Purification and Characterization of Glucosyltransferases from *Streptococcus mutans* 6715

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A water-soluble glucan-synthesizing glucosyltransferase (GTase-S) and a water-insoluble glucan-synthesizing glucosyltransferase (GTase-I) were purified from culture supernatant of *Streptococcus mutans* 6715 (serotype g) by ammonium sulphate precipitation, chromatofocusing on a Polybuffer exchanger PBE 94 column, and subsequent phenyl-Sepharose CL-4B or hydroxyapatite column chromatography. The GTase-S and GTase-I activities were purified 4019- and 4714-fold, respectively, and the molecular weights were calculated to be 160 000 and 165 000, respectively. GTase-S had a pH optimum of 5.0, a K_m of 8.8 mM for sucrose in the presence of 20 μ M-dextran T10, and an isoelectric point of pH 4.3. GTase-I had two pH optima of 5.0 and 7.0, K_m values of 4.9 mM (at pH 5.0) and 7.0 mM (at pH 7.0), and an isoelectric point of pH 4.9. Methylation analysis indicated that the water-soluble glucan produced by GTase-S was a highly branched 1,6- α -linked D-glucan with 1,3-linked glucose residues, and that the water-insoluble glucan synthesized by GTase-I was composed of 1,3- α -linked glucose units.

INTRODUCTION

Streptococcus mutans has been strongly implicated as a causative organism of dental caries (Hamada & Slade, 1980). This organism produces both water-soluble and water-insoluble glucans from sucrose by its constitutive enzymes, glucosyltransferases (GTase; EC 2.4.1.5). *De novo* synthesis of the water-insoluble glucan is involved in the firm attachment and accumulation of *S. mutans* cells on tooth surfaces. The glucan has a highly branched structure with 1,3- α -linked and 1,6- α -linked D-glucose residues (Hamada & Slade, 1980).

Many purification procedures for *S. mutans* GTases have been proposed (Montville *et al.*, 1978; Ciardi, 1983). However, most of the reported GTases were still not fully purified. They formed molecular aggregates, and were contaminated by impurities such as fructosyltransferase, dextranase, invertase and lipoteichoic acid. Recently, two GTase components were purified by the chromatofocusing method from culture supernatant of *S. mutans* of serotype d (Koga *et al.*, 1983b) and c strains (Kuramitsu & Wondrack, 1983). In this report we describe a simple and reproducible procedure, using the chromatofocusing method, for purification of GTases of *S. mutans* 6715 (serotype g). Some properties of purified GTases are also discussed.

METHODS

Materials. *S. mutans* 6715 (serotype g) was from the stock culture collection in the Department of Dental Research, the National Institute of Health, Tokyo, Japan. Polybuffer exchanger PBE 94, Polybuffer 74, phenyl-Sepharose CL-4B, and dextran T10 were obtained from Pharmacia. Hydroxyapatite was purchased from Seikagaku Kogyo Co., Tokyo. [U - 14 C]Sucrose (4.67 Ci mol $^{-1}$; 0.17 TBq mol $^{-1}$) was obtained from New England Nuclear. Brain Heart infusion broth was from Difco, and invertase (EC 3.2.1.26) was from Wako Pure Chemicals, Osaka, Japan. SDS-PAGE standard proteins were from Bio-Rad.

Abbreviations: GTase-S, soluble glucosyltransferase; GTase-I, insoluble glucosyltransferase.

Enzyme assays. GTase activity was measured as described previously (Koga & Inoue, 1981). Enzyme was allowed to react with 10 mM-[U-¹⁴C]sucrose (0.4 Ci mol⁻¹; 14.8 GBq mol⁻¹) and 20 µM-dextran T10 in 20 µl 0.1 M-potassium phosphate buffer, pH 6.0, containing 0.02% (w/v) sodium azide. After incubation at 37 °C for 1 h, the reaction mixture was spotted on a filter paper square (15 × 15 mm, no. 514, Toyo Roshi, Tokyo). The squares were washed three times with methanol and dried. Radioactivity retained on the paper squares was measured by a scintillation spectrometer to quantify the total glucan synthesized. One unit of GTase activity was defined as the amount of enzyme that transformed 1 µmol of sucrose to glucan per min under the conditions described above.

