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Comparison of the characteristics of a hydrophilic variant of *Streptococcus gordonii* ATCC 10558 and a hydrophilic variant of *Streptococcus mutans* Ingbritt

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Abstract

We isolated the hydrophilic variant of *Streptococcus gordonii* ATCC 10558 by the isolation procedure for the hydrophilic variant of *Streptococcus mutans* Ingbritt. The hydrophobicity of the variant of *S gordonii* ATCC 10558 was lower than that of the parent strain, but adherence to salivary coated hydroxyapatite (S-HA) was not lower The hydrophilic variant of *S gordonii* ATCC 10558 released more quantity of proteins of various molecular weights into the culture supernatant than parent strain.

The findings suggest that the adhesin of *S. gordonii* ATCC 10558 to S-HA differs from the components causing cell surface hydrophobicity in *S mutans* Ingbritt.

Key words · *Streptococcus gordonii*, Hydrophilic variant, Adherence

Introduction

Streptococcus gordonii formerly considered to be a subpopulation of the species *Streptococcus sanguis*[1] is one of the early colonizers of dental plaque[2-5] The adherence of *S. gordonii* to the tooth surface is considered to occur by adhesin of the organism to the salivary component of the pellicle of teeth surfaces[6] The mechanism of adhesion of the organism to saliva coated hydroxyapatite (S-HA) has been suggested to be associated with electrosatatic, lectin-like, and hydrophobic interaction[7-12] *S mutans*, which also adheres to salivary components of the pellicle, is defined as cell surface hydrophobicity correlated well with the adherence to S-HA by using the hydrophilic variant[13-15] There have been two kinds of reports, both that the cell surface hydrophobicity of *S sanguis* is related to the adherence to S-HA[16] and that it is not

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related to the adherence[17]

This study isolated a hydrophilic variant of *S. gordonii* ATCC 10558 (formerly *S. sanguis* ATCC 10558) to define the relationship between the cell surface hydrophobicity and the adherence to S-HA, as well as the hydrophobicity, adherence to S-HA, and release of proteins into the culture supernatant of these strains compared with the hydrophilic variant of *S. mutans* Ingbritt

Materials and Methods

Bacteria used in this experiment were *S. gordonii* ATCC 10558, *S. gordonii* 10558L (hydrophilic variant isolated from strain ATCC 10558), and *S. mutans* Ingbritt, *S. mutans* IBL (hydrophilic variant isolated from strain Ingbritt) The strains were grown anaerobically at 37°C (80%N_2, 10%CO_2 and 10%H_2) in brain heart infusion (BHI) broth or a chemically defined (CD) medium[18] composed of 20 amino acids, selected vitamins, glucose, salts, and phosphate buffer (pH 7.2)

The hydrophilic variants were isolated by the procedures described by Rosenberg and Rosenberg[19] Briefly, cells of *S. mutans* Ingbritt and *S. gordonii* ATCC 10558 were harvested from overnight cultures in BHI broth and washed twice with phosphate buffered saline (PBS) The cells were suspended in 2 ml sterilized PUM buffer with (per liter) $K_2HPO_4 \cdot 3H_2O$, 22 2g, KH_2PO_4, 7 3g, Urea, 1 8g, and $MgSO_4 \cdot 7H_2O$, 0 2g[20] After the addition of 1 ml of sterilized n-hexadecane, the suspensions were mixed on a Vortex mixer for 60 seconds Cells in the aqueous phase were removed and inoculated into fresh BHI broth. This enrichment procedure was performed repeatedly to obtain the low hydrophobic strain The hydrophilic variants of *S. mutans* Ingbritt and *S. gordonii* ATCC 10558 were designated IBL and 10558L, respectively

Hydrophobicity was assayed as described by Rosenberg et al [20] Briefly, organisms were inoculated into BHI broth and incubated at 37°C for 18h The cells were harvested by centrifugation and washed three times with 10 mM phosphate buffer (pH 7 2) These cells were resuspended in the buffer to an absorbance at 436 nm of 0 5 Three ml of this suspension was mixed for 60 seconds with 100 μl of n-hexadecane The mixtures were allowed to stand for 15 min, and then the aqueous phase was removed and the absorbance determined. Hydrophobicity was expressed as the percentage of cells that remained in the aqueous phase compared with that in the control suspension

