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Hydrolysis of Glucobioses by Glucoamylase from *Rhizopus niveus*

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Summary

The rates of hydrolysis of eleven glucobioses by crude and purified glucoamylase from *Rhizopus niveus* were determined. The rates of hydrolysis relative to maltose were as follows.

Crude glucoamylase: maltose (100)>laminaribiose (1.45)> gentiobiose (1.00)> β,β-trehalose (0.99) > cellobiose (0.75) > isomaltose (0.72)> α,β-trehalose (0.60) > sophorose (0.51)>nigerose (0.39). α,α-Trehalose and kojibiose were not hydrolyzed.

Purified glucoamylase: maltose (100) >isomaltose (0.50) >α,β-trehalose (0.14) >nigerose (0.12). The other glucobioses were not hydrolyzed.

When gentiobiose was hydrolyzed by crude glucoamylase, a new spot which seemed to be a transglucosylation product was detected after about three hours' incubation.

Glucoamylase is known to hydrolyze starch almost completely into glucose. Phillips and Caldwell (1) suggested that purified glucoamylase from *Rhizopus delemar* by-passed the α-1,6 glucosidic linkage of starch but did not hydrolyze them. Tsujisaka and Fukumoto (2), however, proved that crystalline glucoamylase from *Rhizopus delemar* was capable of hydrolyzing α-1,6 glucosidic bond as well as α-1,4 bond of starch. They also indicated that this enzyme had no effect on isomaltose. It has since been suggested (3,4) that isomaltose is hydrolyzed, although at a rate much less than that for maltose or starch. But the hydrolyzing activity of glucoamylase from genus *Rhizopus* to the other glucobioses such as α-1,1 α-1,2, α-1,3 linked glucobioses was not so far examined.

The present study was aimed to examine the hydrolyzing activity of crude and purified glucoamylase from *Rhizopus niveus* with the glucobioses of different linkages. Eleven kinds of glucobioses were used as the substrates and the rates of hydrolysis were determined both by crude and purified enzyme.

Materials and Methods

Materials

Maltose and cellobiose were commercial products from Tokyo Chemicals Industry Co., Ltd. α,α-Trehalose (5) was prepared from commercial baker's yeast. Kojibiose (6) and nigerose (7) were prepared from dextran produced by

Leuconostoc mesenteroides NRRL B-1299 strain and B strain, respectively. Laminaribiose (8) was prepared from the acetolyzate of Pachyman. Isomaltose and gentiobiose were separated from sweet potato starch hydrol (9). α,β-Trehalose (10), β,β-trehalose (10) and sophorose (11) were chemically synthesized. These glucobioses showed single spot by paper chromatography.

The crude enzyme used in this experiment was a commercial product from the Amano Seiyaku Co., Ltd., Nagoya, Japan. The purified enzyme was also a commercial product from Seikagaku Kogyo Co., Ltd., Tokyo, Japan, which was homogeneous by ultracentrifugal and electrophoretic analysis.

General methods

Paper chromatography was performed on Toyo No. 2 (qualitative) and No. 51 (quantitative) filter paper by three times ascending method with a mixture of n-butanol-pyridine-water (6:4:3). The reagents used for the detection of the compounds were aniline hydrogen phthalate (A.H.P.) (12) and silver nitrate (13).

The rates of hydrolysis of eleven glucobioses by crude and purified glucoamylase were determined by the paper chromatographic method. The reaction product was spotted on Toyo No. 51 filter paper. After irrigating the chromatogram with the above solvent, guide strips were cut off from the both sides of the chromatogram and the position of the sugars were located by A.H.P..

The zones corresponding to sugars were cut off and eluted with water. The eluted sugars were determined by the Anthrone method (14).

Results

Definition of Glucoamylase Activity

One milliliter of enzyme solution was added to the mixture of 5 ml of 1 per cent soluble starch solution and 4 ml of 0.1 M acetate buffer solution (pH 5.0) previously incubated at 40°C. After the mixture was incubated for 30 minutes, Fehling solution was added to the reaction mixture to inactivate the enzyme. The glucose formed was determined by the modified Bertrand method (15). One unit of glucoamylase was defined as the activity of enzyme producing 1 mg of glucose under the above conditions.

One milligram of crude and purified enzyme showed 13 and 115 units of glucoamylase activity, respectively.

Qualitative Analysis of Reaction Product by Paper Chromatography

The substrates used were eleven kinds of glucobioses. The enzyme solution used was buffered to pH 5.0 with 0.2 M acetate buffer.

