Synthesis of Specifically Modified Maltooligosaccharides by Enzymatic Degradation of Cyclodextrin Derivatives. Substrate-Based Investigation of the Active Site of Taka-Amylase

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Enzymatic hydrolysis of 2-, 3-, or 2,3-substituted cyclodextrin (**1–11**) by Taka-amylase gave selectively a modified oligomaltosaccharide (**12–20**) as a main product in good yield. These results suggest the importance of the interaction between the C-2–OH of the oligosaccharide and the subsites R and S of Taka-amylase and also suggest the trivial importance of the interaction between the hydroxyl and the other subsites, including T. The important interaction of the C-3–OH with the subsites S, T, and R is also suggested.

There is increasing need for specifically modified oligosaccharides in many fields.[1] Syntheses of maltooligosaccharide derivatives by condensation of two oligosaccharides are more difficult than those of β-linked D-glucosyl species.[2] Although very effective methods for synthesis of compounds containing α-linkages have been developed recently,[3] syntheses of specifically modified maltoolgosaccharides are still troublesome. Direct modification of maltooligosaccharides at specific positions seems almost impossible because of the production of many isomers, although a few examples have been successful.[3b,4] Melton and Slessor reported a unique type of synthesis by use of α-cyclodextrin derivatives.[5] They found that hydrolysis of 6-monosubstituted α-cyclodextrins by Taka-amylase A (TAA) specifically gave 6′-substituted maltose. We have also described the specific synthesis of 6′,6″-disubstituted maltotriose by Taka-amylolysis of 6A,6B-disubstituted cyclodextrins.[6] We report here useful applications of this method to the syntheses of modified oligosaccharides.

In order to obtain information about interaction between TAA and substrates, substrate specificity has been successfully studied by use of partially O-methylated amylose[7] and specifically modified phenyl maltosides.[4b,8] Studies using the latter substrates have shown that a modification on either C-3′ or C-4′ influences cleavage of the phenyl glucoside bond to only a minor extent, but that a modification on one of the other carbons (C-2, C-3, C-6, and C-2′) completely inhibits enzymatic liberation of phenol. Since information available so far is limited to the subsites[9] R and S of TAA shown in Figure 1, availability of variety of specifically modified amyloses is required to provide information about subsites other than R and S. Cyclodextrins can be modified easily and specifically at the desired position (C-6, C-3, or C-2).[6,10] Since cyclodextrins are substrates of TAA,[11] the modified cyclodextrin can be regarded as a kind of specifically modified amylose.[5,6] Therefore, studies on the interaction between TAA and modified cyclodextrins will hopefully provide the information concerning all subsites including R and S.

Many chemists have been interested in construction of enzyme mimics through specific modification of cyclodextrins.[6,10] One of the most interesting and challenging problems pertaining to construction of better enzyme mimics from cyclodextrins is the structure determination of disubstituted cyclodextrins. For example, when a cyclodextrin is modified on the C-2 carbons with two different substituents, there are five (2A2B, 2A2C, ... and 2A2F), six (2A2B, 2A2C, ... and 2A2G), and seven (2A2B, 2A2C, ... and 2A2H) isomers for α-, β-, and γ-cyclodextrins, respectively. Spectral differences among them, if any, do not aid in structure determination. In this situation, if enzymatic hydrolysis of a modified cyclodextrin were to give specifically a simple linear oligosaccharide, it would be very useful in structure determination.[12]

We describe here TAA hydrolyses of secondary sulfonates and epoxides of cyclodextrins which provide new information about interactions between the subsites and substrates, useful clues for determining the isomeric structure of disubstituted cyclodextrins, and methods of preparation of specifically substituted maltooligosaccharides.

