Studies on α -Glucosidase in Rice

Part I. Isolation and Some Properties of α -Glucosidase I and α -Glucosidase II

By Nobuyuki TAKAHASHI, Tokuji SHIMOMURA and Seiya CHIBA

Department of Agricultural Chemistry, Faculty of Agriculture, Hokkaido University

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Two kinds of α -glucosidase which were homogeneous in disc electrophoretic and ultracentrifugal analysis were isolated from rice seeds by means of ammonium sulfate fractionation and CM-cellulose, Sephadex G-100 and DEAE-cellulose column chromatography and designated as α -glucosidase I and α -glucosidase II.

Both α -glucosidases hydrolyzed maltose and soluble starch to glucose and showed same optimal pH (4.0) on the both substrates. In addition, both enzymes acted on various α -linked gluco-oligosaccharides and soluble starch but little or not on α -linked hetero-glucosides and α -1,6-glucan (dextran).

Activity of the enzymes on maltose and soluble starch was inhibited by Tris and erythritol. α -Glucosidase II was more sensitive to the inhibitors than α -glucosidase I.

K_m value for maltose was 1.1 mM for α -glucosidase I and 2.0 mM for α -glucosidase II.

Studies on α -glucosidase have been extensively made with microorganisms,^{1~6)} animals^{7~9)} and vegetables^{10,11)} and it is known

that the substrate specificity of the enzyme differs from each other according to its origin.

On the other hand, the existence of several different α -glucosidases has been reported in mammalian tissues. For instance, two α -glucosidases¹²⁾ usually described as acid and neutral α -glucosidases and distinguished by their optimal pH, have been found in a dog liver, and five α -glucosidases¹³⁾ in a human intestinal mucosa through heat inactivation experiment. As for plant kingdom, alfalfa α -glucosidase¹⁴⁾ has been reported to exist as a mixture of isoenzymes which were detected by continuous electrophoresis. However, attempts^{13~15)} to separate each α -glucosidase from a mixture of several different isoenzymes has not been suc-

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cessful, and thus the properties of each α -glucosidase have not been clear.

The present paper deals with the isolation of two α -glucosidases from rice seeds and some of their properties.

MATERIALS AND METHODS

Rice seeds. Ungerminated rice seeds (Shinsetsu species) were harvested in Hokkaido in 1969.

Carbohydrates. Maltose (Difco. Lab.), sucrose (Koso Chemical Co. Ltd.), turanose (Nakarai Chemicals Ltd.) phenyl- α -D-glucoside (Nakarai Chemicals Ltd.), glycogen (Nakarai Chemicals Ltd.), dextran (mol. wt. 197,000; Nakarai Chemicals Ltd.), methyl- α -D-glucoside (Tokyo Kasei Co. Ltd.), erythritol (Tokyo Kasei Co. Ltd.) and soluble starch (Junsei Chemicals Co. Ltd.) were obtained from commercial sources. Maltotriose and maltotetraose were prepared from the partial acid-hydrolysate of potato starch by carbon column chromatography. Isomaltose and isomaltotriose were prepared from the partial acid-hydrolysate of dextran (Tokyo Kasei Co. Ltd.) according to the method described by Turvey *et al.*¹⁶⁾ Panose was kindly supplied by Dr. S. Sugawara. Nigerose³⁾ and kojibiose¹⁷⁾ were prepared from maltose by the transglucosylation action of α -glucosidase. Amylose and amylopectin were obtained from potato starch according to the method of E. J. Wilson *et al.*¹⁸⁾

Enzyme assay. The α -glucosidase activity was determined by measuring the rate of formation of glucose from maltose (denoted as maltase activity) or of glucose from soluble starch (indicated as glucoamylase activity in this paper) by Somogyi's copper reagent method with some modification given by Kobayashi *et al.*¹⁹⁾ Reaction mixture for the assay of α -glucosidase activity consisted of 2 ml of 1% substrate (maltose or soluble starch), 1 ml of 0.1 M acetate buffer (pH 4.0) and 1 ml of enzyme solution and was incubated at 37°C for 10–30 min. One unit of maltase or glucoamylase

activity is defined as the amount of enzyme liberating 1 mg of glucose from maltose or soluble starch per 10 min under the conditions described above respectively.

One unit of β -amylase is defined as the amount of enzyme to liberate 0.1 mg of maltose from the reaction mixture containing 2 ml of 1% soluble starch, 1 ml of 0.1 M acetate buffer (pH 4.7) and 1 ml of enzyme solution per 10 min at 37°C. Maltose was measured by a modification of Somogyi's copper reagent method.

