INFECTION AND IMMUNITY, Mar. 1991, p. 1093–1099
0019-9567/91/031093-07$02.00/0
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Vol. 59, No. 3

Nucleotide Sequence of a Gene Coding for a Saliva-Binding Protein (SsaB) from *Streptococcus sanguis* 12 and Possible Role of the Protein in Coaggregation with Actinomyces

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Received 16 August 1990/Accepted 21 December 1990

The nucleotide sequence of a 2.9-kb streptococcal DNA fragment which codes for two proteins with M_rs of 36,000 (*Streptococcus sanguis* adhesin B [SsaB]) and 20,000 has been determined. The *ssaB* gene is 927 bp and codes for a 34,684-Da protein. The open reading frame coding for the 20-kDa protein is 489 bp and codes for a protein of 17,885 Da. The SsaB protein has a putative hydrophobic 19-amino-acid signal sequence resulting in a 32,620-M_r secreted protein, whereas the 20-kDa protein has no signal sequence. Both proteins are hydrophilic, and neither appears to have a hydrophobic membrane anchor sequence in the carboxy-terminal region. A DNA sequence homology of 73% exists between the cloned fragment containing the *ssaB* gene from *S. sanguis* 12 and the cloned fragment containing the type 1 fimbrial gene of *S. sanguis* FW213 (J. C. Fenno, D. J. LeBlanc, and P. Fives-Taylor, Infect. Immun. 57:3527–3533, 1989). Amino acid comparisons of the SsaB and type 1 fimbrial proteins show 87% homology, indicating a close similarity of the two proteins. Antiserum raised against the cloned SsaB protein cross-reacts with a 38-kDa protein identified from *Streptococcus gordonii* (*S. sanguis*) PK488 which was proposed to mediate coaggregation with *Actinomyces naeslundii* PK606 (P. E. Kolenbrander and R. N. Andersen, Infect. Immun. 58:3064–3072, 1990). The SsaB adhesin may play a role in oral colonization by binding either to a receptor on saliva or to a receptor on actinomyces.

Streptococcus sanguis is one of the first bacteria to adhere to teeth and may help initiate dental plaque formation (1, 29). *S. sanguis* constitutes about 8 to 15% of the organisms isolated from mature dental plaque (11). Coaggregation of *S. sanguis* with other plaque bacteria such as actinomyces may contribute to the formation and accumulation of dental plaque (2). Studies with oral veillonellae and streptococci have established that coaggregation properties of oral bacteria may play a critical role in colonization of specific oral surfaces (13, 22).

Ionic, hydrophobic (10, 26), and lectin-like mechanisms (27, 28) have been proposed to mediate binding of *S. sanguis* to saliva-coated hydroxyapatite beads (S-HA) (3). Recently, a 36,000-M_r protein (SsaB) was shown to inhibit attachment of *S. sanguis* 12na to S-HA (9). Antibodies against fimbriae isolated from *S. sanguis* FW213 block adherence of the streptococcus to S-HA (5). A fimbrial gene (type 1) encoding a 34,349-Da protein has been cloned in *Escherichia coli* (8) and sequenced (6). These studies did not resolve whether the fimbrial subunit and the adhesin are distinct proteins, as has now become evident from studies of *E. coli* pap and type 1 fimbriae (19, 23). To help answer this question, we have sequenced the previously cloned 2.9-kb fragment from *S. sanguis* 12, which expresses the *ssaB* gene product, a 36-kDa adhesin, as well as a 20-kDa protein (9).

The DNA sequence of the *ssaB* gene presented here enabled a determination of the relatedness of the sequence of this gene to the reported sequence of the fimbrial gene of *S. sanguis* FW213 (6). We also report the antigenic cross-reactivity of the SsaB protein and the *S. gordonii* PK488 38,000-M_r protein, which appears to mediate coaggregation between streptococci and *Actinomyces naeslundii* PK606 (16). The possible role of these proteins in initial colonization of oral streptococci is discussed.

