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Effect of Dextranase on the Extracellular Polysaccharide Synthesis of *Streptococcus mutans*: Chemical and Scanning Electron Microscopy Studies

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A dextranase preparation (AD17) partially purified from a culture liquor of *Spicaria violacea* strain IFO 6120 significantly inhibited the formation of artificial dental plaque on a steel wire or on an extracted tooth surface. Changes in the surface morphology of *Streptococcus mutans* cells due to AD17 action were studied using scanning electron microscopy. *S. mutans* cells grown in 5% sucrose-containing broth were coated with sticky amorphous capsule-like material, whereas cells grown in sucrose in the presence of AD17 or in glucose instead of sucrose did not synthesize such capsular material. AD17 degraded commercially available dextrans of molecular weight 7×10^4 and 2×10^6 to liberate glucose and various oligosaccharides, including isomaltose. On the other hand, AD17 hydrolyzed the extracellular polysaccharides (mainly glucan in nature) of some strains of *S. mutans* to a limited degree. Only 15 to 36% of the total polysaccharides were hydrolyzed by AD17 with little release of isomaltose. Prolonged incubation of the polysaccharides from *S. mutans* with AD17 did not release additional reducing sugars, which indicates that AD17 did not contain α-1,3-glucanase activity. These results suggest that glucosidic linkages which are susceptible to AD17 may play an important role in the adherence of *S. mutans* cells to smooth surfaces.

Special attention has been focused on the production of insoluble extracellular polysaccharides from sucrose by *Streptococcus mutans* (10, 14). Synthesis of insoluble polysaccharides has been considered a virulence factor which is responsible for the cariogenicity of *S. mutans* (6, 36). The polysaccharides are mainly composed of a glucan; however, some strains of *S. mutans* also produce variable amounts of fructan (10, 24, 31). The water-insoluble glucan has been shown to be predominantly α-1,3-linked (3, 11, 20). On the other hand, water-soluble glucan which is also synthesized by *S. mutans* is exclusively linear α-1,6-linked and appears essentially identical to dextran produced by *Leuconostoc* sp. (9).

Although some research workers stress the importance of the water-insoluble glucan, the relationship of water insolubility and stickiness (or adhesive properties) of the glucan has not been clarified (10, 11, 13).

We found that a dextranase preparation from *Spicaria violacea* strain IFO 6120 inhibited the development of dental caries in rats (unpublished data) and hamsters (S. Hamada et al., submitted for publication), but the dextranase degraded the insoluble glucans to a limited extent in in vitro experiments. This paper describes the effects of dextranase on the extracellular glucans from *S. mutans* and the morphological changes of *S. mutans* cells resulting from dextranase.

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MATERIALS AND METHODS

Enzyme preparation. Dextranase (EC 3.2.1.11; α-1,6-glucan 6-glucanohydrolase) lot AD17 was partially purified from a culture supernatant of *S. violacea* strain IFO 6120 as previously described (26). The reaction mixture for the determination of dextranase activity consisted of 1.0 ml of 1% dextran (clinical dextran, Meito Co., Nagoya, Japan), 400 μl of 0.1 M acetate buffer, pH 5.8, and 100 μl of enzyme solution. After incubation at 37 C for 30 min, the release of the reducing sugars was determined. One dextranase unit was defined as that amount of enzyme which liberates 1 μmol of reducing sugar per min. The activity of AD17 preparation was estimated to be about 500 U per mg of dry weight preparation. One unit by this method is equivalent to 7.4 Tsuchiya units (37).

Bacterial strains. The strain designation and origin of the organisms used in this study are listed in Table 1. Stab cultures were maintained by monthly transfer in Trypticase soy (TS) agar medium (BBL). The MT-labeled strains of *S. mutans* were isolated from dental plaque of children at Osaka University Dental Hospital, Osaka, Japan. The cariogenic potential of strains MT6R, MT615R, and MT703R has been confirmed in conventional Sprague-Dawley rats fed high sucrose diet 2000 (unpublished data). data).

Inhibition of artificial dental plaque with dextranase AD17. Artificial dental plaques on steel wires were formed according to a modified method of McCabe et al. (22) and Fitzgerald et al. (8). Orthodontic wires (0.8 by 90 mm, Sankin Kogyo, Osaka) inserted in silicone stoppers were suspended in test tubes (10 by 100 mm) containing 4 ml of TS broth containing 0.5% yeast extract (Difco) and 5% sucrose (TSYS broth). One-tenth milliliter of filter-sterilized AD17 solution (400 U/ml) was added in the test groups. Control groups received an equal amount of heat-inactivated (100 C, 10 min) AD17 solution or no addition of AD17 solution. A fresh TS broth was inoculated with 0.1 ml of an overnight culture of the test strain, and the wire was transferred each day for 5 successive days. The artificial plaque formed was fixed by suspending the wires in 10% formalin solution and scored from − to 4+.

Removal of preformed plaque with AD17. The artificial plaque was formed as described above, and the wire was suspended in 0.1 N acetate buffer, pH 5.8, containing 10 or 50 U of AD17 per ml. After overnight incubation at 37 C, visual inspections were done of all samples. The test tubes arranged on the same rack were gently shaken for 5 s, and the residual plaque attached to the wire surface was scored as described above.