Glucose assay. The determination of glucose which was released from substrate by enzyme action was made with 0.5 M Tris-glucose oxidase (Sigma) reagent method described by Dahlqvist²⁰⁾ and potassium ferricyanide reagent method described by O. Schales *et al.*²¹⁾

Protein assay. Protein was measured by the method of Itzhaki *et al.*,²²⁾ using bovine serum albumin as standard.

Polyacrylamide disc electrophoresis. This was performed as described by Reisfeld *et al.*²³⁾ using β -alanine-acetic acid buffer (pH 4.5) for 90 min with a current of 3 mA per tube at 4°C. Gel was stained for protein with 1% Amido Black 10B in 7% acetic acid.

Ultracentrifugal analysis. Ultracentrifugal analysis was performed with a Spinco Model E ultracentrifuge.

CM-cellulose column chromatography. CM-cellulose (Mitsubishi Chemical Co. Ltd.) was equilibrated with M/20 acetate buffer (pH 6.2) and packed into a glass column. A linear gradient elution was carried out with the same buffer in the range of M/20 to M/2 at pH 6.2.

Sephadex G-100 column chromatography. Sephadex G-100 (Pharmacia Fine Chemicals) which was allowed to swell in M/20 acetate buffer (pH 4.7) for 2–3 days was packed into a column (3.2 × 64 cm). Elution was carried out with the same buffer at the rate of 10 ml per hr. To know a void volume and total volume of the column, a mixture of blue dextran (mol. wt. 2,000,000; Pharmacia Fine Chemicals) and sodium

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chloride was applied to the column. The blue dextran was assayed by measuring optical density at $630\text{ m}\mu$ and sodium chloride by conductometry. The void volume and the total volume of the column were determined to be 152 ml and 440 ml, respectively.

DEAE-cellulose column chromatography. DEAE-cellulose (Midori Juji Co. Ltd.) was equilibrated with $M/40$ phosphate buffer (pH 8.3) and packed into a glass column. A linear gradient elution was carried out with potassium chloride in the range of 0 to 0.25 M in the buffer.

All the column chromatographic experiments were performed at 4°C .

The paper chromatographic methods adopted were those described by S. Chiba *et al.*³⁾

RESULTS

1) Purification and isolation of α -glucosidases from rice seeds

Rice seeds were hulled and pulverized. The flour (5 kg) was extracted with 12 liters of $M/15$ phosphate buffer (pH 7.6) at room temperature for 8 hr under slow stirring. The extract was filtered through Hyflo Super-Cel and 10 liters of the clear filtrate was obtained. Solid ammonium sulfate was slowly added to the filtrate to make 70% saturation, keeping the pH 6.8~7.2 with 5N ammonia. The precipitate was dissolved in 1000 ml of distilled water and the solution was fractionated with solid ammonium sulfate under the pH range mentioned above. The precipitate obtained in a range of 20 to 40% saturation was dissolved in 300 ml of distilled water and dialyzed overnight against $M/20$ acetate buffer (pH 4.7) at 4°C . The precipitate in the dialyzate was removed by centrifugation. To the clear supernatant, solid ammonium sulfate was added to make 70% saturation and an aqueous solution of the resulting precipitate was dialyzed overnight against $M/20$ acetate buffer (pH 6.2) at 4°C . A portion of the dialyzate was applied on a CM-cellulose column treated with $M/20$ acetate buffer (pH 6.2) in advance, and the linear gradient elution was carried out with acetate

FIG. 1. CM-Cellulose Column Chromatography of the Precipitate with Ammonium Sulfate from the Extract of Rice Flour.

Column size; $3 \times 30\text{ cm}$ equilibrated with $M/20$ acetate buffer (pH 6.2). Applied protein; 92 mg. Elution; linear gradient with acetate buffer (pH 6.2). Each fraction; 15 ml. Flow rate; 45 ml/hr.

(○—○) optical density at $280\text{ m}\mu$
 (●—●) maltase activity
 (△—△) glucoamylase activity
 (□—□) β -amylase activity
 (— — —) concentration of acetate buffer

FIG. 2. Sephadex G-100 Column Chromatography of Fraction B.

Column size; $3.2 \times 64\text{ cm}$ equilibrated with $M/20$ acetate buffer (pH 4.7). Applied protein; 92 mg. Flow rate; 10 ml/hr. Each fraction; 4 ml.

