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PRODUCTION AND SOME PROPERTIES OF BRANCHED CYCLO-MALTO-OLIGOSACCHARIDES

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ABSTRACT

Cyclomalto-oligosaccharides (cyclodextrins, CDs) with maltosyl and panosyl branches were produced from maltose or panose and CDs by the reverse action of pullulanase. Purification on columns of octadesylated silica gave maltosylcyclomalto-hexaose and -heptaose and panosylcyclomalto-hexaose and -heptaose. The solubility of maltosylcyclomaltoheptaose in aqueous 80% ethanol was higher than that of cyclomaltoheptaose in water. The relative rates of degradation of maltose, maltosylcyclomaltohexaose, and maltosylcyclomaltoheptaose with glucoamylase were 1:3.6:5.0, and those of panose, panosylcyclomaltohexaose, and panosylcyclomaltoheptaose were 1:3.0:2.2. The rates of degradation of branched and unbranched malto-oligosaccharides were markedly different, as were those of maltosyl- and panosyl-cyclomalto-oligosaccharides.

INTRODUCTION

Cyclomalto-oligosaccharides (cyclodextrins, CDs) have been used as complexing agents for the stabilization of oily unstable substances and for modifying the physical properties of insoluble and volatile materials such as flavors, and are used widely in the food industry[1]. However, cyclomalto-hexaose (αCD), -heptaose (βCD), and -octaose (γCD) have low solubilities in water[2]. Glucosyl-CDs have extremely high water solubility[3] and methods for the production of such branched CDs have been developed[4–7]. A mixture containing αCD, βCD, γCD, and their maltosyl and dimaltosyl derivatives, which is available commercially, is very water-soluble and is used widely in liquid and paste types of food.

Malto-oligosaccharides can be attached to HO-6 of CDs by the reverse action of pullulanase, and the separation and analysis of the resulting branched CDs have been reported[8], as have their solubilities[6,7]. The preparation of maltosyl and panosyl derivatives of αCD and βCD is now reported.

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The degradation of glucosylcyclomalto-hexaose and -heptaose by the alpha-amylase from *Aspergillus oryzae* has been reported[3]. The susceptibility of the branching moiety of the above branched CDs to glucoamylase is now described.

EXPERIMENTAL

Maltose (purity 99%) and panose (purity 97%) were commercial products, and αCD and βCD were kindly donated by Ensuiko Sugar Refining Co., Ltd., Promozyme (thermostable pullulanase, 200 U/g), kindly donated by Novo Industry Japan Ltd., was dialyzed against 0.1M acetate buffer (pH 5.0) in order to remove the stabilising sugars that inhibit the reverse action of the enzyme, and concentrated to 200 U/mL before use.

Pullulanase (from *Aerobacter aerogenes*, crystalline, suspended in 2.7M ammonium sulfate), kindly dotaned by Hayashibara Co., Ltd., was dialyzed against 0.1M acetate buffer (pH 5.0) before use.

Preparation of panosylcyclomalto-hexaose and -heptaose. — To a solution of maltose (200 g) in 0.1M citrate–phosphate buffer (300 mL, pH 7.0) was added 60 IU of transglucosidase powder (TG "Amano" 100 U/g), kindly donated by Amanoseiyaku Co., Ltd. (Nagoya), and the mixture was kept at 55° for 22 h. The enzyme was inactivated at ~100° for 15 min, then removed by centrifugation. Distilled water (500 mL), yeast cake (50 g for bread making), and calcium carbonate (5 g) were added to the supernatant solution, and fermentation was carried out at 40° for 48 h in order to eliminate glucose and maltose. The mixture was centrifuged, and the supernatant solution was decolorised and passed through Amberlite MB-3 resin, to give panose (60% purity) contaminated with glycerol and isomalto-oligosaccharides containing 2–5 glucose residues.

Cyclomalto-hexaose or -heptaose (10 g) and dialyzed Promozyme (1,600 IU, 8 mL) were added to 100 mL of the above 60% panose preparation. The mixture was shaken at 60° and pH 5–5.5 for 48 h. The enzyme was inactivated at ~100° for 10 min, and the mixture was decolorised and filtered to give a clear panosyl-CD preparation. The conversion of cyclomaltohexoase into panosylcyclomaltohexaose was 38%, and that of cyclomaltoheptaose into panosylcyclomaltoheptaose was 30%.

