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ISOLATION AND SOME PROPERTIES OF EXTRACELLULAR D-GLUCOSYLTRANSFERASES AND D-FRUCTOSYLTRANSFERASES FROM *Streptococcus mutans* SEROTYPES *c*, *e*, AND *f*

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ABSTRACT

Extracellular D-glucosyltransferases (GTase) and D-fructosyltransferases (FTase) were isolated from *Streptococcus mutans* IB (serotype *c*), B14 (*e*), and OMZ175 (*f*) by chromatofocusing, followed by hydroxyapatite column chromatography. The GTases isolated from serotypes *c*, *e*, and *f* are basic proteins (pI 7.4). The serotype *c* and *e* enzymes have two protein components having M_r 173 000 and 158 000 and the enzyme of the serotype *f* one component having M_r 156 000. The GTases of all the serotypes showed a K_m value for sucrose of 10–14mM and an optimum pH 5.5–6.0 for enzyme activity, and their activities were enhanced by the presence of primer Dextran T10. The α-D-glucans synthesized by the purified GTases are water soluble and primarily consist of (1→6)-α-D-glucosidic linkage (41–66 mol/100 mol) and α-D-(1→3,6)-branch linkage (6–20 mol/100 mol), but significant proportions of α-D-(1→3), α-D-(1→4), and α-D-(1→3,4) linkages (11, 6, and 14 mol/100 mol, respectively) were detected in the serotype *c* α-D-glucan. The isolated FTases of the serotypes *c*, *e*, and *f* are acidic enzymes (pI 4.6) and consist of two components having M_r 84 000 and 76 000 for the serotype *c* enzyme, and 106 000 and 84 000 for the serotypes *e* and *f* enzymes, respectively. The K_m value for sucrose was 6, 10, and 17mM for the serotypes *c*, *e*, and *f* enzymes, respectively, and the optimum pH of enzymic activity 5.5–6.0. Reactivity with Concanavalin A, susceptibility to acid hydrolysis, and paper chromatography of the hydrolyzates suggested that the water-soluble β-D-fructans synthesized by the purified FTases were of the inulin-type and had chemical structures somewhat different among the serotypes.

INTRODUCTION

Streptococcus mutans is a cariogenic microorganism having the specific ability to colonize smooth tooth-surfaces. The streptococcus produces extracellular water-

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insoluble and water-soluble polysaccharides from sucrose by action of its constitutive enzymes, D-glycosyltransferase (GTase; EC 2.4.1.5) and D-fructosyltransferase (FTase; EC 2.4.1.10). Tenacious, insoluble α-D-glucans play an essential role in the adherence of *S. mutans* to tooth surfaces, and soluble α-D-glucans and β-D-fructans act as an extracellular energy-storage for this organism[1–3].

Production and properties of the extracellular polysaccharides vary considerably among the groups of *S. mutans* serotypes. The serotypes *a*, *d*, and *g* synthesize primarily insoluble and soluble α-D-glucans, and the types *b*, *c*, *e*, and *f* soluble α-D-glucans (a major product) and β-D-fructans. We have recently demonstrated that *S. mutans* serotype could be divided into four subgroups (serotype *a*; types *d* and *g*; type *b*; and types *c*, *e* and *f*) on the basis of the synthesis and properties of the extracellular insoluble α-D-glucans[4,5]. This is consistent with the grouping of serotypes previously suggested by comparisons of the serologic, genetic, biochemical, and phenotypic properties of the streptococcus[2].

Over the past decade, many laboratories have attempted to purify the enzymes involved in the synthesis of the streptococcal polysaccharides[6,7]. Extracellular GTases produced by several strains, mostly of the serotypes *a*, *d*, and *g* strains, have been resolved, by various techniques, into two major, catalytic components, one mediating the synthesis of insoluble α-D-glucans and another catalyzing the synthesis of soluble α-D-glucans. Some of the purified GTase components have been characterized in detail. However, knowledge of the polysaccharide-synthesizing enzymes from serotypes *c*, *e*, and *f* is rather limited so far[8–11].

We describe herein the isolation and some properties of the GTase and FTase of *S. mutans* strains IB (*c*), B14 (*e*), and OMZ175 (*f*), and the characterization of the products of enzymic activity.

EXPERIMENTAL

Preparation of extracellular crude enzymes[12]. — *S. mutans* strains IB (*c*), B14 (*e*), and OMZ175 (*f*) were cultured for 24 h at 37° in BHI broth (BBL Microbiology System, Cockeysville, MD 21030). The cells were removed by centrifugation at 15 000 *g* for 10 min at 4°, and the supernatant solution was precipitated with $(NH_4)_2SO_4$ at 50% saturation for 20 h at 4°. The pellets obtained by centrifugation were dialyzed against mM phosphate buffer (pH 6.0).

Purification procedures. — The crude enzyme (60 mL) was equilibrated with a ten-fold diluted equilibration buffer (2.5mM histidine · HCl, pH 6.2) and applied to a column (0.9 × 25 cm) of Polybuffer exchanger PBE94 (Pharmacia Fine Chemicals, S-751 04 Uppsala, Sweden), equilibrated with 25mM histidine · HCl buffer (pH 6.2). The column was washed with ten-fold diluted Polybuffer 74, previously adjusted to pH 4.0 with HCl, and subsequently with a linear gradient of 0 to 1.0M NaCl solution at a flow rate of 70 mL/h. The active fractions obtained by chromatofocusing were pooled for each peak, and precipitated with 80% saturated $(NH_4)_2SO_4$. After dialysis against mM phosphate buffer (pH 6.8), the enzyme (5–10

mL) was applied to a hydroxyapatite column (0.9 × 25 cm), equilibrated with mM phosphate buffer (pH 6.8), and eluted with a linear gradient of 1 to 200–300mM phosphate buffer (pH 6.8) at a flow rate of 70 mL/h.