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MATERIALS AND METHODS

Bacterial strains and culture conditions. *S. sanguis* 12 and its non-saliva-aggregating variant 12na, *Streptococcus gordonii* PK488 and its coaggregation-defective mutant PK1804, and *A. naeslundii* PK606 have been described previously (16, 21). All strains were grown under anaerobic conditions (GasPak system; BBL Microbiology Systems, Cockeysville, Md.) at 37°C in a medium containing tryptone, yeast extract, Tween 80, and glucose buffered to pH 7.5 with K_2HPO_4 (16). Cells were harvested by centrifugation, washed, and stored in coaggregation buffer (1 mM Tris [pH 8.0], 0.1 mM $CaCl_2$, 0.1 mM $MgCl_2$, 0.02% NaN_3, 0.15 M NaCl).

E. coli SA2 (9) was grown in LB broth at 37°C. When solid medium was required, agar (1.5% [wt/vol]) was added to LB broth. Ampicillin (100 μg/ml) was added when required.

Nucleotide sequence determination. The DNA sequence was determined by the Sanger dideoxy-chain termination method (32) with a T7 DNA polymerase sequencing kit, as recommended by the manufacturer (Pharmacia LKB Biotechnology Inc., Piscataway, N.J.). [α-^{35}S]dATP (specific activity, 39.3 TBq/mmol; Dupont NEN Research Products, Boston, Mass.) was used to label the reaction products, and the DNA fragments were fractionated in Hydrolink DNA sequencing gels (AT Biochem, Malvern, Pa.). Initially, the universal and reverse primers of pUC18 were used. Synthetic primers (20-mers) were then made by using either a Pharmacia Gene Assembler (Pharmacia LKB Biotechnology Inc.) or the PCR-MATE DNA Synthesizer, model 391 (Applied Biosystems, Foster City, Calif.). Primers were

made at approximately 100- to 150-bp intervals on both strands as the new DNA sequence became available. The sequences of both strands were determined independently at least two times. The double-stranded plasmid DNA templates (pSA2) for sequencing were prepared from *E. coli* SA2 by alkaline lysis and CsCl density gradient centrifugation as described by Maniatis et al. (20). For sequence analysis, the University of Wisconsin Genetics Computer Group software package was used on a VAX-VT100 computer (4).

Electrophoretic methods. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was done with 10% polyacrylamide gels (18). Samples for SDS-PAGE were boiled in 2% SDS and 5% β-mercaptoethanol for 5 min. Prestained molecular weight standards were purchased from Bio-Rad Laboratories (Richmond, Calif.), and their apparent molecular weights were 110,000 (phosphorylase *b*), 84,000 (bovine serum albumin), 47,000 (ovalbumin), 33,000 (carbonic anhydrase), 24,000 (soybean trypsin inhibitor), and 16,000 (lysozyme). Gels were stained with Coomassie brilliant blue. Alternatively, proteins were transferred electrophoretically (33) to nitrocellulose for Western immunoblotting. Western blots were then incubated with either anti-PK488 immunoglobulin G (IgG) which had been absorbed with a coaggregation-defective mutant PK1804 (16) or anti-SsaB IgG (9) and visualized with an alkaline phosphatase-conjugated anti-rabbit IgG and dye indicator supplied by Promega, Madison, Wis.

Antisera. Antisera against *S. gordonii* PK488 (16) and SsaB protein (9) were prepared in New Zealand White rabbits as described previously. IgG was purified either by using a protein A-Sepharose CL-4B (Sigma Chemical Co., St. Louis, Mo.) column or by $(NH_4)_2SO_4$ precipitation (0 to 55%) and ion-exchange chromatography (DE-52; Whatman, Inc., Clifton, N.J.).

