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Purification and Properties of Glucosyltransferase Responsible for Water-Insoluble Glucan Synthesis from *Streptococcus mutans*

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A glucosyltransferase responsible for water-insoluble glucan synthesis was purified from the culture fluids of *Streptococcus mutans* 6715-15 strain by column chromatography on Toyopearl HW-60 and subsequently on hydroxyapatite. The enzyme preparation gave a single band on analysis by polyacrylamide gel electrophoresis. The pH dependency of the activity showed two optimal peaks at 5.8 and 7.3, and the K_m values for sucrose were 1.4 and 3.3 mM at the respective optimal pHs. The molecular weight determined by sodium dodecyl sulfate gel electrophoresis was 180,000. Although the enzyme scarcely synthesized water-insoluble and water-soluble glucans from sucrose, water-insoluble glucan formed from sucrose in the presence of dextran T10 consisted of over 93% α-1,3-glucosidic linkage. Analysis of the structure of water-insoluble glucan indicated that the enzyme catalyzed the formation of branch points in α-1,6-glucan (dextran) and transferred the glucosyl moiety of sucrose to the C-3 position of the branching glucose residue of dextran. Since this enzyme has not yet been registered, we named it mutansynthetase (EC 2.4.1.?).

The extracellular polysaccharides synthesized by *Streptococcus mutans* from sucrose appear to play an important role in the induction of dental caries on the smooth surfaces of teeth (12). Although many investigators have reported that there are two glucosyltransferases responsible for the production of water-insoluble glucan (ISG) and water-soluble glucan (SG) in the culture fluid of *S. mutans* (2, 6, 7, 8, 10, 13, 15, 17), an ISG-synthesizing enzyme has not been purified to electrophoretic homogeneity, and the properties of the enzyme and ISG formed have not been characterized. It is difficult to isolate the enzyme because of its occurrence as part of high-molecular-weight aggregates. Schachtele et al. (18) demonstrated that the aggregates of the enzyme in the culture fluid of *S. mutans* 6715 grown in a chemically defined medium with fructose as the carbon source could be dissociated to a low-molecular-weight form when eluted from a Bio-Gel column in the presence of 1 M NaCl. When this finding was applied to chromatography of Toyopearl HW-60 (Toyo Soda Manufacturing Co., Tokyo, Japan), which was used as a supporter of gel filtration, we were able to isolate the ISG-synthesizing enzyme. This paper describes the purification procedures and some properties of the enzyme of *S. mutans* 6715-15.

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MATERIALS AND METHODS

Bacterium and growth conditions. *S. mutans* 6715-15 was obtained from M. Mori (Osaka Dental University, Osaka, Japan) and grown aerobically for 14 h at 37°C in the 0.2% fructose chemically defined synthetic medium of Terleckyj et al. (21). Under growth conditions, the doubling time was about 1 h. The absorbance of the cell suspension at 660 nm after growth was approximately 1.0.

Crude enzyme preparation. The culture was centrifuged at 10,000 × *g* for 15 min. The culture supernatant fluids were filtered with a membrane filter (0.45 μm; Millipore Corp., Bedford, Mass.) to remove the residual cells, brought to 55% saturation with ammonium sulfate, and allowed to stand for 3 days at 4°C. The precipitates were collected, dissolved in 0.01 M acetate buffer, pH 5.5, and dialyzed against the same buffer. The concentrated fraction was employed as the starting material for further purification. When the polysaccharides synthesized by the crude enzyme preparations from sucrose were hydrolyzed at 0.1 N HCl at 100°C, glucose was detected on paper chromatography of the hydrolysates, but little fructose was detected (data not shown). Therefore, the fructosyltransferase activity might be disregarded in the present study.

Enzyme assay. The reaction mixture for standard assay contained enzyme solution, 0.1 ml of 0.5 M

potassium phosphate buffer, pH 6.0, and 0.1 ml of 0.5 M sucrose. The final volume was adjusted to 0.4 ml with distilled water. Incubation was carried out for 1 h at 37°C. The reaction was stopped by addition of 0.1 ml of 0.24 N HCl. The enzyme activity was determined in duplicate by measuring the amount of reducing sugar released and of ISG and total glucan formed. After termination of the reaction, the reaction mixtures were diluted with 2.5 ml of distilled water and centrifuged at 2,000 × *g* for 5 min. The amount of reducing sugar in the supernatant solution was determined by the method of Somogyi (20), with glucose as a standard. The precipitates were washed three times with 3.0 ml of distilled water and lyophilized. The dried materials were dissolved with 1.5 ml of 0.5 N NaOH. By using aliquots of the alkaline solution, the amount of ISG was determined by the phenol-sulfuric acid method (5), with glucose as a standard. For the determination of total glucan, 5 volumes of ethanol was added to the reaction mixtures to terminate the reaction, and then the amount of total glucan was determined by the same method described above, except for washing with 75% ethanol. SG was determined by subtracting the amount of ISG from that of total glucan. Enzyme activities were expressed as micromoles of reducing sugar released or micromoles of glucose polymerized into glucan from sucrose.

