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Isolation and structural analyses of positional isomers of $6^1,6^m$-di-*O*-α-D-mannopyranosyl-cyclomaltooctaose ($m = 2$–5) and 6-*O*-α-(*n*-*O*-α-D-mannopyranosyl)-α-D-mannopyranosyl-cyclomaltooctaose ($n = 2$, 3, 4, and 6)

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Abstract

Eight positional isomers of $6^1,6^m$-di-*O*-α-D-mannopyranosyl-cyclomaltooctaose (γCD) ($m = 2$–5) and 6-*O*-α-(*n*-*O*-α-D-mannopyranosyl)-D-mannopyranosyl-γCD ($n = 2$, 3, 4, and 6) in a mixture of products from γCD and D-mannose by condensation reaction of α-mannosidase from jack bean were isolated by HPLC. The structures of four isomers of 6-*O*-α-(*n*-*O*-α-D-mannopyranosyl)-D-mannopyranosyl-γCD were elucidated by NMR spectroscopy. On the other hand, four positional isomers of $6^1,6^m$-di-*O*-α-D-mannopyranosyl-γCD were determined by LC–MS analysis of degree of polymerization of the branched oligosaccharides produced by enzymatic degradation with bacterial saccharifying α-amylase (BSA), and combination of BSA and glucoamylase. Similarly cyclomaltodextrin glucanotransferase also digested these isomers.

Keywords: Dimannosyl cyclomaltooctaose (γCD); Positional isomer; Separation; Enzymatic degradation; Structural analysis; HPLC; LC–MS; NMR spectroscopy

1. Introduction

In recent years, it has come to be known that multibranched cyclomaltooligosaccharides (cyclodextrins; CDs) exist as minor components in mixtures of branched CDs prepared by enzymatic transglycosidation or condensation. These multibranched CDs contain many positional isomers. We have previously reported that the structures of many positional isomers of multibranched

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cyclomaltohexaoses (αCDs) and cyclomaltoheptaoses (βCDs) were readily determinable using an enzymatic degradation method [1–5], while it has not been confirmed yet with respect to those of multibranched cyclomaltooctaoses (γCDs), which contain more positional isomers than either the α and β CD series.

In this study, eight positional isomers of dimannosyl-γCD [6^1,6^m-di-*O*-α-D-mannopyranosyl-γCDs (6^1,6^m-$(Man)_2$-γCDs); $m=2$–5 and 6-*O*-α-(*n*-*O*-α-D-mannopyranosyl)-D-mannopyranosyl-γCDs (Man(1→*n*)Man(1→6) γCDs); $n=2$, 3, 4, and 6], which two mannosyl residues, were substituted on γCD with α-mannosidase [EC 3.2.1.24] from jack bean, were purified by HPLC, and the complicated structures of these isomers were elucidated using FABMS, NMR, and enzymatic degradation methods.

2. Experimental

Materials.—A mixture of mannosyl-γCDs was prepared according to the previous paper [6]. Briefly, mannose (2.75 g) and γCD (2.75 g) were dissolved in 4.5 mL of 10 mM acetate buffer (pH 4.5) and incubated with jack bean α-mannosidase (42 U) at 60 °C for 9 days. Then, the reaction mixture was heated at 100 °C for 10 min to stop the enzyme action. The fraction of a mixture of mannosyl-γCDs was obtained from the reaction mixture using a ODS column with water and 1:9 ethanol–water. Purified bacterial saccharifying α-amylase (BSA, EC 3.2.1.1) (400 U/mg) from *Bacillus subtilis* [7] and cyclomaltodextrin glucanotransferase (CGTase, EC 2.4.1.19) (2000 U/mL) from *B. circulans* [8] were prepared and purified by the previously reported method, respectively. A crystalline glucoamylase (EC 3.2.1.3) (37.8 U/mg) was obtained from Seikagaku Kogyo Co., Ltd. (Tokyo, Japan). All reagents were of analytical grade. Reagent-grade organic solvents used for chromatography were dried and freshly distilled before use. Water employed in HPLC methods was distilled, deionized, and redistilled.

Isolation of dimannosyl-γCDs.—The fraction of dimannosyl-γCDs was separated from a mixture of mannosyl-γCDs which were comprised of mono-, di-, and tri-substituted mannosyl-γCDs by HPLC on a TSK gel Amide-80 column (300×21.5 mm i.d.) (TOSOH) with 53:47 acetonitrile–water.

General methods.—HPLC was performed with a Jasco PU 980 pump, a Rheodyne 7125 injector, and a Shodex RI-71 monitor (Showa Denko). HPLC analyses at constant temperature were conducted with an SSC 3510C column oven (Senshu Scientific Co.). The columns employed were a YMC-Pack SH-343-5 ODS (250×20 mm i.d.) (YMC), a DAISOPAK SP-120-5-ODS-BP (150×6 mm i.d.) (DAISO), and a Hypercarb S (100×4.6 mm i.d.) (Shandon).

LC–MS was carried out using a Hewlett–Packard HP1050 series Model HP79852A pump interfaced to a Finnigan TSQ-7000 triple-stage quadrupole mass spectrometer (Finnigan MAT Instruments Inc., San Jose, CA, USA) fitted with the Finnigan electrosplay ionization (ESI) interface. The mass spectrometer was operated in the positive-ion mode; the ESI voltage was set to 4.5 kV, and the ESI current was 0.34 μA. The capillary temperature was 230 °C. The pressure of the sheath gas was 70 psi, and the auxiliary gas was 15 unit. Total ion monitoring was done by scanning covered the range m/z 500–1800 with a scan rate of 3 s/scan.

FABMS was performed in the negative-ion mode using a JEOL JMS-DX 303 mass spectrometer with Xe having a kinetic energy equivalent to 6 kV at an accelerating voltage of 3 kV. The mass marker was calibrated with perfluoroalkylphosphazine (Ultra Mark), and glycerol was used as the matrix solution.

