Carbohydrate Research, 104 (1982) 334–337
Elsevier Scientific Publishing Company, Amsterdam – Printed in The Netherlands

Note

A simple procedure for the preparation of [^{3}H]cycloheptaamylose

RANDALL J. WESELAKE AND ROBERT D. HILL
Department of Plant Science, University of Manitoba, Winnipeg, Manitoba R3T 2N2 (Canada)
Received June 19th, 1981; accepted for publication in revised form, September 8th, 1981)

Cycloamyloses are known inhibitors of a number of bacterial, plant, and animal amylases[1–3]. In addition, their ability to include certain aromatic compounds within the ring formed by the glucose oligomer[4] has been extensively used in research and has potential industrial application. For many studies involving these compounds, the availability of a radioactive form would be an advantage. A method for the preparation of ^{14}C-labelled cycloamyloses has been published[5] and has been used successfully in equilibrium-dialysis studies[6]. It is, however, a long process requiring the preparation of radioactive starch by feeding potatoes with $^{14}CO_2$ followed by treatment with *Bacillus macerans* amylase to produce cycloamyloses. The cycloamylose of choice must then be fractionated from the mixture of products.

We have developed a method for preparing [^{3}H]cycloheptaamylose from a commercial preparation of the nonradioactive compound. The procedure is based on oxidation of C-6 of the glucose residues by chromium(VI) oxide[7], followed by reduction with sodium borotritide. The method avoids the lengthy procedure involved in the preparation of [^{14}C]cycloamyloses.

EXPERIMENTAL

Oxidation–reduction of cycloheptaamylose. — The oxidation step is an adaptation of the Jones oxidation for allylic alcohols by chromic acid[7]. The oxidizing solution was prepared by dissolving chromium(VI) oxide (2.7 g) in concentrated sulfuric acid (2.3 mL) followed by dilution to 10 mL with distilled water. Cycloheptaamylose (Sigma, 100 mg) was dissolved in 10 mL of distilled water. The glucosyl content, determined by the phenol–sulfuric acid method[8], was 86.8 mg. The oxidizing solution (0.15 mL) was added to the solution of cycloheptaamylose, and the mixture was kept for 18 h at room temperature. An excess of solid barium carbonate was then added to precipitate sulfate and chromate and to neutralize any organic acid formed during the reaction. Further deionization was performed after precipitation with barium carbonate by passing the supernatant solution through three columns of

0008-6215/82/0000–0000/S 02.75, © 1982 — Elsevier Scientific Publishing Company

mixed-bed ion-exchanger containing 5 mL each of Rexyn 300 (Fisher Chemical Company) having an exchange capacity of 0.82 meq/mL. Reducing activity of the column effluent was estimated by the Nelson adaptation of the Somogyi method for the determination of glucose[9], using reagents recommended by Robyt and Whelan[10].

The partially oxidized carbohydrate (15 mL) was reduced with tritiated sodium borohydride according to a modification of the method of McLean *et al.*[11]. Sodium borotritide (New England Nuclear) was added to a solution containing sodium borohydride (26 mg), prepared in 5 mL of 80mM potassium hydroxide, to give a final specific activity of 1.27×10^7 d.p.m./μmol. The radioactive sodium borohydride was added gradually to the oxidized cycloheptaamylose. The reaction was carried out in a fume hood. After 18 h, the solution was treated with ~2.5 mL of 0.6M hydrochloric acid to terminate the reaction. The slightly acidic solution was then adjusted to neutrality with 0.1M potassium hydroxide, and the neutral solution evaporated to dryness with a rotary evaporator. The dried material was redissolved in 30 mL of distilled water and evaporated to dryness a second time. The material was redissolved in distilled water (5.75 mL) and applied to a column (2.5 × 28 cm) of Biogel P-4 (200–400 mesh) equilibrated with distilled water. An upward flow of 20 mL/h was used to elute the column, and 6-mL fractions were collected. The fractions were assayed for radioactivity and carbohydrate. Radioactivity was measured by using a Searle Analytic Mark III liquid scintillation spectrometer. The radioactive carbohydrate peak was pooled and lyophilized for storage.