(○—○) optical density at $280\text{ m}\mu$
 (●—●) maltase activity
 (△—△) glucoamylase activity

buffer (see the section of Materials and Methods). As shown in Fig. 1, nonadsorbed fraction A contained β -amylase, whose optimal was pH 4.7. Activities of both maltase and glucoamylase were found in fraction B (see Fig. 1).

To fraction B was added solid ammonium sulfate to make 70% saturation. The resulting precipitate was dissolved in a small amount of distilled water and then applied on a Sephadex G-100 column which was previously equilibrated with M/20 acetate buffer (pH 4.7), and elution was carried out with the same buffer. As shown in Fig. 2, maltase and glucoamylase were eluted in fraction C (fraction number 38~55). When fraction C was subjected to disc electrophoresis, two zymograms were always observed in several experiments as seen in the center tube (Fig. 4).

Solid ammonium sulfate was added to fraction C to make 70% saturation, adjusting the pH to 6.8~7.2 with 1N ammonia. The precipitate was dissolved in a small amount of M/40 phosphate buffer (pH 8.3) and dialyzed overnight against the same buffer at 4°C. The dialyze was applied on a DEAE-cellulose column previously equilibrated with M/40 phosphate buffer (pH 8.3). Elution was carried out with the same buffer until the enzyme activity was no more detected in an effluent, and then the adsorbed component was eluted linearly with potassium chloride in the buffer. As shown in Fig. 3, two peaks, early and late fractions, of protein having α -glucosidase activity were observed and designated as α -glucosidase I and α -glucosidase II, respectively.

Both fractions of α -glucosidase I and α -glucosidase II were dialyzed against distilled water at 4°C for 24 hr and concentrated by ultra-filter (Diaflo), and then used for further experiments.

A summary for the process of purification and isolation of the enzymes from rice seeds is shown in Table I. Total amount of α -glucosidase II as protein was 4 times more than

FIG. 3. DEAE-Cellulose Column Chromatography of Fraction C.

Column size; 1.9×18 cm equilibrated with M/40 phosphate buffer (pH 8.3). Applied protein; 30 mg. Elution; linear gradient with KCl in the buffer. Each fraction; 4 ml.

- (○—○) optical density at 280 mμ
- (●—●) maltase activity
- (△—△) glucoamylase activity
- (— — —) concentration of KCl

FIG. 4. Disc Electrophoresis of α -Glucosidases.

Conditions: β -alanine—glacial acetic acid buffer (pH 4.5), 3 mA/column, 90 min, 4°C.

left: α -glucosidase I

right: α -glucosidase II

center: a mixture of both α -glucosidases

TABLE I. PURIFICATION AND ISOLATION OF α -GLUCOSIDASES FROM RICE SEEDS

	Total volume (ml)	Total maltase activity (unit)	Total protein (mg)	Specific activity (unit/mg of protein)
Flour of rice seeds (5 kg)				
↓ extracted with 12 liters of M/15 phosphate buffer (pH 7.6) for 8 hr filtered through Hyflo Super-Cel				
Clear filtrate	10,000		39,600	
↓ added with amm. sulfate to 70% saturation at pH 6.8~7.2 filtered				
Precipitate				
↓ dissolved in 1000 ml of water fractionated with amm. sulfate between 20~40% saturation at pH 6.8~7.2	1,000	51,100	20,100	2.5
↓ Precipitate				
↓ dissolved in 300 ml of water dialyzed against M/20 acetate buffer (pH 4.7) at 4°C overnight centrifuged	300	45,000	4,600	9.6
↓ Supernatant				
↓ added with amm. sulfate to 70% saturation at pH 6.8~7.2	310	33,770	2,690	12.6
↓ Precipitate				
↓ dissolved in 200 ml of M/20 acetate buffer (pH 6.2) dialyzed against the same buffer at 4°C overnight				
↓ Dialyze				
↓ chromatographed on CM-cellulose				
↓ Eluate (Fraction B)	750	21,720	260	83.2
↓ added with amm. sulfate to 70% saturation at pH 6.8~7.2				
↓ Precipitate				
↓ dissolved in a small amount of water chromatographed on Sephadex G-100				
↓ Eluate (Fraction C)	75	15,270	92	166.0
↓ added with amm. sulfate to 70% saturation at pH 6.8~7.2				
↓ Precipitate				
↓ dissolved in a small amount of M/40 phosphate buffer (pH 8.3) dialyzed against the same buffer at 4°C overnight				
↓ Dialyze				
↓ chromatographed on DEAE-cellulose				
→ α -glucosidase I	40	249	10.1	24.6
→ α -glucosidase II	180	7,649	41.8	182.9

that of α -glucosidase I. The specific activity of α -glucosidase II was 7.3 times greater than that of α -glucosidase I.

