Carbohydrate Research, 168 (1987) 285–294
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

FORMATION OF 6-*O*-α-MALTOSYLCYCLOMALTO-OLIGOSACCHARIDES BY TRANSFER ACTION OF THREE DEBRANCHING ENZYMES

YOSHIMICHI YOSHIMURA*, SUMIO KITAHATA†, AND SHIGETAKA OKADA
Osaka Municipal Technical Research Institute, 1-6-50, Morinomiya, Jyoto-ku, Osaka 536 (Japan)

(Received March 9th, 1987; accepted for publication in revised form, April 29th, 1987)

ABSTRACT

O-Maltosylcyclomaltohexaoses (G_2-cG_6) were formed in yields of 24.3 and 23.2 mmol from 40 mmol of α-maltosyl fluoride (α-G_2F) and 90 mmol of cyclomaltohexaose (cG_6) by the transfer action of pullulanase from *Aerobacter aerogenes* (A-pullulanase) and isoamylase from *Pseudomonas amyloderamosa*, respectively. These yields were three times that given by pullulanase from *Bacillus acidopullulyticus* (B-pullulanase). The yields of *O*-maltosylcyclomalto-oligosaccharides were changed according to the origin of the enzymes and the kind of cyclomalto-oligosaccharide (cG_6, cG_7, or cG_8) used as the acceptor. By the reaction with 40mmol of α-G_2F and 90 mmol of cG_6, 20 mmol of α-G_2F and 30 mmol of cG_7, or 40 mmol of α-G_2F and 90 mmol of cG_8, the amounts of *O*-maltosylcyclomalto-oligosaccharides produced and the transfer ratios of α-G_2F to the acceptors were as follows. By A-pullulanase, 24.3 mmol of G_2-cG_6 was produced in a 60.8% transfer ratio, whereas the yields of G_2-cG_7 and G_2-cG_8 were 1.7 mmol (8.5%) and 8.4 mmol (21.0%), respectively. The yields of G_2-cG_6, G_2-cG_7, and G_2-cG_8 by B-pullulanase were 8.8 mmol (22.0%), 1.2 mmol (6.0%), and 11.7 mmol (29.3%), respectively. In the case of isoamylase, G_2-cG_7 (9.2 mmol, 46.0%) and G_2-cG_8 (20.9 mmol, 52.3%) were produced, as much as for G_2-cG_6 (23.2 mmol, 58.0%). It was suggested that the difference in the amounts of G_2-cG_6 produced by these three debranching enzymes is based on the difference in the mode of action on the α-G_2F used as the substrate, either a transfer action or a hydrolytic action.

INTRODUCTION

Branched cyclomalto-oligosaccharides (cyclodextrins, cycloamyloses) are homogeneous oligosaccharides in which glucose and such maltooligosaccharides as maltose, maltotriose, and so on, are bound to cyclomalto-oligosaccharides by (1→6)-α-D-glucosidic linkages[1]. Recently, they have become of interest because of their high solubility compared with that of the cyclomalto-oligosaccharides[2,3], and

*Fujisawa Research Laboratory, Tokuyama Soda Co., Ltd., 2051 Endo, Fujisawa 252, Japan.
†To whom correspondence should be addressed.

0008-6215/87/$ 03.50

several methods for preparing them have been reported. *O*-D-Glucosylcyclomalto-oligosaccharides are produced by the action of cyclodextrin glucanotransferase[1,2,4,5] (EC 2.4.1.19) on amylopectin, following digestion by glucoamylase (EC 3.2.1.3). *O*-Maltosyl- or *O*-maltotriosyl-cyclomalto-oligosaccharides are formed by condensation of maltose or maltotriose with cyclomalto-oligosaccharides in the presence of pullulanase[6,7] (EC 3.2.1.41) or isoamylase[8] (EC 3.2.1.68), respectively.

We had previously[9] shown that *O*-maltosylcyclomalto-oligosaccharides are efficiently produced from α-maltosyl fluoride (α-G_2F) and cyclomalto-oligosaccharides by the transfer action of pullulanase from *Bacillus acidopullulyticus* (B-pullulanase). Recently, we found that pullulanase from *Aerobacter aerogenes* (A-pullulanase) and isoamylase from *Pseudomonas amyloderamosa* produce *O*-maltosyl-cyclomalto-oligosaccharides more efficiently than does B-pullulanase. We now describe the differences in reactivities of these three debranching enzymes.

