Carbohydrate Research, 131 (1984) 175–179
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

Note

Isolation and characterisation of 6-α-D-glucosylcyclomaltoheptaose

JUN-ICHI ABE, YASUHITO TAKEDA, SUSUMU HIZUKURI*, KAZUE YAMASHITA, AND NAOKO IDE
Department of Agricultural Chemistry, Kagoshima University, Korimoto-1, Kagoshima 890 (Japan)
(Received October 5th, 1983; accepted for publication, January 19th, 1984)

Cyclomaltaoses (cyclodextrins, cycloamyloses) have been widely studied because of their unique structures and properties[1]. However, there have been only limited studies of branched cyclomaltaoses since they were discovered by French *et al.*[2]. These cyclomaltaose derivatives have a D-glucose or a (1→4)-α-D-glucan branch attached to the main cyclic structure by an α-(1→6) linkage. Taylor and Whelan[3,4] reported that 6-α-D-glucosylcyclomaltohexaose and 6-α-D-glucosylcyclomaltoheptaose (GcG_7) are suitable substrates for determining the activity of amylo-1,6-glucosidase (EC 3.2.1.33). They obtained a mixture of these glucosylcyclomaltaoses from the glucoamylase-treated mother liquor of a large-scale preparation of the non-branched cyclomaltaoses from starch by p.c., or separated them by Sephadex G-15 chromatography followed by p.c. Kobayashi *et al.* prepared[5] the branched cyclomaltaoses by fractionation with organic solvents followed by p.c., and reported some properties[6]. In these studies, however, the purity and the structure of the product were not reported in detail. We have isolated pure GcG_7 from a commercial starch syrup (Celdex) and analysed its structure in detail.

Gel-permeation chromatography was the main technique used for the isolation. The commercial starting-material, Celdex, is a concentrated mother liquor remaining after the separation of crystalline cyclomaltoheptaose (cG_7), and contains D-glucose, oligosaccharides, non-cyclic dextrins, and cyclomaltaoses in addition to branched cyclomaltaoses. This mixture was treated twice with crude glucoamylase to hydrolyse the non-cyclic dextrins and oligosaccharides and to shorten the branches of the branched cyclomaltaoses. After removing the D-glucose with baker's yeast, the remaining material was subjected to chromatography on Sephadex G-15 (Fig. 1), and the fractions (50–68) that produced glycerol on Smith degradation and had almost no reducing power were combined. Most of the non-cyclic dextrins and cG_7 were removed by using this column. Cyclomaltohexaose (cG_6) was then separated from the branched cyclomaltaoses by chromatography on

*To whom correspondence should be sent.

0008-6215/84/$ 03.00

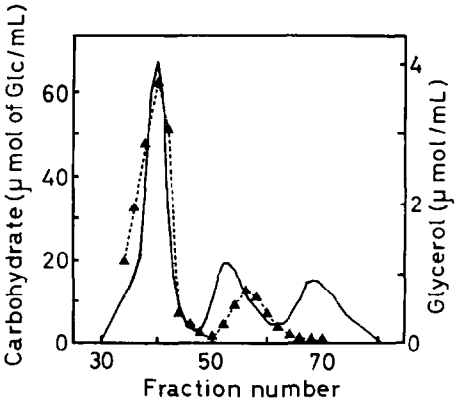

Fig. 1. Gel chromatography on a column (2.6 × 100 cm) of Sephadex G-15 of partially purified GcG_7 (product A, see Experimental) by elution with water at 20 mL/h (5-mL fractions). The carbohydrate-containing peaks were due to dextrins, cG_6, and cG_7, respectively: ———, carbohydrate; ----▲----, glycerol produced on Smith degradation.

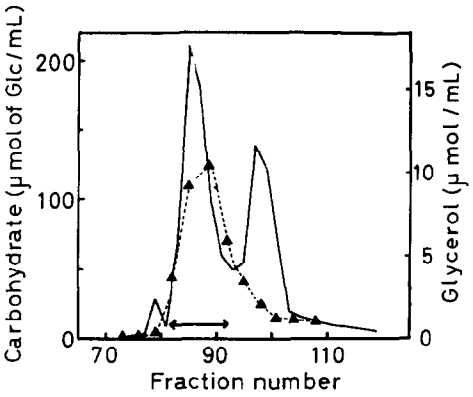

Fig. 2. Gel chromatography on a column (2.6 × 100 cm) of Toyopearl HW-40S of partially purified GcG_7 (product B, see Experimental) by elution with water at 7.8 mL/h (3.9-mL fractions): ———, carbohydrate; ----▲----, glycerol produced on Smith degradation. Fractions 82–92 contained GcG_7.

