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# Roles of Sortase in Surface Expression of the Major Protein Adhesin P1, Saliva-Induced Aggregation and Adherence, and Cariogenicity of *Streptococcus mutans*

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**Sortase is a newly discovered transpeptidase that covalently links LPXTGX-containing surface proteins to the gram-positive bacterial cell wall. In this study, the sortase gene (*srtA*) was isolated from *Streptococcus mutans* NG8 by PCR. The gene encoded a 246-amino-acid protein, including a 40-amino-acid signal peptide. The *srtA* gene was insertionally inactivated by a tetracycline resistance cassette. P1, a major surface protein adhesin previously shown to anchor to the peptidoglycan by the LPXTGX motif, was secreted into the culture medium by the *srtA* mutant. In contrast, the wild-type P1 remained cell wall associated. Complementation of the mutant with *srtA* restored the P1 surface expression phenotype. P1 produced by the mutant, but not that produced by the wild type and the *srtA*-complemented mutant, was recognized by an antibody raised against the hydrophobic domain and charged tail C terminal to the LPXTGX motif. These results suggest that the failure to anchor P1 to the cell wall is due to the lack of cleavage of P1 at the LPXTGX motif. The *srtA* mutant was markedly less hydrophobic than the wild type and the complemented mutant. The *srtA* mutant failed to aggregate in the presence of saliva or salivary agglutinin and adhered poorly to saliva- or salivary agglutinin-coated hydroxylapatite. In rats, the *srtA* mutant colonized the teeth poorly when sucrose was absent. When sucrose was present, the *srtA* mutant colonized the teeth but less effectively and induced significantly less caries ($P < 0.05$) than the wild-type strain. In conclusion, the sortase enzyme in *S. mutans* is responsible for anchoring P1 to the cell surface and plays a role in modulating the surface properties and cariogenicity of *S. mutans*.**

The major cell surface protein P1, also known as antigen I/II (ca. 185 kDa), from *Streptococcus mutans* is an adhesin that interacts with a high-molecular-weight salivary agglutinin and has been implicated in the adherence of the bacterium to the tooth surface (16). Sequence analysis of the P1 gene reveals common features at the C terminus of the protein, as found in several hundreds of surface proteins from many gram-positive coccal bacteria (17). These features include a hydrophilic (wall-associated) region, a highly conserved hexapeptide LPXTGX, a hydrophobic (membrane-spanning) domain, and a charged tail (22, 23).

In our previous studies, we have demonstrated the requirement of the C-terminal domains in P1 surface localization in *S. mutans* (10, 11). Site-directed mutagenesis analysis showed that the Thr residue within the LPXTGX motif plays a crucial role in enzymatic cleavage and anchoring of P1 to the cell wall (15).

A transpeptidase termed sortase (SrtA) was identified initially in *Staphylococcus aureus* (27) and recently in *Streptococcus gordonii* (5), *Streptococcus suis* (20), *Streptococcus pyogenes* (2), and *Listeria monocytogenes* (4, 9). The enzyme is responsible for covalently anchoring protein A to the cell wall (27) and plays a role the pathogenesis of *S. aureus* (12, 18). Recently, the role of SrtA in virulence was also implicated in *L. monocytogenes* (4, 9). In streptococci, although SrtA has been shown to anchor proteins to the cell surface, its role in pathogenesis has not been demonstrated. In this study, the gene coding for the sortase homologue was isolated from the oral pathogen S. *mutans*, and the biological function of SrtA including its role in cariogenicity was studied.

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## MATERIALS AND METHODS

**Bacteria and growth conditions.** *S. mutans* NG8 (serotype c) and mutants were grown in Todd-Hewitt broth, or unless indicated otherwise, at 37°C without agitation. When needed, tetracycline and kanamycin were added to the medium at 10 and 500 μg/ml, respectively. *Escherichia coli* was cultured in Luria-Bertani medium at 37°C. Ampicillin, tetracycline, and kanamycin were used at 100, 15, and 50 μg/ml, respectively.

