CHEMISTRY LETTERS, pp. 743-746, 1990.

Enzymatic Preparation of Specifically Modified Linear Maltooligosaccharides through Porcine Pancreatic Amylase-Catalyzed Hydrolyses of Substituted γ-Cyclodextrins

Kahee FUJITA,* Tsutomu TAHARA,+ and Toshitaka KOGA+
Faculty of Pharmaceutical Sciences, Fukuyama University,
Sanzo, Higashimura-cho, Fukuyama 729-02
+Daiichi College of Pharmaceutical Sciences,
Tamagawa, Minami-ku, Fukuoka 815

Specifically modified linear oligosaccharides were prepared by enzymatic hydrolyses of modified γ-cyclodextrins with porcine pancreatic amylase.

Enzymatic hydrolyses of α-, β-, or γ-cyclodextrin derivatives by Taka amylase A (TAA) provided a synthetic method for the preparation of specifically modified maltooligosaccharides as well as information about interactions between substrates and the binding subsites of TAA.[1] Since other α-amylases have different number and different nature of subsites from those of TAA, the enzymatic hydrolyses of substituted cyclodextrins must give novel methods for the preparation of specifically modified maltooligosaccharides which are not available by the TAA-catalyzed hydrolysis. Porcine pancreatic amylase (PPA) has been studied with respect to its structure[2] and its enzymatic action in hydrolyses of maltooligosaccharides[3] and 6-O-glucosylmaltooligosaccharides.[4] These studies suggested the existence of five subsites in the active site which is different from that of TAA having seven subsites.[5] PPA hydrolyzes γ-cyclodextrin,[6] but does not affect α- and β-cyclodextrins. This nature is also different from that of TAA. Therefore, the combination of PPA and substituted γ-cyclodextrins can be reasonably expected to provide new modified oligosaccharides which cannot be obtained by the TAA method. We report here the enzymatic hydrolyses of some γ-cyclodextrin derivatives **1**-**10** by PPA.[7]

Table 1 shows the conditions and the results of PPA-catalyzed hydrolysis of modified cyclodextrins. The products were isolated by reverse-phase column chromatography.[8] Their fast-atom bombardment mass spectra (FABMS) contained the corresponding peaks of molecular ions. The structure of **11**a-c, **13**a, or **15** was determined by comparing its reverse-phase HPLC[8]

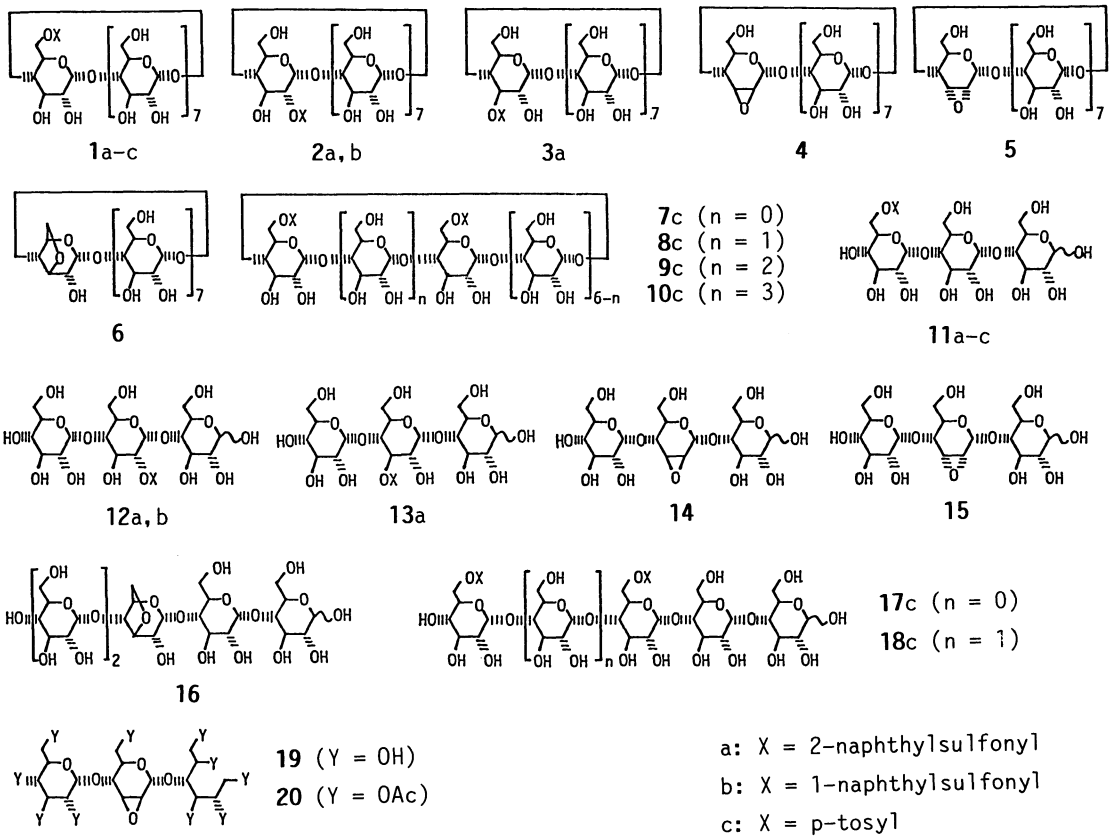
Table 1. Hydrolyses of γ-Cyclodextrin Derivatives by PPA at 40 °C