EXPERIMENTAL

Materials. — Both crystalline and partially purified preparations of pullulanase from *Bacillus acidopullulyticus*[10] were donated by Novo Industri Japan Ltd. Pullulanase from *Aerobacter aerogenes*, and isoamylase from *Pseudomonas amyloderamosa*, were purchased from Seikagaku Kogyo Co., Ltd.

Cyclomaltohexaose, cyclomaltoheptaose, cyclomalto-octaose, and pullulan were supplied by Hayashibara Biochemical Laboratories, Inc. Amylopectin from potato was purchased from Sigma Chemical Co.

Hepta-*O*-acetyl-α-maltosyl fluoride, m.p. 173.5–177°, $[\alpha]_D^{25}$ +111.8° (*c* 0.8, chloroform), was synthesized by treating maltose octaacetate with cold, anhydrous hydrogen fluoride, according to the procedures previously described[11,12]. α-G_2F was generated by deacetylating the heptaacetate at 0° with fresh sodium methoxide in dry methanol. Solutions, of known concentration in dry methanol, were kept in a desiccator at −20°.

Assay of pullulanase and isoamylase activity. — For the assay of pullulanase activity, a reaction mixture containing 0.3 mL of 4% pullulan in 0.1M acetate buffer (pH 5.6 for A-pullulanase or pH 5.0 for B-pullulanase) and 0.3 mL of enzyme solution was incubated at 40°. In the case of isoamylase, 0.3 mL of 4% amylopectin in 0.1M acetate buffer (pH 4.0) was used as substrate. After 30 min, the reaction was stopped by adding 0.9 mL of 0.5M carbonate buffer (pH 10.0), and the reducing sugars released were measured in 0.5-mL aliquots by the Somogyi–Nelson method[13,14], with D-glucose as the standard. One unit of the activity was defined as the amount which liberated one μmol of reducing sugars as D-glucose per minute under the aforementioned conditions.

Assay for inhibition of debranching enzymes by cG_6. — Reaction mixtures containing 0.2 mL of 4% pullulan in acetate buffer (final concentration 70mM; pH 5.6 for A-pullulanase, or pH 5.0 for B-pullulanase), 0.2 mL of pullulanase solution (0.04 U), and 0.2 mL of various concentrations of cG_6 (in the range of 0.1–10mM)

were incubated at 40°. After 20 min, the amount of reducing sugars liberated was determined by the Somogyi–Nelson method. The inhibition of isoamylase action on amylopectin by cG_6 was determined at pH 4.0 in a similar manner.

Thin-layer chromatography. — Thin-layer chromatography (t.l.c.) of the reaction products was conducted on HPTLC NH_2F_{254s} plates (Merck Co., Ltd.; length 10 cm), using 13:7 (v/v) acetonitrile–water as the solvent, with two developments. The carbohydrates on t.l.c. plates were revealed by heating at 110–120° after spraying with sulfuric acid–methanol.

Liquid chromatography (l.c.). — Liquid chromatography was performed under the following conditions; column, Polygosyl 10-NH_2 (4 × 300 mm); solvent system, 13:7 (v/v) acetonitrile–water; flow rate, 2 mL/min; and detector, Shodex RE-11 refractometer.

Fluoride determination. — Fluoride anion concentrations were measured in the presence of TISAB buffer [M acetate buffer, pH 5.2; M sodium chloride; 0.4% of 1,4-cyclohexanebis(dinitrilotetraacetic acid) monohydrate] with a specific fluoride-ion probe (Iwaki specific ion meter, Model 225, and Orion combination fluoride electrode, Model 96-09).