**Cloning and sequencing of the *srtA* gene from *S. mutans* NG8.** A 1,035-bp DNA fragment carrying the *srtA* gene was amplified from the chromosome of *S. mutans* NG8 by PCR using *Taq* DNA polymerase and the primers SL177 (CC TCTAGATTAAAATGATATTTGATTATAGGACTG; *Xba*I site underlined) and SL189 (CTGAATTCTACCCGACTAAAGGACG; *Eco*RI site underlined). The primer sequences were derived from BLAST searching the *S. mutans* UAB159 genome at the University of Oklahoma (www.genome.ou.edu) using the *S. aureus srtA* gene as the query (GenBank accession no. AF162657). The forward primer SL189 was designed to include ca. 300 bp of upstream sequence from *srtA*. The reverse primer included the *srtA* sequence to the stop codon. The PCR conditions were as previously described (10). The PCR product was cloned into the dephosphorylated *Sma*I restricted pUC18. The resulting plasmid, pSrtA/18, was maintained in *E. coli* XL-1 Blue. The cloned DNA fragment was sequenced with M13 forward and reverse primers (Applied Biosystems/Dalhousie University-NRC Joint Laboratory Facilities).

**Inactivation of *srtA* and complementation.** The DNA coding for the mature sortase (741 bp) was amplified from the *S. mutans* NG8 genome by PCR using the primers SL177 and SL176 (CTGAATTCATGAAAAAAGAACGTCAATC TAGGA; *Eco*RI site underlined). The PCR product was initially cloned into the

*Eco*RI and *Xba*I sites of pUC18 and was subsequently subcloned as an *Eco*RI-*Hin*cII fragment into the *Eco*RI-*Nru*I sites of the suicide vector pVA981 (Tet$^r$) (26). The resulting plasmid, pSrtA/981, was transformed into *S. mutans* NG8 via natural transformation as previously described (10). Transformants were selected on tetracycline–Todd-Hewitt agar.

To create a construct for complementation, the DNA coding for the *srtA* gene plus the upstream sequence from pSrtA/18 was subcloned into the *E. coli*-streptococcus shuttle vector pDL276 (Kan$^r$) (8). This was achieved by ligating the 1.0-kb *Kpn*I-*Hin*cII fragment from pSrtA/18 into the same sites of pDL276. The resulting plasmid, pSrtA/276, was introduced into the *srtA* mutant by natural transformation. Transformants were selected on Todd-Hewitt agar containing tetracycline and kanamycin. pSrtA/276 was reisolated from the complemented mutant by a method similar to that described previously (30). The reisolated plasmid was restricted with *Kpn*I and *Hin*cII.

**Southern hybridization.** The isolation of chromosomal DNA from *S. mutans* and blotting of *Hin*dIII digested DNA onto nylon membranes were performed as described previously (30). DNA probes were prepared from pVA981 and the 736-bp sortase PCR product using the ECL direct labeling and detection system (Amersham Pharmacia Biotech. Inc., Baie d'Urfé, Quebec, Canada). Southern hybridization and signal detection were performed according to the manufacturer's instructions.

**ELISA.** Enzyme-linked immunosorbent assay (ELISA) was used to determine amounts of P1 in culture supernatants and intact cells and was performed as described previously (15). In brief, mid-exponential-phase cultures grown in the chemically defined FMC medium (25) were diluted to an optical density at 600 nm ($OD_{600}$) of 0.5 and centrifuged. Cells were washed in phosphate-buffered saline (PBS) and twofold-diluted in PBS. Culture supernatants were similarly diluted. Cells and supernatants were assayed for P1 on microtiter plates using the anti-P1 monoclonal antibody 4-10A (1/5,000, [1]).

**SDS-PAGE and Western immunoblotting.** Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed on 7.5% gels as described by Laemmli (14). Western immunoblotting was performed according to the method of Towbin (28). The nitrocellulose blots were probed with the monoclonal anti-P1 antibody 4-10A (1/5,000) and a rabbit polyclonal antibody (1/100) raised against the C-terminal hydrophobic domain and charged tail of P1 (15).

