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Tohoku Journal of Agricultural Research
Vol. 20, No 3. Oct. 1969
Printed in Japan

Hydrolysis of Glucobioses by Glucoamylase from *Endomyces* sp.

Sugio KAWAMURA, Toshiyuki WATANABE and Kazuo MATSUDA

Department of Agricultural Chemistry, Faculty of Agriculture, Tohoku University, Sendai, Japan.

(Received, July 21, 1969)

Summary

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

Crude glucoamylase: maltose (100)>β,β-trehalose (10.6)>laminaribiose (9.93)>gentiobiose (7.15)>sophorose (7.13)>cellobiose (3.39)>isomaltose (0.53)>nigerose (0.29)>α,β-trehalose(0.21). α,α-Trehalose and kojibiose were not hydrolyzed.

Purified glucoamylase: maltose (100) >isomaltose(1.01)>nigerose(0.39)>α,β-trehalose (0.26)>β,β-trehalose (0.20). The other glucobioses were not hydrolyzed.

When β-linked glucobioses were hydrolyzed by crude glucoamylase, several tri- or tetrasaccharides which seemed to be a transglucosylation product were detected.

In the previous paper (1), the rates of hydrolysis of eleven kinds of glucobioses by crude and purified glucoamylase from *Rhizopus niveus* were examined in order to compare the hydrolyzing activity of both glucoamylase.

Properties of purified glucoamylase from *Endomyces* sp. has been studied by Fukumoto et al. (2), and Fukui and Nikuni (3). Hattori and Takeuchi (4) qualitatively examined the hydrolyzing activity of crude and purified glucoamylase from *Endomycopsis fibliger* to glucobioses. The hydrolysis of starch and gluco-oligosaccharides by *Endomyces* sp. glucoamylase was reported by the same authors (5).

The present paper deals with the examination of the hydrolyzing activity of crude and purified glucoamylase from *Endomyces* sp. IFO 0111 with eleven glucobioses.

Materials and Methods

Materials

Maltose and cellobiose were commercial products from Tokyo Chemicals Industry Co., Ltd. α,α-Trehalose (6) was prepared from commercial baker's yeast. Kojibiose (7) and nigerose (8) were prepared from dextran produced by

Leuconostoc mesenteroides NRRL B-1299 strain and B strain, respectively. Laminaribiose (9) was prepared from the acetolyzate of Pachyman. Isomaltose and gentiobiose were separated from sweet potato starch hydrol (10). α,β-Trehalose (11), β,β-trehalose (11) and sophorose (12) were chemically synthesized. These glucobioses showed single spot by paper chromatography.

The crude enzyme used in this experiment was a commercial product, Matulase G, from Matsutani Kagaku Kogyo Co., Ltd., Itami, Japan. The purified enzyme was kindly given by Dr. T. Fukui, Osaka University, which was homogeneous by ultracentrifugal and electrophoretic analysis.

General methods

Paper chromatography was carried out 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.) (13) and silver nitrate (14).

The rates of hydrolysis of eleven glucobioses by crude and purified *Endomyces* sp. glucoamylase were determined by paper chromatographic method. The reaction product was spotted on Toyo No. 51 filter paper. After developing 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 (15).

Results

Determination 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 (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 (16). One unit of glucoamylase was defined as the amount of enzyme which forms 1 mg of glucose under the above conditions.

One milligram of crude and purified enzyme had 11 and 211 units of glucoamylase activity, respectively.

