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Note

A convenient preparation of maltooctaose and maltononaose by the coupling reaction of cyclomaltodextrinase

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Malto-oligosaccharides, linear (1 → 4)-linked α-D-glucopyranosyl-oligosaccharides, have become valued commodities due to their various uses [1–3] as food additives (sweeteners, gelling agents, viscosity modifiers), fermentation feedstocks, synthons for pharmaceuticals, and experimental substrates for amylases. The malto-oligosaccharides, maltose (G2) [4], maltotriose (G3) [4], maltotetraose (G4) [5], maltopentaose (G5) [6], and maltohexaose (G6) [7] have been produced from starch by enzymatic digestion using malto-oligosaccharide-forming amylases. We reported the preparation of maltoheptaose (G7) and maltooctaose (G8) from cyclomaltoheptaose (β-cyclodextrin, βCD) and cyclomaltooctaose (γ-cyclodextrin, γCD), respectively, using cyclomaltodextrinase (EC 3.2.1.54) from *Bacillus sphaericus* E-244 [8]. We obtained G7 in good yield, but the yield of G8 was not as good. The preparation of G8 by mild acid hydrolysis of γCD was recently reported, but the yield was only 8% [9]. Moreover, no effective methods for preparation of maltononaose (G9) have ever been established.

We found that the cyclomaltodextrinase has not only cyclodextrin-hydrolyzing activity (decycling of cyclodextrins) but also coupling activity (transfer of D-glucose to cyclodextrins with decycling reaction). In this report we describe a new enzymatic synthesis of G8 and G9 from cyclodextrins (CDs) and D-glucose by the coupling reaction of the cyclomaltodextrinase.

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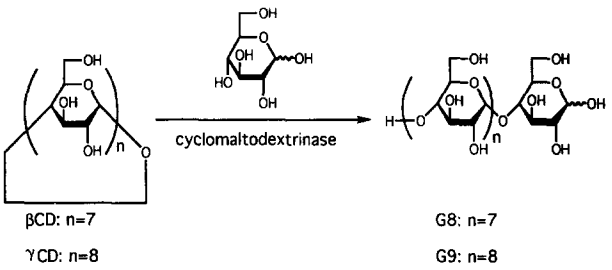

Scheme 1.

The synthetic route of G8 and G9 is shown in Scheme 1. Fig. 1 shows the time course of the coupling reaction using cyclomaltodextrinase, βCD, and D-glucose. The concentration of G8 reached a maximum after 6 h and then gradually decreased. The formation of G8 was 53% [calculated from high-performance liquid chromatography (HPLC) data] and the isolated yield of G8 using a column of active carbon was 39%. Of the other products, G2, G3, G5, G6, and G7 were analyzed on HPLC, but G4 and βCD could not be measured exactly because they overlapped. The time course of the coupling reaction of the cyclomaltodextrinase acting on γCD and D-glucose is shown in Fig. 2. The concentration of G9 reached a maximum after 2 h and then gradually decreased, and G3 was preferentially produced. The yield of G9 was 26% (calculated from HPLC data) and the isolated yield of G9 using a column of active carbon was 20%. Of the other products, G2, G3, G4, G6, and G7 were analyzed, but G5 and γCD could not be measured exactly because they overlapped.

The structures of G8 and G9 were established by hydrolysis tests using glucoamylase (EC 3.2.1.3), spectral data, and elemental analyses. G8 was identified by comparison with an authentic sample [9]. When isolated G8 or G9 and glucoamylase [α-(1 → 4) and α-(1 → 6) glycosidic linkage hydrolytic enzyme] were incubated, D-glucose was the only

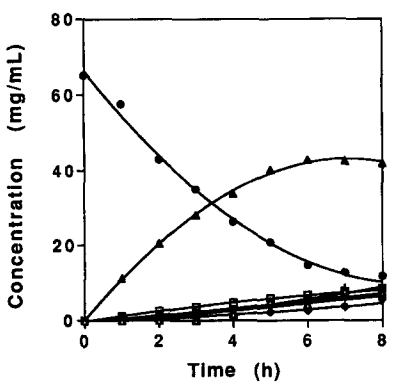

Fig. 1. Time course of the coupling reaction of cyclomaltodextrinase with βCD and D-glucose. The mixtures were incubated in 10 mM phosphate buffer (pH 7.5) at 40 °C. Symbols: ●, βCD; △, G2; +, G3; ◆, G5; ▽, G6; □, G7; ▲, G8.

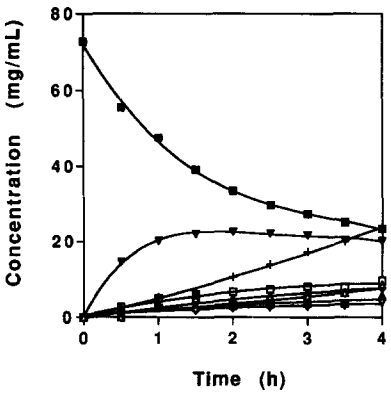

Fig. 2. Time course of the coupling reaction of cyclomaltodextrinase with γCD and D-glucose. The mixtures were incubated in 10 mM phosphate buffer (pH 7.5) at 40 °C. Symbols: ■, γCD; △, G2; +, G3; ◇, G4; ▽, G6; □, G7; ▲, G8; ▼, G9.

product. We determined the degree of polymerization of oligosaccharides and α-(1 → 4) linkage from the ^{1}H NMR signals at δ 4.65 (*J* 7.8–8.1 Hz) and 5.20 (*J* 3.4–3.9 Hz) which were assigned to the reducing end H-1 (β and α, respectively), and at δ 5.35 (*J* 3.9 Hz) assigned to non-reducing H-1.