Isolation of ISG synthesized by purified mutansynthetase in the presence of dextran T10. ISG synthesized by the purified mutansynthetase from sucrose in the presence of dextran T10 was termed ISG-PM. ISG-PM was synthesized in a reaction mixture containing 0.125 M phosphate buffer, pH 6.0, 9 μg of purified enzyme per ml, 1.0 mg of dextran T10 (Pharmacia Fine Chemicals, Uppsala, Sweden) per ml, and 0.125 M sucrose in a total volume of 16 ml. After incubation at 37°C for 24 h, ISG-PM was collected by centrifugation and washed twice with 20 ml of distilled water. After the precipitates were dissolved by adding 10 ml of 0.5 N NaOH, ISG-PM was collected by adding 3 volumes of ethanol to the alkaline solution. The precipitates were washed twice with distilled water and lyophilized.

Partial acid hydrolysis of ISG-PM. A 10-mg amount of ISG-PM was heated for 10 min in a boiling water bath in 0.15 ml of 98% formic acid in a sealed ampoule, and then 1.5 ml of 0.4 N HCl maintained at 100°C was added. Heating was continued for 30 min and then stopped. The solution was rapidly cooled, deionized with Dowex-1 ($HCOO^-$), and evaporated. The disaccharides in the partial acid hydrolysates were isolated by column chromatography of the hydrolysates on Sephadex G-15 (1 by 93 cm). The hydrolysates and the disaccharides were subjected to paper chromatography as described previously (7).

Periodate oxidation of ISG-PM followed by controlled Smith degradation. Periodate oxidation of ISG-PM was carried out as follows: 5.6 mg of ISG-PM was oxidized in 2.8 ml of 0.01 M sodium metaperiodate at 4°C for 9 days in darkness. After oxidation, the consumption of periodate was estimated by iodometric microtitration (16). The oxidized ISG-PM which was still water insoluble was washed three times with distilled water to remove the residual periodate and then reduced with about 10 mg of sodium borohydride at 20°C for 18 h. After the reduced ISG-PM was washed three times with distilled water, it was hydrolyzed in 0.1 N HCl at 20°C for 24 h. After controlled Smith degradation, the remaining ISG-PM was again washed with distilled water and then dissolved in 0.5 N NaOH for estimation of molecular weight.

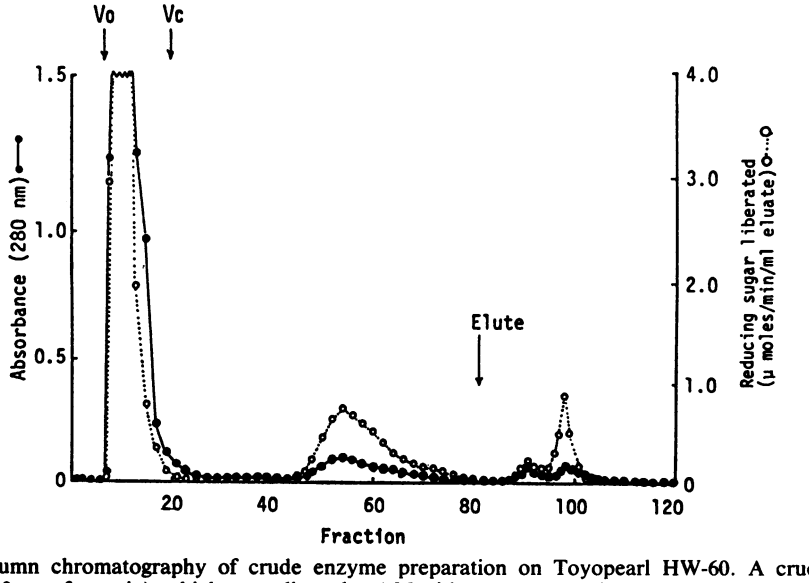

FIG. 1. Column chromatography of crude enzyme preparation on Toyopearl HW-60. A crude enzyme preparation (130 mg of protein), which was adjusted to 1 M with respect to NaCl concentration, was applied to a Toyopearl HW-60 column (2.6 by 35 cm) equilibrated with 0.01 M acetate buffer, pH 5.5, containing 1 M NaCl and eluted with the same buffer, followed by 0.2 M glycine-NaOH buffer, pH 9.0 (arrow). Flow rate, 20 ml/h. Fraction volume, 10 ml each. V_0 and V_c indicate the void volume and column volume, respectively. Symbols: ●, absorbance at 280 nm; ○, enzyme activity.

TABLE 1. Enzyme activities of fractions obtained by Toyopearl HW-60 column chromatography in the absence and presence of dextran T10

Fraction no.	Enzyme activity (μmol/ml of eluate per min)				
	Without dextran T10			With dextran T10[a]	
	Reducing sugar	ISG	SG	Reducing sugar	ISG
9	8.032	0.005	5.000	ND	ND
55	0.768	0.048	0.002	2.191	1.630
98	0.628	0.044	0.008	0.719	0.044

[a] A 100-μg amount of dextran T10 was added to the reaction mixture; ND, not done.