Isolation of G_2-cG_6 and $(G_2)_2$-cG_6. — Four milliliters of 100mM acetate buffer (pH 5.6) containing 40mM α-G_2F, 90mM cG_6 and 3 U of A-pullulanase per mL was incubated for 1 h at 40°. To inactivate the enzyme and decompose the G_2 released, the reaction mixture was heated in boiling water for 30 min after adjusting the pH to 11.5–12.0 with M sodium hydroxide[15,16]. Then, the solution was decolorized, and de-ionized by means of an ion-exchange resin. The de-ionized solution was concentrated to ~4 mL and the concentrate was applied to a chromatographic column (2.2 × 120 cm) of Toyopearl HW-40S, and eluted with water at a flow-rate of 18 mL/h. G_2-cG_6 and $(G_2)_2$-cG_6 fractions were pooled separately, and lyophilized: the yields of G_2-cG_6 and $(G_2)_2$-cG_6 were 105 mg and 25 mg, respectively.

Identification of multi-branched cyclomalto-oligosaccharides. — Such multi-branched cyclomalto-oligosaccharides as $(G_2)_2$-cG_6, $(G_2)_3$-cG_6, $(G_2)_2$-cG_7, $(G_2)_3$-cG_7, and $(G_2)_2$-cG_8, formed by debranching enzymes, were isolated by l.c. After each branched cyclomalto-oligosaccharide had been digested by glucoamylase and pullulanase, the amounts of products in the digests were determined by l.c., and the structures of the branched cyclomalto-oligosaccharides were identified by calculating the ratios of products as previously described[9].

RESULTS

Action of debranching enzymes on α-G_2F in the presence of cG_6. — Debranching enzymes were separately incubated with the mixture of α-G_2F and cG_6.

As shown in Table I, there was no difference in the amount of G_2-cG_6 produced, as between purified and crude enzyme preparations. A-Pullulanase and isoamylase had about three times the ability to form G_2-cG_6 as B-pullulanase. The yield of G_2-cG_6, the main transfer-product, was ninety times that given by the condensation reaction of cG_6 and maltose (G_2) with A-pullulanase.

TABLE I

FORMATION OF BRANCHED cG_6 BY DEBRANCHING ENZYMES[a]

Debranching enzymes		G_2-cG_6 *(mM)*
Name	*State*	
A-Pullulanase	crystalline	25.0
	partially purified	25.0
B-Pullulanase	crystalline	9.0
	partially purified	9.4
Isoamylase	crystalline	25.7
	partially purified	27.4
Condensation[b]	crystalline	0.28

[a]Each enzyme preparation (3 U/mL) was incubated with a mixture of α-G_2F (40mM) and cG_6 (90mM) for 1 h at 40°. [b]G_2 (40mM) and cG_6 (90mM) were used for the condensation reaction by A-pullulanase.

When amylase activity (in the enzyme preparations) that hydrolyzes α-G_2F to maltose was checked with maltopentaose (G_5) as the substrate, a slight activity was found only in the crude preparation of A-pullulanase. These results show that such differences in reactivity among these debranching enzymes are not based on the purity of the enzymes.

In the following experiments, however, crystalline preparations of three of the enzymes were used in order to avoid an interfering factor.

Effect of concentration of cG_6 on branched cG_6 formation. — For the purpose of investigation of the effect of cG_6 concentration on branched cG_6 formation, each debranching enzyme and α-G_2F were incubated with various concentrations of cG_6. After 1 h, the amounts of G_2 and branched cG_6 produced in the reaction mixtures (0.2 mL) were determined by l.c.

The amounts of G_2-cG_6 formed by A-pullulanase and B-pullulanase attained constant values at ~90mM cG_6, as shown in Fig. 1. These two enzymes formed $(G_2)_2$-cG_6 more than G_2-cG_6 under the conditions of low concentration of cG_6. A-Pullulanase also produced a slight amount of $(G_2)_3$-cG_6. On the other hand, in the case of isoamylase, the amounts of G_2-cG_6 increased linearly with increase of cG_6 concentration up to 60mM, and then gradually continued to increase, even at 150mM cG_6. The amounts of G_2-cG_6 formed from 40mM α-G_2F and 90mM cG_6 by A-pullulanase, B-pullulanase, and isoamylase were 24.0 mmol, 7.1 mmol, and 25.1 mmol, respectively.

The G_2-cG_6/$(G_2)_2$-cG_6 ratios at 90mM cG_6 of A-pullulanase, B-pullulanase and isoamylase were respectively calculated to be 5.2, 8.9, and 10.0 from the results shown in Fig. 1.

