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Fractionation and Properties of Glucans Produced by *Streptococcus mutans*

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Water-insoluble (ISG) and water-soluble (SG) fractions of glucans produced by cell-free glucosyltransferase of *Streptococcus mutans* AHT (serotype *g*) were isolated by centrifugation at 20,000 × *g* for 15 min. No further resolution of slightly sonicated ISG was observed with gel filtrations on any Bio-Gel beads, including A-50m. Bio-Gel P-100 filtration subdivided SG into two fractions with higher and lower molecular weights (designated SG-A and SG-B, respectively). SG-A was further resolved into two subfractions, SG-A-I and SG-A-II, by 10 to 40% and 50 to 80% ethanol precipitation, respectively. Relative amounts of ISG, SG-A-I, SG-A-II, and SG-B were 66.3:9.4:4.4:19.9. The molecular sizes of these fractions were $>1.5 \times 10^7$, $\geqq 1.5 \times 10^7$, $\leqq 5 \times 10^6$ ($>1 \times 10^5$), and $\leqq 1 \times 10^4$ daltons, and their α-1,3 glucosidic linkage contents were approximately 35, 35, 16, and 4% for fractions ISG, SG-A-I, SG-A-II, and SG-B, respectively. Both ISG and SG-A-I were resistant to hydrolysis by dextranase and possessed the ability to aggregate with concanavalin A and to agglutinate *S. mutans* cells. SG-A-II had extremely low dextranase susceptibility and significant agglutinating activities, whereas SG-B showed high dextranase sensitivity and neither aggregating nor agglutinating activity. These results indicate that SG of *S. mutans* AHT consists of three types of glucans with distinctly different molecular sizes and chemical structures and strongly suggest that the ISG and SG-A-I fractions are different physical states of an inherently identical glucan. Preliminary observations suggest that the glucans produced by other *S. mutans* strains of several serotypes may be similarly classified.

Glucans produced from sucrose by *Streptococcus mutans* are usually classified into two fractions, water insoluble and water soluble (5, 9). Recently, several investigators have presented results indicating that the water-soluble glucan fraction (SG) from some *S. mutans* strains is very polydisperse (1, 3, 16, 20) and can be subdivided into two fractions with different molecular sizes and chemical structures (1, 19). However, detailed information on the properties of these fractions is limited, and a full classification of *S. mutans* glucans has not yet been established.

In the course of a study on the mechanism of adherence of *S. mutans*, we obtained preliminary results indicating that water-soluble glucans produced by several *S. mutans* strains can be resolved into more than two fractions and that one of the subfractions possesses properties similar to a water-insoluble glucan obtained simultaneously (15). Therefore, we have attempted an extensive reinvestigation in order to classify the glucans of *S. mutans*. The present paper describes the fractionation of *S. mutans* AHT (serotype *g*) glucans into one water-insoluble fraction (ISG) and three SG fractions and describes several important properties of the isolated fractions.

(Preliminary reports of this work were presented at the 26th Annual Meeting of the Japanese Division, International Association for Dental Research, Odawara, Japan, 2-3 December 1978.)

MATERIALS AND METHODS

Bacterial strains and cultural conditions. *S. mutans* AHT (serotype *g*; 11) was mainly used. In some experiments, representative strains of serotypes *a* through *g* listed in Table 6 were also used. They were provided through the courtesy of D. D. Zinner (University of Miami, Miami, Fla.), R. J. Fitzgerald (National Institute of Dental Research, Bethesda, Maryland), B. Krasse (University of Lund, Malmö, Sweden), B. Guggenheim (University of Zurich, Zurich, Switzerland), R. J. Gibbons (Forsyth Dental Cen-

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ter, Boston, Mass.), and S. Hamada (Osaka University, Osaka, Japan).

The strains were grown in a Trypticase (BBL Microbiology Systems)-salt broth (7) supplemented with glucose at a final concentration of 0.5% or in broth in which glucose was replaced by 5.0% sucrose (13).

Synthesis and fractionation of glucans. Cell-free supernatant solution was obtained from glucose-grown cultures of the strains by centrifugation (6,000 × *g*, 20 min, 4°C) and adjusted to pH 6.0 with a few drops of 4 N NaOH or HCl. Two milliliters of the cell-free enzyme preparation was allowed to react with 300 mg of sucrose in 6 ml of 0.1 M potassium phosphate buffer (pH 6.0) containing 0.02% Merthiolate. After incubation at 37°C for 16 h, the assay digest was centrifuged (20,000 × *g*, 15 min, 4°C) to harvest ISG and water-insoluble fructan (Fig. 1). SG and water-soluble fructan were precipitated by the addition of 2.5 volumes of ethanol to the supernatant solution followed by storage for 2 h in a freezer (13). Both of the polysaccharide fractions were washed three times with 0.05 M phosphate buffer (pH 6.0) by centrifugation or by 70% ethanol precipitation, respectively, and then suspended or solubilized in the buffer to give a concentration of 1 mg of glucose or fructose equivalent per ml.

Three classes of glucans were produced by sucrose-grown *S. mutans* AHT: (i) extracellular, water soluble (ESG); (ii) 1 N NaOH soluble, cell associated; and (iii) 1 N NaOH insoluble, cell associated; they were prepared according to the procedure of Freedman and Tanzer (4).

