Sucrose-dependent Cell Adherence and Cariogenicity of Serotype *c* *Streptococcus mutans*

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Four strains of serotype *c* *Streptococcus mutans* differing in glucosyltransferase (GTase) and fructosyltransferase (FTase) activities were examined. These strains had been made resistant to streptomycin. FTase activity of an *S. mutans* clinical variant, MT6801R, which forms large mucoid colonies on sucrose-containing agar, was considerably higher than that of a typical serotype *c* strain, MT8148R, which forms small, rough colonies on the same agar. Two mutants, NG14 and NG7183, were induced from strain MT6801R by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine, and were found to be streptomycin-resistant. GTase and FTase activities of mutant NG14 were similar to those of the typical serotype *c* strain, while in mutant NG7183 the two enzyme activities were very low. Growing cells of these strains (except NG7183) adhered firmly to a glass surface in sucrose broth. Resting cells of all strains attached in small numbers to saliva-coated hydroxyapatite in the absence of sucrose. On the other hand, the presence of sucrose markedly enhanced the attachment of cells of strains MT8148R, MT6801R and NG14, but not NG7183. Cell-surface hydrophobicity and acid production of all strains were similar. Both strain MT8148R and NG14 colonized tooth surfaces and produced significant dental caries in specific-pathogen-free rats. Strain MT6801R had lower colonization ability and cariogenicity when compared with strains MT8148R and NG14. Furthermore, mutant NG7183 was able to colonize the tooth surfaces in small numbers, but failed to cause dental caries. These results indicate that sucrose-dependent cell adherence mediated by *de novo* glucan synthesis is necessary for the accumulation of serotype *c* *S. mutans* cells on the tooth surface and the induction of dental caries.

INTRODUCTION

Streptococcus mutans has been strongly implicated as a causative organism of dental caries. Strains of *S. mutans* are divided serologically into eight types, *a* to *h*. Among these serotypes, serotype *c* strains are most frequently isolated from human dental plaque (Hamada & Slade, 1980; Cohen *et al.*, 1983).

S. mutans produces both water-soluble and water-insoluble polysaccharides from sucrose by the action of multiple forms of glucosyltransferase (GTase) and fructosyltransferase (FTase) (Hamada & Slade, 1980). *De novo* synthesis of water-insoluble glucan is involved in the firm attachment and accumulation of serotype *d* and *g* *S. mutans* to tooth surfaces (Mukasa & Slade, 1973; Hamada, 1977; Koga & Inoue, 1978). In addition, the glucan firmly coats the tooth surface, forming a barrier which prevents the diffusion of acids produced by these serotypes.

Abbreviations: FTase; fructosyltransferase, GTase; glucosyltransferase, GTase-S; water-soluble glucan-synthesizing enzyme, GTase-I; water-insoluble glucan-synthesizing enzyme, MS; mitis-salivarius, BHI; Brain Heart Infusion, PAS; periodic acid-Schiff, S-HA; saliva-coated hydroxyapatite; MNNG, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine.

However, GTases of serotype *c* *S. mutans* are immunologically and biochemically different from the enzymes of serotypes *d* and *g* (Smith & Taubman, 1977; Russell, 1979a; Tsumori *et al.*, 1983). Moreover, serotype *c* *S. mutans* produces considerably less water-insoluble glucan than serotypes *d* and *g*. The biochemical and morphological properties of water-insoluble glucan of the former are very different from those of the glucan of the latter (Yakushiji *et al.*, 1984).

Therefore, the role of polysaccharides in cell adherence and cariogenicity of serotype *c* *S. mutans* should be clarified. In this study, we selected two clinical strains of serotype *c* *S. mutans* and two mutants derived from one of the strains. The mutants differed in their ability to produce glucan and fructan; their ability to adhere to hard surfaces and to form acid, as well as their cariogenicity in rats were compared. In addition, the properties of cell-free and cell-bound proteins of these strains are discussed in terms of their adherence and cell-surface hydrophobicity.