Preparation of maltosylcyclomalto-hexaose and -heptaose. — The pH of a solution of maltose (300 g), and cyclomalto-hexaose or -heptaose (100 g) in distilled water (300 mL) was adjusted to 5–5.5. Dialyzed Promozyme solution (80 mL, 16,000 U) was added and the mixture was shaken at 60° for 48 h. After inactivation of the enzyme, decolorisation, and filtration, clear maltosyl-CD preparations were obtained. The conversion of cyclomaltohexaose into maltosyl-cyclomaltohexaose was 42%, and that of cyclomaltoheptaose into maltosyl-cyclomaltoheptaose was 32%.

Column chromatography on ODS (octadesylated silica). — ODS-100 (150A silica), kindly donated by Japan Organo Co., Ltd., was suspended in aqueous 50%

ethanol in a glass column (2.9 × 100 cm) and was washed with distilled water at 100 mL/h overnight. Each branched cyclomalto-hexaose or -heptaose preparation (400 mL) was applied to the column and eluted first with water (300 mL) to remove acyclic oligosaccharides. Thereafter, gradient elution (1 L of water–1 L of aqueous 20% ethanol) was performed at room temperature.

The total sugar in each fraction was determined by the phenol–sulfuric acid method, and the purity of branched CD in the fractions was checked by h.p.l.c. using a JASCO 880-PU pump equipped with an 880-51 degasser, an 860-CO column oven, a 7125 Rheodyne injector, an 830-RI differential refractometer, and an SIC chromatocorder. The column (4 × 250 mm) contained LiChrospher NH_2 (5 μm, Merck); solvent system, aqueous 55–60% acetonitrile at 0.8 mL/min at 20°; column pressure, <100 kg/cm^2. The refractometer was maintained at 35°. The peak area of the sugars corresponded to the dry weight of the sugars.

Solubility. — Excess of each branched CD was shaken with 200 μL of various concentrations of aqueous ethanol at room temperature for 2 h. After centrifugation, the amount of solute was determined by h.p.l.c. Solubilities are reported as g/100 mL.

Action of glucoamylase on branched CDs. — Glucoamylase powder [from *Rhyzopus niveus,* pure grade, 35 U/mg, Lot No. 76470, Seikagaku Kogyo Co., Ltd. (Tokyo)] was dissolved in 0.11M acetate buffer (pH 5.20) to give 10 U/mL. Substrate solution (2 mL, 0.1 mol/L) and glucoamylase solution (100 μL) were mixed and stirred at 40°. At intervals, an aliquot (200 μL) was removed and heated at ~100° for 8 min to inactivate the enzyme, and 10 μL was subjected to h.p.l.c. Degradation connotes 100 × (peak area of degraded sugar)/(peak area of total sugar).

RESULTS AND DISCUSSION

Purification of branched CDs. — Unreacted cyclomaltoheptaose (βCD) was precipitated after storage of the Promozyme-generated mixture from maltose and βCD at 4° for 2–3 days, and then removed. Fig. 1B shows the h.p.l.c. profile of the mother liquor (starting solution), and Fig. 1A shows the starting solution for maltosylcyclomaltohexaose, from which unreacted αCD was not removed. The structures of the components were determined by the action of pullulanase. The component in each peak was isolated and treated with pure pullulanase, and the products were analyzed by h.p.l.c. and p.c. Maltose and CD were formed from the components of peaks G_2-αCD and G_2-βCD and from $(G_2)_2$-αCD and $(G_2)_2$-βCD in the molar ratios 1:1 and 2:1, respectively. Only maltose was formed from $G_2 \rightarrow G_2$, the mobility in p.c. of which was between those of maltotetraose and maltopentaose (1-butanol–pyridine–water, 6:4:4). Thus, these components were indicated to be maltosylcyclomalto-hexaose and -heptaose, dimaltosylcyclomalto-hexaose and -heptaose, and 6^2-α-maltosylmaltose. The structures of panosyl-CDs were also determined in a similar manner.