In conclusion, we have developed a convenient method of preparing large quantities of G8 and G9 from CDs and D-glucose, using the coupling reaction of cyclomaltodextrinase.

1. Experimental

Reagents and materials.—All chemicals were reagent grade unless otherwise noted. Cyclomaltodextrinase was prepared and purified as described previously [10]. When incubated with βCD, one unit of the enzyme activity was defined as the amount liberating 1 μmol of G7 per min [10]. Glucoamylase (from *Rizopus sphaericus*) was purchased from Toyobo Co., Ltd., Japan. D-Glucose and a series of malto-oligosaccharides (from G2 to G7) used as standards for HPLC analysis were purchased from Wako Chemicals, Japan, and Seishin Pharmaceuticals, Japan. Standard G8 was prepared by the modified method of Hotchkiss et al. [9].

Apparatus.—All melting points were determined on a Yanagimoto micro-melting point apparatus and are uncorrected. Optical rotations were determined with a Jasco DIP-360 digital polarimeter at 25 °C. IR spectra were recorded with a Jasco A-202 spectrometer. The ^{1}H NMR spectra were recorded at 199.5 MHz with a JEOL JNM-FX200 spectrometer and Me_4Si as an internal standard. HPLC was performed on a TSK gel Amide-80 column (4.6 mm i.d. × 250 mm) with a flow rate of 1.0 mL/min using a Jasco pump (880-PU) and a refractive index (RI) detector (Shodex RI SE-71) at room temperature. Column chromatography was performed on active carbon (Seisei shirasagi, from Takeda Chemical Industries, Ltd., Japan).

Maltooctaose (G8).—Cyclomaltodextrinase (534U) was added to a stirred solution of βCD (100 g, 88.1 mmol) and D-glucose (666 g, 3.7 mol) in 10 mM phosphate buffer (pH 7.5, 1.0 L) and the mixture was stirred at 40 °C for 6 h. The solution was boiled for 1 h to stop the reaction. After cooling, toluene (20 mL) was added to the solution and the mixture was stirred at room temperature for 5 h; the resulting βCD including toluene and precipitated enzyme were removed by filtration with a short pad of Celite. The filtrate was then evaporated under reduced pressure to 300 mL and the solution was passed through a column of active carbon. G8 was eluted using an aq EtOH gradient of 0–30% and the appropriate fractions eluted with 25–30% were freeze-dried to give maltooctaose (45.0 g, 34.2 mmol, 39%); mp 223–225 °C dec.; $[\alpha]_D$ +180° (*c* 1.0, H_2O); ν_{max} 3280 (s, br), 2930 (s), 1650 (m), 1420 (w), 1360 (w), 1150 (w), 1080 (w) cm^{-1}; ^1H NMR (D_2O): δ 3.28 ~ 4.01 (m, 48 H, H-2a–h, 3a–h, 4a–h, 5a–h, 6a–h), 4.65 (d, 0.5 H, *J* 7.8 Hz, H-1aβ), 5.23 (d, 0.5 H, *J* 3.4 Hz, H-1aα), 5.35 (d, 7 H, *J* 3.9 Hz, H-1b–h); t_R (detector: RI; eluent: 6:4 MeCN–H_2O): 18.9 min. Anal. Calcd for $C_{48}H_{82}O_{41}$: C, 43.84; H, 6.28. Found: C, 43.64; H, 6.30.

Maltononaose (G9).—Cyclomaltodextrinase (71U) was added to a stirred solution of γCD (20 g, 15.4 mmol) and D-glucose (111 g, 616 mmol) in 10 mM phosphate buffer (pH 7.5, 0.2 L) and the mixture was stirred at 40 °C for 2 h. The solution was boiled for 1 h to stop the reaction. Toluene (4 mL) was added and the mixture was stirred at room temperature for 5 h; the remaining γCD and precipitated enzyme were removed by filtration with a short pad of Celite. Then the solvent was evaporated under reduced pressure to 50 mL and the solution was passed through a column of active carbon. G9 was eluted using an aq EtOH gradient of 0–45% and the appropriate fractions eluted with 40–45% were freeze-dried to give maltononaose (4.55 g, 3.08 mmol, 20%); mp 231–235 °C dec.; $[\alpha]_D$ +181° (*c* 1.0, H_2O); ν_{max} 3300 (s, br), 2930 (s), 1650 (m), 1420 (w), 1360 (w), 1150 (w), 1080 (w) cm^{-1}; ^1H NMR (D_2O): δ 3.28–4.01 (m, 54 H, H-2a–i, 3a–i, 4a–i, 5a–i, 6a–i), 4.65 (d, 0.5 H, *J* 8.1 Hz, H-1aαβ), 5.23 (d, 0.5 H, *J* 3.9 Hz, H-1aα), 5.35 (d, 8 H, *J* 3.9 Hz, H-1b–i); t_R (detector: RI; eluent: 6:4 MeCN–H_2O): 21.0 min. Anal. Calcd for $C_{54}H_{92}O_{46}$: C, 43.90; H, 6.28. Found: C, 43.79; H, 6.31.

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