Purification of the SsaB and 20-kDa proteins. Both proteins were purified from *E. coli* SA2 by gel filtration and ion-exchange chromatography as described previously (9). A tryptic peptide of SsaB was isolated by incubating SsaB with trypsin (10 μg/ml) for 18 h and then purifying the tryptic peptide on a Superose-12 column (Pharmacia FPLC system; Pharmacia, Uppsala, Sweden) with 0.05 M Tris (pH 8.0)–0.1 M NaCl buffer. Fractions were analyzed by SDS-PAGE for the presence and purity of the tryptic peptides of SsaB. The N-terminal amino acid sequences of the 20-kDa protein and the tryptic peptide of SsaB were determined by automated Edman degradation by using an Applied Biosystems model 470A gas-phase sequenator utilizing the resident sequencing program. The amino acid residues were analyzed by reverse-phase high-pressure liquid chromatography. These analyses were provided by the University of Victoria protein sequencing facility. The amino acid compositions of intact recombinant proteins were also determined at the protein sequencing facility at the University of Victoria, Victoria, British Columbia, Canada.

Sonic lysates of streptococci. An amount of 600 mg (wet weight) of streptococcal cells was resuspended in 3 ml of distilled water and was sonicated in an ice bath for 1 min at maximum power (50 W) by using a Kontes micro ultrasonic cell disruptor (Kontes, Vineland, N.J.). The sonicated cell suspension was centrifuged at 20,000 × *g* for 10 min, and the supernatant was stored frozen until used.

Protein determination. Protein concentrations were determined with the Bio-Rad protein assay kit.

Nucleotide sequence accession number. The GenBank accession number for the *S. sanguis* 12 *ssaB* gene is M37189.

RESULTS

DNA sequence analysis. The nucleotide sequence of the 2.9-kb fragment which codes for the *ssaB* gene contained two open reading frames (Fig. 1). The *ssaB* gene is 927 bp and is located between nucleotides 1049 and 1975 on the cloned fragment. The deduced *ssaB* gene product is 309 amino acids and has a molecular weight of 34,684, as calculated from the translated nucleotide sequence. A putative signal peptide of 19 amino acids is indicated by the dashed line starting with nucleotide 1049 in Fig. 1. Cleavage of this leader peptide between alanine and cysteine, according to the Von Heijne parameters (34), would result in an SsaB protein with an M_r of 32,620. A hydropathy plot computed from the sliding average of the hydropathy values (17) supports the presence of a signal peptide and indicates that the mature protein is hydrophilic (data not shown). The N-terminal sequence of the recombinant SsaB protein could not be determined because the N-terminal region was blocked. The N-terminal amino acid sequence determined from the tryptic peptide (35 kDa) of the SsaB protein starts with residue 24 (Fig. 1, asparagine marked by asterisk). The calculated molecular weight of this peptide is 31,946. The deduced isoelectric point of the molecule is about 5.3.

A typical ribosome-binding site (RBS) sequence, GGAGG, similar to *Bacillus* (24, 25), *Streptococcus* (7), and *E. coli* (12) RBS sequences, is present 6 bases upstream from the ATG translation initiation codon. Two identical potential −10 region TAAAAT sequences are located 5 and 10 bases upstream from the RBS sequence (31). They are preceded by two putative −35 regions, TTAAAA and TTGCCC, located 20 and 39 bases, respectively, upstream from the proposed −10 promoter region located between nucleotides 1021 and 1027; however, transcription studies are needed to determine the actual promoter sequences.

A second open reading frame which corresponds to the previously identified 20-kDa protein (9) is located between nucleotides 2130 and 2618. It encodes a 163-amino-acid protein with a calculated molecular weight of 17,885. The N-terminal sequence determined from the purified 20-kDa protein is indicated in Fig. 1 by asterisks starting with nucleotide 2133. In contrast to the SsaB protein, the 20-kDa protein does not appear to have a typical signal sequence. The 20-kDa protein also is hydrophilic and has a deduced isoelectric point of 5.0.