METHODS

Bacterial strains. The source of *S. mutans* MT8148 (serotype *c*) and MT6801 (serotype *c*) was described by Okahashi *et al.* (1984). Spontaneous streptomycin-resistant mutants from strains MT8148 and MT6801 were isolated and termed MT8148R and MT6801R, respectively. These strains showed the same colonial morphology on mitis-salivarius (MS) agar (Difco), and the same biochemical properties, as their parent strains. In the present study, we used these streptomycin-resistant strains together with two mutants (NG14 and NG7183) induced by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) (Adelberg *et al.*, 1965) and differing in colonial morphology on MS agar from strain MT6801R. These mutants were confirmed to be resistant to streptomycin.

Quantitative determination of glucan and fructan. Strains of *S. mutans* were grown for 18 h at 37 °C in Brain Heart Infusion (BHI; Difco) broth. The culture was then centrifuged and the cell-free culture supernatant was adjusted to pH 6.0 with 1 M-NaOH. The cells were washed with 0.1 M-potassium phosphate buffer, pH 6.0, and suspended in the same buffer (volume equivalent to that of the broth in which they were grown originally). GTase and FTase activities were measured by the method of Koga & Inoue (1981). For the measurement of GTase activity, the enzyme was allowed to react with 10 mM-[¹⁴C-glucose]sucrose [0.4 Ci mol⁻¹ (14.8 GBq mol⁻¹) New England Nuclear] and 20 µM-dextran T10 in 20 µl 0.1 M-potassium phosphate buffer, pH 6.0. For the measurement of FTase activity, the enzyme was allowed to react with 10 mM-[¹⁴C-fructose]sucrose [0.4 Ci mol⁻¹ (14.8 GBq mol⁻¹) New England Nuclear] in 0.1 M-potassium phosphate buffer, pH 6.0. 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, Japan). The squares were washed three times with methanol and dried. Radioactivity retained on the paper squares was measured with a Packard model 3385 scintillation spectrometer to quantify the polysaccharide synthesized. One unit (U) of GTase and FTase activities was defined as the amount of enzyme that transferred 1 µmol of sucrose to polysaccharide min⁻¹ under the conditions described above.

Synthesis of polysaccharide by growing cells was determined as follows. *S. mutans* was cultured in BHI broth containing 1% (w/v) sucrose for 18 h at 37 °C. Three fractions of polysaccharides, (i) extracellular, water-soluble, (ii) 1 M-NaOH-soluble, cell-associated and (iii) 1 M-NaOH-insoluble, cell-associated, were prepared from the culture according to the procedure of Freedman & Tanzer (1974). The glucan and fructan contents of these fractions were quantified differentially by the cold anthrone method (Handel, 1967), using glucose and fructose as the standards.

Adherence of growing cells to a glass surface in sucrose broth. *S. mutans* was grown at 37 °C at a 30° angle for 18 h in 3 ml BHI broth containing 1% (w/v) sucrose. The number of adherent cells was determined turbidimetrically and expressed as a percentage of the total cell mass (percentage cell adherence) as described by Hamada *et al.* (1981).

Adsorption of resting cells to saliva-coated hydroxyapatite. Spheroidal hydroxyapatite beads (5 mg) (BDH) were incubated with 160 µl clarified whole saliva for 1 h at 37 °C, and washed three times with buffered KCl (Eifert *et al.*, 1984). *S. mutans* was grown at 37 °C for 18 h in BHI broth containing [6-³H]thymidine [21.0 Ci mmol⁻¹ (777 GBq mmol⁻¹) Amersham] at a final concentration of 10 µCi ml⁻¹. [³H]Thymidine-labelled bacteria (2 × 10⁸) were allowed to react with the saliva-coated hydroxyapatite beads (5 mg) in the presence or absence of 1% (w/v) sucrose in 160 µl buffered KCl (Eifert *et al.*, 1984). After mixing with a micro-mixer (Taiyo Kagaku Kogyo, Tokyo, Japan) at 37 °C for 30 min, the reaction mixtures in microtitre plates were allowed to stand at 37 °C for 0.5 to 6.5 h. After incubation, the beads were washed three times in buffered KCl. The radioactivity associated with the S-HA beads was then determined. The number of bacteria adsorbed was determined from the calculated specific radioactivity of the bacteria.