2) Homogeneity of α -glucosidase I and α -glucosidase II

Disc electrophoretic analysis. Both α -glucosidase preparations were subjected to disc electrophoresis. As shown in Fig. 4, each α -glucosidase gave a single band and α -glucosidase II migrated faster toward cathode than α -glucosidase I under the conditions used above.

Ultracentrifugal analysis. Homogeneity of the α -glucosidases was also investigated by ultracentrifugation. The sedimentation patterns obtained were shown in Fig. 5. The patterns indicated apparent homogeneity of the preparations.

3) Properties of α -glucosidase I and α -glucosidase II

Effect of pH on the activity. The pH-activity curves on maltose and soluble starch are shown in Fig. 6. The pH-optimum of α -glucosidase I (Fig. 6-a) was 4.0~4.3 and α -glucosidase II (Fig. 6-b) 3.7~4.0.

Effect of temperature on the activity. The temperature-activity curves on maltose and soluble starch are shown in Fig. 7. Optimum temperature on maltose was 55°C and soluble starch 50°C, in each α -glucosidase.

Substrate specificity. The specificity of the α -glucosidase toward various glucosides was examined. The results are shown in Table II as a relative rate to hydrolysis of maltose. The relative ratio of glucose-liberating rate from α -1,4-glucosides were as follows; maltose :

FIG. 5. Sedimentation Patterns of α -Glucosidase I (a) and α -Glucosidase II (b).

Protein concentration; 0.68% (α -glucosidase I) and 0.89% (α -glucosidase II) in phosphate buffer (pH 6.1, ionic strength 0.10). Photographs were taken at indicated times after reaching 56,100 rpm. Temperature was 22.0°C.

FIG. 6. pH-Activity Curves of α -Glucosidase I (a) and α -Glucosidase II (b).

The reaction mixture containing 2 ml of 1% substrate (maltose or soluble starch), 2 ml of McIlvaine buffer at various pH and 1 ml of enzyme (α -glucosidase I; 3.5 unit as maltase or 3.1 unit as glucoamylase activity, α -glucosidase II; 3.4 unit as maltase or 4.2 unit as glucoamylase activity) was incubated for 15 min at 37°C. The activity was determined by a modification of Somogyi's copper reagent method.

(●—●) activity on maltose, (△—△) activity on soluble starch.

FIG. 7. Temperature Activity Curves of α -Glucosidase I (a) and α -Glucosidase II (b).

After a reaction mixture containing 0.2 ml of 0.5% substrate (maltose or soluble starch) and 0.2 ml of M/20 acetate buffer (pH 4.0) was preincubated for 5 min at various temperature, 0.1 ml of enzyme (α -glucosidase I; 0.04 unit as maltase or 0.03 unit as glucoamylase activity, α -glucosidase II; 0.05 unit as maltase or 0.04 unit as glucoamylase activity) was added and kept for 10 min at various temperature. The activity was determined by 0.5 M Tris-glucose oxidase reagent method.

(●—●) activity on maltose, (△—△) activity on soluble starch.

TABLE II. RATES OF HYDROLYSIS OF VARIOUS α -GLUCOSIDES BY α -GLUCOSIDASE I AND α -GLUCOSIDASE II

The reaction mixture containing 1 ml of substrate (5 mg/ml), 1 ml of M/10 acetate buffer (pH 4.0) and 1 ml of enzyme (α -glucosidase I; 0.18 unit, α -glucosidase II; 0.20 unit as maltase activity) was incubated for 10 min at 37°C. The liberated glucose was measured by 0.5 M Tris-glucose oxidase and (*) potassium ferricyanide reagent method.