NMR spectra data were recorded for 5–10% solutions in D_2O at 50 °C with a JEOL GSX-500 spectrometer. Chemical shifts were expressed in ppm downfield from the signal of Me_4Si referenced to external 1,4-dioxane (67.4 ppm). The other conditions for ^{13}C NMR, ^1H–^1H COSY and ^1H–^{13}C COSY measurements were the same as in the previous paper [9].

Structural analysis by enzymatic degradation.—Each sample (0.2 mg) of 6^1,6^m-$(Man)_2$-γCDs (**A1, A2, B**, and **D**) in 60 μL of 10 mM acetate buffer was individually incubated with BSA (0.8 mg, 320 U) or CGTase (20 μL, 40 U) at 40 °C for 48 h in order to digest completely. The enzyme was then inactivated by placing it in a boiling water bath for 10 min. Each hydrolysate with enzyme was analyzed by HPLC and LC–MS on a YMC-Pack Polyamine-II (150×4.6 mm i.d.). Further, **A2** and **B** (each 0.2 mg) in 100 μL of 10 mM acetate buffer (pH 6.0) were individually treated with both BSA (0.6 mg, 240 U) and crystalline glucoamylase (0.2 mg, 7.56 U) at 40 °C, and their reaction products were analyzed at regular intervals.

3. Results and discussion

Separation.—Fig. 1 shows the chromatogram on an ODS column of the fraction of dimannosyl-γCDs produced from γCD and D-mannose through condensation reaction with α-mannosidase from jack bean. Seven components were detected, and the ratio of **A**:**B**:**C**:**D**:**E**:**F**:**G** was 29:10:37:14:7:1:2. **F** and **G** tended to escape detection since they had very long retention times and their amounts were very small compared to those of **A–E**. The separation of **A–D** was much improved by decreasing of methanol content in the eluent, which resulted in separation of **A** into **A1** and **A2** with 0.5:95.5 methanol–water, although it was difficult owing to their very long retention times. Moreover, the better separation of **A1** and **A2** was examined on a graphitized carbon column, a Hypercarb S. **A1** and **A2** were separated better with an ethanol–water system than with an acetonitrile–water, methanol–water, or n-propanol–water system. On a Hypercarb S with an ethanol–water system, **A2** was eluted earlier than **A1** in reverse order on an ODS column, and the ratio of **A1:A2** was approximately 1:1. **A–F** were purified on a YMC-Pack SH-343-5 using individual optimum methanol concentration of eluents, and then

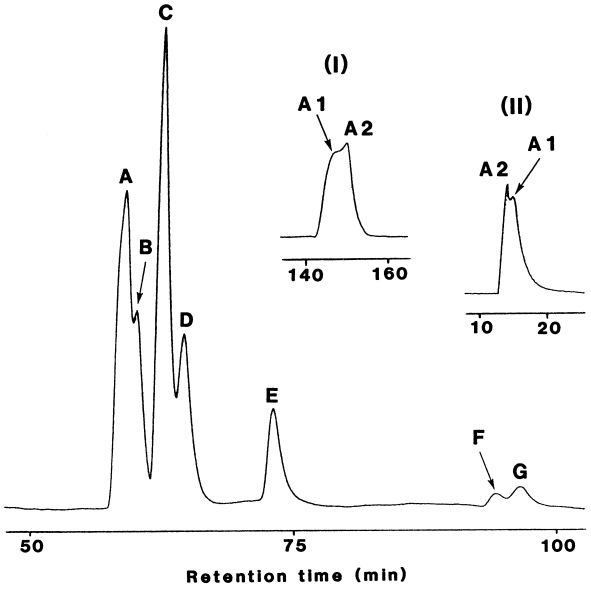

Fig. 1. Elution profile of dimannosyl-γCDs. Chromatographic conditions: column, YMC-Pack SH-343-5 ODS (250×20 mm i.d.); eluent, 3:97 CH_3OH–H_2O; flow rate, 2 mL/min; temperature, 30 °C. Special conditions for separation of **A1** and **A2**: (I) eluent, 0.5:99.5 CH_3OH–H_2O; other conditions are same. (II) column, Hypercarb S (100×4.6 mm i.d.); eluent, 18:82 C_2H_5OH–H_2O; flow rate, 0.5 mL/min; temperature, 30 °C.

A was separated into **A1** and **A2** on a Hypercarb S column with 18:82 ethanol–water. However, purification of **G** was fairly difficult because of **G** was eluted immediately after **F** on this ODS column. Fortunately, **G** was eluted earlier than **F** on another ODS column, a DAISOPAK (150×6 mm i.d.) with 3:97 methanol–water, although the elution orders of other **A–D** were unaltered.

FABMS.—A molecular ion of *m*/*z* 1619 was observed in each negative-ion FABMS spectrum of **A1, A2, B, C, D, E, F**, and **G**, and it was confirmed that all of them were the positional isomers of dimannosyl-γCD in which two mannosyl residues were substituted on γCD. Further, on the basis of the difference of the fragment ions, these were classified in two groups. In the spectra of **A1, A2, B**, and **D**, only one fragment ion *m*/*z* 1457 $[M-Man-H]^-$, which formed through cleavage of either of two mannosyl side chains, was observed. On the other hand, additionally fragment ion *m*/*z* 1295 $[M-2Man-H]^-$ was detected in the spectra of **C, E, F**, and **G**, which formed through cleavage of one mannobiosyl side chain. These phenomena suggest that the members of the former group are the positional isomers of doubly branched mannosyl-γCDs in which two mannosyl side chains are attached directly to the γCD ring, while those of the latter group are the mannobiosyl-γCDs each of which have one side chain of mannobiose linked by different linkage modes.