Scanning electron microscopic observations of S. mutans cells. Cells were grown in TS broth (group 1), TS broth containing 5% sucrose (TSS, group 2), and TSS containing filter-sterilized AD17 (final concentration 10 U/ml of broth, group 3) for 24 to 48 h at 37 C. After washing with saline and water, cells were suspended with water. One drop of the suspension was smeared on a clean cover slip and air dried. Cells were fixed for 1 h with 2.5% glutaraldehyde in 50 mM phosphate buffer, pH 7.0, at 0 C and washed in several changes of saline and water. The cover slip was air dried and coated with gold (ca. 10-nm thickness) in vacuo. The specimens were examined and photographed in a JEM scanning electron microscope (model SM2, Nihon Denshi, Tokyo).

Scanning electron microscopic observation of artificial plaque on the extracted tooth surface. Deciduous premolars were kept in cold saline after extraction because of orthodontic treatment. Teeth which had intact labiobuccal enamel surface were polished with pumice and zinc flowers, and cubes having approximately 5- by 5-mm intact enamel surfaces were made. The cubes were then drilled through the dentin, suspended with wire in 5 ml of saline, and autoclaved at 120 C for 15 min. The sample was transferred to TSS broth and/or TSS broth containing dextranase AD17 which had been inoculated with a test strain and incubated for a defined time. Samples were transferred daily to make artificial plaques on the enamel surface as described above. In other experiments, preformed plaques on the extracted tooth surface were treated with AD17 (50 U/ml). The samples were fixed in 2.5% phosphate-buffered glutaraldehyde for 3 h at

TABLE 1. *Strain designation*

Strain	Serotype[a]	Source	Obtained from:
Streptococcus mutans			
HS6	*a*	Hamster mouth	R. J. Fitzgerald
AHT	*a*	Human mouth	D. D. Zinner
FA1	*b*	Rat mouth	R. J. Fitzgerald
BHT	*b*	Human mouth	D. D. Zinner
DB13	b^{-b}	Mutant of FA1	D. Bratthall
MT6R[c]	*c*	Human mouth	Authors
MT118R	*c*	Human mouth	Authors
OMZ 176	*d*	Human mouth	B. Guggenheim
MT615R	*d*	Human mouth	Authors
LM7	*e*	Human mouth	R. J. Gibbons
MT703R	*e*	Human mouth	Authors
SE11	*f*	Human blood	B. Perch
OMZ 175	*f*	Human blood	B. Perch
K1R	*g*	Human mouth	H. Suginaka
S. salivarius			
SS2		Human mouth	R. J. Gibbons
HHT		Human mouth	D. D. Zinner

[a] Serotype determination of the MT-labeled strains isolated by us was carried out by fluorescent antibody technique and/or immunodiffusion test (S. Hamada et al., Jpn. J. Microbiol., in press). The determination of the other strains was followed according to the description of Perch et al. (29).

[b] A mutant of strain FA1 which has lost *b* antigenicity.

[c] R, Streptomycin-resistant strain.

0 C, washed with phosphate buffer and then water, and dried in vacuo. They were cemented onto the metal stubs using a metal-containing glue and then vapored with gold to a thickness of ca. 10 nm. If the blocks were heavily coated with plaques, they were precoated with carbon to make the coating with gold easier. The specimens were examined as described above.

Preparation of extracellular polysaccharides. Organisms were cultured in 100 ml of TSYS broth for 48 h at 37 C. Cells were removed by centrifugation at 10,000 × *g* for 30 min at 4 C, and 2 volumes of ethanol was then added to the supernatant. After standing overnight at 4 C, ethanol-insoluble precipitates were collected by centrifugation at 10,000 × *g* for 30 min. The precipitate was washed with 67% ethanol three times and dissolved in 50 ml of water. After the suspension was maximally vibrated with a sonifier (Tomy model UP 150P, Tomy Works, Tokyo) and incubated at 60 C for 30 min, it was centrifuged at 10,000 × *g* for 60 min. The supernatant was precipitated with 2 volumes of ethanol (fraction 1, water-soluble polysaccharide). The sediment was dissolved in 20 ml of 0.2 N potassium hydroxide, incubated at 60 C for 30 min, and centrifuged at 10,000 × *g* for 60 min. The supernatant was separated from the sediment and was precipitated by adding 2 volumes of ethanol (fraction 2, alkaline-soluble polysaccharide). The sediment was called fraction 3 (alkaline-insoluble polysaccharide). Fractions 1, 2, and 3 were washed with 67% ethanol three times and lyophilized. The cells were washed with saline two times, and the cell-associated polysaccharides were extracted with 20 ml of 0.2 N potassium hydroxide at 60 C for 30 min. The suspension was neutralized with 2 N HCl and was centrifuged at 10,000 × g for 60 min. Two volumes of ethanol was added to the supernatant, and the resultant precipitate was defined as fraction 4.

Synthesis of polysaccharides in the presence of dextranase AD17. To examine the effect of dextranase on polysaccharide synthesis, cells were grown in TSS and Todd Hewitt-5% sucrose (THS) broth with filter-sterilized dextranase AD17 at a final concentration of 10 U per ml. Control cells were cultured without dextranase. After incubation at 37 C for 48 h, the cultures were centrifuged to separate supernatant and cells. The supernatant was precipitated with 2 volumes of ethanol (fraction A). Cells were washed twice with saline and were extracted with 0.2 N potassium hydroxide as described above (fraction B). Both polysaccharides were washed with 67% ethanol three times and lyophilized. To make a quantitative comparison, both fractions A and B were suspended in defined volumes of distilled water and sonified to get an even suspension. The hexose and/or fructose contents were estimated by chemical analyses.

