BIOTECHNOLOGY LETTERS
Volume 16 No.11 (Nov.1994) p.1151-1156
Received as revised 16th September

REACTION MECHANISM OF ISOMALTOOLIGOSACCHARIDES SYNTHESIS BY α-GLUCOSIDASE FROM *Aspergillus carbonarious*

Kow Jen Duan*, Dey Chyi Sheu, Ming Tse Lin and Hsiao Chiang Hsueh

Department of Bioengineering, Tatung Institute of Technology, Taipei, Taiwan, R.O.C.

SUMMARY

An α-glucosidase from *Aspergillus carbonarious* CCRC 30414 was employed for investigating the enzymatic synthesis of isomaltooligosaccharides from maltose. The enzyme transferred a glucose unit from the nonreducing end of maltose and other α-linked glucosyl oligosaccharides to glucose and other glucosyl oligosaccharides which function as accepting co-substrates. The transfer of a glucose unit occurs most frequently to the 6-OH position of the nonreducing end of acceptor, but transfer to 4-OH position also occurs. Treatment of 30 % (w/v) maltose with the enzyme under optimum conditions afforded more than 50 % isomaltooligosaccharides.

INTRODUCTION

The previous paper described the purification and several properties of the α-glucosidase from *Aspergillus carbonarious* CCRC 30414 (Sheu *et al.*, 1994). The α-glucosidases from various organisms catalyse not only the hydrolysis of an α-glucosidic linkage but also the transglucosylation of an α-glucosyl residue to various glucosyl co-substrates resulting in the synthesis of new oligosaccharides. The transfer of a glucosyl moiety to various positions on the nonreducing ends of acceptors differed with various microorganisms. The *Sacch. logo* (Chiba *et al.*, 1973), *Asp. niger* (Benson *et al.*, 1982; Chiba *et al.*, 1979; Pazur *et al.*, 1978, 1986; Sheu *et al.*, 1993), and *Asp. oryzae* (Pazur *et al.*, 1951) α-glucosidases catalysed the transglucosylation to the 6-OH of the accepting glucose unit and yielded the oligosaccharides with an α-D-(1,6) linkage including isomaltose, panose, isomaltotriose, and tetrasaccharides which are defined as isomaltooligosaccharides. The isomaltooligosaccharides are of special interest to food industry, in the light of the fact that they are stimulating materials to *Bifidobacteria* of human intestines (Kanno, 1990). The paper is to investigate the reaction mechanism of isomaltooligosaccharides production from maltose catalysed by the α-glucosidase from *Aspergillus carbonarious* CCRC 30414.

MATERIALS AND METHODS

Materials The standard oligosaccharides and other chemicals were purchased from commercial sources. The purified α-glucosidase from *Aspergillus carbonarious* CCRC 30414 was obtained as described in the previous paper (Sheu *et al.*, 1994).

Transglucosylating activity Maltose, 0.1 g, in 1 ml sodium-acetate buffer (*p*H 5.0) was incubated with 10 µl enzyme solution at 37°C for 60 min. One unit of transglucosylating activity was defined as the amount of enzyme that produced one µmole panose per min under the described conditions. Panose was analysed by HPLC using Supelco LC-NH_2 column (4.6x250 mm), the mobile phase was acetonitrile/water (75:25, v/v) at 1.0 ml/min.

RESULTS

Reaction mechanisms With maltose as the initial substrate, glucose and panose are produced in large amounts as compared to maltotriose produced as shown in Table 1. The results provide the following transglucosylating reactions of the enzyme to maltose.