^{13}C NMR spectroscopy.—(1) Doubly branched mannosyl-γCDs. Fig. 2 shows the ^{13}C NMR spectra of **A** (mixture of **A1** and **A2**), **B**, and **D** in D_2O at 50 °C. Assignments of signals in their spectra were made by comparison with those in the spectrum of 6-*O*-α-D-mannopyranosyl-γCD [6]. It is known that a substituent on the oxygen atom attached to any carbon atom of the sugar moiety affects the chemical shift of the carbon atom, moving it downfield by 8–11 ppm [10]. The assignments of the C-6 signals were confirmed by the distortionless enhancement by polarization transfer (DEPT) method [11]. The signals for the C-6s of the six γCD ring glucoses (G-6, δ 61.1–61.3), C-6 signals of two mannosyl side chains (M-6, δ 61.6–61.9), and the large downfield-shifted two C-6 signals of γCD ring glucoses (G′-6, δ 67.2–67.4) were observed. The relative signal intensities of three kinds of signals were 6:2:2. On the other hand, those of the C-1 signals of γCD ring glucoses (G-1, δ 101.9–102.5) and α-(1→6)-linked C-1 signals of two mannosyl side chains (M-1, δ 101.2–101.4)

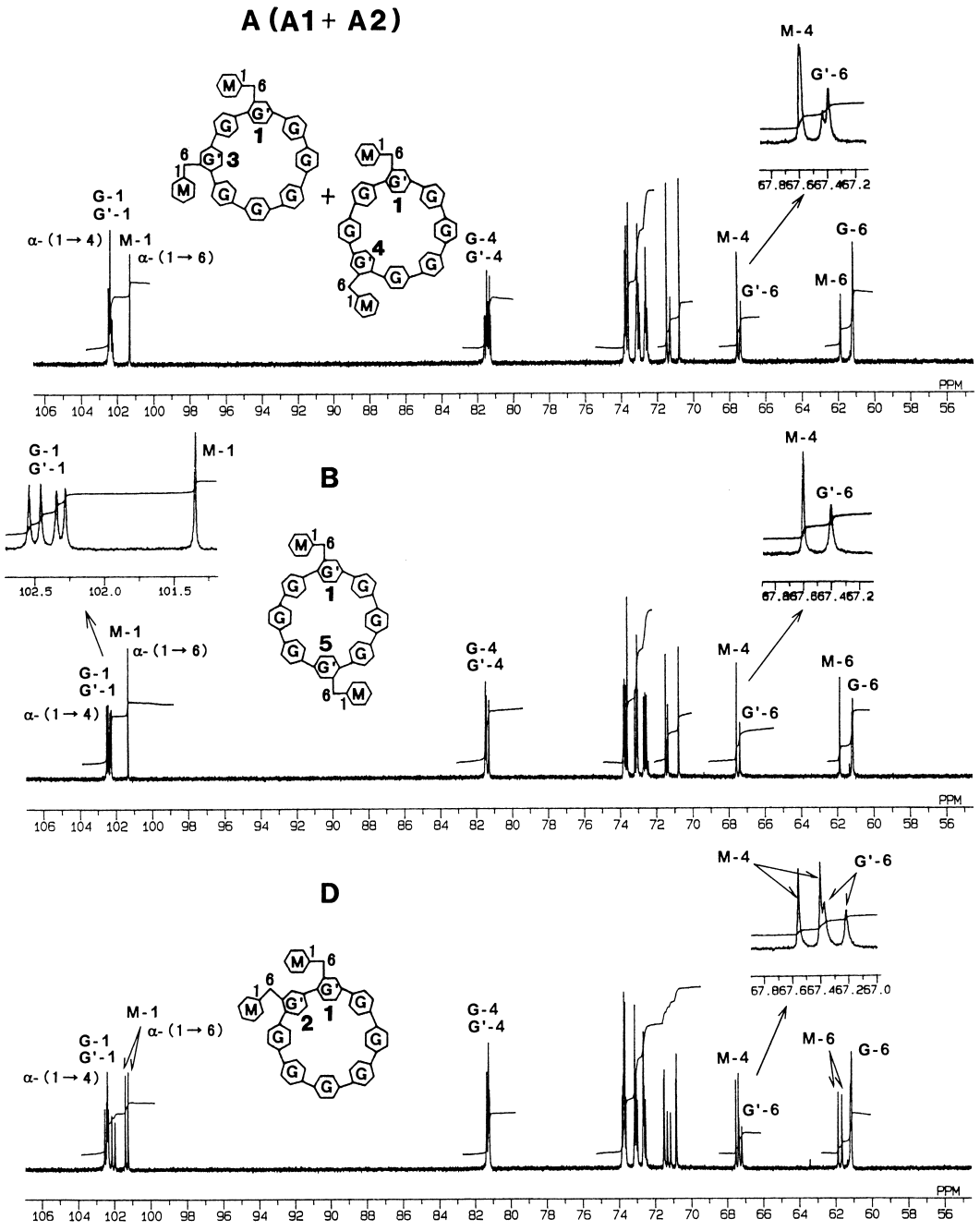

Fig. 2. ^{13}C NMR spectra of $6^1,6^m$-$(Man)_2$-γCDs (**A, B**, and **D**) in D_2O at 50 °C. G-1, -4, and -6 are signals of C-1, -4, and -6 atoms of the γCD ring D-glucopyranose units. G′ is the γCD ring D-glucopyranose unit on which mannosyl residue is α-(1→6)-linked. M-1, -4, and -6 are signals of C-1, -4, and -6 atoms of the D-mannopyranosyl side chain (M) units.

appeared at higher field than G-1 were 8:2. Therefore, it was confirmed that **A, B**, and **D** were the positional isomers of $6^1,6^m$-di-*O*-α-D-mannopyranosyl-γCD in which two mannosyl side chains were attached to γCD ring by α-(1→6)-linkage. Moreover, their spectra were compared in detail in order to estimate each structure of $6^1,6^2$-, $6^1,6^3$-, $6^1,6^4$-, and $6^1,6^5$-isomers. In the even-extended spectrum of **B**, M-1, M-4, M-6, and G′-6 signals were observed as single peak, respectively, while the G-1 signal was split into four peaks, indicating 1:1:1:1 of the relative signal intensities, which was a good reflection of the symmetry between two mannosyl side chains substituted on γCD ring. In contrast to the case of **B**, each M-1, M-4, M-6, and G′-6 signal in that of **D** was split into two peaks owing the strong interaction between two adjacent mannosyl side chains substituted on γCD ring.

