Acid-Catalyzed Hydrolysis of Maltosyl-β-cyclodextrin

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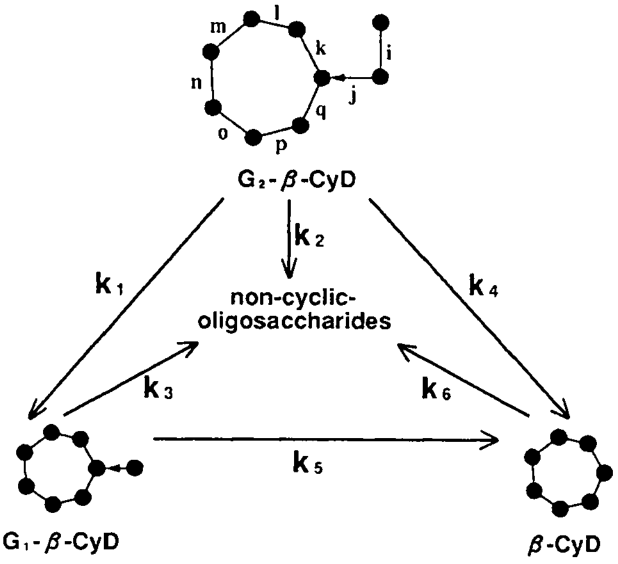
Abstract ☐ Maltosyl-β-cyclodextrin was hydrolyzed via two pathways in acidic solution: (1) ring opening to give noncyclic oligosaccharides and (2) cleavage of maltose in the branched residue to give glucosyl-β-CyD and glucose. Ring opening was ~2–3 times faster than maltose cleavage because of the multiple hydrolysis sites of the β-cyclodextrin (β-CyD) ring (seven glycosidic linkages) compared with only one reaction site of the maltose residue in the branch. Values of the enthalpy and entropy of activation of the hydrolyses were positive and in the range reported for maltose, a result indicating that the hydrolyses proceeded according to the A-1 mechanism (i.e., unimolecular decomposition). The α-1,6-glycosidic bond of branched β-CyDs connecting β-CyD and branched sugar moieties resisted hydrolysis; this property is a potential pharmaceutical advantage because the parent β-CyD, which has low aqueous solubility, would not precipitate after hydrolysis.



Scheme I—Proposed hydrolysis pathways of G_2-β-CyD. Key: (●) glucose unit; (●——●) α-1,4-glycosidic linkage; (●——→●) α-1,6-glycosidic linkage.

Branched cyclodextrins (CyDs) are highly hydrophilic derivatives that have sugar moieties substituted at primary hydroxyl groups via an α-1,6-glycosidic bond and that form inclusion complexes with various drug molecules.[1,2] Branched CyDs are promising candidates as fast-dissolving carriers for poorly water soluble drugs, because their aqueous solubility (>50%, w/v at 25 °C) and biocompatibility are superior to those of the parent β-CyD.[3–5] Reports on the chemical stability of branched CyDs are scarce.[3] From a kinetic point of view, branched CyDs are of interest, because they have three types of glycosidic linkage that are susceptible to acid-catalyzed hydrolysis: (1) α-1,4-bonds in the CyD ring, (2) α-1,6-bonds at a junction between the ring and the branched sugars, and (3) α-1,4-bonds in the branched sugar moieties. In this study, the kinetics of the acid-catalyzed hydrolysis of maltosyl-β-CyD (G_2-β-CyD; Scheme I) was investigated.

Experimental Section

Materials—β-CyD was supplied by Nihon Shokuhin Kako Company (Tokyo, Japan) and was recrystallized from water. Highly purified G_2-β-CyD and glucosyl-β-CyDs (G_1-β-CyDs; >99.9%) were donated by Ensuiko Sugar Refinning Company (Yokoyama, Japan). All other materials and solvents were of analytical reagent grade. Deionized, double-distilled water was used.

