

Short communication

Deletion in sortase gene of *Streptococcus mutans* Ingbritt

Igarashi T. Deletion in sortase gene of *Streptococcus mutans* Ingbritt.
Oral Microbiol Immunol 2004; 19: 210–213. © Blackwell Munksgaard, 2004.

Our previous studies on *Streptococcus mutans* have demonstrated that surface proteins containing a C-terminal sorting signal, such as surface protein antigen (Pac), glucan-binding protein C (GbpC) and dextranase (Dex), are anchored to the cell wall by a sortase (SrtA). In this study we found that, unlike other strains of *S. mutans*, strain Ingbritt did not exhibit cell wall-anchoring of Pac, GbpC and Dex. It is speculated that the SrtA of strain Ingbritt did not function in the cell wall-anchoring process of these surface proteins. Sequence analysis revealed a deletion of an 11-bp nucleotide sequence in the *srtA* gene of strain Ingbritt, resulting in the generation of a new termination codon, resulting in production of an incomplete SrtA enzyme protein. As a result, strain Ingbritt showed a localization change of Pac, GbpC and Dex in the cell, implying that strain Ingbritt loses the biological functions mediated by the cell surface-associated proteins of *S. mutans*. These results suggest that strain Ingbritt could be less cariogenic than other strains of *S. mutans*.

T. Igarashi

Department of Oral Microbiology, Showa University School of Dentistry, Tokyo, Japan

Key words: biofilm; cell wall anchoring; deletion; LPXTG motif; sortase; *Streptococcus mutans* Ingbritt

Takeshi Igarashi, Department of Oral Microbiology, Showa University School of Dentistry, 1-5-8 Hatanodai, Shinagawa-ku, Tokyo 142-8555, Japan
Tel.: +81 3 3784 8166;
fax: +81 3 3784 8012;
e-mail: igatakes@dent.showa-u.ac.jp
Accepted for publication December 5, 2003

Streptococcus mutans is a gram-positive oral bacterium and the principal etiologic agent of human dental caries (12, 20). Formation of dental plaque biofilm is an important biological process associated with the attachment of oral bacteria, in particular *S. mutans*, on the tooth surface. Proteins that *S. mutans* displays on the cell surface contribute to biofilm formation, and some of these surface proteins possess a C-terminal sorting signal which consists of a conserved LPXTG motif, a hydrophobic domain, and a positively charged tail (13, 26).

A series of studies by Schneewind and colleagues identified a transpeptidase called sortase (SrtA) in *Staphylococcus aureus* (22, 26, 34) and demonstrated that the SrtA is involved in anchoring of some proteins with an LPXTG motif to the *S. aureus* cell wall and plays an important role in the virulence of *S. aureus* (17, 22, 23, 26). At present, SrtA and surface proteins with an LPXTG motif have been found in many Gram-positive bacteria such as *S. aureus*, *S. mutans*, *Streptococcus pyogenes*, *Streptococcus suis*, *Streptococcus gordonii* and *Listeria monocytogenes*

(3, 4, 11, 13, 15, 16, 26–28), playing an important role in the pathogenesis of gram-positive bacterial infection (11, 17, 23, 26). In *S. mutans* it is known that at least six different proteins possess a C-terminal sorting signal: surface protein antigen (Pac), wall-associated protein antigen A (WapA), cell wall protein (WapE), glucan-binding protein C (GbpC), dextranase (Dex) and fructanase (FruA) (1, 6, 13, 24, 26, 29, 32). In recent studies, we have determined the nucleotide sequence of the *srtA* gene of *S. mutans* GS5 and have demonstrated that the *S. mutans* SrtA is involved in the cell wall sorting reaction of Pac, GbpC and Dex and that cariogenic properties mediated by these surface proteins are lost by insertional inactivation of the *srtA* gene of *S. mutans* (14–16).

In this study we found that strain Ingbritt of *S. mutans* lacked the ability to anchor Pac, GbpC and Dex to the cell wall and clarified that this phenomenon was caused by a deletion of an 11-bp nucleotide sequence in the *srtA* gene.