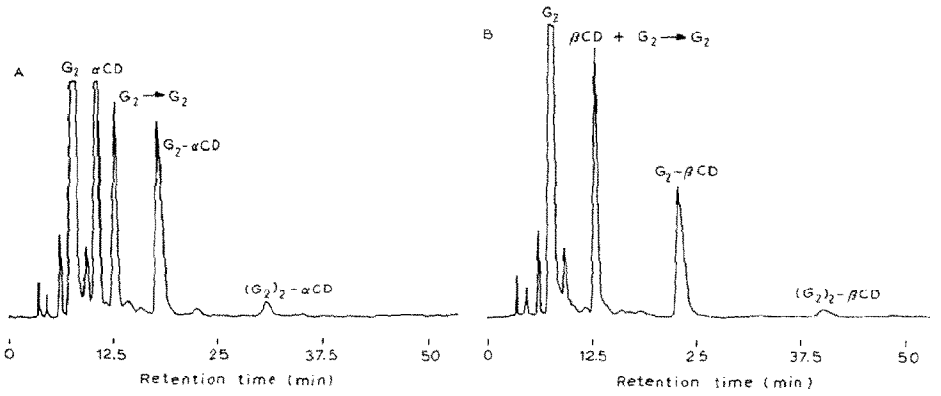

Fig. 1. H.p.l.c. of the starting solutions for A, maltosylcyclomaltohexaose; and B, maltosylcyclomaltoheptaose: G_2, maltose; $G_2 \rightarrow G_2$, 6²-α-maltosylmaltose.

Maltosyl-cyclomaltohexaose was separated from the mixture of products by gradient on ODS with aqueous ethanol (Fig. 2A). Fractions of >95% purity were combined and freeze-dried to give maltosylcyclomaltohexaose (13 g, purity 98%, from 100 g of αCD). Fractions before 42 contained mainly dimaltosylcyclomaltohexaose, and pure material (1 g, purity 98%, from 100 g of αCD) was obtained by rechromatography on ODS by elution with aqueous 4% ethanol. The branched cyclomaltoheptaose was desorbed more readily from ODS than was branched cyclomaltohexaose (Fig. 2B), and even dimaltosylcyclomaltoheptaose was desorbed by elution with water (~300 mL). Accordingly, maltose contaminated

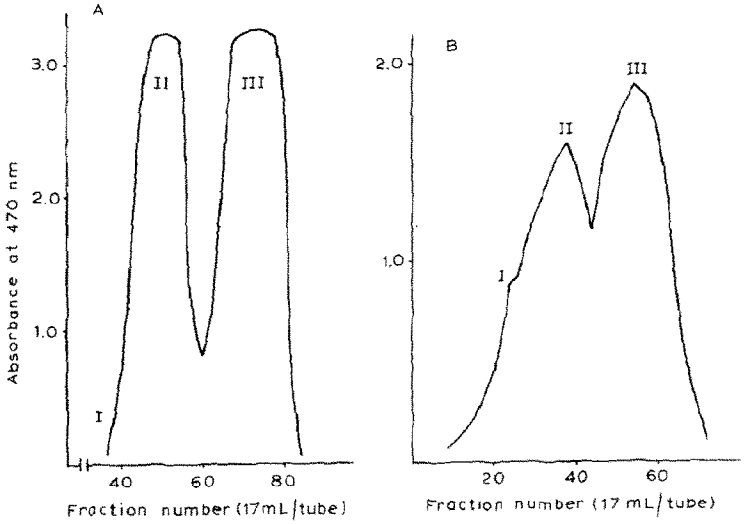

Fig. 2. Elution profiles for column chromatography on ODS; A, maltosylcyclomaltohexaose; and B, maltosylcyclomaltoheptaose: I, dimaltosylcyclomalto-hexaose or -heptaose, II, maltosylcyclomaltohexaose or -heptaose; III, cyclomalto-hexaose or -heptaose (see EXPERIMENTAL).

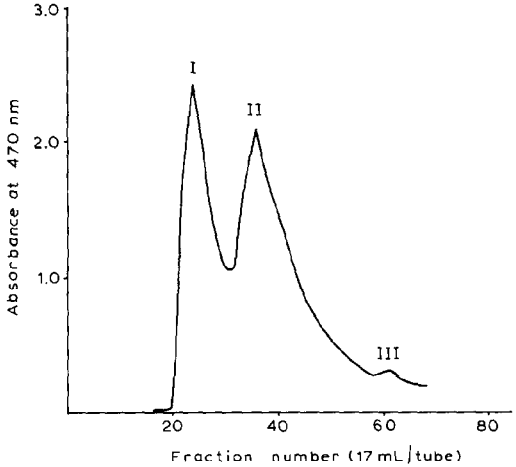

Fig. 3. Rechromatography on ODS of the fractions mainly containing dimaltosylcyclomaltoheptaose (see EXPERIMENTAL for details of the column). The concentrated fraction (20 mL, 40%) was eluted with aqueous 4% ethanol at 100 mL/h, and 10-μL aliquots of the fractions were analysed. The symbols are the same as in Fig. 2.

the fractions before 20. This contamination was reduced by elution at <25°. On elution at >65°, the branched CDs were desorbed easily, but the ODS was damaged at >80°.