The amounts of G_2 released (hydrolyzate of α-G_2F) decreased with an increase of cG_6 concentration. Especially in the case of isoamylase, the amount of G_2

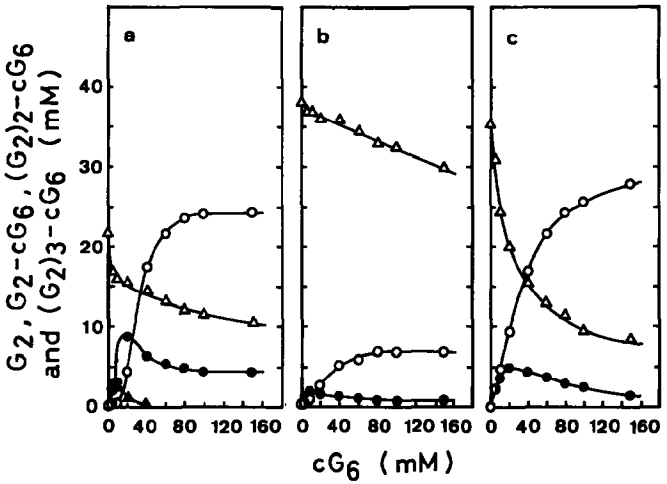

Fig. 1. Effects of concentration of cG_6 on branched cG_6 formation. [Each debranching enzyme (3 U/mL) and α-G_2F (40mM) were incubated with various concentrations of cG_6 for 1 h at 40°: (a) A-pullulanase; (b) B-pullulanase; (c) isoamylase; ○, G_2-cG_6; ●, $(G_2)_2$-cG_6; ▲, $(G_2)_3$-cG_6; and △, G_2.]

released was changed drastically from 35.5 mmol (in the absence of cG_6) to 8.6 mmol (at 150mM cG_6).

Effect of α-G_2F concentration on formation of branched cG_6. — An enzyme (A-pullulanase, B-pullulanase, or isoamylase) was incubated with various concentrations of α-G_2F in the presence of cG_6. The amounts of G_2 and branched cG_6 produced in the reaction mixtures (0.2 mL) were determined by l.c. (see Fig. 2).

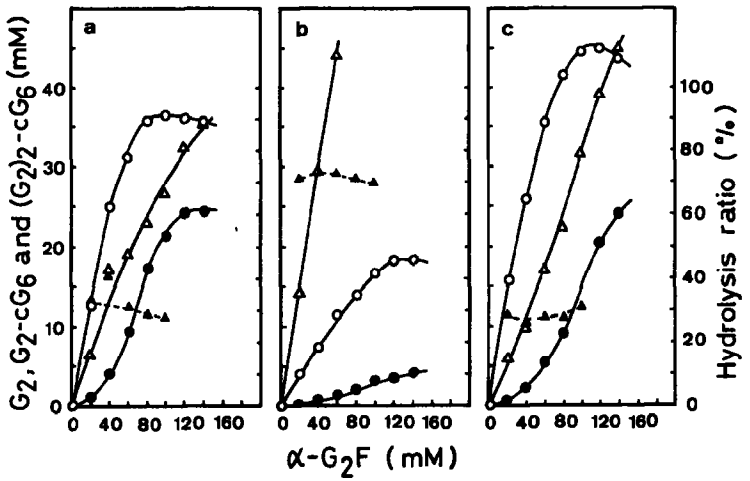

Fig. 2. Effects of concentration of α-G_2F on branched cG_6 formation. [Each debranching enzyme (3 U/mL) was incubated with various concentrations of α-G_2F in the presence of cG_6 (90mM) for 1 h at 40°: (a) A-pullulanase; (b) B-pullulanase; (c) isoamylase; ○, G_2-cG_6; ●, $(G_2)_2$-cG_6; △, G_2; and ▲, hydrolysis ratio (G_2 released/α-G_2F used).]

In each case, the amounts of G_2-cG_6 attained a maximum at 100–120mM α-G_2F. The maximum yield of G_2-cG_6 formed by A-pullulanase, B-pullulanase, and isoamylase was 36.5, 18.5, and 44.9 mmol, respectively. The ratio of hydrolysis (G_2 released/α-G_2F used) of the three enzymes was not affected by the change of α-G_2F concentration in the range of 20–100mM. This ratio of B-pullulanase was an extremely high level (70–75%) compared with those of A-pullulanase (~30%) and isoamylase (25–30%).

