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Purification and Properties of α-Glucosidase from Slugs

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Three forms of α-glucosidase (EC 3.2.1.20), designated as I, II, and III, have been isolated from slugs by a procedure including fractionation with ammonium sulfate, Sephacryl S-200 HR column chromatography, CM-cellulose column chromatography, and preparative disc gel electrophoresis. The three enzymes readily hydrolyzed maltose and malto-oligosaccharides, but hydrolyzed isomaltose more slowly. α-Glucosidase III hydrolyzed soluble starch at a faster rate than maltose, but α-glucosidase I hydrolyzed soluble starch more slowly.

Key words: Slug, *Incilaria bilineata*, α-glucosidase.

INTRODUCTION

Slugs are distributed throughout Japan, and eat higher plants by halves. Slugs readily digest polysaccharides, such as starch, contained abundantly in plants. The in-vivo significance of glucose production from starch in plant tissue by α-glucosidases is not yet clear, although some authors[1,2] have suggested that the enzyme forms a part of the non-phosphorolytic pathway for the breakdown of starch, and is functioning in seed germination by hydrolyzing the oligosaccharides produced by α- and β-amylases. α-Glucosidases from rice seeds, germinated green gram, sugar-beet seeds, and millet seeds readily hydrolyze soluble starch, liberating glucose[3)-6)]. Moreover, α-glucosidases from barley seeds[7)] and millet seeds[6)] can hydrolyze native starch granules isolated from each plant. There are few reports on α-glucosidases, which can hydrolyze native starch. It is, therefore, of interest to investigate how α-glucosidases of the slug is involved in the hydrolysis of starch. We deal with the purification and properties of the α-glucosidases of slugs.

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MATERIALS AND METHODS

Materials. Maltose (HHH), maltotriose, maltotetraose, maltopentaose, maltohexaose, maltoheptaose, and isomaltose (Hayashibara Biochemical Laboratories, Inc.), soluble starch (Ishizu Pharmaceutical Co., Ltd.), and Sephacryl S-200 HR (Pharmacia Biotech Co. Ltd.) were obtained from commercial sources.

Enzyme assay. α-Glucosidase activity was determined as follows. The reaction mixture containing 0.1 ml of 1% maltose, 0.25 ml of 0.1 M acetate buffer, pH 4.5, and enzyme solution in a final volume of 0.5 ml was incubated at 37℃ for 1 hr. After incubation, the reaction was stopped by boiling for 5 min. The amount of glucose formed was measured by the method of Papadopoulos et al.[8], as modified by Dahlqvist[9]. One unit of α-glucosidase activity was defined as the amount of enzyme which liberates 1 μmol/hr of glucose from maltose under the conditions described above.

Xylan, avicel-hydrolyzing activities were determined by measuring the amount of products liberated from substrates according to the method of Somogyi[10].

Determination of protein. Protein was determined by the method of Warburg et al.[11]. The protein profiles in column chromatography were followed by measuring the absorbance of eluates at 280 nm.

RESULTS AND DISCUSSION

Isolation of α-glucosidase

Slugs (*Incilaria bilineata*) 64.2 g were suspended in 50 mM acetate buffer, pH 5.3, and homogenized by a mixer (National electric mixer MX-V 253, Matsushita Electric Industries) for 30 sec. The homogenate was centrifuged at 8,000 xg for 10 min, and the supernatant was collected. The supernatant was brought to 0.9 saturation with ammonium sulfate. The precipitate was collected by centrifugation, and dissolved in 50 mM acetate buffer, pH 5.3. Carbohydrate-hydrolyzing activity was abundant in the solution (Table 1). After reaction of the solution with soluble starch, malto-oligosaccharides were detected on a paper chromatogram. This indicates that slugs possess suitable enzymes for degrading polysaccharides, such as starch and cellulose, which is ubiquitous in plants. The solution was loaded on a Sephacryl S-200 HR column (2.8×93 cm). The column was eluted with 20 mM acetate buffer, pH 5.3, containing 150 mM sodium chloride. Two peaks (I and II) having

