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Short Communication

Separation of functionalized dextrins by reversed-phase high-performance liquid chromatography

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ABSTRACT

Functionalized linear dextrins were separated on a preparative scale by high-performance liquid chromatography on RP-18 phases with water as the eluent. These dextrins were obtained by the transfer of α-cyclodextrin to glucosides by means of a cyclodextrin-glycosyltransferase of *Klebsiella pneumoniae* M5 aI. Product recovery was readily achieved owing to the simple eluent used.

INTRODUCTION

Functionalized dextrins can be produced by enzymatic transfer of cyclodextrins (CD_n containing n units of glucose) to an acceptor. The reaction is catalyzed by cyclodextrin-glycosyltransferase (CGT) and may be schematically described as:

$$CD_n + A \rightleftharpoons A\text{-}G_n \quad (1)$$

$$A\text{-}G_n + A \rightleftharpoons A\text{-}G_i + A\text{-}G_{(n-i)} \quad (2)$$

where $A\text{-}G_i$ are coupling products which consist of an acceptor molecule (A) and i, n units of glucose (G).

The CGT from *Klebsiella pnemoniae* catalyzed only the transfer of CD_6 (α-CD) to an acceptor molecule which contains D-glucose with the reducing C-1 position blocked and the C-4 position underivatized [1]. The transfer products may be used for different applications. The series of substituted malto-oligosaccharides (for example *p*-nitrophenyl α-malto-oligosaccharides) is used for determining glucosidases and amylases [2–4]. Erlose, a transglycosylation product from the transfer of CD_6 to sucrose, is a low-cariogenic sweetener [5]. Glycosylmoranoline derivatives are used as antidiabetes drugs [6].

The separation and analysis of dextrins from enzymatic or chemical digests according to their degree of polymerization (DP) is important in the food industry. Liquid chromatography using water or aqueous solutions as the eluent is usually applied. Different approaches may be distinguished: low-pressure gel-permeation chromatography using a polyacrylamide gel [7] or a cross-linked dextran gel [8] as the stationary phase; low- [9] and high-pressure liquid chromatography [10] on cation- [9–12] or anion- [13,14] exchange resins; high-performance liquid chromatography (HPLC) using aminopropyl-bonded silica as the stationary phase [12,15,16]. Preparative separation of malto-oligosaccharides has been described for HPLC using C_{18}-bonded and aminopropyl silica gel [12]. Recently, reversed-phase columns in HPLC have been used to separate normal and branched cyclodextrins [17].

Reversed-phase chromatography with pure water

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as the eluent combines two advantages: the use of an eluent that is both non-toxic and cheap.

The aim of this study was to test reversed-phase HPLC with respect to its suitability for the preparative separation of functionalized dextrins.

EXPERIMENTAL

Apparatus

The HPLC system consisted of a Hewlett-Packard Model 1084A pump (Palo Alto, CA, USA)