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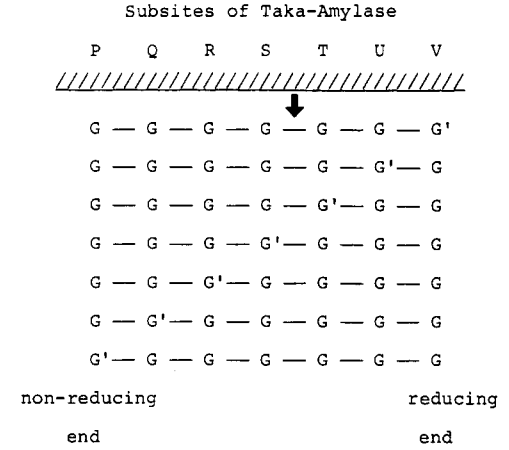


Figure 1. Binding patterns of modified oligosaccharide to Taka-amylase: P–V, subsite; G, glucose unit; G′, modified glucose unit; ↓, cleavage point of glucosidic bond.

Experimental Section

General. ^{1}H NMR spectra were determined with a JEOL FX-90Q (90 MHz), a JEOL FX-100 (100 MHz), or a JEOL JNM GX-270 (270 MHz) spectrometer. ^{13}C NMR spectra were obtained with a JEOL FX-90Q (22.5 MHz), a JEOL FX-100 (25 MHz), or a JEOL JNM GX-270 (67.5 MHz) spectrometer. Fast atom bombardment mass (FABMS), field desorption mass (FDMS), and electron impact mass (EIMS) spectra were recorded with a JEOL JMS DX-300, DX-303, or D-300/JMA 3500 data system. Thin-layer chromatography (TLC) was run with precoated silica gel plates (Merck, Art 5554). Spot detection was carried out by UV light and/or staining with 0.1% 1,3-dihydroxynaphthalene in EtOH–H_2O–H_2SO_4 (200:157:43 V/V/V). A solvent of TLC development was n-C_3H_7OH–AcOEt–H_2O (7:7:5 V/V/V). A Merck Lobar prepacked column (LiChroprep RP18 column, 25 × 310 mm) was used for reversed-phase column chromatography. High-performance liquid chromatography (HPLC) was performed on a Shimadzu LC3A with a Tskgel ODS-120T column (4.6 × 250 mm, 5 μm, Toyo Soda, Japan) or with a Chemcosorb 5-ODS-H column (4.6 × 100 mm, 5 μm, Chemco, Japan).

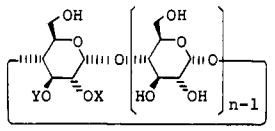
Materials. Crystalline Taka-amylase (TAA, EC 3.2.1.1 α-1,4-glucan-4-glucanohydrolase) was supplied by Sankyo Co. Ltd. (Japan). The preparations and structural assignments of 3-*O*-(β-naphthylsulfonyl)-β-cyclodextrin (**1**),[10j] 3-*O*-(β-naphthylsulfonyl)-α-cyclodextrin (**2**),[10g] 3-*O*-(*p*-tosyl)-α-cyclodextrin (**3**),[10k] 2-*O*-(α-naphthylsulfonyl)-α-cyclodextrin (**5**),[10g] 2-*O*-(β-naphthylsulfonyl)-α-cyclodextrin (**6**),[10g] 2-*O*-(*p*-tosyl)-α-cyclodextrin (**7**),[10k] 2,3-anhydro-(3*R*)-β-cyclodextrin (**8**),[10j] 2,3-anhydro-(3*R*)-α-cyclodextrin (**9**),[10k] 2,3-anhydro-(2*S*)-β-cyclodextrin (**10**),[10l] and 2,3-anhydro-(2*S*)-α-cyclodextrin (**11**)[10k] have been reported before. 2-*O*-(α-Naphthylsulfonyl)-β-cyclodextrin (**4**) was prepared and structurally assigned as follows. Powdered α-naphthalenesulfonyl chloride (3.5 g) was added in one portion to 50 mL of aqueous solution (pH 12.0, adjusted by addition of aqueous NaOH) of β-cyclodextrin (3.5 g). The suspension was vigorously stirred at room temperature and the pH of the suspension was allowed to decrease rapidly. After the mixture became neutral, it was filtered and applied on a reversed-phase column. A gradient elution from water (1 L) to 10% aqueous CH_3CN (1 L) gave pure **4** (121.4 mg, 3.0%).

4: ^{13}C NMR (25 MHz, Me_2SO-d_6, absorptions other than aromatic carbons) δ 59.7, 69.1, 71.9, 72.2, 72.8, 80.5, 80.8, 81.4, 97.9, 101.7; FABMS, m/z 1325 (M + H$^+$).