The release of reducing sugar and glucose, and the synthesis of water-insoluble glucan from sucrose by GTases were also estimated. The reaction mixture consisted of 30 mM-sucrose with appropriate amounts of enzyme solution and dextran T10 (0, 0.5 or 10 mg ml⁻¹) in a total volume of 3 ml 0.1 M-phosphate buffer, pH 6.0, containing 0.02% (w/v) sodium azide. After incubation at 37 °C for 4 h, the amounts of reducing sugar and glucose released from sucrose were measured by the method of Somogyi (1945) and the glucose oxidase method (Blood Sugar GOD Perid Test; Boehringer-Mannheim), respectively, with glucose as a standard. To determine the water-insoluble glucan, the glucan formed in the reaction mixture was dispersed by sonic oscillation, and A₅₅₀ was measured with a double-beam spectrophotometer (Hamada & Torii, 1980).

Culture conditions and preparation of culture supernatant. Brain heart infusion broth (185 g) was pretreated with invertase (2 g) to avoid contamination of sucrose, and dialysed against distilled water. The volume of the dialysate was adjusted to 4 l with distilled water. The medium was autoclaved at 120 °C for 15 min, and 1 l filter-sterilized 1.5% (w/v) glucose solution was added. *S. mutans* 6715 was grown in this medium at 37 °C for 18 h. The cell-free culture supernatant was collected by centrifugation at 10000 g for 20 min at 4 °C.

Purification of GTases. This was done at 4 °C unless otherwise specified. (1) Ammonium sulphate precipitation. Crude GTase was precipitated from the culture supernatant by adding solid ammonium sulphate to 50% saturation. The precipitate was collected by centrifugation, dissolved in 25 mM-piperidine/HCl buffer, pH 5.5, and dialysed against the buffer. Insoluble material was removed by centrifugation at 10000 g for 30 min.

(2) Chromatofocusing column chromatography. This was done as described previously, with some modifications (Koga *et al.*, 1983b). The crude GTase (30 ml) was applied to a column (1.5 × 30 cm) of Polybuffer exchanger PBE 94 equilibrated with 25 mM-piperidine/HCl buffer, pH 5.5. The enzyme was first eluted with fivefold diluted Polybuffer 74 (pH 4.1, 300 ml), followed by elution with a linear gradient of 0.1 to 0.4 M-NaCl (300 ml). Fractions of 10 ml were collected at a flow rate of 15 ml h⁻¹. A water-insoluble glucan-synthesizing GTase (GTase-I) and a water-soluble glucan-synthesizing GTase (GTase-S) were eluted at 0.15 and 0.31 M-NaCl, respectively. Active fractions were pooled for each peak, concentrated by precipitation with 80% saturated ammonium sulphate, and dialysed against 10 mM-phosphate buffer, pH 6.8.

(3) Hydroxyapatite column chromatography. The GTase-I fraction (10 ml) separated by chromatofocusing column chromatography was applied to a column (0.7 × 8 cm) of hydroxyapatite equilibrated with 10 mM-phosphate buffer, pH 6.8. The column was washed with the buffer (20 ml), and then the enzyme was eluted with a linear gradient of 10 to 500 mM-phosphate buffer (pH 6.8, 90 ml). Fractions of 3 ml were collected at a flow rate of 18.6 ml h⁻¹ at 25 °C. Active fractions were pooled, concentrated with an Amicon CF-25 membrane, and dialysed against 10 mM-phosphate buffer, pH 6.8.

(4) Phenyl-Sepharose CL-4B column chromatography. The GTase-S fraction (10 ml) separated by chromatofocusing column chromatography was dialysed against 1 M-ammonium sulphate in 10 mM-phosphate buffer, pH 6.8. The fraction was applied to a column (1.5 × 10 cm) of phenyl-Sepharose CL-4B equilibrated with the same buffer. Proteins were eluted with a linear gradient of 1 to 0 M-ammonium sulphate in 10 mM-phosphate buffer, pH 6.8 (120 ml). Fractions of 3 ml were collected at a flow rate of 36 ml h⁻¹ at 25 °C. Active fractions were pooled, concentrated with an Amicon CF-25 membrane, and dialysed against 10 mM-phosphate buffer, pH 6.8.