From these facts, it was estimated that **B** and **D** were $6^1,6^5$- and $6^1,6^2$-di-*O*-α-D-mannopyranosyl-γCDs, respectively.

(2) Mannobiosyl-γCDs. Fig. 3 shows the ^{13}C NMR spectra of **C, E, F**, and **G** in D_2O at 50 °C. NMR resonances in the spectra of these compounds were assigned using ^1H–^1H COSY and ^1H–^{13}C COSY methods. Compounds **C, E, F**, and **G** are the positional isomers of mannobiosyl-γCD in which one more mannosyl residue is attached to side chain mannose of 6-*O*-α-D-mannopyranosyl-γCD.

In the spectrum of **C**, four kinds of signals for the C-6 were observed, the relative intensities of C-6 signals of γCD ring glucoses (G-6, δ 61.1–61.2), and one C-6 signal (M′-6, δ 61.8) of the terminal mannosyl residue (M′), and the large downfield-shifted two C-6 signals (G′-6 and M-6, δ 67.4 and 66.5) of the branched points of γCD ring glucose and the another mannosyl residue (M) were 7:1:1:1. Moreover, two α-(1→6)-linked C-1 signals (M-1 and M′-1) of mannobiosyl side chains appeared at δ 101.2 and 100.2, respectively, more upfield than G-1 (δ 102.1–102.5), and the relative intensities of G-1, M-1, and M′-1 were 8:1:1. Consequently, **C** was thought to be 6-*O*-α-(6-*O*-α-D-mannopyranosyl)-D-mannopyranosyl-γCD [Man(1→6)Man(1→6)γCD].

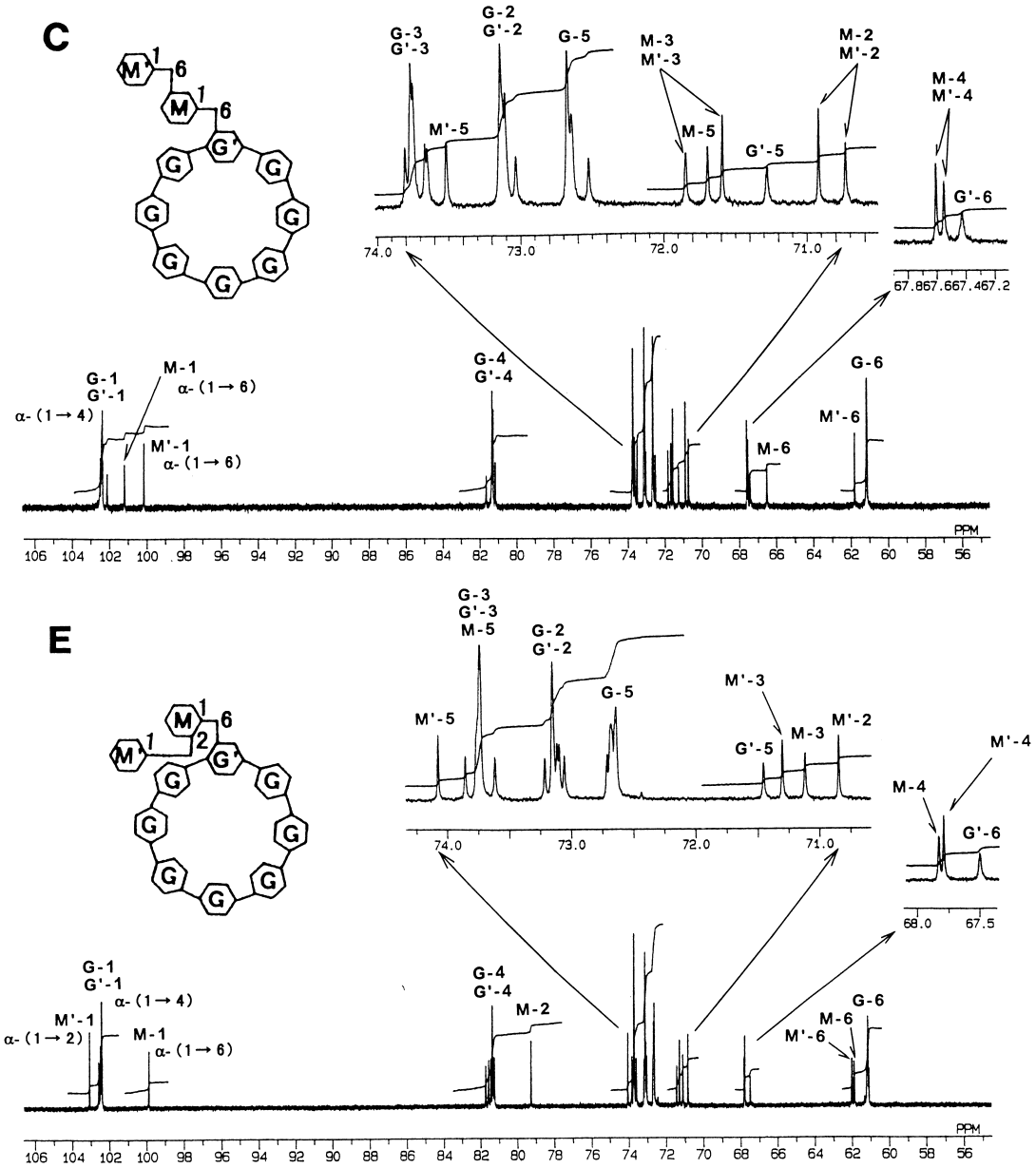

Fig. 3. ^{13}C NMR spectra of Man(1→*n*)Man(1→6) γCDs (**C, E, F** and **G**) in D_2O at 50 °C. M′-1, -4, and -6 are signals of C-1, -4, and -6 atoms of the terminal D-mannopyranosyl residue (M′) unit. Other abbreviations are the same as in Fig. 2.