Assay for enzyme activity. — The enzyme was treated with 10mM sucrose containing 3.7 kBq [U-C^{14}]sucrose (133 MBq/mmol; New England Nuclear, Boston, MA 02118) in the presence of 0.02% Dextran T10 (M_r 10 000; Pharmacia Fine Chemicals) in 0.1M phosphate buffer (20 μL, pH 6.0). After incubation for 30 min at 37°, the radioactivity incorporated into the polysaccharides was estimated by the filter paper method[13]. For the differential quantitative determination of GTase and FTase activities, 1.9 kBq of D-[U-^{14}C]glucose–sucrose (12.1 GBq/mmol) or D-[U-^{14}C]fructose–sucrose (11.7 GBq/mmol) was used instead of [U-^{14}C]sucrose. One unit of enzyme activity is defined as the amount of enzyme that transforms 1 μmol of sucrose into polysaccharides per min under the conditions defined. For the determination of optimum pH, the enzymic reaction was performed in a half-strength McIlvain (50mM citric acid–0.1M Na_2HPO_4) buffer (pH 4.5–7.5) in the presence (for GTase activity) or absence (for FTase activity) of 0.01% Dextran T10.

Determination of K_m *value.* — The substrate-saturation kinetics of the purified transferases were determined by the method of Lineweaver and Burk[14]. The enzyme (1.4–2 mU) was treated with 0.8–50mM sucrose containing 3.7 kBq of [U-^{14}C]sucrose in 0.1M phosphate buffer (pH 6.0) at 37°. After an incubation of 30–60 min, the amount of D-glucose or D-fructose polymerized was estimated as described earlier.

$NaDodSO_4$–polyacrylamide-gel electrophoresis (SDS–PAGE). — The enzyme proteins (~3–20 μg) were treated with 1% $NaDodSO_4$ in the presence of 20% glycerol and 1% 2-mercaptoethanol in 10mM Tris · HCl buffer (pH 6.8) for 30 min at 37°, and then examined by SDS–PAGE (7.5% gel concentration) at a constant current of 10 mA/slab for 12 h at 4° in the buffer system of Laemmli[15]. The gel was incubated with 5% sucrose in 50mM phosphate buffer (pH 6.0) containing 1% Tween 80 for 24–48 h at 37°. α-D-Glucans formed were examined macroscopically and stained with the periodic acid–Schiff base reagent[16]. To estimate the relative molecular mass of the transferase components, the proteins were stained with Coomassie Brilliant Blue. Heat-stable RNA-polymerase B, having 5 subunits with M_r as follows: β′ (180 000), β (140 000), X (100 000), α (42 000), and Z (39 000) (SDS–PAGE Marker I, Seikagaku Kogyo Co. Ltd. Chuo-ku, Tokyo, Japan) was used as standard.

Isoelectric focusing. — Analytical isoelectric-focusing was performed on a 1% Agarose-gel slab with Pharmalyte (pH 3–10; Pharmacia Fine Chemicals) containing 12% sorbitol. Aliquots (14–38 mU) of the enzymes were applied on pieces of glass-fibre filter paper placed on the gel surface, and the proteins focused at 15 W for 1.5 h at 15–18°. The pH of the gel was measured with a pI calibration kit (pH range, 3–10; Pharmacia Fine Chemicals). To locate the enzyme activities, the gel was incubated, after the focusing, for 10 h at 37° in 5% sucrose in 0.1M phosphate

buffer (pH 6.0) containing 0.02% Merthiolate and stained with the periodic acid–Schiff reagent[16].

Characterization of the products of enzymic activity. — The polysaccharides were synthesized by incubating 5% sucrose in 0.1M phosphate buffer (pH 6.0) with GTase or FTase for 4 days at 37° and harvested by precipitation with 70% (v/v) ethanol. The α-D-glucan and β-D-fructan in the products were quantitatively determined by the anthrone methods of Halhoul and Kleinberg[17].

The α-D-glucans (1–5 mg) were methylated by the method of Hakomori[18], hydrolyzed with 90% formic acid for 6 h at 100°, and subsequently with 2M trifluoroacetic acid for 6 h at 100°. The methylated β-D-fructans were hydrolyzed first with 90% formic acid for 10 min at 100°, and then with 9% formic acid for 3–5 h at 100°. The methylated derivatives were analyzed as alditol acetates by g.l.c.[19].

The inulin or levan-type of the β-D-fructans was determined by the agar gel-diffusion technique[20] in 1% Noble agar (Difco Laboratories, Detroit, MI 48232) containing 0.1M phosphate-buffered (pH 7.2) 0.15M NaCl. Concanavalin A (3 mg/mL; Sigma Chemical Co., St. Louis, MO 63178) was placed in the center well and treated for 16 h at 25° with the D-fructans (10 mg/mL) placed in the peripheral wells located radially 8 mm from the center well. Inulin and levan, used as references, were purchased from Wako Pure Chemical Industries Ltd. (Higashi-ku, Osaka, Japan) and Sigma Chemical Co., respectively.