SDS-PAGE. SDS-polyacrylamide slab gels were prepared as described by Laemmli (1970). Enzyme proteins (20 µg) were heated at 100 °C for 5 min in 10 mM-Tris/HCl buffer, pH 6.8, containing 1% (w/v) SDS, 1% (w/v) 2-mercaptoethanol and 20% (w/v) glycerol. Electrophoresis was performed at 10 mA per gel at 4 °C for 16 h using a 7.5% (w/v) resolving and a 3% (w/v) stacking gel (14 × 11 × 0.2 cm) containing 0.1% SDS. Proteins were stained with Coomassie brilliant blue R-250. Ovalbumin (mol. wt 45000), bovine serum albumin (66200), phosphorylase B (92500), β-galactosidase (116250) and myosin (200000) were used as molecular weight standards.

Electrophoretic blotting. Enzyme proteins (20 µg) were electrophoresed in SDS-polyacrylamide slab gels as described above, and transferred to a nitrocellulose sheet by the electrophoretic blotting technique (Towbin *et al.*, 1979). After confirmation of the transfer of the proteins by staining of a portion of the sheet with Coomassie R-250, another portion was treated with 1G-3E7 monoclonal antibody which reacted specifically with GTase-I (Furuta *et al.*, 1983). The antibody bound to the immobilized replica proteins on the sheet was detected by solid-phase immunoassay with horseradish peroxidase-conjugated rabbit anti-mouse immunoglobulin.

Analytical isoelectric focusing. Enzyme proteins (20 µg) were subjected to isoelectric focusing on an LKB Ampholine polyacrylamide gel plate with a pH range of 3.5 to 9.5 and run in an LKB Multiphor electrophoresis chamber. After focusing at 300 V for 2 h, the gel was incubated in 10 mM-phosphate buffer, pH 6.0, containing 5%

(w/v) sucrose, 50 μ M-dextran T10 and 0.02% (w/v) sodium azide at 37 °C for 24 h. Glucans formed on the gel were stained by the periodic acid-Schiff base method (Russell, 1979; Zacharius *et al.*, 1969).

Determination of K_m value. The reaction mixture (20 μ l) consisted of McIlvain (citrate/ Na_2HPO_4) buffer at the appropriate pH, appropriate amount of enzymes, and 2 to 10 mM-[U- ^{14}C]sucrose in the presence or absence of 20 μ M-dextran T10. After incubation at 37 °C for 30 min, the total glucan synthesized was determined as described above. The K_m values were estimated by using the initial velocity obtained at various substrate concentrations by the method of Eadie and Hofstee.

Amino acid analysis. Enzyme proteins (0.2 mg) were hydrolysed in 6 M-HCl, *in vacuo*, at 110 °C for 24 h, and then analysed by a Hitachi 835-50 amino acid analyser (Spackman *et al.*, 1958). Tryptophan was determined after hydrolysis in 3 M-2-mercaptoethanesulphonic acid.

Methylation analysis. Enzyme samples (400 mU) were allowed to react with 30 mM-sucrose in a total volume of 3 ml 0.1 M-phosphate buffer, pH 6.0, containing 0.02% (w/v) sodium azide. After incubation at 37 °C for 72 h, the synthesized glucans were precipitated by the addition of 3 vols ethanol, and the resulting precipitates were collected by centrifugation. The glucans were washed three times with 75% (v/v) ethanol, and the precipitates were lyophilized.

Glucans (1 mg) were methylated by the method of Hakomori (1964) with sodium methylsulphonyl methanide and methyl iodine in methyl sulfoxide, and analysed as alditol acetates in a gas chromatograph as described by Hisamatsu *et al.* (1980).

Protein determination. Protein was measured by the Lowry method, with bovine serum albumin as a standard. The protein content of column effluents was monitored at 280 nm with a double-beam spectrophotometer (Model UV-190; Shimadzu Works, Kyoto, Japan).