$$\underset{\text{(maltose)}}{\alpha\text{-D-Glcp}(1\rightarrow4)\text{-D-Glc}} + E \longrightarrow Glc^{*}E + Glc \qquad (1)$$

$$Glc^{*}E + \alpha\text{-D-Glcp}(1\rightarrow4)\text{-D-Glc} \longrightarrow E + \underset{\text{(panose)}}{\alpha\text{-D-Glcp}(1\rightarrow6)\text{-}\alpha\text{-D-Glcp}(1\rightarrow4)\text{-D-Glc}} \qquad (2)$$

$$Glc^{*}E + \alpha\text{-D-Glcp}(1\rightarrow4)\text{-D-Glc} \longrightarrow E + \underset{\text{(maltotriose)}}{\alpha\text{-D-Glcp}(1\rightarrow4)\text{-}\alpha\text{-D-Glcp}(1\rightarrow4)\text{-D-Glc}} \qquad (3)$$

The transfer of a glucosyl residue from maltose occurs most frequently to the 6-OH of the acceptor, and yields panose as the major transglucosylating product.

Table 1. Changes of each reaction component for 15 % (w/v) of maltose as the substrate catalysed by α-glucosidase from *Aspergillus carbonarious*.

Time (hr.)	Glucose (g/l)	Maltose (g/l)	Panose (g/l)	Maltotriose (g/l)
0	0.	150.	0.	0.
1	15.8	111.4	17.4	4.8
2	23.6	88.7	29.4	5.4

The reaction was carried out at 37°C in 0.1 M sodium-acetate buffer *p*H (5.0), 0.5 unit of enzyme was added to 0.5 ml of maltose solution.

When isomaltose was employed as the initial substrate, glucose and isomaltotriose were produced in a large amount as shown in Table 2. Isopanose was produced in a relatively small amount. The principal reactions are proposed as follows:

$$\underset{\text{(isomaltose)}}{\alpha\text{-D-Glcp}(1\rightarrow6)\text{-D-Glc}} + E \longrightarrow Glc^{*}E + Glc \quad (4)$$

$$Glc^{*}E + \alpha\text{-D-Glcp}(1\rightarrow6)\text{-D-Glc} \longrightarrow E + \underset{\text{(isomaltotriose)}}{\alpha\text{-D-Glcp}(1\rightarrow6)\text{-}\alpha\text{-D-Glcp}(1\rightarrow6)\text{-D-Glc}} \quad (5)$$

$$Glc^{*}E + \alpha\text{-D-Glcp}(1\rightarrow6)\text{-D-Glc} \longrightarrow E + \underset{\text{(isopanose)}}{\alpha\text{-D-Glcp}(1\rightarrow4)\text{-}\alpha\text{-D-Glcp}(1\rightarrow6)\text{-D-Glc}} \quad (6)$$

The transfer of a glucosyl residue from isomaltose occurs again most frequently to the 6-OH of the acceptor, so that isomatotriose is the main product and isopanose is a minor product.

Table 2. Changes of each reaction component for 15 % (w/v) of isomaltose as the substrate catalysed by α-glucosidase from *Aspergillus carbonarious*.

Time (hr.)	Glucose (g/l)	Isomaltose (g/l)	Isomaltotriose (g/l)	Isopanose (g/l)
0	0.	150.	0.	0.
1	14.3	115.5	16.4	3.8
2	26.9	91.7	21.8	7.1

The reaction was carried out at 37°C in 0.1 M sodium-acetate buffer *p*H (5.0), 1.0 unit of enzyme was added to 0.5 ml of isomaltose solution.

When panose was employed as the initial substrate, two kinds of tetrasaccharides as well as glucose and maltose were produced as shown in Table 3. The reactions are supposed to be:

$$\alpha\text{-D-Glcp}(1\rightarrow6)\text{-}\alpha\text{-D-Glcp}(1\rightarrow4)\text{-D-Glc} + E \longrightarrow Glc^{*}E + \alpha\text{-D-Glcp}(1\rightarrow4)\text{-D-Glc} \quad (7)$$

$$Glc^{*}E + \alpha\text{-D-Glcp}(1\rightarrow6)\text{-D-Glcp}(1\rightarrow4)\text{-D-Glc} \longrightarrow E + \underset{\text{(6-}\alpha\text{-D-glucosyl panose)}}{\alpha\text{-D-Glcp}(1\rightarrow6)\text{-}\alpha\text{-D-Glcp}(1\rightarrow6)\text{-}\alpha\text{-D-Glcp}(1\rightarrow4)\text{-D-Glc}} \quad (8)$$