Determination of molecular weight of ISG-PM and its side chain. ISG-PM dissolved in 0.5 N NaOH was applied to a Toyopearl HW-60 column (1 by 97 cm) equilibrated with 0.5 N NaOH. The column was eluted with 0.5 N NaOH. The molecular weight exclusion limit of the gel for dextran was estimated to be about 3×10^5. The molecular weights of the side chains obtained by controlled Smith degradation from ISG-PM were determined by the same procedure. Molecular weights of marker dextran (Sigma Chemical Co., St. Louis, Mo.) were 20,800, 82,000, and 264,000.

PAGE. Polyacrylamide gel electrophoresis (PAGE) of the purified enzyme was carried out essentially by the method of Davis (4) without the use of sample and stacking gels. Protein in the gel was stained with Coomassie brilliant blue (Sigma).

Sodium dodecyl sulfate (SDS)-PAGE was carried out essentially by the method of Weber and Osborn (23) with 5% gel. Protein sample was heated for 2 min at 100°C in the presence of 1% SDS, 1% 2-mercaptoethanol, and 4 M urea before being layered onto the gel. Coomassie blue was used to detect protein band.

^{13}C NMR spectra of ISG-PM. ^{13}C nuclear magnetic resonance (NMR) spectra of ISG-PM and dextran T10 were recorded with a JEOL JNM-PFT-100 spectrometer at 60°C. ^{13}C chemical shifts were expressed in parts per million from external sodium 3-(trimethylsilyl) propionate-d_4. The assignments of ISG-PM and dextran T10 were based on that of α-1,3- and α-1,6-glucans given by Colson et al. (3). FT-NMR conditions were as follows: spectral width, 4 kHz; pulse flipping angle, 90°; acquisition time, 0.4 s; number of data points, 4,096; transient time, 1.0 s; number of transients, 2,000 for dextran T10 and 42,500 for ISG-PM; concentration of glucan, 100 mg/ml (0.24 M NaOH-D_2O) for dextran T10 and 40 mg/ml (0.88 M NaOH-D_2O) for ISG-PM.

Protein determination. Protein was determined spectrometrically at 280 nm or by the method of Lowry et al. (14), with bovine serum albumin as a standard.

Material. Toyopearl gels (Toyo Soda Manufacturing Co.) are a totally porous semirigid spherical gel synthesized from hydrophilic vinyl polymer. They are the same material as Fractogel STK available from E. Merck AG, Darmstadt, West Germany.

RESULTS

Enzyme purification. (i) Chromatography on Toyopearl HW-60. The crude enzyme preparations were applied to a Toyopearl HW-60 column (2.5 by 90 cm) equilibrated with 0.01 M acetate buffer, pH 5.5, which was eluted with the same buffer. The fraction eluted at or near the void volume of the column mainly produced ISG from sucrose, indicating that this enzyme was the aggregated form having a molecular weight of over 1,000,000, and the subsequent fractions synthesized the SG (data not shown). The results coincided with those of the previous reports (7, 13, 17, 19). Schachtele et al. (18) demonstrated the dissociation of aggregated forms of ISG-synthesizing glucosyltransferase

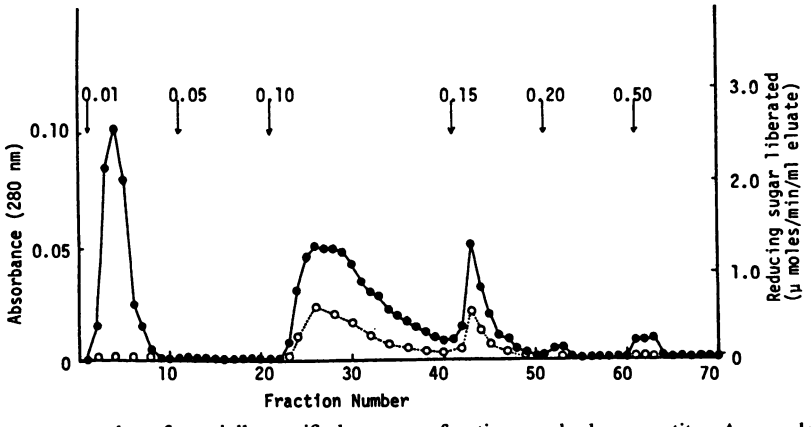

FIG. 2. Chromatography of partially purified enzyme fraction on hydroxyapatite. A sample (4.5 mg of protein) of enzyme fraction prepared by chromatography on Toyopearl HW-60 was applied to a hydroxyapatite column (1.6 by 5.5 cm). Elution was carried out with 0.01, 0.05, 0.10, 0.15, 0.20, and 0.50 M potassium phosphate-buffered saline, pH 6.8. Flow rate, 2.5 ml/h. Fraction volume, 5.3 ml. Symbols: ●, absorbance at 280 nm; ○, enzyme activity.

