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Note

Synthesis of α-D-glycopyranosyl α-L-sorbofuranoside, and its use as a D-glucosyl donor

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Sucrose phosphorylase from *Pseudomonas saccharophila* has been used to synthesize a variety of disaccharides from α-D-glucopyranosyl phosphate plus each of a variety of ketoses[1]. The enzyme forms an enzyme–substrate complex with the D-glucopyranosyl group from either sucrose, α-D-glucopyranosyl phosphate[2], or α-D-glucopyranosyl fluoride[3]. The D-glucopyranosyl group can then be transferred to either arsenate, phosphate, a ketose, or water.

α-D-Glucopyranosyl α-L-sorbofuranoside has been prepared by use of sucrose glucosyltransferase and either α-D-glucopyranosyl phosphate[4] or sucrose[5] as the D-glucopyranosyl donor. In the latter method, the excess of sucrose was removed by fermentation, and the D-glucosyl L-sorboside was recovered from the spent medium. We now describe the synthesis of α-D-glucopyranosyl α-L-sorbofuranoside from either sucrose or α-D-glucopyranosyl fluoride as the D-glucopyranosyl donor.

EXPERIMENTAL

Materials. — Yeast invertase (β-D-fructofuranoside fructohydrolase, EC 3.2.1.26), D-glucose oxidase–peroxidase, α-D-glucopyranosyl phosphate (Glc-1-P), and protamine sulfate were obtained from Sigma Chemical, St. Louis, Missouri. Gel-permeation media were obtained from Bio-Rad, Richmond, California. *Pseudomonas saccharophila* (No. 15946 Doudoroff) was obtained from the American Type Culture Collection, and *Streptococcus mutans* (FA1) and *Streptococcus salivarius* (SS2) were obtained from R. J. Gibbons, Forsyth Dental Center, Boston, Massachusetts. All chemical compounds used were of reagent grade.

Sucrose glucosyltransferase [disaccharide D-glucosyltransferase (nonspecific) EC 2.4.1.7] was prepared from *P. saccharophila* according to the method of Silverstein *et al.*[6] and was kept frozen until it was used. The broth levansucrase–dextransucrase [(2→6)-β-D-fructan: D-glucose 6-fructosyltransferase, EC 2.4.1.10, and (1→6)-α-D-glucan: D-fructose 2-glucosyltransferase, EC 2.4.1.5] was prepared from *S. mutans* (FA1) by the method of Scales[7], and was stored in the lyophilized state at

−20°. The cell-bound levansucrase [(2→6)-β-D-fructan: D-glucose 6-fructosyltransferase, EC 2.4.1.10] from *S. salivarius* (SS2) was prepared according to Garszczynski and Edwards[8], and was stored in the frozen state at −20° until it was used.

Tetra-*O*-acetyl-α-D-glucopyranosyl fluoride was prepared by the reaction of α-D-glucopyranose pentaacetate with liquid hydrogen fluoride[9,10]. The crystalline product had m.p. 108° (reported[9] m.p. 108°), and the n.m.r. spectrum agreed with published values[10]. The tetraacetate fluoride was catalytically deacetylated with freshly prepared sodium methoxide[11], neutralized with ion-exchange resin [IR-120 (H^+)], and the fluoride used without further purification.

Methods. — Total carbohydrate content was determined by the phenol–sulfuric acid method[12], reducing sugar by Somogyi's method[13], ketohexose by the cysteine–sulfuric acid method[14], and D-glucose by the D-glucose oxidase–peroxidase method[15]. Phosphate was determined by the Fiske–Subbarow method[16].

Descending paper-chromatography was performed with Whatman No. 1 paper and 25:36:10:10 propyl alcohol–benzyl alcohol–85% formic acid–water. The sugars were detected with the aniline–diphenylamine–phosphoric acid spray reagent[17]. Carbon–Celite chromatography was performed by using equal amounts of Darco G-60 carbon and acid-washed Celite 545. The column (5 × 45 cm) was eluted with water, and then with 5% and 10% ethanol in water[18].

