Effects of selected surfactants on purified glucosyltransferases from mutans streptococci and cellular adherence to smooth surfaces

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Summary. The inhibitory effect of non-ionic, anionic, cationic and ampholytic surfactants on cellular growth of *Streptococcus mutans* MT8148 and *S. sobrinus* 6715, on glucan synthesis by the purified glucosyltransferase (GTase) from these organisms, and on bacterial adherence to glass surfaces was examined *in vitro*. Cationic surfactants exhibited marked bactericidal activities. Anionic and ampholytic compounds were less strongly bactericidal and non-ionic surfactants produced only slight inhibition of cell growth under the conditions tested. Some non-ionic compounds had no effect on this. Glucan synthesis by GTase from mutans streptococci was inhibited by anionic and cationic surfactants. Among various GTase proteins, insoluble glucan synthesising GTases, i.e., *S. mutans* CA-GTase and *S. sobrinus* GTase-I were those most effectively inhibited by these agents. However, it was noted that whereas lower concentrations of cationic surfactants enhanced these GTase activities, higher concentrations of the surfactants were inhibitory. Non-ionic detergents stimulated soluble glucan synthesis from *S. mutans* CF-GTase and cationic and ampholytic surfactants enhanced or inhibited glucan synthesis depending on the concentrations of the surfactants. Sucrose-dependent cellular adherence of resting cells of mutans streptococci to glass surfaces was inhibited by the addition of surfactants that annulled the GTase activities.

Introduction

There is strong evidence that synthesis of adherent, water-insoluble glucans from sucrose by glucosyltransferases (GTases) of mutans streptococci, including *Streptococcus mutans* and *S. sobrinus*, is an important factor in the induction of dental caries.¹ When mutants of these organisms lose their ability to synthesise such glucans they exhibit markedly decreased cariogenicity in experimental animals.^{2, 3} It is, therefore, logical to consider that to inhibit glucan formation in mutans streptococci is a valid strategy for restricting adherence and persistent colonisation by these caries-inducing organisms.⁴

A number of substances have been reported as possible inhibitors of GTases. Natural compounds, e.g., cocoa extract⁵ and tea extracts^{6, 7} are known to contain GTase-inhibitory constituents, and it has also been shown that some amino sugar derivatives, such as acarbose and nojirimycin, and glycyrrhizin, a saponin of liquorice, inhibit the synthesis of insoluble glucans by GTase from mutans streptococci.^{8, 9} Oligosaccharides containing α -linked glucosidic linkages such as maltose, isomaltose and panose,^{10, 11} dextrins,¹² structural isomers of sucrose such as

palatinose and trehalulose,¹³ and maltitol, a disaccharide sugar alcohol that contains α (1 $\rightarrow$ 4) linkage¹⁴ also suppress GTase activity significantly.

Dental plaque accumulation can be controlled by topical applications of dentifrices or the use of mouth rinses that contain surfactants of detergents, whose surface activity is produced by the hydrophilic (polar) and hydrophobic (hydrocarbon or non-polar) moieties of their molecules.^{15, 16} Surfactants have been classified most commonly as anionic, cationic, ampholytic and non-ionic, depending on the nature of their electrical charges.¹⁷ Some surfactants exhibit anti-enzyme and antimicrobial activities.¹⁸⁻²⁰

No comparison has been attempted, however, to determine the effects of detergents of different formulations on highly purified GTases, cellular growth and adherence of mutans streptococci. This paper describes the effects of representative detergent compounds on these factors.

Materials and methods

Organisms

S. mutans MT8148 (serotype c) and *S. sobrinus* 6715 (serotype g) were selected from the culture collection at the Department of Oral Microbiology, Osaka University Faculty of Dentistry, Suita-Osaka. They were

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Table 1. Minimum inhibitory concentrations (MICs) of selected surfactants for *S. mutans* MT8148 and *S. sobrinus* 6715

Surfactant	MIC ($\mu\text{g/ml}$)*	
	MT8148	6715
Anionic		
Sodium dodecyl sulphate (SDS)	40.0 (14.1)	50.0 (14.1)
Sodium di 2-ethylhexyl sulphosuccinate	70.0 (17.5)	167.7 (58.9)
Cationic		
Cetyltrimethylammonium bromide (CTAB)	0.030 (0.016)	0.030 (0.016)
Cetylpyridinium chloride (CPC)	0.030 (0.011)	0.050 (0.035)
Ampholytic		
Lauryldimethylamino-acetic acid betaine	450.0 (141.4)	250.0 (70.7)
Alkyl diaminoethyl glycine	40.0 (14.1)	35.0 (18.7)
Non-ionic		
Octylphenol-ethylene oxide (Nonidet P-40)	450.0 (212.1)	350.0 (187.0)
POE (20) Sorbitan monolaurate (Tween 20)	NI	NI

* Data are expressed as the mean (SD) of triplicate experiments. NI, not inhibitory.

grown in Todd-Hewitt Broth (Difco) supplemented with glucose 0.5% w/v and incubated at 37°C for 18 h. Cells were harvested by centrifugation, washed three times with distilled water, and lyophilised.