RESULTS

Purification of GTases

Crude GTase, prepared by ammonium sulphate precipitation from the cell free culture supernatant of *S. mutans* 6715, was applied on a column of Polybuffer PBE 94 equilibrated with 25 mM-piperidine/HCl buffer, pH 5.5. Two active peaks were eluted by NaCl, at 0.15 M- and 0.31 M-NaCl (Fig. 1). The enzyme eluted at 0.15 M-NaCl (GTase-I) produced a water-insoluble glucan from sucrose, whereas the enzyme eluted at 0.31 M-NaCl (GTase-S) produced a water-soluble glucan.

The GTase-I fraction was further chromatographed on a hydroxyapatite column (Fig. 2). No GTase activity was found in the major protein peak eluted with 10 mM-phosphate buffer. The enzyme was eluted with approximately 0.1 M-potassium phosphate. Active fractions (fractions 15 to 22) were pooled, concentrated, and dialysed against 10 mM-phosphate buffer, pH 6.8.

The GTase-S fraction separated by the chromatofocusing method was applied to a column of phenyl-Sepharose CL-4B equilibrated with 1 M-ammonium sulphate in 10 mM-phosphate

Fig. 1. Separation of GTase-I and GTase-S from the ammonium sulphate fraction of *S. mutans* 6715 extracellular glucosyltransferases by chromatofocusing on a Polybuffer exchanger PBE 94 column. Column dimension, 1.5 $\times$ 30 cm; flow rate, 15 ml h $^{-1}$; volume per fraction, 10 ml. —, Absorbance at 280 nm. Glucosyltransferase was assayed with 10 mM-[U- ^{14}C]sucrose in the presence (○) or absence (●) of 20 μ M-dextran T10. I, GTase-I; II, GTase-S., pH gradient; ---, NaCl gradient.

Fig. 2

Fig. 2. Purification of GTase-I by hydroxyapatite column chromatography. Column dimension, 0.7×8 cm; flow rate, 19 ml h^{-1} ; volume per fraction, 3 ml. —, Absorbance at 280 nm. Glucosyltransferase (○) was assayed with 10 mM -[U- ^{14}C]sucrose in the presence of $20 \mu\text{M}$ -dextran T10. ---, Phosphate buffer gradient.

Fig. 3

Fig. 3. Purification of GTase-S by phenyl-Sepharose CL-4B column chromatography. Column dimension, 1.5×10 cm; flow rate, 36 ml h^{-1} ; volume per fraction, 3 ml. —, Absorbance at 280 nm. Glucosyltransferase (○) was assayed with 10 mM -[U- ^{14}C]sucrose in the presence of $20 \mu\text{M}$ -dextran T10. ---, Ammonium sulphate gradient.

Table 1. Purification of glucosyltransferase from *S. mutans* 6715

Step	Total protein (mg)	Total activity (U)	Specific activity (U mg^{-1})	Recovery (%)	Purification* (fold)
1. Culture supernatant	42400	23.9	0.00056	100	1
2. 50% Ammonium sulphate	491	21.0	0.0427	87.9	76
3. Chromatofocusing					
GTase-S	27.5	6.09	0.221	25.5	395
GTase-I	25.2	1.54	0.611	6.44	109
4. Phenyl-Sepharose CL-4B					
GTase-S	1.22	2.75	2.25	11.5	4019
5. Hydroxyapatite					
GTase-I	0.24	0.63	2.63	2.64	4714

* Measurement of protein concentration in the crude broths usually results in a gross overestimate because of the presence of a number of interfering small molecules. Consequently the true purification values are probably smaller than those listed.

buffer, pH 6.8 (Fig. 3). No GTase activity was found in non-adsorbed proteins washed from the resin with starting buffer, or in bound proteins eluted by lowering the ammonium sulphate concentration (1 to 0 M). The enzyme was eluted by subsequent irrigation with 10 mM-phosphate buffer, pH 6.8. Active fractions (fractions 52 to 58) were pooled and concentrated.