Strains Ingbritt, 109c (15, 32), and *srtA* mutant (15) of *S. mutans* were grown in

Todd-Hewitt broth (Difco Laboratories, Detroit, MI). *Escherichia coli* JM109 is routinely used as a plasmid host and grown in Luria-Bertani broth (13).

Chromosomal DNA of *S. mutans* was isolated by CsCl-ethidium bromide density gradient centrifugation (13). Plasmid DNA was extracted with a Wizard miniprep purification kit (Promega, Madison, WI). Polymerase chain reaction was performed as previously described (13). *srtA*-deficient mutant of *S. mutans* 109c was prepared by insertional inactivation as reported previously (15). The *srtA* gene of *S. mutans* Ingbritt was polymerase chain reaction-amplified by oligonucleotide primers, SmSrt229F and SmSrt1129R, cloned into pT7Blue T-vector (Novagen, Madison, WI), and sequenced with an ABI prime cycle sequencing kit and a Model 373S automated DNA sequencer (Applied Biosystems, Foster City, CA) as reported previously (13). The primers were designed on the basis of the sequence of flanking regions of the strain GS5 *srtA* gene (15). The nucleotide sequences of SmSrt229F and SmSrt1129R were as follows: SmSrt229F, 5'-GGT GTC AAA GTG

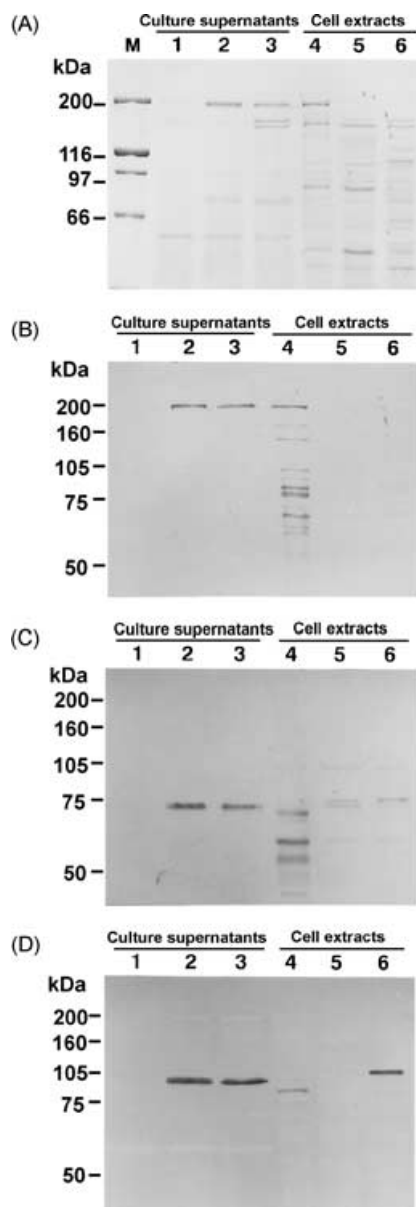


Fig. 1. Detection of cell wall-anchored proteins in *S. mutans*. Cell extracts and culture supernatants were prepared from *S. mutans* cells cultured in Todd-Hewitt broth and were subjected to SDS-PAGE. Equal amounts of proteins were added to each lane (see Material and methods). A) Protein staining with Coomassie blue. B, C and D) Western blots with specific anti-serum: B) anti-Pac serum, C) anti-GbpC serum, D) anti-Dex serum. Lanes 1 and 4, wild-type strain 109c; lanes 2 and 5, *srtA* mutant; lanes 3 and 6, strain Ingbritt, lane M, size marker.

ATG CGC CT -3' and SmSrt1129R, 5'-ATG CTC ATG AGA CCT CAC AG -3'.

Whole cells (30 mg of the cell pellet) were suspended in 600 μ l of 1% sodium dodecyl sulfate-1% 2-mercaptoethanol, heated at 100°C for 5 min and centrifuged as reported previously (15, 24). The resulting supernatant was used as the cell extract. The culture supernatant was adjusted to a final OD₂₈₀ of 15. The cell extract (15 μ l for each sample) and the culture supernatant (30 μ l for each sample) were analyzed by SDS-PAGE and Western blotting with anti-Pac (15), anti-GbpC (16) or anti-Dex (14) serum.