Hydrophobicity. *S. mutans* strains were grown at 37 °C for 18 h in BHI broth. The relative surface hydrophobicity of the cells was determined by their adsorption to hexadecane and their aggregation in salt solution. The ability of the strains to adsorb to hexadecane was estimated as described by Rosenberg *et al.* (1980).

The cells were washed twice and suspended in PUM buffer (Rosenberg *et al.*, 1980) to an OD₅₅₀ of 0.6. Triplicate samples (3 ml) of the bacterial suspensions were placed in test-tubes, and 300 µl hexadecane was added. The tubes were then mixed with a vortex mixer for 1 min and allowed to stand until the phases separated. The OD₅₅₀ of the lower aqueous phase was measured. Adsorption was calculated as the percentage loss in optical density relative to that of the initial cell suspension. Salt aggregation ability was determined by the procedure of Fives-Taylor & Thompson (1985). Cells were washed twice and suspended in 2 mM-potassium phosphate buffer, pH 6.8, to a concentration of 4×10^9 cells ml⁻¹. Dilutions of ammonium sulphate, pH 6.8, from 4.0 to 0.2 M were made in 2 mM-potassium phosphate buffer, pH 6.8, in 0.2 M increments. The bacterial suspensions (30 µl) were added to 30 µl of the appropriate salt dilution in 96-well, V-bottomed microtitre plates. The plates were rocked gently for 2 min at room temperature, and aggregation was read macroscopically. The data are represented as the lowest ammonium sulphate concentration causing bacterial aggregation.

SDS-PAGE. *S. mutans* strains were grown in dialysate medium of BHI broth at 37 °C for 18 h. The cell-free culture supernatant was collected by centrifugation at 10000 *g* for 20 min at 4 °C. Extracellular proteins were precipitated from the culture supernatant by adding solid ammonium sulphate to 50% saturation. The precipitate was collected by centrifugation, dissolved in 5 mM-potassium phosphate buffer, pH 6.8, and dialysed against the buffer. The concentrated culture supernatant was incubated at 37 °C for 5 min in 10 mM-Tris/HCl buffer, pH 6.8, containing 2% (w/v) SDS and 1% (w/v) 2-mercaptoethanol. The whole cells (50 mg dry weight ml⁻¹) were suspended in 2% (w/v) SDS and 1% (w/v) 2-mercaptoethanol and heated at 100 °C for 30 min. The SDS extract was clarified by centrifugation. SDS-polyacrylamide slab gels were prepared as described by Laemmli (1970). The culture supernatant (0.5 mg protein) and the cell extract (30 µl) were electrophoresed 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% (w/v) SDS. Ovalbumin (*M*_r 43000), catalase (60000), bovine serum albumin (67000), phosphorylase B (94000) and ferritin (220000) were used as *M_r* standards. Proteins were stained with Coomassie brilliant blue R-250. To detect GTase and FTase activities, the gels were incubated at 37 °C for 18 h with 5% (w/v) sucrose and 0.1% (w/v) dextran T10 (Pharmacia) in 50 mM-potassium phosphate buffer, pH 6.0. In addition, the gels were incubated at 37 °C for 48 h with 5% (w/v) raffinose in 50 mM-potassium phosphate buffer, pH 6.0, to detect FTase (Russell, 1979*a*). Polysaccharides formed were stained with the periodic acid-Schiff (PAS) reagent by the method of Zacharius *et al.* (1969).