Adherence of parent strains and hydrophilic variants to the experimental pellicle were measured as reported elswhere[21] The following procedure was used Washed HA (30 mg, Bio-Rad) with HEPES buffer were put into 1.5 ml microtubes and coated with 1 ml of whole saliva for 1 h The whole saliva coated HA was designated S-HA. After masking of the uncoated surface of S-HA with 1 ml of bovine serum albumin (BSA, 30 mg/ml), 1 ml of organisms was added to these S-HA in the microtubes and the microtubes were rotated for 2

h at room temperature. Unattached organisms were removed with 6 washes in HEPES buffer and 0.2 ml of 2 N HCl and 2.3 ml of HEPES buffer were added to dissolve the HA. The absorbance at 540 nm was measured

The culture supernatant of the organisms was separated from the cells which were grown in CD medium at 37°C for 18 hr and the culture supernatant was obtained by centrifugation at 10, 000 g for 30 min. The supernatant was dialyzed against distilled water, freeze-dried, and suspended in H_2O at 1/50 of the original Ten μl (about 600 μg/ml protein) of this 50-fold concentrated supernatant was applied to 4-20% gradient SDS-polyacrylamide gel and electrophoresis was performed by the method of Laemmli[22] The gels were stained for protein with Coomassie brilliant blue. The protein patterns were analyzed with Image Master (Pharmacia, Biotech)

The immuno gold labelling of the cell of *S mutans* Ingbritt and IBL was performed with the method of Amako et al.[23] The bacteria were washed and resuspeded with PBS. The bactreial suspensions were adsorbed on Formvar-coated copper grids. After the bacterial suspension on the gride were absorbed with filter paper, the grids were placed on a drop of anti-Antigen B antibody (1 . 10 dilution with PBS-1% tween 20-2% BSA) which was prepared from purified Antigen B immunized to rabbit in a previous experiment[21], and incubated for 20 min at 37°C The grids were washed 4 times with PBS-tween 20-BSA The grids were placed on a drop of protein A-gold colloidal (1 : 10 dilution, diluted with PBS-tween 20-BSA, particles 5 nm, E Y. labs, Inc.), and incubated at room temperature for 5 min The gride were washed 4 times with PBS-tween 20-BSA, and then were washed 2 times with 2% ammonium

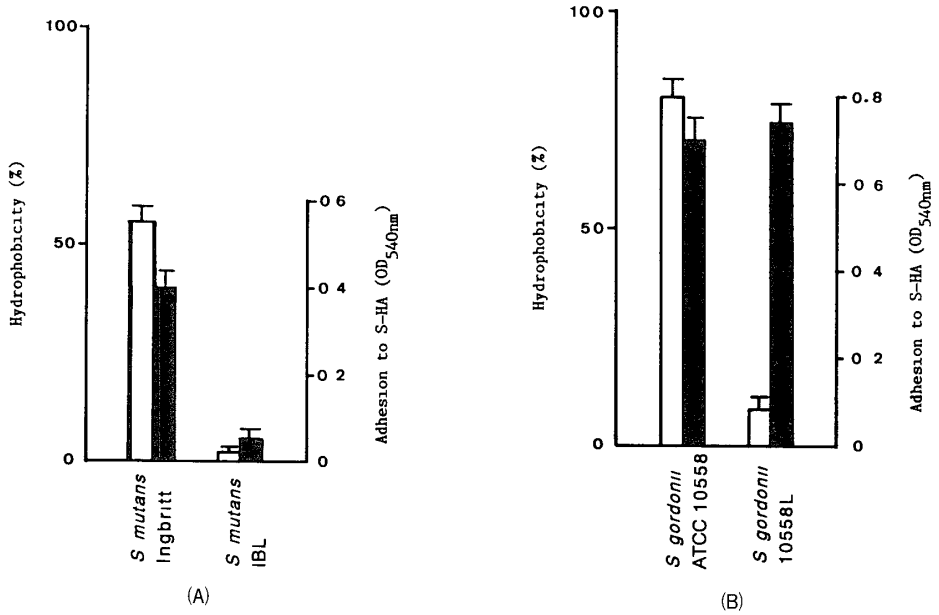


Fig 1

Cell surface hydrophobicity and adhesion to S-HA (Mean ± S D) (A) *S mutans* Ingbritt and *S mutans* IBL (B) *S gordonii* ATCC 10558 and *S gordonii* 10558L