Degradation of polysaccharides with dextranase AD17. The actions of dextranase AD17 on the four polysaccharide fractions were determined according to the description of Murayama et al. (26). One milligram of each polysaccharide fraction in 0.1 N acetate buffer, pH 5.8, was added with 20 U of dextranase AD17 dissolved in the same buffer. After incubation at 37 C for 24 h, the release of reducing sugars was estimated. Sodium azide was added in a final concentration of 0.1% as a preservative.

Chemical analyses. Total hexoses were determined by the anthrone method (34) using glucose as a standard. Fructose was estimated according to the methods of Handel (15) and Roe (32). Reducing sugars were estimated as previously described (26) using glucose as a standard. Protein was determined with Folin phenol reagent (21). To identify the component sugars in the polysaccharides, samples were hydrolyzed with 1.5 N HCl at 100 C for 0.5, 1, 2, and 4 h for thin-layer chromatography. Thin-layer plates were made from Kieselgel G (Merck) dissolved in 0.02 M sodium acetate and from Kieselguhr G (Merck) in water. The former plate was developed twice with solvent consisting of acetone-water (2:1) (30). The latter was developed three times with the solvent of ethyl acetate-isopropanol-water (16:6:3) (4). Spots of the component sugars were located using diphenylamine-aniline reagent (1).

RESULTS

Effect of dextranase AD17 on the artificial plaque of S. mutans. As shown in Table 2, artificial dental plaque formations by *S. mutans* strains were significantly inhibited when dextranase AD17 was incorporated into the culture medium at a final concentration of 10 and/or 50 U per ml. Plaque formation by many strains including HS6 (serotype *a*), MT615R (*d*), and OMZ 176 (*d*) was almost completely prevented (Fig. 1a), but some of the strains were considerably resistant to the action of dextranase AD17 (Fig. 1b). The preformed plaques on wires were incubated overnight in 0.1 N acetate buffer, pH 5.8, or TSYS broth containing 10 U of dextranase AD17 per ml. No significant change in the plaque was observed macro-

TABLE 2. *Inhibition of artificial plaque formation on wires by S. mutans cells with dextranase AD17*

S. mutans strain	Degree of plaque deposition on wires	
	Control (no AD17)	10 or 50 U of AD17/ml
HS6	4+	−
AHT	4+	±
FA1	2+	−
BHT	3+	−
DB13	2+	−
MT6R	3+	±
MT118R	3+	±
OMZ 176	4+	−
MT615R	4+	−
LM7	4+	2+
MT703R	4+	±
SE11	4+	3+
OMZ 175	4+	2+
K1R	4+	−

scopically. However, the plaques of many strains detached from wire surface when the test tubes were gently agitated as compared with those of the control groups without the enzyme (Table 3).

Effect of dextranase AD17 on the cellular morphology of S. mutans and the artificial dental plaque. Figure 2 shows the scanning electron microscopic photograph of *S. mutans*

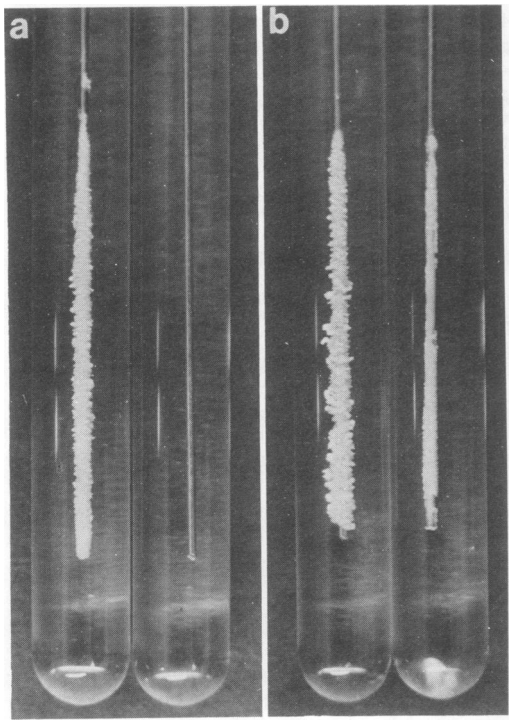

FIG. 1. *Inhibitory effect of dextranase on artificial plaque of S. mutans strains OMZ 176 (a) and LM7 (b). Each figure shows positive control plaque (left) and experimental plaque formed in the presence of 50 U of dextranase AD17 per ml of culture medium (right).*

TABLE 3. *Removal of preformed artificial plaque by S. mutans with dextranase AD17*

S. mutans strain	Residual plaque on wire		
	Control	Treated in acetate buffer with AD17[a]	Treated in TS broth with AD17[a]
MT6R	3+	−	±
MT118R	3+	+	+
OMZ 176	4+	4+	4+
MT615R	4+	2+	3+
LM7	4+	−	±
MT703R	4+	±	±

[a] 10 U of AD17/ml of buffer or broth.