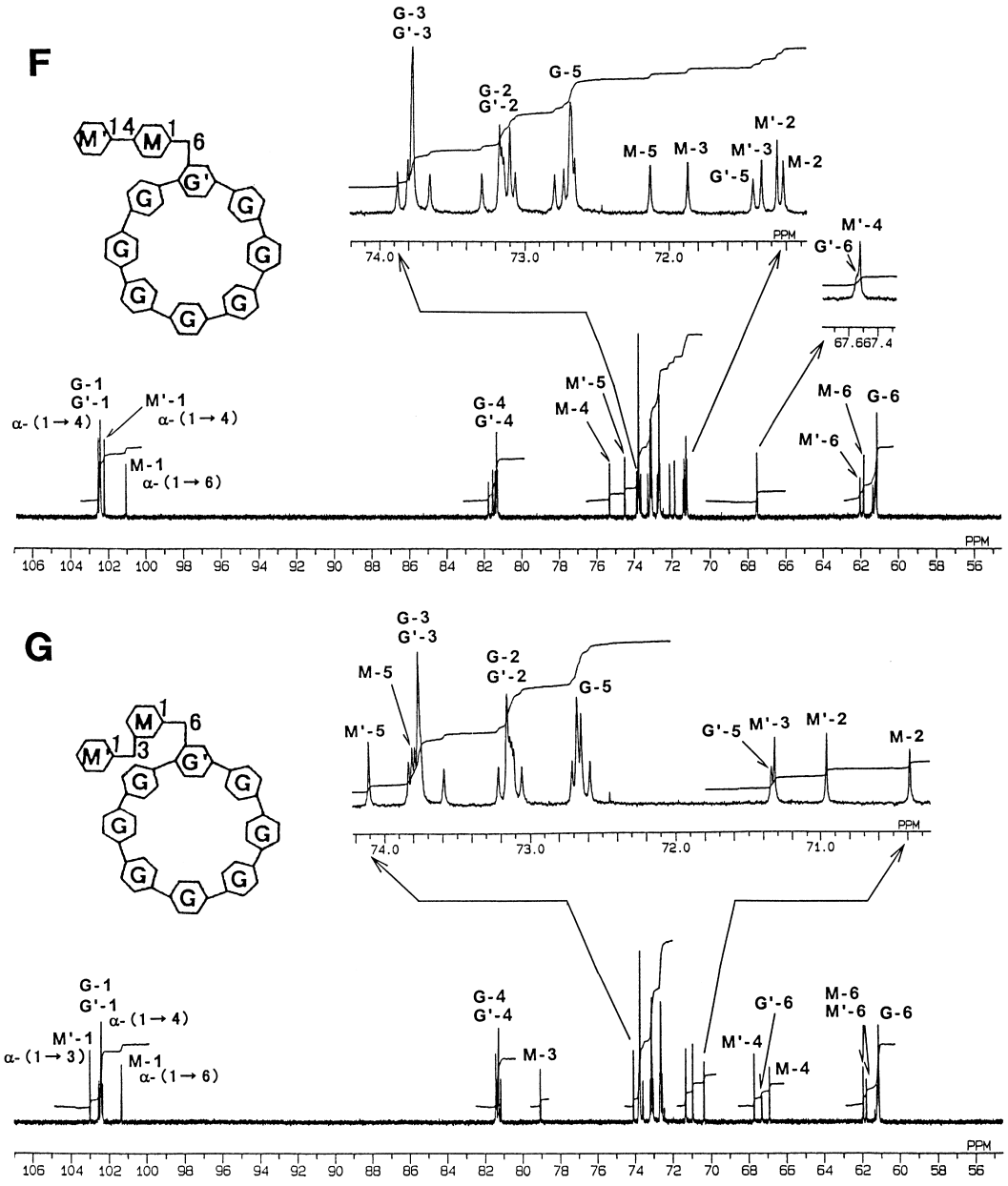

Fig. 3. cont'd.

In the spectrum of **E**, two C-6 signals of mannobiosyl side chain (M-6 and M′-6, δ 61.9 and 62.0) were observed near of G-6 (δ 61.1–61.2), and mannobiosyl side chain substituted C-6 signal (G′-6) shifted downfield to δ 67.5; the relative intensities of G-6, M-6, M′-6, and G′-6 were 7:1:1:1. M′ substituted C-2 signal (M-2) shifted downfield to δ 79.3, the adjacent α-(1→6)-linked C-1 signal (M-1) shifted upfield to δ 99.9 under the influence of M′ substitution on C-2 (β effect), and the α-(1→2)-linked C-1 signal (M′-1, δ 103.1) was observed more downfield than G-1. The relative intensities of G-1, M-1, and M′-1 were 8:1:1. Therefore, it was determined that **E** was 6-*O*-α-(2-*O*-α-D-mannopyranosyl)-D-mannopyranosyl-γCD [Man(1→2)Man(1→6)γCD].

Compound **F** showed the characteristic spectrum for 6-*O*-α-(4-*O*-α-D-mannopyranosyl)-D-mannopyranosyl-γCD [Man(1→4)Man(1→6)γCD]. That is, the mannobiosyl-substituted C-6 signal (G′-6) of the glucose ring and the M′ substituted C-4 signal (M-4) of another mannose shifted downward to δ 67.5 and 75.3, respectively. Moreover, G-1 and α-(1→4)-linked C-1 signal (M′-1) of M′ and α-(1→6)-linked C-1 signal (M-1) of M were observed at δ 102.3–102.5, 102.2, and 101.1, respectively, and the relative intensities of G-1, M′-1, and M-1 were 8:1:1.