Formation of branched cyclomalto-oligosaccharides from cG_6, cG_7, and cG_8. — In order to investigate the acceptor specificity of the debranching enzymes, three debranching enzymes were incubated with α-G_2F and an acceptor (cG_6, cG_7, or cG_8). Appropriate aliquots (0.02–0.05 mL) of the reaction mixtures were removed, in order to analyze them by l.c. (see Table II).

A-Pullulanase formed 24.3mM G_2-cG_6 from 40mM α-G_2F, and the ratio of transfer (G_2-cG_6 produced/α-G_2F used) was 60.8%, whereas this enzyme formed 1.7mM G_2-cG_7 from 20mM α-G_2F and 8.4mM G_2-cG_8 from 40mM α-G_2F in the lower ratio of transfer (8.5 and 21.0%). B-Pullulanase produced G_2-cG_6, G_2-cG_7, and G_2-cG_8 in the lower ratio of transfer (22.0, 6.0, and 29.3%). In the case of iso-

TABLE II

BRANCHED CYCLOMALTO-OLIGOSACCHARIDES FORMATION FROM cG_6, cG_7, AND cG_8[a]

Debranching enzyme	*α-G_2F (mM)*	*Acceptor (mM)*	*G_2-cG_6 (mM)*	*$(G_2)_2$-cG_6 (mM)*	*G_2-cG_7 (mM)*	*$(G_2)_2$-cG_7 (mM)*	*G_2-cG_8 (mM)*	*$(G_2)_2$-cG_8 (mM)*
A-Pullulanase								
	20	cG_6 30	8.7	0.9				
	40	90	24.3	4.0				
	20	cG_7 30			1.7	1.2		
	20	cG_8 30					ND[b]	ND[b]
	40	90					8.4	1.5
Condensation[c] (G_2	40,	cG_6 90)	0.3	ND[b]				
B-Pullulanase								
	20	cG_6 30	4.2	0.3				
	40	90	8.8	0.7				
	20	cG_7 30			1.2	0.6		
	20	cG_8 30					4.3	0.4
	40	90					11.7	1.6
Condensation[c] (G_2	40,	cG_6 90)	0.3	ND[b]				
Isoamylase								
	20	cG_6 30	8.7	0.7				
	40	90	23.2	2.0				
	20	cG_7 30			9.2	2.0		
	20	cG_8 30					6.1	1.5
	40	90					20.9	3.0
Condensation[c] (G_2	40,	cG_6 90)	0.2	ND[b]				

[a]Each debranching enzyme (20 U/mL, 0.03 mL) was incubated with 0.17 mL of a reaction mixture containing α-G_2F (20mM or 40mM) and cG_6 (30mM or 90mM), cG_7 (30mM), or cG_8 (30mM or 90mM) for 1 h at 40°. [b]Not detected. [c]G_2 (40mM) and cG_6 (90mM) were used for the condensation reaction.

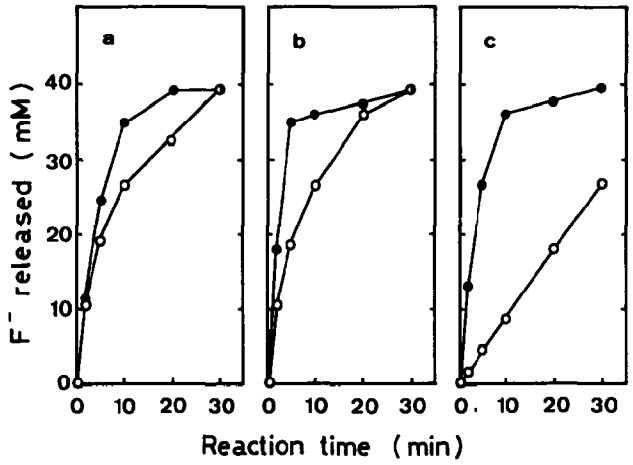

Fig. 3. Effects of addition of cG_6 on the release of fluoride anion from α-G_2F. [Each debranching enzyme (3 U/mL) was incubated with α-G_2F (40mM) in the presence or absence of cG_6 (90mM) at 40°: (a) A-pullulanase; (b) B-pullulanase; (c) isoamylase; ○, in the absence of cG_6; and ●, in the presence of cG_6 (90mM).]

amylase, G_2-cG_7 (9.2mM, 46.0%) and G_2-cG_8 (20.9mM, 52.3%) were produced as much as G_2-cG_6 (23.2mM, 58.0%).