Chemicals

Sodium dodecyl sulphate, cetylpyridinium chloride, and polyoxyethylene (20) sorbitan monolaurate [POE (20)] were purchased from Wako Pure Chemicals, Osaka, Japan. Cetyltrimethylammonium bromide was purchased from Nakarai Chemicals, Kyoto, Japan, and octylphenol-ethylene oxide was from Sigma. [^{14}C -glucose]sucrose was obtained from New England Nuclear Corp., MA, USA. Sodium di 2-ethylhexyl sulphosuccinate, lauryldimethylaminoacetic acid betaine and alkyl diaminoethyl glycine were kindly supplied by Y. Yamamoto, Sunstar Inc., Osaka, Japan.

Preparation of glucosyltransferases (GTases)

S. mutans MT8148 and *S. sobrinus* 6715 were grown in 8 L of diffusate medium of TTY broth for 18 h and 37°C.²¹ The supernate from each culture was concentrated by passing it through an ultrafiltration module (Model ACL-1010, Asahi Chemical Industry, Tokyo, Japan), and salted out by 50% saturated ammonium sulphate. The precipitate was dialysed against 10 mM potassium phosphate buffer (pH 6.0), and used as a crude cell-free GTase preparation.

The crude cell-free GTase (CF-GTase) of *S. mutans* MT8148 was further purified by the chromatofocussing method with a Polybuffer exchanger PBE94

(Pharmacia LKB Biotechnology, Uppsala, Sweden).²² This enzyme produced mainly water-soluble glucan (SG) from sucrose. Cell-associated GTase (CA-GTase) synthesising water-insoluble glucan (IG) was extracted from whole cells of *S. mutans* MT8148 by treatment with 8 M urea at 25°C for 1 h, and the extract was purified by DEAE-Sephacel (Pharmacia) column chromatography as described previously.²³

GTase-I synthesising IG, and two GTases synthesising SG (GTase-Sa and GTase-Sb) were isolated from the crude cell-free GTase of *S. sobrinus* 6715 by the chromatofocussing methods as described previously.²⁴ It was noted that GTase-Sa was primer-independent and GTase-Sb primer-dependent.²⁵

Minimum inhibitory concentrations (MICs) of surfactants

Two-fold dilutions of the surfactants were made to provide a range of concentrations from 1 to 0.00001%; 100 μl of each solution was placed into wells of the flat-bottomed 96-well plate (Corning Glass Works, Corning, NY, USA). Subsequently, 100 μl containing 1×10^6 cfu of an overnight culture of organisms suspended in $2 \times$ concentrated Brain Heart Infusion Broth (Difco) was inoculated into each well. After the plate was incubated at 37°C for 48 h, the MIC was determined as the lowest concentration of surfactant that caused complete inhibition of growth of the indicator organisms.

Glucan synthesis in the presence of surfactants

Synthesis of IG was measured turbidimetrically by increased absorbance at 550 nm after IG-synthesising GTase (10–20 mU) was incubated in 2 ml of 50 mM sodium phosphate buffer (pH 6.0; hereafter designated NaPB) containing sucrose 1%, sodium azide 0.05% and surfactants in the presence or absence of primer Dextran T10 (20 μM , Pharmacia) at 37°C for 18 h.

For the measurement of SG synthesis, SG-synthesising GTase was allowed to react with 10 mM [^{14}C -glucose]sucrose (1.85 GBq/mol) and various concentrations of surfactants, with or without Dextran T10 in 0.1 M NaPB, pH 6.0. After incubating at 37°C for 1 h, this was spotted onto a filter paper (7.5 $\times$ 15.0 mm, no. 514A; Toyo Roshi, Tokyo, Japan), washed three times with methanol 100% and dried. Radioactivity of [^{14}C]glucan synthesised was quantitated by placing the filter paper in a scintillation counter (Model 1214, Pharmacia) as described by Koga *et al.*² One unit of GTase activity was defined as the amount of enzyme that transformed 1 μmol of sucrose to glucan/min under the conditions described above.