**Aggregation assays.** Aggregation experiments were conducted as described previously (16). Saliva used was freshly prepared clarified unstimulated whole saliva. Salivary agglutinin was prepared by the affinity method of Rundegren and Arnold (21) with modifications as described previously (29). *S. mutans* cells grown for 16 h in Todd-Hewitt broth were washed twice with KPBS (2.7 mM KCl, 1.5 mM $KH_2PO_4$, 137 mM NaCl, 6.5 mM $Na_2HPO_4$, pH 7.2) and resuspended to an $OD_{700}$ of approximately 1. Assay mixtures using whole saliva contained 800 μl of cells and 200 μl of fresh clarified saliva, while assays using agglutinin contained 800 μl of cells, 50 μl of agglutinin (2 μg/ml), 5 μl of 0.1 M $CaCl_2$, and 145 μl of KPBS. The mixtures were mixed by inversions at time zero and incubated at room temperature without further mixing. The $OD_{700}$s of the mixtures were measured with a spectrophotometer (model 8452A diode array; Hewlett-Packard, Waldbronn, Germany) at time intervals as indicated.

**Adherence assays.** The adherence of [methyl-$^3$H]thymidine (specific activity, 6.7 Ci/mmol; NEN Life Science Products, Inc., Boston, Mass.)-labeled cells to hydroxylapatite coated with clarified whole saliva (1/10 diluted) and salivary agglutinin (1 μg/ml) was conducted as described previously (16). Briefly, radiolabeled *S. mutans* cells were washed once in adherence buffer (50 mM KCl, 1 mM $CaCl_2 \cdot H_2O$, 38.3 mM $MgCl_2 \cdot 6H_2O$, 0.78 mM $KH_2PO_4$, 1.22 mM $K_2HPO_4$, pH 7.2) and dechained to single or double cells by rapid passages through a 27-gauge needle. The cells were resuspended to an $OD_{600}$ of 0.15. In the adherence assay, triplicates of 200 μl of cells (ca. 17,000 cpm) were incubated with saliva- or agglutinin-coated hydroxylapatite granules (type III; Sigma-Aldrich Canada Ltd., Oakville, Ontario, Canada) at 37°C. After 1 h, the hydroxylapatite was allowed to settle under gravity, and 150 μl of the liquid above the hydroxylapatite was removed for liquid scintillation counting. Percent adherence of cells adhered the hydroxylapatite was calculated as follows: [(control counts − test counts)/(control counts)] × 100, where control counts were counts from tubes from which hydroxylapatite was omitted.

**Hydrophobicity.** The absorption of cells to hexadecane was determined as described previously (16). Briefly, cells from late-exponential-phase cultures were washed once in PBS. The cells were resuspended in PBS and adjusted to an $OD_{436}$ of ca. 0.6. Hexadecane (0.2 ml, Sigma-Aldrich) was added to 1 ml of cell suspensions. The mixtures, in triplicate, were vortexed for 60 s, and the aqueous phase was allowed to settle under gravity for 15 min. The cell density in the aqueous phase was then measured at 436 nm. The percentage of cells adsorbed to hexadecane was taken as 1 − (aqueous-phase cell density after adsorption/aqueous-phase cell density before adsorption).

**Rat model of colonization and caries.** Specific-pathogen-free Sprague-Dawley rats (19-day-old females) upon arrival from the supplier (Charles River Laboratories, St. Constant, Quebec, Canada) were fed kanamycin (500 μg/ml) water to lower the microbial load. At the same time the animals received a 5% (wt/vol) sucrose diet (diet D01122001; Research Diet, Inc., New Brunswick, N.J.). On day 3, a group of animals ($n = 20$) was inoculated with $1.2 \times 10^8$ CFU of *S. mutans* NG8(pDL276) by pipetting 100 μl of an overnight culture in Todd-Hewitt broth to the oral cavity and nostrils. A second group of animals ($n = 21$) was similarly inoculated with the same number of *S. mutans srtA*(pDL276) mutants. A third group of animals ($n = 20$) was given Todd-Hewitt broth as the uninfected control. Following the inoculation, the kanamycin in the drinking water was lowered to 250 μg/ml. On day 4, the animals received a second inoculation as described above. Immediately following the second inoculation, the animals received normal (kanamycin-free) water and the sucrose diet for the remainder of the experiment. After 6 weeks, the animals were euthanized and the mandible was removed from each rat and submerged in 95% ethanol for 48 h. Excess tissue around the teeth was then carefully removed and the teeth were stained in 0.4% (wt/vol) murexide for 24 h. Carious lesions were scored as described by Keyes (13), and the results were analyzed by Student's $t$ test, with a $P$ of $<0.05$ considered statistically significant.