Table 1. Glycosidases from *Incilaria bilineata*

Substrate	Relative rate of hydrolysis (%)	
	pH 5.3	pH 6.8
Maltose	100	25.3
Cellobiose	56.0	53.4
Lactose	41.2	31.6
Sucrose	29.3	21.1
Soluble starch	64.1	16.2
Avicel[#1]	49.0	
Xylan[#1]	4.0	

The reaction mixture (0.5 ml) containing enzyme solution, 0.1 ml of 1% substrate and 50 mM acetate buffer, pH 5.3 (or phosphate buffer, pH 6.8), was incubated at 37℃ for 30 min. Glucose liberated was determined by the glucose oxidase-peroxidase method.

[#1], reducing sugars liberated were determined by the method of Somogyi.

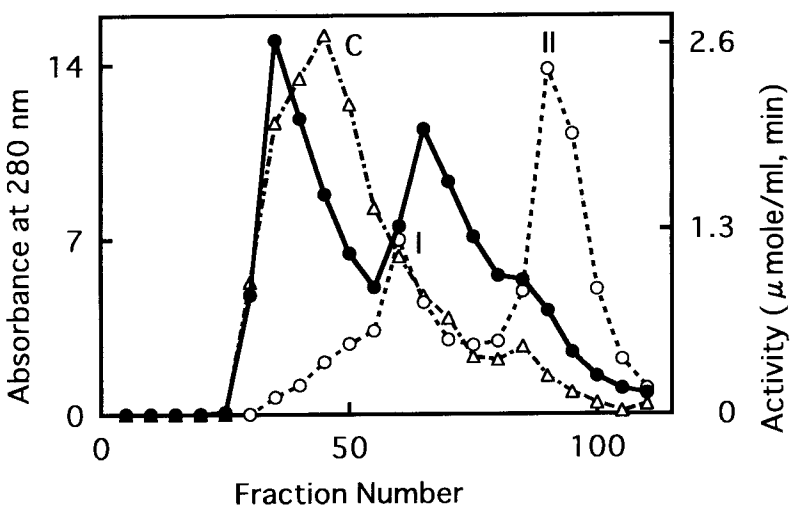

Fig. 1. Sephacryl S-200 HR column chromatography of α-glucosidases from slugs. The column was eluted with 20 mM acetate buffer, pH 4.5, containing 150 mM sodium chloride. The eluate was collected in 5.7 ml fractions. ●——●, Absorbance at 280 nm; ○·····○, maltose-hydrolyzing activity; △·····△, cellobiose-hydrolyzing activity; I, α-glucosidase I; II, α-glucosidase II; C; β-glucosidase.

α-glucosidase activity were obtained, and separated from β-glucosidase (Fig. 1). To the eluates containing α-glucosidases I and II was added ammonium sulfate to give 0.9 saturation. Each precipitate was collected by the same procedure of centrifugation as described above, and dissolved in 50 mM acetate buffer, pH 5.3. Each solution was separately put on a Sephacryl S-200 HR column (2.8×93 cm), and the column was eluted with the same

buffer as described above. The active fractions were collected, and brought to 0.9 saturation with ammonium sulfate. The precipitate was collected by centrifugation, and dissolved in 50 mM acetate buffer, pH 5.3. Each solution was dialyzed overnight against 20 mM acetate buffer, pH 4.5. The two enzyme solutions were separately put on a CM-cellulose column (1.6×10 cm) equilibrated with 20 mM acetate buffer, pH 4.5. Enzyme solution I was divided into two fractions (I -1, I -2). The three enzyme solutions (I -1, I -2, II) were brought to 0.9 saturation with ammonium sulfate. The precipitate was collected by centrifugation, and dissolved in 50 mM acetate buffer, pH 5.3. Each solution was dialyzed overnight against 20 mM acetate buffer, pH 4.5. Enzyme solutions I -1 and I -2 were subjected to preparative disc gel electrophoresis, pH 9.0. The gel and the electrode buffer were prepared by the method of Reisfeld et al.[12]. After electrophoresis, the gel was cut into 3 mm sections and crushed in 50 mM acetate buffer, pH 4.5. The active fractions were collected, and concentrated to 3 ml using an Amicon ultrafiltration device (PM-10 membrane ; Amicon Co.). The concentrate was dialyzed overnight against 20 mM acetate buffer, pH 4.5, and used as an α-glucosidase preparation to determine the enzymatic properties. Table 2 summarizes the purification procedures.