Preparation of α-D-glucopyranosyl α-L-sorbofuranoside. — A. With α-D-glucopyranosyl phosphate as the D-glucopyranosyl donor. Sucrose phosphorylase (150 ml, 27 U/ml) in citrate buffer (pH 7.0, 50mM) was incubated with L-sorbose (11.0 g) and α-D-glucopyranosyl phosphate (14.0 g) for 18 h at 37°. The mixture was deionized by passage through a column of Amberlite IR-45 (OH^-), and the neutral material was then placed on a carbon–Celite column. The column was washed with water (2 litres) and then eluted with 1:19 ethanol–water[18]. The fractions (10–12 ml) containing the D-glucosyl L-sorboside were pooled, evaporated *in vacuo* at 40°, and crystallized by the addition of absolute ethanol. Yields of ~2 g were obtained.

B. With sucrose as the D-glucopyranosyl donor. Sucrose phosphorylase (150 ml, 27 U/ml) in citrate buffer (pH 7.0, 50mM) was incubated at 37° with L-sorbose (11.0 g) and sucrose (8.0 g) until the optical rotation became constant (~4–6 h). The mixture was heated for 5 min at 100°, and cooled; then, yeast invertase (50 mg, 250 U/mg) was added, and the hydrolysis of the excess of sucrose was monitored by the change in optical rotation, which became constant after 3–5 h. The mixture was then heated (to inactivate the invertase) and the mixture was applied to a carbon–Celite column (5 × 45 cm) and eluted as already described. About 2.5 g of sugar was isolated.

C. With α-D-glucopyranosyl fluoride as the D-glucopyranosyl donor. Sucrose phosphorylase (100 ml, 24 U/ml) in citrate buffer (pH 7.0, 50mM) was incubated with L-sorbose (16 g) and α-D-glucopyranosyl fluoride (4.3 g). The mixture was incubated at 37°, the reaction being monitored by the change in optical rotation. When the optical rotation became constant (~5 h), the mixture was heat-inactivated, applied to a carbon–Celite column, and eluted as already described. The excess of glucosyl

fluoride was not completely removed from the column by the water elution. The initial phase of the disaccharide elution by 5% ethanol in water gave an eluate that contained glucosyl fluoride. Rechromatography of the material on a carbon–Celite column (2.5 × 20 cm) was necessary in order to resolve the two compounds completely. This procedure yielded about 700 mg.

RESULTS

Characterization of the glucosyl sorboside. — The glucosyl sorboside prepared by the three methods had identical chemical properties. The disaccharide was non-reducing and had m.p. 184–186°; a 1:1 mixture of the disaccharide with sucrose (m.p. 188°) had m.p. 169–175°. It was readily hydrolyzed with sulfuric acid (0.05M for 15 min at 100°) to yield two moles of reducing sugar per mole of disaccharide. The disaccharide had $[\alpha]_D$ +32.6–33.9° (*c* 5, water); after hydrolysis with sulfuric acid, the resulting mixture of sugars had $[\alpha]_D$ +8.0° (*c* 5, water); a 1:1 mixture of D-glucose and L-sorbose had a specific optical rotation of +7.08°. Paper chromatography revealed one component migrating like sucrose (R_{Glc} 0.60) and, after acid hydrolysis, two components migrating like glucose and sorbose (R_{Glc} 1.00, 1.21). There were equimolar amounts of glucose and ketose in the disaccharide, as determined by (*a*) the action of D-glucose oxidase after acid hydrolysis, and (*b*) analysis by the cysteine–sulfuric acid method. The presence of sorbose was shown by the characteristic ratio of the 605 nm:412 nm absorbances (*A*) in the cysteine–sulfuric acid assay. Both sorbose and the disaccharide had A_{605}/A_{412} ratios of 2.15–2.20:1, whereas fructose gave a ratio of 0.29:1. The physical and chemical data found are in agreement with data previously published[1,4].