In the spectrum of **G**, the relative intensities of G-6 (δ 61.1–61.2), M-6 (δ 61.9) and M′-6 (δ 61.8) of the mannobiosyl side chain and of G′-6 (δ 67.3) were 7:1:1:1. Further, the M′ substituted C-3 signal (M-3) of M shifted downward to δ 79.1, while on the contrary, the adjacent C-4 signal (M-4) shifted upfield to δ 66.9 by the β effect, and M′-4 was observed at δ 67.7. On the other hand, the α-(1→3)-linked C-1 signal (M′-1), the α-(1→4)-linked C-1 signal (G-1), and the α-(1→6)-linked C-1 signal (M-1) were observed at δ 103.0, 102.3–102.5, and 101.4, respectively, and the relative intensities of G-1, M-1, and M′-1 were 8:1:1. These results suggest that **G** is 6-*O*-α-(3-*O*-α-D-mannopyranosyl)-D-mannopyranosyl-γCD [Man(1→3)Man(1→6)γCD].

Structural analysis.—It is well known that BSA and CGTase hydrolyze α-(1→4)-glucosidic linkages except for the first and second glucosidic linkages toward the reducing end from the branching point (α-(1→6)-glucosidic linkage) in the starch and branched dextrins, and produce 6^3-*O*-α-D-glucosylmaltotriose (the degree of polymerization (dp) 4) as the smallest branched oligosaccharides. Using this fact we already elucidated the structures of many positional isomers of several multibranched α and β CDs. The highly rigid αCD ring which has the smallest cavity size is decomposed with CGTase, while it keeps intact with BSA. On the other hand, the βCD ring is decomposed with BSA as well as CGTase due to its more flexible structure and larger cavity size than αCD ring. Therefore, $6^1,6^m$-$(Man)_2$-γCDs would be digested with either of them through the most flexible structure and the largest cavity size of γCD ring among three kinds of CD rings. 6-*O*-α-D-Mannopyranosyl-γCD(Man-γCD), which is the fundamental compound for $6^1,6^m$-$(Man)_2$-γCDs, was finally decomposed to dp 4 of the branched oligosaccharide and glucose with BSA, and also the same final degradation products were detected in the digest of it with CGTase. On the basis of these results, the models of reaction of $6^1,6^m$-$(Man)_2$-γCDs with BSA are shown together with that of Man-γCD in Fig. 4. With BSA the $6^1,6^2$- and $6^1,6^3$- isomers are decomposed to branched oligosaccharides of dp 6 and dp 7, respectively, and to glucose. On the other hand, the same degradation products, that is, branched oligosaccharide of dp 4 and glucose are produced from the $6^1,6^4$- and $6^1,6^5$- isomers. Accordingly, the $6^1,6^2$- and $6^1,6^3$- isomers among $6^1,6^m$-$(Man)_2$-γCDs will be easily elucidated by enzymatic degradation methods with BSA. It would give all the same results in the case of using CGTase as BSA. Fig. 5 shows the chromatograms by RI detection and the mass chromatograms of the enzymatic degradation products of **A1, A2, B**, and **D** with BSA. In the LC–MS analysis, the mass chromatograms at special *m*/*z* 684, 1008, and 1170 for **1–3** corresponded to the enzymatic digests of **A1, A2, B**, and **D**, respectively, all these were monitored as adduct ions with ammonium ion $[M + NH_4]^+$ in the positive-ion ESI mode. Compounds **1** and **3** detected in the degradation products of **A1** and **D** were branched oligosaccharides of dp 7 and dp 6, respectively, since their adduct ions were observed at *m*/*z* 1170 and 1008. Thus, **A1** and **D** corresponded to $6^1,6^3$- and $6^1,6^2$-di-*O*-α-D-mannopyranosyl-γCDs, respectively. On the other hand, the same degradation product **2** of dp 4 (*m*/*z* 684) was obtained from both **A2** and **B**; therefore, they should be either of $6^1,6^4$- and $6^1,6^5$-di-*O*-α-D-mannopyranosyl-γCDs. For the distinction between $6^1,6^4$- and $6^1,6^5$- isomers, intermediates of the enzymatic

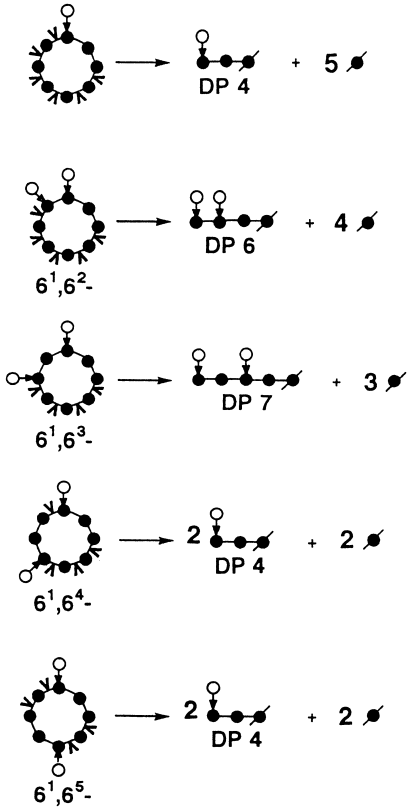

Fig. 4. Models of reaction on four positional isomers of $6^1,6^m$-$(Man)_2$-γCDs and Man-γCD with BSA. Symbols: ●, glucose; ●, glucose with reducing end; ○, mannose; ●—●, α-(1→4)-glucosidic linkage; ○, α-(1→6)-mannosidic linkage; ∧ , attack point of BSA.