Table 2. Summary of purification of three α-glucosidases from slugs

Procedure	Total protein (mg)	α-Glucosidase activity		
		Total activity (U)	Specific activity (U/protein, mg)	Yield (%)
$(NH_4)_2SO_4$ fractionation	3,790	18,900	5.0	100
First Sephacryl S-200 HR column chromatography				
I	408	2,440	6.0	12.9
II	169	10,380	61.4	54.9
Second Sephacryl S-200 HR column chromatography				
I	95	1,480	15.6	7.8
II	76	10,000	131.6	52.9
CM-cellulose column chromatography				
I -1	30	640	21.3	3.4
I -2	13	150	11.5	0.8
II	38	4,980	131.1	26.3
Preparative disc gel electrophoresis				
I -1	0.36	30	83.3	0.2
I -2	0.12	11	91.7	0.1

General properties of enzymes

The pH optima of the three α-glucosidases were found to be 4.5 (I -1, II) and 5.5 (I -2). After a 20-hr preincubation at 30℃ with 50 mM McIlvaine's buffer, the three enzymes were stable in the pH range 3.0-8.0. The temperature optima of the α-glucosidases were 55℃ (I -1, II) and 45℃ (I -2). After a 15-min preincubation with 50 mM acetate buffer, pH 4.5, at various temperatures, the enzymes were stable up to 55℃ (I -1, II) and 45℃ (I -2).

Substrate specificity

The activity of the three enzymes on various substrates was examined. Table 3 shows the relative rates of hydrolysis. The three enzymes readily hydrolyzed maltose and malto-oligosaccharides, but hydrolyzed isomaltose weakly. Since we measured the amount of glucose liberated from the substrate as described in MATERIALS AND METHODS, the hydrolysis values of maltose and isomaltose were twice as high as those of the other substrates for the same degree of hydrolysis. α-Glucosidase II had higher substrate specificity for maltooligosaccharide and soluble starch than for maltose.

α-Glucosidase II was the most abundant enzyme of α-glucosidases contained in slugs. In conclusion, α-glucosidase II may play an important role for the breakdown of starch in slugs.

Table 3. Substrate specificity of three α-glucosidases from slugs

	Relative rate of hydrolysis (%)		
	I -1	I -2	II
Maltose	100	100	100
Isomaltose	11.6	8.9	15.5
Maltotriose	62.1	50.6	84.9
Maltotetraose	59.4	38.7	113.5
Maltopentaose	58.2	46.4	72.9
Maltohexaose	44.1	35.4	64.9
Maltoheptaose	39.5	30.4	64.4
Soluble starch	10.7	28.5	66.9

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ナメクジのα-グルコシダーゼの精製と性質

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ナメクジには多くのグリコシダーゼが存在し，摂取する植物多糖の代謝に関与している。その中で著量に存在し，澱粉代謝に関わっているα-グルコシダーゼについて研究を行った。ナメクジを破砕し，得られたα-グルコシダーゼを硫安分画，セファクリルS-200HRカラムクロマトグラフィー，CM-セルロースカラムクロマトグラフィー，調製用ディスク電気泳動により精製した。本酵素はセファクリルS-200HRカラムクロマトグラフィーで2種(I，II)に分画された。I画分はCM-セルロースカラムクロマトグラフィーでさらに2種(I-1，I-2)に分画された。得られた3種のα-グルコシダーゼはマルトースやマルトオリゴ糖によく作用したが，イソマルトースに対する作用は弱かった。3種の中で最も多量に存在するα-グルコシダーゼIIは他の酵素（I-1，I-2）よりも可溶性澱粉に対する作用が顕著で，マルトースよりも強く作用した。

キーワード：ナメクジ，*Incilaria bilineata*，α-グルコシダーゼ