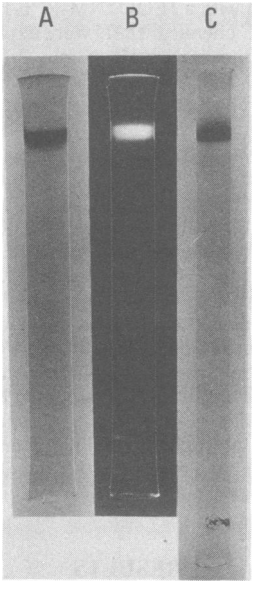

FIG. 3. PAGE and SDS-PAGE of purified enzyme. Purified enzyme (7.5 μg) was applied to each gel. (A) PAGE gel stained with Coomassie brilliant blue; (B) PAGE gel incubated overnight at 37°C with 0.125 M sucrose; (C) SDS-PAGE gel stained with Coomassie brilliant blue.

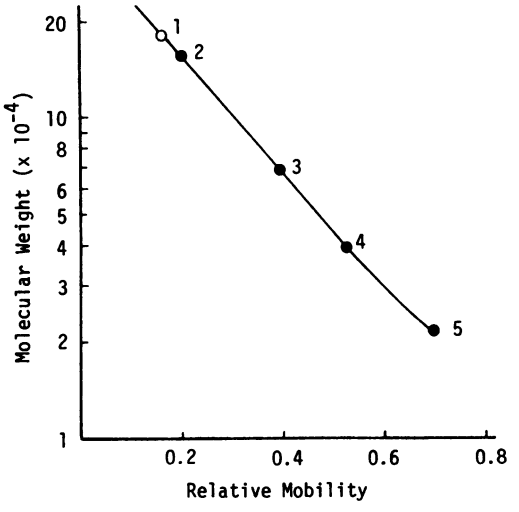

FIG. 4. Molecular weight estimation of purified enzyme by SDS-PAGE. A 7.5-μg amount of enzyme was applied to a gel. The marker proteins and the enzyme are identified by the following numbers: 1, purified enzyme; 2, β subunit of RNA polymerase (molecular weight, 155,000); 3, bovine serum albumin (molecular weight, 68,000); 4, α subunit of RNA polymerase (molecular weight, 39,000); 5, trypsin inhibitor (molecular weight, 21,000).

of *S. mutans* 6715 in the presence of 1 M NaCl. To confirm their findings, the crude enzyme solution was adjusted to contain 1 M NaCl, and then the enzyme sample was applied to a Toyopearl HW-60 column (2.6 by 35 cm) equilibrated with 0.01 M acetate buffer, pH 5.5, containing 1 M NaCl. The enzymes were eluted with the same buffer. As shown in Fig. 1, enzyme activities determined by the release of reducing sugars were detected at the fractions eluted before the column volume and at the delayed fractions eluted over the column volume. Enzyme activity was also detected by eluting with 0.2 M glycine-NaOH buffer, pH 9.0. These enzymes were characterized in the absence and presence of dextran T10 (Table 1). The first enzyme fraction (fraction no. 9) synthesized SG from sucrose but not ISG, suggesting that this enzyme might be dextransucrase. The second (fraction no. 55) and third (fraction no. 98) fractions synthesized more ISG than SG in the absence of dextran T10. However, in the presence of dextran T10, the activities of the second fraction were greatly increased, especially with respect to synthesis of ISG. On the other hand, the activities of the third fraction were not increased by the addition of dextran T10. Figures and Edwards (6) have demonstrated that there are two kinds of enzymes synthesizing ISG in *S. mutans* 6715. The activity of one enzyme is accelerated by the addition of dextran, but the other is not. In our case, the third fraction was eluted by alkaline solution, indicating that the enzyme was firmly bound to the Toyopearl gel. Since the enzyme that eluted at pH 9 might have denatured in the alkaline medium, the second enzyme peak (fraction no. 46 to 75) was collected and subjected to hydroxyapatite column chromatography.

(ii) Hydroxyapatite column chromatography. The fractions of the second activity peak were pooled and concentrated with an ultrafilter of Millipore immersible CX-PTGC and dialyzed against 0.01 M potassium phosphate-buffered saline, pH 6.8. The dialyzed material was applied to a column (1.6 by 5.5 cm) of hydroxyapatite preequilibrated with the same buffered saline. Elution was carried out with various concentrations of potassium phosphate-buffered saline as shown in Fig. 2. Enzyme activity was eluted in 0.10 M and 0.15 M phosphate-buffered saline. Since both enzyme fractions showed the same properties with respect to PAGE, SDS-PAGE, and the primer dependence of reactions, both fractions were combined, concentrated with ultrafiltration, and dialyzed against 0.01 M potassium phosphate buffer, pH 6.0. This dialyzed preparation was referred to as purified mutansynthetase and was employed in all subsequent experiments.