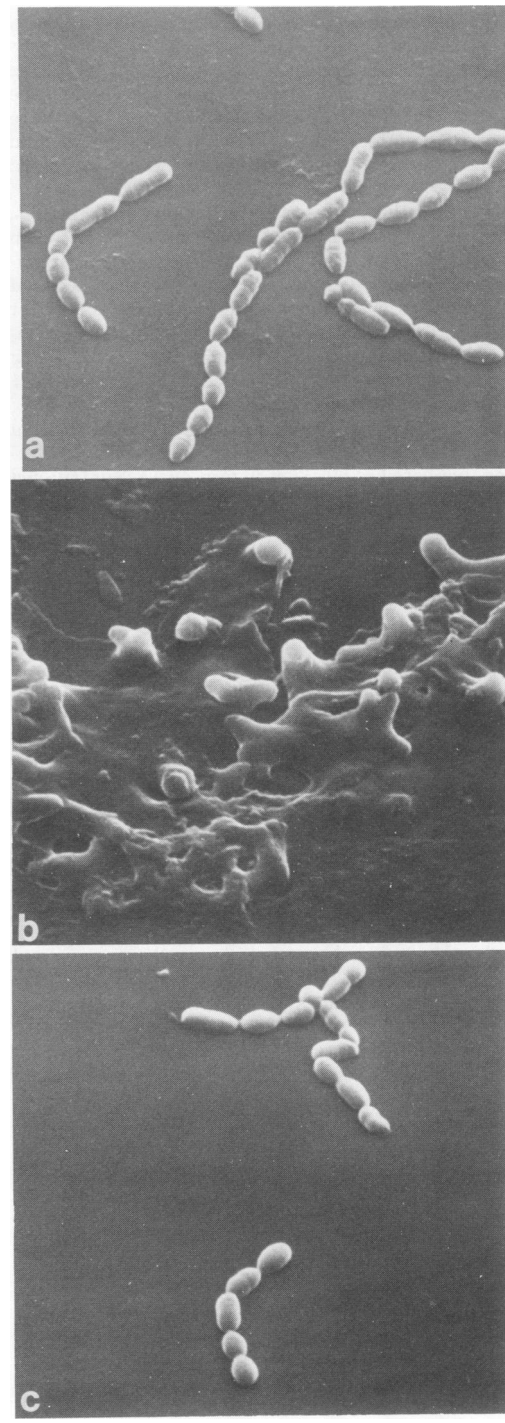

FIG. 2. *Scanning electron micrograph of S. mutans strain OMZ 176 grown in TS broth (a), TSS broth (b), and TSS broth containing dextranase (c). Addition of dextranase into sucrose-containing TSS medium prevented the formation of amorphous capsular material. ×6,800.*

strain OMZ 176 which had been cultivated under different conditions. TS-grown cells of *S. mutans* OMZ 176 show typical streptococcal morphology on the cover slip. Individual organisms could be well observed (Fig. 2a). On the other hand, if the cells were grown in TSS broth, the cells were covered with amorphous capsule-like material of heavy thickness. In most areas, the coating almost obscured the contours of cell morphology and embedded the organisms in it (Fig. 2b). However, the incorporation of dextranase AD17 into the TSS broth prevented the formation of the amorphous outer coat of the organisms (Fig. 2c). The shape of the organisms cultured in TSS broth containing dextranase AD17 was essentially similar to that grown in TS broth. The similar morphological variations were observed in strains MT6R, MT615R, and MT703R (data not shown), although formation of the capsule-like outer coat by these strains was not as prominent as that of strain OMZ 176.

Figure 3a and b illustrates the artificial dental plaque formed on the cleaned extracted tooth surface by serial transfers of *S. mutans* strain OMZ 176 in TSS broth. Not only confluent growth on the surface but also three-dimensional expansion is observed (Fig. 3a). Figure 3b demonstrates the cumulonimbus-like structure of strata which is composed of packed streptococcal cells and their extracellular polysaccharide capsular material. As shown in Fig. 4a and b, the addition of dextranase AD17 to TSS broth significantly inhibited the formation of artificial dental plaque on the extracted tooth surface. Figure 4 shows a visual field of scanning electron microscopy where some *S. mutans* cells adhered to the tooth surface even when the dextranase had been added. But most of the visual field of the sample (Fig. 4b) was found to be almost organism free.

Similar effects of dextranase AD17 were confirmed in the other *S. mutans* strains. Figure 5a illustrates the artificial dental plaque by strain MT6R on the extracted tooth surface. This plaque is somewhat different in morphology from that of strain OMZ 176. The microcolonies of strain MT6R preferably grow on the same plain in contrast to the three-dimensional growth of strain OMZ 176. Dextranase AD17 prevented the formation of artificial plaque (Fig. 5b). Furthermore, preformed plaque on the tooth surface could be significantly removed by the dextranase treatment (Fig. 5c).

Inhibition of polysaccharide synthesis by S. mutans in the presence of dextranase AD17. *S. mutans* strains MT6R and OMZ 176 were cultivated in THS and TSS broths with or without dextranase AD17. The cultures without dextranase AD17 showed heavy precipitation on the bottom of the culture flasks, whereas those with the enzyme grew with relatively homogeneous turbidity of the culture broth. Phase-contrast microscopic observation revealed that the organisms in THS broth formed large aggregates, but those cultivated in the presence of the enzyme were chain forming. Table 4 shows that the polysaccharide production by the *S. mutans* strains in the culture supernatant (fraction A) was significantly reduced in the presence of dextranase AD17. The polysaccharides produced were water insoluble. However, cell-associated polysaccharide synthesis was not influenced by the action of the dextranase (fraction B). On the other hand, dextranase AD17 did not inhibit the polysaccharide production by *Streptococcus salivarius* strains HHT and SS2 (Table 5). The polysaccharides