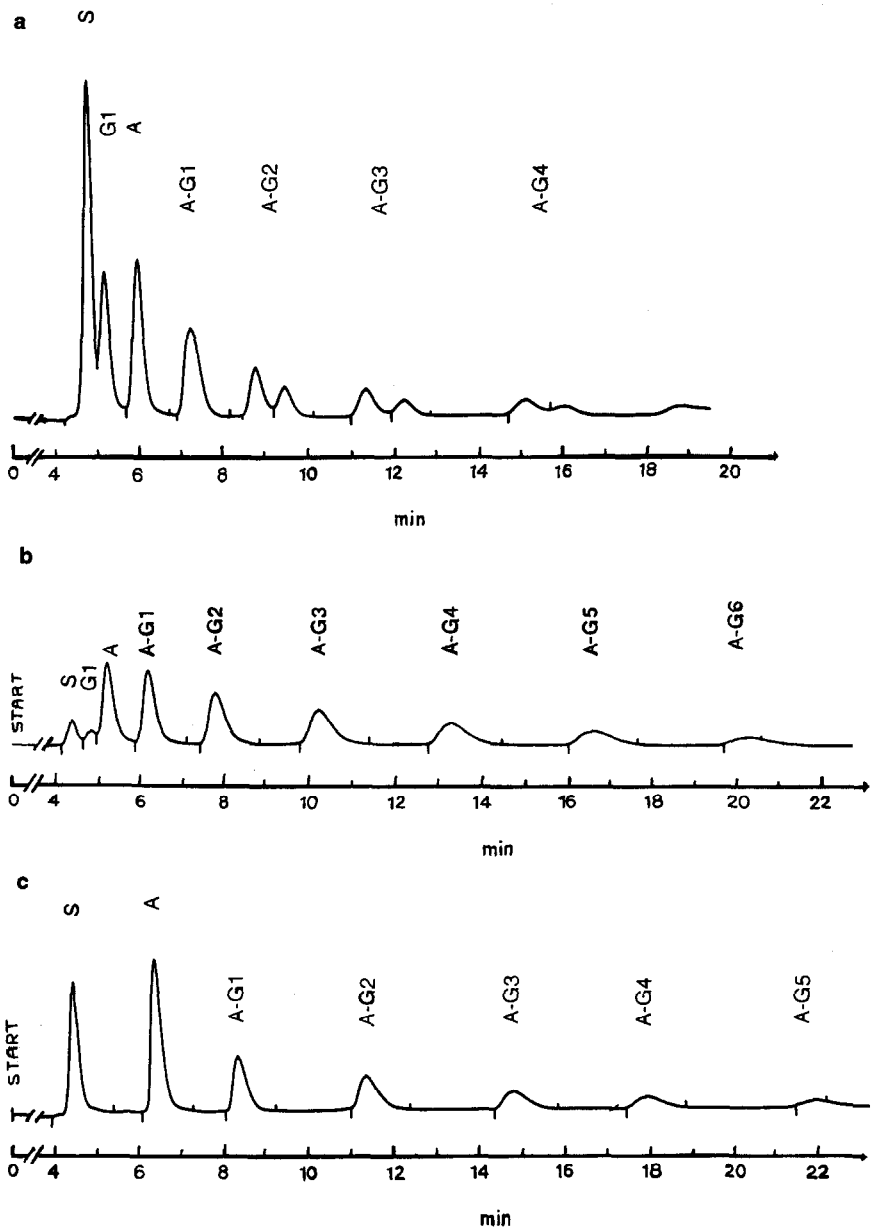

Fig. 1. Separation of functionalized dextrins from transfer reactions of α-CD on various acceptors (A). Detector attenuation, 32; sample size, 500 μl. S, salt; $[\alpha\text{-CD}]_0$, initial concentration of α-CD; $[A]_0$, initial concentration of acceptor. (a) A = maltose ($[\alpha\text{-CD}]_0$ = 9.05 m*M*; $[A]_0$ = 20.03 m*M*). (b) A = maltitol ($[\alpha\text{-CD}]_0$ = 8.93 m*M*; $[A]_0$ = 18.52 m*M*). (c) A = saccharose ($[\alpha\text{-CD}]_0$ = 8.74 m*M*; $[A]_0$ = 25.14 m*M*).

with an integrator, a Model ASI 45 automatic sampler (Kontron, Zürich, Switzerland), a Rheodyne Model 7000 sample injection valve (Berkeley, CA, USA) with a 500-μl loop and an Erma Optical Works Model 7510 refractive index detector (Tokyo, Japan). A commercial column from Macherey Nagel (Oensingen, Switzerland), 250 × 10 mm I.D., was packed with Nucleosil 7 C_{18}: particle diameter, 7 μm; pore diameter, 10 nm. As a precaution, a 2-μm filter from Rheodyne was placed between the sampler and the column.

Materials

The transfer reactions catalyzed by CGT from *K. pneumoniae* M5 aI (EC 2.4.1.19) were performed in a strirred glass vessel equipped with a thermostat and a magnetic stirrer. Before the addition of enzyme (0.49 kU/l) (determined by a cyclization test) [18], a volume of 100 ml of an aqueous solution containing α-CD (kindly provided by Consortium für Elektrochemische Industrie, Munich, Germany) and an acceptor was equilibrated at 40°C and adjusted to pH 7. After incubation for 25 min, the reaction was stopped by decreasing the pH from 7 to 3 by addition of 0.1 *M* hydrochloric acid.

The acceptors used were maltose from Carl Roth (Karlsruhe, Germany), maltitol from Fluka (Buchs, Switzerland) and saccharose from Serva (Heidelberg, Germany).