Substrate	α -Glucosidase I Hydrolysis		α -Glucosidase II Hydrolysis	
	Glucose liberated (μ g/ml/10 min)	Relative rate (%)	Glucose liberated (μ g/ml/10 min)	Relative rate (%)
Maltose	60.0	100.0	72.0	100.0
Maltotriose	56.0	93.3	33.0	45.8
Maltotetraose	54.0	90.0	29.6	41.1
Soluble starch	6.2	10.3	4.0	5.5
Isomaltose	+		1.9	2.6
Isomaltotriose	+		0.5	0.7
Panose	+		2.2	3.0
Nigerose	50.0	83.3	61.1	84.9
Kojibiose	2.6	4.4	11.1	15.4
Sucrose	+		+	
Turanose	+		+	
Methyl- α -D-glucoside	—		—	
Phenyl- α -D-glucoside*	—		—	

+ weak, — negligible.

maltotriose : maltotetraose : soluble starch were 100 : 93.3 : 90.0 : 10.3 for α -glucosidase I and 100 : 45.8 : 41.1 : 5.5 for α -glucosidase II. Namely, α -glucosidase II is more specific toward maltose than α -glucosidase I. Both α -glucosidases showed little activity on the substrate containing α -1,6-glucosidic linkage, *e.g.* isomaltose, isomaltotriose and panose, and especially the activity of α -glucosidase I toward these substrates was almost negligible. Nigerose, α -1,3-glucoside, was hydrolyzed to a similar extent, 83.3 and 84.9, and kojibiose α -1,2-glucoside 4.4 and 15.4, by α -glucosidase I and α -glucosidase II, respectively. An order of the hydrolysis rate toward various α -linked glucosaccharides was α -1,4- > α -1,3- > α -1,2- > α -1,6-

TABLE III. RATES OF HYDROLYSIS OF VARIOUS POLYSACCHARIDES BY α -GLUCOSIDASE I AND α -GLUCOSIDASE II

The reaction mixture containing 2 ml of substrate (2 mg/ml), 1 ml of M/10 acetate buffer (pH 4.0) and 1 ml of enzyme (α -glucosidase I; 0.24 unit, α -glucosidase II; 0.25 unit as glucoamylase activity) was incubated for 10 min at 37°C. The liberated glucose was measured by 0.5 M Tris-glucose oxidase reagent method.

Substrate	α -Glucosidase I Hydrolysis		α -Glucosidase II Hydrolysis	
	Glucose liberated (μ g/ml/10 min)	Relative rate (%)	Glucose liberated (μ g/ml/10 min)	Relative rate (%)
Soluble starch	62.0	100.0	70.0	100.0
Amylose	20.0	32.3	36.3	51.8
Amylopectin	84.8	137.0	88.0	125.7
Glycogen	62.9	101.5	60.0	85.7
Dextran	—		—	

— negligible.

glucosidic linkage. However, the rice α -glucosidases acted on α -linked heteroglucosides very weakly or almost negligibly.

Rates of hydrolysis of various polysaccharides was also investigated. As shown in Table III, order of hydrolysis rate was amylopectin > soluble starch $\geq$ glycogen > amylose. By paper chromatography, it was verified that glucose was the sole product having low molecular weight. On the other hand, no products were detected in paper chromatography from dextran even in a prolonged incubation (24 hr).

Effect of tris (2-amino-2-hydroxymethylpropane-1,3-diol) and erythritol

Tris²⁴⁾ and erythritol²⁴⁾ were known to inhibit maltase activity. The effects of these inhibitors on maltase and glucoamylase activity of the rice α -glucosidases were studied at a final concentration 100 mM. The results are presented in Table IV. In the glucose assay,

24) B. B. Jørgensen and O. B. Jørgensen, *Biochim. Biophys. Acta*, **146**, 167 (1967).

TABLE IV. EFFECT OF TRIS AND ERYTHRITOL ON THE MALTASE AND THE GLUCOAMYLASE ACTIVITY OF α -GLUCOSIDASES

The reaction mixture containing 0.5 ml of 0.1% substrate (maltose or soluble starch) in M/20 acetate buffer (pH 4.0), 0.5 ml of 300 mM inhibitor and 0.5 ml of enzyme (α -glucosidase I; 0.08 unit as maltase activity or 0.08 unit as glucoamylase activity, α -glucosidase II; 0.1 unit as maltase activity or 0.09 unit as glucoamylase activity) was incubated for 20 min at 37°C. In control experiment, 0.5 ml of distilled water was used in place of the inhibitor. The liberated glucose was measured by 0.5 M Tris-glucose oxidase reagent method.

		Inhibition (%)	
		Tris (100 mM)	Erythritol (100 mM)
α -Glucosidase I	maltase activity	48.6	14.8
	glucoamylase activity	50.9	13.7
α -Glucosidase II	maltase activity	80.7	54.4
	glucoamylase activity	81.8	45.6

FIG. 8. Lineweaver-Burk Plot of α -Glucosidases Activity with Maltose as Substrate.