Open box, hydrophobicity (%), Closed box, adhesion to S-HA (OD_{540nm})

acetate The preparations were then negatively stained with 1% phosphotangustate and examined in a transmission electron microscope

Results

The hydrophilic variant of *S mutans* Ingbritt could be isolated by 6 times repetition of the isolation procedure However, the hydrophilic variant of *S gordonii* ATCC 10558 was more difficult to isolate, and the isolation procedures were repeated 35 times The biological characteristics of these hydrophilic variants were found to identical to the parent strains with Minitek Numerical Identification System, and the morphology of the colony on Mitis-Salivarius agar were the same as the parent strains (data not shown) Hydrophobicity and adherence to S-HA of the *S mutans* IBL cells was 2%, and 0 05, and of the parent strain 55% and 0 40 (Fig 1A). In *S mutans*, the decrease of hydrophobicity of the cell surface was accompanied by a decrease in adherence to S-HA The hydrophobicity of the cells of *S gordonii* 10558L showed a lower value (8%) than the parent strain (80%) However, the adherence of *S gordonii* 10558L to S-HA did not decrease (Fig 1B) The adherence of these cells to S-HA was also confirmed with microscopic observations, and almost the same number of organisms of S-HA which were treated with the cell of *S gordonii* ATCC 10558 and *S gordonii* 10558L were observed (data not shown) These results indicate that cell surface hydrophobicity may take part in the adherence of the cell of *S mutans* Ingbritt to S-HA, but that cell surface hydrophobicity does not take part in the adherence of the cell of *S gordonii* ATCC 10558

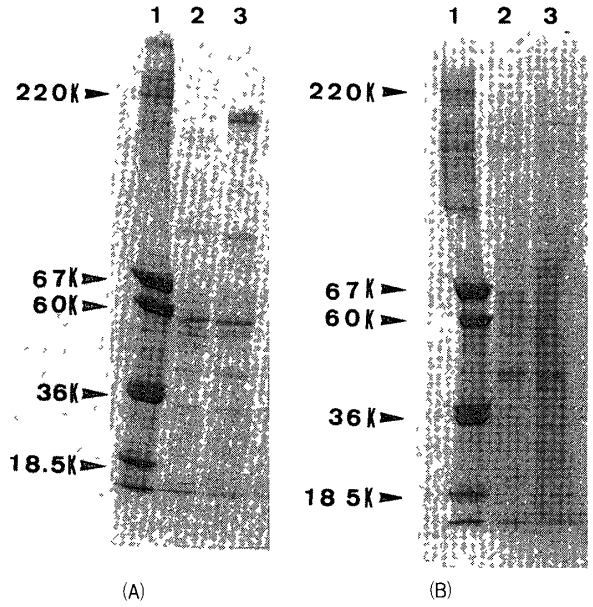


Fig 2
SDS-PAGE analysis of supernatant of *S mutans* Ingbritt, IBL, *S gordonii* ATCC 10558, and 10558L (A) SDS-PAGE pattern of supernatant of *S mutans* Ingbritt and IBL lane 1, molecular weight makers, ferritin (half unit), 220,000, albumin, 67,000, catalase, 60,000, lactate dehydrogenase, 36,000, ferritin, 18,500, lane 2, culture supernatant of *S mutans* Ingbritt, lane 3, culture supernatant of *S mutans* IBL (B) SDS-PAGE pattern of supernatant of *S gordonii* ATCC 10558 and *S gordonii* 10558L lane 1, molecular weight makers as in (A), lane 2, culture supernatant of *S gordonii* ATCC 10558, lane 3, culture supernatant of *S gordonii* 10558L
Samples were electrophoresed on a 4-20% SDS-polyacrylamide gradient gel and stained with Coomassie brilliant blue