Purity and properties of purified mutansynthe-

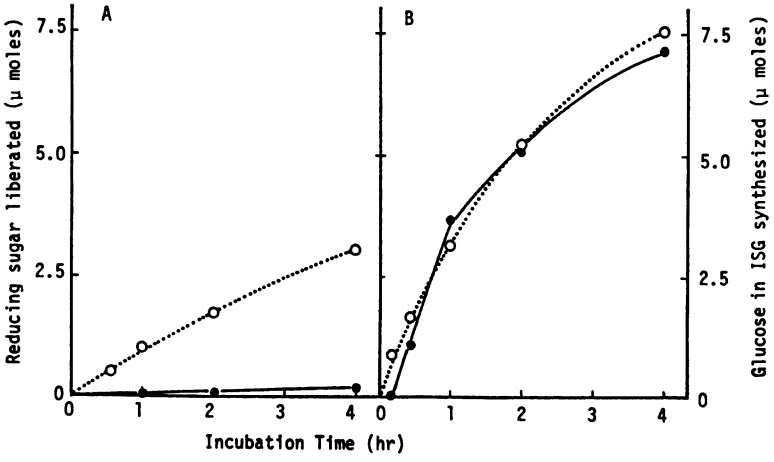

FIG. 5. Time course of purified enzyme activity in the absence (A) and presence (B) of dextran T10; 3.7 μg of enzyme and 0.1 mg of dextran T10 were used under standard assay conditions. Enzyme activities were determined with release of reducing sugar (○) and synthesis of ISG (●).

tase. The purity and activity of purified mutansynthetase were investigated by 7.5% PAGE (Fig. 3). The enzyme showed a single band staining with Coomassie brilliant blue. The protein band corresponded with the white band of ISG which appeared in the gel after incubation with 0.125 M sucrose overnight at 37°C. In addition, the purified enzyme showed a single component on SDS-PAGE (Fig. 3), and the molecular weight of the enzyme, determined from logarithmic plots (Fig. 4), was about 180,000.

The time course of the enzymatic reaction is shown in Fig. 5. In the absence of dextran (Fig. 5A), the amount of ISG formed was less than 1/10 that of released reducing sugar, although both activities were linear with time. After 24 h of incubation, ISG formed was less than 1 μmol, and SG precipitated with 75% ethanol was not detected (data not shown). In the presence of 0.1 mg of dextran T10 in the reaction mixture (0.6 μmol as glucose residue), synthesis of ISG was comparable to the release of reducing sugar within 4 h of incubation, with the exception of the initial lag of ISG synthesis for 15 min, as shown in Fig. 5B. The above results indicated that the purified enzyme specifically catalyzed the synthesis of ISG from sucrose in the presence of dextran.

ISG-PM synthesized by purified mutansynthetase from sucrose in the presence of dextran T10 was analyzed by the following procedures. ISG-PM obtained after incubation for 24 h was used. The partial acid hydrolysate of ISG-PM showed glucose and some oligosaccharides on paper chromatography, but the separation of the oligosaccharides was not clear (data not shown). To determine the structure of the oligosaccharides, the disaccharides in the partial acid hydrolysate were isolated from Sephadex G-15 column chromatography and subjected to paper

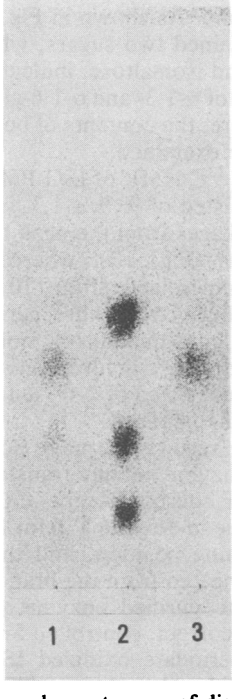

FIG. 6. Paper chromatogram of disaccharides isolated from partial acid hydrolysates of ISG-PM on Sephadex G-15 column chromatography. Reducing sugars were stained with silver nitrate reagent. Lane 1, isolated disaccharides (5 μg); lane 2, standard samples of glucose (10 μg) , isomaltose (20 μg), and isomaltotriose (30 μg) in that order from top to bottom; lane 3, standard sample of nigerose (10 μg).