To monitor *S. mutans* colonization, animals were inoculated as described above. At 6 h after the second inoculation, the oral mucosa and the tongue of five animals from each of the groups were swabbed with cotton swabs. Each swab was put in 3 ml of PBS and vortexed for 1 min. The broth was serially diluted and plated on to mitis salivarius agar (Becton Dickinson, Sparks, Md.) containing kanamycin (500 μg/ml). The plates were incubated for 48 h in candle jars. Typical colonies were Gram stained and verified to be *S. mutans* by biochemical tests (3). At 1 week after the second inoculation, the same five animals from each group were similarly swabbed, and the bacteria were inoculated onto plates. The mandibles from these animals were removed and briefly sonicated (20 s, 20% of maximum output; Vibra Cells, Sonics & Materials Inc., Danbury, Conn.) in 3 ml of PBS. The dislodged *S. mutans* cells were similarly inoculated onto plates. At 6 weeks, the *S. mutans* on the mandibles from 10 animals was similarly inoculated onto plates. In a parallel experiment of colonization, cohorts of rats were given a normal sucrose-free diet (Prolab RMH 3000) instead of a sucrose diet but were similarly inoculated with NG8 and the *srtA* mutant for comparison.

**Nucleotide sequence accession number.** The sequence described in this study can be obtained from GenBank under accession number AF542085.

## RESULTS

**Cloning and analysis of *srtA*.** The *srtA* gene was identified by BLAST-searching the *S. mutans* UA159 genome at the University of Oklahoma site using the *S. aureus srtA* gene as the query sequence. The *srtA* gene, including a 300-bp upstream sequence, was cloned from *S. mutans* NG8. Sequence analysis showed that the *S. mutans* SrtA is a 246-amino-acid-protein that includes a 40-amino acid-signal sequence. The putative active site (VTLVTCTD) is located at the C terminus (27). The upstream sequence contains a putative promoter region and an inverted repeat sequence. The −10 region of the promoter lies within the inverted repeat sequence. The promoter and predicted transcriptional start site lie within an open reading frame, which is highly homologous to the DNA gyrase A protein.

**Characterization of an *srtA* mutant and a complemented mutant.** An *srtA* mutant of *S. mutans* NG8 was constructed by the insertion of pVA981 through homologous recombination using pSrtA/981. Following transformation of *S. mutans* NG8 with pSrtA/981, seven tetracycline-resistant transformants were obtained. Western immunoblotting showed that the transformants elaborated the 185-kDa P1 into the culture supernatant (data not shown). One of the transformants was further assayed for the relative distribution of P1 in the extracellular and the cell surface-bound fractions by ELISA. As

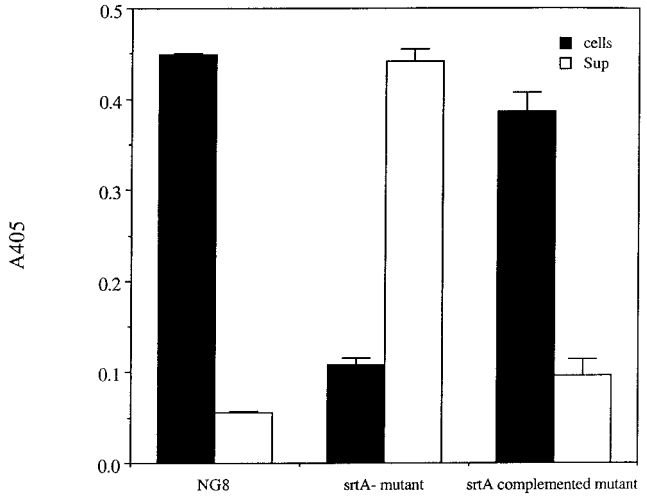


FIG. 1. Distribution of P1 in whole cells and culture supernatants (Sup) determined by ELISA using the anti-P1 monoclonal antibody 4-10A. Error bars, standard deviations.

shown in Fig. 1, P1 from the *srtA* mutant was mainly found in the culture supernatant, and in contrast, the P1 from the wild-type NG8 was mainly cell surface bound.

