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Enzymatic Preparation of Specifically Modified Linear Maltooligosaccharides through Taka-amylase A-catalyzed Hydrolysis of 6-O-Arenesulfonyl-γ-cyclodextrins

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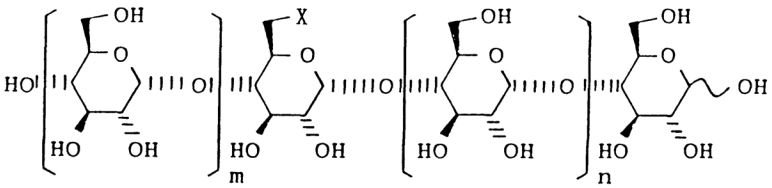
6-O-Arenesulfonyl-γ-cyclodextrins were hydrolyzed enzymatically by Taka-amylase A to give 6"-O-arenesulfonylated maltotetraoses and 6"-O-arenesulfonylated maltotrioses.

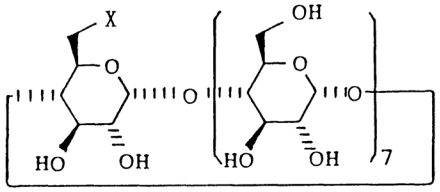
Cyclodextrins are enzymatically hydrolyzed by some α-amylases including Taka-amylase A.[1] Melton and Slessor reported that 6-O-substituted α-cyclodextrins were hydrolyzed by Taka-amylase A to give selectively 6'-O-substituted maltoses.[2] We also reported that 6-O-substituted α- or β-cyclodextrins,[3] 2-O-substituted α- or β-cyclodextrins,[4] 3-O-substituted α- or β-cyclodextrins,[4] or $3^A,6^A$-anhydro-β-cyclodextrin[5] gave 6'-O-substituted maltoses, 2"-O-substituted maltotrioses, 3'-O-substituted maltotrioses (and/or 3"-O-substituted maltotetraose), and 3",6"-anhydromaltotetraose, respectively. These studies provided new information about interactions between substrates and the active sites of Taka-amylase A as well as a novel synthetic method for the preparation of specifically modified (or activated) oligosaccharides.

However, these experiments using α- or β-cyclodextrin derivatives give only the final products and therefore very limited information about the subsites of the active site. The reason is that the slow rate of hydrolytic cleavage of the cyclodextrin macrocyclic ring does not allow accumulation of intermediate linear oligosaccharides in enough amount to be isolated.

Since γ-cyclodextrin is hydrolyzed much faster than α- or β-cyclodextrin,[1] enzymatic hydrolysis of γ-cyclodextrin derivatives by large amount (not catalytic amount) of Taka-amylase A can reasonably be expected to give new specifically modified linear oligosaccharides (new intermediates) which can not be isolated in the enzymatic hydrolysis of modified α- or β-cyclodextrins. This result will also provide information concerning the importance of the subsites.

Table 1 shows the condition and result of the Taka-amylase A-catalyzed hydrolysis of 6-O-arenesulfonyl-γ-cyclodextrin **1**a-c which was easily prepared by the reaction of γ-cyclodextrin with the corresponding arenesulfonyl chloride in pyridine.[6]





1a: X = β-NsO

1b: X = α-NsO

1c: X = p-TsO

Ns = naphthalenesulfonyl

Ts = toluenesulfonyl

2a: m = 1, n = 1, X = β-NsO

2b: m = 1, n = 1, X = α-NsO

2c: m = 1, n = 1, X = p-TsO

3a: m = 0, n = 1, X = β-NsO

3b: m = 0, n = 1, X = α-NsO

3c: m = 0, n = 1, X = p-TsO

4a: m = 0, n = 0, X = β-NsO

4b: m = 0, n = 0, X = α-NsO

4c: m = 0, n = 0, X = p-TsO

5 : m = 0, n = 0, X = SC_6H_5

The products **2**a-c, **3**a-c, and **4**a-c were isolated by reverse-phase column chromatography.[7] Their FAB mass spectra showed the corresponding molecular ions. By treatment with aqueous alkali, the products **2**a-c were converted to 3",6"-anhydromaltotetraose whose structure was determined by comparing its HPLC retention time and its FAB mass spectrum with those of the authentic compound.[5] The products **3**a-c were reduced at their reducing ends with $NaBH_4$ followed by complete acetylation with acetic anhydride in pyridine. From analysis of the EI mass spectra of the resultant undecaacetates, the position of the sulfonylated glucose unit in **3**a-c was determined. The products **4**a-c were converted to 6'-S-phenyl-6'-thiomaltose **5** whose structure was determined by comparing the HPLC retention time and the FAB mass spectrum with those of the authentic specimen.[3]