Crude enzyme

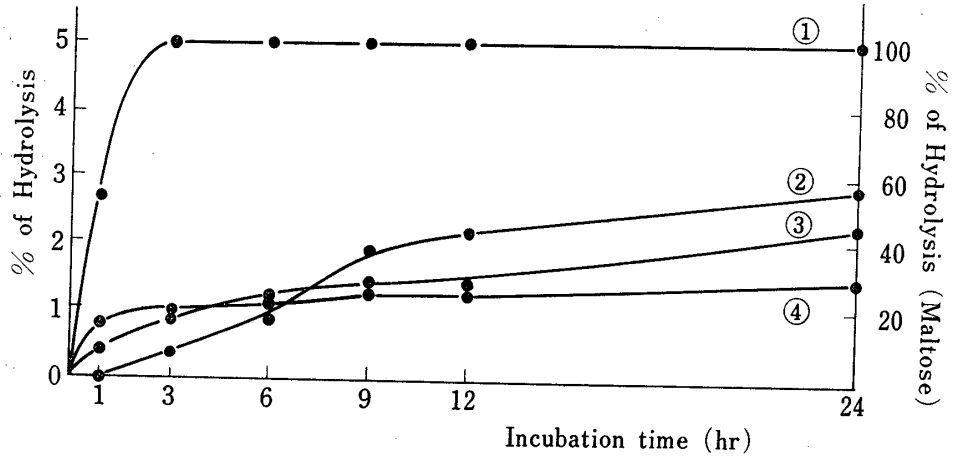
One milliliter of enzyme solution (26 units/ml) was mixed with the substrate

solution (20 mg/ml) and incubated at 55°C. After incubation periods of 0, 6, 12 and 24 hours, 0.2 ml of reaction products were pipetted and heated at 80°C for 5 minutes to inactivate the enzyme.

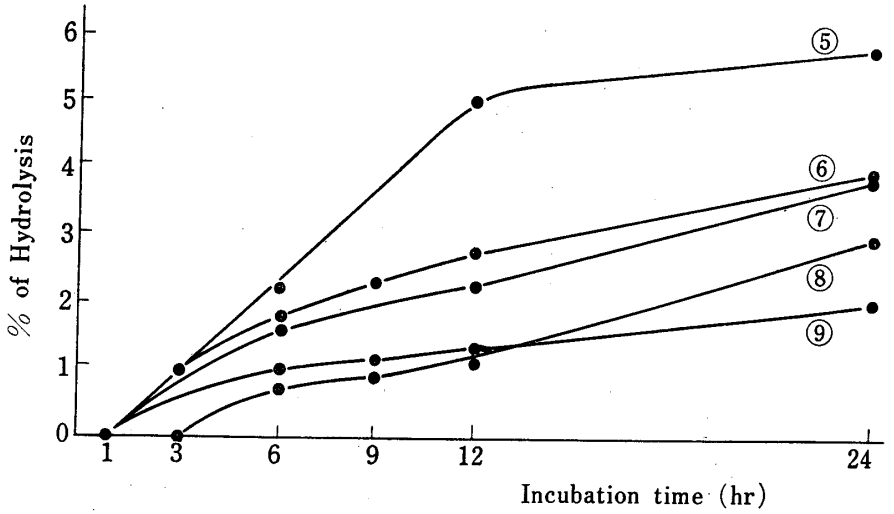
The reaction product was examined by paper chromatography. Kojibiose and α,α-trehalose were not hydrolyzed.

Purified enzyme

The enzyme solution (0.2 ml, 13 units/ml) was added to 2 mg of substrate and incubated at 55°C. After 24 hours, the reaction mixture was heated at 80°C for 5 minutes and examined by paper chromatography. Kojibiose, α,α-trehalose, β,β-trehalose and β-linked glucobioses were not hydrolyzed.



(a) Hydrolysis of α-linked glucobioses



(b) Hydrolysis of β-linked glucobioses

Fig. 1. Hydrolysis of glucobioses by crude glucoamylase
① Maltose, ② Isomaltose, ③ α,β-Trehalose, ④ Nigerose, ⑤ Laminaribiose, ⑥ Gentiobiose, ⑦ β,β-Trehalose, ⑧ Cellobiose, ⑨ Sophorose

Determination of the Reaction Products

The rates of hydrolysis of eleven glucobioses by crude and purified glucoamylase were determined.

Crude enzyme

The aliquots of 0.5 ml of substrate solution (100 mg/5 ml) were put into the test-tubes and incubated until the solution reached 55°C. The enzyme solutions (0.5 ml, 26 units/ml) were added into each test-tube and incubated at 55°C. After incubation periods of 0, 1, 3, 6, 9, 12 and 24 hours, each test-tube was heated at 80°C for 5 minutes to inactivate the enzyme. The same experiment was carried out with each substrate.

