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Note

A simplified method for preparing linear isomalto-oligosaccharides

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In the course of work on dextran-degrading enzymes, it became important to have isomalto-oligosaccharides as reference compounds for analyses by liquid chromatography (l.c.). Dextransucrase (EC 2.4.1.5) has been employed to prepare methyl α-isomaltoside and its homologs from sucrose by use of methyl α-D-glucoside as the glycosyl acceptor[1]. Alternatively, if maltose is the glycosyl acceptor, almost quantitative yields of panose (6^2-*O*-α-D-glucopyranosylmaltose) can be obtained under special conditions by the action of dextransucrase on sucrose[2]. Leucrose (5-*O*-α-D-glucopyranosyl-D-fructopyranose), palatinose (isomaltulose; 6-*O*-α-D-glucopyranosyl-D-fructofuranose), and dextran are also formed. Apparently, sucrose or its component sugars, D-glucose and D-fructose, serve as glycosyl acceptors in these syntheses[3].

Koepsell *et al.*[4] tested a variety of sugars and sugar derivatives as glycosyl acceptors for the dextransucrase reaction. They found maltose and isomaltose (6-*O*-α-D-glucopyranosyl-D-glucopyranose) to be especially efficient as glycosyl acceptors, and the formation of dextran was severely limited in their presence. The products of the reaction with isomaltose as the acceptor were low-molecular-weight oligosaccharides of the isomaltose series. With maltose, a series of oligosaccharides terminating in panose at the reducing end would be formed. D-Glucose would also be expected to give rise to a series of isomalto-oligosaccharides, but, under the conditions described by Koepsell *et al.*[4], the yield was quite low.

Walker[5,6] prepared isomalto-oligosaccharides with dextransucrase from *Streptococcus mutans* OMZ 176 by use of isomalto-oligosaccharides as acceptor for the labelled D-glucose contained in [^{14}C]sucrose. Under these conditions, high yields of isomalto-oligosaccharides containing one D-glucose unit more than the acceptor were obtained. This excellent method is limited for general use, due to the commercial unavailability of acceptor isomalto-oligosaccharides.

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In the present work, D-glucose was chosen instead of isomaltose as the glycosyl acceptor, because it is readily available and far less costly. Also, commercial yeast can be used to remove the excess of D-glucose after termination of the dextransucrase reaction.

EXPERIMENTAL

Material. — Dextransucrase was prepared from *Leuconostoc mesenteroides* NRRL B-512F at the Northern Regional Research Center by A. Kazenko, as described by Hehre[7]. It assayed at 37.3 units/mg. D-Glucose was purchased from Difco laboratories. Sucrose was common table sugar. Isomalto-oligosaccharides were isolated from *Penicillium funiculosum* digests[8] of B-512F dextran by Dr. Allene Jeanes and her co-workers of this laboratory.

Methods. — The reaction mixture, in 50 mL of 0.01M acetate buffer, pH 5.0, consisted of 0.3M sucrose (5.13 g), 0.4M D-glucose (3.6 g), and 1400 units of dextransucrase. Incubation was conducted for 24 h at 4°, as low-temperature incubation favors formation of oligosaccharides over that of dextran[1]. The reaction was terminated by addition of methanol (50 mL) to precipitate any dextran that might have been formed. The suspension was centrifuged to remove polysaccharide, and the supernatant liquor was concentrated under vacuum to 25 mL to remove methanol. To each 5 mL of the concentrate was added Fleischmann's active, dry yeast (200 mg). The yeast was allowed to work at 30° until evolution of CO_2 ceased (~6 h). This procedure removes[9] any excess of D-glucose, sucrose, and leucrose. The yeast cells were removed by centrifugation, and the supernatant liquor contained ~6% of isomalto-oligosaccharides.

L.c. analyses were conducted in a Water Model 201 liquid chromatograph equipped with a μBondapak carbohydrate column, and a differential refractometer for detection of the individual oligosaccharides. Elution times and relative concentrations of each oligosaccharide were determined by computer analysis.

A Partisil Pac preparative column from Whatman, Inc. was used in order to obtain sufficient quantities of individual isomalto-oligosaccharides for methylation analyses. At least 2 mg of each oligosaccharide was collected and tested for purity before being subjected to methylation analysis.

Methylation. — Permethylation of the isomalto-oligosaccharides was conducted by the method of Hakomori[10]. The samples were first reduced with $NaBD_4$, and then the excess of borate was removed from the decationized solution by repeated evaporation with methanol before alkylation. Upon completion of the methylation, the product was extracted by partitioning between 1:2 $CHCl_3$–H_2O. The resulting, permethylated oligosaccharides were hydrolyzed in the following sequence: (*a*) in 90% formic acid for 1 h at 100°; (*b*) removal of formic acid by vacuum evaporation; (*c*) 4 h at 80° in Stellner's reagent[11]; and (*d*) 4 h at 80° in Stellner's reagent diluted 1:1 with water. Following hydrolysis, the sulfate ions were removed by treatment with Dowex-1 X-8 (OAc^-) resin. The samples were dried in a rotary evaporator at

39° with the aid of absolute ethanol. Per-*O*-acetylated aldononitrile (PAAN) derivatives were prepared as described by Seymour *et al.*[12]. The mixture of 6-*O*-acetyl-penta-*O*-methyl-D-glucitol-*1-d* and PAAN derivatives was then analyzed by combined gas–liquid chromatography–mass spectrometry[12].

