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PRODUCTION AND PARTIAL CHARACTERIZATION OF THE EXTRACELLULAR POLYSACCHARIDES FROM ORAL *Streptococcus salivarius*

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ABSTRACT

The production of polysaccharides from sucrose by extracellular enzymes from oral *Streptococcus salivarius* isolates and the physico-chemical properties of water-insoluble products (IPs) were investigated. Extracellular enzymes from all the 18 strains tested produced insoluble α-D-glucans (IGs) as well as soluble β-D-fructans, and formed adhering deposits on glass. Generally, the IPs (mostly IGs) of *S. salivarius* strains differed from the *S. sobrinus* IPs by (a) containing significant proportions of α-D-(1→4)-, in addition to α-D-(1→3)- and α-D-(1→6)-glucosyl linkages, and much higher proportions of α-D-(1→3) than α-D-(1→6) linkages, (b) being more susceptible to hydrolysis by mutanase than by dextranase, (c) possessing low or no streptococcal cell-agglutinating ability, and (d) showing weaker adhesion to a glass surface. The degree of the polysaccharide adherence differed greatly among the *S. salivarius* strains and, therefore, they were divided into three groups of adherence producers; heavy, moderate, and slight. The IPs of the three groups contained, generally in descending order, a higher proportion of higher-molecular-weight fractions, and consisted of higher proportions of IG containing higher proportions of -(1→6)-α-D and -(1→4)-α-D glucosyl linkages and (1→3,6) branches, but showed higher susceptibility to hydrolysis by mutanase as well as dextranase. Thus, the production and the properties of extracellular insoluble α-D-glucans from sucrose differ considerably between oral *S. salivarius* and cariogenic *S. sobrinus*.

INTRODUCTION

Streptococcus salivarius, predominant in the oral cavity, is well known as one of the β-D-fructan (levan) producers. Since the first publication by Niven *et al.*[1,2] in 1941, several investigators have reported[3–9] that certain strains of the streptococcus species produce α-D-glucans, water-insoluble as well as soluble, from sucrose by the action of its constitutive enzyme, D-glucosyltransferase (dextransucrase, EC 2.4.1.5). However, little attention, even by oral microbiologists, has

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been given to this aspect of *S. salivarius*. One of the reasons for this lack of attention is probably that its preferential habitat is the saliva and the tongue, and not the tooth surface[10].

It has been argued that one of the most important factor of virulence of the cariogenic "*mutans*" streptococci is the synthesis of tenacious, water-insoluble α-D-glucans from dietary sucrose[11–14]. By analogy with the insoluble α-D-glucans produced by the cocci, it is very likely that insoluble α-D-glucans of *S. salivarius* are also involved in the attachment and accumulation of the microorganism on tooth surfaces, and in the subsequent development of tooth decay. Indeed, it has been demonstrated that certain strains of *S. salivarius* possess the ability to adhere to solid surfaces in the presence of sucrose *in vitro*[8,15,16], and to induce tooth decay in rodents reared on a sucrose diet[4,17–22].

Recently, we have isolated a considerable number of *S. salivarius* strains from the human mouth[23]. They form relatively large rough or mucoid colonies on sucrose agars, similar to those of cariogenic "*mutans*" streptococci, and produce insoluble α-D-glucans from sucrose in various amounts. The morphological characteristics of these colonies have been shown to be due to their insoluble α-D-glucan-synthesizing capacity. We describe herein the production and some of the properties of the extracellular polysaccharides of these oral *S. salivarius* strains with special emphasis on insoluble α-D-glucans.

EXPERIMENTAL

Bacterial strains. — Eighteen strains of *S. salivarius* isolated from the human mouth[23] were used. Table I lists their colonial types, rough (R), mucoid rough (M), or smooth (S), on sucrose agars. For some strains changes in colonial form, mostly M to, or from S or R, had occasionally occurred during storage. Strains were routinely stored by adding sterile glycerol (5.0 mL) to an overnight culture grown in Brain Heart Infusion broth (BHI; Difco, Detroit, MI; 5.0 mL), and freezing these cell suspensions at −10 to −15°.

Preparation of extracellular enzymes. — The organisms were grown in BHI broth at 37° overnight. The culture supernatant was obtained by centrifugation at 10 000*g* for 20 min at 4°, and precipitated with $(NH_4)_2SO_4$ at 50% (w/v) saturation overnight at 4°. The precipitates were collected by centrifugation, dissolved in a small volume of 5mM sodium phosphate buffer (pH 6.0), and dialyzed against the buffer.

The polysaccharide-synthesizing activity of the enzyme preparations was assayed by allowing adequate amounts of each enzyme to react with [U-^{14}C]sucrose (0.37 kBq, 74 GBq mol^{-1}; New England Nuclear, Boston, MA) in 0.1M phosphate buffer (pH 6.0, 20 μL) for 30 min at 37°. The radioactivity incorporated into the polysaccharides was measured by the filter-paper method described previously[24]. One unit of enzyme activity was defined as the amount of enzyme that transforms 1 μmol of sucrose into polysaccharide per min under the conditions described. Protein content was determined by the method of Lowry *et al.*[25].

