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Hydrolysis of Glucobioses by *Aspergillus niger* Glucoamylase

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Summary

The rates of hydrolysis of eleven glucobioses by crude glucoamylase from *Aspergillus niger* were determined. The rates of hydrolysis relative to maltose were as follows: Maltose (100)>α,β-trehalose (4.13)>isomaltose (3.74)> kojibiose (3.52) > nigerose (3.48) > laminaribiose (0.28) > β,β-trehalose (0.26) > cellobiose(0.25)>sophorose(0.13)>gentiobiose(0.12). α,α-Trehalose was not hydrolyzed.

This enzyme was different from crude glucoamylase from *Rhizopus niveus* and *Endomyces* sp., specifically in the kojibiase activity.

In the previous papers (1, 2), the rates of hydrolysis of eleven kinds of glucobioses by glucoamylases made from *Rhizopus niveus* and *Endomyces* sp. were studied. In this paper, the hydrolysis of eleven glucobioses by glucoamylase made from *Aspergillus niger* was exmained and the rates of hydrolysis were compared with those by glucoamylases made from *Rhizopus niveus* and *Endomyces* sp..

Materials and Methods

Materials

Maltose and cellobiose were commercial products from the Tôkyo Chemicals Industry Co., Ltd. α,α-Trehalose (3) was prepared from commercial baker's yeast. Kojibiose (4) and nigerose (5) were prepared from dextran produced by *Leuconostoc mesenteroides* NRRL B-1299 and B, respectively. Laminaribiose (6) was prepared from the acetolyzate of Pachyman. Isomaltose and gentiobiose were separated from sweet potato starch hydrol (7), while α,β-trehalose (8), β,β-trehalose (8) and sophorose (9) were chemically synthesized. These glucobioses showed single spots by paper chromatography.

The crude enzyme was provided by Kyôwa Tôka Kôgyo Co., Ltd., Chiba. This enzyme was prepared with alcohol precipitation from the culture broth of *Aspergillus niger* mutant 19.

The determination of glucoamylase activity was as follows: One milliliter of enzyme solulion 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) which was incubated at 40°C before the addition. After the mixture was incubated for 30 minutes, the Fehling solution was added to the reaction mixture to inactivate the enzyme. The amount of glucose formed was determined by the modified Bertrand method (10).

One unit of glucoamylase was defined as the amount of enzyme required to form 1 mg of glucose under the above conditions. One milligram of crude enzyme had 9.6 units of glucoamylase activity.

General methods

The paper chromatography was carried out with Tôyo No. 2 (qualitative) and No. 51 (quantitative)filter paper using the three times ascending method with a solvent mixture of pyridine: n-butanol:water (4:6:3). The sugars were detected on the chromatogram using aniline hydrogen phthalate (A.H.P.) (11) and silver nitrate (12).

The degree of hydrolysis of the eleven glucobioses was determined by the paper chromatographic method. The hydrolyzate was spotted on Tôyo No. 51 filter paper. After developing the chromatogram with the above solvent, guide strips were cut off from both sides of the chromatogram and the position of the sugars were located by A.H.P. and silver nitrate. The zones corresponding to the sugars were cut off and eluted with water. The eluates were determined by the Anthrone method (13).