Kinetics—G_2-β-CyD (2.5×10^{-3} M) was hydrolyzed in 0.1 M HCl–KCl buffer [ionic strength (μ) = 0.2, pH 1.1] at various temperatures. At timed intervals, the reaction solution (0.5 mL) was sampled and neutralized with 0.1 M NaOH (0.5 mL) containing α-CyD (2.5×10^{-3} M) as an internal standard for analysis by high-performance liquid chromatography (HPLC). The neutralized solution (20 μL) was analyzed by HPLC for simultaneous determination of G_2-β-CyD, G_1-β-CyD, and parent β-CyD. The HPLC system consisted of a pump (Hitachi 655A, Tokyo, Japan), a detector (Shodex SE-51 differential refractometer, Tokyo, Japan), a column (ERC-NH-1181, 6 mm diameter × 250 mm; Erma Optical Works, Tokyo, Japan), a mobile phase of acetonitrile:water (65:35, v/v), and a flow rate of 1.2 mL/min. The three β-CyDs were well separated (Figure 1),[6,7] and linear calibration curves were obtained over the concentration range (1.0×10^{-4}–2.5×10^{-3} M) of β-CyDs. The following data were obtained from plots of concentrations of β-CyD, G_1-β-CyD, and G_2-β-CyD, respectively, versus peak height ratios (β-CyDs:internal standard): slopes of 7.244×10^{-3}, 8.343×10^{-3}, and 8.951×10^{-3}; intercepts of 9.980×10^{-5}, 2.297×10^{-4}, and 1.300×10^{-4}; and correlation coefficients of 0.995, 0.991, and 0.993. Rate constants were determined from an average of three measurements, which were within 5% of each other.

Results and Discussion

Glycosides may be degraded by hydrolysis, epimerization, and dehydration. Among these reactions, acid-catalyzed hydrolysis of glycosidic linkages predominates in dilute solutions of substrates at temperatures <100 °C.[8] Because G_2-β-CyD has three types of glycosidic linkage in the molecule, hydrolysis under acidic conditions proceeds according to Scheme I: (1) hydrolysis of α-1,4-bond in the branch to yield G_1-β-CyD, (2) hydrolysis of α-1,6-bond to yield parent β-CyD, and (3) hydrolysis of α-1,4-bond in the ring to yield noncyclic oligosaccharides. The HPLC chromatogram of partially hydrolyzed products of G_2-β-CyD (Figure 2) indicates that, as the reaction progressed, the intensity of the G_2-β-CyD peak decreased, and those of G_1-β-CyD, glucose (retention time, 8.12 min; capacity factor, 0.68), and maltose (retention time, 9.82 min; capacity factor, 1.04) appeared and increased gradually. The peak of parent β-CyD was not observed during the reaction; the same result was obtained for G_1-β-CyD hydrolysis. Unfortunately, noncyclic saccharides, except for glucose and maltose, were not quantitatively determined perhaps because of (1) poor sensitivity of detection [the refractive index of noncyclic saccharides was much lower (<1/2) than that of CyDs] and (2) further rapid hydrolysis of noncyclic saccharides to maltose and glucose.

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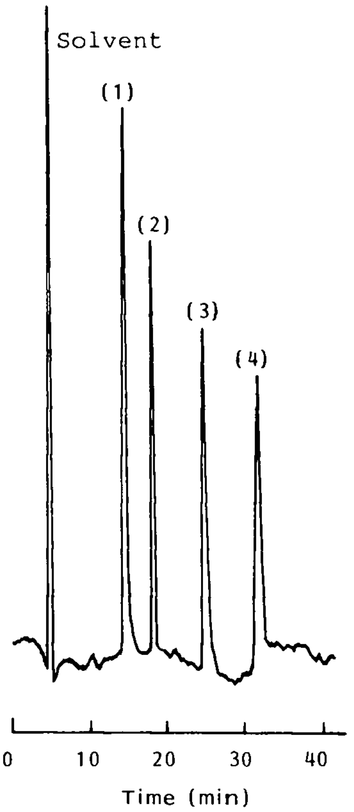


Figure 1—Liquid chromatogram of α-CyD (**1**, internal standard), β-CyD (**2**), G_1-β-CyD (**3**), and G_2-β-CyD (**4**).

Figure 2—Liquid chromatogram of partially hydrolyzed products of G_2-β-CyD. Key: (**1**) glucose; (**2**) maltose; (**3**) G_1-β-CyD; (**4**) G_2-β-CyD. The increase in the baseline at ~11 min was due to the elution of salts such as KCl and NaCl.