TABLE I

EXTRACELLULAR ENZYMES PREPARED FROM D-GLUCOSE-GROWN *S. salivarius* STRAINS

Strain	*Colonial form on sucrose agar*[a]	*Polysaccharide synthesizing activity (mU mL^{-1})*	*Protein (mg mL^{-1})*	*Specific activity (mU mg^{-1})*	*Activation of enzyme activity by Dextran T10 (fold)*
Y4	R (R)	3716.4	22.8	163.0	0.89
Y5	R (R)	3740.5	27.9	134.1	0.92
S4	R (R)	1098.2	13.4	82.0	0.96
S3	R (R)	440.5	10.9	40.4	1.29
M4	R (R)	255.6	8.6	29.7	1.30
Y6	S/R (R)	675.2	31.3	21.6	0.82
M5	S (M)	204.9	13.8	14.8	2.68
M6	S (M)	158.9	13.3	11.9	2.92
Y3	S (M)	543.6	36.9	14.7	1.16
M2	S (S)	100.1	13.5	7.4	2.94
M1	S (S)	108.3	8.4	12.9	3.23
S1	S/M (S)	52.7	13.3	4.0	2.17
S2	S (S)	63.4	9.2	6.9	2.01
M3	S (R)	86.6	6.9	12.5	1.05
S5	M/S (M)	270.3	13.9	19.4	1.31
S6	S (M)	206.4	13.9	14.8	0.80
Y1	S (S)	305.0	44.5	6.9	2.45
Y2	S (S)	534.5	35.1	15.2	0.80

[a]R, gelatinous rough; M, mucoid rough; and S, mucoid smooth. In parentheses, original classification when strains were first isolated[23].

The enzyme activities and protein contents of the preparations used are summarized in Table I. Usually, the enzymes of R colony-forming strains gave higher activity than those of S or M colony-forming strains. The enzyme activities of the seven strains, M1, Y1, M2, M6, M5, S2, and S1, were elevated a few times by the presence of Dextran T10 (Pharmacia Fine Chemicals, Uppsala) at a final concentration of 20μM in the reaction mixture.

Adherence of polysaccharides to glass. — The reaction mixture consisted of enzymes (30 mL) and sucrose (150 mg) in 0.1M sodium phosphate buffer (pH 6.0, 3.0 mL). The mixture was incubated at 37° in a glass test-tube (12 × 105 mm) kept at an angle of 30°. The polysaccharides adherent to the glass surface after a 16-h incubation were washed by hand twice with 5mM phosphate buffer (pH 6.0, 3.0 mL) and once with de-ionized water, and then suspended in de-ionized water (3.0 mL). The amount of polysaccharides was estimated by the anthrone method[26] with D-glucose as a standard.

Synthesis of polysaccharides. — Sucrose (200 mg) and extracellular enzyme (40 mU) in 0.1M sodium phosphate buffer (pH 6.0, 4.0 mL) were incubated for 18 h at 37°. The mixture was centrifuged (20 000*g*, 15 min, 4°) to harvest water-insoluble products (IPs), and the IPs obtained were then washed by centrifugation twice with 5mM phosphate buffer (pH 6.0) and once with de-ionized water. Water-

soluble products (SPs) were precipitated from the supernatant solution by addition of ethanol (2.5 vols.), followed by storage for 2 h in a freezer. They were then washed twice in 70% ethanol solution. The washed polysaccharides (IPs and SPs) were exhaustively dialyzed against de-ionized water. Differential quantitative determination of α-D-glucan and β-D-fructan in the fractions was performed by the anthrone method[27] with D-glucose and D-fructose as standards.

The IPs preparations of *S. sobrinus* AHT and OMZ176 were obtained in the same manner as described above.

Methylation analysis. — The IPs (1–3 mg) were methylated by the method of Hakomori[28]. The methylated polysaccharide was treated with 90% (v/v) formic acid for 6 h at 100°, and then hydrolyzed with 2M trifluoroacetic acid for 6 h at 100° under N_2. Alditol acetate derivatives of partially methylated hexoses were analyzed by g.l.c.[29]. The glass column (0.3 × 200 cm) used contained 0.3% OV-275 and 0.4% GEXF-1150 on Uniport HP (80–100 mesh; Gas-Chro Kogyo Co., Tokyo). A gas-liquid chromatograph with H_2-flame-ionization detector (GC-4CPF; Shimadzu works, Kyoto) was used, with N_2 as carrier gas (50 mL min^{-1}) and a column temperature of 120 to 180° (1° min^{-1}). Commercial dextran T40 (Pharmacia) and glycogen (Nakarai Chemicals, Kyoto) were used as standards.

Hydrolysis by D-*glucanases.* — Mutanase [(1→3)-α-D-glucan 3-glucanohydrolase, EC 3.2.1.84], from a *Pseudomonas* strain was a gift from Dr. Y. Yamamoto (Sunstar Inc., Takatsuki, Osaka). The following D-glucanases were commercially obtained: dextranase [(1→6)-α-D-glucan 6-glucanohydrolase, EC 3.2.1.11, from a *Penicillium* strain; Seikagaku-kogyo Co., Tokyo]; isoamylase (glycogen 6-glucanhydrolase, EC 3.2.1.68, from a *Pseudomonas amyloderamosa* strain; Funakoshi Pharmaceutical Co., Tokyo); glycoamylase [(1→4)-α-D-glucan glucohydrolase, EC 3.2.1.8, from a *Rhizopus* strain; Funakoshi]; pullulanase (amylopectin 6-glucanohydrolase, EC 3.2.1.41, from *Aerobacter aerogenes* ATCC 9621; Hayashibara Biochemical Laboratories Inc., Shimoishi, Okayama); and laminaranase [(1→3)-(1→3;1→4)-β-D-glucan 3(4)-glucanohydrolase, EC 3.2.1.6, from a mollusk, Sigma Chemical Co., St. Louis, MO]. One unit of enzyme activity was defined as the amount that would release 1 μmol of sugar, as D-glucose, per min under the specified conditions[30,31].