Toyopearl HW-40S (Fig. 2). The residual reducing sugars were hydrolysed with pure glucoamylase, and the resulting D-glucose was removed by gel filtration on Bio-gel P-2. The 6-α-D-glucosylcyclomaltoheptaose (GcG_7), thus obtained, was free from any reducing sugar, gave a single brownish-yellow spot (R_{cG_7} 0.79) in p.c. on detection with iodine vapor, and gave 1 mol of glycerol per 8.01 mol of D-glucosyl residues on Smith degradation. Application in sequence to GcG_7 of methylation, hydrolysis, reduction, and acetylation gave 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol, 1,4,5-tri-*O*-acetyl-2,3,6-tri-*O*-methyl-D-glucitol, and 1,4,5,6-tetra-*O*-acetyl-2,3-di-*O*-methyl-D-glucitol in the molar ratios 1:5.88:1.05. GcG_7 was hydrolysed easily with amylo-1,6-glucosidase, as reported by Taylor and Whelan[3,4], and yielded the theoretical amount of D-glucose. The final products were D-

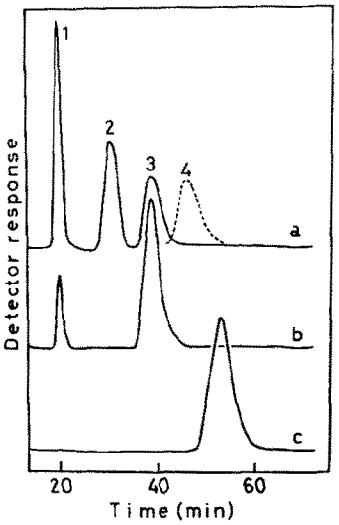

Fig. 3. H.p.l.c. of cyclomaltaoses and their hydrolysed products: (*a*) 1, D-glucose; 2, cG_6; 3, cG_7; 4, cG_8; (*b*) products of hydrolysis of GcG_7 with amylo-1,6-glucosidase; (*c*) GcG_7.

glucose and cG_7 in the molar ratio 1:1.01 as judged by h.p.l.c. (Fig. 3); GcG_7 gave a single peak (*T* 54 min), and the hydrolysed products gave peaks for D-glucose and cG_7 at *T* 20 and 40 min. D-Glucose, cG_6, cG_7, and cyclomalto-octaose (cG_8) are well separated by h.p.l.c. (Kitahata *et al.*[7]), and GcG_7 was eluted after cG_8.

The D-glucosyl branch-linkage of GcG_7 was resistant to glucoamylase (*Aspergillus niger*, purified) under conditions (3.63 U/mg of substrate, 24 h, 40°) where >98% of isomaltose was hydrolysed. The solubility of GcG_7 is higher than that[8] (18.5 mg/mL, at room temperature) of cG_7, and a concentrated GcG_7 solution (150 mg/mL) gave no crystals at 0°. The $[\alpha]_D^{25}$ value of +178° (*c* 0.3, water), which is a little higher than that[8] (+162.5°) of cG_7, is very similar to that[8] (+177.4°) of cG_8.

EXPERIMENTAL

Materials. — Celdex and cG_6, cG_7, and cG_8 were gifts from Nihon Shokuhin Kako Co. *Rhizopus delemar* glucoamylase (crude) was donated by Shin Nihon Kagaku. Pure glucoamylase of *Aspergillus niger* was prepared as previously described[9]. Amylo-1,6-glucosidase was prepared[10] from rabbit muscle and further purified[11] to remove a trace amount of contaminating alpha-amylase.

Methods. — Carbohydrate was determined by the anthrone–sulfuric acid method[12]. Reducing sugars were determined routinely by the Somogyi–Nelson method[13,14], and, for examination of the purified GcG_7, a modified[15] Park–Johnson method was used because of its high sensitivity. D-Glucose was measured by the D-glucose oxidase method[16]. The non-reducing, terminal residue was determined by rapid Smith-degradation[17], but a longer period (3 h) was required than

for non-cyclic dextrins for complete oxidation. Optical rotations were measured with a JASCO J-20 automatic recording spectropolarimeter.

Specimens were methylated twice (Hakomori[18]) and then hydrolysed; the products were converted[19] into the corresponding alditol acetates and analysed[20] in a Hitachi 663-30 gas chromatograph fitted with a flame-ionisation detector and a column (3 mm × 2 m) of 0.3% of OV-275–0.4% of GE XF 1150 on Uniport HP (80–100 mesh).

H.p.l.c. was conducted at room temperature with a JASCO Trirotar liquid chromatograph equipped with a differential refractometer Shodex SE-11 and a column of Shodex S-614 (Showa Denko Co.), and elution with acetonitrile–water (65:35) at 1 mL/min.

P.c. was performed on Toyo No. 50 filter paper with 1-butanol–1-propanol–water (3:5:5) and detection with iodine vapor.

Preparation of GcG_7. — Celdex solution (477 g of carbohydrate, 1250 mL) was treated with 25,000 units of crude glucoamylase of *Rhizopus delemar* at 40° and pH 4.5 for 24 h. The resulting D-glucose was removed (D-glucose oxidase–peroxidase test) using baker's yeast (1 kg) under vigorous aeration at 30°. The yeast was removed by centrifugation and the supernatant solution was concentrated under reduced pressure. The solution still contained considerable amounts of reducing sugars (probably mainly branched oligosaccharides), which were treated with 11,000 units of crude glucoamylase at 40° for 24 h. The solution was subjected to gel filtration on a column (5.6 × 42 cm) of Bio-gel P-2, and the fractions free from D-glucose were combined and concentrated to give product A (5.30 g of carbohydrate, 8 mL).