The naphthalenesulfonate **4** (50 mg) was dissolved in 5 mL of aqueous K_2CO_3 (pH 12) and kept at room temperature for 30 min. After neutralization by addition of HCl, the solution was applied on a reversed-phase column. A gradient elution from water (1 L) to 10 % aqueous MeOH (1 L) gave pure **10** (37.2 mg, 88. 4 %).

10: ^{13}C NMR (67.5 MHz, D_2O, characteristic absorptions) δ 51.82, 56.66, 62.72, 62.87, 62.96, 63.51, 100.27, 103.81, 104.09, 104.25, 104.33, 104.39; FABMS, m/z 1139 (M + Na$^+$).

Hydrolysis by Taka-Amylase. A $^1/_{50}$-fold amount by the weight of TAA in relation to that of the modified cyclodextrin was used in the hydrolysis.[13] A solution of TAA and a modified



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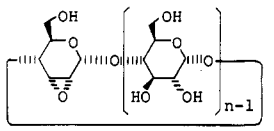
2 : n = 6, X = H, Y = β-Ns
3 : n = 6, X = H, Y = Ts
4 : n = 7, X = α-Ns, Y = H
5 : n = 6, X = α-Ns, Y = H
6 : n = 6, X = β-Ns, Y = H
7 : n = 6, X = Ts, Y = H

Ns ≡ naphthalenesulfonyl
Ts ≡ p-toluenesulfonyl



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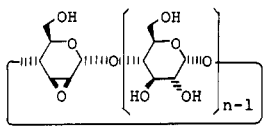

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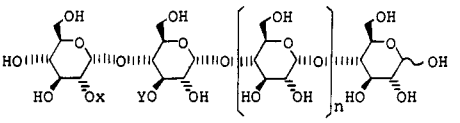


12 : n = 1, X = H, Y = β-Ns
13 : n = 0, X = H, Y = β-Ns
14 : n = 1, X = H, Y = Ts
15 : n = 0, X = H, Y = Ts
16 : n = 0, X = α-Ns, Y = H
17 : n = 0, X = β-Ns, Y = H
18 : n = 0, X = Ts, Y = H

cyclodextrin (**4**–**11**) in acetate buffer solution (pH 5.5, 0.2 M) containing calcium chloride (0.01 M) was kept at 40 °C to give a modified oligosaccharide (**16**–**20**) as a main product. The progress of the enzymatic reaction was monitored by use of silica gel TLC. Usually the particular oligosaccharide obtained was the main product from the initial stage of reaction to the final stage. Exceptionally, an intermediate product **12** or **14** was observed in the case of the amylolyses of **1** and **2** to **13**, or **3** to **15** and was isolated, respectively. In the case of **1** and **2**, the enzymatic hydrolysis gave one main product (**12**) other than glucose and maltose at the initial stage of reaction, and **12** was completely changed to **13** after 2 days. A similar result was obtained in Taka-amylolysis of **3**. After elimination of TAA through a Sephadex G-25 column, the solution was applied on a reversed-phase column or charcoal (Darco G-60)–Celite (1:1) column. After an elution with water to eliminate inorganic salts, a gradient elution with methanol was applied to the column to give a pure product. The reaction conditions and the yields are shown in Table I.

12: ^{13}C NMR (25 MHz, D_2O, characteristic absorptions other than aromatic absorptions) δ 63.1, 87.8, 94.6, 98.4, 100.9, 102.1, 102.5; FABMS, m/z 857 (M + H$^+$), 879 (M + Na$^+$), 895 (M + K$^+$).

13: ^{13}C NMR (25 MHz, D_2O, characteristic absorptions other than aromatic absorptions) δ 63.1, 87.8, 94.6, 98.4, 100.8, 102.1; FABMS, m/z 717 (M + Na$^+$), 733 (M + K$^+$).

14: ^{13}C NMR (25 MHz, D_2O, characteristic absorptions other than aromatic absorptions) δ 63.0, 87.7, 94.6, 98.4, 100.8, 102.5; FABMS, m/z 843 (M + Na$^+$).