The IP samples (0.4 mg) and D-glucanase (2.0 mU) in 0.1M acetate buffer (pH 5.2 for laminaranase and pH 6.0 for the other enzymes, 0.4 mL) were incubated for 16 h at 37°, and the release of reducing sugars was measured by the Somogyi and Nelson method[32] with D-glucose as a standard.

Cell-agglutinating activity. — The IPs from strains Y4, S3, S2, and S6 were tested for their ability to agglutinate streptococcal cells by the method described previously[31]. The IP (IG) of *S. sobrinus* AHT has been prepared previously[33] and Dextran T500 was a product of Pharmacia Fine Chemicals (Uppsala, Sweden). Resting cells of these streptococcal strains were prepared from the BHI broth cultures mentioned above.

Molecular size distribution. — The molecular size distribution of IPs was examined by ultrafiltration[31] on Diaflo membrane XM300 (Amicon Corp., Lexington, MA).

RESULTS

Synthesis and adherence of polysaccharides. — The macroscopic appearance of the polysaccharides produced by *S. salivarius* enzymes basically resembled those of *"mutans"* streptococci, particularly *S. sobrinus* (not shown). Insoluble products precipitated, forming flocculates or granular aggregates. The IPs of the R colony-forming strains were more viscous and, once collected by centrifugation, became somewhat difficult to disperse homogeneously, whereas those of the M or S colony-forming strains were readily dispersible.

It is noteworthy that enzymic products were occasionally stringy, which occurred readily among the S colony formers (not shown). In addition, some

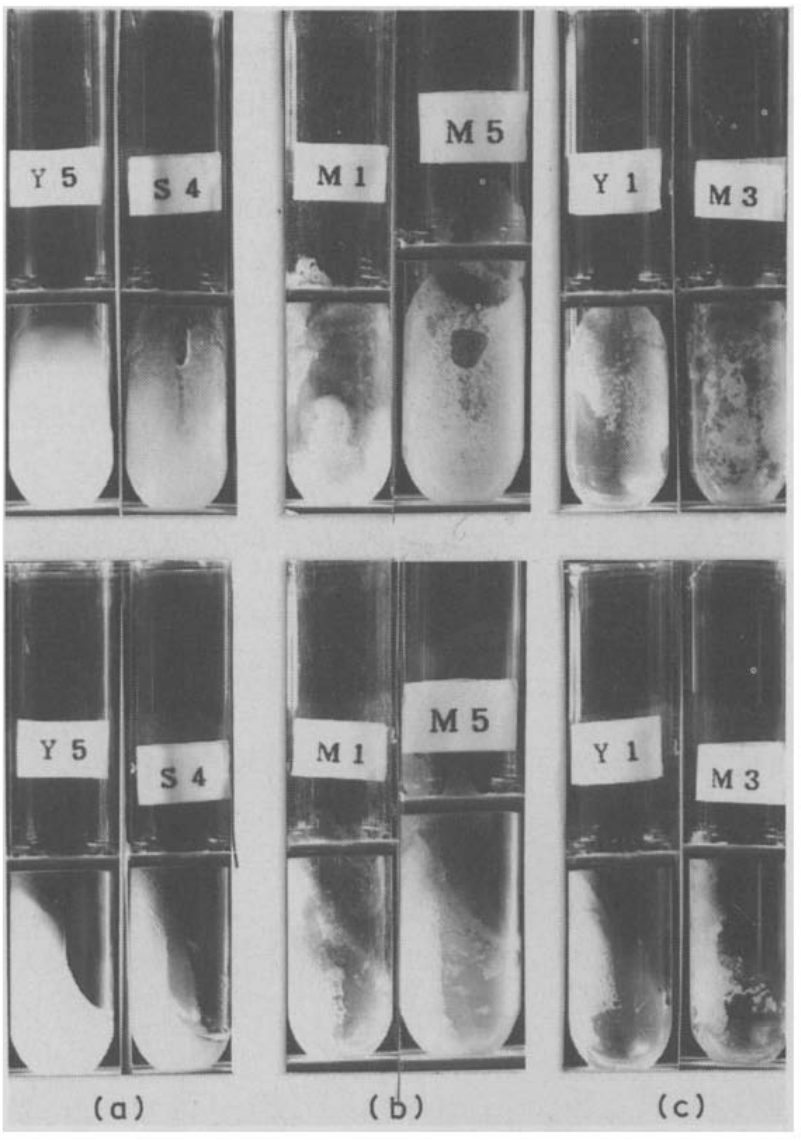

Fig. 1. Typical appearance of the adherence of insoluble polysaccharide on a glass surface produced by the enzyme of: (a) Strains Y5 and S4 (heavy adherence); (b) M5 and M1 (moderate adherence); and (c) Y1 and M3 (slight adherence). Extracellular enzyme (30 mU) and sucrose (150 mg) were allowed to react in 0.1M phosphate buffer (pH 6.0, 3 mL) at 30° for 16 h in a test tube (12 × 105 mm) kept at an angle of 30°. Adherent deposits on glass surface were washed three times by hand with 3 mL of the buffer or distilled water.