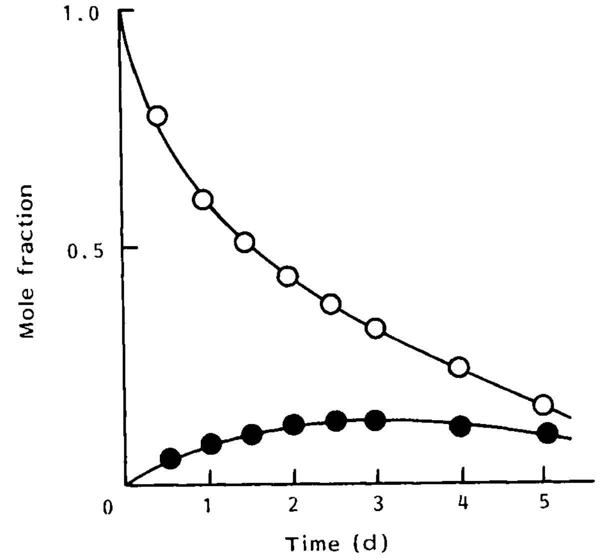


Figure 3—Reaction profiles for hydrolysis in 0.1 M HCl–KCl buffer (pH 1.1, μ = 0.2) at 60 °C. Key: (○) G_2-β-CyD; (●) G_1-β-CyD.

In this study, therefore, we followed changes in the concentrations of G_2-β-CyD and G_1-β-CyD. Figure 3 shows curves of concentration of G_2- and G_1-β-CyDs in 0.1 M HCl–KCl buffer at 60 °C versus time (concentration–time curves). With an exponential decrease in the concentration of G_2-β-CyD, the concentration of G_1-β-CyD increased initially and then subsequently decreased. Therefore, the reaction profiles were analyzed according to eqs 1 and 2 with a least-squares method to obtain the rate constants (k_1, k_2, and k_3). The rate constants in eqs 1 and 2 were treated as pseudo-first-order rate constants, because the concentration of HCl was much higher than those of the substrates:

$$d[G_2]/dt = -(k_1 + k_2)[G_2] \tag{1}$$

$$d[G_1]/dt = k_1[G_2] - k_3[G_1] \tag{2}$$

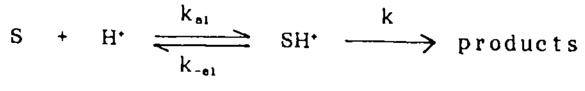
In eqs 1 and 2, $[G_2]$ and $[G_1]$ are the concentrations of G_2- and G_1-β-CyD, respectively.

Acid-catalyzed hydrolysis of glycosidic bonds proceeds according to the A-1 mechanism (unimolecular decomposition)[8–10] via the Arrhenius complex (SH^+, $k_{-a1} >> k$), not via the van't Hoff complex ($k_{-a1} << k$), as shown by Scheme II (S and SH^+ are the substrate and protonated substrate, respectively; k_{a1} and k_{-a1} are rate constants for the protonation of S and the deprotonation of SH^+, respectively; and k is the rate constant for the degradation of SH^+ to products). Because the rate-determining step in Scheme II is the decomposition of SH^+, the rate of hydrolysis (v) of S can be written as in eq 3, where K is the dissociation constant of SH^+, as defined by eq 4:

$$v = k[SH^+] = (k/K)\,[S][H^+] \tag{3}$$

$$K = k_{-a1}/k_{a1} = [S][H^+]/[SH^+] \tag{4}$$

Because G_2-β-CyD has nine glycosidic oxygens (from i to q in



$$S + H^+ \underset{k_{-a1}}{\overset{k_{a1}}{\rightleftharpoons}} SH^+ \xrightarrow{k} \text{products}$$

Scheme II

Scheme I) in the molecule, eq 5 can be derived for the overall hydrolysis rate:

$$v = \Big(\sum_{i} (k_i/K_i)\Big)[S][H^+] + \Big(\sum_{i}\sum_{j} (k_{ij}/K_{ij})\Big)[S][H^+]^2 +$$

$$\Big(\sum_{i}\sum_{j}\sum_{k} (k_{ijk}/K_{ijk})\Big)[S][H^+]^3 +$$

$$\Big(\sum_{i}\sum_{j}\sum_{k}\sum_{l} (k_{ijkl}/K_{ijkl})\Big)[S][H^+]^4 +$$

$$\cdots\cdots\Big(\sum_{i}\sum_{j}\sum_{k}\sum_{l}\sum_{m}\sum_{n}\sum_{o}\sum_{p}\sum_{q}(k_{ijklmnopq}/$$

