sedimentation coefficient ($s_{20,w}$) was calculated to be 5.06 S for α -glucosidase and 3.36 S for glucoamylase.

IV. Properties of enzyme

1) *Molecular weight.* Molecular weight was determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. From the data of Fig. 5, the molecular weights of α -glucosidase and glucoamylase were calculated to be 51,000 and 55,000, respectively.

2) *pH optimum, pH stability, temperature optimum and heat stability.* pH optimum, pH stability, temperature optimum and heat stability are shown in Table II.

3) *Substrate specificity.* The activities of

FIG. 5. Determination of the Molecular Weight of α -Glucosidase and Glucoamylase.

SDS-polyacrylamide gel electrophoresis was performed as described in MATERIALS AND METHODS.

I, α -glucosidase; II, glucoamylase; (1), lysozyme; (2), myoglobin; (3), chymotrypsinogen A; (4), ovalbumin; (5), bovine serum albumin.

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

	α -Glucosidase	Glucoamylase
pH Optimum	5.0	4.5
pH Stability ^a	4.0~6.0	3.5~5.5
Temperature optimum	50°C	50°C
Heat stability ^b	up to 40°C	up to 40°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.

the α -glucosidase and the glucoamylase were assayed with various substrates (Table III). The α -glucosidase readily hydrolyzed maltose, phenyl α -maltoside, maltotriose, amylose and soluble starch, and weakly isomaltose, and very slightly panose, amylopectin and glycogen. The glucoamylase hydrolyzed amylose, amylopectin, glycogen, soluble starch, and maltotriose. Although the glucoamylase hydrolyzed maltose, the rate of the hydrolysis was much lower than those for amylaceous polysaccharides. The limits of hydrolysis for polysaccharides were also tested (Table III).

4) *Patterns of action on phenyl α -maltoside and amylose.* As shown in Fig. 6, the α -glucosidase and the glucoamylase hydrolyzed phenyl α -maltoside into glucose and phenyl α -glucoside. The two enzymes produced glucose from amylose, and glucose was the

TABLE III. SUBSTRATE SPECIFICITY

Substrate	Relative rate of hydrolysis* (%)		Km value (mg/ml)		Limit of hydrolysis (%)	
	I ^a	II ^b	I ^a	II ^b	I ^a	II ^b
Amylopectin	1.6	74.4	0.250		100	
Amylose	45.6	100	0.400	0.160	100	100
Glycogen	trace	54.8	0.210		100	
Soluble starch	22.8	84.4	0.134		100	
Maltotriose	58.2	54.0				
Panose	2.6	3.7				
Phenyl						
α-maltoside	50.9	13.3				
Isomaltose	4.9	trace				
Maltose	100	13.6	0.190	(5.55 × 10 ⁻⁴ M)		
Phenyl						
α-glucoside	trace	trace				
Sucrose	0	0				
Cellobiose	0	0				
Lactose	0	0				

* 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. Enzyme solution used was as follows: α-glucosidase (0.582 units) and glucoamylase (0.583 units).

^a α-Glucosidase; ^b Glucoamylase.

only detectable hydrolysis product. A linear relationship was observed between the degrees of dextrinization and hydrolysis of amylose. The degree of dextrinization was determined by the iodine color reaction.²³⁾

5) *Raw starch digestion.* The glucoamylase preparation was examined for glucoamylase activity toward boiled soluble starch, rice starch, buckwheat starch, corn starch, and potato starch (Table IV). For comparison, data for a glucoamylase from *Rh. niveus* are also included.

6) *Activities on oxidized amylose.* The α-glucosidase and the glucoamylase were examined for the enzyme activity toward amylose and oxidized amylose (Table V). For comparison, data for an α-glucosidase from *M. javanicus*, a glucoamylase from *Rh. niveus*, and the mixed solution of glucoamylase from *Rh. niveus* and α-amylase from *B. subtilis* (liquefying type) are also included. While all the enzyme preparations released glucose from

FIG. 6. Pattern of Action on Phenyl α-Maltoside.