TABLE II

ADHERENCE OF POLYSACCHARIDES PRODUCED BY THE EXTRACELLULAR ENZYMES[a]

Strain	*Concentration (mg mL^{-1})*	*Strain*	*Concentration (mg mL^{-1})*	*Strain*	*Concentration (mg mL^{-1})*
Y4	14.8	M5	4.5	S1	0.7
Y5	26.0	M6	3.1	S2	0.8
S4	13.0	Y3	0.03[b]	M3	0.1
S3	9.4	M2	1.7	S5	0.8
M4	8.5	M1	1.4	S6	0.1
Y6	11.0			Y1	0.07
				Y2	0.2

[a]At a concentration of 10 mU mL^{-1}. [b]Sometimes produces rather heavy adherence[23].

reaction mixtures would set as a fragile jelly-like lump, and the resultant clot would not slide out when the test tubes were inverted. This was often found with the R colony formers (not shown).

Adherence of polysaccharides to glass is shown in Fig. 1. The polysaccharides synthesized by the enzyme from the R colony-producing strains formed, on the bottom surface of tilted test tubes, a large, thick, and turbid clot which retained much moisture. The adhesion of the IPs of even these *S. salivarius* strains appeared weaker and looser when compared to those of *S. sobrinus*. The IPs of the M or S

TABLE III

PRODUCTION OF WATER-INSOLUBLE AND -SOLUBLE POLYSACCHARIDES BY THE EXTRACELLULAR ENZYMES

Strain	*Insoluble*		*Soluble*		*Ratio of insoluble to soluble polysaccharides*	*Ratio of total D-glucan to D-fructan*
	D-Glucan (mg mL^{-1})	*D-Fructan*	*D-Glucan (mg mL^{-1})*	*D-Fructan*		
Y4	3.89	0.28	0.03	0.06	46.3	11.5
Y5	5.53	0.32	0.05	0.04	65.0	15.5
S4	3.59	0.28	0.02	0.17	20.4	8.02
S3	3.59	0.28	0	0.37	10.5	5.52
M4	5.61	0.24	0.03	0.11	41.8	16.1
Y6	2.87	0.35	0.04	0.07	29.3	6.93
M5	2.05	0.24	0.05	0.32	6.19	3.75
M6	2.46	0.16	0	2.16	1.21	1.06
Y3	0.51	0.10	0	0.74	0.82	0.61
M2	0.64	0.10	0.04	0.24	2.64	2.00
M1	0.71	0.07	0.02	0.25	2.89	2.28
S1	1.05	0.17	0.02	1.29	0.93	0.73
S2	1.18	0.19	0.15	3.06	0.43	0.41
M3	0.78	0.24	0	2.55	0.40	0.28
S5	0.49	0.20	0.06	1.19	0.55	0.40
S6	0.21	0.17	0.08	1.57	0.23	0.17
Y1	0.34	0.06	0	0.90	0.44	0.35
Y2	0.11	0.07	0.02	0.70	0.25	0.17

colony-producing strains adhered more weakly to glass surfaces as a thin film or remnant patches of film.

The amount of polysaccharide adhering to glass also greatly differed among the strains (Table II). The differences were 10-fold or more between the highest and the lowest amount. By taking the polysaccharide-adherence ability shown in table as a temporary standard, the 18 *S. salivarius* strains were tentatively separated into three goups: the heavy- (film), moderate-, and slight-adherence producers, *i.e.*, the top six strains, the succeeding five, and the final seven in Table II, respectively.

As summarized in Table III, the bulk of the IPs were α-D-glucans (IGs) and most SPs were β-D-fructans (SFs) for all strains. However, the total amounts of polysaccharides and their harvested water-insoluble and water-soluble fractions differed greatly among strains, although the same unit (radioisotopically determined) of enzyme activity was employed for the synthesis. The difference between maximal and minimal production was about 6-fold for the total amount of polysaccharides, 50-fold for the IGs, and 75-fold for the SFs.

The relative amounts of insoluble and soluble α-D-glucans and β-D-fructans in a polysaccharide sample also varied among strains (Table III and Fig. 2): the polysaccharides synthesized by the enzymes from the heavy-adherence-producing strains contained high proportions of IGs (85% or more of total polysaccharides produced, Fig. 2a), whereas more than 75% of total polysaccharides produced by most of the slight-adherence producers were SFs (Fig. 2b); the polysaccharides produced by the enzymes of the moderate-adherence producers consisted of nearly equal proportions of α-D-glucans and β-D-fructans. The ratio of IG to IF (insoluble