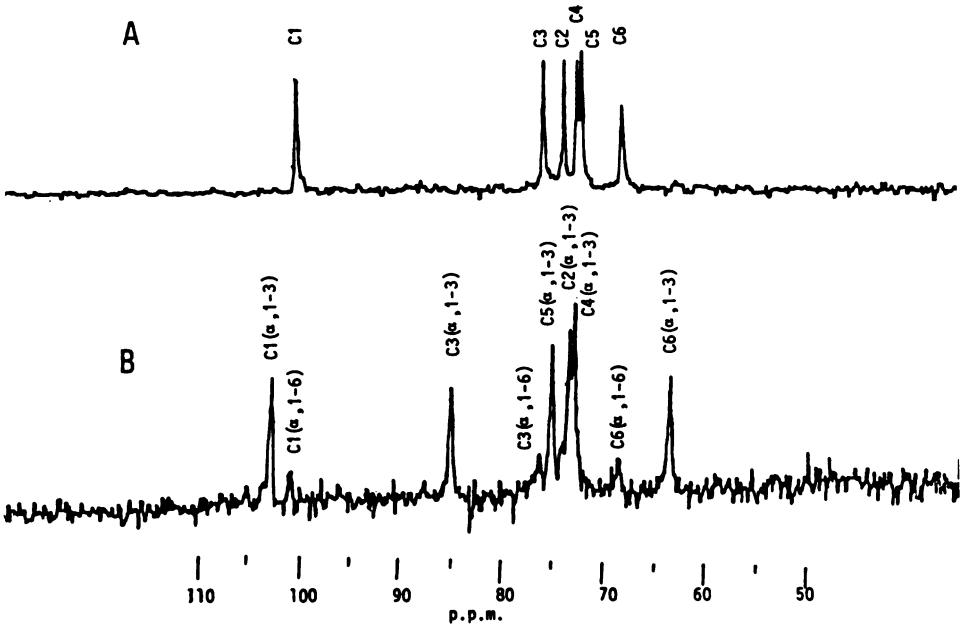

FIG. 7. ^{13}C NMR spectra of dextran T10 (A) and ISG-PM (B). The details of the experiments are described in the text. The assignments were based on those of α-1,3- and α-1,6-glucan reported by Colson et al. (3).

chromatography. As shown in Fig. 6, the disaccharides contained two sugars, which migrated as nigerose and isomaltose, indicating that ISG-PM consisted of α-1,3- and α-1,6-glucosidic linkages. Therefore, the contents of both linkages in ISG-PM were examined.

Analysis of ^{13}C NMR of ISG-PM showed that ISG-PM consisted of 93% α-1,3- and 7% α-1,6-glucosidic linkages from the peak height of anomeric carbons (Fig. 7B), whereas ^{13}C NMR spectra of commercial dextran T10 showed no α-1,3 linkage (Fig. 7A). Under periodate oxidation, ISG-PM consumed 0.148 mol of periodate per mol of glucan anhydroglucose, indicating that ISG-PM consisted of 7.4% α-1,6- and 92.6% α-1,3-glucosidic linkages.

From these results, we have postulated that the purified enzyme initially transfers the glucosyl moiety of sucrose to the C-3 position of glucose residue in dextran T10 for the formation of the branching position, and then the α-1,3 linkage is elongated from the branching glucose residue by the purified enzyme. If the above hypothesis is correct, controlled Smith degradation of the periodate oxidized ISG-PM might release the side chains containing the α-1,3 linkage. Accordingly, the molecular weights of ISG-PM and its controlled Smith degradation products were determined by Toyopearl HW-60 (1 by 97 cm) chromatography (Fig. 8). The mean values of their molecular weights were 240,000 and 36,000, respectively. From this result, it was calculated that ISG-PM had 6.4 side chains per dextran T10 molecule and contained 4.2% α-1,6 linkage originating from dextran T10. This percentage of α-1,6 linkage was in good agreement

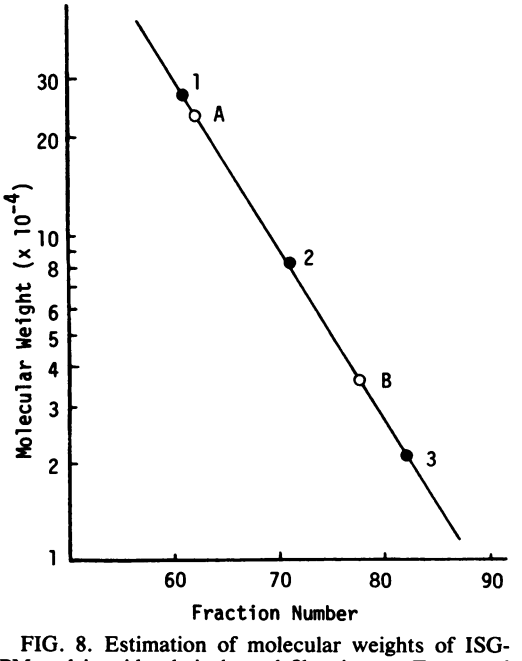

FIG. 8. Estimation of molecular weights of ISG-PM and its side chain by gel filtration on Toyopearl HW-60. Molecular weights of marker dextrans were (1) 264,000, (2) 82,000, and (3) 20,800. (A) ISG-PM; (B) ISG-PM side chain.

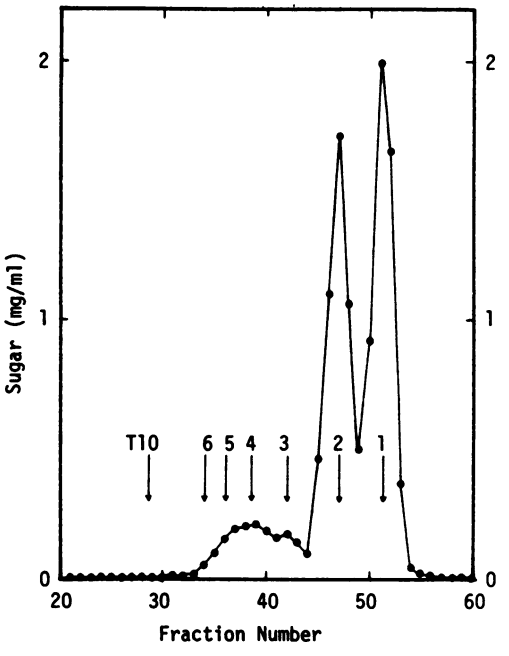

FIG. 9. Gel filtration of soluble fraction of reaction mixture after 24 h of incubation of the purified enzyme with sucrose and dextran T10. After incubation, the soluble fraction (0.5 ml) was applied to Sephadex G-15 column (1 by 96 cm). Elution was carried out with distilled water. Fractions of 1.0 ml were collected, and sugars were determined by the phenol-sulfuric acid method. Arrows numbered 1 to 6 and T10 show the eluted positions of mono, di, tri, tetra, penta, and hexasaccharides and dextran T10 (void volume), respectively.