The reaction mixture (5 ml) containing 0.25 ml of the α-glucosidase preparation (7.276 units), 1 ml of 1% substrate and 3.75 ml of 0.05 M acetate buffer, pH 5.3, was incubated at 37°C. A 0.3 ml portion of the enzyme solution (33.028 units) was used for glucoamylase. For the estimation of glucose liberated, a portion of the reaction mixture was pipetted out at various time intervals, and heated in a boiling water bath for 5 min. For the estimation of phenol liberated, a portion of the reaction mixture was pipetted out at various time intervals, and the reaction was stopped by pouring into 2 ml of 5% Na₂CO₃. ○—○, glucose liberated by α-glucosidase; ●—●, phenol liberated by α-glucosidase; ○---○, glucose liberated by glucoamylase; ●---●, phenol liberated by glucoamylase.

TABLE IV. ACTIVITIES OF GLUCOAMYLASES ON RAW STARCH AND BOILED SOLUBLE STARCH

The reaction mixture containing 10 mg of raw starch, glucoamylase solution, and 0.05 M acetate buffer, pH 5.3, in a final volume of 5.0 ml was incubated at 37°C for 60 min under shaking conditions. Soluble starch dissolved in deionized water in a concentration of 1% was used as the substrate. The amounts of glucoamylase and glucoamylase from *Rh. niveus* (pure type) used were 0.5 ml (8.250 units) and 0.04 ml (12.420 units), respectively.

Enzyme Substrate	Glucoamylase (relative rate of hydrolysis (%))	
	Glucoamylase	Glucoamylase (<i>Rh. niveus</i>)
Boiled soluble starch	100	100
Rice starch	2.2	7.2
Buckwheat starch	1.1	4.6
Corn starch	2.7	3.7
Potato starch	0.6	0.4

amylose in the yield of approximately complete hydrolysis, there was a considerable difference in the release of glucose from the

TABLE V. ACTIVITIES OF α -GLUCOSIDASE AND GLUCOAMYLASE ON OXIDIZED AMYLOSE

The reaction mixture containing 0.2 ml of 1% oxidized amylose solution (or amylose solution), enzyme solution, 0.1 ml of 0.5 M acetate buffer, pH 5.3, and deionized water in a final volume of 1.0 ml was incubated at 37°C. A portion of the reaction mixture was pipetted out at various time intervals and stopped by heating the mixture in a boiling water bath for 5 min. The amount of glucose formed was determined by the same method as described in MATERIALS AND METHODS. The amounts of enzymes used were as follows: α -glucosidase (7.420 units), glucoamylase (2.600 units), *M. javanicus* α -glucosidase (15.349 units), *Rh. niveus* glucoamylase (8.458 units), *B. subtilis* α -amylase (0.2 units).

Enzyme		<i>L. edodes</i> (Berk.) Sing.		<i>M. javanicus</i>	<i>Rh. niveus</i> glucoamylase	
		α -Glucosidase %	Glucoamylase %	α -Glucosidase %	+ none %	+ <i>B. subtilis</i> α -amylase %
Amylose	1. 15 min	23.0	26.0	72.3	82.4	66.8
	2. 30 min	33.5	43.7	79.1	94.7	73.0
	3. 3 hr	98.7	100.2	97.5	97.4	108.6
Oxidized amylose	1. 15 min	20.3	24.0	42.0	42.9	44.5
	2. 30 min	29.6	40.0	39.2	41.7	49.8
	3. 60 min	37.2	40.7	42.0	50.0	54.3
	4. 3 hr	39.5	41.0	43.2	48.8	66.8
	5. 5 hr	49.0	41.3	46.0	47.9	64.0
	6. 9 hr	49.2	43.7	46.0	54.2	69.2
	7. 23 hr	49.5	45.6	46.0	55.0	76.1

%; hydrolysis degree of substrate.

oxidized amylose between a glucoamylase from *Rh. niveus* and the other enzymes. The glucoamylase from *Rh. niveus* and the mixed solution of *Rh. niveus* glucoamylase and *B. subtilis* α -amylase continued to release glucose from the oxidized amylose after approximately 50% hydrolysis. In contrast the release of glucose by the enzyme preparations from *L. edodes* (Berk.) Sing. and *M. javanicus* diminished to a negligibly low rate after approximately 50% hydrolysis of the oxidized amylose. It is assumed that the enzyme preparations from *L. edodes* (Berk.) Sing. and *M. javanicus* did not contain α -amylase.