By the condensation reaction of each enzyme with 40mM G_2 and 90mM cG_6, a slight amount of G_2-cG_6 (0.2-0.3 mmol) was formed. $(G_2)_2$-cG_6, $(G_2)_2$-cG_7, and $(G_2)_2$-cG_8 were also produced. The ratios of these products [$(G_2)_2$-cG_6/G_2-cG_6, $(G_2)_2$-cG_7/G_2-cG_7 and $(G_2)_2$-cG_8/G_2-cG_8] lay almost between 1:10 to 2:10, except

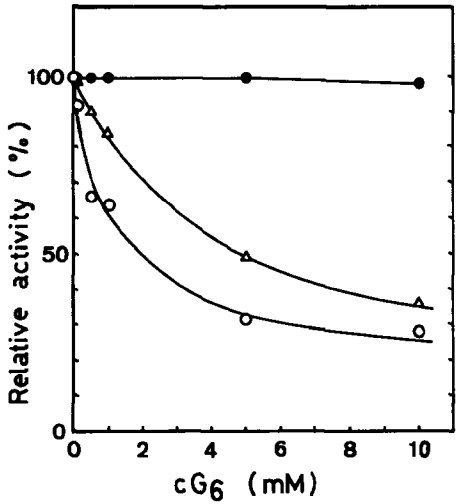

Fig. 4. Inhibition of debranching enzymes by cG_6. [The hydrolysis rates of pullulan by A-pullulanase and B-pullulanase, and of amylopectin by isoamylase, were measured in reaction mixtures containing substrate (1.3%) and cG_6 (0.1–10mM) as an inhibitor: ○, A-pullulanase; △, B-pullulanase; and ●, isoamylase.]

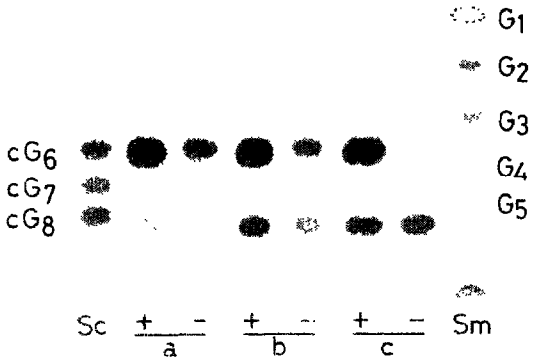

Fig. 5. Thin-layer chromatogram of the reaction products from G_2-cG_6 by debranching enzymes. [Debranching enzymes (2.9 U/mL) were incubated with G_2-cG_6 (5.7mM) in the presence (+) or absence (−) of cG_6 (43mM) for 1 h at 40°: (a) A-pullulanase; (b) B-pullulanase; (c) isoamylase; Sc, standards of cyclomaltohexaose (cG_6), cyclomaltoheptaose (cG_7), and cyclomaltooctaose (cG_8); Sm, standards of malto-oligosaccharides: G_1, G_2, *etc.* are D-glucose, maltose, *etc.*]

that the $(G_2)_2$-cG_7/G_2-cG_7 ratios using A-pullulanase and B-pullulanase were in significantly high level: 7:10 and 5:10, respectively.

Effect of cG_6 on release of fluoride anion from α-G_2F. — Each debranching enzyme was incubated with α-G_2F in the presence or absence of cG_6. After 2, 5, 10, 20, and 30 min, 0.1-mL aliquots of the reaction mixture were removed for examination of the concentration of fluoride anion released (see Fig. 3).

In the absence of cG_6, A-pullulanase and B-pullulanase decomposed α-G_2F and released fluoride anion more rapidly than isoamylase. By the addition of cG_6, the release of fluoride anion was stimulated, especially in the case of isoamylase, and the rates became almost the same.