15: ^{13}C NMR (25 MHz, D_2O, characteristic absorptions other than aromatic absorptions) δ 63.0, 87.8, 94.6, 98.4, 100.8, 102.2; FABMS, m/z 659 (M + H$^+$), 681 (M + Na$^+$).

16: ^{13}C NMR (25 MHz, D_2O, characteristic absorptions other than aromatic absorptions) δ 63.0, 82.6, 94.7, 96.3, 96.7, 98.5, 103.0; FABMS, m/z 695 (M + H$^+$), 717 (M + Na$^+$).

17: ^{13}C NMR (25 MHz, D_2O, characteristic absorptions other than aromatic absorptions) δ 62.9, 82.3, 94.6, 97.8, 98.0, 98.4, 102.5;

(13) Commercially available TAA (α-amylase Type X-A, Sigma) is convenient for the large-scale syntheses. In these cases, the enzyme is used in the same amount to that of the cyclodextrin derivatives, where the result is very similar to that obtained by use of pure TAA.

Table I. Taka-Amylolysis of Modified Cyclodextrins at 40 °C[a]

cyclodextrin derivative (unit, mg)	reaction condition		product
	volume of solvent, mL	reaction period	
1 (200)	20	30 min	**12** (103, 1 mg, 79.4%)
1 (100)	10	48 h	**13** (33, 2 mg, 63.4%)
2 (100)	10	12 h	**12** (20.5 mg, 27.8%) + **13** (36.7 mg, 61.5%)
2 (200)	20	44 h	**13** (80.5 mg, 67.4%)
3 (100)	10	13 h	**14** (51.2 mg, 70.3%) + **15** (12 mg, 20.5%)
3 (100)	10	7 days	**15** (26 mg, 59.8%)
4 (50)	5	1 h	**16** (18.8 mg, 71.8%)
5 (50)	4.5 + Me_2SO (0.5)	24 h	**16** (23.8 mg, 79.6%)
6 (50)	5	24 h	**17** (27 mg, 90.3%)
7 (50)	4.5 + Me_2SO (0.5)	15 h	**18** (28.5 mg, 97.6%)
8 (50)	5	1 h	**19** (27.1 mg, 93.4%)
9 (70)	7	7 days	**19** (31 mg, 65%)
10 (50)	5	2 h	**20** (27.7 mg, 95.4%)
11 (100)	10	7 days	**20** (32 mg, 47%)

[a] Solvent, 0.2 M acetate buffer (pH 5.5) containing calcium chloride (0.01 M). A $^1/_{50}$-fold amount by weight of Taka-amylase in relation to that of the modified cyclodextrin was used.

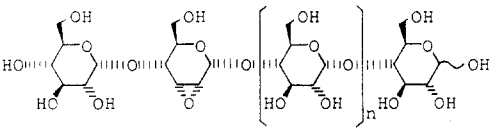
FABMS, m/z 717 ($M + Na^+$), 733 ($M + K^+$).

18: ^{13}C NMR (25 MHz, D_2O, characteristic absorptions other than aromatic absorptions) δ 23.5, 62.9, 82.3, 94.6, 98.0, 98.1, 98.4, 102.2; FABMS, m/z 659 ($M + H^+$), 681 ($M + Na^+$).

19: ^{13}C NMR (22.5 MHz, D_2O, characteristic absorptions) δ 54.1, 58.0, 63.1, 63.2, 94.6, 96.9, 98.5; FABMS, m/z 671 ($M + Na^+$).

20: ^{13}C NMR (25 MHz, D_2O, characteristic absorptions) δ 52.3, 55.7, 63.0, 63.3, 63.6, 68.2, 94.5, 98.4, 98.7, 99.4, 102.0; FABMS, m/z 649 ($M + H^+$), 671 ($M + Na^+$).