The nucleotide sequence of the *srtA* gene of *S. mutans* Ingbritt determined in this study has been deposited in the DDBJ, GenBank and EMBL databases under the accession number AB105440.

The distribution of cell-associated proteins Pac, GbpC and Dex in strains Ingbritt, 109c, and *srtA* mutant of *S. mutans* were examined by Western blot analysis with specific anti-serum (14–16). As shown in Fig. 1, these surface proteins of strain Ingbritt were found in the culture supernatant, but not in the cell extract (Fig. 1B–D, lanes 3 and 6). In contrast, Pac, GbpC, and Dex of the wild-type 109c were associated with the cell surface, but not detected in the culture supernatant (Fig. 1B–D, lanes 1 and 4). As compared with strain Ingbritt and the *srtA* mutant, cell localization of Pac, GbpC and Dex in strain Ingbritt was the same as that of the *srtA* mutant (Fig. 1B–D, lanes 2 and 3, 5 and 6) (14–16). The Pac (200 kDa), GbpC (75 kDa) and Dex (102 kDa) released in the culture supernatant of strain Ingbritt revealed slightly slower migration than those (200, 72, 92 kDa, respectively) in the cell extract of the wild-type 109c (Fig. 1B–D, lanes 3 and 4). Multiple bands that are due to degradation of the cell wall-anchored proteins were visible in the cell extract of the wild type (Fig. 1B–D, lane 4). In contrast, the bands from cell extracts of *srtA* mutant and Ingbritt were of higher molecular mass than in the corresponding supernatants (Fig. 1C, lanes 5, 6 and Fig. 1D, lane 6). We speculate that higher molecular mass may be due to aggregation

products with some other components. Details should be further analyzed.

To determine why strain Ingbritt lost the surface attachment of Pac, GbpC and Dex, we cloned and sequenced the *srtA* gene of strain Ingbritt. The nucleotide sequence of the *srtA* gene of strain Ingbritt was 730 bp long, which was shorter than that (741 bp) of strain GS5 (15). The sequence homology between the *srtA* genes of Ingbritt and GS5 was 97.0%. Sequence analysis also exhibited a deletion of an 11-bp nucleotide sequence in the *srtA* gene of strain Ingbritt. The deleted sequence was AAGGATTAGAT, which was located between 326 and 336 on the *srtA* gene of strain GS5 (Fig. 2). The deletion resulted in generation of a new termination codon, TGA, at position 364 on the *srtA* gene sequence of strain Ingbritt (Fig. 2), implying that strain Ingbritt is unable to produce an intact SrtA enzyme protein.

Our previous studies demonstrated that the *srtA*-deficient mutant of *S. mutans* failed to retain Pac, GbpC and Dex on the cell surface and lost surface protein-mediated physiological phenomena such as adherence, aggregation and modification of extracellular glucan (14–16). In this study we found that *S. mutant* Ingbritt lacked the ability of cell wall-anchoring of surface proteins with an LPXTG motif (Fig. 1). At present, *srtA* genes of *S. mutans* are reported in strains GS5, NG8 and UA159 (1, 15, 18). As comparison of the nucleotide sequences of these *srtA* genes showed, NG8 and UA159 had exactly the same sequences, and GS5 shared 98.9% homology with NG8 and UA159. However, only the Ingbritt *srtA* sequence showed deletion of the 11-bp AAGGATTAGAT between position 325 and 337 (Fig. 2). This deletion generates a new termination codon, resulting in production of an incomplete SrtA enzyme protein. Western blots showed that both strain Ingbritt and the *srtA* mutant failed to retain Pac, GbpC and Dex on the cell surface (Fig. 1), whereas the wild-type 109c substantially retained these surface proteins on the cell surface. These results suggest that the failure in the surface expression of Pac, GbpC, and Dex in