A typical enzyme purification is summarized in Table 1. Purification of GTase-I was 4714-fold with an overall yield of 2.6%, and that of GTase-S was 4019-fold with an overall yield of 11.5%.

Properties of purified GTases

SDS-PAGE showed homogeneity of the purified GTase preparations (Fig. 4a). The molecular weights of purified GTase-I and GTase-S were estimated to be 165000 ± 3000 and 160000 ± 3000 (mean $\pm$ SD from five independent experiments), respectively.

Analytical isoelectric focusing of purified GTases revealed that the pI values of GTase-I and GTase-S were 4.9 ± 0.2 and 4.3 ± 0.1 (mean $\pm$ SD from three separate experiments), respectively.

Fig. 4. SDS-PAGE and electrophoretic blotting of purified GTase-I and GTase-S. (a) SDS-polyacrylamide gel electrophoretic pattern. Electrophoresis was performed with 7.5% acrylamide, containing 20 μ g of each enzyme. The gel was stained with Coomassie brilliant blue. Molecular weight markers used were ovalbumin (mol. wt. 45000), bovine serum albumin (66200), phosphorylase B (92500), β -galactosidase (116250) and myosin (200000). (b) IG-3G7 monoclonal antibody bound to an immobilized antigen on a nitrocellulose sheet transferred by an electrophoretic blotting procedure. The antibody was detected by solid-phase immunoassay with horseradish peroxidase-conjugated rabbit anti-mouse immunoglobulin. (a) Lane 1, molecular weight markers; lane 2, GTase-S; lane 3, GTase-I. (b) Lane 1, GTase-S; lane 2, GTase-I.

Table 2. *Amino acid compositions of purified glucosyltransferases from S. mutans 6715*

Amino acid	Composition (mol%)	
	GTase-I	GTase-S
Aspartic acid	9.02	10.05
Threonine	5.39	5.83
Serine	14.73	12.47
Glutamic acid	12.10	12.72
Proline	4.11	4.73
Glycine	15.22	13.59
Alanine	8.76	11.59
Valine	3.83	4.01
Methionine	0.90	1.01
Isoleucine	2.30	2.67
Leucine	4.42	4.34
Tyrosine	2.66	3.16
Phenylalanine	2.76	2.54
Lysine	8.71	6.99
Histidine	2.41	1.87
Arginine	2.34	2.05
Cysteine	0.23	0.32
Tryptophan*	0.19	0.10

* Determined after hydrolysis in 3 M-2-mercaptoethanesulphonic acid.

The pH optimum of GTase-S was 4.5 to 5.5, whereas GTase-I showed two pH optima of 5.0 and 7.0 (data not shown). The Eadie-Hofstee plots demonstrated that the K_m values of GTase-I for sucrose at pH 5.0 and 7.0 in the presence of 20 μ M-dextran T10 were 4.9 and 7.0 mM, respectively. The K_m value of GTase-S for sucrose at pH 5.0 in the absence and presence of 20 μ M-dextran T10 were 2.2 and 8.8 mM, respectively.

The amino acid compositions of the two GTases were similar (Table 2). The content of acidic amino acids was greater than that of basic amino acids, which explains the low pI values of these enzymes.

Fig. 4(b) shows the reactivity of IG-3E7 monoclonal antibody to the two GTases. The IG-3E7 antibody was bound to GTase-I, but did not react with GTase-S, indicating that no GTase-I was present in the GTase-S preparation.

Effects of dextran T10 on GTase activities

GTase-I (9.8 mU) synthesized only trace amounts of glucan from sucrose in the absence of dextran T10, but the presence of the dextran strongly enhanced the total glucan synthesis (Fig. 5). The degree of stimulation of total glucan synthesis increased with increasing amounts of dextran T10 up to 5 mg ml⁻¹ but decreased at dextran concentration above 5 mg ml⁻¹; maximum stimulation was approximately 16-fold. The activity of GTase-S (9.5 mU) also increased with increasing amounts of soluble dextran, but the degree of stimulation of total glucan synthesis was considerably less than that observed for GTase-I.