Adherence of resting cells to a glass surface

To assess the inhibition of sucrose-dependent adherence of mutans streptococci to the surface of a glass

Fig. 1. Effects of surfactants on water-insoluble glucan synthesis of CA-GTase (**A**) and on water-soluble glucan synthesis of CF-GTase (**B**) from *S. mutans* MT8148. CA-GTase was assayed without 10 μ M Dextran T10; CF-GTase was assayed with 10 mM [14 C-glucose]sucrose in the presence of 10 μ M Dextran T10. Anionic surfactants—sodium dodecyl sulphate (—○—), sodium di-2-ethylhexyl sulphosuccinate (—●—); cationic—cetyltrimethylammonium bromide (—○—), cetylpyridinium chloride (—●—); ampholytic—lauryldimethylaminoacetic acid betaine (—○—), alkyl di-aminoethyl glycine (—●—); non-ionic—octylphenol-ethylene oxide (—○—), POE (20) sorbitan monolaurate (—●—).

tube, resting cell suspensions (1.5 mg/3 ml of 50 mM NaPB containing sodium azide 0.05% tube) were incubated in sucrose 1% and various concentrations of surfactants at 37°C for 18 h at an angle of 30°. The quantity of cells that adhered to the glass surface was determined turbidimetrically and expressed as percentage adherence.¹²

Results

Four different surfactants were ranked with regard to their inhibitory effect on the growth of mutans streptococci as follows: cationic $\gg$ anionic $\geq$ ampholytic $>$ non-ionic surfactants (table I). There was no significant difference in MICs of these surfactants between the two strains of *S. mutans* and *S. sobrinus*. Non-ionic detergents such as POE (20)

sorbitan monolaurate (Tween 20) (table I), POE (20) sorbitan monooleate (Tween 80), POE (20) nonyl-phenyl ether, and POE (40) monostearate did not inhibit mutans streptococci. Octylphenol-ethylene oxide (Nonidet P-40) was slightly inhibitory to the growth of mutans streptococci (table I).

In the assessment of the inhibitory effects of various surfactants on glucan synthesis from sucrose by GTases of mutans streptococci, it was found that anionic surfactants were the most effective in inhibiting water-insoluble glucan synthesis by CA-GTase from *S. mutans*. Cationic surfactants also exhibited strong inhibitory effects on CA-GTase. However, these agents activated enzyme activity at a lower concentration, i.e., 0.001%. Although ampholytic and non-ionic surfactants did not influence GTase activity significantly, alkyl diaminoethyl glycine produced a

Fig. 2. Effects of surfactants on glucan synthesis of extracellular GTases from *S. sobrinus* 6715: a, sodium dodecyl sulphate; b, sodium di-2-ethylhexyl sulphosuccinate; c, cetyltrimethylammonium bromide; d, cetylpyridinium chloride; e, lauryldimethylaminoacetic acid betaine; f, alkyl diaminoethyl glycine. Crude GTase (▨) and GTase-Sa (▤) were assayed without 10 μ M Dextran T10; GTase-I (▧) and GTase-Sb (■) were assayed with 10 μ M Dextran T10. Data (means of triplicate experiments) are expressed as the ratio of the quantities of glucan synthesised in the presence of surfactant and those without.

two-fold rise in insoluble glucan synthesis at a final concentration of 0.1 % (fig. 1A). Water-soluble glucan synthesis due to CF-GTase of *S. mutans* was markedly inhibited by anionic and cationic surfactants at concentrations of 0.1–1 %. Cationic, ampholytic and non-ionic surfactants enhanced glucan synthesis at lower concentrations, but this was suppressed at higher concentrations (fig. 1B).

Fig. 2 shows the effects of various surfactants on glucan synthesis by crude GTase, GTase-Sa, GTase-Sb, and GTase-I of *S. sobrinus* 6715. In general, cationic and anionic surfactants markedly inhibited insoluble glucan synthesis; GTases synthesising water-

soluble glucans were less sensitive to these surfactants. Non-ionic surfactants did not inhibit GTase activity of *S. sobrinus* 6715. Inhibition of insoluble glucan synthesis by crude GTase upon addition of anionic or cationic detergent was due to specific inhibition of GTase-I present in the crude enzyme preparation.