Epoxidation of 12–18. A solution of **12** (80 mg) in 0.1 N NaOH (5 mL) or 0.1 N $Ba(OH)_2$ (5 mL) was stirred at room temperature for 3 h and neutralized with HCl or CO_2, respectively. After filtration, the filtrate was chromatographed by a reversed-phase column with an elution of water to give **19** (56.5 mg, 93.2%). Similarly, **13** (33.2 mg), **14** (15 mg), **15** (26 mg), **16** (45.9 mg), **17** (15 mg), or **18** (10 mg) gave **21** (24.3 mg, 99.2%), **19** (11.8 mg, 99.6%), **21** (18 mg, 93.7%), **22** (21.1 mg, 65.7%), **22** (8.2 mg, 78.1%), or **22** (6.4 mg, 86.5%), respectively.



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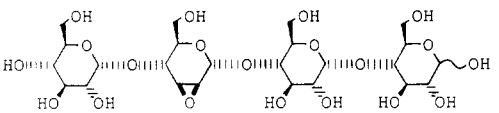

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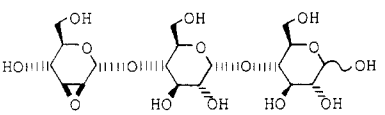

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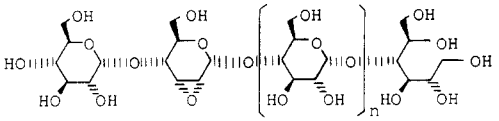


23 : n = 1
24 : n = 0

21: ^{13}C NMR (25 MHz, D_2O, characteristic absorptions) δ 54.1, 58.0, 63.1, 63.2, 94.6, 96.9, 97.7, 98.5; FABMS, m/z 509 ($M + Na^+$).

22: ^{13}C NMR (25 MHz, D_2O, characteristic absorptions) δ 52.6, 58.3, 63.2, 63.6, 94.5, 98.4, 98.6, 102.9; FABMS, m/z 487 ($M + H^+$), 509 ($M + Na^+$).

$NaBH_4$ Reduction of 18–21. A solution of **18** (46 mg) in 1% aqueous $NaBH_4$ (5 mL) was kept at room temperature for 20 h. After neutralization of the solution by addition of dilute HCl, the mixture was chromatographed by a reversed-phase column with an elution of water to give pure **25** (40 mg, 86.7%). Similarly, **19** (50 mg), **20** (32 mg), or **21** (25 mg) gave **23** (36.2 mg, 72.2%), **26** (16 mg, 49.8%), or **24** (16.1 mg, 64.1%), respectively.

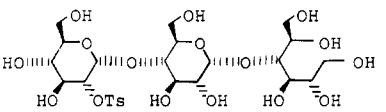
23: ^{13}C NMR (25 MHz, D_2O, characteristic absorptions) δ 54.0, 58.0, 63.0, 63.2, 65.0, 65.5, 84.6, 97.0, 98.5, 103.1; FABMS, m/z 673 ($M + Na^+$).

24: ^{13}C NMR (25 MHz, D_2O, characteristic absorptions) δ 54.0, 58.1, 63.1, 65.3, 65.6, 65.8, 79.6, 98.4, 102.1, 102.4; FABMS, m/z 489 ($M + H^+$).

25: ^{13}C NMR (25 MHz, D_2O, characteristic absorptions other than aromatic absorptions) δ 23.5, 62.9, 64.8, 65.4, 82.3, 84.7, 98.4, 102.9; FABMS, m/z 661 ($M + H^+$), 683 ($M + Na^+$).

26; FABMS m/z 651 ($M + H^+$), 673 ($M + Na^+$).

Acetylation of 21 and 23–26. A solution of **23** (5 mg) and acetic anhydride (0.5 mL) in pyridine (0.5 mL) was allowed to stand at room temperature for 48 h and concentrated by evaporation of volatile materials together with a stream of nitrogen. After dry chloroform (0.5 mL) was added to the residue, the evaporation was repeated. This procedure was carried out two more times. The crude product were purified by reversed-phse HPLC with a gradient elution from 50% aqueous CH_3CN to 80% aqueous CH_3CN to give pure **27** (4.5 mg, 48.9%). Similarly, **21**, **24**, **25**, or **26** gave **29**, **28**, **30**, or **31**, respectively, in similar yield. The EIMS spectral fragmentations of **27–31** observed are shown in Figure 2.