Results

Qualitative Analysis of the Reaction Product by Paper Chromatography

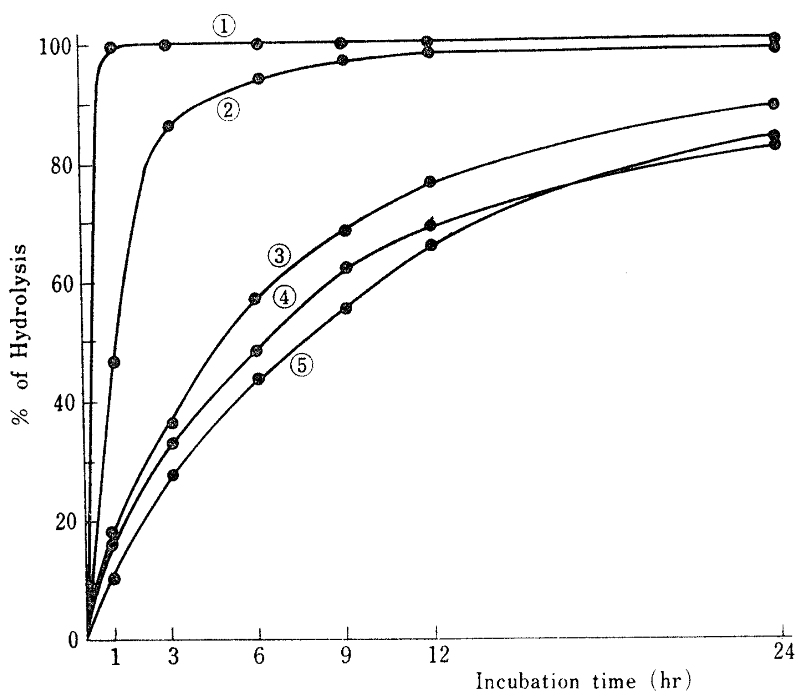
The substrates used were eleven kinds of glucobioses. The enzyme solution was buffered with pH 5.0, 0.2 M acetate buffer to make 26 units/ml of enzyme solution.

The mixture of the enzyme solution (0.25 ml) and the substrate solution (5 mg/0.25 ml) was incubated at 55°C for 24 hours. The mixture was heated at 85°C for 5 minutes to inactivate the enzyme and then examined by paper chromatography. α,α-Trehalose was not hydrolyzed.

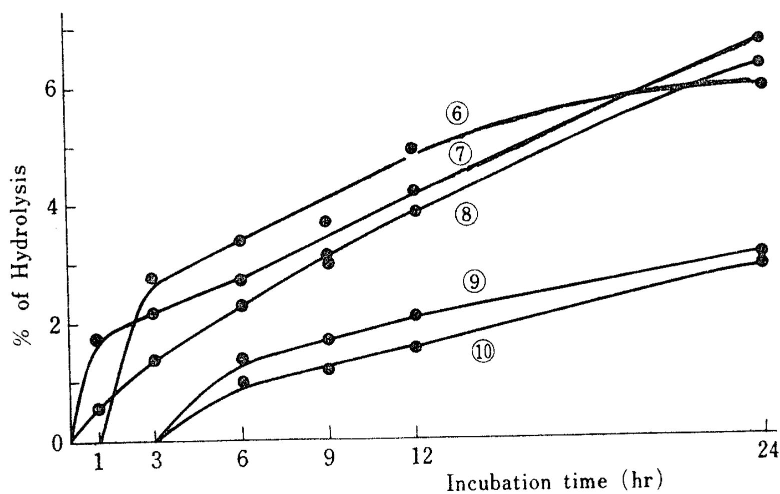
Determination of the Reaction Product

The rates of hydrolysis of ten of the glucobioses, except α,α-trehalose, were determined.

Two milliliters of the enzyme solution were mixed with the substrate solution (40 mg/2 ml) and incubated at 55°C. After incubation periods of 0, 1, 3, 6, 9, 12 and 24 hours, 0.5 ml of the mixture was pipetted and heated at 85°C for 5 minutes



(a) Hydrolysis of α-linked glucobioses



(b) Hydrolysis of β-linked glucobioses

FIG. 1. Hydrolysis of glucobioses by *Aspergillus* glucoamylase
① Maltose, ② α,β-Trehalose, ③ Isomaltose, ④ Nigerose, ⑤ Kojibiose, ⑥ Cellobiose, ⑦ Laminaribiose, ⑧ β,β-Trehalose, ⑨ Sophorose, ⑩ Gentiobiose

to inactivate the enzyme. The same experiments were carried out with each substrate.

The reaction products were determined by paper chromatography followed by the Anthrone method. The results are shown in Fig. 1. The rates of hydrolysis at 24 hours relative to maltose are shown in Table 1.