GS5-aa	101	L	K	I	N	L	P	I	F	K	G	L	D	N	V	G	L	T	Y	G	A	G	T	M	K	N	D	Q	V	M	G	E	131
GS5-nt	301	TTAAAAATCAATTTACCAATCTTCAAAGGATTAGATAATGTTGGCTTAACATATGGTGTGGAACGATGAAAAATGACCAAGTCATGGGAGAA																												393			
		: :																															

Fig. 2. Deletion of nucleotides in the *srtA* gene of *S. mutans* Ingbritt. Nucleotide (nt) and amino acid (aa) sequences of the *srtA* gene from strain Ingbritt were aligned with those of strain GS-5. Nucleotides deleted in the *srtA* gene of strain Ingbritt are indicated by dashed lines. Termination codon, a TGA, in strain Ingbritt is underlined. nt, nucleotide sequence. aa, amino acid sequence.

strain Ingbritt, as well as in the *srtA* mutant, is due to the lack of SrtA activity. The fundamental difference between strain Ingbritt and the *srtA* mutant is a deletion in the *srtA* gene instead of insertional inactivation of the *srtA* gene. Although it is not clear why the 11-bp nucleotide sequence in the *srtA* gene was deleted, there are several reports suggesting that the SrtA enzyme of strain Ingbritt does not function normally (2, 10, 31, 35). For example, Russell reported a release of antigen B (also known as P1, PAc and I/II) into the growth medium of strain Ingbritt (31), and Forester et al. and Ayakawa et al. also reported that strain Ingbritt excreted large amounts of antigen P1 into the culture medium (2, 10), although these proteins are representative surface-associated proteins in *S. mutans* (5, 19, 26, 31). In addition, Walker et al. reported that strain Ingbritt, which is able to release Dex in the culture medium, is the best producer of Dex (35). These reports strongly suggest that strain Ingbritt was defective in the ability to anchor P1 (or B) and Dex to the cell wall.

In addition to the deletion of the sortase gene of Ingbritt, there have been several reports of genetic variations in strains of *S. mutans*. Murakami et al. found a frameshift mutation in the *pac* gene of strain GS-5, resulting in premature termination and loss of cell wall-anchoring of the PAc antigen (24). Another mutation in strain GS-5 was detected in the *gfpC* gene by Sato et al. (33). The mutation in the *gfpC* gene generated a new termination codon, resulting in production of an incomplete GbpC protein and loss of dextran-dependent aggregation. These genetic changes of the *pac* and *gfpC* genes in GS-5 have been linked to its low cariogenicity. Chromosomal deletions in *S. mutans* have also been described by Ferretti et al. (9) and Robinson et al. (30). It is not yet clear, however, whether these genomic changes are consequences of long-term subculture in the laboratory or just a reflection of variation within the species.

Genetic information reveals that *S. mutans* has at least five surface proteins with a C-terminal sorting signal; PAc, GbpC, Dex, WapA and FruA (13, 15, 26). Use of the isogenic mutant of each of these proteins in *S. mutans* showed that these surface proteins were deeply involved in cariogenic properties such as adherence to tooth surfaces, dextran-dependent aggregation, modification of extracellular glucan and supply of nutrients (5–8, 19, 21, 25, 29). In addition, our recent study using the *srtA*-deficient

mutant of *S. mutans* revealed that an anchoring process of surface proteins involving SrtA is essential for biological events of surface proteins in *S. mutans* (14–16). However, strain Ingbritt lacking SrtA could not anchor surface proteins to the cell wall, resulting in loss of multiple cariogenic properties mediated by the surface proteins. Therefore, *S. mutans* Ingbritt could be less cariogenic than other strains of *S. mutans*. As *S. mutans* Ingbritt is a strain that has been widely used in various studies as a reference strain, researchers should be cautious in using strain Ingbritt as a cariogenic pathogen.

Acknowledgments

I thank Ms Emiko Asaga for her excellent technical assistance. This work was supported in part by a Grant-in-Aid for Scientific Research (no. 14571798 and no. 14571749) from the Ministry of Education, Science, Sports and Culture of Japan.