The β-D-fructans were also characterized by paper-chromatography analysis of the partial acid hydrolyzates of samples[21]. The β-D-fructan (5 mg/mL) was hydrolyzed with 10mM H_2SO_4 for 20 min at 70°, portions (~50 μg) of the hydrolyzate were deposited onto Whatman No. 1 paper (20 × 20 cm), and the paper was developed 4 times with 6:4:3 (v/v) 1-butanol–pyridine–water for 3 h at room temperature in ascending chromatography. The spots were made visible with the silver nitrate spray reagent and their R_F values determined.

Cell-agglutinating activity. — The ability of the α-D-glucans to agglutinate *S. mutans* AHT-k (serotype *g*) cells was determined as described previously[22]. The soluble α-D-glucan produced by the *S. mutans* OMZ176 (serotype *d*) GTase-S was prepared as described previously[12]. The highest serial, two-fold dilution of the α-D-glucan solutions (1 mg/mL) to give a significant (+) cellular agglutination was determined.

RESULTS

Preparation of GTase and FTase. — All the crude enzymes from *S. mutans* IB (*c*), B14 (*e*), and OMZ175 (*f*) showed both GTase and FTase activity (Table I). Similar chromatographic separation patterns were obtained for the extracellular GTases and FTases from the serotypes *c*, *e*, and *f* strains. The chromatofocusing method separated GTase from FTase. GTase (CF-I) was eluted as a single peak, at ~pH 4.9, by irrigation with Polybuffer 74, and FTase (CF-II) as a broad peak by subsequent irrigation with 0–1M NaCl gradient [see Fig. 1 for OMZ175 (*f*) strain].

TABLE I

PURIFICATION OF THE EXTRACELLULAR GTASE AND FTASE OF *S. mutans* SEROTYPE *c*, *e*, AND *f* STRAINS

Purification step	*Specific activity (U/mg of protein[a])*	*Recovery (%)*	*Purification (fold)*	*Activation by Dextran T10 (fold)*	*Ratio of α-D-glucan to β-D-fructan[b]*
S. mutans IB (*c*)					
Culture supernatant	0.005	100	1	1.5	3:2
$(NH_4)_2SO_4$ precipitation	0.08	47	18	1.9	51:49
Chromatofocusing					
CF-I	0.29	16	60	7.4	99:1
CF-II	0.07	11	14	1.3	19:81
Hydroxyapatite column					
CF-I-HA	7.8	6	1615	10.9	99:1
CF-II-HA	0.7	7	140	1.2	3:42
S. mutans B14 (*e*)					
Culture supernatant	0.006	100	1	1.5	1:1
$(NH_4)_2SO_4$ precipitation	0.17	45	29	1.5	23:27
Chromatofocusing					
CF-I	0.40	13	69	4.4	99:1
CF-II	0.17	8	29	1.0	2:23
Hydroxyapatite column					
CF-I-HA	5.4	4	918	7.6	100:0
CF-II-HA	0.80	4	134	1.0	1:19
S. mutans OMZ175 (*f*)					
Culture supernatant	0.001	100	1	1.5	7:18
$(NH_4)_2SO_4$ precipitation	0.26	49	26	1.4	31:69
Chromatofocusing					
CF-I	0.54	15	54	9.8	49:1
CF-II	0.23	13	23	1.1	1:19
Hydroxyapatite column					
CF-I-HA	7.1	3	710	11.2	97:3
CF-II-HA	1.2	5	122	1.0	1:99

[a][U-^{14}C]sucrose was used for the determination of enzyme activity. [b]D-Glucose–[U-^{14}C]sucrose and D-fructose–[U-^{14}C]sucrose were used to differentiate quantitatively the α-D-glucan and β-D-fructan synthesized.

The GTase and FTase were further purified by hydroxyapatite column chromatography: GTase (CF-I-HA) was eluted at a phosphate concentration of 0.21M and FTase (CF-II-HA) of 0.12M [see Fig. 2 for OMZ175 (*f*) strain]. The purified GTase and FTase produced almost exclusively α-D-glucans and β-D-fructans, respectively (Table I). The purification of the transferases of the strains IB (*c*), B14 (*e*), and OMZ175 (*f*) is summarized in Table I. The GTases were purified 710–1615-fold with an overall yield of 3–6%, and the FTases 122–140-fold with an overall yield of 4–7%.