RESULTS

In Fig. 1 is depicted a typical chromatogram of the synthate prepared as described in the Methods section. Oligosaccharides having a degree of polymerization (d.p.) of 11 are readily discernible by this technique. Isomaltose (peak 2) is the preponderant oligosaccharide, accounting for ~37% of the products. The relative amounts of isomalto-oligosaccharides of d.p. 2 to 11, expressed as percentages are: 36.6, 9.5, 13.1, 12.8, 6.2, 4.6, 5.3, 5.1, 4.1, and 2.7, respectively.

Linearity and d.p. of the oligosaccharides were confirmed by methylation analysis[13]; *i.e.*, no dimethyl ether PAAN derivatives could be detected, and the molar ratios of the 2,3,4,6-tetra- to 2,3,4-tri-methyl ether derivatives accorded with the

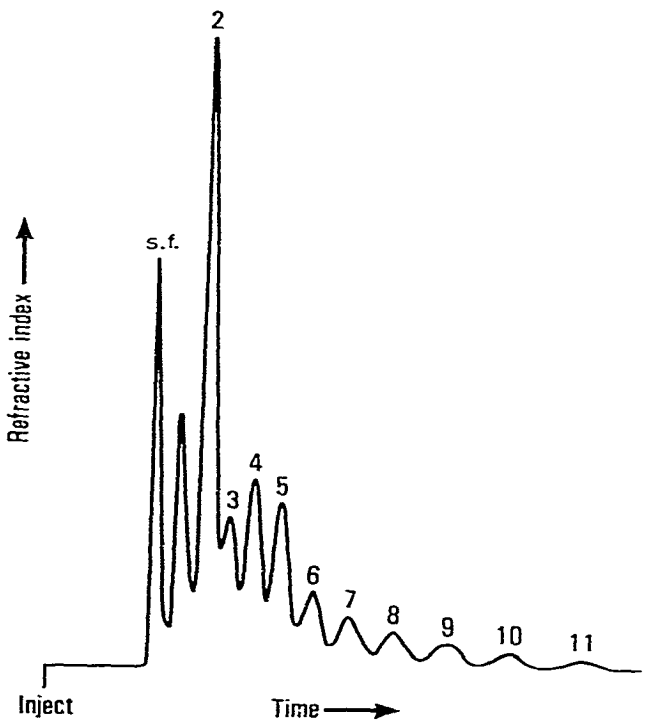

Fig. 1. Liquid chromatogram of isomalto-oligosaccharides formed by the action of dextransucrase on sucrose, using D-glucose as the glycosyl acceptor. [Waters μBondapak carbohydrate column; solvent, 13:7 acetonitrile–H_2O at 1.6 mL/min. Numbers above peaks = degree of polymerization; s.f. = solvent front. The unmarked peak that was eluted before isomaltose could not be identified as being a hexose. Time, in minutes.]

TABLE I

PEAK RETENTION-TIMES OF ISOMALTO-OLIGOSACCHARIDES

Degree of polymerization	*Oligosaccharides from*	
	Dextransucrase synthate	P. funiculosum *digest of dextran B-512F*
	Retention time (min)	
2	3.03	3.06
3	3.46	
4	3.91	3.89
5	4.45	4.26
6	5.08	4.81
7	5.81	5.58
8	6.68	6.32
9	7.74	7.15
10	8.86	
11	10.26	

presumed d.p. values (oligosaccharides of d.p. 9 and 11 were not analyzed) and exclusive (1→6) linkage.

The procedure described here offers a convenient and economical method for preparation of linear isomalto-oligosaccharides.

Table I lists the peak retention-times of the dextransucrase synthate, as well as of some isomalto-oligosaccharides derived from enzymically hydrolyzed dextran. Good agreement is found through d.p. 4. From d.p. 5 through 9, the oligosaccharides from the dextran hydrolyzate are eluted significantly faster than their counterparts from the synthate. Because B-512F dextran, like all bacterial dextrans, is branched[13], it is reasonable that the faster-eluted oligosaccharides are branched. Methylation analysis of the d.p. 4 oligosaccharide revealed it to be linear, but with interior (1→3)- and (1→6)-linked residues; *i.e.*, 1,2,3,4,5-penta-*O*-methylalditol-*1-d* monoacetate and PAAN derivatives of 2,3,4,6-tetra-, 2,3,4-tri-, and 2,4,6-tri-*O*-methyl-D-glucose were obtained in equimolar amounts. All of the (1→3)-linked residues in B-512F dextran occur at points of branching. Consequently, release of the tetrasaccharide must have entailed cleavage of a (1→6) linkage appended to a backbone-chain residue involved in branching.

The l.c. peak for the apparent d.p. 5 component of the dextran digest was unsymmetrical. Methylation-fragmentation analysis revealed the presence of a 1,3,6-tri-*O*-substituted glucose residue, in addition to the components found in the tetrasaccharide. The major components were nonreducing end-groups and unbranched (1→6)-linked residues. A branched oligosaccharide appears to be the principal constituent of the d.p. 5 fraction.

Branching of the d.p. 6 oligosaccharide isolated from the enzymic digest was also confirmed by methylation analysis. Penta-*O*-methylalditol, 2,3,4,6-tetra-, 2,3,4-

tri-, and 2,4-di-*O*-methyl derivatives were obtained in the respective molar ratios of 1:2:2:1.

These results demonstrate the sensitivity of l.c. retention-times to subtle changes in oligosaccharide structure. The d.p. 4 oligosaccharide, for example, consistently displays a retention time slightly shorter than that of synthetic isomaltotetraose.

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