The reaction mixture containing 0.5 ml of substrate at various concentration in M/20 acetate buffer (pH 4.0) and 0.5 ml of enzyme was incubated for 10 min at 37°C. The liberated glucose was measured by 0.5 M Tris-glucose oxidase reagent method.

(○—○) α -glucosidase I, (●—●) α -glucosidase II.

0.5M-Tris buffer (pH 7.0) used in the glucose oxidase reagent suppressed the activities of both α -glucosidases completely.

Michaelis constant. Maltose was incubated at various concentrations with α -glucosidases

and the initial rate of glucose formation was measured. Lineweaver-Burk plot obtained is shown in Fig. 8. K_m value for maltose was calculated to be 1.1 mM for α -glucosidase I and 2.0 mM for α -glucosidase II.

DISCUSSION

The present study was carried out to examine the activity on maltose and soluble starch. Some plant α -glucosidase preparations^{10,11)} were reported to hydrolyze not only maltose but also soluble starch to glucose. However, it is not certain whether the activity on soluble starch is caused by α -glucosidase only or by the coexistence of α -glucosidase (maltase) with a slight amount of β -amylase, because the reported enzyme preparations were not proved to be homogeneous ones. Two kinds of α -glucosidase prepared here are freed from β -amylase and purified as homogeneous samples in both disc electrophoretic (Fig. 4) and ultracentrifugal analyses (Fig. 5). Therefore, the activity of the α -glucosidases toward soluble starch is not caused by the cooperative action of maltase with β -amylase. An activity ratio of the α -glucosidases on maltose and soluble starch was not changed by pH (Fig. 6) and the presence of the inhibitors employed here (Table IV), but changed a little by temperature (Fig. 7). These facts suggest that both activities are due to the same protein, but both activities are not always catalyzed at the same active site.

The α -glucosidases obtained here acted on α -linked gluco-oligosaccharides but little or not on α -linked hetero-oligosaccharides (Table II). These properties in substrate specificity are similar to those of alfalfa¹⁴⁾ and barley malt α -glucosidase.¹⁰⁾ However, the relative rates of hydrolysis of substrate are different from each other, for instance, in the case of alfalfa α -glucosidase, relative activity is maltose : 100, nigerose : 24, kojibiose : 24, isomaltose : 26 and sucrose : 121 and in the case of barley malt α -glucosidase, maltose : 100, and isomaltose : 6.

These values may be expressed as a sum of cooperation of several enzymes because the alfalfa and the barley malt enzyme employed were crude or partially purified preparation.

The rice α -glucosidases could hydrolyze α -1,6-glucosidic linkage of oligosaccharide to some extent (Table II), whereas the release of glucose from dextran was not observed (Table III). Moreover, the rice α -glucosidases were eluted within a total volume range of the Sephadex G-100 column (Fig. 2). In contrast, mammalian acid α -glucosidase⁷⁾ was reported to hydrolyze dextran and to be eluted after the passage of a total volume of a Sephadex G-100 column.

Erythritol is glucose analogue and tris is not. However, the latter was more inhibitory than the former against the α -glucosidase activities. α -Glucosidase II was more sensitive to the inhibitors used here than α -glucosidase I.

K_m value for maltose was 1.1 mM for α -glucosidase I and 2.0 mM for α -glucosidase II. These values are similar to K_m values for barley malt (2.0 mM)¹⁰⁾ and alfalfa α -glucosidase (0.83 mM).¹⁴⁾ On the other hand, V_{max} was

found to be changed by ionic composition (this point and physical properties will be discussed in the next paper).

As above mentioned, the α -glucosidases I and II have the similar general properties (Tables II and III) but differ from each other in the behavior on DEAE-cellulose column chromatography (Fig. 3), the mobility in disc electrophoresis (Fig. 4), the sensitivity to the inhibitors (Table IV) and the affinity for maltose (Fig. 8). From these data, it is presumed that these enzymes may be "isozymes" because the term "isoenzyme"²⁵⁾ is used to denote enzymatically active proteins, catalyzing the same reaction and existing in the same species but different in some of their physicochemical properties.

Recently, Marshall *et al.*²⁶⁾ reported that three acid α -glucosidases, which were presumed as isoenzymes, were isolated from sweet corn. However, the properties of these α -glucosidases were not published at present.

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