Sonic treatment of ISG. A 1-mg (glucose equivalent)/ml solution of ISG in 0.05 M phosphate buffer (pH 6.0) was sonicated at 150 W (5 A) for the appropriate time at 4°C with a Super Sonic Vibrator (UR-150P, Tominaga Sheisakusho Ltd., Tokyo, Japan).

Gel filtration of glucans. A portion (0.4 ml) of an approximately 1-mg (glucose equivalent)/ml solution of SG and ESG or a sonicated suspension of ISG (small aggregates and all) was put on a small column (9 by 250 mm) of various types of Bio-Gel beads (Fig. 1). The column was eluted with 0.05 M phosphate buffer (pH 6.0) at a flow rate of 0.17 ml/min at room temperature. Fractions of 0.55 to 0.6 ml were collected and quantitated for glucan and fructan contents as described below. Gel beads used were Bio-Gel P-100 (100 to 200 mesh; fractionation range, 5,000 to 100,000 daltons; Bio-Rad Laboratories, Richmond, Calif.), A-1.5m (<10,000 to 1,500,000 daltons), A-5m (10,000 to 5,000,000 daltons), A-15m (40,000 to 15,000,000 daltons), and A-50m (100,000 to 50,000,000 daltons). The columns were standardized by filtration of a mixture (0.4 ml) consisting of Dextran T2000 (Pharmacia Fine Chemicals AB, Uppsala, Sweden) and glucose at concentrations of 0.5 mg/ml each.

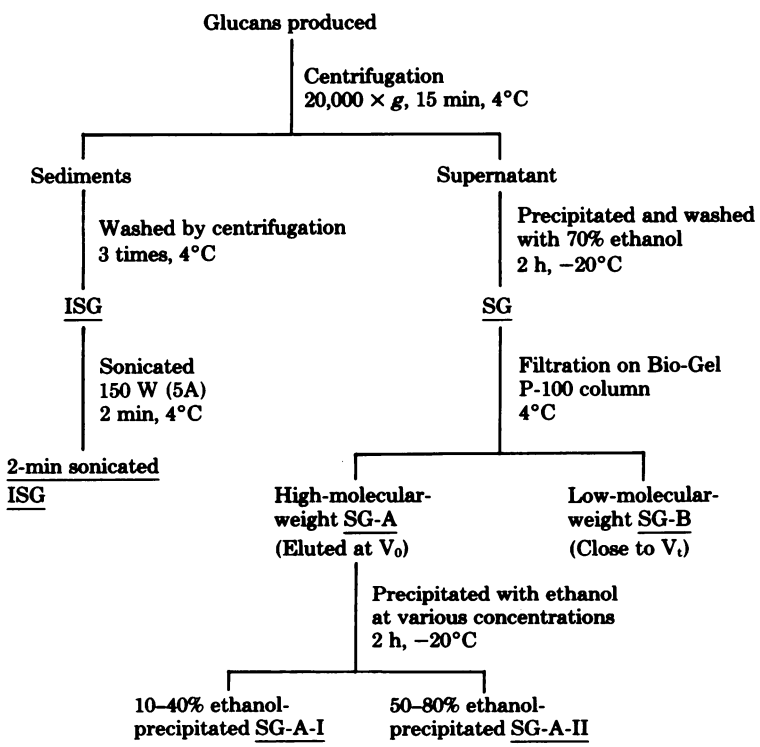

FIG. 1. *Flow diagram of fractionation of glucans. Two milliliters of cell-free glucosyltransferase of S. mutans AHT was allowed to react with 300 mg of sucrose in 6 ml of 0.1 M potassium phosphate buffer (pH 6.0) containing 0.02% Merthiolate at 37°C for 16 h. All subsequent procedures were done by using 0.05 M phosphate buffer (pH 6.0).*

Precipitation of glucans with ethanol. One-tenth milligram of glucose equivalent of SG and ESG was solubilized in 1 ml of 0.05 M phosphate buffer (pH 6.0) and reprecipitated as described above by the addition of ethanol to give final concentrations ranging from 0 to 80% (Fig. 1). Precipitates were recovered by centrifugation (20,000 × *g*, 15 min, 4°C), dried in vacuo, and redissolved in 1 ml of buffer.

Aggregation of glucans with concanavalin A. Aggregation of glucans was measured by the method described by Goldstein et al. (8). Various amounts (0 to 400 μg of glucose equivalent) of glucans were allowed to react with 2 mg of concanavalin A (type III, Sigma Chemical Co., St. Louis, Mo.) in 0.4 ml of 0.1 M citrate buffer (pH 5.2) and 0.1 phosphate buffer (pH 7.0). After incubation at 25°C for 10 min, the turbidity yielded was measured at 420 nm with a Hitachi-Perkin-Elmer spectrophotometer (model 139, Hitachi Seisakusho, Tokyo, Japan).

Cell-agglutinating activity and sensitivity to dextranase. Ability of the glucan fractions to agglutinate *S. mutans* cells and susceptibility to the hydrolytic action of dextranase were determined by the method reported earlier (15). Resting cells used for the agglutination test were prepared from a glucose-grown culture of *S. mutans* AHT. A preparation of dextranase (Andard-Mount Co., Middlesex, England) was a gift from K. Yokogawa of Dainippon Pharmaceutical Co. Ltd., Osaka, Japan.

Analysis of glucosidic linkages. Periodate oxidation and borohydride reduction of glucans were performed by the method of Hamilton and Smith (12). Degraded end products were trimethylsilylated in the usual way and analyzed by gas-liquid chromatography as described elsewhere (13).

