NOTE

Nature of the Fructan of Streptococcus mutans OMZ 176

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The fructan of Streptococcus mutans OMZ 176 was shown to have a levan structure by comparing the chromatographic mobilities of the saccharides produced by partial acid hydrolysis of the fructan and known levan and inulin. This was confirmed by using concanavalin A as a lectin in a double-diffusion gel technique.

Streptococcus mutans OMZ 176 is a cariogenic organism that produces glucans and a fructan from sucrose. Several studies have been made on the structure of the glucans (6, 9, 14, 15, 17), but essentially nothing is known about the structure of the fructan. Althoughlevans (β-2→6-linked fructans) are elaborated by bacteria (3) and inulins (β-2→1-linked fructans) are usually elaborated by plants (1), the fructans of several S. mutans strains (JC-1, JC-2, Ingbritt A, CH7, BHT, and AHT) have been shown to have an inulin structure (4, 7, 8, 19). The fructans produced by Streptococcus salivarius strains (S1, SS2, HHT, and ATCC 13419), however, have been shown to be levans (10, 12, 16). Therefore, we thought that it would be of interest to determine the nature of the fructan elaborated by S. mutans OMZ 176. The structure was studied using paper chromatographic analyses of partial acid hydrolysates of the fructan and known levan and inulin and agar-gel double-diffusion plates with concanavalin A as a precipitating lectin.

Test levans were prepared from Aerobacter levanicum NRRL B-1678 levansucrase and sucrose (2) and from Leuconostoc mesenteroides NRRL B-512F culture supernatant fluid and raffinose. S. mutans OMZ 176 fructan was obtained by reacting a 40 to 50% saturated ammonium sulfate cut (18) of the culture supernatant fluid with a 0.1 M raffinose solution buffered at pH 7.2. Raffinose is a specific substrate for forming fructans whose synthesizing enzymes are contaminated with glucansucrases (5). The fructan was obtained from the raffinose digest by precipitating it with 2 volumes of ethanol. It was further purified by dialysis and re-precipitation with ethanol. A sample of inulin was obtained from Matheson, Coleman, and Bell.

Solutions of levan, inulin, and S. mutans fructan (50 mg/ml) were prepared and hydrolyzed for 20 min with 0.01 M sulfuric acid at 70°C. The reaction was stopped by cooling and neutralizing with sodium hydroxide. The solutions were desalted by Amberlite MB-3 columns, concentrated, and freeze-dried.

Fig. 1. Ascending paper chromatograph of the partial acid hydrolysates of fructans: L, levan from A. levanicum; I, inulin; S, fructan from S. mutans OMZ 176; Fru, fructose; 2 to 6, degree of polymerization of the oligosaccharides. In = inulin oligosaccharides and Ln = levan oligosaccharides with n monomer units.

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trated to dryness, and dissolved in 67% ethanol. Samples were spotted onto Whatman no. 1 paper (23 by 33 cm) for ascending chromatography, using 1-butanol-pyridine-water (6:4:3, vol/vol) with four ascents at 35°C. Saccharides were detected with silver nitrate, and their mobilities were determined as $R_m$. French-Wild plots (11) were made to compare the mobilities of the saccharides produced from the different fructans. Figure 1 shows a paper chromatogram, and Fig. 2 shows the French-Wild plot. The results show that the saccharides produced from S. mutans OMZ 176 fructan are identical to the saccharides obtained from levan and are dissimilar to those obtained from inulin.

This result was further tested by the double-diffusion agar plate procedure of Goldstein and So (13). Concanavalin A, obtained from Sigma Chemical Co., was added to the center wells in concentrations of 5 to 25 mg/ml; the fructan solutions (1 to 50 mg/ml) were added radially 5 mm from the center well. The results are shown in Fig. 3. Concanavalin A gave a continuous precipitin band with A. levanicum levan (C), L. mesenteroides levan (D), and S. mutans fructan (B), but failed to give any precipitate with inulin. This experiment, utilizing a very different technique from the chromatographic analyses of the partial acid hydrolysates, confirms the results obtained by the chromatographic analyses, namely, that S. mutans OMZ 176 fructan has a levan structure and not an inulin structure.

Of the various strains of S. mutans investigated, OMZ 176 is apparently unique in this

![Fig. 3. Agar-gel double-diffusion test for interaction of fructans with concanavalin A. (A) Inulin; (B) S. mutans OMZ 176 fructan; (C) A. levanicum levan; (D) L. mesenteroides levan; (1 to 3) concanavalin A. All fructans were at a concentration of 50 mg/ml. The concanavalin A concentrations were as follows: (1) 5 mg/ml; (2) 10 mg/ml; and (3) 25 mg/ml.]

![Fig. 2. French-Wild plot (11) of the partial acid hydrolysates of fructans. Symbols: , oligosaccharides from levan from A. levanicum; , oligosaccharides from unknown fructan from S. mutans OMZ 176; , oligosaccharides from inulin. $\alpha' = R_f/(1 - R_f)$, where $\alpha$ = the number of ascents for an ascending chromatogram; in this case, $\alpha = 4$.]
respect as five other stains produce inulins. Furthermore, in this regard, S. mutans OMZ 176 is more similar to the four stains of S. salivarius that have been shown to elaborate levans. The possible significance of this finding to the involvement of S. mutans OMZ 176 in dental plaque formation and dental caries is not clear at this time, but it does indicate a biochemical difference and similarity between this organism and other very closely related organisms in the oral cavity.

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LITERATURE CITED