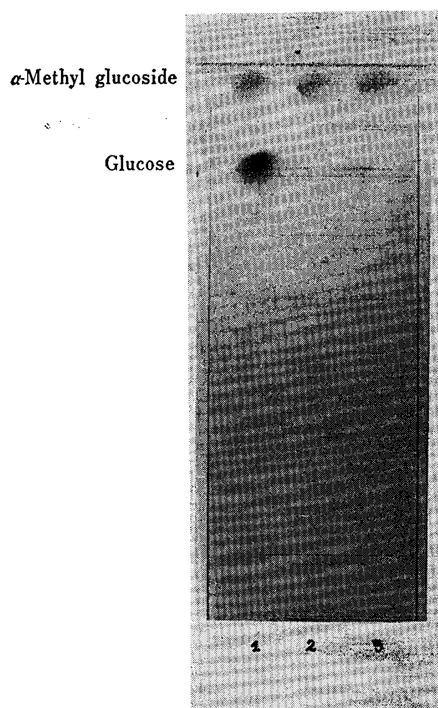


FIG. 2. Chromatogram of hydrolyzate of α-methyl glucoside by three glucoamylases 1 *Aspergillus niger*, 2 *Rhizopus niveus*, 3 *Endomyces* sp.

TABLE 1 *Rate of Hydrolysis Relative to Maltose*

	Aspergillus niger glucoamylase	*Rhizopus niveus* glucoamylase	*Endomyces* sp. glucoamylase
α,α-Trehalose	0	0	0
α,β-Trehalose	4. 13	0. 60	0. 21
Kojibiose	3. 52	0	0
Nigerose	3. 48	0. 39	0. 29
Maltose	100	100	100
Isomaltose	3. 74	0. 72	0. 53
β,β-Trehalose	0. 26	0. 99	10. 6
Sophorose	0. 13	0. 51	7. 13
Laminaribiose	0. 28	1. 45	9. 93
Cellobiose	0. 25	0. 75	3. 39
Gentiobiose	0. 12	1. 00	7. 15

Discussion

As shown in Fig. 1, only α,α-trehalose was not hydrolyzed by *Aspergillus niger* glucoamylase. Unlike the glucoamylases made from *Rhizopus niveus* and *Endomyces* sp., the *Aspergillus niger* glucoamylase had kojibiase activity (see Table 1). In this respect glucoamylase made from *Aspergillus niger* was distinctly different from the others. Moreover, the rates of hydrolysis of α-linked glucobioses by glucoamylase made from *Aspergillus niger* were much higher than those by glucoamylases made from *Rhizopus niveus* and *Endomyces* sp..

As shown in Fig. 2, crude glucoamylase made from *Aspergillus niger* was

capable of hydrolyzing α-methyl glucoside, while the glucoamylases made from *Rhizopus niveus* and *Endomyces* sp. were devoid of this activity. It has been reported that purified glucoamylase made from *Aspergillus niger* had no α-methyl glucosidase activity (15, 16). On the other hand, fungal transglucosylase is a typical α-glucosidase which can hydrolyze α-methyl glucoside (17). These results suggest that the high rates of hydrolysis of α-linked glucobioses by crude *Aspergillus niger* glucoamylase may be due to the coexistence of transglucosylase and glucoamylase. The existence of transglucosylase activity in crude glucoamylase made from *Aspergillus niger* was already suggested in the previous paper (14).

S.A. Barker et al. (15) and Ueyama et al. (18) exmained chromatographically the hydrolysis of several glucobioses using glucoamylase made from *Aspergillus niger*. The hydrolysis of glucobioses by *Aspergillus oryzae* glucoamylase was examined by Sawasaki (19). M. Abdullah et al. (20), Okazaki (21) and Watanabe and Fukimbara (22, 23) determined the rates of hydrolysis of glucobioses using glucoamylases made from *Aspergillus niger*, *Aspergillus oryzae* and *Aspergillus awamori*, respectively. In each case, α,α-trehalose was not hydrolyzed but β,β-trehalose was hydrolyzed by glucoamylase made from *Aspergillus awamori* (22). Moreover, the ease of hydrolysis of maltose, isomatlose and nigerose was different between *Aspergillus awamori* (22) and *Aspergillus niger* (18, 20).

As described above, there seemed to be some differences in the hydrolyzing activity of glucobioses among the *Aspergillus* glucoamylases.

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