γ-Cyclodextrin derivative	PPA[a]	Solvent[b]	Reaction time/d	Product
1a (50 mg)	VI (100 mg)	A (5 mL)	1	**11**a (14.1 mg, 60.4%)
1b (20 mg)	VI (40 mg)	A (2 mL)	1	**11**b (5.10 mg, 54.6%)
1c (50 mg)	VI (100 mg)	A (5 mL)	1	**11**c (19.1 mg, 84.1%)
2a (50 mg)	VI (100 mg)	A (5 mL)	1	**12**a (16.0 mg, 68.4%)
2b (50 mg)	VI (100 mg)	A (5 mL)	1	**12**b (17.8 mg, 76.2%)
3a (50 mg)	VI (100 mg)	A (5 mL)	1	**13**a (10.3 mg, 44.0%)
4 (44 mg)	VI (88 mg)	A (4.4 mL)	1	**14** (12.5 mg, 74.7%)
5 (100 mg)	VI (200 mg)	A (10 mL)	1	**15** (21.5 mg, 56.6%)
6 (189 mg)	I (378 μL)	A (18.9 mL)	3	**16** (85.9 mg, 71.7%)
7c (70 mg)	VI (140 mg)	B (17.5 mL)	4	**17**c (30.3 mg, 71.3%)
8c (100 mg)	VI (200 mg)	B (25 mL)	4	**18**c (53.0 mg, 74.9%)
9c (100 mg)	VI (200 mg)	B (25 mL)	4	**11**c (62.1 mg, 75.7%)
10c (100 mg)	VI (200 mg)	B (25 mL)	4	**11**c (70.0 mg, 85.3%)

a) Commercially available PPA (Sigma) was used. VI; α-amylase Type VI-A, I; α-amylase Type I-A. b) Solvent A ; 0.06 mol dm^{-3} aqueous NaCl containing $CaCl_2$ (0.02 mol dm^{-3}), Solvent B; a 4:1 (v/v) mixture of Solvent A and dimethyl sulfoxide.

retention time and FABMS with those of the authentic compound.[1g,h] The sulfonates **12**a,b were converted to **14** by treatment with aqueous alkali and then reduced with $NaBH_4$ to give **19**, which was completely acetylated to give **20**. The fragmentation pattern of the electron-impact mass spectrum of **20** was used to determine the position of the modified glucose unit in the oligosaccharides. Following the method similar to that described above, the structures of **16**, **17**c, and **18**c were determined.

The present enzymatic hydrolyses by PPA gave modified maltooligosaccharides selectively. The product **13**a can be also prepared by TAA-catalyzed hydrolysis of 3-O-(2-naphthylsulfonyl)-α(or β)-cyclodextrin.[1g] Although **11**a-c are produced as the intermediate products together with other products in the reaction of **1**a-c with TAA,[1h] the compounds were obtained as major and final products in the present system. This is also shown in the results that **11**c was a major product in the reaction of 6^A,6^D- and 6^A, 6^E-di-O-(p-tosyl)-γ-cyclodextrins (**9**c and **10**c). The 2'-O-arylsulfonylmaltotrioses **12**a,b, which were major products from **2**a,b in the present system, can not be obtained by the TAA-catalyzed hydrolysis which gives the corresponding 2"-O-arylsulfonylmaltotrioses.[1h] (2'S)-2',3'-Anhydromaltotriose **14** cannot be prepared by the TAA reaction of **4**, which afforded



(2"S)-2",3"-anhydromaltotetraose.[1g] Also, (3'R)-2',3'-anhydromaltotriose **15** cannot be prepared by the TAA reaction of **5**, which provided (3"R)-2",3"-anhydromaltotetraose.[1g] While the TAA reaction of **6** produced 3",6"-anhydromaltotetraose,[1i] the hydrolysis by PPA gave a different product (**16**, 3",6"-anhydromaltopentaose).[7]

This enzymatic hydrolysis method is applicable to preparation of di-substituted oiligosaccharides. The specifically disulfonylated oligosaccharides (**17c** and **18c**) were obtained from $6^A,6^B$- and $6^A,6^C$-di-O-(p-tosyl)-γ-cyclodextrins (**7c** and **8c**). These compounds cannot be prepared by the TAA reaction and are difficult and troublesome to prepare by stepwise and purely chemical reactions.