Electrophoretic blotting. The culture supernatant (0.5 mg protein) was electrophoresed in SDS-polyacrylamide slab gels as described above, and transferred to a nitrocellulose sheet by the electrophoretic blotting technique (Burnette, 1981). The sheet was treated with a monoclonal antibody which reacted with a water-soluble glucan-synthesizing enzyme (GTase-S) purified by the method of Sato *et al.* (1984). 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 (Furuta *et al.*, 1983).

Acid production. *S. mutans* strains were grown aerobically and anaerobically (Gas Pack; BBL) for 48 h in BHI broth supplemented with 1% (w/v) glucose. Growth of the culture was measured as the OD₅₅₀. The final pH of the broth was measured with a PHM 82 standard pH meter (Radiometer). The amount of lactic acid in the culture supernatant was determined by the method of Gutmann & Wahlefeld (1974).

Cariogenicity. The ability of streptomycin-resistant *S. mutans* strains to induce dental caries in specific-pathogen-free Sprague-Dawley rats was determined as described by Ooshima *et al.* (1983). In brief, weanling rats (15 d old) were given both an ordinary powdered diet MF (Oriental Yeast Co., Osaka, Japan) containing tetracycline [4 mg (g diet)⁻¹] and deionized water containing penicillin G [4000 U (ml water)⁻¹] *ad libitum*. After treatment with antibiotics for 2 d, *S. mutans* cells (1×10^9) were orally inoculated in groups of 10 to 12 rats on six consecutive days; the rats were then maintained on diet 2000 containing 56% (w/w) sucrose (Keyes & Jordan, 1964). Dental plaque samples were obtained from the surfaces of the molar teeth of the rats using a cotton applicator, which was then shaken in a test tube containing 1 ml sterile saline (0.85% NaCl). Suspended samples (0.1 ml) were spread on MS agar containing streptomycin (500 µg ml⁻¹). The plates were incubated at 37 °C for 2 d, and the number of colonies recovered was determined. At 86 d after the initial infection, the animals were killed, and the jaws were removed. The caries lesions were scored by the modification by Ooshima *et al.* (1983) of the method of Keyes (1944).

RESULTS

Colonial morphology and polysaccharide production

S. mutans MT8148R, a typical serotype *c* strain, formed small, raised, rough, pale blue colonies on MS agar, while *S. mutans* MT6801R, a clinical variant from human dental plaque, formed large mucoid colonies on the same agar (Fig. 1*a, b*). Two mutants (NG14 and NG7183), differing in colonial morphology from the parent strain MT6801R, were induced by MNNG.

Fig. 1. Colonial morphology of serotype *c* *S. mutans* strains on MS agar. (a) Strain MT8148R; (b) strain MT6801R; (c) strain NG14; (d) strain NG7183.

Table 1. *GTase and FTase activities of serotype c S. mutans strains*

Strains of *S. mutans* were grown at 37 °C for 18 h in BHI broth. Cell-free and cell-bound enzyme activities were determined as described in Methods. Values are the mean $\pm$ SD of triplicate cultures.

Strain	Enzyme activity (mU ml ⁻¹)			
	Cell-free		Cell-bound	
	GTase	FTase	GTase	FTase
MT8148R	15.7 $\pm$ 4.8	21.6 $\pm$ 1.7	1.4 $\pm$ 0.5	0.8 $\pm$ 0.2
MT6801R	12.9 $\pm$ 1.6	84.4 $\pm$ 2.1	1.1 $\pm$ 0.1	2.3 $\pm$ 0.6
NG14	11.2 $\pm$ 2.4	9.9 $\pm$ 0.4	1.1 $\pm$ 0.1	0.5 $\pm$ 0.1
NG7183	0.8 $\pm$ 0.1	0	0.1 $\pm$ 0.1	0

The colonial morphology of mutant NG14 was similar to that of the typical serotype *c* *S. mutans* strain MT8148R, while mutant NG7183 formed significantly smaller smooth colonies (Fig. 1c, d).