Colorimetric quantitation of glucans and fructans. The amount of glucans was determined by the anthrone method (21). Differential quantitation of glucan and fructan was performed with anthrone by the procedure described by Halhoul and Kleinberg (10), using glucose and fructose as standards, respectively.

RESULTS

Fractionation of glucans. Glucans produced from sucrose by cell-free glucosyltransferase of *S. mutans* AHT were separated by centrifugation (20,000 × *g*, 15 min) into two fractions, ISG and SG. The relative amount of ISG to SG was 66.3:33.7.

A suspension of ISG in 0.05 M phosphate buffer (pH 6.0) formed various sizes of aggregates. Vigorous vibration with a Vortex mixer did not disaggregate the clumps. Sonic oscillation for 2 min provided a macroscopically homogeneous suspension and was accompanied by only slight (ca. 13%) "solubilization" (not sedimented by centrifugation). The 2-min sonicated ISG consistently produced a single peak at the void volume of columns when fractionated on various kinds of Bio-Gel beads (Fig. 2a–e). Recoveries of ISG were 51, 91, 84, 69, and 59% for Bio-Gel P-100, A-1.5m, A-5m, A-15m, and A-50m columns, respectively (data not shown). This 2-min treatment did not significantly degrade ISG molecules since they remained highly active in the aggregation of concanavalin A and the agglutination of resting *S. mutans* cells (see below). Longer sonic treatment for 5 to 30 min solubilized 40 to 70% of ISG (data not shown) with the formation of a low-molecular-weight fraction (Fig. 2f). The 30-min sonication markedly diminished the ability of ISG to aggregate concanavalin A and destroyed all cell-agglutinating activity (data not shown). For these reasons, ISG used throughout the experiments was sonicated for 2 min.

A Bio-Gel P-10 filtration subdivided SG into two fractions, one being eluted at the void volume and the second close to the total volume of the column (designated SG-A and SG-B, respectively; Fig. 2a). Gel filtration on columns of Bio-Gel A-1.5m through A-50m also showed separation of SG into two major peaks with high and low molecular weights, each corresponding to the SG-A and SG-B fractions obtained with the P-100 column (Fig. 2b to e). Recoveries of SG from the columns were higher than 84% (Table 1). Relative amounts of the high-molecular-weight fraction in total recovered SG were approximately 41, 37, 28, 27, and 18% for Bio-Gel P-100, A-1.5m, A-5m, A-15m, and A-50m columns, respectively (Table 1), suggesting that 65 to 69% of SG-A has a molecular weight higher than 15×10^6 and 31 to 35% has a molecular weight lower than 5×10^6, when it is assumed that all of the SG applied was recovered. This was confirmed by the observation that fraction SG-A obtained by the P-100 gel filtration chromatography was further resolved into two fractions by precipitation with ethanol at various concentrations (Fig. 3). Approximately 68% of SG-A was precipitated at 10 to 40% ethanol concentrations (SG-A-I), and the remaining 32% was recovered at 50 to 80% concentrations (SG-A-II). SG-B having a distinctly smaller molecular size than the SG-A fraction remained soluble in ethanol solutions of less than 40%, but was precipitated in increasing amounts as the ethanol concentration was increased up to 80% (Fig. 3). The proportions of SG-A-I and SG-A-II are nearly identical to those of the high- and low-molecular-weight fractions of SG-A calculated from the results obtained by gel filtration (Fig. 2). Fraction SG-A-I (precipitated at a 20% ethanol concentration) was eluted in the void volume of the Bio-Gel A-15m column, whereas SG-A-II (precipitated at a 50% ethanol concentration) was distributed as a single broad peak between the V_0 and V_t of the column (data not shown).