A potential RBS sequence, GGAG, is present 7 bases upstream from the ATG translation initiation codon (12). Two potential −10 regions, TATCAA and TACAAT, are located 8 and 19 bases upstream from the RBS sequence (31). Two probable −35 regions, TTCGAA and TTCAAA, are located 18 and 39 bases respectively, away from one of the −10 regions (TACAAT). Translation of the 20-kDa protein terminates at nucleotide 2618. This sequence is followed by an inverted repeat capable of stem-loop formation which, as determined by methods of Zuker and Stiegler (37), has a negative free energy of −13.7 kcal (1 cal = 4.184 J) per mol. This stem-loop structure would consist of a perfectly matched 12-base stem separated by a 6-base loop.

The hydropathy plots (17) indicate that neither the SsaB protein nor the 20-kDa protein appears to have a hydrophobic membrane anchor sequence in its carboxy-terminal region (data not shown). Amino acid compositions of the deduced SsaB and 20-kDa proteins are very similar to those determined for the SsaB and 20-kDa proteins purified from *E. coli* (Table 1).

Comparison of *ssaB* with an *S. sanguis* type 1 fimbrial gene.

```
CTGCAGGAAAGATTATCCTGATTGTCCACCATGATCTGAGTAAGGTTGTTGATTATTTCG     60
PstI
ACCAAATTATCCTGCTCAATCGTAAGCTGATTGCCTTTGGTCCTACCAAGACCAGCTTTA    120

CCAAGGACAATATGAAAAAGACTTACGGCTCCCAGCTCTTTATGAATGGAGGTGCCTAGG    180

ATGATTTTAGAATTTTTCCAAGGGCTGCGGGACTTTCACTTTCTGCAAAACGCCCTCATC    240

ACAGCCATCGTCATTGGGATTGTTGCTGGAGCTGTCGGCTGCTTTATCATTCTGCGCGCG    300

GCATGTCGCTTATGGGAGACGCTATCTCTCATGCGGTTCTGCCTGGTGTGGCCCTGTCCT    360

ACATTTTAGGGATTAATTTCTTTATCGGAGCCATCACTTTCGGACTTTTGGCCTCGATTA    420

TTATTACATACATCAAGGGCAATTCCATTATCAAAAGCGATACGGCCATTGGGATTACCT    480

TTTCTTCCTTCCTAGCTCTGGGTGTAATCCTGATCCGGTTGCCAATAGCTCCACTGATCT    540

CTTTCATATTCTCTTCGGGAATATCTTAGCCGTGCAGGATATTGATATGTGGATTAGTAT    600

TGGAGTCGGAATAGCTGTGCTCTTAATCATCACCCTCTTTTTCAAGCAGCTATTGATTAC    660

CTCCTTTGACCCCCTGCTGCTCAAGCCATGGGCATGCCGGTCAGCTTCTATCACTATCTG    720

CTGATGATTCTTTTGACCTTGGTCTCTGTGACAGCCATGCAGAGTGTGGGAACCATTCTG    780

ATTGTCGCTCTCTTGATAACGCCAGCAGCAACGGCCTACCTCTATGCCAACAGTCTGAAA    840

ACTATGATTCTGCTGTCCTCAGGGCTGGGGCCTCTAGCTTCTGTACTGGGACTCTTTATC    900

GGCTACAGCTTTAATGTCGCTGCAGGATCCAGCATTGTCCTGACCTCAGCCCTCATCTTT    960
                    PstI
CTCATCTCTTTCTTTATTGCCCCTAAGCAACGCTATTTAAAACTTAAAAACCGACCTAAA   1020
                    -35                -35
CTAAAATAAAATTTAATGGAGGAATCCCATGAAAAAATTAGGTTTTTTATCCCTGCTTTT   1080