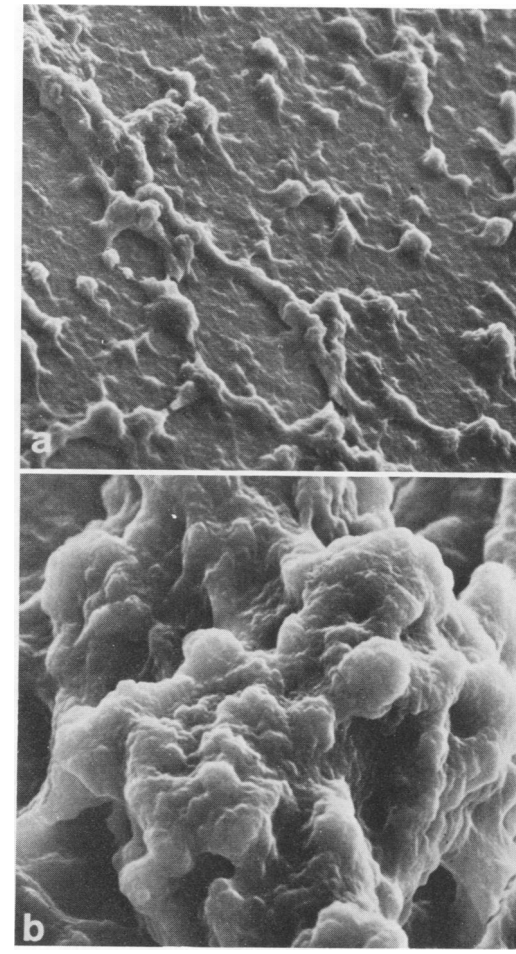

FIG. 3. *Artificial plaque formation by S. mutans* strain OMZ 176 on the enamel surface of an extracted *tooth. (a) ×680; (b) ×2,040.*

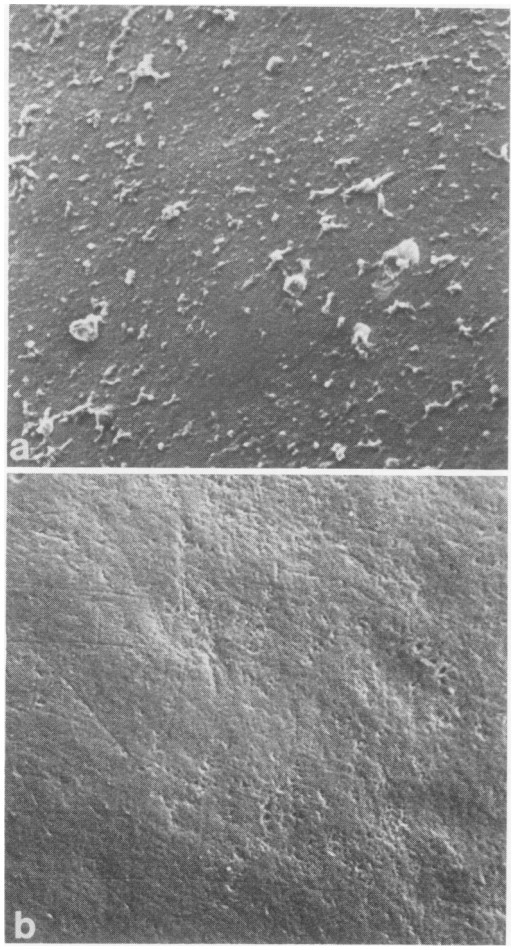

FIG. 4. *Inhibitory effect of dextranase AD17 on the plaque formation on enamel surface. Although most areas were essentially bacteria free, S. mutans cells still adhered to enamel surface but marked decrease in plaque formation was observed as compared to Fig. 3a. ×200.*

synthesized by *S. salivarius* were mainly composed of fructose (Table 5).

Chemical composition of the isolated polysaccharide fractions and susceptibility to dextranase AD17. Four polysaccharide fractions were obtained from each of *S. mutans* strains MT6R, MT118R, OMZ 176 and LM7. Hydrolysates of all fractions were developed with thin-layer chromatography to determine the component sugars. Most of the polysaccharides were mainly composed of glucose. However, significant amounts of fructose were occasionally found in the hydrolysates from some of the polysaccharide fractions, for example fraction 4 of strain LM7 (Fig. 6). Prolonged hydrolysis of the polysaccharide preferentially destroyed fructose. No fructose was detected in the fractions from strain OMZ 176. Total hexoses, fructose, and proteins were estimated colorimetrically, and the values are presented in Table 6. Because the sugar components were found to be only glucose and fructose, glucose content could be expressed as a difference value between total hexose and fructose content.