$$Glc^*E + \alpha\text{-D-Glcp}(1\rightarrow6)\text{-}\alpha\text{-D-Glcp}(1\rightarrow4)\text{-D-Glc} \longrightarrow$$
$$E + \alpha\text{-D-Glcp}(1\rightarrow4)\text{-}\alpha\text{-D-Glcp}(1\rightarrow6)\text{-}\alpha\text{-D-Glcp}(1\rightarrow4)\text{-D-Glc} \quad (9)$$
(4-α-D-glucosyl panose)

It is noted that the enzyme cleaved the α-(1,6) rather than α-(1,4) linkage of the nonreducing end of the panose, and so yields maltose as shown in Equation 7. More 6-α-D-glucosyl panose is produced than 4-α-D-glucosyl panose.

Table 3. Changes of each reaction component for 15 % (w/v) of panose as the substrate catalysed by α-glucosidase from *Aspergillus carbonarious* .

Time (hr.)	Glucose (g/l)	Maltose (g/l)	Panose (g/l)	6-α-glucosyl panose (g/l)	4-α-glucosyl panose (g/l)
0	0.	0.	150.	0.	0.
1	5.4	9.5	118.2	10.7	6.3
2	9.9	12.6	103.2	15.6	8.9

The reaction was carried out at 37°C in 0.1 M sodium-acetate buffer *p*H (5.0), 0.50 unit of enzyme was added to 0.5 ml of panose solution.

To determine whether glucose is a glucosyl acceptor or not, glucose and maltose were employed as the substrates, and a large amount of isomaltose was produced (data are not shown). In the reaction without adding glucose, however, only panose and glucose were produced as shown in Table 1. The isomaltose is synthesized by the direct transfer of a glucosyl residue to another glucose as the following equation.

$$Glc^*E + Glc \longrightarrow \alpha\text{-D-Glcp}(1\rightarrow6)\text{-D-Glc} + E \quad (10)$$

Water can also act as acceptor for the glucosyl residue as shown in the following equation.

$$Glc^*E + H_2O \longrightarrow Glc + E \quad (11)$$

When the solution was diluted, more water competed with various glucosyl co-substrates for acceptor resulting in a lower yield of transglucosylation products.

Formation of isomaltooligosaccharides Formation of isomaltooligosaccharides catalysed by α-glucosidase of the *Aspergillus carbonarious* CCRC 30414 at a maltose concentration of

300 g/l is shown in Figure 1. Maltose was rapidly converted into glucose and panose according to Equations 1 and 2 in the beginning of reaction. Maltotriose was cleaved at a high rate resulting in the concentration of maltotriose increasing to a maximum value, and then decreasing when the concentration of maltose was less than 120 g/l. HPLC analysis of a reaction solution is shown in Figure 2.

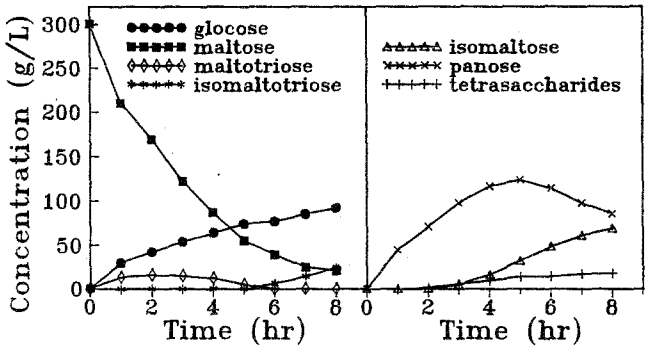

Fig. 1 Concentration change of each component for 30% (w/v) of maltose as the sole substrate catalysed by α-glucosidase from *Aspergillus carbonarious*. The reaction was carried at 37°C in 0.1 M sodium-acetate buffer (*p*H 5.4), 1.0 unit of enzyme was added to 0.5 ml of maltose solution.