Radiochemical purity was assessed by t.l.c. on silica gel G, based on a method by Weindenhof[12] for separating cyclohexaamylose, cycloheptaamylose, glucose, and maltose. Radioactive cycloheptaamylose (5–10 μg) was spotted onto plates (20 cm × 20 cm) of silica gel G (Fisher Scientific Company). The plate was first irrigated with 6:3:1 1-butanol–acetic acid–water. After the solvent front had migrated 12.5 cm beyond the origin, the plate was removed and dried. The plate was developed a second time in the same direction using 6:3:1:2:4 1-butanol–acetic acid–water–pyridine–*N*,*N*-dimethylformamide to a point 6 cm past the origin. The plates were dried and developed with iodine vapor[13]. The lane containing the radioactive cycloheptaamylose was collected in 1-cm sections, suspended in a gel prepared from Aquassure (New England Nuclear Corporation) and water, and assayed for radioactivity. Recovery of radioactivity was >95%. In another experiment, designed to check for contamination by linear malto-oligosaccharides, only the first solvent-system was developed. The plate was then dried and irrigated a second time in the same direction with the same solvent-system. Radioactive cycloheptaamylose was compared with the same material that had been exposed to sweet-potato beta amylase (Sigma). Both maltose and maltotriose were used as standards in separate lanes and were made visible by the detection reagent of Stahl and Kaltenbach[14], which contained 95% ethanol (9 mL), anisaldehyde (0.5 mL), and a few drops of acetic acid. The plate was developed by placing it for 30 min in an oven at 100°.

TABLE I

RECOVERY OF PRODUCTS DURING THE REACTIONS FORMING [^{3}H] CYCLOHEPTAAMYLOSE

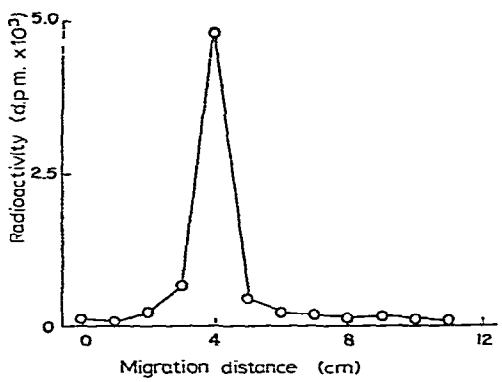
	Oxidation by CrO_3	*Reduction by NaB^3H_4*
Yield, mg (%)	31.5 (38)	29.9 (95)
Reducing activity (μmol glucose)	4.68	n.d.*
Specific activity (d.p.m./μmol)	—	1.08×10^6

*Not detectable.

RESULTS AND DISCUSSION

The Jones oxidation with chromic acid[15] is frequently used for oxidation of allylic alcohols. It is performed under relatively mild conditions that would not be expected to hydrolyze the α-(1→4) linkages in cycloheptaamylose.

The recovery of carbohydrate for this stage of the reaction was 38% (Table I), based on the determination of total carbohydrate as glucosyl unit in the effluent from ion-exchange chromatography. It is apparent, therefore, that a considerable proportion of the reaction proceeds beyond the aldehyde stage to form the corresponding glucuronic acid. This product would be removed through ion-exchange chromatography. The amount of reducing activity was 4.68 μmol of glucose, indicating that ~2.5% of the available glucose equivalents in the product had been oxidized. The carbohydrate yield after reduction by borohydride was 94% and the product had



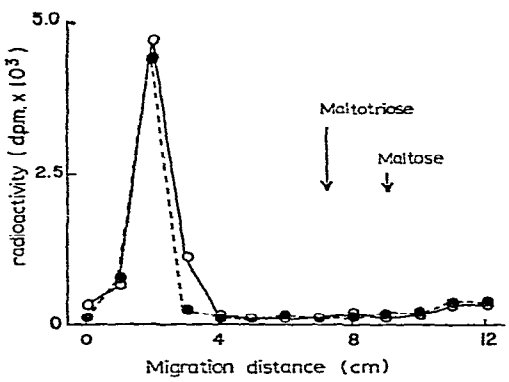


Fig. 1. Thin-layer chromatography of [^{3}H]cycloheptaamylose on silica gel G. The chromatogram was first irrigated with 6:3:1 1-butanol–acetic acid–water to a point 12.5 cm past the origin, followed by irrigation with 6:3:1:2:4 1-butanol–acetic acid–water–pyridine–*N*,*N*-dimethylformamide to a point 6.0 cm past the origin.

