Note
Action of Levan Fructotransferase of Arthrobacter ureafaciens on a Mixture of Branched Levanpentasaccharides

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The levan fructotransferase of Arthrobacter ureafaciens has been reported to produce, in addition to di-o-fructofuranose 2, 6’:2’, 6 dihydride (fructotetraose IV) as the main product, a significant amount of 1-O-β-D-fructofuranosyl difructose anhydride IV from levan.1-3 To investigate the formation of this trisaccharide, the possibility of its enzymatic formation from a mixture of branched levanpentasaccharides was examined.

A mixture of branched levanpentasaccharides was prepared from the partial acid hydrolysis of levan after successive fractionations on carbon-Celite and cellulose powder columns. It was derived from pooled samples of the fractions 36-40 in cellulose powder column chromatography, which are given in Fig. 2-D of our previous experiment.9 The sample was concentrated at 40°C and precipitated by adding ethanol, washed with ethanol and diethyl ether, and dried in a desiccator over silica gel, yielding about 54 mg of 4 g of levan. The sample (about 100 mg) was fractionated by repeating the cellulose powder column chromatography and dried by lyophilization, yielding about 17-20 mg of a dried material.

The ratio of reducing sugar to total ketohexose of the sample was calculated to be 1:5.01 by the method of Nelson40 and Somogyi41 and the resorcinol hydrochloric acid method,42 respectively, using fructose as the standard. Its [α]D20 = -36.05° (c=0.48, in H2O) was recorded. If the all (ten) kinds of branched levanpentasaccharides are recovered in the mixture, their methylation analysis should give a 4:6:1:1.5 molar ratio of 1,3,4,6-tetra-O-methyl-, 1,3,4-tri-O-methyl-, 3,4,6-tri-O-methyl- and 3,4-di-O-methylfructoses. Methylation analysis of the sample and its acid hydrolysis were done as described previously.1,4,8 and the methylated fructose derivatives were measured by thin-layer chromatography using Densitrol (Advantec DMU-3C type) with a width of slit 0.5 x 5 mm at 440 nm, after twice repeated ascending chromatographies of 2 μl of the sample with the Si-BzEWB-NS system.5 Calibration curves of the methylated fructose derivatives were obtained in the range of 0.6 to 4.6 μg/spot (2 μl). The results predicted the ratio of 4:5.5:1:4.2, suggesting the sample would contain all the ten kinds of branched levanpentasaccharides.

The levan fructotransferase preparation was prepared and purified as described earlier.9 Its specific activity was 27.4 U/mg of protein at an equal level of the previous value. Action of the enzyme on the mixture of branched levanpentasaccharides was examined with a reaction mixture containing 5 μl of 1% levanpentasaccharides, 5 μl of 0.2 M acetate buffer (pH 6.0), and 10 μl of enzyme solution containing 0.014 unit, incubated at 20°C. Two-μl portions of the reaction mixture were withdrawn and analyzed by thin-layer chromatography with the Si-BeW-NS system.13 The chromatogram is shown in Fig. 1. The enzymatic digest gave five fructooligosaccharide spots including the spot of the original sample. The second spot in the order of higher mobility showed the same mobility as the trisaccharide. The other three spots indicate production of fructooligosaccharides of principally DP 2 to 4 including difructose anhydride IV by the action of the enzyme on some of branched levanpentasaccharides.

Isolation of this sugar was done from a reaction mixture which was prepared by incubation of 15 mg of branched levanpentasaccharide with 3 ml of enzyme solution containing 0.35 unit in a total 6.0 ml of 50 mM acetate buffer (pH 6.0) at 20°C for 240 min. The reaction was stopped by heating in a boiling water bath for 5 min. Its 5.5-μl portion was fractionated on a small carbon-Celite column by distilled water(100ml)-17% ethanol(100ml) gradient elution, under the same conditions as described.13 The trisaccharide fractions were pooled and concentrated. The sample obtained was fractionated again on a cellulose powder column under the conditions described.42 In these fractions 20-24 were pooled and concentrated to dryness and dissolved in 1 ml of distilled water. The yield was about 330 μg as fructose.

The product had the same mobility as the authentic trisaccharide under six different thin-layer chromatographic conditions; the Si-BEW-NS, Si-BPW-NS, Si-PEW-NS, Av-BEW-AP, Av-BPW-AP, and Av-PEW-AP systems.8 Only when the chromatography was done with the Si-BeW-NS system, the sample showed contamination of a small amount of another fructooligosaccharide with a lower mobility. The product (53 μg) showed a little reducing property by the Nelson and Somogyi's method, probably due to contaminating sugar. Hydrolytic susceptibility of the product toward yeast β-fructofuranosidase was examined. A mixture of 50 μg of the product and 0.1 μg of enzyme (Boehringer Mannheim GmbH, Darmstadt, Germany Lot 7040108) in 20 μl of 25 mM acetate buffer (pH 6.0) was incubated at 25°C for 60 min. The reaction mixture was examined by thin-layer chromatography with Av-BPW-NS system, in which the anthrone phosphoric acid spray reagent (AP) of the Av-BPW-AP system was replaced with the naphthoresorcinol sulfuric acid reagent (NS). The enzymatic digest of the product gave two spots corresponding to fructose and difructose anhydride IV.

These results strongly suggest that the trisaccharide (1-O-β-D-fructofuranosyl difructose anhydride IV) could be produced by the intramolecular levanfructosyl transfer (ILFT) reaction)18 of levan fructotransferase on one or some branched levanpentasaccharides included in the substrate mixture. That is, the formation from a levan molecule would result, as suggested previously,2 in the ILFT reaction on a special end group or groups at the branched region produced by the enzymic degradation of a levan molecule, such as O-β-D-Fru(1-2)-O-[(β-D-Fru(1-2)-O-β-D-Fru(1-2)-O-β-D-Fru(1-2)], O-β-D-Fru(1-2)-O-[(β-D-Fru(1-2)-O-β-D-Fru(1-2)-O-β-D-Fru(1-2)].

![Fig. 1. Thin-layer Chromatogram Showing Formation of Several Fructooligosaccharides from a Mixture of Branched Levanpentasaccharides by the Action of Levan Fructotransferase.](image-url)
References