Table 7 presents the results of the hydrolytic activity of dextranase AD17 against various fractions of *S. mutans* polysaccharides. As positive controls, two kinds of dextrans of molecular weights of 2×10^6 (Pharmacia, Uppsala, Sweden) and 7×10^4 (Nakarai Chemicals, Kyoto, Japan) were used. Both dextrans were prepared from *Leuconostoc mesenteroides*. After 24 h of incubation of the polysaccharides with dextranase AD17, nearly half of the glycosidic linkages were split in the control dextrans (Table 7). Thin-layer chromatography revealed that enzyme hydrolysates of the commercial dextrans produced mainly glucose (Fig. 7), small amounts of isomaltose, and significant amounts of isomaltodextrin, probably isomaltotriose. However, isomaltose was not found in the enzymatic hydrolysates of the polysaccharide fractions from *S. mutans* strains MT6R and OMZ 176, except fraction 4 of OMZ 176 polysaccharide (Fig. 7). Only glucose and oligosaccharide were detected in these fractions. Moreover, the release of reducing sugars after enzymatic degradation of *S. mutans* polysaccharides was limited (15 to 36% of the total polysaccharides) as compared to that of commercial dextrans. Similar results were obtained in the polysaccharide fractions from strains MT118R and LM7. In general, the cell-associated fraction of each polysaccharide was more resistant to the action of dextranase AD17, except that from OMZ 176. Fraction 4 of strain OMZ 176 shows a similar hydrolytic pattern to that of commercial dextran in thin-layer plates and better release of reducing sugars (Table 7). Prolonged reaction (up to 264 h) did not release the reducing sugars from the cell-associated polysaccharide fractions. Isomaltose was not susceptible to dextranase AD17 (Table 7).

DISCUSSION

Several investigators (1, 5, 11, 28) demonstrated that dextranase had the limited ability to degrade the extracellular polysaccharides produced by *S. mutans*. The relative resistance of these glucans to dextranase, in contrast to the dextrans from *L. mesenteroides*, may depend on the high content of α-1,3-glucosidic linkages rather than α-1,6-linkages in the glucans, as was first suggested by Guggenheim

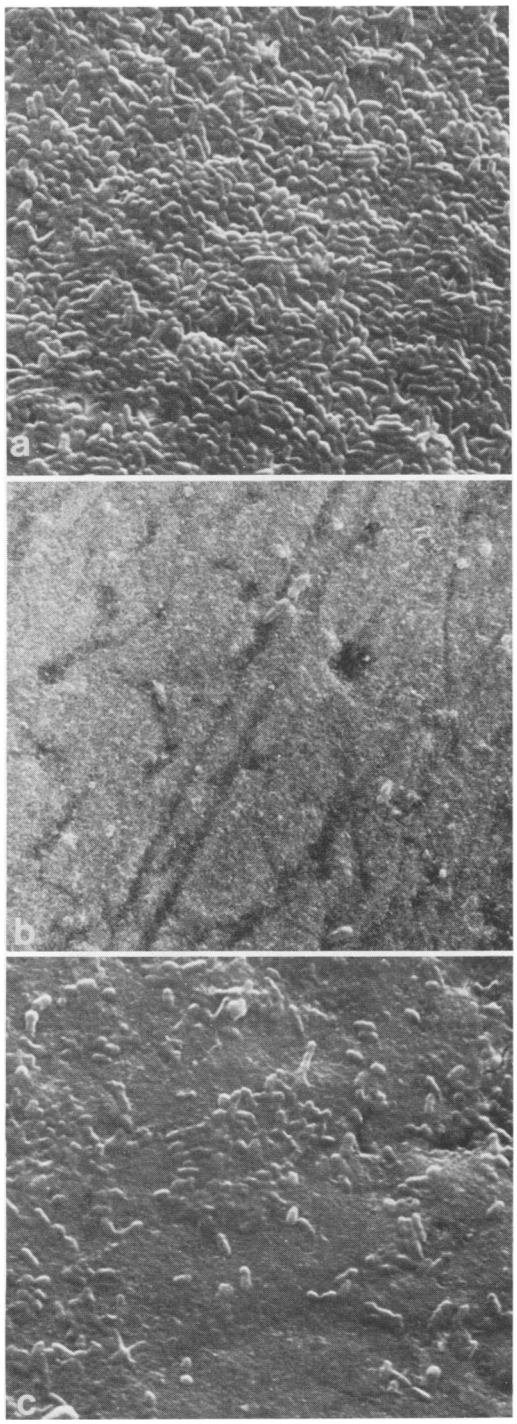

FIG. 5. *Artificial plaque formation by S. mutans strain MT6R on enamel surface (a). Incorporation of dextranase AD17 into culture medium prevented the formation of plaque (b). However, when the enzyme was added to the preformed plaques, significant amounts of plaque remained even after enzyme treatment (c). ×2,040.*

(11). The thin-layer chromatogram of the enzymatic hydrolysates of the polysaccharide fractions supports the previous findings in respect of the limitation of dextranase action against the glucans from *S. mutans*.

Dextranase AD17 split the *S. mutans* polysaccharides into glucose and isomaltodextrin, and only small amounts of isomaltose were detected in the hydrolysates. However, dextran, which is composed of linear α-1,6-glucosidic linkages, was degraded into glucose, isomaltose, and isomaltodextrins. The difference in the end products of the enzymatic hydrolysates under the same condition should be a reflection of the structural differences between dextran and *S. mutans* polysaccharides. In this connection, Minah et al. (23) suggested that dental plaque samples contained little dextran because no release of isomaltose was detected after treatment of plaque materials with dextranase 110 from *Penicillium funiculosum* NRRL 1768. However, it should be noted that dextranase does not necessarily split glucans, especially those from *S. mutans*, into isomaltose and other components (1, 16, 33).

Furthermore, it must be taken into consideration that chemical modification of the glucans might occur during the synthesis of polysaccharides by *S. mutans* cells. This could occur through the action of their own dextranases, which several investigators have recently reported (7, 12, 35).