Standard solutions of maltodextrins were prepared with glucose from Merck (Darmstadt, Germany), maltotriose from Sigma (St. Louis, USA) and maltotetraose, maltopentaose and maltohexaose from Boehringer (Mannheim, Germany).

TABLE I

CONCENTRATION OF DEXTRINS FROM A TRANSFER REACTION OF α-CD ON MALTITOL

($[\alpha\text{-CD}]_0$ = 20.93 m*M*; $[A]_0$ = 54.43 m*M*).

Oligosaccharide	Concentration (m*M*)
A	18.31 ± 1.09
A-G_1	13.94 ± 0.14
A-G_2	9.66 ± 0.13
A-G_3	6.57 ± 0.12
A-G_4	4.44 ± 0.32
A-G_5	2.99 ± 0.12
A-G_6	< 1.90

Chromatographic conditions and measurements

Distilled and degassed water was used as the eluent in all the experiments. The separations were carried out at ambient temperature and the detection was at 40°C with a flow-rate of 3 ml/min.

Samples were filtered through 0.2-μm filters from Schleicher & Schuell (Dassel, Germany) prior to analysis. Production of these dextrins by enzymatic transfer of α-CD has been studied by analyzing the reaction mixtures by HPLC on an aminopropyl silica column with 65% (v/v) aqueous acetonitrile solution [1]. Maltitol and saccharose series were separated under the same chromatographic conditions in order to identify the composition of the mixtures before using the RP column.

RESULTS AND DISCUSSION

Fig. 1 shows typical chromatograms illustrating the resolution and separation of functionalized dextrins by HPLC. The retention time increased with the degree of polymerization. The α- and β-anomers of maltosaccharides beyond maltotriose (A-G_1) (Fig. 1a) were resolved at ambient temperature. The retention times for oligosaccharides of the same DP were different. Fig. 1b and c shows remarkable differences in the retention of coupling products from maltitol and saccharose. The time needed to separate dextrins up to DP 7 = A-G_5 was 17 and 22 min for derivatives of maltitol and saccharose, respectively.

Table I shows the composition of a solution obtained from a transfer experiment in the presence of α-CD and maltitol. The carbohydrate content was estimated by the external standard method taking maltitol as the reference substance and using relative peak areas.

Table II shows the resolution (R_s) of adjacent peaks for the same transfer products as in Table I but for different dilutions. In general, a satisfactory resolution was obtained for each pair of saccharides ($R_s > 1$) [19]. The least well resolved was the pair maltitol–A-G_1, but resolution was still acceptable for concentrations up to 6.5 mg of each constituent.

CONCLUSIONS

The present study has shown that RP-18 phases may be advantageously applied for the preparative-

TABLE II

RESOLUTION OF FUNCTIONALIZED DEXTRINS OBTAINED FROM A TRANSFER REACTION (TABLE I)

Dilution factor is calculated as $V_s/(V_s + V_w)$, where V_s is the volume of mixture of saccharides from transfer reaction and V_w is the volume of distilled water.

Dilution factor	R_s				
	A->A-G_1	A-G_1->A-G_2	A-G_2->A-G_3	A-G_3->A-G_4	A-G_4->A-G_5
0.167	1.39	2.15	2.73	2.50	2.23
0.200	1.58	2.14	2.66	2.73	2.11
0.250	1.39	2.07	2.25	2.37	2.08
0.333	1.33	1.82	2.22	2.17	2.79
0.500	1.31	1.79	2.11	1.92	1.58
1.0	0.98	1.40	1.71	1.59	1.40
2.0	0.73	1.22	1.28	1.35	1.18

scale separation of functionalized dextrins. The separation is fast, and the dextrins may be recovered entirely when distilled water is used as the mobile phase. To prevent column overloading, an excess of acceptor should be avoided during the enzymatic reaction. Cyclodextrins adsorb strongly on the RP-18 phase. Therefore it is necessary to eliminate them, preferably prior to the chromatographic separation. This can be done by precipitation with organic solvents. Thus, CD_6 was eliminated to a great extent by trichloroethane precipitation. Long-term experiments (5–6 weeks) with repeated injection have revealed a certain instability of the RP-18 phase used in the presence of water. In consequence, an end-capped stationary phase or a self-adjusting column adaptor would be beneficial.

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