Production and Some Properties of Branched Cyclodextrins†

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Cyclodextrins (CDs,†† cyclomaltooligosaccharides) 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 $G_2\text{-}\alpha\text{-CD}$, $G_2\text{-}\beta\text{-CD}$, $\text{Pan-}\alpha\text{-CD}$, and $\text{Pan-}\beta\text{-CD}$.

The solubility of $G_2\text{-}\beta\text{-CD}$ in aqueous 80% ethanol was higher than that of $\beta\text{-CD}$ in water. The relative rates of degradation of maltose, $G_2\text{-}\alpha\text{-CD}$, and $G_2\text{-}\beta\text{-CD}$ with glucoamylase were 1 : 3.6 : 5.0 and those of panose, $\text{Pan-}\alpha\text{-CD}$, and $\text{Pan-}\beta\text{-CD}$ were 1 : 3.0 : 2.2. The rates of degradation of branched and unbranched maltooligosaccharides were markedly different from those of $G_2\text{-CDs}$ and Pan-CDs .

Cyclodextrins (CDs, cyclomaltooligosaccharides) 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, $\alpha\text{-CD}$, $\beta\text{-CD}$, and $\gamma\text{-CD}$ have low solubilities in water.²⁾ $G_1\text{-CDs}$ have extremely high water solubility³⁾ and methods for the production of such branched CDs have been developed.⁴⁻⁷⁾ A mixture containing $\alpha\text{-CD}$, $\beta\text{-CD}$, and $\gamma\text{-CD}$ 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.

Maltooligosaccharides can be attached to C6-OH of CDs by the reverse action of pullulanase and the separation and analysis of the resulting branched CDs has been reported,⁸⁾ as well as their solubilities.^{6,7)} The preparation of maltosyl and panosyl derivatives of $\alpha\text{-CD}$ and $\beta\text{-CD}$ is now reported.

The degradation of $G_1\text{-}\alpha\text{-CD}$ and $G_1\text{-}\beta\text{-CD}$

by the $\alpha\text{-amylase}$ from *Aspergillus oryzae* has been reported.⁹⁾ The susceptibility of the branching moiety of the above branched CDs to glucoamylase is now described.

EXPERIMENTALS

Maltose (purity 99%) and panose (purity 97%) were commercial products, and $\alpha\text{-CD}$ and $\beta\text{-CD}$ 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 0.1 M acetate buffer (pH 5.0) in order to remove the stabilizing sugars which inhibit the reverse action of the enzyme and concentrated to 200 U/ml before use.

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

Preparation of Pan- $\alpha\text{-CD}$ and Pan- $\beta\text{-CD}$.

To a solution of maltose (200 g) in 0.1 M citrate-phosphate buffer (300 ml, pH 7.0), 60 IU of transglucosidase powder [TG "Amano" 100 U/mg, kindly donated by Amanoseiyaku Co., Ltd. (Nagoya)] was added, and the mixture was kept at 55°C for 22 hr. The enzyme was

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†† The symbols G_1 , $(G_1)_2$, G_2 , $(G_2)_2$, Pan- , CD , $\alpha\text{-CD}$, $\beta\text{-CD}$, $\gamma\text{-CD}$, $\delta\text{-CD}$ are abbreviations for glucosyl-, diglucosyl-, maltosyl-, dimaltosyl-, panosyl-, cyclodextrin, cyclomaltohexaose, cyclomaltoheptaose, cyclomaltooctaose, cyclomaltononaose.

inactivated at $\sim 100^\circ\text{C}$ for 15 min, and 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°C for 48 hr in order to eliminate glucose and maltose. The mixture was centrifuged, the supernatant solution was decolorized, and passed through Amberlite MB-3 resin to give panose (60% purity) contaminated with glycerol and isomaltooligosaccharides contained 2–5 glucose units.

α -CD or β -CD (10 g) and dialyzed Promozyme (1600 IU, 8 ml) were added to 100 ml of the above 60% panose preparation. The mixture was shaken at 60°C and pH 5–5.5 for 48 hr. The enzyme was inactivated at $\sim 100^\circ\text{C}$ for 10 min, the mixture was decolorized and filtered to give a clear Pan-CD preparation. The conversion of α -CD into Pan- α -CD was 38%, and that of β -CD into Pan- β -CD was 30%.

Preparation of G_2 - α -CD and G_2 - β -CD. The pH of the solution of maltose (300 g), α -CD or β -CD (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°C for 48 hr. After inactivation of the enzyme, decolorization and filtration, clear G_2 -CD preparations were obtained. Conversion of α -CD into G_2 - α -CD was 42%, and that of β -CD into G_2 - β -CD was 32%.