7) α -Glucosyltransfer products. When the α -glucosidase was incubated with 5% maltose, unknown substances (temporarily named X₁, X₂, and X₃) were detected on a paper chromatogram (Fig. 7). As shown in Fig. 7, the main product, X₂, was presumed to be maltotriose on the basis of the similarities in the paper chromatographic mobility.

8) Anomeric configuration of fission products. Time-course of the reaction of the α -glucosidase and the glucoamylase with

FIG. 7. A Paper Chromatogram of Transglucosylation Products from Maltose.

The reaction mixture (2 ml) containing 0.1 ml of the α -glucosidase preparation (24.12 units), 1 ml of 10% maltose, 0.5 ml of 0.1 M acetate buffer, pH 5.3, and 0.4 ml of deionized water 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.

I, the reaction mixture (10 hr)+isomaltose (100 μ g); II, the reaction mixture (10 hr)+maltotriose (200 μ g); III, glucose (60 μ g)+maltose (150 μ g)+isomaltose (150 μ g)+maltotriose (300 μ g)+panose (300 μ g).

amylose was investigated by following the changes in the optical rotation. Two other amylases, from *B. subtilis* and *Rh. niveus*, were

FIG. 8. Optical Rotation Study.

The mixture (25 ml) containing 5.0 ml of 1% amylose aqueous solution, enzyme solution and deionized water was incubated at 40°C. The measurements of optical rotation were made 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 (173.7 units); ●---●, *Rh. niveus* glucoamylase (77.6 units); ○—○, α-glucosidase (168.2 units); ●—●, glucoamylase (88.4 units).

also studied for comparison. Figure 8 showed the changes in the optical rotation with time of incubation for the two amylases and the two enzymes from *L. edodes* (Berk.) Sing. The upward rotation of the digests upon the addition of alkali indicates that the product from amylose by the glucoamylase from *L. edodes* (Berk.) Sing. has β-configuration. On the contrary, it is indicated that the α-glucosidase from *L. edodes* (Berk.) Sing. produces α-glucose from amylose.

9) *Inhibitor.* Among the metal ions tested, Cu²⁺, Hg²⁺, Pb²⁺ and Zn²⁺ were the potent inhibitors for the α-glucosidase, and Al³⁺, Hg²⁺ and Pb²⁺ for the glucoamylase. They were also inhibited by Tris (Table VI).

DISCUSSION

An α-glucosidase and a glucoamylase from *L. edodes* (Berk.) Sing. were isolated in highly purified forms by a procedure including fractionation with ammonium sulfate, DEAE-cellulose column chromatography and pre-

TABLE VI. EFFECT OF METAL IONS AND SEVERAL ENZYME INHIBITORS

For the assay of the effect of metal ions, enzyme solution, 0.02 ml of 0.1 M metal ion solution in deionized water, and 0.05 M acetate buffer, pH 5.3, in a final volume of 0.4 ml were preincubated at 37°C for 30 min. After preincubation, 0.1 ml of 1% maltose (soluble starch for glucoamylase) was added and the resulting mixture was kept at 37°C for 15 min.

For the assay of the effect of several inhibitors, the enzyme solution, 0.2 ml of 0.1 M acetate buffer, pH 5.3, 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 (soluble starch for glucoamylase) and the resulting mixture was kept at 37°C for 15 min.

Enzyme solution used was as follows: α-glucosidase (0.582 units) and glucoamylase (0.583 units).