Southern analysis showed that the pVA981 probes hybridized to a 7.3-kb *Hin*dIII fragment from *srtA* mutant DNA, while no signal was obtained from the NG8 DNA (data not shown). When the same DNA was hybridized with the *srtA* probe, two fragments of 7.3 and 0.4 kb were obtained for the *srtA* mutant DNA, and a single 0.4-kb band was observed for the NG8 DNA. These hybridization patterns confirmed the insertion of pVA981 into the *srtA* gene of *S. mutans*.

An *srtA*-complemented mutant was obtained by transforming pSrtA/276, which carries the *srtA* gene and the 300-bp upstream sequence, into the *srtA* mutant. Restriction analysis of the plasmid reisolated from the complemented mutant confirmed that the *srtA* gene was maintained as a 1-kb DNA fragment on pDL276 (data not shown). The *srtA*-complemented mutant produced mainly surface-localized P1, similar to the wild-type NG8 (Fig. 1).

P1 produced by NG8, the *srtA* mutant, and the *srtA*-complemented mutant was analyzed for the C-terminal hydrophobic domain and charged tail by Western blotting. As shown in Fig. 2B, P1 produced by the *srtA* mutant, but not the P1 from NG8 and the *srtA*-complemented mutant, reacted with the antibody directed against the C-terminal hydrophobic domain and charged tail. Duplicate samples from the three organisms showed a ca. 185-kDa protein reacted with the anti-P1 monoclonal antibody directed against the middle portion of P1 (Fig. 2A). Interestingly, the *srtA* mutant P1 migrated slightly more slowly than the wild-type and *srtA*-complemented P1.

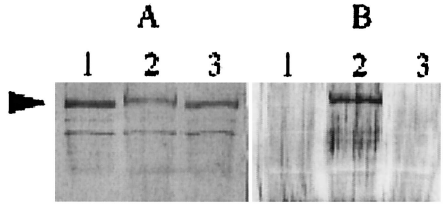


FIG. 2. Western immunoblots of P1 from *S. mutans* NG8, *srtA* mutant, and *srtA*-complemented mutant. P1 was extracted from cells with boiling SDS-PAGE buffer as described by Homonylo-McGavin and Lee (10). P1 was detected with the monoclonal anti-P1 antibody 4-10A (A) and a rabbit polyclonal antibody raised against the C-terminal hydrophobic domain and charged tail of P1 (B). Lane 1, wild-type NG8; lane 2, *srtA* mutant; lane 3, *srtA*-complemented mutant. The arrowhead indicates the ca. 185-kDa P1 protein.

**Roles of SrtA in surface-related biological properties.** The role of SrtA in modulating the cell-surface-related properties of *S. mutans* was investigated in aggregation, adherence, and hydrophobicity assays. In saliva-induced aggregation, NG8 and the *srtA*-complemented mutant aggregated upon incubation with saliva, but the *srtA* mutant failed to aggregate (Fig. 3A). Similar results were observed with salivary agglutinin (Fig. 3B).