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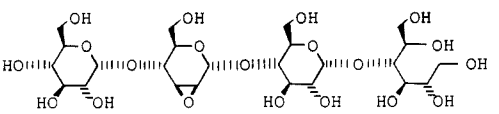

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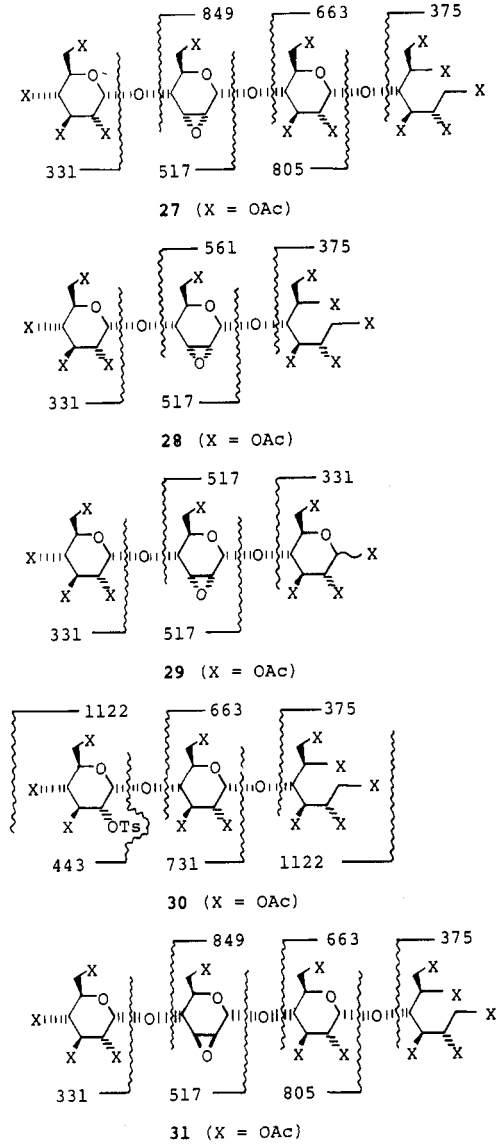


Figure 2. EIMS spectral fragmentation pattern of **27–31**, which showed correct molecular ions in FDMS spectra.

FDMS (m/z) **27**, 1197 ($M + H^+$), 1219 ($M + Na^+$); **28**, 909 ($M + H^+$), 931 ($M + Na^+$); **29**, 865 ($M + H^+$), 887 ($M + Na^+$); **30**, 1123 ($M + H^+$), 1145 ($M + Na^+$); **31**, 1197 ($M + H^+$), 1216 ($M + Na^+$).

Results and Discussion

Cyclodextrins. Preparations and structure determinations of the cyclodextrin derivatives **1–11** except **4** have been reported previously.[10g,j,k,l] The sulfonate **4** was prepared by reaction of β-cyclodextrin with α-naphthalenesulfonyl chloride and separated well from the other minor products by reversed-phase column chromatography. The epoxide **10** was easily obtained from **4** by treatment with alkaline water.[10l] The sulfonylation position (C-2–OH) of **4** was determined by the ^{13}C NMR spectrum which was very similar to that of **5**. Moreover this assignment was confirmed by the ^1H NMR (270 MHz) spectrum of **10** which showed a singlet at δ 5.09 for C-1–H demonstrating the manno epoxide structure.[10k,l] Therefore, the position of sulfonylation was C-2–OH.

Enzymatic Hydrolysis of Modified Cyclodextrins. Enzymatic hydrolyses of **1–11** gave selectively modified maltooligosaccharides in good yields. These are summarized in Table I. The reaction of **1** gave exclusively **12** (G–G′–G–G) after 30 min and also exclusively **13** (G–G′–G) after 48 h. (Hereafter, the following abbreviations are used: G, glucose unit; G′, modified glucose unit.) However, such a clean, selective reaction at the initial stage was not seen with the corresponding α-cyclodextrin isomer **2** or **3**. A single product G–G′–G (**13** or **15**) was obtained after 44 h or 7 days, respectively. Generally, the enzymatic reaction was faster for the β-cyclodextrin derivatives than for the α-cyclodextrin derivatives. This tendency is in accord with the difference in reactivity between α- and β-cyclodextrins for Taka-amylolysis. By the enzymatic reactions described here, 2-, 3-, and 2,3-activated oligosaccharides become easily available, since β-cyclodextrin is a cheap starting material and since the specific modifications on C-2 and C-3 are established as mentioned above.