Qualitative Analysis of the Reaction Product by Paper Chromatography

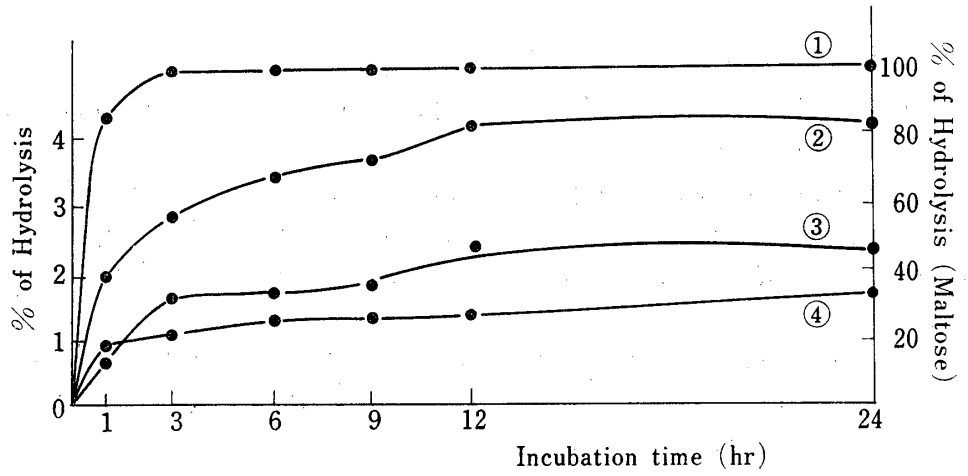
The substrates used were eleven kinds of glucobioses. The enzyme solution used was buffered at pH 5.0 with 0.2 M acetate buffer.

Crude enzyme

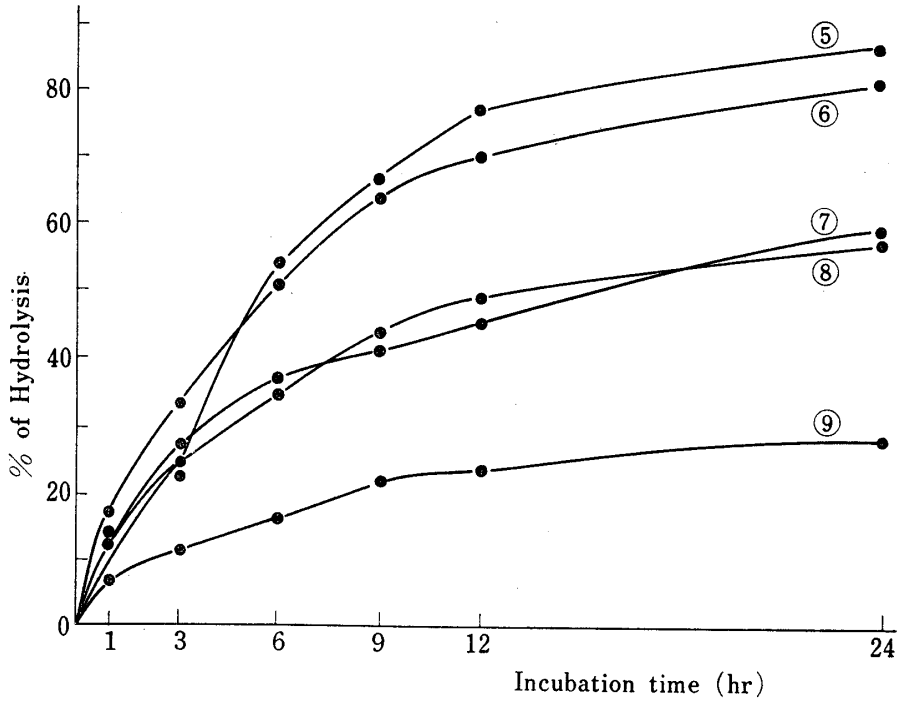
The mixture of enzyme solution (0.25 ml, 26 units/ml) and substrate solution (5 mg/0.25 ml) was incubated at 55°C.

Purified enzyme

The enzyme solution (0.2 ml, 13 units/ml) was added to 2 mg of substrate and the mixture was incubated at 55°C.



(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 Nigerose, ④ α,β-Trehalose ⑤ β,β-Trehalose, ⑥ Laminaribiose, ⑦ Gentiobiose, ⑧ Sophorose, ⑨ Cellobiose

After 24 hours, the reaction mixture was heated at 90°C for 5 minutes to inactivate the enzyme and examined by paper chromatography.

Kojibiose and α,α-trehalose were not hydrolyzed even by crude enzyme. By the purified enzyme, in addition to the above two sugars, β-linked glucobioses were not hydrolyzed except β,β-trehalose.