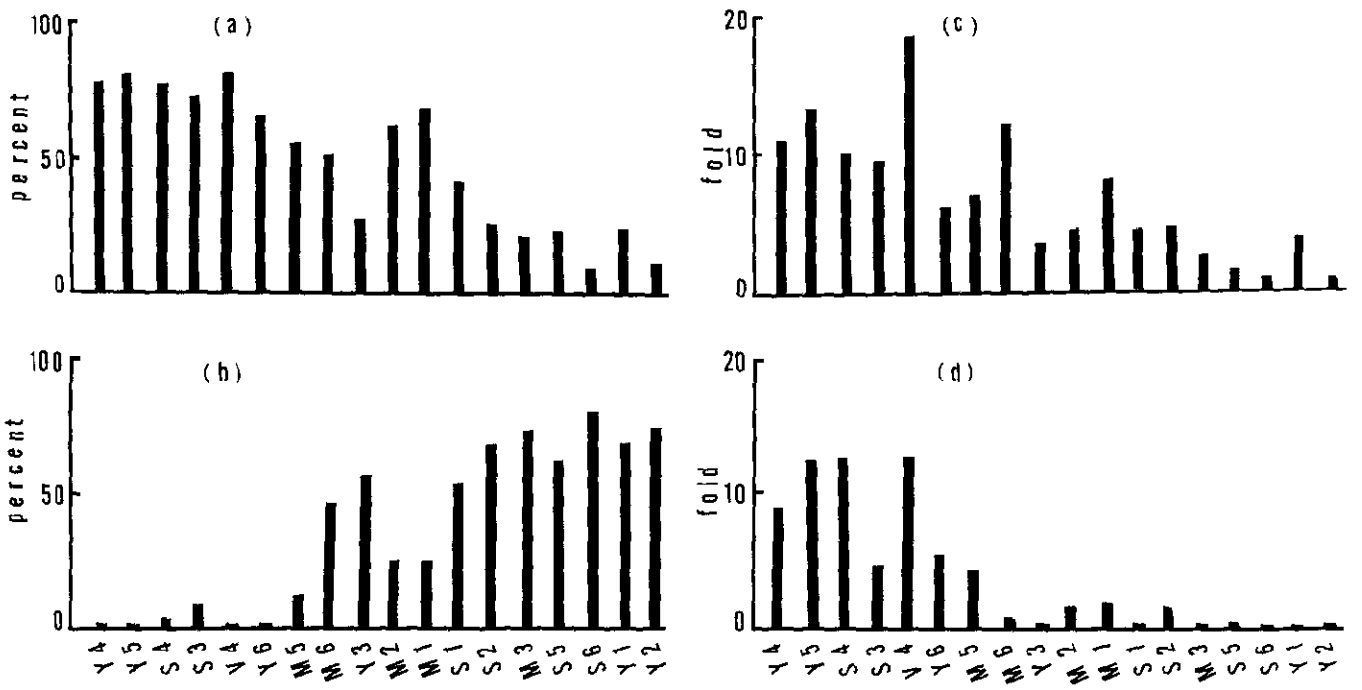

Fig. 2. Relative amounts of water-insoluble and -soluble α-D-glucans and β-D-fructans in the polysaccharide products: (a) Proportion of insoluble α-D-glucans in total polysaccharide; (b) proportion of soluble β-D-fructans in total polysaccharide; (c) ratio of insoluble α-D-glucans to insoluble β-D-fructans; and (d) ratio of total α-D-glucans to total β-D-fructans.

TABLE IV

RETENTION RATE ON ULTRAFILTRATION THROUGH DIAFLO MEMBRANE XM300 OF WATER-INSOLUBLE POLYSACCHARIDES

Strain	*Percent*	*Strain*	*Percent*	*Strain*	*Percent*
Y4	93.4	M5	83.4	S1	95.9
Y5	95.5	M6	88.6	S2	85.8
S4	98.7	Y3	98.8	M3	96.2
S3	95.1	M2	83.6	S5	98.3
M4	96.2	M1	91.9	S6	96.1
Y6	91.3			Y1	85.5
				Y2	83.6

β-D-fructan) was, therefore, remarkably higher for the heavy-adherence-producing strains than for the other strains (Fig. 2c).

Thus, the heavy-, moderate-, and slight-adherence producers generally possessed, in this order, a higher capacity for IP and IG productions, and lower capacity for SF synthesis, and the ratio of α-D-glucans to β-D-fructans in total polysaccharides became lower (Fig. 2d). Comparison of the results shown in Tables II and III clearly indicates that the degree of IP adherence ability of a strain generally corresponds to its IG-synthesizing activity.

Properties of insoluble polysaccharides. — An ultrafiltration study demonstrated that the bulk (more than 80%) of the IPs produced by the enzymes of all the strains were excluded by a XM300 membrane (Table IV). The IPs of all but one of the heavy-adherence producers, and half of the moderate- or slight-adherence producers showed higher retention rates (95% or more).

Streptococcal cell-agglutinating ability was tested with four selected strains (Table V). Unlike *S. sobrinus* IP, none of the *S. salivarius* IPs showed the ability to agglutinate *S. sobrinus* AHT cells, even at the maximum concentration (0.5 mg mL^{-1}). The IPs of the heavy-adherence-producing strains S3 and Y4 agglutinated

TABLE V

STREPTOCOCCAL CELL-AGGLUTINATING ABILITY OF WATER-INSOLUBLE POLYSACCHARIDES

Polysaccharide	*Agglutinating titer[a] of cells of the strains*				
	Y4	*S3*	*S2*	*S6*	*AHT*
Y4	2	[b]	[b]	[b]	[b]
S3	[b]	2	[b]	[b]	[b]
S2	[b]	[b]	[b]	[b]	[b]
S6	[b]	[b]	[b]	[b]	[b]
S. sobrinus AHT	16	8	4	2	128
Dextran T500	[b]	[b]	[b]	[b]	[c]

[a]Reciprocal of the highest dilution of the insoluble polysaccharide suspension at a 0.5 mg.mL^{-1} concentration to give an apparent agglutination of cells. [b]No agglutination occurred even at 0.5 mg.mL^{-1}. [c]Not determined.

only homologous cells, but those of the slight adherence-producing strains S2 and S6 were inactive against any cells, including the homologous ones. It should be noted here that the four *S. salivarius* cells tested agglutinated upon addition of the *S. sobrinus* AHT IPs, although the minimal effective concentration was considerably higher than that for the *S. sobrinus* cells (Table V).