Although dextranase AD17 could not completely degrade the glucans from *S. mutans*, artificial plaque formation on the steel wires and on the extracted human tooth surfaces were markedly inhibited by the enzyme. These results suggest that the adherence-promoting capacities of the extracellular glucans of *S. mutans* might be attributable to α-1,6-glucosidic linkages which are susceptible to the action of dextranase AD17. It should be noted here again that prolonged reaction (264 h) of the dextranase with the insoluble polysaccharides from *S. mutans* did not release the reducing sugars (data not shown). Newbrun (28) clearly demonstrated that, if a dextranase contained trace amounts of α-1,3-glucanase activity, *S. mutans* glucans were decomposed extensively after prolonged incubation. Even in the presence of dextranase, *S. mutans* continued to produce water-insoluble glucans which are considered to be mainly composed of α-1,3-linkages. This sug-

TABLE 4. *Effect of dextranase AD17 on the polysaccharide production by S. mutans*

Polysaccharide fraction	*S. mutans* strain	Culture medium	Total hexose[a] (μg/ml of culture)	
			Control (no enzyme)	With AD17[b]
A (from culture supernatant)	MT6R	TSS	1,750	500
		THS	1,350	450
	OMZ 176	TSS	600	380
		THS	380	250
B (from cells)	MT6R	TSS	9.5	7.5
		THS	21.8	19.5
	OMZ 176	TSS	9.5	ND
		THS	5.5	4.8

[a] Calculated as glucose using anthrone method (34).
[b] 10 U of AD17/ml of culture. ND, Not determined.

TABLE 5. *Effect of dextranase AD17 on the polysaccharide (PS) production by S. salivarius*

Strain	Treatment	PS in culture supernatant		PS from cells: total hexose
		Total hexose	Fructose	
HHT	Control (no enzyme)	8.0	7.4	2.4
	With AD17 (10 U/ml)	8.0	7.6	1.5
SS2	Control (no enzyme)	2.5	2.8	1.8
	With AD17 (10 U/ml)	2.4	2.2	1.4

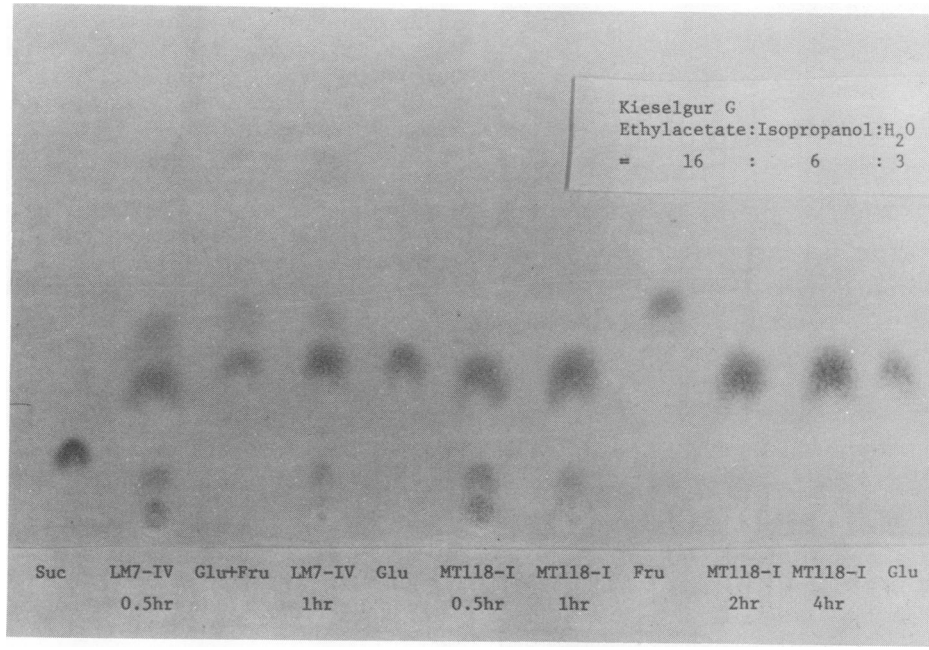

FIG. 6. *Thin-layer chromatography of HCl hydrolysates of S. mutans polysaccharide fractions. Strain, polysaccharide fraction, and hydrolysis time are shown.*

gests that water-insoluble glucans may not be solely responsible for the adherence of *S. mutans*. Similar work has been done by Walker (38). She reported that isomaltodextrins initially synthesized by glucosyltransferase of *S. mutans* were hydrolyzed by dextranase as rapidly as α-1,6-glucans, and the hydrolysis product of the isomaltodextrin would become a com-

TABLE 6. *Chemical analysis of polysaccharide fractions from S. mutans*

Strain	Fraction	Polysaccharide (mg/mg)			
		Total hexose	Glucose[a]	Fructose	Protein
MT6R	1	0.84	0.81	0.03	0.07
	2	0.99	0.96	0.03	0.02
	3	1.10	1.09	0.01	0.01
	4	0.63	0.63	0	0.12
MT118R	1	0.95	0.90	0.05	0.07
	2	0.95	0.79	0.16	0.05
	3	1.06	1.06	0	0.01
	4	0.66	0.64	0.02	0.12
OMZ 176	1	0.78	0.78	0	0.09
	2	0.84	0.84	0	0.06
	3	1.01	1.01	0	0.01
	4	0.85	0.85	0	0.06
LM7	1	0.89	0.86	0.03	0.05
	2	1.00	1.00	0	0.01
	3	0.99	0.99	0	0.01
	4	0.85	0.60	0.25	0.05

[a] The glucose content is expressed as difference between total hexose and fructose values.