Properties of GTases and their products. — All the purified GTases exhibited

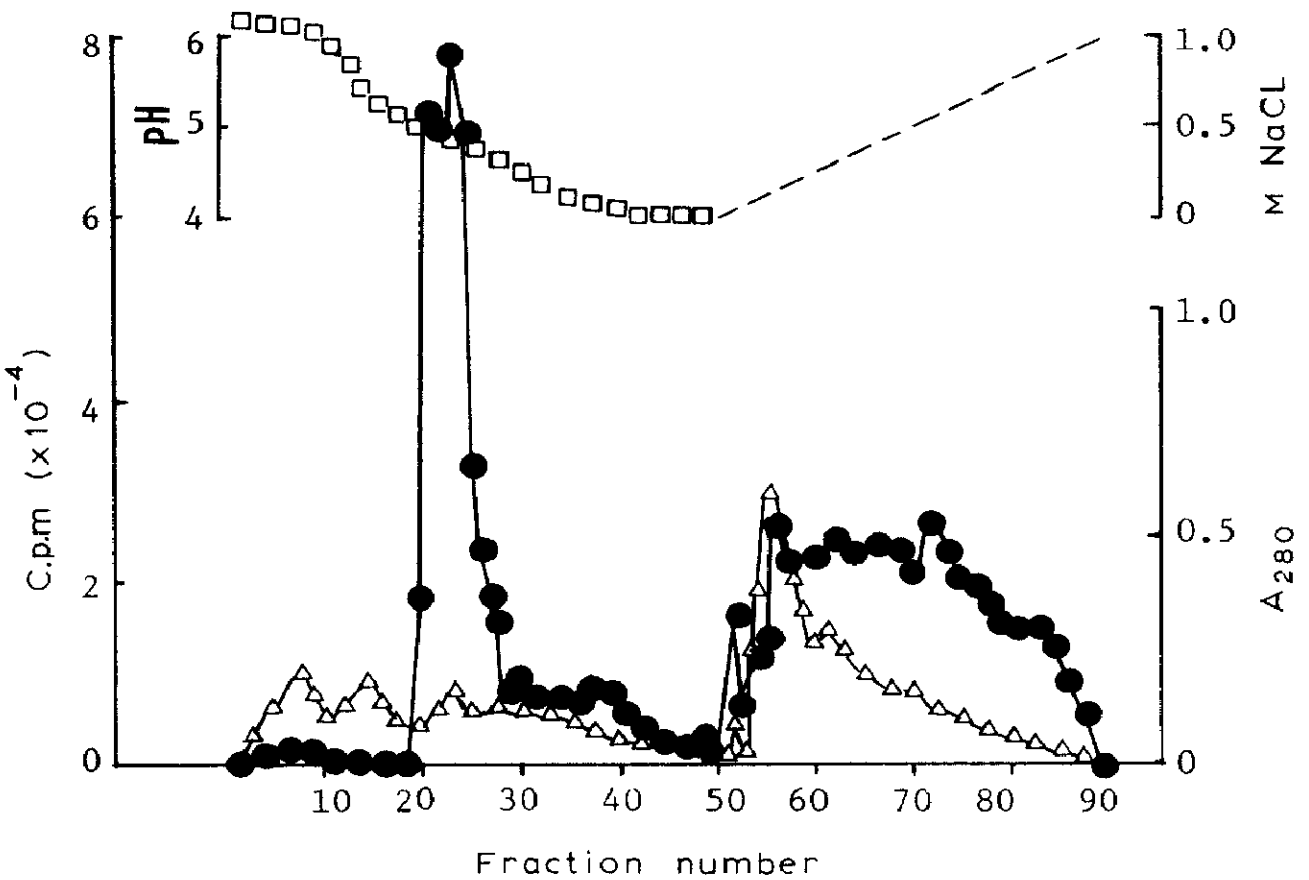
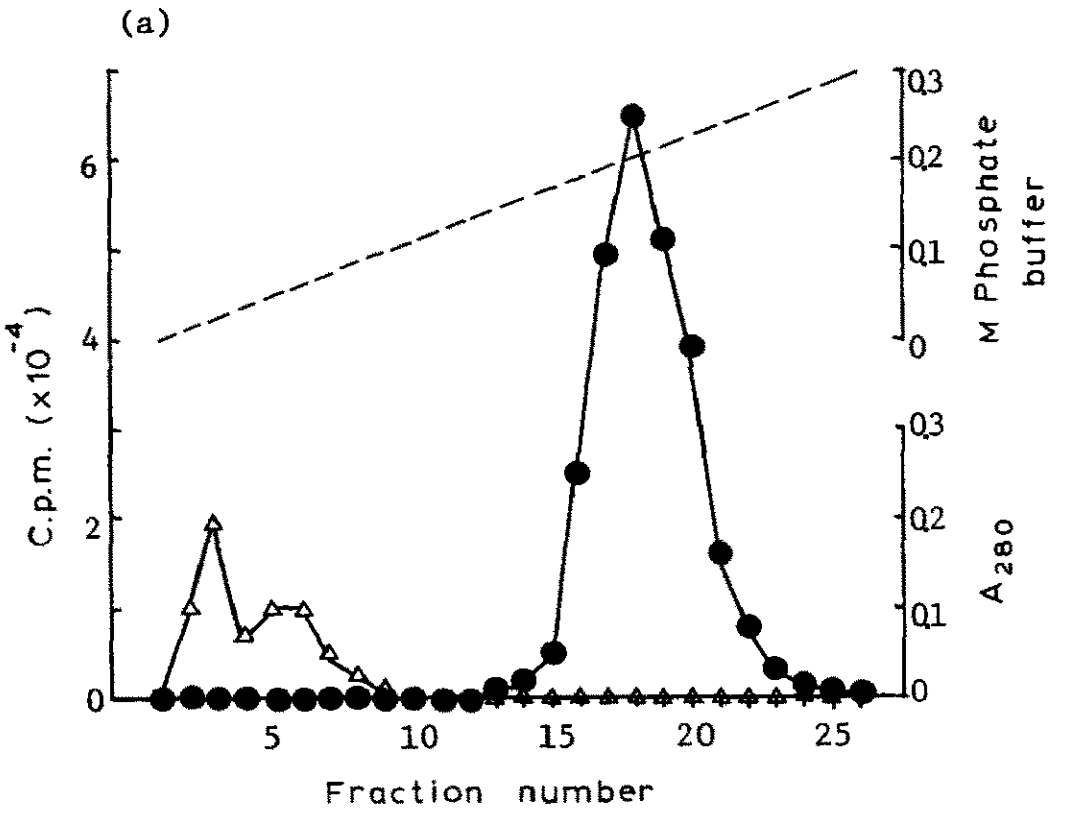