Determination of the Reaction Product

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

The substrate solution (40 mg/2 ml) and enzyme solution (2 ml, 26 units/ml) were mixed and incubated at 55°C. After incubation periods of 0, 1, 3, 6, 9, 12 and 24 hours, 0.5 ml of mixture was pipetted and heated at 90°C for 5 minutes to inactivate the enzyme. The same experiment was carried out with each substrate.

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.

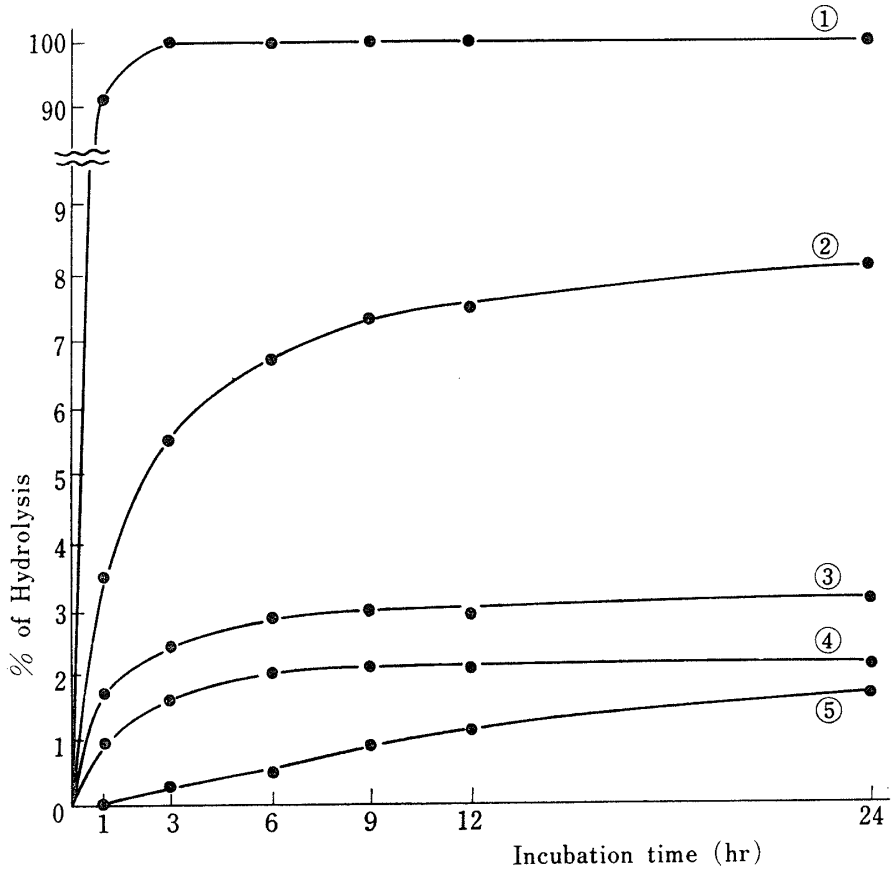


Fig. 2. Hydrolysis of glucobioses by purified glucoamylase.
① Maltose, ② Isomaltose, ③ Nigerose, ④ α,β-Trehalose ⑤ β,β-Trehalose

TABLE 1. *Rate of Hydrolysis Relative to Maltose*

	Endomyces sp. glucoamylase		*Rhizopus niveus* glucoamylase	
	Crude	Purified	Crude	Purified
α,α-Trehalose	0	0	0	0
α,β-Trehalose	0.21	0.26	0.60	0.14
Kojibiose	0	0	0	0
Nigerose	0.29	0.39	0.39	0.12
Maltose	100	100	100	100
Isomaltose	0.53	1.01	0.72	0.50
β,β-Trehalose	10.6	0.20	0.99	0
Sophorose	7.13	0	0.51	0
Laminaribiose	9.93	0	1.45	0
Cellobiose	3.39	0	0.75	0
Gentiobiose	7.15	0	1.00	0

Discussion

As shown in Figs 1 and 2, kojibiose, α,α-trehalose and β-linked glucobioses except β,β-trehalose were not hydrolyzed by purified glucoamylase, while β-linked glucobioses were hydrolyzed by crude glucoamylase. On the contrary, crude and purified glucoamylase from *Endomycopsis fibliger* could hydrolyze β-linked glucobioses (4). β-Glucosidase may be contaminated in this enzyme.