GTase and FTase activities of the above strains grown in BHI broth are shown in Table 1. Most of the activity of the two enzymes was located extracellularly in glucose broth. The FTase activity of strain MT6801R was considerably higher than that of strain MT8148R. These findings indicate that the mucoid material of the MT6801R colonies should be fructan. The GTase activity of mutant NG14 was similar to that of the parent strain MT6801R, and the FTase activity of the mutant was far lower than that of the parent strain. In the mutant NG7183, activities of both enzymes were very low.

Table 2. Extracellular and cell-associated polysaccharides produced by serotype *c* *S. mutans* strains grown in sucrose broth

Strains of *S. mutans* were grown at 37 °C for 18 h in BHI broth containing 1% (w/v) sucrose. Three polysaccharide fractions were prepared from the culture by the procedure of Freedman & Tanzer (1974). The glucan and fructan contents of these fractions were quantified differentially by the cold anthrone method. Values are the mean $\pm$ SD of triplicate cultures.

Strain	Polysaccharide synthesized (mg ml ⁻¹)					
	Extracellular, water-soluble		1 M-NaOH-soluble, cell-associated		1 M-NaOH-insoluble, cell-associated	
	Glucan	Fructan	Glucan	Fructan	Glucan	Fructan
MT8148R	4.25 $\pm$ 0.08	0.75 $\pm$ 0.07	0.62 $\pm$ 0.01	0.09 $\pm$ 0.01	0.17 $\pm$ 0.02	<0.01
MT6801R	1.55 $\pm$ 0.04	1.95 $\pm$ 0.01	0.42 $\pm$ 0.02	0.03 $\pm$ 0.01	0.11 $\pm$ 0.01	<0.01
NG14	1.25 $\pm$ 0.06	0.60 $\pm$ 0.10	0.48 $\pm$ 0.01	<0.01	0.09 $\pm$ 0.01	<0.01
NG7183	0.95 $\pm$ 0.11	<0.01	0.04 $\pm$ 0.01	<0.01	0.10 $\pm$ 0.01	<0.01

Polysaccharides produced by *S. mutans* grown overnight in 1% (w/v) sucrose broth were separated into three fractions and quantified (Table 2). All strains used produced extracellular, water-soluble glucan. Among these strains, the production of the glucan by strain MT8148R was very high. Furthermore, all strains except mutant NG7183 produced 1 M-NaOH-soluble, cell-associated glucan. The amount of extracellular, water-soluble fructan produced by strain MT6801R was lower than would be expected from the FTase activity detected (Tables 1 and 2). This result may be ascribed to the hydrolysis of fructan by fructanase and/or the difference of conditions of fructan synthesis. The fructan content of the 1 M-NaOH-soluble, cell-associated polysaccharide was very low. No significant difference was observed among the strains with regard to 1 M-NaOH-insoluble, cell-associated polysaccharide synthesis.

Adherence of *S. mutans*

Adherence of growing cells of serotype *c* *S. mutans* strains to a glass surface was determined. Values of percentage cell adherence of strains MT8148R, MT6801R, NG14 and NG7183 were 78.7 $\pm$ 5.7, 82.5 $\pm$ 2.5, 85.2 $\pm$ 0.9 and 3.3 $\pm$ 0.9 (mean $\pm$ SD from triplicate cultures), respectively.

The kinetics of adsorption of [³H]thymidine-labelled resting cells (2×10^8) of *S. mutans* MT8148R to S-HA was examined. Adsorption of the cells to S-HA reached a maximum value after 30 min incubation in the absence of sucrose (Fig. 2). However, a 3 h incubation was required to achieve maximum adsorption in the presence of sucrose. Based on these results, the reaction mixtures were incubated for 3 h in subsequent experiments. As shown in Table 3, resting cells of all strains used attached similarly to S-HA in the absence of sucrose. However, addition of sucrose to the reaction mixture markedly enhanced the attachment of strains MT8148R, MT6801R and NG14, but had little effect on the attachment of mutant NG7183.

Surface hydrophobicity

The cell-surface hydrophobicity of serotype *c* *S. mutans* strains was determined by their adsorption to hexadecane and their aggregation in ammonium sulphate. The hydrophobicity of mutants NG14 and NG7183 was slightly higher than that of strains MT8148R and MT6801R.