The susceptibility of IPs to hydrolysis by various D-glucanases is shown in Table VI and Fig. 3. The IPs of all strains were significantly hydrolyzed by mutanase, but their susceptibility to dextranase was low, even negligible for some. The IPs of the heavy-adherence producers appeared to be degraded by these α-D-glucanases more readily than those of most of the other strains. The IPs of certain *S. salivarius* strains tested also appeared to be hydrolyzable by laminaranase, but only to limited extents when compared to the hydrolysis by mutanase (Fig. 4). In contrast, isoamylase, glucoamylase, and pullulanase were relatively inactive against the IPs of most strains; any significant but low level of hydrolysis by these α-D-glucanases was found only sporadically and irrespective of adherence ability.

As summarized in Table VII, the IPs (IGs) of the *S. salivarius* strains consisted of α-D-(1→3) and α-D-(1→6), and α-D-(1→3,6)-branch linkages, and also, unlike the *S. sobrinus* IGs, of significant proportions of (1→4)-α-D-glucosyl linkages and α-D-(1→4,6)-branches, although detection of the branch linkages was

TABLE VI

D-GLUCANASE SUSCEPTIBILITY OF INSOLUBLE POLYSACCHARIDES

Strain	*Reducing sugar released (μg mL⁻¹)*				
	Mutanase	*Dextranase*	*Isoamylase*	*Glucoamylase*	*Pullulanase*
Y4	26.3	3.6	[a]	5.6	[a]
Y5	25.4	3.9	2.5	[a]	[a]
S4	54.9	7.7	[a]	[a]	[a]
S3	42.5	16.2	[a]	4.8	[a]
M4	44.4	5.7	3.5	2.7	[a]
Y6	29.5	2.0	[a]	[a]	0.5
M5	35.3	2.7	[a]	[a]	[a]
M6	30.6	[a]	5.7	0.7	[a]
Y3	15.5	1.0	0.3	0.7	0.6
M2	12.3	[a]	5.0	0.7	1.5
M1	11.0	[a]	2.5	2.6	[a]
S1	23.0	0.3	0.7	0.2	3.1
S2	11.6	2.6	[a]	[a]	[a]
M3	15.0	0.3	2.2	[a]	[a]
S5	17.0	7.5	3.3	[a]	1.8
S6	22.9	[a]	1.5	[a]	2.0
Y1	9.2	0.8	0.5	0.3	0.4
Y2	13.4	[a]	[a]	[a]	0.3
S. sobrinus OMZ 176	7.0	21.7	[a]	[a]	[a]

[a]Not detected.

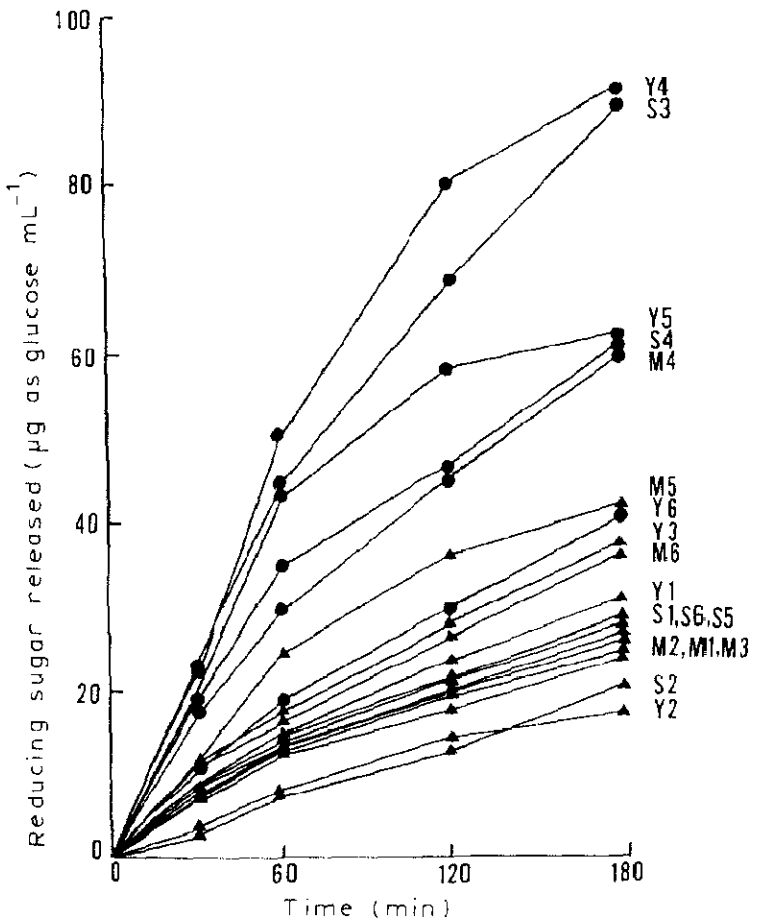

Fig. 3. Hydrolysis of the water-insoluble polysaccharide by mutanase. IP (1.0 mg) was incubated with mutanase (5.0 mU) at 37° in 1.0 mL of 0.1M acetate buffer, pH 6.0.

evident only for the IGs of the seven non-heavy-adherence-producing strains. The contents (49–88 mol/100 mol) of α-(1→3)-linked D-glucosyl residues in *S. salivarius* IGs appeared to be higher, as compared to those of the *S. sobrinus* IGs. Among the *S. salivarius* IPs, the α-D-(1→3) linkage content was considerably lower for IGs of the heavy-adherence producers, whereas most of the other strains contained higher proportions of (1→6)-, (1→4)-, and (1→3,6)-α-D-glucosyl linkages. Therefore, the ratios of α-D-(1→3)- to α-D-(1→6) and α-D-(1→4) linkage, or the total of the last two were generally lower for the heavy-adherence producers (Fig. 5 and Table VII).