Fig. 1. Chromatofocusing of the crude enzyme excreted by *S. mutans* OMZ175 (*f*), at a flow rate of 70 mL/h and a fraction volume of 10 mL: (●—●—●), activity (c.p m. × 10^{-4}); (△—△—△), absorbance at 280 nm; (□), pH; and (-----), molarity of NaCl. Fractions 20–30, CF-I; Fractions 56–85, CF-II.

a maximal activity at pH 5.5–6.0. Significant activity (>50%) was detected at pH 4.5, but not at pH 7.5. The isoelectric point of the enzymes was ~pH 7.4 for all the GTases. The Lineweaver–Burk plots indicated K_m values for sucrose of 11, 10, 14mM for the strains IB (*c*), B14 (*e*), and OMZ175 (*f*), respectively. All the GTases were activated about 10-fold by the presence of Dextran T10 (Table I).

On SDS–PAGE the GTases showed a single or multiple protein bands after mild $NaDodSO_4$ treatment. On incubation with 5% sucrose, opaque, periodic acid–Schiff reactive, swollen α-D-glucan bands appeared at the positions corresponding to one or two of the protein bands (Fig. 3), indicating that the strains IB (*c*) and B14 (*e*) enzymes still contained a few inactive proteins. The M_r values of the enzyme components were estimated to be ~173 000 and 158 000 for each of the strains IB (*c*) and B14 (*e*), and 156 000 for the strain OMZ175 (*f*).

Consistent with the macroscopic appearances of the bands in PAGE gels, the α-D-glucans, produced by the purified GTases from the serotypes *c*, *e*, and *f* strains, were turbid but soluble in water. However, methylation analysis (Table II) revealed that the chemical structure of the α-D-glucan from strain IB (*c*) was very different to the structures of the α-D-glucans from the other two strains, B14 (*e*) and OMZ175 (*f*). The soluble α-D-glucans synthesized by the GTases of strains B14 (*e*) and OMZ175 (*f*) primarily consisted of α-(1→6)- (65–66%) and α-(1→3,6)-linked D-glucose residues (10.5–20%), other linkages including α-(1→3) being virtually absent. In contrast, the α-D-glucan synthesized by the strain IB (*c*) GTase contained significant proportions of α-(1→3)- (10.5%), α-(1→4)- (6.5%), and α-



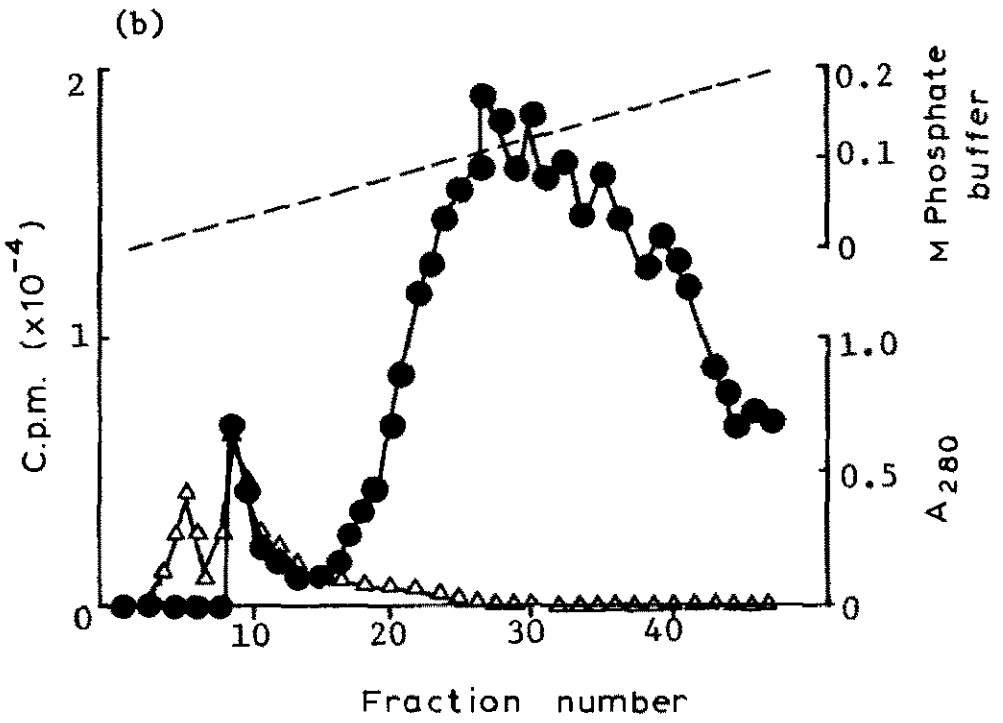


Fig. 2. Hydroxyapatite chromatography of Fractions CF-I (a) and CF-II (b) of the chromatofocusing of the enzyme from *S. mutans* OMZ175 (*f*) (Fig. 1), at a flow rate of 70 mL/h and a fraction volume of 12 mL: (●—●—●) activity (c.p.m. × 10^{-4}); (△—△—△) adsorbance at 280 nm; and (------) molarity of phosphate buffer (pH 6.8). (a) Fractions 16–21, CF-I-HA; (b) Fractions 22–43, CF-II-HA.

(1→3,4)- (14%), in addition to α-(1→6)- (41.5%) and α-(1→3,6)- (6%) linked D-glucose residues.