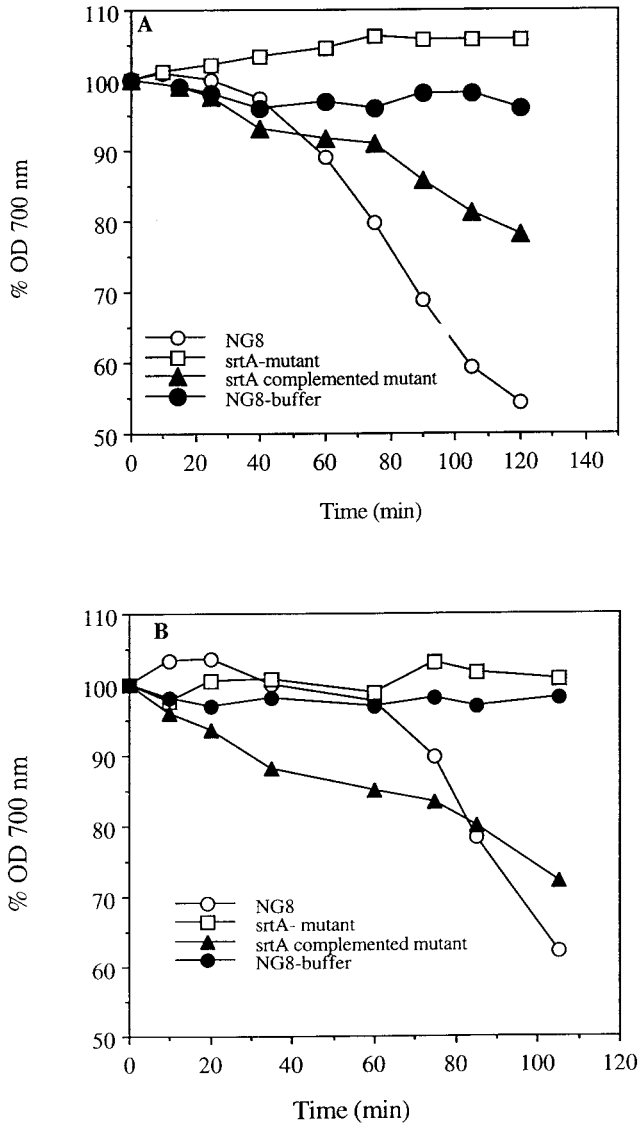


FIG. 3. Saliva (A)- and salivary agglutinin (B)-induced aggregation of *S. mutans* and mutants. NG8-buffer refers to the aggregation assay performed with *S. mutans* NG8 in the absence of saliva or salivary agglutinin.

TABLE 1. Colonization of oral mucosa and teeth by *S. mutans* NG8 and *srtA* mutant in rats

| Colonization site | Time postinoculation | Mean no. of CFU ± SD for mice fed: | | | |
|---|---|---|---|---|---|
| | | Sucrose diet | | Normal diet | |
| | | NG8 | SrtA$^-$ | NG8 | SrtA$^-$ |
| Mucosa[a] | 6 h | 3.11 ± 0.98 | 1.15 ± 0.67 | 3.25 ± 0.65 | 1.09 ± 0.36 |
| | 1 wk | 3.01 ± 1.65 | 0.89 ± 0.41 | 2.95 ± 1.71 | 0.78 ± 0.32 |
| Mandible[b] | 1 wk | 10.40 ± 2.79 | 4.73 ± 2.25 | 13.09 ± 3.38 | 0.33 ± 0.33 |
| | 6 wk | 14.59 ± 2.66 | 5.36 ± 2.76 | —[c] | — |

[a] For mucosa, results are given as CFU ($10^4$).
[b] For mandibles, results are given as CFU ($10^6$).
[c] —, not determined.

When the cells were assayed for adherence to saliva- or agglutinin-coated hydroxylapatite, a striking difference between the *srtA* mutant and NG8 was observed. The *srtA* mutant was nonadherent; the extent of adherence (mean ± SD) to saliva-coated and agglutinin-coated hydroxylapatite was 6.5% ± 5.7% and 1.4% ± 2.1%, respectively. In contrast, NG8 showed 55.7% ± 4.7% and 56.6% ± 2.3% adherence to saliva-coated and agglutinin-coated hydroxylapatite, respectively. The *srtA*-complemented mutant behaved similarly to NG8, with 54.8% ± 3.2% and 55.7% ± 9.1% adherence to the respective surfaces.

The *srtA* mutant was markedly less hydrophobic than NG8 and the *srtA* complemented mutant. The percentages of cells adsorbed to hexadecane (mean ± SD) for the *srtA* mutant, NG8, and the complemented mutant were 2.2% ± 6%, 59.4% ± 7%, and 53.2% ± 9%, respectively.

**Roles of SrtA in a rat model of colonization and caries.** Following inoculation, the presence of *S. mutans* on the oral mucosa and teeth was estimated on selective agar. *S. mutans* was detected from the animals inoculated with NG8 and the *srtA* mutant but not from the uninfected animals. The levels of *S. mutans* on the oral mucosa at 6 h and 1 week postinoculation were similar within the group but were about threefold less for the *srtA* than the NG8 group (Table 1). A 300- to 500-fold-higher *S. mutans* level was found on the teeth than on the mucosa. Again, a lower level of *S. mutans* was detected on the teeth from the *srtA* group than on those from the NG8 group. Within each group, a similar level of *S. mutans* was found on the mandible at 1 and 6 weeks postinoculation. In a parallel study in which the animals were fed a normal diet, the levels of *S. mutans* on the mucosa were similar to those of the sucrose-diet-fed animals. However, the number of *S. mutans* on the mandible was markedly lower for the *srtA* group than the NG8 group. Interestingly, the mandibles from the *srtA* group fed a normal diet contained markedly less *S. mutans* than those fed the sucrose diet.