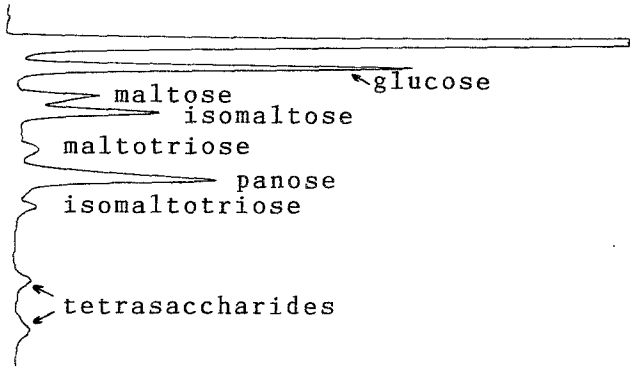

Fig. 2 HPLC analysis of an isomaltooligosaccharides solution.

DISCUSSION

α-glucosidases from various microorganisms perform not only hydrolysis but also transglucosylation to many D-glucosyl donor-substrates and acceptor-substrates. The *Sacch. logos*, *Asp. niger* and *Asp. oryzae* α-glucosidases transferred a glucosyl moiety to the 6-OH

position of an acceptor resulting in the synthesis of panose and isomaltose as the main products from maltose and glucose respectively. An α-1,4-transglucosylase from rat liver catalysed the production of maltotriose as the major product from maltose (Stetten, 1959). Buckwheat α-glucosidase, however, an α-1,2-, α-1,3-, α-1,4-transglucosylase, catalysed the formation of kojibiose, nigerose, and maltose from maltose and soluble starch (Chiba, 1979).

α-glucosidase of the present investigation catalysed the transfer of a glucosyl unit from maltose and other α-linked glucosyl oligosaccharides to glucose and other glucosyl oligosaccharides that functioned as accepting co-substrates. The transfer of glucose unit occurred most frequently to the 6-OH of the acceptor, but transfer to 4-OH also occurred. When a high concentration of maltose was employed as the only substrate, panose and isomaltose were the major transglucosylating products. Production of isomalto-oligosaccharides is generally carried out at a maltose concentration from 30 % to 50 % (W/V). The α-glucosidase of the present study is a suitable enzyme for isomaltooligosaccharides production, since the yield of oligosaccharides was more than 55 %.

ACKNOWLEDGMENTS

The authors are thankful for the financial support provided by the National Science Council through the grant NSC 82-0402-E-036-015, and the part of support provided by the Tatung company was appreciated.

REFERENCES

Benson, C.P., Kelly, C.T. and Fogarty, W.M. (1982), *J. Chem. Tech. Biotechnol.*, 32, 790-798.

Chiba, S., and Shimomura, T. (1973), *Agri. Biol. Chem.*, 37, 1831-1837.

Chiba, S. and Shimomura, T. (1979), *J. Jap. Soc. Starch Sci.*, 26, 59-67.

Kanno, T. (1990), *Denpun Kagaku*, 37, 87-97.

Pazur, J.H. and French, D. (1951), *J. Am. Chem. Soc.*, 73, 3536.

Pazur, J.H., Tominaga, Y., DeBrosse, C.W. and Jackman, L.M. (1978), *Carbohydrate Research*, 61, 279-290.

Pazur, J.H., DeHoff, D.K., Miskiel, F.J. and Baumrucker, C.R. (1986), *Carbohydrate Research*, 149, 137-147.

Sheu, D.C., Wang, S.S., Huang, S.F., Wang, H.F., and Duan, K.J. (1993), *J. of The Chin. Agri. Chem. Soc.*, 31(6), 740-751.

Sheu, D.C., Duan, K.J., and Lin, C.T. (1994), *Biotechnology Technique*, 8, 515-520.

Stetten, M.R. (1959), *J. Am. Chem. Soc.*, 81, 1437-1471.