Table 1. Enzymatic Hydrolysis of 6-O-Substituted γ-Cyclodextrin by Taka-amylase A[a)]

Cyclodextrin derivative	Temp/°C	Time	Product(Yield/%)[b)]
1a	10	2 h	**2**a (67.4)[c)]
	10	2 d	**2**a (28.8), **3**a (28.9)
	40	3 h	**4**a (88.3)
1b	10	1.5 h	**2**b (35.5), **3**b (54.4)
	40	4 h	**4**b (61.1)
1c	10	2 h	**2**c (31.7), **3**c (43.6)
	10	2 d	**4**c (99.4)

a) The solvent was 0.1 mol dm^{-3} acetate buffer (pH 5.5) containing $CaCl_2$ (0.01 mol dm^{-3}). Taka-amylase A(α-amylase from Aspergillus oryzae) which is commercially available as α-amylase Type X-A (Sigma) was used. The concentrations of the cyclodextrin derivative and Taka-amylase A were each 10 mg/(1 mL of the solvent). b) This represents the major product(s) having the arenesulfonyl group. Needless to say, D-glucose was another major product. c) The progress of the reaction was stopped just before **3**a began to form.

The specifically activated (sulfonylated) oligosaccharides **2**a-c and **3**a-c could not be isolated from the enzymatic hydrolysis of 6-O-arenesulfonyl-α- or β-cyclodextrins, even when Taka-amylase A was used in an amount equal to that of the cyclodextrin derivative [2,3)] and/or the reaction was carried out at 5 °C or 10 °C. On the other hand, purely chemical methods for the preparation of these compounds from smaller sugars will need troublesome repeats of activation, protection, and deprotection of hydroxyl groups of the sugars. One-step chemical synthesis of specifically modified linear oligosaccharides from the corresponding linear oligosaccharides is also impossible. This method will require tedious separation of many regioisomeric monosulfonylated oligosaccharides.

Since there does not exist any regiochemical isomer, 6-O-arenesulfonyl-γ-cyclodextrins can be easily prepared and easily isolated as a pure material.[6)] As shown above, they can be smoothly hydrolyzed to give one or two modified oligosaccharide(s) at 10 °C. Furthermore, the products can be effectively separated by reverse-phase column chromatography.[7)] Therefore, the selective chemical modification of γ-cyclodextrin followed by the Taka-amylase A-catalyzed hydrolysis has several advantages for preparation of specifically modified oligosaccharides.

Although the reaction of **1**a at 10 °C for 2 h afforded only the 6"-O-substituted maltotetraose (**2**a), the hydrolysis at 10 °C for longer reaction time (2 d) or at 40 °C gave a mixture of **2**a and **3**a or exclusively **4**a, respectively.

The hydrolysis of **1**b at 10 °C for 1.5 h gave a mixture of **2**b and **3**b. The reaction of **1**c at 10 °C for 2 h afforded a mixture of **2**c and **3**c. However, the hydrolysis of **1**b or **1**c at higher reaction temperature and/or for longer reaction time gave **4**b or **4**c, respectively. These show that the 6"-O-substituted maltotetraose (**2**a-c) and the 6"-O-substituted maltotriose (**3**a-c) were the intermediates and that the 6"-O-substituted maltotetraose was, at least in the reaction of **1**a, the precursor of the 6"-O-substituted maltotriose, which led to the 6'-O-substituted maltose.

These results will be useful for substrate-based investigation on interaction between hydroxyl groups of substrates and the enzyme's active site which consists of seven subsites.[8] This will be reported in the near future together with other results.

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6) For example, the tosylate (**1**c) was prepared as follows. p-Toluenesulfonyl chloride (12 g) was added to a solution of γ-cyclodextrin (10 g) in pyridine (800 mL) and the mixture was stirred for 1 h. After addition of water (10 mL), the mixture was concentrated in vacuo and chromatographed by a reverse-phase column (LiChroprep RP18 column, 25 mm x 310 mm, Merck) with gradient elution from water (1 L) to 50% aqueous ethanol (1 L) to give the monotosylate (3.5 g, 31%). The other arenesulfonates **1**a,b were similarly prepared and isolated in similar yields.
7) A Merck prepacked column (see Ref. 6) or a Kusano prepacked ODS column (CPO-HS-221-20, 22 mm x 100 mm) was used for reverse-phase column chromatography. After elution with water to eliminate inorganic salts and glucose, gradient elution from water to aqueous methanol or aqueous acetonitrile was used for separation of the products having the arenesulfonyl group.
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