SDS-PAGE analysis of whole cells and culture supernatants

SDS extracts of serotype *c* *S. mutans* strains were analysed by SDS-PAGE. Gels stained with Coomassie brilliant blue showed that mutant NG7183 did not possess a protein of M_r 160000 (protein B) which was present in strains MT8148R, MT6801R and NG14 (Fig. 3). Extracts of all the strains contained a high- M_r protein (M_r 200000; protein A). Strain MT6801R produced large amounts of extracellular proteins with M_r values of 96000 (protein C) and 83000 (protein

Fig. 2. Adsorption to S-HA of resting cells of *S. mutans* MT8148R. [^3H]Thymidine-labelled bacteria (2×10^8) were allowed to react with S-HA (5 mg) in the presence (●) or absence (▲) of 1% (w/v) sucrose for 15 min to 7 h at 37°C.

Table 3. Adsorption to S-HA of resting cells of serotype *c* *S. mutans* strains

[^3H]Thymidine-labelled bacteria (2×10^8) were allowed to react with S-HA (5 mg) in the presence or absence of 1% (w/v) sucrose for 3 h at 37°C. Values are the mean $\pm$ SD of triplicate assays.

Strain	10 ⁻⁶ $\times$ No. of cells adsorbed per 5 mg S-HA	
	Without sucrose	With sucrose
MT8148R	25.4 $\pm$ 3.0	87.3 $\pm$ 5.1
MT6801R	25.2 $\pm$ 1.1	74.4 $\pm$ 1.3
NG14	34.2 $\pm$ 3.2	96.9 $\pm$ 7.8
NG7183	26.8 $\pm$ 0.9	29.4 $\pm$ 2.2

D) (Fig. 4a) which produced fructan from sucrose (Fig. 4b) and raffinose (data not shown). In addition, several FTase bands were noted in this strain. On the other hand, no FTase band was detected in mutant NG7183. The quantity of the M_r 160000 protein (protein B) produced by mutant NG7183 was much less than that of the same protein produced by strains MT8148R, MT6801R and NG14. The protein B band synthesized opaque white material from sucrose and reacted with anti-GTase-S monoclonal antibody (Fig. 4c), but did not synthesize polysaccharide from raffinose (data not shown). These results indicate that the protein B band is GTase.

Acid production

Acid production of serotype *c* *S. mutans* was determined in 1% (w/v) glucose broth under aerobic and anaerobic conditions. Acid production by mutants NG14 and NG7183 was comparable to that by the parent strain (MT6801R) and strain MT8148R in terms of final pH of the culture broth and concentration of lactate in the culture supernatant under both conditions (Table 5). No difference in relative growth rates of all the strains was detected under aerobic or anaerobic conditions.

Cariogenicity and colonization ability

Serotype *c* *S. mutans* strains were tested for their pathogenicity in specific-pathogen-free Sprague-Dawley rats reared on a high sucrose diet. Strain MT8148R and mutant NG14 induced significant dental caries, while strain MT6801R, which produced a large amount of fructan, had diminished colonization ability and decreased cariogenicity as compared to strain MT8148R (Table 6). Mutant NG7183, which had lost the ability to form adhesive deposits on a glass surface in sucrose broth, was able to colonize the tooth surface of rats, but caused little decay. In addition, strains MT8148R, MT6801R and NG14 produced both smooth surface caries and fissure caries, but mutant NG7183 produced only fissure caries (data not shown).

Fig. 3. SDS-PAGE of SDS extracts of serotype *c* *S. mutans* strains. Whole cells were suspended in 2% (w/v) SDS and 1% (w/v) 2-mercaptoethanol, and heated at 100 °C for 30 min. The SDS extract was clarified by centrifugation, and analysed by SDS-PAGE. The gel was stained with Coomassie brilliant blue. M_r markers used were ovalbumin (M_r 43 000), catalase (60 000), bovine serum albumin (67 000), phosphorylase B (94 000) and ferritin (220 000). Lane 1, strain MT8148R; lane 2, strain MT6801R; lane 3, strain NG14; lane 4, strain NG7183. Protein A is a high- M_r protein (M_r 200 000); protein B is a GTase protein (M_r 160 000).