The soluble α-D-glucans produced by the GTases of the serotypes *c*, *e*, and *f* strains showed *S. mutans* cells-agglutinating activity. The minimal concentration to give significant agglutination (+) was 0.78, 6.25, and 3.13 μg/mL for the α-D-glucans of the strains IB (*c*), B14 (*e*) and OMZ175 (*f*), respectively. These values are

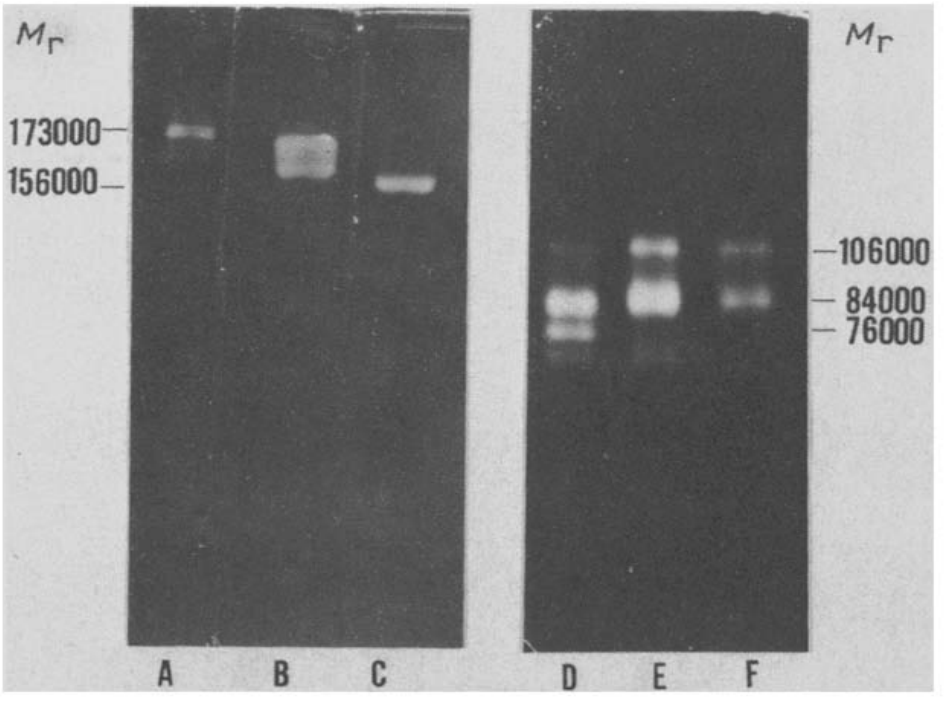


Fig. 3. SDS-PAGE of the purified GTase (CF-I-HA) and FTase (CF-II-HA). The GTases from *S mutans* IB (*c*, 19mU), B14 (*e*, 38mU), and OMZ175 (*f*, 20mU) were applied to the gels A, B, and C, respectively. The FTases from the strains IB (*c*, 14mU), B14 (*e*, 16mU), and OMZ175 (*f*, 15mU) were applied to the gels D, E, and F, respectively After electrophoresis (see Experimental section), the gels were incubated with 5% sucrose for 20 h at 37°.

higher than that of 0.195 μg/mL given by the soluble α-D-glucans[12] of strain OMZ176 (*d*) and by Dextran T250.

Properties of FTases and their products. — The FTases of all the strains showed maximal activity at pH ~5.5–6.0, and were virtually inactive at pH 4.5 and 7.5. None of the FTases was activated by Dextran T10. All the enzymes possessed similar pI values, ~4.6. The K_m values for sucrose were 6, 10, and 17mM for the enzymes for strains IB (*c*), B14 (*e*), and OMZ175 (*f*), respectively.

SDS–PAGE separated the FTase of each strain into two components. Both synthesized opaque periodic acid–Schiff-reactive β-D-fructans on incubation with sucrose. The M_r values of the enzyme components were shown to be 106 000 and 84 000 for the strains B14 (*e*) and OMZ175 (*f*), and 84 000 and 76 000 for the strain IB (*c*) (Fig. 3).

The β-D-fructans produced by the purified FTases from the strains were all water soluble. The agar-gel, double-diffusion technique (Fig. 4) showed that the streptococcal β-D-fructans did not form precipitation lines with concanavalin A, which reacts specifically with levan. On paper chromatography, the oligosaccharides, obtained by partial hydrolysis of the β-D-fructans, showed R_F values identical with those of the inulin hydrolyzates (Fig. 5). However, the OMZ175 (*f*) β-D-fructan was the most susceptible to acid hydrolysis and the IB (*c*) β-D-fructan the most resistant. Similarly, preliminary g.l.c. analysis of the methylated D-fructans revealed that the OMZ175 (*f*) β-D-fructan yielded one major (90%) peak that corresponded to 3,4,6- (or 1,3,4)-tri-*O*-methyl-β-D-fructose, whereas the β-D-fructans of the strains IB (*c*) and B14 (*e*) gave two closely adjacent peaks, one being that just mentioned (not shown).

TABLE II

METHYLATION ANALYSIS OF THE D-GLUCANS PRODUCED BY THE PURIFIED GTASES FROM *S. mutans*.