$$K_{ijklmnopq})\Big)[S][H^+]^9 \qquad (5)$$

In eq 5, each variable from i to q has a value of 1 to 9, $i<j<k<l<m<n<o<p<q$, and subscripts i–q stand for the nine glycosidic oxygens. The first term of eq 5 is the rate equation for the monoprotonated species, each protonated glycosidic linkage being hydrolyzed at the rate of k_i/K_i. The second term of eq 5 is the rate equation for the biprotonated species, one of two protonated sites being hydrolyzed with the rate constant of k_{ij}/K_{ij}. The third to ninth terms are, similarly, those for the tri- to nonaprotonated species, respectively. Equation 5 can be simplified to eq 6 on the basis of the following results and assumptions. (1) The second to ninth terms of eq 5 are negligible, because the rate of hydrolysis of G_2-β-CyD was first-order dependent on hydronium ion concentration (Figure 4). The pH values in Figure 4 can be regarded as those of the Hammett acidity function (H_0),[11] because of the low acid concentration (<0.1 M HCl). (2) No appearance of parent β-CyD was observed, a result indicating that the α-1,6-glycosidic linkage is resistant to hydrolysis, and thus, k_j is negligible. (3) $k_k = k_q$, $k_l = k_p$, and $k_m = k_o$ from the symmetry relationship of the ring. (4) The ring-opening rate was assumed to be same ($k_k = k_m = k_n$ to simplify the kinetic treatment and to correlate eq 1 with eq 5.

$$v = (k_i/K_i + 2 \cdot k_k/K_k + 2 \cdot k_l/K_l +$$

$$2 \cdot k_m/K_m + k_n/K_n)[S][H^+]$$

$$\approx (k_i/K_i + 7 \cdot k_k/K_k)[S][H^+] \qquad (6)$$

$$k_1 = (k_i/K_i)[H^+] \qquad (7)$$

$$k_2 = 7\ (k_k/K_k)[H^+] \qquad (8)$$

$$k_3 = 7\ (k_k/K_k)[H^+] \qquad (9)$$

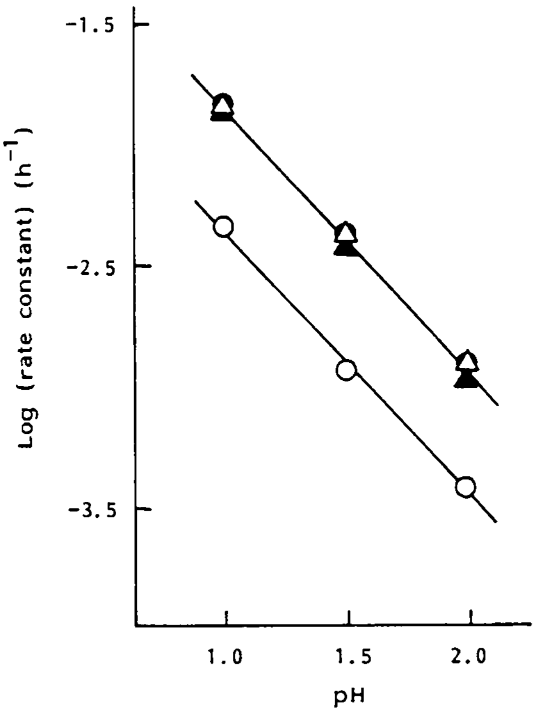


Figure 4—Profiles of pH versus hydrolysis rates of G_2-β-CyD, G_1-β-CyD, and parent β-CyD at 60 °C. Key: (○) k_1; (●) k_2; (△) k_3; (▲) k_6.