Relative amounts of isolated fractions of glu-

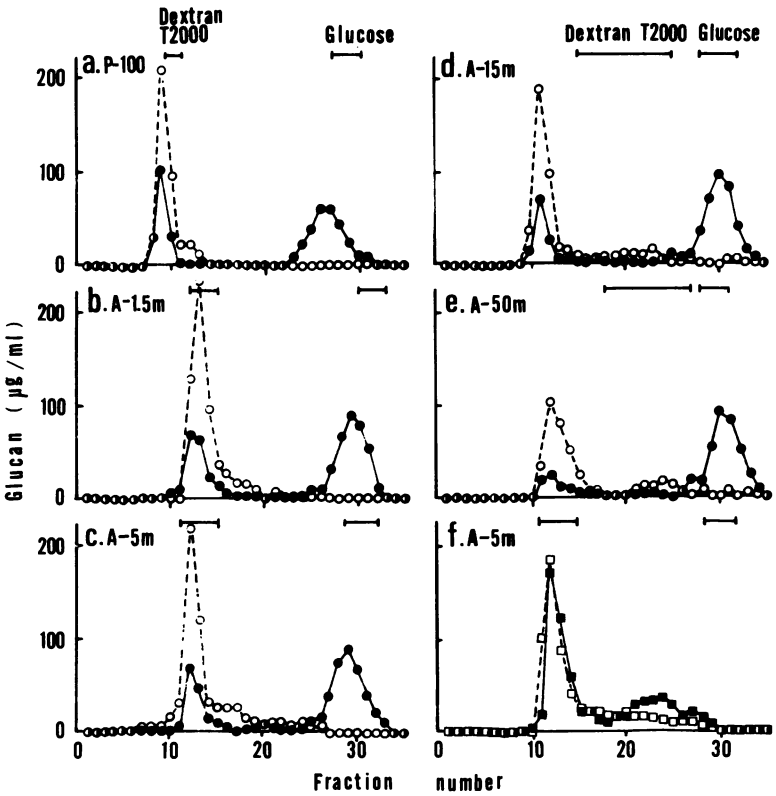

FIG. 2. *Gel filtration with various types of Bio-Gel beads of the ISG and SG produced by cell-free enzyme of S. mutans AHT. One milligram (glucose equivalent) of the ISG or SG fraction was suspended or dissolved in 1 ml of 0.05 M phosphate buffer (pH 6.0). A suspension of ISG was sonicated for 2 to 30 min at 150 W (5A) at 4°C. A portion (0.4 ml) of the suspension or solution was put on a column (9 by 250 mm) of the Bio-Gel beads indicated. The column was eluted with 0.05 M phosphate buffer (pH 6.0) at a flow rate of 0.17 ml/min at room temperature, and fractions of 0.6 ml were collected. Symbols: a through e—○, ISG sonicated for 2 min; ●, SG; (f) ISG sonicated for 5 (□) and 30 (■) min. The high- and low-molecular-weight fractions of SG obtained with the P-100 column were designated SG-A and SG-B, respectively.*

TABLE 1. *Fractionation of SG-A on various types of Bio-Gel beads and distribution of molecular size of glucans contained in the SG-A fraction*

Type of Bio-Gel beads	Recovery from column (%)	Amt of fractions in void volume		Distribution in molecular wt of SG-A	
		% of recovered SG	% of SG-A	Molecular wt ($\times 10^6$)	Relative amt (%)
P-100	84.4	40.8	100[a]	0.1-1.5	10.3
A-1.5m	100.9	36.6	89.7	1.5-5	20.8
A-5m	102.8	28.1	68.9	5-15	3.4
A-15m	98.1	26.7	65.4	15-50	20.3
A-50m	85.4	18.4	45.1	>50	45.1

[a] By definition, SG-A is the glucan peak excluded from Bio-Gel P-100 beads.

cans produced by cell-free glucosyltransferase are summarized in Table 2. Amounts of the three classes of glucans isolated from a 5% sucrose-grown culture of strain AHT are also shown. Alkali-soluble, cell-associated glucan and ESG were obtained in proportions of about 76:24, indicating that a significant difference exists between SG produced by cell-free enzyme (34%) and the corresponding fraction ESG produced by growing cells (24%; the amount of alkali-insoluble, cell-associated glucan fraction has been ignored, since it includes cellular components of *S. mutans*). Like SG, ESG was resolved into two peaks by Bio-Gel P-100 column chromatography (the relative amounts of the high[ESG-A]- to low-molecular-weight peaks were 26:74), but unlike SG, almost all (89%) was recovered in the low-molecular-weight peak when fractionated on Bio-Gel A-15m (Fig. 4), only 11% appearing in the excluded high-molecular-weight fraction. ESG-A was not precipitated in 10 to 30% ethanol solutions (Fig. 3). Thus, ESG produced by sucrose-grown cells was devoid of a fraction cor-

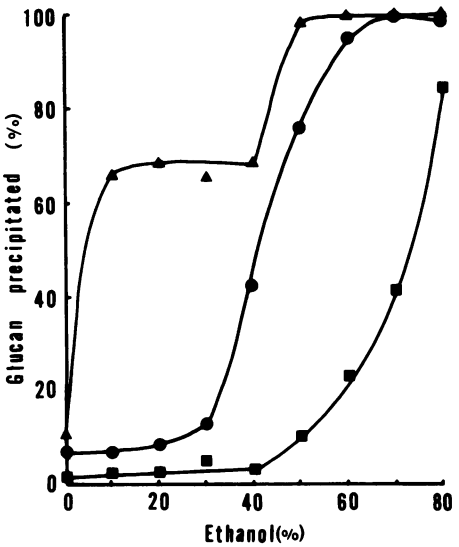

FIG. 3. *Precipitation of fractions SG-A, SG-B, and ESG-A at various concentrations of ethanol. One-tenth milligram (glucose equivalent) of the fractions, SG-A (▲), SG-B (■), and ESG-A (●), was dissolved in 1 ml of 0.05 M phosphate buffer (pH 6.0), reprecipitated by the addition of ethanol to give the final concentrations indicated, and then stored at −20°C for 2 h. Precipitates were harvested by centrifugation (20,000 × g, 15 min, 4°C) and redissolved in 1 ml of buffer. The fraction precipitated at a 20% ethanol concentration from SG-A and the fraction precipitated from the resulting supernatant at a 50% concentration were designated SG-A-I and SG-A-II, respectively.*