Structure Determination of the Taka-Amylolysis Products. This is summarized in Scheme I. FABMS spectra of the Taka-amylolysis products **12–20** showed the correct molecular ions. Treatment with aqueous alkali converted the sulfonates to the corresponding epoxides **19–22** which showed the correct molecular ions in FABMS spectra. HPLC and spectral data demonstrated that the epoxide (**21**) obtained from **13** was identical with that from **15**, that the epoxide (**19**) obtained from **12** was identical with those from **8**, **9**, and **14** and that the epoxide (**22**) obtained from **16** was identical with those from **17** and **18**. The oligosaccharides **19**, **20**, **21**, and **18** were reduced at their reducing ends with aqueous $NaBH_4$ to give the glucitol derivatives, **23**, **26**, **24**, and **25**, which were completely acetylated to **27**, **31**, **28**, and **30**, respectively. The epoxide **21** was also acetylated to give **29**. The acetylated compound **27–31** showed the correct molecular ions in their FDMS spectra. The fragmentation patterns of the EIMS spectra of **27–31** demonstrated the positions of the modified glucose units in the oligosaccharides as shown in Figure 2.

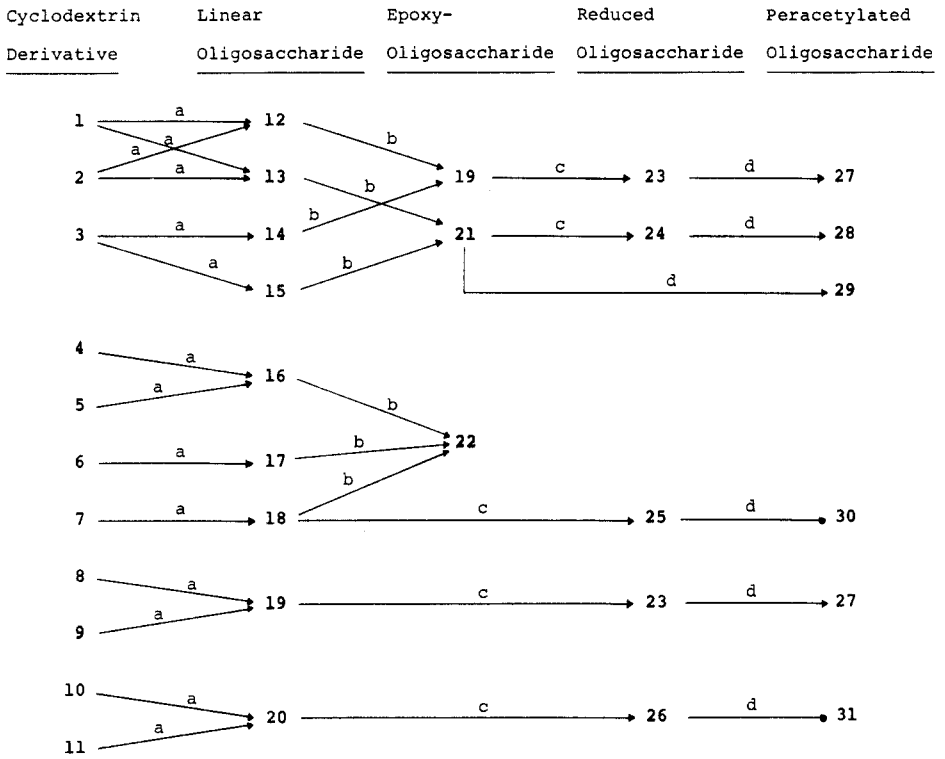
Interaction of Taka-Amylase Subsites with Modified Cyclodextrins. Substituted linear oligosaccharides are produced by the first cleavage of a glucosidic bond in the substituted cycloodextrin **1–11**. The possible types of interaction of the substituted linear oligosaccharides with TAA are depicted in Figure 1. Production of G′-G-G (**16–18**) from **4–7** demonstrated that the interactions between the hydroxyls on C-2 of the saccharide and the subsites R and S were important for glucosidic bond cleavage at the active site, although interaction of the hydroxyl groups with the other subsites including T would possess trivial importance. The significant role played by interactions between the C-2–OH and subsites R and S is consistent with results reported earlier by the TAA hydrolysis of 2 or 2′-substituted phenyl maltosides.