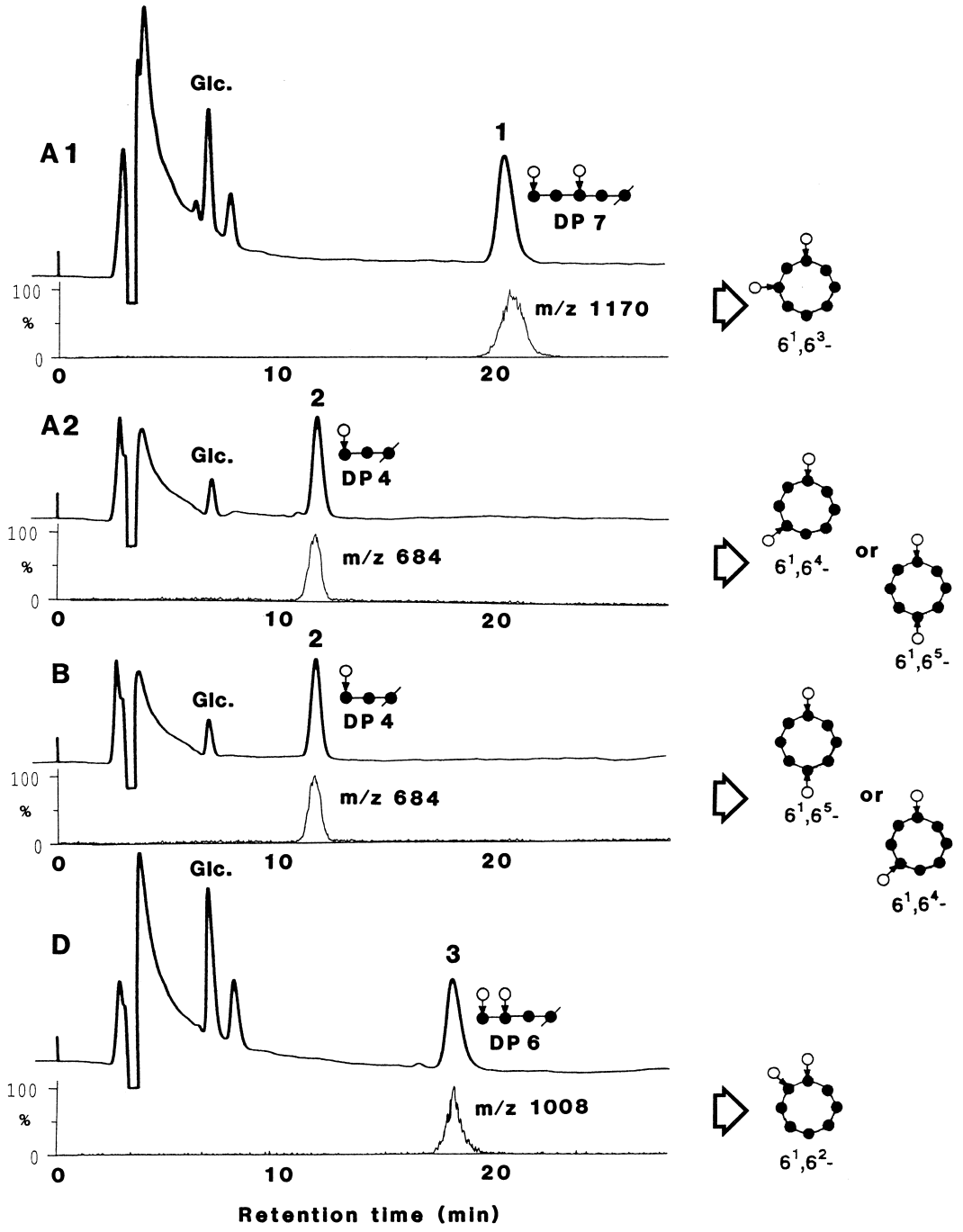

Fig. 5. Chromatograms of degradation products from **A1, A2, B**, and **D** with BSA and mass chromatograms of their ammonium adducts. Chromatographic conditions: column, YMC-Pack Polyamine-II (150×4.6 mm i.d.); eluent, 57:43 CH_3CN–10 mM CH_3COONH_4; flow rate, 0.5 mL/min; temperature, 30 °C. Each upper chromatogram was detected by RI.

degradation products of two isomers with combination of a small amount of BSA and a large amount of glucoamylase were examined. BSA is able to cleave eight α-(1→4)-glucosidic linkages of $6^1,6^4$- and $6^1,6^5$- isomers ((**1**)–(**8**)), but the bond (**4**) in Fig. 6 is cleaved with a little difficulty. Glucoamylase hydrolyzes α-(1→4)-glucosidic linkages immediately from the non-reducing end to the α-(1→6)-mannosidic linkage, after γCD rings of $6^1,6^4$- and $6^1,6^5$- isomers are opened with BSA. Therefore, a branched oligosaccharide of dp 8 is produced as the intermediate from the $6^1,6^4$-isomer, while it cannot been obtained from the $6^1,6^5$-isomer. Fig. 7 showed that the branched oligosaccharide of dp 8 (*m*/*z* 1332) was produced in the initial degradation products of **A2** with BSA and

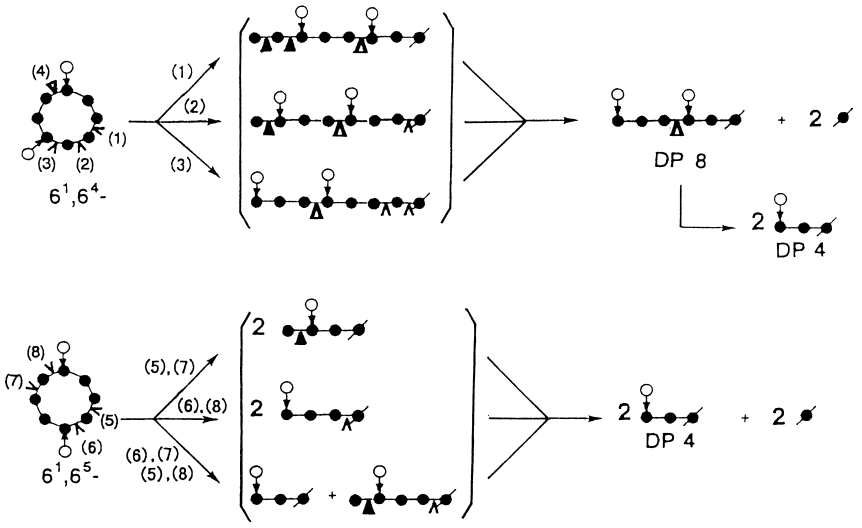

Fig. 6. Models of reaction on $6^1,6^4$- and $6^1,6^5$-$(Man)_2$-γCDs with combination of BSA and glucoamylase. Symbols: △, attack point of BSA with a little difficulty; ▲, attack point of BSA and glucoamylase; other symbols as in Fig. 4.