D-*Glucans*	*Retention time*[a]	*Linkage* (α-D-*1→*)	*Relative proportion (mol/100 mol)*
Dextran	1	Nonreducing group	4.6
	1.38	3	0.9
	1.48	6	86.9
	1.87	3,6	7.6
Glycogen	1	Nonreducing group	6.8
	1.43	?	4.7
	1.48	6	1.9
	1.54	4	77.4
	1.96	4,6	9.3
S. mutans IB (*c*)	1	Nonreducing group	21.3
	1.33	3	10.6
	1.48	6	41.3
	1.53	4	6.5
	1.73	3,4	14.0
	1.87	3,6	6.2
S. mutans B14 (*e*)	1	Nonreducing group	11.8
	1.34	3	1.7
	1.42	?	1.5
	1.48	6	65.2
	1.87	3,6	19.8
S. mutans OMZ175 (*f*)	1	Nonreducing group	19.7
	1.33	3	3.7
	1.48	6	65.9
	1.87	3,6	10.6

[a]Relative to that of 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucose-D-glucitol.

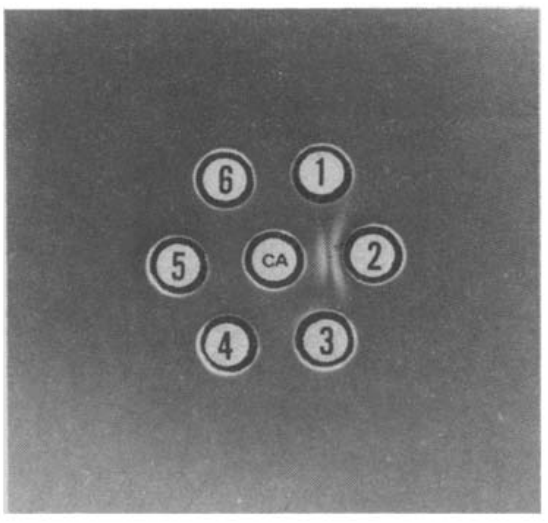


Fig. 4. Reactivity of the streptococcal β-D-fructans with concanavalin A in agar-gel diffusion. Center well: concanavalin A. Peripheral wells: 1, inulin; 2, levan; 3, D-fructan synthesized by the IB (*c*) FTase; 4, D-fructan synthesized by the B14 (*e*) FTase; 5, D-fructan synthesized by the OMZ175 (*f*) FTase; and 6, distilled water.

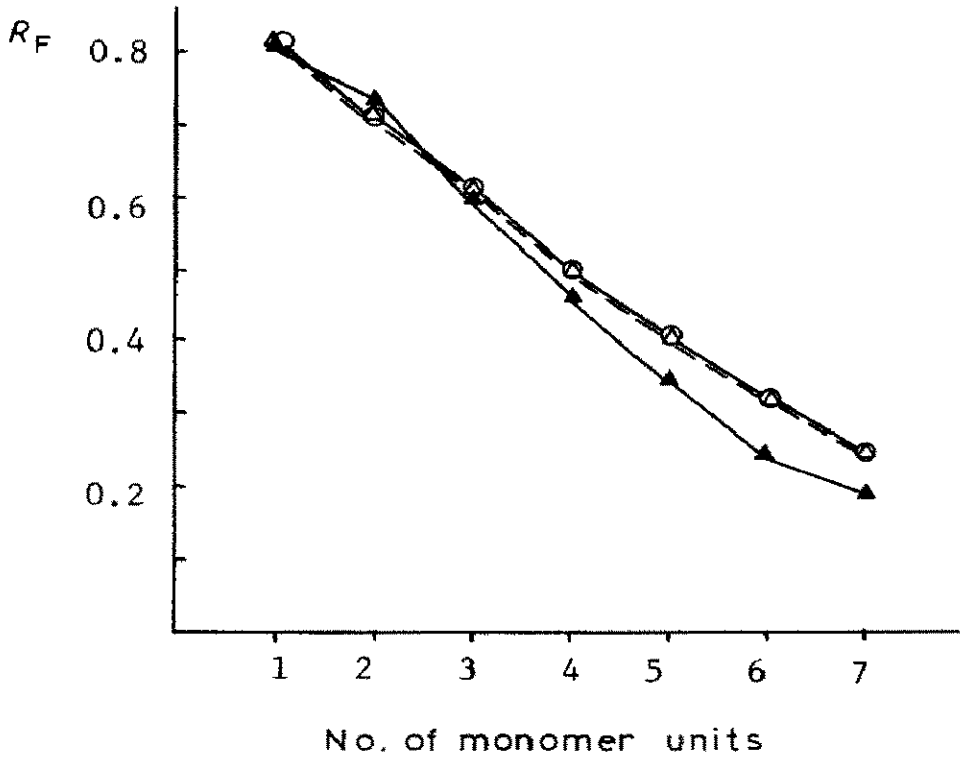


Fig. 5. Ascending paper chromatogram of the partial acid hydrolyzates of D-fructans: (△—△) inulin; (▲—▲) levan; (○—○) D-fructan synthesized by the OMZ175 (*f*) FTase.

DISCUSSION

We have previously demonstrated that the extracellular enzymes of *S. mutans* serotypes *c*, *e*, and *f* produce primarily water-soluble α-D-glucans and β-D-fructans, but little insoluble α-D-glucans[4]. Application of the same purification procedures separated (Table I) the soluble α-D-glucan-synthesizing enzyme (GTase-S) from the soluble β-D-fructan-synthesizing enzyme (FTase-S) of the strains IB (*c*), B14 (*e*), and OMZ175 (*f*). An insoluble α-D-glucan-synthesizing activity (GTase-I), previously detected in the crude preparations[4], was lost during purification. The GTases of the 3 serotypes possessed similar properties with respect to pH dependency of reaction, K_m value for sucrose, isoelectric point, periodic acid–Schiff-reactivity, and production of opaque α-D-glucans. A preliminary study demonstrated that the enzyme preparations were immunologically identical.