Product A was divided into eight portions which were chromatographed on Sephadex G-15 (Fig. 1). The first carbohydrate peak contained non-cyclic dextrins, since it was reducing and yielded D-glucose on treatment with glucoamylase. P.c. showed that the main components of the second and third peaks were cG_6 and cG_7, respectively. Fractions 50–68 gave glycerol on Smith degradation and were non-reducing, indicating the presence of branched cyclomaltaoses. Fractions 52–62 were combined, concentrated to give product B (1.49 g of carbohydrate, 5 mL), and subjected to chromatography on a column of Toyopearl HW-40S (Fig. 2). The first carbohydrate peak nearly coincided with that of the glycerol-producing fraction noted above, and fractions 82–92 (shown by the double-headed arrow in Fig. 2) were combined and concentrated (2 mL). The yield was 370 mg as cyclomaltaoses. The second carbohydrate peak was cG_6, because it stained purple with iodine in p.c. and was non-reducing. After concentration of this fraction, a small amount of reducing sugars (1.4 mg as glucose) was detected. Therefore, the fraction was treated overnight with 400 units of pure glucoamylase at 40°, and the resulting D-glucose was removed by chromatography on Bio-gel P-2. The yield of GcG_7 was 270 mg.

ACKNOWLEDGMENTS

We thank Professor I. Satake for his help in the measurement of optical rotation and Dr. F. Yagi for helpful advice on h.p.l.c. This work was supported in part by a grant-in-aid for Scientific Research from the Ministry of Education, Science and Culture of Japan (Grant No. 58470109).

REFERENCES

1 M. L. BENDER AND M. KOMIYAMA, *Reactivity and Structure Concepts in Organic Chemistry*, Vol. 6, *Cyclodextrin Chemistry*, Springer–Verlag, New York, 1978.
2 D. FRENCH, A. O. PULLEY, J. A. EFFENBERGER, M. A. ROUGVIE, AND M. ABDULLAH, *Arch. Biochem. Biophys.*, 111 (1965) 153–160.
3 P. M. TAYLOR AND W. J. WHELAN, *Arch. Biochem. Biophys.*, 113 (1966) 500–503.
4 P. M. TAYLOR AND W. J. WHELAN, in W. J. WHELAN (Ed.), *Control of Glycogen Metabolism*, Academic Press, New York, 1967, pp. 101–112.
5 S. KOBAYASHI, K. KAINUMA, AND S. SUZUKI, *Nippon Nogei Kagaku Kaishi*, 51 (1977) 691–698; *Chem. Abstr.*, 89 (1978) 60016g.
6 S. KOBAYASHI, N. SHIBUYA, D. FRENCH, H. OKEMOTO, H. KIMURA, AND K. KAINUMA, *Abstr. Pap. Ann. Meet. Agric. Chem. Soc. Jpn.*, 1983, p. 304.
7 S. KITAHATA, S. YOSHIKAWA, AND S. OKADA, *Denpun Kagaku*, 25 (1978) 19–23; *Chem. Abstr.*, 91 (1979) 122224a.
8 D. FRENCH, M. L. LEVINE, J. H. PAZUR, AND E. NORBERG, *J. Am. Chem. Soc.*, 71 (1949) 353–356.
9 J. ABE, Y. TAKEDA, AND S. HIZUKURI, *Biochim. Biophys. Acta*, 703 (1982) 26–33.
10 R. C. WHITE, C. J. RUFF, AND T. E. NELSON, *Anal. Biochem.*, 115 (1981) 388–390.
11 Y. TAKEDA, J. ABE, AND S. HIZUKURI, unpublished work.
12 L. H. KOEHLER, *Anal. Chem.*, 24 (1952) 1576–1579.
13 M. SOMOGYI, *J. Biol. Chem.*, 195 (1952) 19–23.
14 N. NELSON, *J. Biol. Chem.*, 153 (1944) 375–380.
15 S. HIZUKURI, Y. TAKEDA, M. YASUDA, AND A. SUZUKI, *Carbohydr. Res.*, 94 (1981) 205–213.
16 D. M. KILBURNE AND P. M. TAYLOR, *Anal. Biochem.*, 27 (1969) 555–558.
17 S. HIZUKURI AND S. OSAKI, *Carbohydr. Res.*, 63 (1978) 261–264.
18 S. HAKOMORI, *J. Biochem.* (Tokyo), 55 (1964) 205–208.
19 J. H. SLONEKER, *Methods Carbohydr. Chem.*, 6 (1972) 20–24.
20 M. HISAMATSU, J. ABE, A. AMEMURA, AND T. HARADA, *Agric. Biol. Chem.*, 44 (1980) 1049–1055.