Table I summarizes the rate constants k_1, k_2, and k_3 at 50, 60, and 70 °C; these constants are correlated with those of the elemental steps by eqs 7–9. In the hydrolysis of G_1-β-CyD under the same conditions, no appearance of parent β-CyD was observed, and the degradation rate constant of G_1-β-CyD was in good agreement with the k_3 value determined from G_2-β-CyD. The apparent ring-opening rate (5.21×10^{-2} h^{-1} at 70 °C) of parent β-CyD was of the same order of magnitude as that (2.4×10^{-2} h^{-1} at 70 °C) reported by Schönberger et al.[12]; the higher rate constant obtained in this study may be due to the presence of potassium chloride, because the intermediate of the A-1 mechanism is a positively charged oxonium ion.[10] On the other hand, the rate constant (1.14×10^{-2} h^{-1} at 0.115 N HCl and 80 °C) reported by Szejtli et al.[13,14] seems to be slightly underestimated because of the indirect monitoring of CyD concentrations. The hydrolysis rate (4.87×10^{-3} h^{-1} at 60 °C) of maltose in the branch of G_2-β-CyD was in agreement with that (3.76×10^{-3} h^{-1} in 0.1 N HCl at 60 °C) of maltose calculated with the data of Wolfrom et al.[15]

The apparent ring-opening rates (k_2 and k_3) of β-CyDs were ~2–3 times faster than the hydrolysis rate (k_1) of the glycosidic bond in the branch of G_2-β-CyD (Table I) because of the multiple reaction sites (seven glycosidic linkages) of the ring compared with one reaction site of the maltose residue in the branch. Therefore, the rate of the one-bond cleavage was ~2–3 times slower for the ring compared with the linear branch. The ring-opening rate constants of G_1- and G_2-β-CyD were almost the same as that of parent β-CyD.

Figure 5 shows Arrhenius plots for the hydrolysis rates (k_1, k_2, k_3, and k_6) of G_2-β-CyD and parent β-CyD, and Table II summarizes the thermodynamic activation parameters. Both enthalpy (ΔH^*) and entropy (ΔS^*) values for the one-bond cleavage of β-CyDs were positive (133–148 kJ · mol^{-1} and 34–88 J · K^{-1} · mol^{-1}, respectively). The positive ΔS^* values

Table I—Rate Constants for Hydrolysis of G_2-β-CyD in 0.1 M HCl–KCl Buffer (pH 1.1, μ = 0.2)

Rate Constant	Value[a] at Indicated Temperature × 10^3, h^{-1}		
	50 °C	60 °C	70 °C
k_1	0.89	4.87	23.8
k_2	2.25 (0.32)[b]	14.7 (2.10)	45.6 (6.51)
k_3	2.49 (0.36)	14.7 (2.10)	48.7 (6.96)
k_3[c]	2.65 (0.38)	14.3 (2.04)	49.7 (7.10)
k_6[d]	2.72 (0.39)	14.1 (2.00)	52.1 (7.44)

[a] Average values of triplicate runs, which were within 5% of each other. [b] The values in parentheses are rate constants for the one-bond cleavage. [c] Determined from the hydrolysis of G_1-β-CyD as a starting substrate. [d] Determined from the hydrolysis of parent β-CyD.

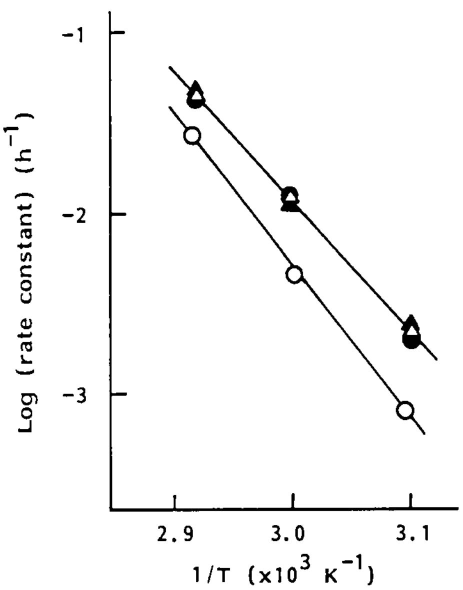


Figure 5—Arrhenius plots for hydrolysis rates of G_2-β-CyD, G_1-β-CyD, and parent β-CyD in 0.1 M HCl–KCl buffer (pH 1.1, μ = 0.2). Key: (○) k_1; (●) k_2; (△) k_3; (▲) k_6.