Inhibition of adherence by the surfactants was tested with resting cells of mutans streptococci cultured in glucose-containing Todd-Hewitt broth. With the exception of two non-ionic compounds, surfactants inhibited significantly sucrose-dependent adherence of *S. mutans* and *S. sobrinus* cells to glass surfaces; adherence of *S. mutans* was enhanced in the presence of

Table II. Effects of surfactants on sucrose-dependent adherence of resting cells of *S. mutans* MT8148

Concentration of surfactant (%)	Mean percentage (SD) adherence* after treatment with surfactants							
	anionic		cationic		ampholytic		non-ionic	
	a	b	c	d	e	f	g	h
0	63.8 (1.6)	63.8 (1.6)	63.6 (1.6)	63.6 (1.6)	63.6 (1.6)	63.6 (1.6)	63.6 (1.6)	63.6 (1.6)
0.00001	60.3 (2.0)	58.3 (1.8)	56.3 (3.7)	57.5 (0.4)	61.8 (6.3)	63.3 (2.7)	61.7 (1.4)	62.5 (1.6)
0.0001	52.7 (6.0)	57.6 (4.1)	56.0 (2.8)	49.2 (3.0)	61.8 (3.1)	60.6 (5.0)	62.4 (5.3)	61.1 (1.5)
0.001	44.0 (4.1)	56.7 (5.0)	45.4 (3.8)	29.1 (2.1)	57.9 (2.2)	53.7 (3.9)	63.5 (6.2)	62.3 (2.5)
0.01	55.3 (5.4)	54.8 (2.8)	25.6 (9.2)	25.4 (1.7)	61.6 (4.8)	40.8 (4.9)	67.8 (4.5)	64.4 (6.2)
0.1	2.0 (0.2)	1.5 (0.4)	1.2 (0.3)	2.1 (0.5)	35.1 (3.1)	61.5 (2.3)	66.2 (3.0)	72.0 (2.8)†
1	2.6 (1.0)	1.5 (0.2)	0.4 (0.1)	3.7 (0.7)	17.1 (2.3)	1.9 (0.5)	65.5 (6.1)	71.8 (5.5)

* The ratio of cells adhering to the glass surface compared with total cells; mean of triplicate experiments; a: Sodium dodecyl sulphate, b: sodium di 2-ethylhexyl sulphosuccinate, c: cetyltrimethylammonium bromide, d: cetylpyridinium chloride, e: lauryldimethylaminoacetic acid betaine, f: alkyl diaminoethyl glycine, h: octylphenol-ethylene oxide, g: POE(20) sorbitan monolaurate.

† Statistical analysis was carried out by the Student's *t* test between control and the other group (0.00001–1 %); for non-ionic surfactants (g, h), *p* < 0.05.

Table III. Effects of surfactants on sucrose-dependent adherence of resting cells of *S. sobrinus* 6715

Concentration of surfactant (%)	Mean percentage (SD) adherence after treatment with surfactants							
	anionic		cationic		ampholytic		non-ionic	
	a	b	c	d	e	f	g	h
0	81.7 (0.9)	81.7 (0.9)	81.7 (0.9)	81.7 (0.9)	81.7 (0.9)	81.7 (0.9)	81.7 (0.9)	81.7 (0.9)
0.00001	80.1 (0.6)	80.5 (0.5)	76.5 (1.1)	81.2 (1.5)	79.5 (3.7)	75.8 (1.0)	77.3 (2.2)	77.6 (1.0)
0.0001	80.5 (0.7)	82.5 (0.6)	77.0 (0.6)	79.7 (1.0)	78.7 (1.9)	71.5 (5.3)	74.3 (5.6)	79.2 (1.9)
0.001	80.2 (1.0)	80.8 (1.4)	78.3 (0.7)	82.6 (0.3)	75.7 (1.1)	75.0 (2.0)	75.6 (1.3)	83.3 (0.8)
0.01	65.2 (0.7)	60.0 (1.2)	79.7 (1.4)	81.2 (4.4)	75.7 (1.5)	78.7 (2.1)	81.8 (1.2)	81.2 (2.8)
0.1	0.5 (0.2)	0.4 (0.4)	2.0 (0.6)	6.2 (0.8)	82.8 (3.7)	7.8 (3.1)	80.3 (0.3)	78.1 (0.8)
1	0.3 (0.1)	0.5 (0.4)	0.7 (0.1)	2.5 (0.4)	50.4 (2.6)	1.8 (0.7)	77.6 (1.0)	78.1 (1.5)

See footnote to table II.