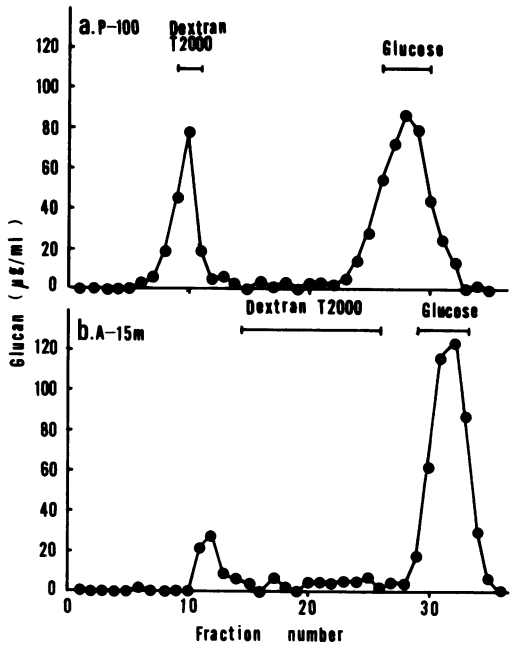

FIG. 4. *Gel filtration of fraction ESG obtained from a sucrose-grown culture of S. mutans AHT. Fraction ESG was prepared from a 5% sucrose-grown culture of S. mutans AHT according to the method described by Freedman and Tanzer (4). Conditions for elution were the same as those for Fig. 1. Fractions of 0.58 ml were collected. The high-molecular-weight fraction obtained with the P-100 column was designated ESG-A.*

TABLE 2. *Relative amounts of various classes of glucans produced by S. mutans AHT*

Glucans synthesized:	Fraction	mg[a]/ml of enzyme or culture	% of total glucan
By cell-free enzyme	ISG	0.52	66.3
	SG	0.26	33.7
	SG-A-I	0.07	9.4
	SG-A-II	0.03	4.4
	SG-B	0.16	19.9
In sucrose-grown culture	Cell associated	2.10	80.5 (—)[b]
	1 N NaOH insoluble	0.47	18.0 (—)
	1 N NaOH soluble	1.63	62.5 (76.2)
	ESG	0.55	19.5 (23.8)

[a] Milligrams of glucose equivalent.
[b] Alkali-insoluble fraction not included. Numbers in parentheses are percentage of 1 N NaOH-soluble glucan plus ESG.

responding to SG-A-I produced by cell-free glucosyltransferase.

Properties of the glucan fractions. ISG and SG-A-I contained a large proportion (approximately 35%) of α-1,3-linked glucose residues (Table 3). SG-A-II also contained a significant amount (ca. 16%) of α-1,3 linkage. Almost all glucose residues (ca. 96%) of SG-B were α-1,6 linked.

Figure 5 depicts the sensitivity of the glucan fractions to dextranase. SG-B was almost completely degraded by dextranase. Hydrolysis of SG-A-II by the enzyme was evident, but only to an extremely limited extent. Hydrolysis of ISG and SG-A-I was not detected.

Both ISG and SG-A-I exhibited high aggregation activity with concanavalin A (Fig. 6). SG-A-II aggregated with the lectin to a lower extent, but SG-B had almost no aggregating activity.

SG-A-I possessed greatest ability to agglutinate resting *S. mutans* cells (Table 4). ISG sonicated for 2 min was a little less active than SG-A-I, but when sonicated for only 1 min it was equally as active (data not shown). The cell-

TABLE 3. *Glucosidic linkage contents of various classes of glucans produced by cell-free enzyme of S. mutans AHT*

Glucan fraction	Glucosidic linkage content (%)		
	α-1,6 α-1	α-1,4 α-1,2	α-1,3 α-1,3,6
ISG[a]	64.5	0.5	35.0
SG-A-I	64.2	0.4	35.4
SG-A-II	83.9	0.1	16.0
SG-B[b]	95.8	0.2	4.0

[a] Sonicated for 2 min at 150 W (5 A).

[b] Calculated on the basis of the glucosidic linkage contents of fractions SG and SG-A (not shown) and the relative amounts of fractions SG-A and SG-B (Table 2).

agglutinating activity of SG-A-II also was high, but that of SG-B was extremely low.

Gel filtrations of water-soluble polysaccharides produced by cell-free enzyme of several *S. mutans* strains. In preliminary studies of other *S. mutans* strains belonging to the same or different serotypes, gel filtration profiles of the water-soluble polysaccharide fraction produced by selected strains of serotypes *a* through *g* were compared.

Amounts of water-insoluble and -soluble glucans and fructans produced by cell-free enzyme obtained from a glucose-grown culture of the strains tested are summarized in Table 5. The enzymes of all the strains except BHT (serotype *b*), which has no ability to adhere to glass when grown in a sucrose broth (unpublished observation), produced significant amounts of ISG and all synthesized SG. Fructan was not detected in any of the insoluble products, but significant amounts of water-soluble fructan were produced by enzymes of serotype *b*, *c*, *e*, and *f* strains.

The water-soluble polysaccharide fractions were fractionated on Bio-Gel P-100 and A-15m columns. Typical elution profiles are depicted in Fig. 7. The soluble glucan of HS-6 (serotype *a*) was resolved into two peaks with high and low molecular weights on both columns, as was SG of AHT (serotype *g*; Fig. 7a). A similar profile was obtained with the soluble glucan of another type *g* strain, K1-R, and a type *d* strain, OMZ176 (not shown). The soluble glucan of strain OMZ175 of serotype *f* also seemed to belong to this category (Fig. 7b). In contrast, the SG produced by strains IB (serotype *c*) and LM7 (serotype *e*) were resolved into several peaks distributed between the V_0 and V_t of the A-15m column, though they were separated into only two fractions with different molecular sizes on the P-100 column (Fig. 7c). The glucan of BHT (serotype *b*) produced many peaks even with the P-100 column (Fig. 7b). Water-soluble fructans synthesized by several strains of some serotypes were also resolved into distinctly different high- and low-molecular-weight peaks (see Fig. 7b and d).