The culture supernatant of *S mutans* IBL contained larger amounts of Antigen B (MW 190,000) than the culture supernatant of the parent strain and the relative concentration of Antigen B of the supernatant of *S mutans* IBL was about 4 5 times that of *S mutans* Ingbritt by analysis with Image Master (Fig 2A and Fig. 3A) To confirm whether *S mutans* IBL released the Antigen B of the cell

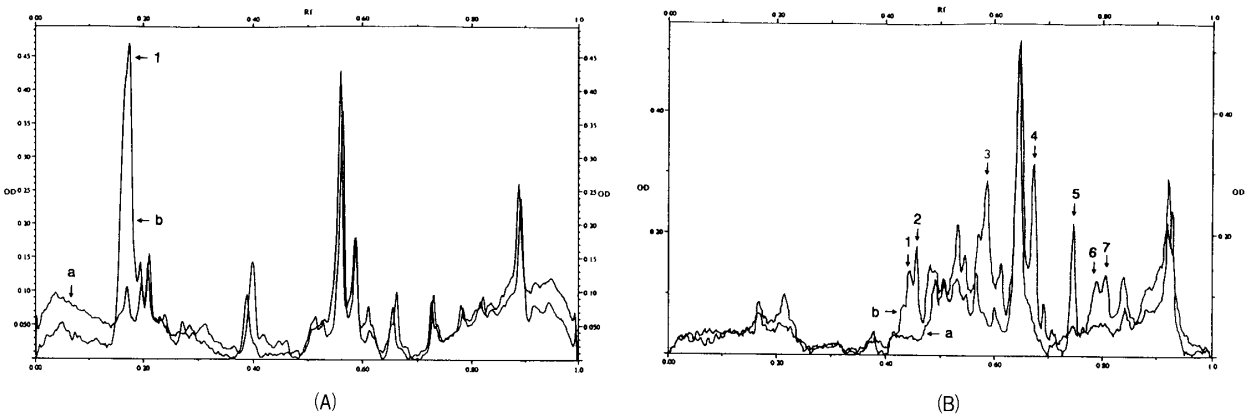


Fig 3

Densitogram of protein bands of the SDS-PAGE films in Fig 2 (A) Densitograme of supernatant of *S mutans* Ingbritt and *S mutans* IBL line, a supernatant of *S mutans* Ingbritt, line b, supernatant of *S mutans* IBL, peak 1, Antigen B (MW 190,000) (B) Denstitogram of supernatant of *S gordonii* ATCC 10558 and *S gordonii* 10558L line a, supernatant of *S gordonii* ATCC 10558, line b, supernatant of *S gordonii* 10558L, peak 1, Mw 80,000 protein, peak 2, Mw 77,000 protein, peak 3, Mw 54,500 protein, peak 4, Mw 43,000 protein, peak 5, Mw 35,000 protein, peak 6, Mw 28,500 protein, peak 7, Mw 26,500 protein