Thus, the characteristic of this PPA reaction is that it provides a convenient and selective one-step method for preparation of substituted oligosaccharides which cannot be obtained easily by other methods.

We thank Japan Maize-Products Co. Ltd. for a generous gift of γ-cyclodextrin.

References

1) a) K. Fujita, A. Matsunaga, and T. Imoto, Tetrahedron Lett., 25, 5533

(1984); b) K. Fujita, A. Matsunaga, and T. Imoto, J. Am. Chem. Soc., 106, 5740 (1984); c) I. Tabushi, T. Nabeshima, K. Fujita, A. Matsunaga, and T. Imoto, J. Org. Chem., 50, 2638 (1985); d) K. Fujita, A. Matsunaga, Y. Ikeda, and T. Imoto, Tetrahedron Lett., 26, 6439 (1985); e) K. Fujita, S. Nagamura, T. Imoto, T. Tahara, and T. Koga, J. Am. Chem. Soc., 107, 3233 (1985); f) K. Fujita, T. Tahara, T. Imoto, and T. Koga, ibid., 108, 2030 (1986); g) K. Fujita, T. Tahara, S. Nagamura, T. Imoto, and T. Koga, J. Org. Chem., 52, 636 (1987); h) K. Fujita, T. Tahara, T. Imoto, and T. Koga, Chem. Lett., 1988, 1329; i) K. Fujita, H. Yamamura, T. Imoto, and I. Tabushi, ibid., 1988, 543 ; j) L. D. Melton and K. N. Slessor, Can. J. Chem., 51, 327 (1973).

2) E. H. Fischer and P. Bernfeld, Helv. Chem. Acta, 31, 1831 (1948); Y. Sakano, S. Takahashi, and T. Kobayashi, Denpun Kagaku, 30, 30 (1983); I. Kluh, FEBS Lett., 136, 231 (1981); F. Payan, R. Haser, M. Pierrot, M. Frey, J. P. Astier, B. Abadie, E. Duee, and G. Buisson, Acta Crystallogr., Sect. B, 36, 416 (1980).

3) J. F. Rebyt and D. French, J. Biol. Chem., 245, 3917 (1970).

4) K. Kainuma, and French, FEBS Lett., 6, 182 (1979); D. French, A. B. Pulley, M. Abdullah, and J. C. Linden, J. Chromatogr., 24, 271 (1966).

5) K. Hiromi, Biochem. Biophys. Res. Commun., 41, 1 (1970); J. A. Thoma, C. Brothers, and J. E. Spradlin, Biochemistry, 9, 1768 (1970); J. A. Thoma, G. V. K. Rao, C. Brothers, J. E. Spradlin, and L. H. Li, J. Biol. Chem., 246, 562 (1971).

6) M. Abdullah, D. French, and J. F. Robyt, Arch. Biochem. Biophys., 144, 595 (1969).

7) Commercially available PPA (Type VI-A or Type I-A, Sigma) was used for the enzymatic reaction. The amylase Type I-A is a material recrystallized two times. The experimental results using this enzyme were very similar to those with the amylase Type VI-A except for the case of **6**. Since the amylase Type VI-A is much cheaper than the anylase Type I-A, the experiments using the amylase Type VI-A are described in Table 1. In the case of **6**, the amylase Type I-A afforded **16** as a major product, but the amylase Type VI-A gave a mixture of **16** and 3",6"-anhydromaltotetraose. The former result is described in Table 1.

8) A Merck Lobar prepacked column (LiChroprep RP18 column, 25 mm x 310 mm) or a Kusano prepacked ODS column was used for reverse-phase column chromatography. High-performance liquid chromatography (HPLC) was performed on a Shimadzu LC3A with a Tskgel ODS 120T column (4.6 mm x 150 mm, 5 μm, TOSOH), with a Zorbax ODS column (4.6 mm x 250 mm, 5 μm, DuPont), or with a Cosmosil 5PE column (4.6 mm x 100 mm, 5 μm, Nacalai).

(Received February 13, 1990)