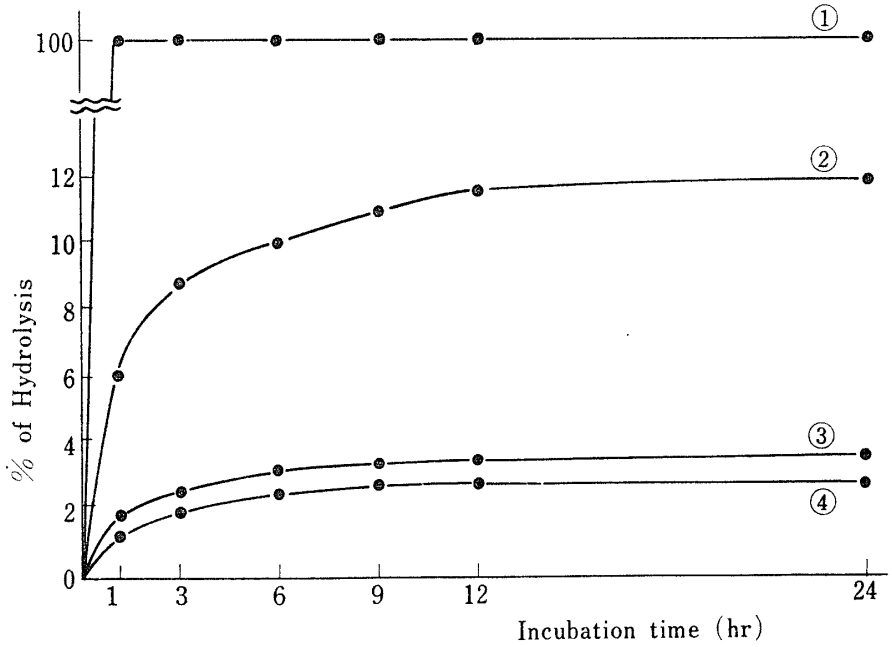


Fig. 2. Hydrolysis of glucobioses by purified glucoamylase
① Maltose ② Isomaltose ③ α,β-Trehalose ④ Nigerose

TABLE 1. *Rate of Hydrolysis Relative to Maltose*

	Rate relative to maltose	
	Crude glucoamylase	Purified glucoamylase
α,α-Trehalose	0	0
α,β-Trehalose	0.60	0.14
Kojibiose	0	0
Nigerose	0.39	0.12
Maltose	100	100
Isomaltose	0.72	0.50
β,β-Trehalose	0.99	0
Sophorose	0.51	0
Laminaribiose	1.45	0
Cellobiose	0.75	0
Gentiobiose	1.00	0

Purified enzyme

The substrate solution (40 mg/2 ml) and enzyme solution (2 ml, 26 units/ml) were mixed and incubated at 55°C. After the incubation periods of 0, 1, 3, 6, 9, 12 and 24 hours, 0.5 ml of mixture was pipetted and heated at 80°C for 5 minutes.

The reaction products were determined by paper chromatography followed by the Anthrone method. The results are shown in Figs. 1 and 2. The rates of hydrolysis relative to maltose are shown in Table 1.

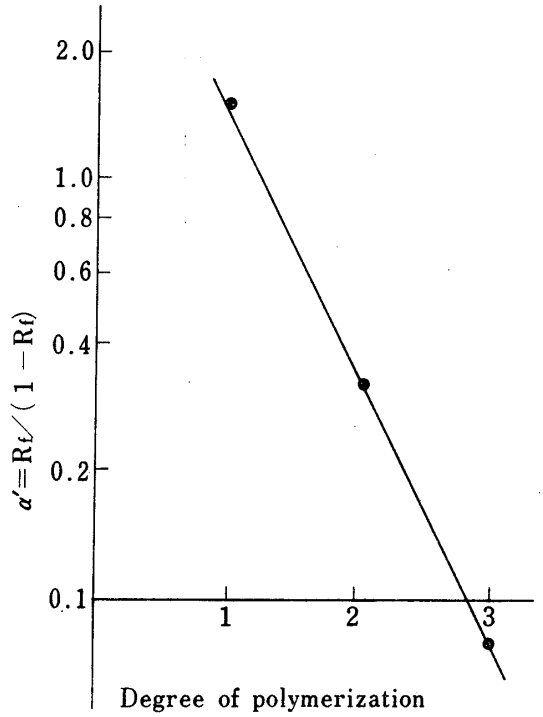


Fig. 3 Relation between α' value and DP.

Discussion

As shown in Figs. 1 and 2, β-linked glucobioses were not hydrolyzed by purified glucoamylase, while β-linked glucobioses were hydrolyzed by crude glucoamylase. The hydrolysis rates of α-linked sugars by crude glucoamylase, as compared with those of β-linked sugars, were smaller. These results suggest the contamination of β-glucosidase in the crude glucoamylase.

As to the 1,1-linked trehaloses, α,β-trehalose was hydrolyzed but α,α-trehalose was not. The hydrolysis of trehalose therefore seems to be different from that of other glucobioses.

When gentiobiose was hydrolyzed by crude glucoamylase, a new spot which seemed to be a transglucosylation product was detected after about three hours' incubation. From the R_f value on paper chromatogram this sugar seemed, most probably, to be a trisaccharide. And if it is assumed that this sugar is a trisacc-

haride. plot of log $R_f/(1-R_f)$ of this sugar, gentiobiose and glucose against degree of polymerization is in a linear relationship. These results strongly suggest that this sugar is gentiotriose (Fig. 3).

Acknowledgement

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