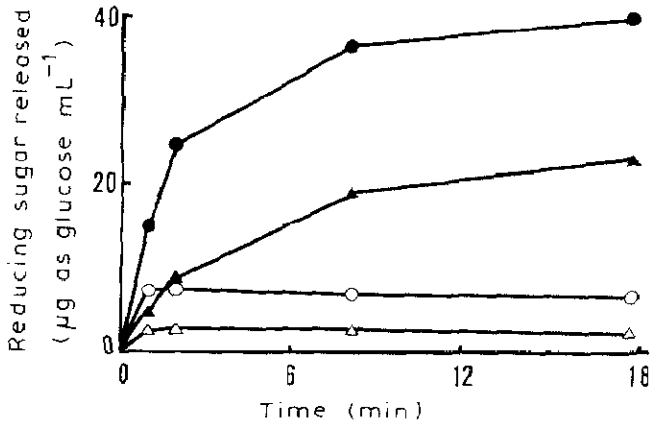

Fig. 4. Susceptibility to hydrolysis by laminaranase of the water-insoluble polysaccharides of strain Y4 (●,○) and M2 (▲,△). Assay conditions were the same as given in the legend to Fig. 3, except at pH 5.2: (●,▲) mutanase and (○,△) laminaranase.

TABLE VII

METHYLATION ANALYSIS OF WATER-INSOLUBLE D-GLUCANS

Strain	O-*Methyl*-D-*glucose (mol per 100 mol)*[a]					
	2,3,4,6- (1.0)	*2,4,6- (1.38)*	*2,3,4- (1.51)*	*2,3,6- (1.57)*	*2,6- (1.82)*	*2,4- (1.92)*
Y4	10.3	51.1	14.8	10.1	[b]	10.7
Y5	13.8	49.4	16.3	1.3	[b]	10.1
S4	10.2	68.3	8.8	8.3	[b]	4.4
S3	7.4	71.5	11.0	5.0	[b]	5.1
M4	7.9	73.7	7.5	6.0	[b]	4.9
Y6	12.2	61.6	20.1	3.7	[b]	2.4
M5	3.0	80.2	11.3	1.6	0.4	3.6
M6	3.2	82.9	6.9	1.9	0.8	4.3
Y3	2.6	86.3	6.2	1.4	0.4	3.1
M2	4.5	81.8	5.7	5.8	[b]	2.2
M1	2.7	88.4	3.1	4.5	[b]	1.3
S1	3.2	82.4	6.0	3.5	[b]	4.9
S2	4.3	75.5	9.8	6.5	[b]	3.9
M3	2.3	87.1	4.0	2.8	0.6	3.1
S5	5.6	74.8	12.1	1.2	1.1	5.2
S6	2.2	85.5	8.1	1.5	[b]	2.2
Y1	1.1	93.0	2.0	1.1	0.6	2.2
Y2	1.2	85.1	4.0	2.2	0.9	7.6
S. sobrinus						
OMZ 176	18.6	48.5	16.7	[c]	[c]	16.1

[a]In parentheses, relative retention time. [b]Trace. [c]Not detected.

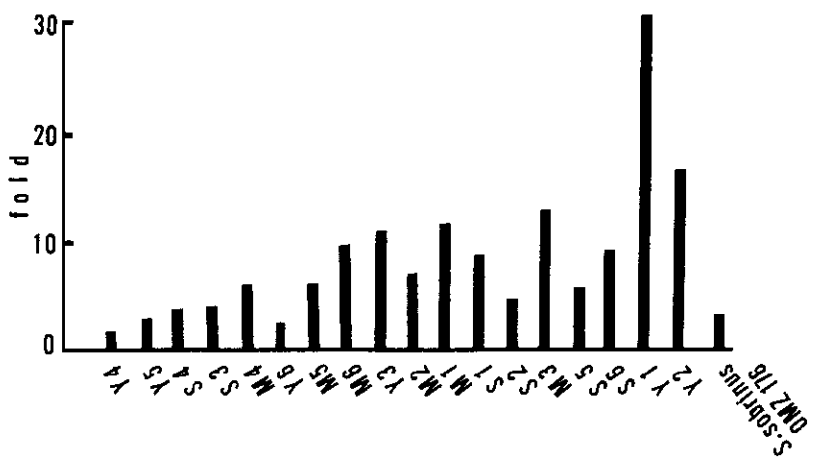

Fig. 5. Molar ratio of (1→3)- to the sum of (1→6)- and (1→4)-α-D-glucosyl linkages in the water-insoluble polysaccharides.

DISCUSSION

The water-insoluble α-D-glucan-synthesizing strains of oral *S. salivarius* were tentatively divided into three groups, based on the ability to produce insoluble deposits on glass (Table II). All but one of the heavy-adherence-producing strains were the R colony formers (see Table I) which possessed a higher ability to synthesize IGs (Table III and Fig. 2). Previous studies have demonstrated that the R colony formers, when grown in sucrose broth, produce firmer adhering and heavier microbial cell deposits on wire or glass, as compared to the M or S colony formers of lower IG-synthesizing ability[23]. The present observations confirm that sucrose-dependent *in vitro* "plaque" formation of *S. salivarius* strains directly depends on the ability to synthesize insoluble α-D-glucans from sucrose[3,4,8,16], in analogy to the cariogenic "*mutans*" streptococci.