The material in fractions 20–28, mainly dimaltosylcyclomaltoheptaose, was

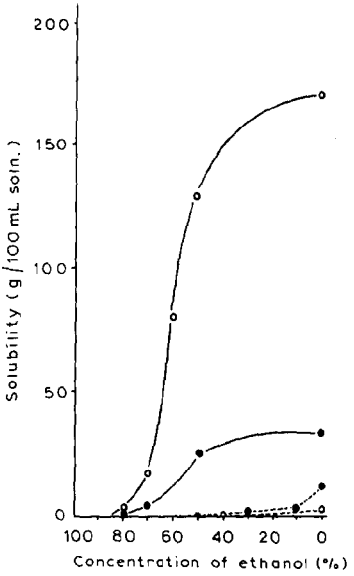

Fig. 4. Solubility of branched CDs in aqueous ethanol (see EXPERIMENTAL): —●—, maltosylcyclomaltohexaose; —○—, maltosylcyclomaltoheptaose; -----●-----, cyclomaltohexaose; -----○-----, cyclomaltoheptaose.

refractionated on ODS by elution with aqueous 4% ethanol (Fig. 3) to give 1 g of pure dimaltosylcyclomaltoheptaose (purity 98%).

Panosylcyclomalto-hexaose and -heptaose were also purified as described above and 200 mg of each pure preparation was obtained. The elution volume of these branched CDs was between those of dimaltosylcyclomalto-hexaose and -heptaose, and maltosylcyclomalto-hexaose and -heptaose.