Column chromatography on ODS (octadecylated silica). ODS-100 (150 A 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/hr overnight. Each branched α -CD or β -CD 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 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 HPLC 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 used (4×250 mm) contains LiChrospher NH_2 ($5 \mu\text{m}$, Merck). Solvent system, aqueous 55–60% acetonitrile at 0.8 ml/min at 20°C . Column pressure, <100 kg/cm 2 . The refractometer was maintained at 35°C . The peak area of the sugars corresponded to dry weight of the sugars.

Solubility. Excess amount of each branched CD was shaken with 200 μl of various concentrations of aqueous ethanol at room temperature for 2 hr. After centrifugation, the amount of solute was determined by HPLC. Solubilities are reported as g/100 ml.

Action of glucoamylase on branched CDs. Glucoamylase powder [from *Rhizopus niveus*, pure grade, 35 U/mg, Lot No. 76470, Seikagaku Kogyo Co., Ltd. (Tokyo)] was dissolved in 0.1 M acetate buffer (pH 5.20) to make 10 U/ml. Substrate solution (2 ml, 0.1 mol/l) and glucoamylase solution (100 μl) were mixed and stirred at 40°C . At intervals, an aliquot (200 μl) was removed, heated at $\sim 100^\circ\text{C}$ for 8 min to inactivate the enzyme, and 10 μl was subjected to HPLC. Degradation correlates $100 \times$ (peak area of degraded sugar)/(peak area of

Fig. 1. Solubility of branched CDs in aqueous ethanol.

—●—, G_2 - α -CD; —○—, G_2 - β -CD; ---●---, α -CD; ---○---, β -CD.

Fig. 2. HPLC profiles showing gradual degradation of $G_2\text{-}\alpha\text{-CD}$ by glucoamylase. S, solvent peak; 1, glucose; 2, $G_1\text{-}\alpha\text{-CD}$; 3, $G_2\text{-}\alpha\text{-CD}$.

Fig. 3. HPLC profiles showing gradual degradation of $\text{Pan-}\alpha\text{-CD}$ by glucoamylase. S, solvent peak; 1, glucose; 2, $G_1\text{-}\alpha\text{-CD}$; 3, $G_2\text{-}\alpha\text{-CD}$; 4, $\text{Pan-}\alpha\text{-CD}$.

total sugar).

RESULTS AND DISCUSSION

$G_2\text{-}\alpha\text{-CD}$, $G_2\text{-}\beta\text{-CD}$, $\text{Pan-}\alpha\text{-CD}$, and $\text{Pan-}\beta\text{-CD}$ were purified. The molecular weight of the branched CDs, determined by Secondary Ion Mass Spectrometry⁹⁾ (SIMS, using Hitachi model M-80B), was 1296, 1458, 1458 and 1620 for

$G_2\text{-}\alpha\text{-CD}$, $G_2\text{-}\beta\text{-CD}$, $\text{Pan-}\alpha\text{-CD}$, and $\text{Pan-}\beta\text{-CD}$, respectively.

$[\alpha]_D(C=1.0, H_2O)$, determined by digital polarimeter, model DIP 181 (Japan Spectroscopic Co., Ltd.), was +164.5, +167.8, +171.7 and +176.1 for $G_2\text{-}\alpha\text{-CD}$, $G_2\text{-}\beta\text{-CD}$, $\text{Pan-}\alpha\text{-CD}$, and $\text{Pan-}\beta\text{-CD}$, respectively.

Solubility of the branched CDs. As shown in Fig. 1, solubility of $G_2\text{-}\beta\text{-CD}$ is extraordinary

Fig. 4. Degradation of branched CDs by glucoamylase.

—●—, G₂-α-CD; —○—, G₂-β-CD; ---●---, Pan-α-CD; ---○---, Pan-β-CD. —▲—, maltose; ---▲---, panose.

rily high (170 g/100 ml solution), and even in aqueous 80% ethanol, it is more soluble than β-CD in water. The solubility of G₂-α-CD is not so high. CDs that comprise an odd number of glucose units (β-CD and γ-CD) have low solubilities in water in contrast to those that have even numbers of glucose units (α-CD and γ-CD). However, the trends for the solubility of branched CDs are not clear. From the results reported here, singly-branched β-CD has a solubility higher than that of singly-branched α-CD. Sakano and Shiraishi reported⁶⁾ that, in contrast, doubly-branched β-CD has solubility lower than doubly-branched α-CD. The relation between the structure and solubility requires elucidation.

Action of glucoamylase on the branched-CDs. As shown in Fig. 2, G₂-α-CD was de-

graded gradually by glucoamylase to form glucose and G₁-α-CD. G₂-β-CD was the more susceptible to the glucoamylase, and the relative rates of degradation were 5.0 and 3.6 (cf. 1 for maltose).

The rate of degradation of Pan-α-CD was slightly higher than that of Pan-β-CD (Fig. 3) but much lower than those of the maltosyl analogues. The maltosyl derivatives were formed as intermediates. A considerable amount of the intermediate from Pan-α-CD accumulated, but not for Pan-β-CD.

The rates of degradation by glucoamylase are summarized in Fig. 4.

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