POE (20) sorbitan monolaurate 0.1 % (tables II and III).

Discussion

It is known that various agents can affect release of GTases during growth of mutans and other oral streptococci, or modify the enzymic activity of GTases isolated from cultures of mutans streptococci. For example, non-ionic detergents such as Tween 80 and Triton X-100 have been reported to increase glucan synthesis by GTase(s) of *S. sobrinus* cultures,^{26–29} and similar findings have been obtained with other oral streptococci including *S. salivarius* and *S. sanguis*.^{30–32} The enhanced GTase production in these oral streptococci following addition of Tween 80 may be due either to changes in fatty acid composition of the cytoplasmic membranes, which in turn can lead to perturbations in proton motive force generation,^{31,32} or to simple resolution of GTase molecules from the enzyme aggregation, *via* lipophilic interaction in the presence of Tween 80.³³ However, other investigators postulated strain or species variations in the effect of Tween 80 on GTase synthesis by several oral streptococcal species.³⁴

In the present study non-ionic surfactants including Tween 80 did not affect the activities of GTases from *S. sobrinus*, whereas the activity of *S. mutans* CF-GTase, but not CA-GTase, was stimulated significantly. Thus it may be speculated that enhancement of GTase activities by non-ionic surfactants in mutans streptococci is due to activation of soluble glucan synthesis in *S. mutans*, whereas in *S. sobrinus* it is due to enhanced release of GTases during growth of the organisms.

Both cationic and anionic surfactants inhibit growth of many bacterial species, including oral streptococci, and also inhibit enzymic activities of GTases isolated from mutans streptococci.^{16,18,19,35–37} Generally it is agreed that cationic surfactants exert the most powerful bactericidal activity against mutans streptococci, followed by anionic surfactants;^{16,19,37} non-ionic surfactants do not usually inhibit oral streptococci.³⁷ However, Kabara¹⁶ claimed that the lauryl monoesters possess exceptionally powerful antimicrobial properties. Results obtained in this study (table I) agree with the results reported by other investigators.^{16,18,19,35–37}

Inhibition of glucan synthesis by various surfactants may be a factor in their ability to prevent dental plaque formation and induction of dental caries.¹⁸ It has been reported that anionic or cationic surfactants cause

strong inhibition of GTases prepared from mutans streptococci,^{18,35} but the GTases used in these studies were not properly purified. In this study we used GTases from *S. mutans* and *S. sobrinus* that were highly purified by chromatography and we obtained new findings. In general it was shown that each GTase protein is affected by specific surfactants; e.g., non-ionic detergents did not affect water-insoluble glucan synthesis by CA-GTase of *S. mutans* and GTase-I from *S. sobrinus*, but they profoundly enhanced water-soluble glucan synthesis of *S. mutans* CF-GTase and *S. sobrinus* GTase-Sa (figs. 1 and 2). Furthermore, *S. sobrinus* GTase-I was more sensitive to the inhibitory effect of ionic surfactants than the GTase-Sa or GTase-Sb of the organism.

Other features regarding the effects of surfactants on GTase, particularly in *S. mutans*, are that GTase activities are enhanced in the lower concentrations of cationic or ampholytic surfactants, but this is followed by a sharp decrease in GTases activities at higher concentrations of the same surfactants (fig. 1). A similar profile of GTase activities has been obtained;³⁸ lower concentrations of hydrogen peroxide stimulated

insoluble glucan synthesis of *S. sobrinus* GTases, whereas higher concentrations of this agent caused complete inhibition of glucan production by GTase-S and GTase-I. This phenomenon may be due to crosslinking of the enzyme proteins through tyrosine residues or deamination of lysyl residues of GTase.³⁸ Some surfactants, e.g., cationic or ampholytic, may induce hydrophobic or ionic interactions between GTases and a functional hydrophobic group within the surfactant molecule. It has been reported that some cationic surfactants inhibit experimental caries induction in hamsters,³⁹ and that surfactants such as chlorhexidine digluconate and cetylpyridinium chloride prevented cellular co-aggregation between *Prevotella* spp. and *Streptococcus* spp. or *Actinomyces* spp.⁴⁰

These findings indicate that topical application of aqueous solutions of these surfactants may result in reduced glucan synthesis and plaque accumulation due to inhibition of GTase activities and cell growth; this may eventually lead to a reduction in caries. Some of the surfactants used in this study have been used commercially in dentifrices and gargles.

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