Effect of cG_6 on hydrolysis of pullulan or amylopectin. — It was already known that the hydrolysis of pullulan by pullulanase is inhibited[17] by the addition of cG_6. In order to find a difference in the affinity of A-pullulanase and B-pullulanase for cG_6, the effect of the concentration of cG_6 on the inhibition was examined. As shown in Fig. 4, the addition of 10mM cG_6 inhibited the activity of both pullulanases by >60%, but inhibited the hydrolysis of amylopectin by isoamylase very little. The K_i values of cG_6 for A-pullulanase and B-pullulanase were evaluated from Dixon plots[18] as 0.10mM and 0.32mM, respectively.

Effect of cG_6 on hydrolysis of G_2-cG_6. — Figure 5 shows the thin-layer chromatogram of the hydrolyzates of G_2-cG_6. By A-pullulanase, G_2-cG_6 was completely hydrolyzed to maltose and cG_6 in the absence of cG_6, but in the presence of cG_6 the hydrolysis of G_2-cG_6 was incomplete. The hydrolyzing activity of B-pullulanase for G_2-cG_6 was lower than that of A-pullulanase, and the hydrolysis was almost completely inhibited by the addition of cG_6. In the case of isoamylase, G_2-cG_6 was scarcely hydrolyzed even in the absence of cG_6.

These results show that the low yield of G_2-cG_6 produced by B-pullulanase is not due to hydrolysis of the G_2-cG_6 formed.

DISCUSSION

Three debranching enzymes gave branched cyclomalto-oligosaccharides in different yields. These enzymes seem to have different reactivities towards α-G_2F, and different acceptor specificities towards cyclomalto-oligosaccharides. The yields of G_2-cG_6 produced by A-pullulanase and isoamylase were about three times that obtained by B-pullulanase.

The reason why B-pullulanase gave a low yield of G_2-cG_6 was not explained by contamination by amylase in the enzyme preparation and not by the hydrolytic activity of this enzyme for the G_2-cG_6 formed. No significant difference in the affinity of pullulanases for cG_6 was found even though the K_i value of A-pullulanase was different from that given in the literature[17].

B-Pullulanase hydrolyzed 95% of α-G_2F in the absence of cG_6, and 70–80% of α-G_2F even in the presence of 100mM cG_6. In contrast, A-pullulanase exhibited high transfer-activity for α-G_2F even in the absence of cG_6. The transfer products were presumed to be fluorides of branched oligosaccharides, such as *O*-α-maltosyl-(1→6)-α-maltosyl fluoride and *O*-α-maltosyl-(1→6)-[*O*-α-maltosyl-(1→6)]$_n$-α-maltosyl fluoride (n = 1, 2, 3, ...) by their R_F values in t.l.c. (data not shown herein). The amount of these products was decreased by the addition of cG_6; however, it was no longer detectable at 40mM cG_6. In other words, transfer products in the presence of cG_6 are only branched cG_6.

In the case of isoamylase, the mode of action on α-G_2F was drastically changed by the addition of cG_6, from a hydrolytic reaction to a transfer reaction.

These results suggest that the difference in the amounts of G_2-cG_6 production by debranching enzymes is based on the difference in the mode of action on α-G_2F used as the substrate, either a transfer action or a hydrolytic action.

G_2-cG_6 is formed by such mechanisms as follow. At first, cG_6 stimulates the α-G_2F decomposition and the G_2-cG_6 production by debranching enzymes, and then residual cG_6 inhibits the hydrolysis of the G_2-cG_6 formed.

The yields of branched cyclomalto-oligosaccharides are changed not only by the origin of the enzymes but also by the kinds of cyclomalto-oligosaccharides. It is noteworthy that isoamylase efficiently produces G_2-cG_7, little of which was produced by pullulanases. G_2-cG_8 is also produced in high yield, as well as G_2-cG_6, by isoamylase. Therefore, isoamylase is the most suitable enzyme for the purpose of the formation of *O*-maltosylcyclomalto-oligosaccharides.