DISCUSSION

The glucans produced from sucrose by cell-free glucosyltransferase of *S. mutans* AHT (serotype *g*) were separated into one water-insoluble fraction and three water-soluble fractions by the combination of differential centrifugation at 20,000 × *g* for 15 min, gel filtration on a Bio-Gel P-100 column, and precipitation with 10 and 50% ethanol.

Fractions ISG and SG-A-I were similar in all the properties examined except water solubility. Both possessed a molecular weight >15 × 10^6 (Fig. 2), contained high proportions (ca. 35%) of α-1,3-linked glucose residues (Table 3), and were totally resistant to the action of dextranase (Fig. 5). They were almost equivalent in their abilities to rapidly aggregate concanavalin A (Fig. 6) and

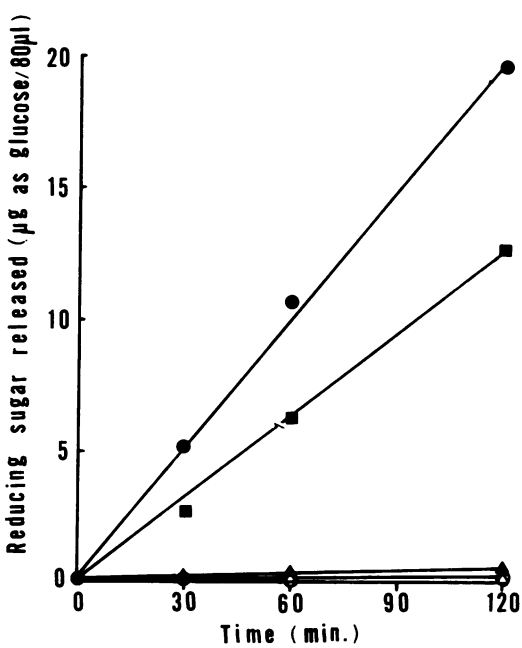

FIG. 5. *Hydrolysis of various classes of glucans by dextranase. The glucans (80 μg of glucose equivalent) were incubated with 2.8 mU of dextranase in 80 μl of 0.1 M citrate buffer (pH 5.5) at 37°C. After an appropriate incubation time, the enzyme reaction was terminated by the addition of 20 μl of 0.5 N NaOH, and reducing sugar released was determined by the Somogyi-Nelson method. Symbols: ○, ISG sonicated for 2 min; △, SG-A-I; ▲, SG-A-II; ■, SG-B; ●, Dextran T250 (Pharmacia).*

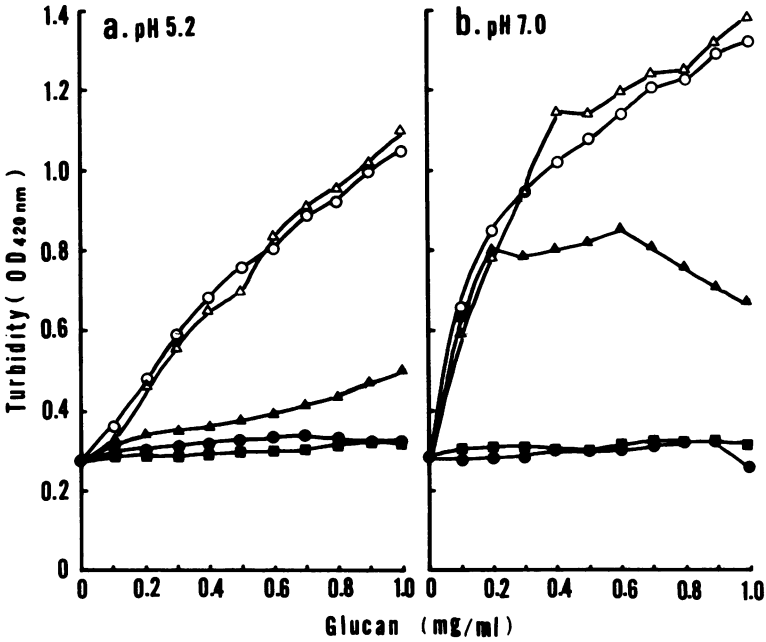

FIG. 6. *Aggregation of various classes of glucans with concanavalin A. Various amounts (0.04 to 0.4 mg of glucose equivalent) of the glucans were allowed to react with 2 mg of concanavalin A (type III, Sigma) in 0.4 ml of (a) 0.1 M citrate buffer (pH 5.2) or (b) 0.1 M phosphate buffer (pH 7.0) at 25°C for 10 min. Turbidity was read at 420 nm (OD, optical density). For symbols, see legend to Fig. 5.*

TABLE 4. *Abilities to agglutinate S. mutans cells of various classes of glucans produced by cell-free enzyme of S. mutans AHT*

Glucan fraction	Agglutination titer[a] at glucan concn (mg of glucose equivalent/ml) of:												
	2	2^0	2^{-1}	2^{-2}	2^{-3}	2^{-4}	2^{-5}	2^{-6}	2^{-7}	2^{-8}	2^{-9}	2^{-10}	2^{-11}
ISG[b]	3+	3+	2+	1+	1+	1+	1+	1+	1+	–	–	–	–
SG-A-I	3+	3+	3+	3+	3+	2+	2+	2+	1+	1+	1+	±	–
SG-A-II	2+	2+	1+	1+	1+	1+	1+	1+	1+	1+	–	–	–
SG-B	1+	±	–	–	–	–	–	–	–	–	–	–	–