Table 4. *Hydrophobicity of serotype c S. mutans strains*

Cells were grown in BHI broth. The relative surface hydrophobicity of the cells was determined by their adsorption to hexadecane and their aggregation in salt. The bacterial suspension (3 ml) was mixed with 300 μ l hexadecane. Adsorption was calculated as the percentage loss in optical density relative to that of the initial cell suspension (see Methods). The value of salt aggregation ability represents the lowest ammonium sulphate concentration (M) causing bacterial aggregation. Values are the mean $\pm$ SD of triplicate assays.

Strain	Hydrophobicity	
	Bacteria adsorbed to hexadecane	Bacterial aggregation (M)
MT8148R	27.2 $\pm$ 4.7	1.2
MT6801R	21.1 $\pm$ 2.0	1.2
NG14	38.3 $\pm$ 3.6	1.1
NG7183	32.7 $\pm$ 3.8	1.1

DISCUSSION

S. mutans adheres to tooth surfaces by sucrose-independent and sucrose-dependent mechanisms. The former mechanism is via electrostatic, lectin-like and hydrophobic interactions, and hydrogen bonding (Gibbons, 1984), whereas the latter is due to *de novo*

Fig. 4. SDS-PAGE and electrophoretic blotting of culture supernatants of serotype *c S. mutans* strains. Culture supernatants were concentrated, and treated at 37 °C for 5 min in 2% (w/v) SDS and 1% (w/v) 2-mercaptoethanol, and analysed by SDS-PAGE. (a) Gel stained with Coomassie brilliant blue. (b) Gel incubated at 37 °C for 18 h with 5% (w/v) sucrose and 0.1% (w/v) dextran T10 in 50 mM-potassium phosphate buffer, pH 6.0. Polysaccharides formed were stained with PAS reagent. (c) Anti-GTase-S 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. Lane 1, strain MT8148R; lane 2, strain MT6801R; lane 3, strain NG14; lane 4, strain NG7183.

Table 5. Acid production by serotype *c* *S. mutans* strains

Cells were grown aerobically and anaerobically in BHI broth containing 1% (w/v) glucose for 48 h at 37 °C. Values are the mean $\pm$ SD of triplicate cultures.

Strain	Growth (OD ₅₅₀)		Final pH		Lactate production [$\mu\text{mol (ml culture)}^{-1}$]	
	Aerobic	Anaerobic	Aerobic	Anaerobic	Aerobic	Anaerobic
MT8148R	3.0 $\pm$ 0.3	2.8 $\pm$ 0.4	4.2 $\pm$ 0.1	4.0 $\pm$ 0.2	45.2 $\pm$ 5.0	51.4 $\pm$ 2.7
MT6801R	3.0 $\pm$ 0.1	2.7 $\pm$ 0.4	4.2 $\pm$ 0.1	4.1 $\pm$ 0.1	45.0 $\pm$ 5.9	52.7 $\pm$ 2.0
NG14	2.9 $\pm$ 0.3	2.7 $\pm$ 0.2	4.3 $\pm$ 0.1	4.1 $\pm$ 0.1	42.7 $\pm$ 5.7	50.2 $\pm$ 1.8
NG7183	2.8 $\pm$ 0.3	2.7 $\pm$ 0.3	4.3 $\pm$ 0.2	4.2 $\pm$ 0.1	48.9 $\pm$ 3.0	48.4 $\pm$ 1.8

Table 6. Cariogenicity of serotype *c* *S. mutans* strains in specific-pathogen-free Sprague-Dawley rats