TABLE 7. *Enzymatic hydrolysis of S. mutans polysaccharide fractions with dextranase AD17*

Substrate		Reducing sugars released (μg [as glucose]/mg of substrate)
Dextran T2000 (Pharmacia)		563
Dextran (Nakarai)		537
MT6R Fraction	1	360
	2	349
	3	305
	4	196
OMZ 176	1	257
	2	206
	3	228
	4	351
MT118R	4	211
LM7	4	149
Isomaltose		3

petitive acceptor against sucrose in the reaction system. Thus the synthesis of the polysaccharides was significantly restricted.

It was also demonstrated that heat-killed *S. mutans* cells retained the ability to adhere to a glass surface when crude glucosyltransferase and sucrose were added (24). However, if the cells were pretreated with dextranase, the adherence ability decreased significantly (25; Hamada and Slade, J. Dent. Res., in press). It was suggested that this might be due to degradation of surface-located glucans which would be responsible for the glucosyltransferase receptor. Dextranase AD17 may likewise destroy or prevent the synthesis of the receptor glucans on the cell surface, which resulted in the loss of adherence ability of *S. mutans* cells to smooth surfaces. This may explain the inability to form capsule-like sticky polysaccharides on the *S. mutans* cell surface under the influence of dextranase AD17, as shown in Fig. 2c. This explanation is supported by the scanning electron microscopic observations of Johnson et al. (18). These workers found that sucrose-grown cells of a mutant of *S. mutans* strain GS5, which had lost the ability to adhere to a glass surface, could not form aggregates and microcolonies, whereas the wild-type strain formed them. These properties of the mutant may be comparable to those of cells grown in medium containing both sucrose and dextranase.

On the other hand, from their examination of ultrathin sections, Nalbandian et al. (27) demonstrated two different kinds of extracellular structural components, sucrose-dependent fibrillar (with some globular) glucans of irregular morphology and sucrose-independent, surface-associated fuzzy coat which was probably protein in nature. The extracellular fibrillar structure was destroyed by the action of dextranase, which is in agreement with our findings in the scanning electron microscopic study.

The effects of dextranase on the formation of artificial dental plaque by *S. mutans* strain 6715 (serotype *d*) have been reported by Hoffman et al. (17). In their scanning electron microscopic studies, they described honeycombed demineralization patterns on the enamel surface just underneath the artificial plaque after incubation for 138 h. However, this type of demineralization pattern could not be observed in our experiment. This may be due to the difference

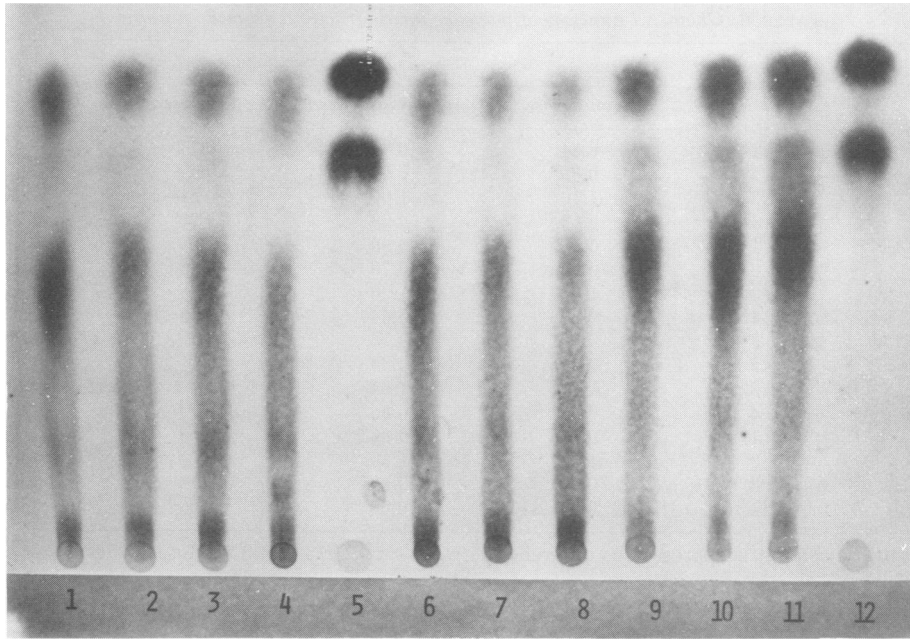

FIG. 7. *Thin-layer chromatogram of dextranase hydrolysates of S. mutans polysaccharide fractions: (1–4) polysaccharides from strain MT6R fractions 1 to 4; (6–9) polysaccharides from strain OMZ 176; (10, 11) commercial dextrans of molecular weight 7 × 10⁴ and 2 × 10⁶ each; (5, 12) standards glucose and isomaltose each.*

in incubation time of the tooth surface in the bacterial cultures. Hoffman et al. (17) reported neither the morphological changes of *S. mutans* cells grown in sucrose broth nor the depression of the synthesis of the polysaccharide with dextranase treatment.

Although there is no agreement at present on the effectiveness of dextranase in experimental dental caries in animals and in human clinical trials, administration of dextranse AD17 into the diet and drinking water markedly inhibited the development of dental caries in conventional hamsters (Hamada et al., submitted for publication) and rats (unpublished data) which had been inoculated with *S. mutans* strains and fed high-sucrose-containing cariogenic diet 2000.

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