Carious lesions were detected on the smooth (buccal and lingual) and sulcal surfaces of the molars in the three groups of animals (Table 2). The caries scores for the NG8 group were significantly higher than those for the uninfected group. The NG8 group showed significantly higher sulcal caries scores than the *srtA* group. For the smooth-surface caries, the enamel lesion was higher for the NG8 than the *srtA* group. Between the *srtA* and the uninfected group, the *srtA*-infected rats showed a similar caries score for all types of lesions except the enamel and slight dentinal lesions on the smooth surface, which were higher in the *srtA* group.

## DISCUSSION

In the present study, the *srtA* gene was isolated from *S. mutans* and its biological function was investigated. The entire *srtA* gene, including its promoter, was found on a 1,035-bp DNA fragment. Immediately upstream from the coding sequence of *srtA* is an open reading frame with high homology to DNA gyrase A. The putative *srtA* promoter and predicted transcriptional start site lie within the DNA gyrase A gene. The −10 region of the *srtA* promoter lies within an inverted repeat,

TABLE 2. Caries scores for *S. mutans* NG8 and SrtA$^-$ mutant in a rat model

| Strain | No. of rats | Mean rat wt ± SE (g) | Mean caries score ± SE[a] | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | Smooth surface | | | Sulcal | | |
| | | | E | Ds | Dm | E | Ds | Dm |
| NG8 | 20 | 375.2 ± 6.2 | 21.5 ± 0.90 | 6.90 ± 1.37 | 0.50 ± 0.29 | 22.6 ± 0.84 | 12.1 ± 0.83 | 3.15 ± 1.05 |
| SrtA$^-$[b] | 21 | 364.1 ± 6.1 | 18.4 ± 0.84 ($P = 0.0304$) | 6.76 ± 0.89 ($P = 0.4420$) | 0.10 ± 0.10 ($P = 0.0954$) | 18.5 ± 0.72 ($P = 0.0016$) | 6.52 ± 1.28 ($P = 0.0521$) | 0.24 ± 0.17 ($P = 0.0042$) |
| None[c] | 20 | 363.1 ± 6.0 | 9.45 ± 1.32 ($P < 0.0001$; $P = 0.0014$) | 1.90 ± 0.89 ($P = 0.0006$; $P = 0.0007$) | 0 ($P = 0.0274$; $P = 0.1750$) | 15.7 ± 1.21 ($P = 0.0015$; $P = 0.3969$) | 5.60 ± 0.95 ($P < 0.0001$; $P = 0.2383$) | 0.45 ± 0.33 ($P = 0.0074$; $P = 0.3477$) |

[a] Mean caries scores on molar surfaces. The animals did not develop any extensive dentinal lesions or lesions on the proximal surface. Abbreviations for lesion types: E, enamel; Ds, slight dentinal; Dm, moderate dentinal
[b] *P* values from *t* test between NG8 and SrtA$^-$ mutant.
[c] None, uninfected control. The first *P* values are from *t* tests between NG8 and uninfected control, and the second *P* values are from *t* tests between SrtA$^-$ mutant and uninfected control.

suggesting that the expression of *srtA* may be regulated. The *srtA* promoter is functional, as evidenced by the complementation study. Sequence analysis showed that the size of *S. mutans* SrtA (206 amino acids) is similar to those from *S. aureus* (27), *S. gordonii* (5), and *L. monocytogenes* (4). The putative active site (VTLVTCTD) found on the *S. aureus* SrtA was similarly located at the C terminus of the protein.