Strain	No. of rats	Caries score (mean $\pm$ SD)	Recovery of <i>S. mutans</i> [log (c.f.u.) (mean $\pm$ SD)]
MT8148R	10	23.9 $\pm$ 7.4	6.73 $\pm$ 0.43
MT6801R	11	17.7 $\pm$ 2.8	4.73 $\pm$ 0.44
NG14	10	25.1 $\pm$ 7.7	6.31 $\pm$ 0.56
NG7183	11	1.7 $\pm$ 1.6	4.17 $\pm$ 0.57
None	12	1.8 $\pm$ 1.3	0

synthesis of water-insoluble glucan by GTases from sucrose (Hamada & Slade, 1980). In this study, we isolated from *S. mutans* MT6801R (serotype *c*) a mutant, NG7183, that formed small, smooth colonies on MS agar. We found that mutant NG7183 could not adhere to a glass surface in sucrose broth, although the mutant was able to colonize the surfaces of teeth *in vivo*. Clearly, there is some disparity between *in vitro* sucrose-dependent adherence and *in vivo* adherence, indicating that sucrose-dependent adherence does not appear to be the whole story in *in vivo* experiments. Whole cells of mutant NG7183 attached to S-HA in the absence of sucrose, suggesting that serotype *c* *S. mutans* can colonize the surfaces of teeth by a sucrose-independent mechanism. However, its attachment was enhanced in the presence of sucrose. In addition, mutant NG7183 failed to cause dental caries in spite of having the same ability to produce acid and the same growth rate as strains MT8148R, MT6801R and NG14. These results imply that the sucrose-dependent adherence is required for caries induction by serotype *c* *S. mutans*.

Recently, the role of cell-surface hydrophobicity in mediating bacterial adherence to host surfaces has received considerable attention by many investigators (Olsson & Westergren, 1972; Nesbitt *et al.*, 1982; Weiss *et al.*, 1982; Westergren & Olsson, 1983). Gibbons & Etherden (1983) reported that highly hydrophobic oral bacteria attach in significantly higher numbers to experimental pellicles than do less hydrophobic or hydrophilic bacteria. McBride *et al.* (1984) have indicated that hydrophobic *S. mutans* strains possess a protein of M_r 190000, similar to antigen I/II (Russell *et al.*, 1980) and antigen B (Russell, 1979*b*), in the cell wall. In this study, the hydrophobicity of mutants NG14 and NG7183 and their ability to attach to S-HA in the absence of sucrose was slightly higher than that of the parent strain MT6801R (Tables 3 and 4). SDS extracts from whole cells of all strains used in this study contained the high- M_r protein (Fig. 3), which was confirmed by the electrophoretic blotting method to react with anti-antigen B serum (data not shown). The increase of hydrophobicity of the mutants may result from exposure of new hydrophobic components due to a change in the hydrophilic proteins on the cell surfaces.

The high-fructan-producing strain MT6801R adhered firmly to a glass surface in sucrose broth. However, the number of cells of strain MT6801R that adsorbed to experimental pellicles

in the presence of sucrose was lower than that of strains MT8148R and NG14. In addition, strain MT6801R had lower colonization ability and cariogenicity when compared with strains MT8148R and NG14 (Table 6). These results suggest that fructan is unable to act as a mediator in the attachment and accumulation of *S. mutans* to tooth surfaces or as a barrier to prevent the diffusion of acids produced by the organism. The decrease in the colonization ability and cariogenicity of strain MT6801R might be attributable to production of large amounts of fructan as well as water-insoluble glucan. The presence of significant amounts of fructan in water-insoluble glucan may disturb firm adherence of the glucan to the tooth surface, since fructan can be easily removed by dissolving in saliva.

In summary, the present results indicate (i) that serotype *c* *S. mutans* adheres to tooth surfaces by sucrose-independent and sucrose-dependent mechanisms, (ii) that the sucrose-dependent adherence is required for caries induction by serotype *c* *S. mutans*, as well as serotype *d* and *g* strains and (iii) that fructan does not act as a virulence factor of serotype *c* *S. mutans*.

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