Metal ion or inhibitors	Concentration mm	Hydrolysis (residual activity, %)	
		α-Glucosidase	Glucoamylase
None		100	100
AlCl ₃	5	71.3	66.3
BaCl ₂ ·2H ₂ O	5	81.2	98.1
CaCl ₂	5	83.7	99.1
CoCl ₂	5	70.6	87.2
Cu(CH ₃ COO) ₂	5	21.3	81.9
HgCl ₂	5	2.5	70.7
KCl	5	81.2	100.5
MgCl ₂	5	87.2	97.0
MnCl ₂	5	90.1	100.5
NaCl	5	94.7	92.3
NiCl ₂	5	85.5	88.8
Pb(CH ₃ COO) ₂	5	15.2	74.4
ZnCl ₂	5	11.3	96.7
EDTA	5	83.7	82.6
Monoiodo-acetic acid	0.05	105.0	117.9
PCMB	0.05	59.6	78.9
Tris	50	53.8	50.4
Turanose	14.6	50.0	109.8

parative gel electrofocusing. The two enzymes were homogeneous as judged by polyacrylamide gel electrofocusing and in ultracentrifugation. The α-glucosidase readily hydrolyzed maltose, maltotriose, phenyl α-maltoside, amylose, soluble starch, and weakly isomaltose. α-Glucosidases from plants,²³⁻²⁵ *M. javanicus*,²⁶ *M. racemosus*,²⁷ *Pen. purpurogenum*,³ *Pen. oxalicum*,⁴ and pig liver²⁸ are known to

hydrolyze soluble starch, whereas α -glucosidase from *Asp. niger*⁸⁾ has no or very slight hydrolytic activity toward the substrate. In the substrate specificity, the α -glucosidase from *L. edodes* (Berk.) Sing. seems to be similar to the α -glucosidases from *Mucor* sp. and *Penicillium* sp., but different from those obtained from *Aspergillus* sp. Like α -glucosidases from *M. javanicus*,²⁶⁾ *M. racemosus*,²⁷⁾ *Pen. purpurogenum*,³⁾ pig liver,²⁸⁾ and *B. cereus*,²⁹⁾ α -glucosidase from *L. edodes* (Berk.) Sing. formed maltotriose as the main α -glucosyltransfer product from maltose. In contrast, isomaltose was the main α -glucosyltransfer product, when α -glucosidases from *Asp. niger*,⁸⁾ *Asp. oryzae*,⁹⁾ *Asp. awamori*,⁷⁾ *Schizosaccharomyces pombe*,³⁰⁾ and buckwheat²³⁾ were incubated with maltose. From the experimental results reported here and in the previous papers, it has been found that α -glucosidases from fungi can be classified into at least three groups according to their starch-degrading activities and their modes of transglucosylation action. The enzymes of the first 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 second group is composed of α -glucosidase from *Pen. oxalicum* which catalyzes the transfer of the α -glucosyl group from maltose to give isomaltose and panose, in addition to its hydrolytic activity for soluble starch. The third group includes α -glucosidases from *Asp. niger*, *Asp. oryzae*, *Asp. fumigatus*³¹⁾ and *Asp. awamori*. They hydrolyze soluble starch very weakly or not, and catalyze the transfer of the α -glucosyl residue of maltose predominately to the primary alcohol groups of the acceptor saccharides to produce isomaltose and panose.

The glucoamylase from *L. edodes* (Berk.) Sing. readily hydrolyzed amylose, amylopectin, glycogen, and soluble starch, converting them almost quantitatively into glucose. The complete hydrolysis of amylopectin and glycogen strongly suggests that the enzyme has the

ability to cleave α -1,6-glucosidic linkage of higher molecular substrates. The enzyme hydrolyzed maltose at a much lower rate than amylaceous polysaccharides. It hydrolyzed phenyl α -maltoside into glucose and phenyl α -glucoside, and degraded the raw starch preparations at the lower rate than glucoamylase from *Rh. niveus*. The enzyme from *L. edodes* (Berk.) Sing. hydrolyzed corn starch at the most rapid rate of the raw starch tested, whereas glucoamylases from *Rh. niveus*, *Pen. oxalicum*⁴⁾ and *Asp. awamori*⁷⁾ hydrolyzed rice starch most rapidly.

The changes in the optical rotation of the amylose digests upon the addition of alkali indicated that the α -anomer of glucose was produced when the α -glucosidase hydrolyzed amylose liberating glucose. On the other hand, the glucoamylase hydrolyzed amylose liberating β -glucose. α -Glucosidase and glucoamylase seem to resemble each other in the hydrolytic pattern of amylose, but they are very different in the configuration of the anomeric carbon atom of glucose which they produce.