However, the production and properties of the insoluble α-D-glucans of *S. salivarius* appear to be not completely identical to those of "*mutans*" streptococci. We have previously shown that the D-glucan-synthesizing enzyme (D-glucosyltransferase) activity of *S. salivarius* is detected only in an extracellular state but not in a cell-bound form[23]. In addition, the amount and ability of *S. salivarius* IPs to adhere to surfaces were lower (Table III), and none of these IPs possessed the ability to agglutinate cells of the "*mutans*" streptococci and *S. salivarius* except homologous one (Table V).

The most striking difference between the IGs of these two species of streptococci is the presence of α-D-(1→4) linkage and probably α-D-(1→3,4) branch linkage in *S. salivarius* IGs (Table VII). However, these α-D-glucosyl linkages, in addition to the α-D-(1→6), -(1→3), and (1→3,6) linkages found in common with the IGs of "*mutans*" streptococci, might not be present in one polysaccharide molecule. This preliminary study has demonstrated that one of the partially purified glucosyltransferase isoenzymes synthesizes insoluble α-D-glucan consisting only of α-D-(1→3) and -(1→6) linkages together with α-D-(1→3,6) branches which, as a major component of the *S. salivarius* IPs as suggested by the results of methylation analysis (Table VII), is basically similar to the tenacious insoluble α-D-(1→3)-glucans of "mutans" streptococci. If this is the case, then another component, which might contain α-D-(1→3) and -(1→4) linkages with α-D-(1→3,4) branches, would be a minor component unique to *S. salivarius*. Furthermore, the (1→3)-α-D-glucosyl linkage content was remarkably higher for the *S. salivarius* IGs than for the "*mutans*" IGs (Table VII); the α-D-(1→3) linkage content of the IGs of the moderate- and slight-adherence producers was higher than the highest value (66 mol per 100 mol for the strain 6715) of the "*mutans*" IGs[33], and even the lowest value, which was found for the heavy-adherence producers, is almost equal to the average value (55 mol per 100 mol) for the "*mutans*" IGs[33]. These results agree with the finding that susceptibility to hydrolysis by mutanase was clearly higher for the *S. salivarius* IPs than for the "*mutans*" IGs (Table VI and Fig. 3).

The detection of significant mutanase-susceptibility in all the *S. salivarius* IPs

(Table VI and Fig. 3) strongly suggest that (1→3)-linked α-D-glucopyranosyl residues form a linear sequence in the α-D-glucan molecules. (1→6)-Linked α-D-glucopyranosyl residues also appear to be linearly linked, but the average length of the chain may be considerably shorter than that of the α-D-(1→3)-linked chain, as shown by the lower α-D-(1→6) linkage content (Table VII) and the lower dextranase-susceptibility (Table VI). These observations are highly consistent with the finding that none of the *S. salivarius* IPs tested showed significant *"mutans"* streptococcus cell-agglutinating activity (Table V). A long sequence of α-D-(1→6)-linked glucopyranosyl residues has been thought to be responsible for the agglutinating properties of *"mutans"* α-D-glucans[31,34,35].

IPs (IGs) of the heavy-adherence-producing *S. salivarius* strains and the *"mutans"* streptococcal strain showed nearly equal α-D-(1→3) linkage contents (Table VII), as well as ratios of α-D-(1→3) to α-D-(1→6), and α-D-(1→3) to α-D-(1→4) plus α-D-(1→6) linkages (Fig. 5). However, the adherence of *S. salivarius* cells in sucrose broth[23] and their IP products from sucrose (Table II) on wire, glass, or both was much weaker than that of *"mutans"* streptococci. The observation by Guggenheim[3] that soluble D-fructans (levan) formation might interfere with the colonisation of smooth surfaces by insoluble D-glucan-dependent *"mutans"* streptococci suggests that simultaneous production of polysaccharide molecules having a different structure, such as an α-D-(1→4)-linked and α-D-(1→3,4)-branched one, would inhibit the adherence or adhesion of the *"mutans"* insoluble, α-D-glucan-like, and α-D-(1→3)-linked component of the *S. salivarius* IGs.

An additional explanation for the lower IPs adherence of the moderate- or slight-adherence producers, the IPs of which contains extremely high proportions of α-D-(1→3) linkage and accordingly a lower content of α-D-(1→6) linkage (Table VII), may be the following. It has been demonstrated that the abundant and strong adherence of the insoluble α-D-glucans of "*mutans*" streptococci on surfaces is lost when the proportion of α-D-(1→3) to -(1→6) linkages is shifted to extremely high values, for example, by the presence of dextranase[30,36].

The IGs of the heavy-adherence producers contain, relatively, low proportion of α-D-(1→3) linkage and high proportions of α-D-(1→6) and a D-(1→3,6) linkages (Fig. 2), but showed a higher susceptibility to hydrolysis by both mutanase and dextranase than those of the other strains (Table VII and Fig. 3). These findings suggest differences for other physico-chemical properties of the IPs between the heavy-adherence producers and the others.

Although the same amount of enzyme activity (as determined radioisotopically) was employed for all strains, the total amounts of polysaccharide synthesized varied among the strains (Table III); it was generally lower for the strains showing a higher production of soluble β-D-fructans. This may be due to the fact that a significant proportion of the SPs had such a low molecular size (not shown) that they could not be precipitated by the 75% ethanol solution used for harvest[37]. It should be kept in mind that the structural features and properties of the *S. salivarius* polysaccharides (mainly IGs) discussed herein are not those of a pure, homogeneous

polymer but those of a heterogeneous mixture as might be found in the oral cavity. As an attempt to clarify the detailed features of the polysaccharide components, the study of the products obtained by separate glucan-synthase isoenzymes of one *S. salivarius* strain will be reported elsewhere.

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