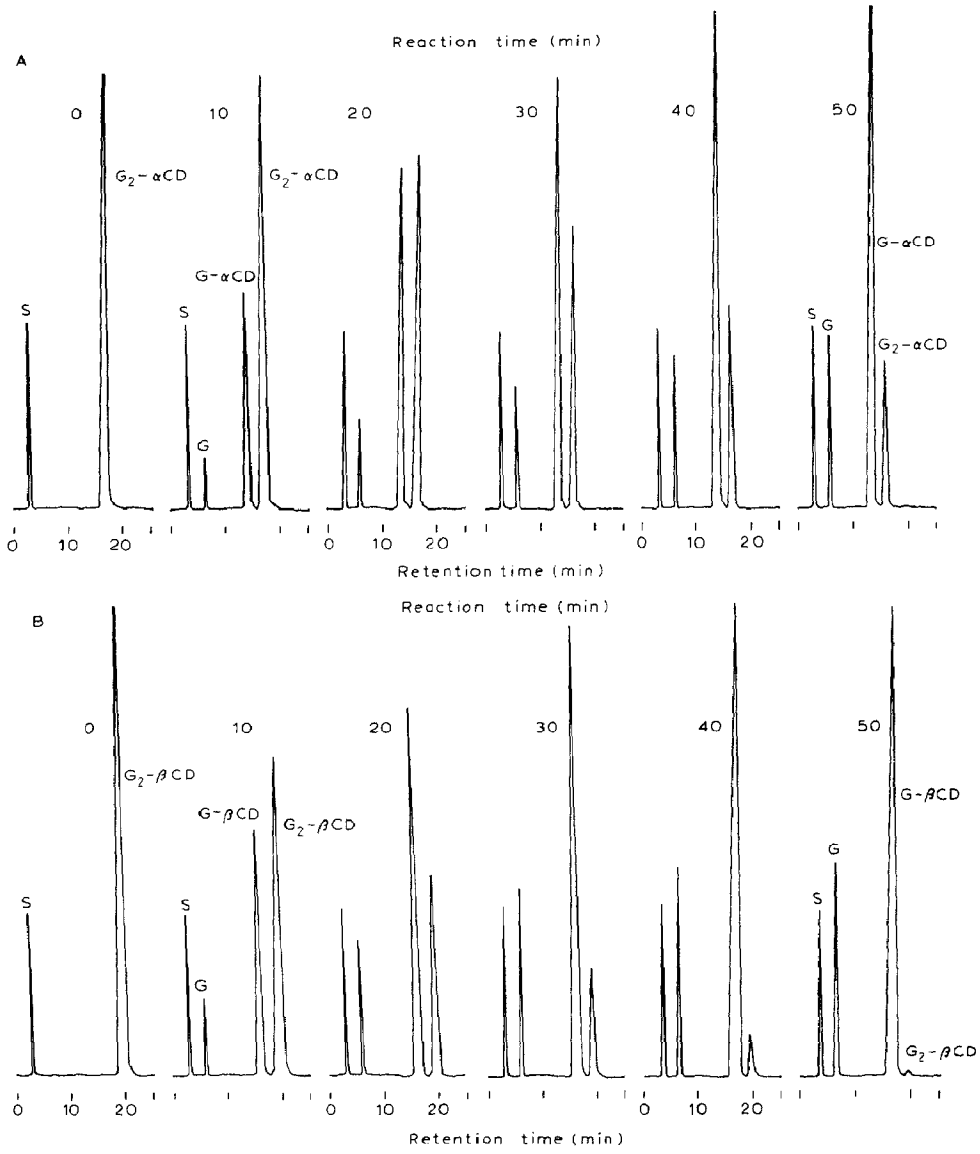

Fig. 5. H.p.l.c. profiles showing gradual degradation by glucoamylase of A, maltosylcyclomalto-hexaose; B, maltosylcyclomaltoheptaose: S, solvent peak; G, glucose; G_2, maltose.

The molecular weights of the branched CDs, determined by secondary ion mass spectrometry[9] (SIMS, using Hitachi model M-80B), were 1,296 and 1.458, and 1.458 and 1,620, for maltosyl- and panosyl-cyclomalto-hexaose and -heptaose, respectively.

Values of $[\alpha]_D^{20}$ (*c* 1, water), determined with a JASCO DIP 181 digital polarimeter, were +164.5° and +167.8°, and +171.7° and +176.1°, for maltosyl- and panosyl-cyclomalto-hexaose and -heptaose, respectively.

Solubility of the branched cyclomaltodextrins. — As shown in Fig. 4, the solubility of maltosylcyclomaltoheptaose is extraordinarily high (170 g/100 mL of solution) and, even in aqueous 80% ethanol, it is more soluble than cyclomalto-heptaose in water. The solubility of maltosylcyclomaltohexaose is not so high.

CDs that comprise an odd number of glucose residues (cyclomaltoheptaose and cyclomaltononaose) have low solubilities in water, in contrast to those that have even numbers of glucose residues (cyclomaltohexaose and cyclomalto-octaose). However, the trends for the solubility of branched CDs are not clear. From the results reported here, singly branched cyclomaltoheptaose has a solubility higher than that of singly branched cyclomaltohexaose. Sakano and Shiraishi[6]

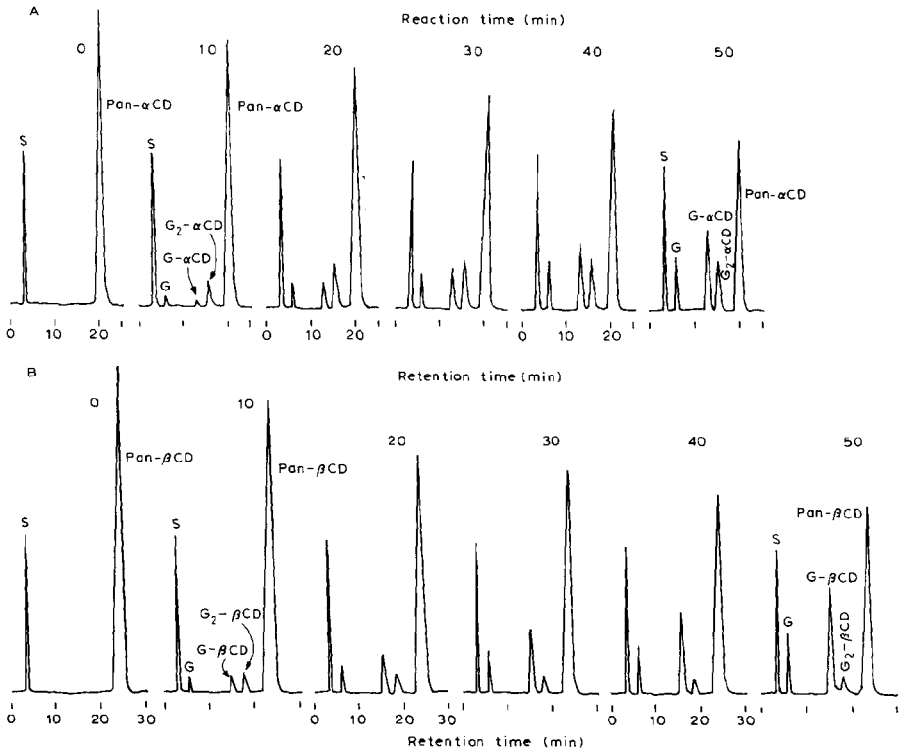

Fig. 6. H.p.l.c. profiles showing gradual degradation by glucoamylase of A, panosylcyclomaltohexaose; B, panosylcyclomaltoheptaose: S, solvent peak; G, glucose; Pan, panose.