GTase-I (40 mU ml⁻¹) synthesized only trace amounts of water-insoluble glucan in the absence of dextran T10, but the synthesis of such glucan was significantly stimulated by the addition of dextran T10 at a concentration of 0.5 mg ml⁻¹ (Table 3). No water-insoluble glucan was synthesized by GTase-S (76 mU ml⁻¹) in the absence or presence of dextran T10.

Both enzymes released free glucose from sucrose (Table 3); the amount released was smaller in the presence of dextran T10. No detectable fructosyltransferase activity was demonstrated, as judged by the incorporation of radioactivity into polysaccharide from 1-*O*-(α -D-glucopyranosyl)- β -D-[U-¹⁴C]fructofuranoside (sucrose labelled in the fructosyl moiety). Furthermore, no dextranase activity was present in these GTases, which was demonstrated by the fact that these enzymes did not release reducing sugars from dextran T2000 after 1 h incubation in 0.1 M-phosphate buffer, pH 6.0, at 37 °C.

Fig. 5. Effect of dextran T10 on activity of GTase-I and GTase-S. Glucosyltransferase (10 mU) was allowed to react with 10 mM-[U-¹⁴C]sucrose and 0 to 10 mg ml⁻¹ dextran T10 in 20 μ l 0.1 M-phosphate buffer (pH 6.0). Activity is presented as the ratio to control activity (no added dextran T10). ▲, GTase-I. △, GTase-S.

Table 3. Effects of dextran T10 on glucosyltransferase activities of *S. mutans* 6715

	Dextran T10 (mg ml ⁻¹)	Sugar release (μ mol ml ⁻¹ min ⁻¹)		Insoluble glucan synthesis (A_{550})*
		Glucose	Reducing sugar	
GTase-I	10	13	93	0.004
	0.5	17	134	0.360
	0	19	103	0.004
GTase-S	10	3	253	0.003
	0.5	5	180	0.003
	0	7	29	0.003

* Determined spectrophotometrically after 4 h.

Linkage analysis of glucans synthesized by GTases

It was shown by methylation analysis of glucans synthesized after 72 h by purified GTases in the absence of dextran T10 that glucan synthesized by GTase-I gave 92% 2,4,6-tri-*O*-methyl-D-glucose, which indicates that the glucan was a 1,3- α -linked D-glucan with little branching. Water-soluble glucan synthesized by GTase-S gave 36 mol% 2,3,4,6-tetra-*O*-methyl-D-glucose, 47 mol% 2,3,4-tri-*O*-methyl-D-glucose and 13 mol% 2,4-di-*O*-methyl-D-glucose, indicating that the product of GTase-S consisted of a low molecular weight highly branched 1,6- α -linked D-glucan with 1,3-linked glucose residues.

DISCUSSION

In this study, GTases of *S. mutans* 6715 were resolved into two fractions by chromatofocusing. One fraction (GTase-I) synthesized water-insoluble glucan from sucrose, and the other (GTase-S) synthesized water-soluble glucan. It should be noted that after using a pH gradient as in orthodox chromatofocusing these GTases were eluted by an ionic strength gradient. However,

no such separation of these GTases could be obtained by a simple ion exchange chromatography on the PBE column. GTase-I and GTase-S were purified to homogeneity by hydroxyapatite chromatography and by phenyl-Sepharose CL-4B chromatography, respectively.

The purified GTase-I had a molecular weight of 165000 ± 3000 , and pH optima at 5.0 and 7.0. Recently, Fukui *et al.* (1982) purified a similar GTase-I from culture fluid of *S. mutans* 6715-15 by column chromatography on Toyopearl HW-60 and subsequently on hydroxyapatite. This purified enzyme had a molecular weight of 180000, and pH optima at 5.8 and 7.3. However, the K_m values of their enzyme (1.4 mM at pH 5.8 and 3.3 mM at pH 7.3) differ from those of GTase-I purified in the present study (4.9 mM at pH 5.0 and 7.0 mM at pH 7.0). This difference in the K_m value may be due to the differences in the enzyme assay method or the purity of the enzyme.