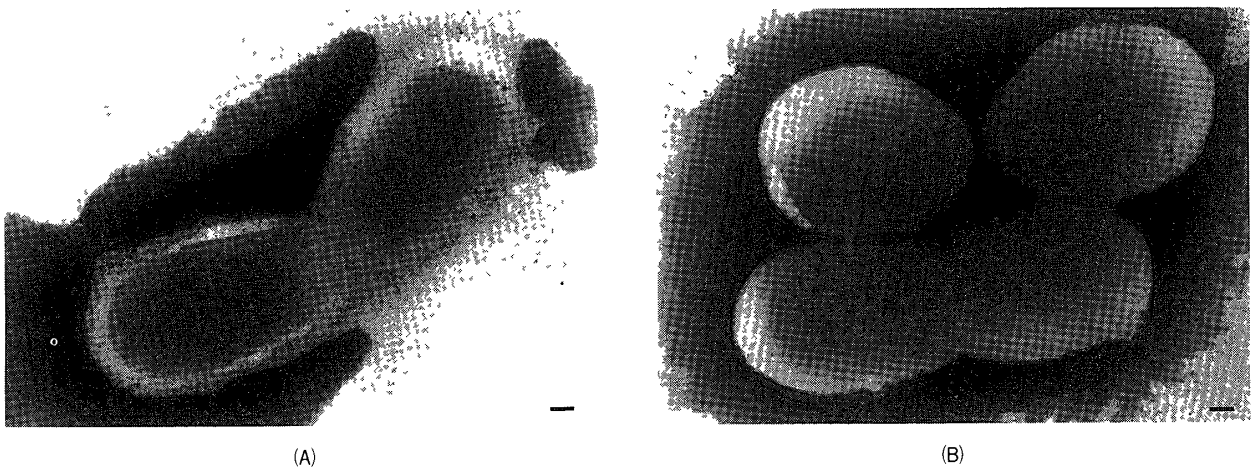
surface into the culture supernatant or whether *S. mutans* IBL retained Antigen B as the parent strain and additionally is the source of the Antigen B in the culture supernatant, the distribution of Antigen B in both cell surfaces were observed by immuno gold labelling. Numerous gold particles were observed on the cell surface of *S mutans* Ingbritt, but only a few gold particles were observed in *S. mutans* IBL (Fig. 4A and B). This observation confirmed that *S mutans* IBL could not retain the Antigen B on the cell surface, and they release almost all Antigen B into the culture supernatant.

S. gordonii 10558L released 7 kinds of protein: 80 KDa, 77 KDa, 54.5 KDa, 43 KDa, 35 KDa, 28 5 KDa, and 26.5 KDa (Fig. 2B and 3B). These were not seen or appeared in smaller quantities in the parent strain (Fig 2B and Fig. 3B). As *S. gordonii* 10558L adhered to S-HA like ATCC 10558, it is considered that *S. gordonii* 10558L still retains the adhesin to S-HA, but *S gordonii* 10558L may release the component for cell surface hydrophobicity into the culture supernatant from the results of the characteristic of *S. mutans* Ingbritt and IBL

Discussion

The hydrophilic variant of *S. mutans* Ingbritt was simply isolated, but isolation of the hydrophilic variant of *S. gordonii* ATCC 10558 was very difficult. Morris et al.[16] also reported that there was a hydrophilic variant of the *S sanguis* strain which was hard to isolate Differences in the frequency of the hydrophilic variant of *S. mutans* Ingbritt and *S. gordonii* ATCC 10558 are not yet clearly defined.

The cell surface adhesin (190 KDa protein) of *S. mutans* to S-HA was purified by a number of researchers, and the adhesin has been designated variously Antigen B, I/II, IF, P1, and PAc[24-28]These are the same adhesin. The relationship between Antigen B and cell surface



(A) (B)

Fig 4

Electron micrographs of gold bead-labeled *S mutans* Ingbritt and IBL Bar markers represent 100 nm *S mutans* Ingbritt (A) and IBL (B) cell were incubated with anti-Antigen B antibody and then reacted with gold beads (5nm diameter) conjugated protein A These preparations were negatively stained with 1% phosphotungstate