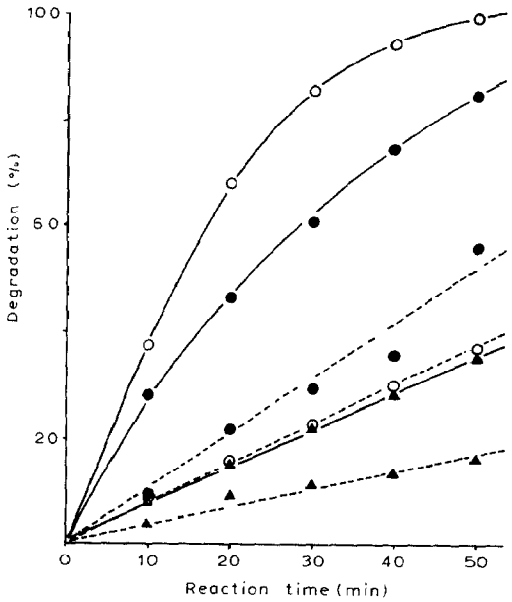

Fig. 7. Degradation of branched CDs by glucoamylase: —●—, maltosylcyclomaltohexaose; —○—, maltosylcyclomaltoheptaose, -----●-----, panosylcyclomaltohexaose; -----○-----, panosylcyclomaltoheptaose; —▲—, maltose; -----▲-----, panose.

reported that, in contrast, doubly branched cyclomaltoheptaose has a solubility lower than that of doubly branched cyclomaltohexaose. The relation between the structure and solubility requires elucidation. Therefore, we plan to prepare a variety of branched CDs on a large scale.

Action of glucoamylase on the branched CDs. — As shown in Fig. 5, maltosyl-cyclomalto-hexaose, and -heptaose were degraded gradually by glucoamylase to form glucose and glucosylcyclomalto-hexaose and -heptaose. Maltosylcyclomalto-heptaose was the more susceptible to the glucoamylase. The relative rates of degradation were 5.0 and 3.6 (*cf.* 1 for maltose).

The rate of degradation of panosylcyclomaltohexaose was slightly higher than that of panosylcyclomaltoheptaose (Fig. 6), but much lower than those of the maltosyl analogues. The maltosyl derivatives were formed as intermediates. A considerable amount of the intermediate from panosylcyclomaltohexaose accumulated, but not for panosylcyclomaltoheptaose. The rates of degradation by glucoamylase are summarized in Fig. 7.

The rates of degradation of dimaltosylcyclomalto-hexaose and -heptaose are shown in Fig. 8. The process involves $(G_2)_2$-CD→G_2,G_1-CD→$(G_1)_2$-CD. The rate of degradation of dimaltosylcyclomaltoheptaose was remarkably higher than that of dimaltosylcyclomaltohexaose. The intermediate from dimaltosylcyclomalto-hexaose accumulated and the amount of diglucosylcyclomaltohexaose formed was significantly low. This result shows that maltosyl-glucosyl-cyclomaltohexaose is

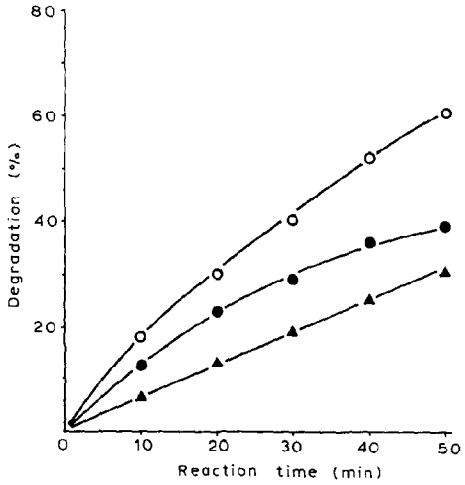

Fig. 8. Degradation of dimaltosylcyclomalto-hexaose and -heptaose by glucoamylase: —●—, dimaltosylcyclomaltohexaose; —○—, dimaltosylcyclomaltoheptaose; —▲— maltose.

resistant to glucoamylase. Thus, branched CDs could be suitable substrates for probing the mechanism of glucoamylase action.

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