Isoamylase catalyzes the splitting of certain α-D-(1→6) linkages in amylopectin, glycogen, and suitable branched oligosaccharides[19,20]. It is well known that pullulan and the (1→6)-α-maltosylic linkage in such G_2-branched oligosaccharides as 6-*O*-α-maltosylmaltrotriose are scarcely hydrolyzed by isoamylase[21–23], whereas α-G_2F is readily decomposed by isoamylase.

The ratios of $(G_2)_2$-cG_6/G_2-cG_6 and $(G_2)_2$-cG_7/G_2-cG_7, produced by A-pullulanase, were higher than those given by B-pullulanase and isoamylase. These results show that A-pullulanase is a suitable enzyme in order to synthesize such

multi-branched cyclomalto-oligosaccharides as $(G_2)_2$-cG_6, $(G_2)_3$-cG_6, $(G_2)_2$-cG_7, and $(G_2)_3$-cG_7.

REFERENCES

1 D. FRENCH, A. O. PULLEY, J. A. EFFENBERGER, M. A. ROUGVIE, AND M. ABDULAH, *Arch. Biochem. Biophys.*, 111 (1965) 153–160.
2 S. KOBAYASHI, N. SHIBUYA, B. M. YOUNG, AND D. FRENCH, *Carbohydr. Res.*, 126 (1984) 215–224.
3 J. ABE, Y. TAKEDA, S. HIZUKURI, K. YAMASHITA, AND N. IDE, *Carbohydr. Res.*, 131 (1984) 175–179.
4 S. KOBAYASHI, K. KAINUMA, AND S. SUZUKI, *Nippon Nougei Kagaku Kaishi*, 51 (1977) 691–698; *Chem. Abstr.*, 89 (1978) 60,016g.
5 K. KOIZUMI, T. UTAMURA, M. SATO, AND Y. YAGI, *Carbohydr. Res.*, 153 (1986) 55–67.
6 M. ABDULAH AND D. FRENCH, *Arch. Biochem. Biophys.*, 137 (1970) 483–493.
7 Y. SAKANO, M. SANO, AND T. KOBAYASHI, *Agric. Biol. Chem.*, 49 (1985) 3391–3398.
8 S. HIZUKURI, J. ABE, N. MIZOWAKI, K. KOIZUMI, AND T. UTAMURA, *Carbohydr. Res.*, 154 (1986) 81–92.
9 S. KITAHATA, Y. YOSHIMURA, AND S. OKADA, *Carbohydr. Res.*, 159 (1987) 303–313.
10 B. E. NORMAN, *Denpun Kagaku*, 30 (1983) 200–211; *Chem. Abstr.*, 99 (1983) 138,302*a*.
11 D. H. BRAUNS, *J. Am. Chem. Soc.*, 51 (1929) 1820–1831.
12 D. S. GENGHOF, C. F. BREWER, AND E. J. HEHRE, *Carbohydr. Res.*, 61 (1978) 291–299.
13 N. NELSON, *J. Biol. Chem.*, 153 (1944) 375–380.
14 M. SOMOGYI, *J. Biol. Chem.*, 195 (1952) 19–23.
15 J. C. SOWDEN, *Adv. Carbohydr. Chem.*, 12 (1957) 35–79.
16 D. FRENCH, M. L. LEVINE, E. NORBERG, P. NORDIN, J. H. PAZUR, AND G. M. WILD, *J. Am. Chem. Soc.*, 76 (1954) 2387–2390.
17 J. J. MARSHALL, *FEBS Lett.*, 37 (1973) 269–273.
18 M. DIXON, *Biochem. J.*, 55 (1953) 170–171.
19 B. MARUO AND T. KOBAYASHI, *Nature (London)*, 167 (1951) 606–607.
20 T. HARADA, K. YOKOBAYASHI, AND A. MISAKI, *Appl. Microbiol.*, 16 (1968) 1439–1444.
21 K. YOKOBAYASHI, A. MISAKI, AND T. HARADA, *Agric. Biol. Chem.*, 33 (1969) 625–627.
22 K. YOKOBAYASHI, A. MISAKI, AND T. HARADA, *Biochim. Biophys. Acta*, 212 (1970) 458–469.
23 K. KAINUMA, S. KOBAYASHI, AND T. HARADA, *Carbohydr. Res.*, 61 (1978) 345–357.