The molecular weight of the purified GTase-S was 160000 ± 3000 (Fig. 4), and the pI value was 4.3 ± 0.1 . Shimamura *et al.* (1982) purified a similar enzyme from the culture supernatant of *S. mutans* 6715, with a molecular weight of 149000 ± 5000 , and a pI of 4.1. Further, the optimum pH in the presence of dextran T10 of their GTase-S are the same as those of our GTase-S.

The activity of GTase-I and GTase-S purified from the culture supernatant of *S. mutans* 6715 in this study was increased up to 16- and 5-fold, respectively, by the addition of dextran T10 to the enzyme assay. Dextran stimulation of *S. mutans* GTase-I activity has been observed by several investigators (Germaine *et al.*, 1977; Hare *et al.*, 1978; Fukui *et al.*, 1982; Shimamura *et al.*, 1982; Koga *et al.*, 1983a). McCabe & Smith (1977) reported that GTase-S from *S. mutans* 6715 is also stimulated by the addition of exogenous soluble dextran. In contrast, it has been shown that a GTase-S unaffected by an exogenous dextran is secreted by *S. mutans* OMZ 176 (Koga *et al.*, 1983b) and 6715 (Shimamura *et al.*, 1983) in medium supplemented with Tween 80. We found no such dextran-independent GTase-S in this study. Perhaps some of surfactants such as Tween 80 stimulate the production of dextran-independent GTase-S.

Luzio & Mayer (1983) observed that GTase purified from the culture fluids of *S. sanguis* ATCC 10558 catalyses the hydrolysis of sucrose to glucose and fructose, and that the formation of glucan competes with the hydrolysis of sucrose. The GTases of *S. mutans* 6715 also could release glucose from sucrose, and the degree of release of glucose in the absence of dextran T10 was higher than that in its presence. The release of glucose from sucrose would occur if fructosyltransferase, dextranase and invertase were present. However, these enzymes were not detected. Moreover, the GTases purified in this study showed a single band with Coomassie brilliant blue on SDS-PAGE. These results indicate that the GTase itself possesses a hydrolytic activity and the release of glucose is due to transfer of glucosyl moieties to water.

Unlike crude GTase, GTase-I or GTase-S alone did not yield a turbid suspension from sucrose. Koga *et al.* (1983a) isolated a dextran-dependent GTase-I and a dextran-independent GTase-S from the culture supernatant of *S. mutans* OMZ 176, and indicated that the combined incubation of GTase-I and GTase-S is required to produce adherent water-insoluble glucan from sucrose. Further investigation will be needed to clarify whether or not water-soluble glucan produced by the GTase-S purified in the present study acts as an intrinsic acceptor for the glucan synthesis by the GTase-I.

In addition, the very low yields of GTases isolated is a common problem with reported purifications of these enzymes and has led to the worry that, against the background of multiple forms of GTases, the isolated species may not be very representative of the total (Ciardi, 1983). Therefore, presence of other species of GTase should also be investigated.