Reaction of the glucosyl sorboside with levansucrase plus dextransucrase. — The glycosyltransferase preparation from *S. mutans* (FA1) spent culture-media contains a dextransucrase as well as a levansucrase, whose activities have not yet been separated[7]. The glucosyl sorboside (0.8 ml, 5% final concentration) was incubated with the dextransucrase–levansucrase from *S. mutans* (77 μg/ml; 0.6 U/ml; 0.2 ml) at pH 6.0 in 50mM phosphate buffer. The mixture was incubated for 12 h at 37° and then exhaustively dialyzed against water. The nondialyzable polymer was assayed for total carbohydrate and for ketose. The total carbohydrate content of the nondialyzable material was 3.14 mg (measured as glucose)/ml. No sorbose, measured by the cysteine–sulfuric acid assay, was detected in the nondialyzable material. Chromatographic analysis of sulfuric acid hydrolyzates (1 h at 100° with 0.05M) did not show the presence of sorbose. Acid hydrolyzates (18 h at 100° with 0.5M) showed only the presence of glucose. The polymer was excluded from a gel-filtration column of Bio-Gel P-60, indicating a molecular weight >60,000. In addition to polymer formation, there was also hydrolysis of the glucosyl sorboside. When a similar experiment was performed with the levansucrase from *S. salivarius*, there was neither hydrolysis of, nor polymer formation from, the glucosyl sorboside.

DISCUSSION

The reversal of enzymic action of sucrose phosphorylase has been used to prepare sucrose analogs wherein the D-fructosyl group has been replaced by the ketosyl group of a variety of ketoses[1]. In the initial synthesis of glucosyl sorboside, Glc-1-P and L-sorbose were allowed to react with sucrose phosphorylase, and the disaccharide was isolated after the removal of the monosaccharides as the phenylosazones[4]. Sucrose as the α-D-glucopyranosyl donor was employed by Rapoport and Dedonder[5], the excess of sucrose and D-fructose being decomposed by fermentation with *Saccharomyces italicus*. The resistance of the glucosyl sorboside to yeast invertase permits hydrolysis of the excess of sucrose, and isolation of the glucosyl sorboside, the only disaccharide remaining, by carbon–Celite chromatography. The extent of the reactions may be monitored by the change in optical rotation.

α-D-Glucopyranosyl fluoride serves as a D-glucosyl donor with sucrose glucosyltransferase, yielding with L-sorbose a disaccharide which is identical to that prepared by the previously published methods[4,5]. This reaction is similar to the enzymic phosphorylation of α-D-glucopyranosyl fluoride with sucrose glucosyltransferase in the presence of inorganic phosphate[3]. The participation of α-D-glucopyranosyl fluoride as a glucosyl donor in the synthesis of a glucan with amylosucrase[19] and of a dextran with dextransucrase[20] has been demonstrated. We have obtained chromatographic evidence of the synthesis of a disaccharide using 2-deoxy-D-*arabino*-hexosyl fluoride; the yield of the overall reaction was, however, greatly lessened due to the lability of the 2-deoxy-D-*arabino*-hexosyl fluoride. Enzymic synthesis of a variety of sucrose analogs by using chemical derivatives of α-D-glucopyranosyl fluoride may now be feasible.

The D-glucosyl L-sorboside was found to be a substrate for the dextransucrase from *S. mutans*, and to yield a glucan of large molecular weight. There was no indication of synthesis of a sorban by the levansucrase from either *S. mutans* or *S. salivarius*. The disposition of the hydroxymethyl group on C-5 of sorbose may not allow it to fit suitably into the active site of the levansucrase to accept the "activated" carbonyl group of the sorbose, even though the rest of the molecule resembles sucrose; this phenomenon has also been observed with the levansucrase from *Bacillus subtilis*[5]. This does not, however, rule out the possibility of exchange reactions of the sorbosyl moiety with other sugars.

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