[a] Cell agglutination was scored as – (no agglutination) to 3+ (marked agglutination).
[b] Sonicated for 2 min at 150 W (5 A).

agglutinate resting *S. mutans* cells (Table 4). In addition, a significant portion of ISG was "solubilized" by mild sonic treatment, and the resulting homogeneous suspension could not be macroscopically differentiated from the SG-A-I solution. A solution of SG-A-I assumed an opaque milk-white color, as did ISG suspensions, and formed minute precipitates during long-term storage in a refrigerator (unpublished observation). These observations strongly indicate that ISG and SG-A-I may be inherently identical and that ISG is derived from SG-A-I by aggregation of the glucan molecules in aqueous solution to form an insoluble precipitate. It has been reported that parts of the water-soluble polysaccharide fraction produced from sucrose by the cell-free glucosyltransferase of many *S. mutans* strains become "insoluble" when redissolved in water ("soluble polysaccharide II"; 2).

Fraction ESG produced by sucrose-grown AHT cells (more than half of the detectable activity of glucosyltransferase in the culture is cell bound; 18) was almost devoid of a subfraction corresponding to the SG-A-I fraction of the glucan produced by cell-free enzyme (Fig. 3 and 4). However, the high cell-agglutinating activity of SG-A-I (Table 4) would almost certainly make it a component of the cell-associated fraction in sucrose-grown cultures, and, indeed, the sum of the SG-A-I and ISG fractions represents a proportion of the total glucan almost identical to that of the alkali-soluble, cell-associated glucan

TABLE 5. *Amounts of water-insoluble and -soluble glucans and fructans produced by cell-free enzyme from various strains of S. mutans*

Strains[a]	Serotype	Polysaccharides produced (μg of glucose equivalent/ml of enzyme)			
		Water insoluble		Water soluble	
		Glucan	Fructan	Glucan	Fructan
HS-6	*a*	969	0	465	36
BHT	*b*	0	0	156	138
IB	*c*	480	0	1,803	186
OMZ176	*d*	342	0	312	9
LM7	*e*	501	0	1,161	459
OMZ175	*f*	156	0	894	630
K1-R	*g*	825	0	246	21
AHT	*g*	519	0	264	9

[a] Strains were kindly provided by: D. D. Zinner (BHT, AHT), R. J. Fitzgerald (HS-6, K1-R), B. Krasse (IB), B. Guggenheim (OMZ176), R. J. Gibbons (LM7), and S. Hamada (OMZ175).

formed in the sucrose-grown culture (Table 2). This supports the identity of the glucans in fractions ISG and SG-A-I. It has been demonstrated that the cell-associated, alkali-soluble glucan fraction obtained from sucrose-grown cultures of several *S. mutans* strains contains a subfraction which becomes "soluble" in water (not reprecipitated by centrifugation) in addition to a water-insoluble subfraction (3, 17). However, it remains possible that despite their similar content of α-1,3 linkages (Table 3), they are glucans possessing distinct structures. Thus, SG-A-I may be an essentially water-soluble polysaccharide similar to the soluble dextran, produced by *Leuconostoc mesenteroides* strain B-1355, which contains a large proportion of α-1,3 linkages alternating with α-1,6-glucosidic linkages (A. Misaki, M. Torii, T. Sawai, and I. Goldstein, Carbohydr. Res., in press).

Fraction SG-A-II is certainly distinct from ISG and SG-A-I in all properties examined in the present study. A solution of this fraction is colorless and translucent. It has a molecular weight ranging from 5×10^6 (Table 1) to 3×10^5, the lower limit being indicated by ultrafiltration of fraction SG-A (15). The significant content of α-1,3-linked glucose residues in addition to α-1,6-linked residues (Table 3) and the relatively high reactivity with concanavalin A of this glucan (Fig. 6) suggest that it has a branched structure. Thus, SG-A-II can be considered to be a water-soluble fraction distinct from the water-soluble SG-B fraction, which is a dextranase-sensitive, linear α-1,6 glucan possessing a molecular weight of approximately 10^4 or less (Fig. 2, 5, and 6; Tables 3 and 4).

These results suggest that glucans of *S. mutans* could be classified into three categories, one water-insoluble fraction of extremely high molecular weight (ISG plus SG-A-I) and two water-soluble fractions with higher and lower molecular weights (SG-A-II and SG-B, respectively). The relative amounts of ISG–SG-A-I–SG-A-II–SG-B obtained under the present conditions were 66.3:9.4:4.4:19.9 (Table 2), but recovery of SG from P-100 was critically poor (ca. 84%, Table 1). Furthermore, a recent study (14) shows that the amounts of ISG and SG produced by a single preparation of *S. mutans* glucosyltransferase are significantly affected by the conditions of glucan synthesis. For example, at acidic pH values the relative amount of ISG is increased, whereas at neutral or alkaline pH values higher amounts of SG are produced and recovered, particularly fraction SG-B. Thus, the proportions of the glucan fractions shown above are tentative and cannot be considered an inherent characteristic of all enzyme preparations from *S. mutans* AHT.