Fig. 2. Thin-layer chromatography of [^{3}H]cycloheptaamylose before (○——○) and after (●——●) exposure tos weet potato beta amylase. The chromatogram was irrigated with two ascents of 6:3:1 1-butanol–acetic acid–water to a point 12.5 cm past the origin.

no detectable reducing activity. The specific activity of the final product was about half that expected from the apparent carbonyl content of the oxidized cycloheptaamylose and the specific activity of the borohydride used.

A number of potential impurities could contaminate the final product. Deionization after oxidation removed most of the charged species, as the conductivity of the solution after ion-exchange chromatography was 7 μmho. Saccharides ranging from D-glucitol to maltoheptaitol could be present after reduction by sodium borohydride. The tritiated cycloheptaamylose was eluted in Biogel P-4 chromatography as a single included peak of constant specific activity. Radioactive contaminants of lower molecular weight and salt ions were eluted after [^{3}H]cycloheptaamylose. T.l.c. of the purified product gave a single spot (Fig. 1). Treatment of radioactive cycloheptaamylose with beta amylase, followed by t.l.c., showed no radioactive spots other than cycloheptaamylose (Fig. 2) indicating that the preparation did not contain linear oligosaccharide.

The procedure is relatively straightforward and avoids the problems in preparing ^{14}C-labelled cycloheptaamylose. The specific activity of the final product may be greatly increased, if necessary, by using NaB^3H_4 of higher specific activity. We have been using the [^{3}H]cycloheptaamylose for equilibrium-binding studies with cereal alpha amylase. It should also be possible to prepare [^{3}H] cyclohexaamylose similarly.

ACKNOWLEDGMENTS

We thank Dr. N. R. Hunter for helpful discussions. The research was supported, in part, by a Canadian Wheat Board Fellowship to R. W. and NSERC Grant Number A 4689.

REFERENCES

1 M. Ohnishi, *J. Biochem. (Tokyo)*, 69 (1971) 181–189.
2 J. A. Thoma and D. E. Koshland, Jr., *J. Am. Chem. Soc.*, 82 (1960) 3329–3333.
3 S. Mora, I. Simon, and P. Elodi, *Mol. Cell. Biochem.*, 4 (1974) 205–209.
4 F. Cramer and H. Hettler, *Naturwissenschaften*, 54 (1967) 625–632.
5 G. H. Anderson, F. M. Robbins, F. J. Dominigues, R. G. Moores, and C. L. Long, *Toxicol. Appl. Pharmacol.*, 5 (1963) 257–266.
6 J. A. Thoma, D. E. Koshland, Jr., J. Ruscica, and R. Baldwin, *Biochem. Biophys. Res. Commun.*, 12 (1963) 184–188.
7 K. E. Harding, L. M. May, and K. F. Dick, *J. Org. Chem.*, 40 (1975) 1664–1665.
8 M. Dubois, K. A. Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith, *Anal. Chem.*, 28 (1956) 350–356.
9 N. Nelson, *J. Biol. Chem.*, 153 (1944) 375–380.
10 J. F. Robyt and W. J. Whelan, in J. A. Radley (Ed.), *Starch and Its Derivatives*, Chapman and Hall 1968, p. 432.
11 C. McLean, D. A. Werner, and D. Aminoff, *Anal. Biochem.*, 55 (1973) 72–84.
12 N. Weidenhof, *J. Chromatogr.*, 15 (1964) 100–102.
13 R. M. Greenway, P. W. Kent, and M. W. Whitehouse, *Research*, 6 (1953) Suppl. No. 1, 63.
14 E. Stahl and U. Kaltenbach, *J. Chromatogr.*, 5 (1961) 351–355.
15 K. Bowden, I. M. Heilbron, E. R. H. Jones, and B. C. L. Weedon, *J. Chem. Soc.*, (1946) 39–45.
16 M. A. Swanson and C. F. Cori, *J. Biol. Chem.*, 172 (1948) 797–804.