   -10  -10         rbs      MetLysLysLeuGlyPheLeuSerLeuLeuLe
                             --------------------------------

GCTAGCAGTCTGCACTCTCTTTGCCTGCTCCAATCAGAAGAACGCGTCCAGTGACTCGTC   1140
uLeuAlaValCysThrLeuPheAlaCysSerAsnGlnLysAsnAlaSerSerAspSerSe
-------------------------                          * * * * * * *

CAAGCTCAAGGTTGTTGCGACCAACTCAATTATCGCTGATATTACCAAAAATATAGCTGG   1200
rLysLeuLysValValAlaThrAsnSerIleIleAlaAspIleThrLysAsnIleAlaGl
  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  *  ?  *  *

CGATAAGATTGACCTGCACAGCATTGTACCAGTCGGCAAAGACCCCCACGAATACGAGCC   1260
yAspLysIleAspLeuHisSerIleValProValGlyLysAspProHisGluTyrGluPr
  *  *

CCTGCCTGAAGATGTCAAAAAGACTTCTCAAGCGGATCTCATCTTCTACAATGGTATCAA   1320
oLeuProGluAspValLysLysThrSerGlnAlaAspLeuIlePheTyrAsnGlyIleAs

CCTAGAAACCGGCGGCAATGCTTGGTTTACCAAGTTGGTCAAAAATGCCAATAAGGAAGA   1380
nLeuGluThrGlyGlyAsnAlaTrpPheThrLysLeuValLysAsnAlaAsnLysGluGl

AAACAAGGACTACTATGCTGTCAGCGATGGCGTTGACGTCATTTACCTTGAAGGTCAAAG   1440
uAsnLysAspTyrTyrAlaValSerAspGlyValAspValIleTyrLeuGluGlyGlnSe

TGAAAAGGGTAAGGAAGACCCTCATGCTTGGCTCAATTTGGAAAACGGTATCATCTATGC   1500
rGluLysGlyLysGluAspProHisAlaTrpLeuAsnLeuGluAsnGlyIleIleTyrAl

GCAAAATATTGCCAAGCGTTTGATCGAAAAAGACCCTGACAATAAGGCTACTTACGAGAA   1560
aGlnAsnIleAlaLysArgLeuIleGluLysAspProAspAsnLysAlaThrTyrGluLy

AAATCTCAAAGCCTATGTAGAAAAGCTAACCGCTTTGGATAAGGAAGCCAAGGAGAAATT   1620
sAsnLeuLysAlaTyrValGluLysLeuThrAlaLeuAspLysGluAlaLysGluLysPh

CAACAATATTCCAGAAGAAAAGAAAATGATTGTGACAAGTGAAGGATGCTTCAAGTACTT   1680
eAsnAsnIleProGluGluLysLysMetIleValThrSerGluGlyCysPheLysTyrPh

CTCTAAGGCCTACAACGTGCCGTCGGCCTACATCTGGGAAATCAACACCGAAGAAGAAGG   1740
eSerLysAlaTyrAsnValProSerAlaTyrIleTrpGluIleAsnThrGluGluGluGl

AACTCCAGACCAAATCAAAAGCTTAGTTGAAAAATTACGCAAGACAAAAGTGCCGTCTCT   1800
yThrProAspGlnIleLysSerLeuValGluLysLeuArgLysThrLysValProSerLe

CTTTGTCGAATCAAGTGTGGACGACCGTCCGATGAAGACTGTTTCTAAAGACACCAACAT   1860
uPheValGluSerSerValAspAspArgProMetLysThrValSerLysAspThrAsnIl

CCCAATCCACGCTAAAATTTTCACTGACTCAATCGCTGATCAAGGCGAAGAAGGCGACAC   1920
eProIleHisAlaLysIlePheThrAspSerIleAlaAspGlnGlyGluGluGlyAspTh

GTACTACAGCATGATGAAATACAATCTAGATAAAATTTCTGAAGGCTTGGCCAAATAATG   1980
rTyrTyrSerMetMetLysTyrAsnLeuAspLysIleSerGluGlyLeuAlaLys

ATAAAAGGTTAGGAAGACAAAATCCTAACCTCTTTATTTTTGGAGAAGGAGCTTCACATG   2040

GAGTCGATTTCAAATTTCCGGTCAAATTATTCGAAAAAAATCCCTATTCGCTGTACAATG   2100
      -35                -35                          -10
AAGCTATCAAACATGAATGGAGACTTCCTATGACAACTTTTCTCGGAAATCCTGTAACCT   2160
  -10              rbs        MetThrThrPheLeuGlyAsnProValThrP