References

- Ajdic D, McShan WM, McLaughlin RE, et al. Genome sequence of *Streptococcus mutans* UA159, a cariogenic dental pathogen. *Proc Natl Acad Sci USA* 2002; **99**: 14434–14439.
- Ayakawa GY, Boushell LW, Crowley PJ, Erdos GW, McArthur WP, Bleiweis AS. Isolation and characterization of monoclonal antibodies specific for antigen P1, a major surface protein of mutans streptococci. *Infect Immun* 1987; **55**: 2759–2767.
- Barnett TC, Scott JR. Differential recognition of surface proteins in *Streptococcus pyogenes* by two sortase gene homologs. *J Bacteriol* 2002; **184**: 2181–2191.
- Bloken TC, Franke CA, Jones KF, et al. Inactivation of the *srtA* gene in *Streptococcus gordonii* inhibits cell wall anchoring of surface proteins and decreases *in vitro* and *in vivo* adhesion. *Infect Immun* 2001; **69**: 75–80.
- Bowen WH, Schilling K, Giertsen E, et al. Role of a cell surface-associated protein in adherence and dental caries. *Infect Immun* 1991; **59**: 4606–4609.
- Burne RA, Chen Y-YM, Wexler DL, Kuramitsu H, Bowen WH. Cariogenicity of *Streptococcus mutans* strains with defects in fructan metabolism assessed in a program-fed specific-pathogen-free rat model. *J Dent Res* 1996; **75**: 1572–1577.
- Colby SM, Whiting GC, Tao L, Russell RRB. Insertional inactivation of the *Streptococcus mutans* *dexA* (dextranase) gene results in altered adherence and dextran catabolism. *Microbiology* 1995; **141**: 2929–2936.
- Crowley PJ, Brady LJ, Michalek SM, Bleiweis AS. Virulence of a *spaP* mutant of *Streptococcus mutans* in a gnotobiotic rat model. *Infect Immun* 1999; **67**: 1201–1206.
- Ferretti JJ, Russell RRB, Dao ML. Sequence analysis of the wall-associated protein precursor of *Streptococcus mutans* antigen A. *Mol Microbiol* 1989; **3**: 469–478.
- Forester H, Hunter N, Knox KW. Characteristic of a high molecular weight extracellular protein of *Streptococcus mutans*. *J Gen Microbiol* 1983; **122**: 2779–2788.
- Garandeau C, Reglier-Poupet H, Dubail I, Beretti JL, Berche P, Charbit A. The sortase SrtA of *Listeria monocytogenes* is involved in processing internalin and in virulence. *Infect Immun* 2002; **70**: 1382–1390.
- Hamada S, Slade HD. Biology, immunology, and cariogenicity of *Streptococcus mutans*. *Microbiol Rev* 1980; **44**: 331–384.
- Igarashi T, Yamamoto A, Goto N. Sequence analysis of the *Streptococcus mutans* Ingbritt *dexA* gene encoding extracellular dextranase. *Microbiol Immunol* 1995; **39**: 852–860.
- Igarashi T, Asaga E, Goto N. Roles of *Streptococcus mutans* dextranase anchored to the cell wall by sortase. *Oral Microbiol Immunol* 2004; **19**: 102–105.
- Igarashi T, Asaga E, Goto N. The sortase of *Streptococcus mutans* mediates cell wall anchoring of a surface protein antigen. *Oral Microbiol Immunol* 2003; **18**: 266–269.
- Igarashi T, Asaga E, Sato Y, Goto N. Inactivation of *srtA* gene of *Streptococcus mutans* inhibits dextran-dependent aggregation by glucan-binding protein C. *Oral Microbiol Immunol* 2004; **19**: 57–60.
- Jonsson IM, Mazmanian SK, Schneewind O, Verdreng M, Bremell T, Tarkowski A. On the role of *Staphylococcus aureus* sortase and sortase-catalyzed surface protein anchoring in murine septic arthritis. *J Infect Dis* 2002; **185**: 1417–1424.
- Lee SF, Boran TL. Roles of sortase in surface expression of the major protein adhesin P1, saliva-induced aggregation and adherence, and cariogenicity of *Streptococcus mutans*. *Infect Immun* 2003; **71**: 676–681.
- Lee SF, Progulsk-Fox A, Erdos GW, et al. Construction and characterization of isogenic mutants of *Streptococcus mutans* deficient in major surface protein antigen P1 (I/II). *Infect Immun* 1989; **57**: 3306–3313.
- Loesche WJ. Role of *Streptococcus mutans* in human dental decay. *Microbiol Rev* 1986; **50**: 353–380.
- Matsumura M, Izumi T, Matsumoto M, Tsuji M, Fujiwara T, Ooshima T. The role of glucan-binding proteins in the cariogenicity of *Streptococcus mutans*. *Microbiol Immunol* 2003; **47**: 213–215.
- Mazmanian SK, Liu G, Ton-That H, Schneewind O. *Staphylococcus aureus* sortase, an enzyme that anchors surface proteins to the cell wall. *Science* 1999; **285**: 760–763.
- Mazmanian SK, Liu G, Jensen ER, Lenoy E, Schneewind O. *Staphylococcus aureus* sortase mutants defective in the display of surface proteins and in the pathogenesis of animal infections. *Proc Natl Acad Sci USA* 2000; **97**: 5510–5515.
- Murakami Y, Nakano Y, Yamashita Y, Koga T. Identification of a frameshift mutation resulting in premature termination and loss of cell wall anchoring of the PAc antigen of *Streptococcus mutans* GS-5. *Infect Immun* 1987; **65**: 794–797.