hydrophobicity and the adherence to S-HA in *S. mutans* were proved with the characteristic of the hydrophilic variant[15,16] and the PAc-defective mutant which was constructed by inserting an erythromycin-resistant gene into the pac gene[28] which is the gene for PAc. This relationship can also be understood from the SDS-PAGE protein pattern and immuno gold labelling for cell surface Antigen B of the parent strain and hydrophilic variant of *S mutans* Ingbritt in this experiment. The hydrophilic variant of *S. gordonii* ATCC 10558 did not decrease the ability to adhere to S-HA Our findings strongly support the findings of Wyatt et al [17] and suggest that the adhesin of *S. gordonii* to S-HA differs in the components of cell surface hydrophobicity. Morris et al [16] also isolated the hydrophilic variant from *S sanguis* 12, and examined aggregation, adherence, and hydrophobicity They indicated that cell surface hydrophobicity was related to adherence to S-HA. There are two differences between the experiments of Morris et al [16] and our experiments. First the procedure to isolate the hydrophilic variant. Morris et al.[16] isolated the hydrophilic variant from an aqueous phase where the bacteria were grown at 37°C overnight in the presence of n-hexadecan. We isolated the hydrophilic variant from the aqueous phase where n-hexadecane was added to the medium after the bacteria were grown. The second difference is the strain used in these experiments Morris et al [16] used *S. sanguis* 12 (serovar type I), while we used *S gordonii* ATCC 10558 (previously *S sanguis* serovar type I/II) The difference in bacterial strain and serovar may be related to differences in the properties of the cell surface components of these organisms. In this experiment, *S gordonii* 10558L still retained the adhesin for S-HA on the cell surface. However, our experiment could not clearly define whether this characteristic of *S gordonii* ATCC 10558 is a common characteristic with *S gordonii* or a strain specific characteristic of *S gordonii* ATCC 10558 Experiments which examine many strains of *S gordonii* may be needed to define this

There have been numerous reports of the putative adhesins of *S. sanguis* These putative adhesins were 160 KDa protein[16], longfibril protein[29], 80-52 KDa protein[30], and a heavier than 150 KDa protein of fimbriae[31] Ganeshkumar et al.[32] reported cloning the gene for 36 KDa protein of the putative adhesin of *S. sanguis* 12, Rosan et al.[33] reported cloning the gene for the 84 KDa protein of the putative adhesin in *S sanguis* G9B, and Fives-Taylor et al.[34] reported cloning of the gene for 30 KDa fimbriae subunit of the putative adhesin of *S. sanguis* FW213 However, restriction maps of these putative adhesin genes were distinct from each other. Three distinct putative adhesin genes have been identified in *S sanguis* Gibbons et al.[6] reported that strains of *S. sanguis* and *S. oralis* did not bind effectively to PRPs treated with HA, whereas almost all strains of *S. gordonii* do. These findings suggest that the adhesin of *S. sanguis* may differ from that of *S. gordonii* We therefore need to clarify the characteristics of the adhesin of *S gordonii*.

The hydrophilic variant of *S mutans* synthesized Antigen B, it did not retain the antigen on the cell surface, and released the antigen into the culture supernatant[15,16] This characteristic of the hydrophilic variant of *S mutans* Ingbritt was also observed in this experiment. *S. gordonii* 10558L also released 7 kinds of proteins in smaller quantities in the culture supernatant of the parent strain. Jenkinson and Carter[35] reported that cell surface proteins, 43 KDa and 45 KDa proteins, were present in larger amounts in the hydrophobic variant of *S sanguis* Challis than in the parent strain and hydrophilic variant. They indicated that the differences in hydrophobicity of the mutants were associated with alternations in amounts and exposure of the surface protein and that these two proteins are involved in cell surfce hydrophobicity and adherence to S-HA. If the hydrophilic variant of *S gordonii* ATCC 10558 also synthesizes the material which acts to cause cell surface hydrophobicity and releases it into the culture supernatant as *S. mutans*, one or more compounds in the SDS-PAGE protein pattern of the supernatant of *S. gordonii* 10558L may cause the components responsible for cell surface hydrophobicity

In conclusion, our findings suggest that the adhesin of *S. gordonii* ATCC 10558 for S-HA differs from the component of the cell surface hydrophobicity

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要　旨

Streptococcus mutans Ingbrittからhydrophilic variantを得るのと同様の方法にて*Streptococcus gordonii* ATCC 10558よりhydrophilic variantを得た。*S. gordonii* ATCC 10558のhydrophilic variantは親株に比較してhydrophobicityは低下していたが，唾液コートハイドロキシアパタイト（S-HA）に対する付着性は低下しなかった。*S. gordonii* ATCC 10558のhydrophilic variantは親株に比較して培地中に多くの種類のタンパク質を産生した。

これらのことより，*S gordonii* ATCC 10558のS-HAに対する付着因子は*S. mutans* Ingbrittの付着因子と異なり菌体表層のhydrophobicityに関与する物質とは異なることが示唆された。