Acknowledgments. The authors wish to express their thanks to Assistant Professor K. Kawazu, Faculty of Agriculture, Okayama University for the use of Yanaco model OR-50 automatic polarimeter and Professor J. Ozawa, the Institute for Agricultural and Biological Sciences, Okayama University for his encouragement. The authors are grateful to Miss A. Mino for her experimental assistance in this work.

REFERENCES

- 1) Y. Yamasaki, T. Miyake and Y. Suzuki, *Agric. Biol. Chem.*, **37**, 131 (1973).
- 2) Y. Yamasaki, Y. Suzuki and J. Ozawa, *ibid.*, **41**, 1553 (1977).
- 3) Y. Yamasaki, Y. Suzuki and J. Ozawa, *ibid.*, **40**, 669 (1976).
- 4) Y. Yamasaki, Y. Suzuki and J. Ozawa, *ibid.*, **41**, 1451 (1977).
- 5) Y. Yamasaki, Y. Suzuki and J. Ozawa, *ibid.*, **41**, 2149 (1977).
- 6) A. Tsuboi, Y. Yamasaki and Y. Suzuki, *ibid.*, **38**, 543 (1974).
- 7) Y. Yamasaki, Y. Suzuki and J. Ozawa, *ibid.*, **41**, 755 (1977).
- 8) Y. Tsujisaka and J. Fukumoto, *Symp. Enz.*

- Chem. Japan*, **13**, 84 (1958).
- 9) S. Sugawara, Y. Nakamura and T. Shimomura, *Bull. Agric. Chem. Soc. Jpn.*, **23**, 156 (1959).
- 10) J. Fukumoto, Y. Tsujisaka and K. Minamii, *Sci. Ind. (Japan)*, **28**, 92 (1954).
- 11) S. Hayashida, *Bull. Agric. Chem. Soc. Jpn.*, **21**, 386 (1957).
- 12) Y. Tsujisaka, J. Fukumoto and T. Yamamoto, *Nature*, **181**, 770 (1958).
- 13) T. Fukui and Z. Nikuni, *Agric. Biol. Chem.*, **33**, 884 (1969).
- 14) N. M. Papadopoulos and W. C. Hess, *Arch. Biochem. Biophys.*, **88**, 167 (1960).
- 15) A. Dahlqvist, *Biochem. J.*, **80**, 547 (1961).
- 16) O. Folin and V. Ciocalteu, *J. Biol. Chem.*, **73**, 627 (1927).
- 17) O. Warburg and W. Christian, *Biochem. Z.*, **310**, 384 (1941).
- 18) C. W. Wrigley, *J. Chromatogr.*, **36**, 362 (1968); *idem*, *Science Tools*, **15**, 17 (1968).
- 19) K. Weber and M. Osborn, *J. Biol. Chem.*, **244**, 4406 (1969).
- 20) N. E. Welker and L. L. Campbell, *J. Bacteriol.*, **86**, 681 (1963).
- 21) J. J. Marshall and W. J. Whelan, *Anal. Biochem.*, **43**, 316 (1971).
- 22) T. Yamamoto, M. Ukawa and J. Fukumoto, *Amylase Symposium*, **4**, 11 (1969).
- 23) M. Takahashi and T. Shimomura, *Agric. Biol. Chem.*, **32**, 929 (1968).
- 24) N. Takahashi, T. Shimomura and S. Chiba, *ibid.*, **35**, 2015 (1971).
- 25) S. Chiba and T. Shimomura, *ibid.*, **39**, 1041 (1975).
- 26) Y. Yamasaki, T. Miyake and Y. Suzuki, *ibid.*, **37**, 251 (1973).
- 27) Y. Yamasaki, Y. Suzuki and J. Ozawa, *ibid.*, **41**, 1559 (1977).
- 28) K. Uchida and Y. Suzuki, *ibid.*, **38**, 195 (1974).
- 29) Y. Yamasaki and Y. Suzuki, *ibid.*, **38**, 443 (1974).
- 30) S. Chiba and T. Shimomura, *ibid.*, **30**, 536 (1966).
- 31) M. J. Rudick and A. D. Elbein, *Arch. Biochem. Biophys.*, **161**, 281 (1974).
-