Some differences were observed among the enzymes from these serotypes. The GTases of the serotypes *c*, *e*, and *f* consisted of isozyme components having different M_r values (Fig. 3). A most striking difference was the presence, in the soluble α-D-glucan produced by the purified GTase of strain IB (*c*), of significant proportions of α-D-(1→4)- and α-D-(1→4,6)-linked residues, which were not detected in the α-D-glucans produced by the serotypes *e* and *f* enzymes (Table II). Earlier studies[23,24] had reported the presence of α-D-(1→4) linkages in the α-D-glucans of some *S. mutans* strains, including IB (*c*). In contrast, our previous study[4] had shown the absence of α-D-(1→4) and α-D-(1→4,6) linkages in the soluble α-D-glucan fraction produced by the crude enzyme of the IB (*c*) strain used in the present study. In addition, the presence of the α-D-(1→4) linkage in the soluble

α-D-glucan synthesized by the isolated GTase from another stam of this same strain was not reported[11]. The reason for these discrepancies is not known.

The M_r values (151 000–173 000), optimum pH values (pH 5.5–6.5), and K_m values for sucrose (4.3–14.0mM) of the GTases-S from the serotypes *c* (IB; ref. 11 and this paper), *e* (B14; this paper), and *f* (OMZ175; this paper) are similar to the values (150 000–174 000; pH 5.4–5.5; 1.3–2.4mM) of those from the serotypes *a* (HS6; ref. 25), *d* (OMZ176; ref. 12), and *g* (6715; ref. 26). The soluble α-D-glucans produced by the enzymes from serotypes *a* (HS6), *d* (OMZ176), *g* (6715), *c* (IB), *e* (B14), and *f* (OMZ175) were highly-branched, periodic acid–Schiff-reactive α-D-(1→6)-glucans. It was also shown that the purified GTase-S of strain GS-5 (*c*), as well as that of strain OMZ176 (*d*), can synthesize insoluble D-glucans in the presence of appropriate amounts of NH_4^+ ion[27]. However, the GTases-S of serotypes *c* (IB, GS-5; ref. 28), *e* (B14), and *f* (OMZ175) are basic proteins (pI 7.4–8.4), whereas those of serotypes *a* (HS6) and *g* (6715) are acidic proteins (pI 4.1–4.5). In addition, the GTases-S of serotypes *c* (IB, GS-5), *e* (B14), and *f* (OMZ175) were highly activated by the presence of exogenous, soluble dextrans, but the soluble α-D-glucan-synthesizing components of serotypes *a* (HS6) and *d* (OMZ176, B13; ref. 28) were not. The soluble α-D-glucans produced by serotypes *c, e,* and *f* possess lower *S. mutans* cell-agglutinating activities than the α-D-glucans of serotype *d* (this paper). Furthermore, our preliminary study has demonstrated that the GTases-S of serotypes *c, e,* and *f* are immunologically related to each other[10], but unrelated to those of serotypes[5], *a, d,* and (or) *g*. Thus, the GTases-S of serotypes *a, d,* and *g*, and those of serotypes *c, e,* and *f* show some similarities and some differences in their properties.

The synthesis of an adherent, insoluble α-D-glucan by *S. mutans* OMZ176 (*d*) and B13 (*d*) is mediated by the concerted action of two GTases, an insoluble α-D-(1→3)-glucan and a branched, soluble α-D-(1→6)-glucan-synthesizing enzyme[29,30]. It was also suggested[31] that, in addition to these two GTases, a third GTase, *i.e.*, a linear α-D-(1→6)-glucan-synthesizing enzyme, may be involved in the production of an adherent α-D-glucan by *S. mutans* serotype *g* (6715, K1-R, OMZ65). It is of interest to know by which alternative system the adherent, insoluble α-D-glucan is synthesized by serotypes *c, e* and *f* strains; unfortunately, a purified GTase-I has not yet been obtained.

The *S. mutans* serotypes *c, e,* and *f*, as well as the serotype *b*, possess a significant ability to produce β-D-fructans[4,5,32]. Studies on the FTases of these serotypes, particularly of the serotypes *e* and *f*, are rather limited[8,11]. A recent, comparative study[5] demonstrated that the extracellular FTases of the serotypes *b, c, e* and *f* are acid proteins. As shown in this paper, the purified FTases from strains IB (*c*), B14 (*e*), and OMZ175 (*f*) show similar properties with respect to optimum pH, K_m value for sucrose, and isoelectric point, and all the β-D-fructans produced by the FTases were of the inulin type (Figs. 4 and 5). This result is consistent with the previous results of the chemical analysis of β-D-fructans produced by extracellular, crude enzymes from the serotypes *b* and *e* strains[33,34]. Although, (a) slight de-

viations in the molecular-size distribution of the isozymes were observed (Fig. 3), and (b) it is probable that the inulin-type β-D-fructans produced have somewhat different chemical structures (as suggested by the different susceptibilities to acid hydrolysis and g.l.c. patterns of the methylated derivatives), it is concluded that the FTases of the *S. mutans* serotype *c*, *e* and *f* are acid inulosucrases (EC 2.4.1.9) possessing largely similar properties. It should be noted, here, that the β-D-fructans produced from raffinose by *S. mutans* OMZ176 (*d*) is of the levan type[21].

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