The role of SrtA in anchoring P1 to the bacterial cell surface was demonstrated through knockout and complementation studies. Results showed that the *srtA* mutant failed to retain P1 on the cell surface and complementing the mutant with the *srtA* gene restored the surface expression of P1. Western immunoblots showed that the *srtA* mutant P1 was recognized by the antibody generated against the C-terminal hydrophobic domain and charged tail, while the wild-type and complemented mutant P1 was not recognized. These results strongly suggest that the failure in the surface expression of P1 in the *srtA* mutant is due to the lack of enzymatic cleavage of the C-terminal hydrophobic domain and charged tail at the LPXTGX motif. It is noteworthy that the *srtA* mutant P1 has a slightly slower migration on SDS-polyacrylamide gels. The slower migration may be indicative of the slightly larger size of P1 because of the retention of the C-terminal domains.

The *srtA* mutant displayed major differences in surface-related properties compared to the wild type and the *srtA*-complemented mutant. The *srtA* mutant was markedly less hydrophobic than the wild type and the *srtA*-complemented mutant. In addition, the *srtA* mutant was nonadherent to hydroxylapatite and nonaggregating in the presence of saliva and salivary agglutinin. A decrease in hydrophobicity, adherence, and aggregation was previously observed, but to a lesser extent, for an isogenic P1-negative mutant of *S. mutans* NG8 (7). The fact that these phenotypes were more prominent in the *srtA* mutant may be due to the loss of other LPXTGX-containing surface proteins in addition to P1. Recent results by Bolken et al. (5), showed that the *srtA* mutant of *S. gordonii* has a decreased adherence to immobilized fibronectin. Collectively, these results suggest that sortase plays a very important role in these surface-related properties by modulating the bacterial cell surface in *S. mutans*, *S. gordonii*, and possibly other oral streptococci.

In rats, the *srtA* mutant showed a decreased ability to colonize the oral mucosa and the teeth. The colonization of the oral mucosa by the *srtA* mutant was about three times less than that by the wild type in mice with or without sucrose in their diet. The *srtA* mutant of *S. gordonii* also showed a similar decrease in colonization of the oral mucosa of mice (5). The most drastic effect of *srtA* inactivation in colonization was observed on the teeth in the absence of sucrose. The *srtA* mutant was almost incapable of colonizing the teeth. At 1 week postinoculation, the bacterium could only be recovered from one of five animals, and in this one animal the *S. mutans* count was 40 times less than that in the wild-type group. In contrast, the *srtA* mutant can colonize the teeth when sucrose is present but to a lesser extent (three times less) than the wild type. The inability to colonize teeth in the absence of sucrose relates well to the adherence results. The colonization of teeth by the *srtA* mutant in the presence of sucrose is most likely achieved through glucan-mediated processes (24). It is interesting that the P1-negative mutant of *S. mutans* was reported to colonize teeth to a lesser extent (ca. 1.8 times less) than the wild-type NG8 in gnotobiotic rats with a 5% sucrose diet (7).

The effect of *srtA* inactivation in the virulence of *S. mutans* was assessed in a rat model of caries. The gnotobiotic rat model developed by Michalek et al. (19) is probably ideal for the study. However, the model entails special requirements, such as specialized facilities and in-house breeding of animals, which are not accessible. In this study, we used the specific-pathogen-free rat model which was described by Bowen et al. (6). In the present study, a group of rats not infected with *S. mutans* was included as a control. The results showed that caries do develop in this control group. However, the *S. mutans* NG8-infected group developed a statistically higher degree of caries ($P < 0.0274$) than the control group. There was no significant difference in caries scores between the *srtA* and control group except the enamel and slight dentinal lesions on the smooth surface. The *srtA* mutant caused less caries than the wild-type NG8 in all lesion types except the slight dentinal lesion on the smooth surface. These results strongly suggest that the *srtA* mutant is less virulent than the wild type. These results are in general agreement with those of Crowley et al. (7), which showed that the P1-negative mutant causes less caries than the NG8 strain in gnotobiotic rats. The fundamental difference between our study and that of Crowley et al. (7) is that the reduction in caries in this case is due to *srtA* inactivation in stead of *spaP* knockout.

Although sortase has been identified in *S. pyogenes* (2), *S. suis* (20), and *S. gordonii* (5) its role in virulence has not been demonstrated. Our animal data provide the first evidence that SrtA plays a role in virulence in streptococci and adds *S. mutans* to the list of two other organisms, *S. aureus* and *L. monocytogenes*, for which SrtA has been implicated in virulence.

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