As compared with crude glucoamylase from *Rhizopus niveus*, the rates of hydrolysis of β-linked glucobioses by crude glucoamylase from *Endomyces* sp. were five to ten times larger than those by the former. This result shows that the β-glucosidase of the latter is much more active than the former. β, β-Trehalose was hydrolyzed by purified glucoamylase from *Endomyces* sp. but was not by purified enzyme from *Rhizopus niveus*. In this point, these two amylases seemed to be different in properties. It is reported (17) that *Aspergillus awamori* var. *fumeus*–1–B 42 produces two glucoamylase; acid-stable and less acid-stable glucoamylases, and that both purified glucoamylase have β,β-trehalase activity. The glucoamylase from *Endomyces* sp. is rather similar to glucoamylases from *Aspergillus awamori* in β, β-trehalase activity.

When β-linked glucobioses were hydrolyzed by crude glucoamylase from *Endomyces* sp., several tri- or tetrasaccharides which seemed to be a transglucosylation product were detected (Fig. 3) in each cases. This crude glucoamylase may have transglucosylation activity to β-linked glucobioses.

These tri- or tetrasaccharides produced by transglucosylation are now investigated.

Acknowledgement

The authors are grateful to Dr. T. Fukui, Osaka University, for the gift of purified glucoamylase from *Endomyces* sp., and also wish to thank Matsutani Kagaku Kogyo Co., Ltd., Itami, for the commercial glucoamylase, Matulase G.

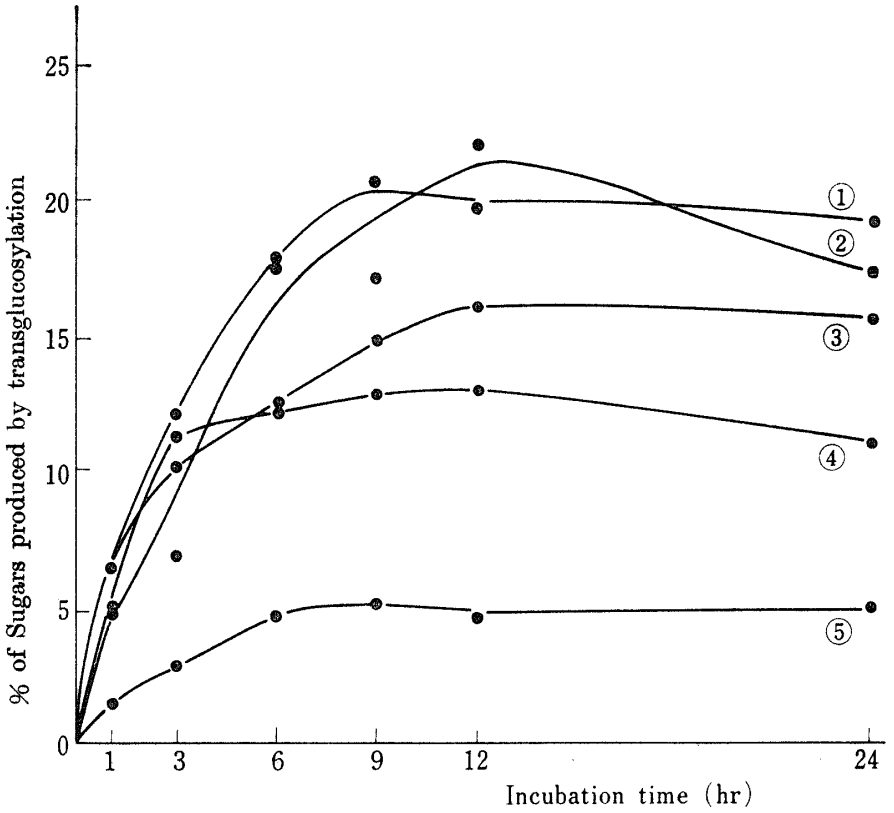


Fig. 3. Sugars produced by transglucosylation from β-linked glucobioses.
① Laminaribiose, ② β,β-Trehalose, ③ Sophorose, ④ Gentiobiose, ⑤ Cellobiose

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