The importance of water-insoluble glucans for firm attachment of *S. mutans* to smooth surfaces has been demonstrated (5, 9). The soluble SG-A-I fraction, which is similar to ISG in many important properties, would likewise contribute to adherence of *S. mutans*. Preliminary results indicate that a mutant strain of *S. mutans* AHT retaining the ability to synthesize the SG-A-I glucan feebly adhered to glass in sucrose broth, whereas other organisms that lacked this ability did not adhere. The roles of water-soluble fractions SG-A-II and SG-B in the adherence and cariogenic virulence of *S. mutans* remain obscure at present. The dextranase-insensitive SG-A-II may induce tight cohesion of *S. mutans* cells through its high cell agglutinating activity and, therefore, it may make an important contribution to plaque formation. SG-B, which has low cell-agglutinating activity, is inactivity in this regard. It is reported that high-molecular-weight dextran induces agglutination of *S. mutans* cells, whereas low-molecular-weight dextran does not (6). It is feasible that SG-B might be utilized as one of the energy sources by dextranase-producing microbes in plaques.

The preliminary results shown in Fig. 7 suggest that the new classification of glucans of *S. mutans* AHT (serotype *g*) can be extended to those produced by several other *S. mutans* strains of different serotypes. Reinvestigations of *S. mutans* glucans on the basis of the proposed criteria should provide further insight into mechanisms of establishment and persistence of *S. mutans* on tooth surfaces and the formation of plaque in vivo.

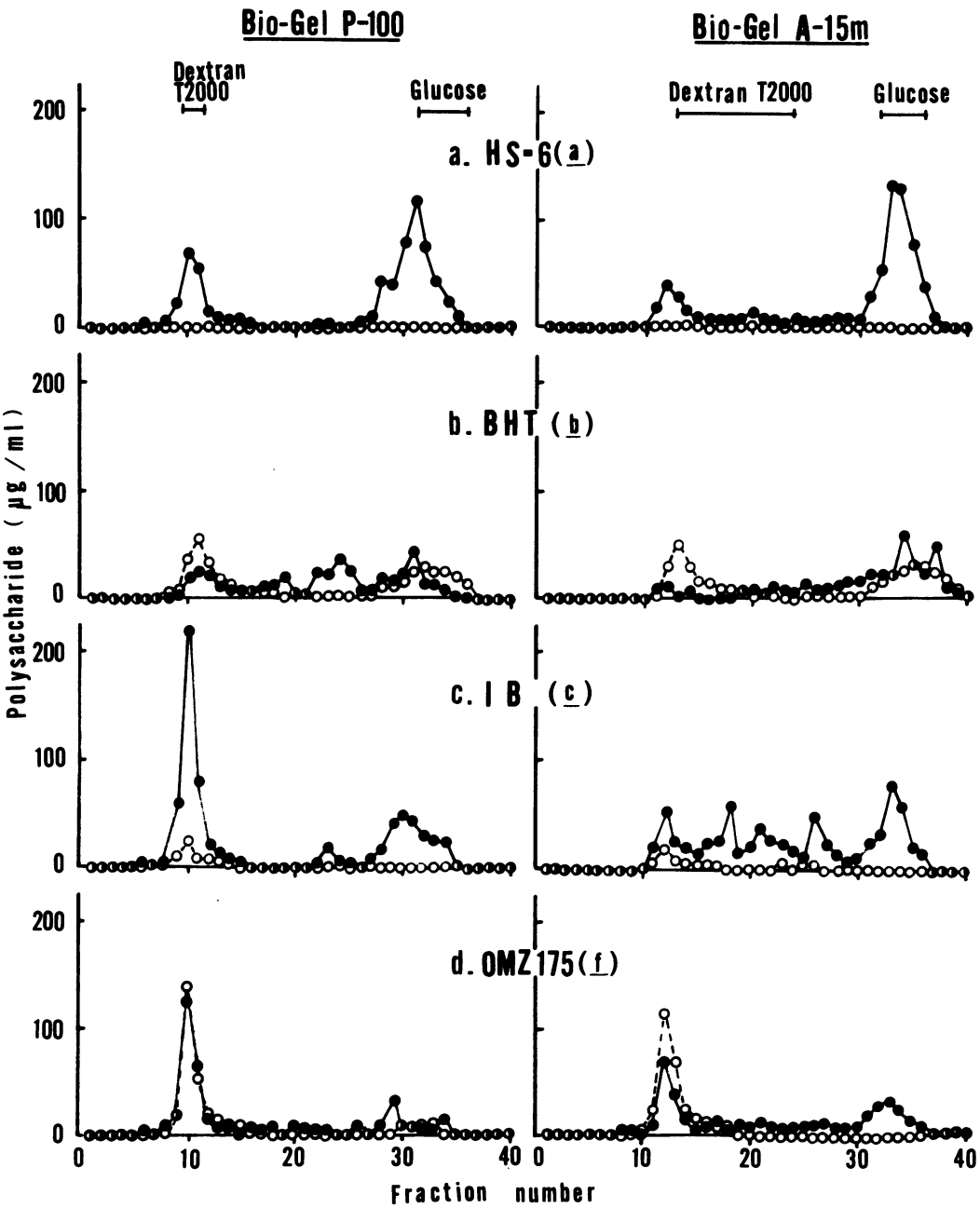

FIG. 7. *Typical gel filtration profiles of water-soluble polysaccharides produced by cell-free enzyme of various S. mutans strains. The water-soluble fraction of polysaccharides synthesized by cell-free enzyme from four strains listed in Table 5 was fractionated on columns (9 by 250 mm) of Bio-Gel P-100 and A-15m under the same conditions as used for Fig. 2, except that fractions of 0.55 ml were collected. Amounts of glucans (●) and fructans (○) were differentially quantitated with anthrone by the method of Haulhoul and Kleinberg (10).*

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