Table II—Thermodynamic Activation Parameters[a] for Hydrolysis of G_2-β-CyD in 0.1 M HCl–KCl Buffer (pH 1.1, μ = 0.2) at 60 °C

Rate Constant	ΔG^*, kJ · mol^{-1}	ΔH^*, kJ · mol^{-1}	ΔS^*, J · K^{-1} · mol^{-1}
k_1	120	148	87.7
k_2	117 (122)[b]	136	57.9 (42.3)
k_3	117 (122)	134	53.4 (35.6)
k_6[c]	117 (122)	133	50.2 (33.5)

[a] Standard errors (n = 3): ΔG^*, ±2 kJ · mol^{-1}; ΔH^*, ±2 kJ · mol^{-1}; and ΔS^*, ±7 J · K^{-1} · mol^{-1}. [b] The values in parentheses are activation parameters for the one-bond cleavage. [c] Determined from the hydrolysis of parent β-CyD.

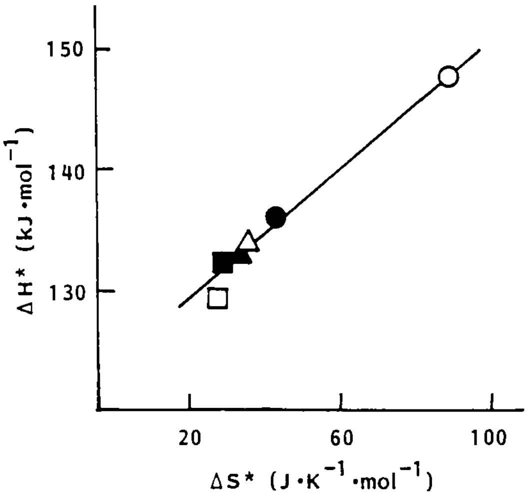


Figure 6—Isokinetic relationship for hydrolysis of G_2-β-CyD, G_1-β-CyD, and parent β-CyD. Key: (○) k_1; (●) k_2; (△) k_3; (▲) k_6; (□) hydrolysis of maltose reported by Wolfrom[15]; (■) hydrolysis of maltose reported by Szejtli.[13,14]

indicate the hydrolysis of β-CyDs according to the A-1 mechanism (i.e., the protonated species, SH^+ in Scheme II, is unimolecularly decomposed).[8] Of course, the positive change of ΔS^* may be at least partly responsible for the positive entropy change of the proton-transfer reaction, because k_1, k_2, and k_3 were functions of the equilibrium constant of the protonation, as shown by eqs 7–9. Activation enthalpies and entropies for the hydrolysis of disaccharides are usually 120–170 kJ · mol^{-1} and 20–75 J · K^{-1} · mol^{-1}, respectively.[10] The activation parameters of the ring opening of β-CyDs were comparable to those (ΔH^* = 129 and 133 kJ · mol^{-1} and ΔS^* = 27 and 31 J · K^{-1} · mol^{-1} in ~0.1 N HCl at 60 °C) of maltose reported by Wolfrom et al.[15] and Szejtli et al.[13,14] On the other hand, the activation parameters of maltose in the branch of G_2-β-CyD were relatively higher. The larger entropy gain observed for the latter may be a result of increased motional freedom when going to the transition state, because of splitting of the bulky macrocyclic ring from the maltose moiety. For ring opening of β-CyDs, less motional freedom may be necessary because of the intramolecular hydrolysis. Figure 6 shows the isokinetic relationship for the hydrolysis of the three kinds of β-CyDs. The plot of ΔH^* versus ΔS^* for one-bond cleavage was linear (correlation coefficient, 0.999); therefore, hydrolysis of the β-CyDs followed the same mechanism. The isokinetic temperature calculated from the slope of the line was 272 K, and the points of ΔH^* and ΔS^* of maltose reported by Wolfrom[15] and Szejtli[13,14] fell well on the straight line.

In conclusion, G_2-β-CyD was hydrolyzed via two pathways in acidic solution: ring opening and cleavage of maltose in the branch. Ring opening was apparently faster (~2–3 times) than maltose cleavage because of the multiple hydrolysis sites of the β-CyD ring (seven glycosidic linkages) compared with one reaction site of maltose residue in the branch. It is a pharmaceutical advantage that the α-1,6-glycosidic bond of branched β-CyDs resists hydrolysis, because no precipitation of parent β-CyD, which has low aqueous solubility (1.8% w/v at 25 °C), after hydrolysis will occur.

In this study, we focused on the hydrolysis of the maltose residue in the branch and the apparent ring opening. To clarify the detailed features of the hydrolysis of branched β-CyDs, particularly after the ring opening, fractional analysis of the hydrolysates of noncyclic oligosaccharides will be necessary.

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