The production of G-G′-G (**13** or **15**) from **1–3** showed that interaction of the C-3–OH with the subsites S and T is important for the amylolysis, while interaction with the other subsites including R was far less important. However, G–G′–G–G (**12** or **14**) was produced from the modified cyclodextrins (**1** and **2**, or **3**) before formation of the G–G′–G (**13** or **15**), respectively, demonstrating that the interaction of the C-3–OH with the subsite R possessed a significant influence.

Although there is considerable steric deformation of a glucose residue (allo or manno epoxide) in **8–11**, and although the structures of the residues differ from one another, the TAA hydrolysis product was of a similar type G–G′–G–G (**19** and **20**). This seems to imply that modifications on both C-2 and C-3 and the steric deformation do not affect interactions between the oligosaccharide and subsites other than R, S, and T.

While the importance of subsites T–V can not be estimated on the basis of studies on TAA hydrolysis of mod-

Scheme I[a]



[a] (a) Taka-amylase; (b) NaOH or $Ba(OH)_2$; (c) $NaBH_4$; (d) Ac_2O/Py.

ified phenyl maltosides, studies on transglucosidation by TAA showed that C-3-OH is the glucosidic hydroxyl responsible for the interaction with the subsite T and also showed that etherification of C-2-OH did not affect its interaction with subsite T.[14] These results agree with the present results on TAA hydrolyses of 1–7.

A complete molecular model of Taka-amylase[15] was recently proposed on a result of an X-ray structure analysis[16,17] at 3-Å resolution and the determination[18] of the complete amino acid sequence. Model fitting[15] of an amylase chain in the catalytic site showed that C-2 hydroxyls of the amylose interact with Glu 35 (subsite P), Asp 340 (Q), Asp 297 and Glu 230 (S), Lys 209 (T), and Asp 233 (U) and also suggests that the C-3 hydroxyls interact with Tyr 79 (Q), Arg 344 (R), His 296 (S), Glu 230 (T), Lys 209 (U), and Asp 233 (V). The importance of these interactions may be compared with our results obtained from hydrolyses of 1–11 by TAA. The interactions of a C-2 hydroxyl with Asp 297 and Glu 230 appear to be more important than the other interactions of a C-2 hydroxyl. Also, the interaction of two C-3 hydroxyls with His 296 and Glu 230 appear to be more important than the other interactions of C-3 hydroxyls. Although Arg 344 (subsite R) was thought to interact with a C-3-OH from the model fitting study,[15] our results with respect to 1–3 demonstrate such an interaction to be trivial. Rather, the results of the hydrolyses of 4–7 indicate that there is an important interaction between a C-2-OH and Arg 344 in subsite R. This was supported by the phenyl maltoside experiment.[8f]

Summary. Our results demonstrate that subsites other than R, S, and T of TAA have far less importance in the interaction with oligosaccharides than subsites R, S, and T. Preliminary studies on Taka-amylolysis[10g] of disubstituted cyclodextrins showed that the cleavage patterns observed in the monosubstituted cyclodextrins also hold supporting the present conclusion as to the importance of the subsites. However, the degree of the trivial interaction between the other subsites and the oligosaccharides could not be estimated in the present study since the slow rate of the enzymatic cleavage of the cyclodextrin ring did not allow accumulation of stable linear oligosaccharide intermediates in amounts sufficient to be isolated.

The present TAA hydrolyses gave a single product, showing that this method will be useful not only for structure determination of disubstituted cyclodextrins but also for preparation of specifically modified oligosaccharides. In the latter case it is important to note that changing the order of the experimental procedures, i.e., Taka-amylolysis before or after epoxidation, gave different epoxyoligosaccharides.

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