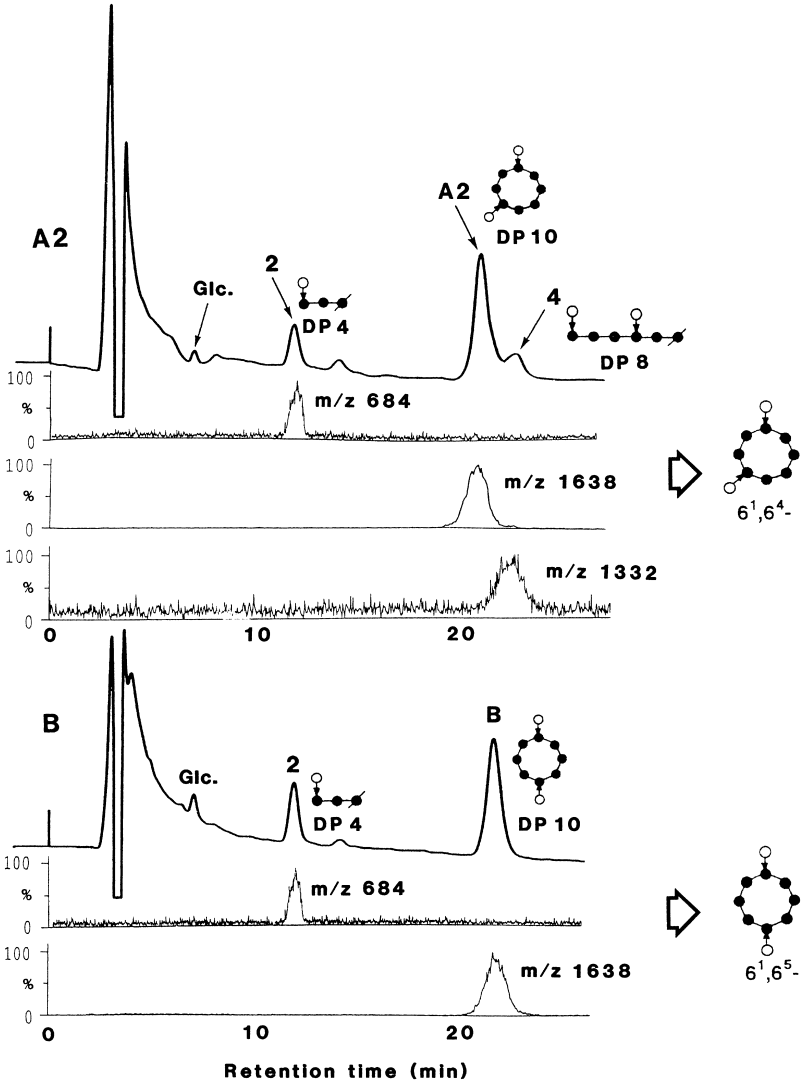

Fig. 7. Chromatograms of initial degradation products from **A2** and **B** with combination of BSA and glucoamylase, and mass chromatograms of their ammonium adducts. Reaction time is 1.5 h. Chromatographic conditions as in Fig. 6.

glucoamylase, while it was not observed in the digests of **B**. This result means that **A2** and **B** are $6^1,6^4$- and $6^1,6^5$-di-*O*-α-D-mannopyranosyl-γCDs, respectively.

In addition, the structures determined with enzymatic degradation methods agreed with those estimated previously by ^{13}C NMR spectroscopy.

4. Conclusions

All the isomers of $6^1,6^m$-$(Man)_2$-γCD and Man(1→*n*)Man(1→6)γCD in a mixture of products from γCD and D-mannose by condensation reaction of α-mannosidase from jack bean were isolated by HPLC. The four isomers of Man(1→*n*)Man(1→6)γCD (*n* = 2, 3, 4, and 6) were purified using an ODS column and a methanol–water system, and their structures were elucidated by NMR spectroscopy. The elution order on a YMC-Pack ODS column was Man(1→6)Man(1→6)γCD, Man(1→2)Man(1→6)γCD, Man(1→4)Man(1→6)γCD, and Man(1→3)Man(1→6)γCD, and the last two isomers were eluted too late compared with other two isomers.

On the other hand, the $6^1,6^3$- and $6^1,6^4$- isomers among four isomers of $6^1,6^m$-$(Man)_2$-γCD (*m* = 2, 3, 4, and 5) were very difficult to separate on the ODS column with the methanol–water system, while the $6^1,6^3$- and $6^1,6^4$- isomers were eluted in that order on a graphitized carbon column with an ethanol–water system. The structures of these isomers were revealed by LC–MS analysis of dps of the branched oligosaccharides produced by the enzymatic degradation with BSA, and combination of BSA and glucoamylase. The structures of the $6^1,6^2$- and $6^1,6^3$- isomers were easily determined, since branched oligosaccharides of dp 6 and dp 7, respectively, were detected in their enzymatic digests. The distinction of the $6^1,6^4$- and $6^1,6^5$- isomers was possible by detection of branched oligosaccharide of dp 8 as the intermediate in the degradation products with combination of BSA and glucoamylase, because of this branched oligosaccharide was obtained from only the $6^1,6^4$-isomer. The $6^1,6^m$-$(Man)_2$-γCDs were decomposed to the same final digests with CGTase and BSA. It is likely that a series of these positional isomers of dimannosyl-γCDs are very useful as the standard samples from the standpoint of the saccharide chains recognition in biochemistry.

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