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REFERENCES

- CIARDI, J. E. (1983). Purification and properties of glucosyltransferases of *Streptococcus mutans*: a review. In *Glucosyltransferases, Glucans, Sucrose and Dental Caries*, pp. 51–64. Edited by R. J. Doyle & J. E. Ciardi. Washington, DC: IRL Press.
- FUKUI, K., MORIYAMA, T., MIYAKE, T., MIZUTANI, K. & TANAKA, O. (1982). Purification and properties of glucosyltransferase responsible for water-insoluble glucan synthesis from *Streptococcus mutans*. *Infection and Immunity* **37**, 1–9.
- FURUTA, T., NISIZAWA, T., CHIBA, J. & HAMADA, S. (1983). Production of monoclonal antibody against a glucosyltransferase of *Streptococcus mutans* 6715. *Infection and Immunity* **41**, 872–875.
- GERMAINE, G. R., HARLANDER, S. K., LEUNG, W. L. S. & SCHACHTELE, C. F. (1977). *Streptococcus mutans* dextranase: functioning of primer dextran and endogenous dextranase in water-soluble and water-insoluble glucan synthesis. *Infection and Immunity* **16**, 637–648.
- HAKOMORI, S. (1964). A rapid permethylation of glycolipid and polysaccharide catalyzed by methylsulfinyl carbanion in dimethyl sulfoxide. *Journal of Biochemistry* **55**, 205–208.
- HAMADA, S. & SLADE, H. D. (1980). Biology, immunology, and cariogenicity of *Streptococcus mutans*. *Microbiological Reviews* **44**, 311–384.
- HAMADA, S. & TORII, M. (1980). Interaction of glucosyltransferase from *Streptococcus mutans* with various glucans. *Journal of General Microbiology* **116**, 51–59.
- HARE, M. D., SVENSSON, S. & WALKER, G. J. (1978). Characterization of the extracellular, water-insoluble α -D-glucans of oral streptococci by methylation analysis, and by enzymic synthesis and degradation. *Carbohydrate Research* **66**, 245–264.
- HISAMATSU, M., ABE, J., AMEMURA, A. & HARADA, T. (1980). Structural elucidation of succinoglycan and related polysaccharides from *Agrobacterium* and *Rhizobium* by fragmentation with two special β -D-glucanases and methylation analysis. *Agricultural and Biological Chemistry* **44**, 1049–1055.
- KOGA, T. & INOUE, M. (1981). Inactivation of D-glucosyltransferases from oral *Streptococcus mutans* and *Streptococcus sanguis* by photochemical oxidation. *Carbohydrate Research* **93**, 125–133.
- KOGA, T., SATO, S., INOUE, M., TAKEUCHI, K., FURUTA, T. & HAMADA, S. (1983a). Role of primers in glucan synthesis by glucosyltransferases from *Streptococcus mutans* strain OMZ176. *Journal of General Microbiology* **129**, 751–754.
- KOGA, T., SATO, S., YAKUSHIJI, T. & INOUE, M. (1983b). Separation of insoluble and soluble glucan-synthesizing glucosyltransferases of *Streptococcus mutans* OMZ176 (serotype d). *FEMS Microbiology Letters* **16**, 127–130.
- KURAMITSU, H. & WONDRAK, L. (1983). Insoluble glucan synthesis by *Streptococcus mutans* serotype c strains. *Infection and Immunity* **42**, 763–770.
- LAEMMLI, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature, London*, **227**, 680–685.
- LUZIO, G. A. & MAYER, R. M. (1983). The hydrolysis of sucrose by dextranase. *Carbohydrate Research* **111**, 311–318.
- MCCABE, M. M. & SMITH, E. E. (1977). Specific method for the purification of *Streptococcus mutans* dextranase. *Infection and Immunity* **16**, 760–765.
- MONTVILLE, T. J., COONEY, C. L. & SINSKEY, A. J. (1978). *Streptococcus mutans* dextranase: a review. *Advances in Applied Microbiology* **24**, 55–84.
- RUSSELL, R. R. B. (1979). Glycosyltransferases of *Streptococcus mutans* strain Ingbritt. *Microbios* **23**, 135–146.
- SHIMAMURA, A., TSUMORI, H. & MUKASA, H. (1982). Purification and properties of *Streptococcus mutans* extracellular glucosyltransferase. *Biochimica et biophysica acta* **702**, 72–80.
- SHIMAMURA, A., TSUMORI, H. & MUKASA, H. (1983). Three kinds of extracellular glucosyltransferases from *Streptococcus mutans* 6715 S (serotype g). *FEBS Letters* **157**, 79–84.
- SOMOGYI, M. (1945). A new reagent for the determination of sugars. *Journal of Biological Chemistry* **160**, 61–68.
- SPACKMAN, D. H., STEIN, W. H. & MOORE, S. (1958). Automatic recording apparatus for use in the chromatography of amino acids. *Analytical Chemistry* **30**, 1190–1206.
- TOWBIN, H., STAHELIN, T. & GORDON, J. (1979). Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proceedings of the National Academy of Sciences of the United States of America* **76**, 4350–4354.
- ZACHARIUS, R. M., ZELL, T. E., MORRISON, J. H. & WOODLOCK, J. J. (1969). Glycoprotein staining following electrophoresis on acrylamide gels. *Analytical Biochemistry* **30**, 148–152.