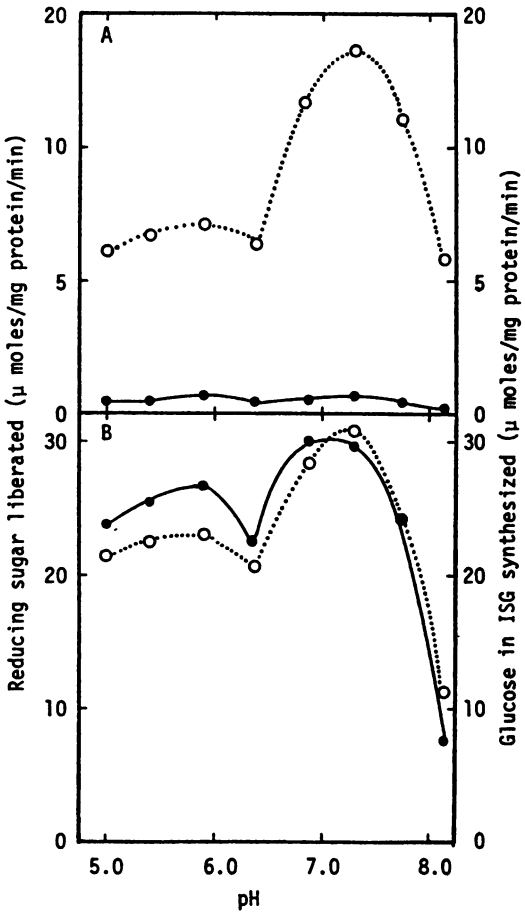

FIG. 10. Effects of pH on purified enzyme activity in the absence (A) and presence (B) of dextran T10. Enzyme activity was determined by the amount of reducing sugar (○) and ISG (●) after 60 min of incubation. A 100-μg amount of dextran T10 was added to the reaction mixture.

with the results of ^{13}C NMR and periodate oxidation analyses.

To examine whether SG was synthesized by the purified enzyme from sucrose in the presence of dextran T10, the soluble fraction of reaction mixture after 24 h of incubation was applied to a column (1 by 93 cm) of Sephadex G-15 (Fig. 9). The eluted sugars were monosaccharide (fructose and glucose), disaccharide (sucrose), and tri- to hexasaccharide. The sample after 0 h of incubation with dextran T10 showed two peaks of dextran T10 and sucrose (data not shown). Therefore, it is evident that the added dextran T10 was incorporated into ISG, and SG larger than hexasaccharide was not synthesized. The characterization of the oligosaccharides synthesized will be described elsewhere.

To determine whether the purified enzyme preparation contained the activity of α-1,6-dextranase, the preparation and dextran T10 were incubated at 37°C for 24 h. After incubation, the reaction mixture was subjected to Sephadex G-15 column chromatography. No oligosaccharide which might be split from dextran T10 was detected (data not shown), indicating that the purified enzyme preparation had no activity of dextranase.

The effects of pH on the activity of purified enzyme in the absence and presence of dextran T10 were examined in potassium phosphate buffer (Fig. 10). The activity showed two pH optima of 5.8 and 7.3 with or without the addition of dextran T10.

The K_m value was determined at a sucrose concentration of between 0.5 and 8.0 mM at pH 5.8 and 7.3. Their values obtained from Lineweaver-Burk plots were 1.4 and 3.3 mM, respectively.

The effects of increasing concentration of dextran T10 on the activity of purified enzyme were examined (Fig. 11). The activity determined by release of reducing sugar increased with the addition of dextran T10 and showed a threefold

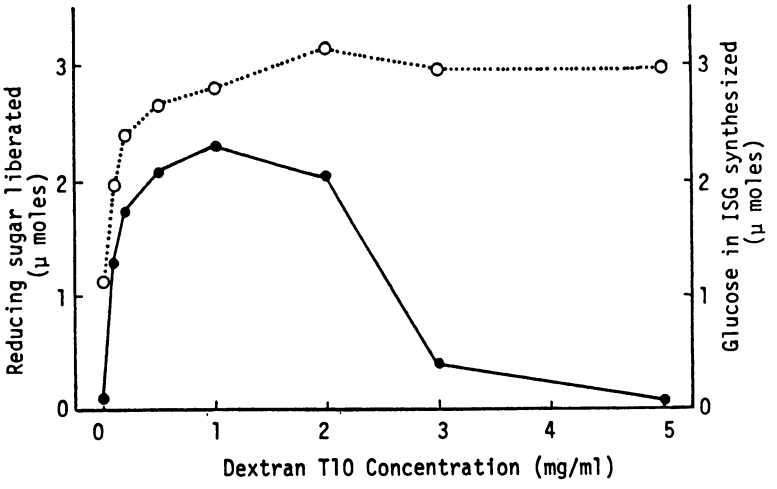

FIG. 11. Effects of dextran T10 on purified enzyme activity. Various amounts of dextran T10 were added to the reaction mixture containing 3.7 μg of enzyme. After 60 min of incubation, amounts of reducing sugar (○) and ISG (●) were determined.