25. Nakano K, Matsumura M, Kawaguchi M, et al. Attenuation of glucan-binding protein C reduces the cariogenicity of *Streptococcus mutans*: analysis of strain isolated from human blood. *J Dent Res* 2002; **81**: 376–379.
26. Navarre WW, Schneewind O. Surface proteins of gram-positive bacteria and mechanisms of their targeting to the cell wall envelope. *Microbiol Mol Biol Rev* 1999; **63**: 174–229.
27. Osaki M, Takamatsu D, Shimojo Y, Sekizaki T. Characterization of *Streptococcus suis* encoding proteins homologous to sortase of gram-positive bacteria. *J Bacteriol* 2002; **184**: 971–982.
28. Pallen MJ, Lam AC, Antonio M, Dunbar K. An embarrassment of sortases – a richness of substrates? *Trends Microbiol* 2001; **9**: 97–101.
29. Qian H, Dao ML. Inactivation of the *Streptococcus mutans* wall-associated protein A gene (*wapA*) results in a decrease in sucrose-dependent adherence and aggregation. *Infect Immun* 1993; **61**: 5021–5028.
30. Robinson WG, Old LA, Shah DSH, Russell RRB. Chromosomal insertions and deletions in *Streptococcus mutans*. *Caries Res* 2003; **37**: 148–156.
31. Russell RRB. Wall-associated protein antigen of *Streptococcus mutans*. *J Gen Microbiol* 1979; **114**: 109–115.
32. Sato Y, Yamamoto Y, Kizaki H. Cloning and sequence analysis of the *gbpC* gene encoding a novel glucan-binding protein of *Streptococcus mutans*. *Infect Immun* 1997; **65**: 668–675.
33. Sato Y, Okamoto K, Kizaki H. *gbpC* and *pac* gene mutations detected in *Streptococcus mutans* strain GS-5. *Oral Microbiol Immunol* 2002; **17**: 263–266.
34. Ton-That H, Liu G, Mazmanian SK, Faull KF, Schneewind O. Purification and characterization of sortase, the transpeptidase that cleaves surface proteins of *Staphylococcus aureus* at the LPXTG motif. *Proc Natl Acad Sci USA* 1999; **96**: 12424–12429.
35. Walker GW, Pulkownik A, Morrey-Jones JG. Metabolism of the polysaccharides of human dental plaque: release of dextranase in batch cultures of *Streptococcus mutans*. *J Gen Microbiol* 1981; **127**: 201–208.

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.