increase at a 1.0-mg/ml concentration of dextran T10. The activity determined by formation of ISG reached a maximum at 1.0 mg of dextran T10 per ml and then declined. During this reactive process, the initial lag phase at which ISG was not detected in the reaction mixture was observed at each concentration of dextran T10. The lag time increased with the addition of dextran T10 (data not shown). As shown in Fig. 11, the lag time at 5 mg of dextran T10 per ml was about 60 min. The fact that the lag phase occurred in the presence of dextran T10 indicates that the occurrence of ISG could be controlled by the length of the side chain elongated from dextran T10.

DISCUSSION

The ISG-synthesizing enzyme aggregates found in the culture supernatants of *S. mutans* 6715 grown in a fructose chemically defined medium could be dissociated with 1 M NaCl as reported by Schachtele et al. (18). The dissociated enzyme had an affinity against a Toyopearl HW-60 gel in the presence of 1 M NaCl. Although the mechanism of the affinity has not been clarified, chromatography of the crude enzyme preparations on Toyopearl HW-60 successfully isolated the ISG-synthesizing enzyme from the SG-synthesizing enzyme (Fig. 1 and Table 1). The ISG-forming enzyme was isolated in a highly purified state by hydroxyapatite column chromatography. However, it was inadequate to calculate the yield of the ISG-synthesizing enzyme at each step of purification. The ISG synthesis by the purified enzyme preparation required the coexistence of an optimal amount of primer dextran (Fig. 11). As far as the enzyme preparation is crude and contains the dextransucrase activity, ISG is synthesized by the preparation without adding primer dextran. It is impossible to separately determine the activities of dextransucrase and mutansynthetase at each purification step because of interaction between the two enzymes.

In the absence of dextran, the purified enzyme synthesized little ISG from sucrose but showed invertase activity, indicating that the sucrose could not be an effective glucose acceptor for the synthesis of ISG .

In the presence of dextran, the purified enzyme synthesized ISG from sucrose and the added dextran, indicating that the enzyme absolutely required the dextran as a substrate (acceptor) for the formation of ISG. ISG-PM formed by the purified enzyme consisted of about 93% α-1,3 linkage de novo synthesized and 7% α-1,6 linkage derived from the added dextran. Determination of molecular weight of ISG-PM and its side chain obtained from controlled Smith degradation of the oxidized ISG-PM proved that the number of branch points of α-1,3-glucan in dextran T10 was 6.4. From these results, we concluded that the purified enzyme initially transferred the glucose moiety of sucrose to position C-3 of the glucosyl residue of dextran T10 and then catalyzed subsequently the α-1,3 linkage to the branched glucosyl residues. The possibility of the existence of this enzyme was suggested earlier by Germaine et al. (9). Since this report is the first of enzyme purified from cariogenic streptococci and since the enzyme has not yet been registered, we recommend that this enzyme be called mutansynthetase (EC. 2.4.1.?) as distinct from dextransucrase (EC 2.4.1.5). Gug-

genheim and Newbrun (11) have named ISG synthesized by *S. mutans* as "mutan."

From the number of branch points in ISG-PM, it was also calculated that one branch point occurred at every 9.6 glucosyl residues of dextran T10; this result showed a close agreement with Walker's observation (22) that isomaltodextrin consisting of 7 to 8 glucosyl residues elicited the branching activity of *S. mutans* OMZ 176 glucosyltransferase. Germaine et al. (9) showed that isomaltodextrin over octaose possessed a primer activity for glucan synthesis of *S. mutans* 6715 glucosyltransferase. This fact also seemed to support the occurrence of a branch point every 9.6 glucosyl residues.

Several reports have shown the isolation of glucosyltransferase responsible for the ISG synthesis from culture fluids of *S. mutans* (1, 2, 6, 10, 11, 13, 15, 17, 19). However, their purified enzyme preparations forming ISG from sucrose seem to contain both dextransucrase and mutansynthetase because the formation of ISG by mutansynthetase absolutely required the exogenous dextran (Fig. 5). In addition, the presence of dextransucrase could raise the production of the dextran which was used as the substrate by mutansynthetase for synthesis of ISG. Although the mechanism of ISG synthesis by mutansynthetase in the presence of sucrose and dextran was demonstrated, the mechanism of glucan synthesis in the coexistence of dextransucrase and mutansynthetase remains to be clarified.

The purified mutansynthetase had two pH optima. It may be that there are two enzymes of the same molecular weight having different pH optima or that the conformational change of an enzyme arising from pH effect occurs. Accordingly, further investigation to clarify this point will be needed.

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