                                 *  *  *  *  *  *  *  *  *
TTACTGGAAAGCAACTGCAAGTCGGCGACACAGCCCACGATTTTAGCCTGACAGCTACTG   2220
heThrGlyLysGlnLeuGlnValGlyAspThrAlaHisAspPheSerLeuThrAlaThrA
 *  *  *  *  *  *  *  *  *  *  *  *  *  ?  *  *  *  *  *  *
ACCTTTCAAAGAAAACTCTGGCTGATTTTGCTGGCAAAAAGAAAGTCCTGAGCATCATCC   2280
spLeuSerLysLysThrLeuAlaAspPheAlaGlyLysLysLysValLeuSerIleIleP
 *  *  *  *  *  *  ?  *  *

CATCTATCGATACTGGTGTATGCTCGACTCAGACTCGCCGTTTCAACCAAGAGCTCTCTG   2340
roSerIleAspThrGlyValCysSerThrGlnThrArgArgPheAsnGlnGluLeuSerA

ACTTGGACAATACCGTTGTTATCACGGTTTCAGTTGACTTGCCTTTTGCTCAAGGCAAGT   2400
spLeuAspAsnThrValValIleThrValSerValAspLeuProPheAlaGlnGlyLysT

GGTGTGCTGCTGAAGGCATTGAAAATGCTGTTATGCTCTCTGACTACTTCGACCATTCTT   2460
rpCysAlaAlaGluGlyIleGluAsnAlaValMetLeuSerAspTyrPheAspHisSerP

TCGGCCGTGACTATGCTGTCCTCATAAATGAATGGCACCTTTTGGCTCGCGCAGTTCTTG   2520
heGlyArgAspTyrAlaValLeuIleAsnGluTrpHisLeuLeuAlaArgAlaValLeuV

TCTTGGACGAAAACAACACTGTGACCTACGCTGAATATGTCGACAATATCAATACCGAGC   2580
alLeuAspGluAsnAsnThrValThrTyrAlaGluTyrValAspAsnIleAsnThrGluP

CTGACTATGATGCGGCGATTGCAGCAGTTAAGAGCTTGTAAGAGATAAAAATACGGACCT   2640
roAspTyrAspAlaAlaIleAlaAlaValLysSerLeu                ---------->

GCTAGTCCGTATTTTTTTGTGCGAAAAACTTCTGCCAAAACAGACGGAACCAAGCCCAGG   2700
    <-----------

AACACAAGAGAAAGGATGGTCGATTCAGTAATTTCTTGAACCGAGCAATCTCTGCCTTTA   2760

CCTGTTTCTGAGCACTCCCGCCCTTTCGAGCATAAGGGCTCTTAGCGAAGCGATAGCCCAG  2820

CGACCACGATTATTCTGAGGTGATTTGAACTTGCGGCCGCCATTTTCAATCGGAAGCCTG   2880

CGAAAGCAAGGATCCCAGACTGCAG                                      2905
                    PstI
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FIG. 1. Nucleotide sequence of the *S. sanguis* 12 *ssaB* gene and adjacent DNA regions. The DNA strand is listed 5′ to 3′, and nucleotides are numbered to the right of each lane. Translations for the *ssaB* gene and for the 20-kDa protein begin at nucleotides 1049 and 2130 and end at nucleotides 1975 and 2618, respectively. The potential signal peptide for the SsaB protein is underlined. The asterisks mark the identical N-terminal sequences obtained by Edman degradation for a tryptic peptide of SsaB and the 20-kDa protein; a question mark means that the amino acid determination was ambiguous at the indicated residue. In the DNA 5′ to the SsaB- and 20-kDa protein-coding regions, potential RBS and −10 and −35 consensus sequences of two potential promoter regions are indicated. The *Pst*I restriction site in the 2.9-kb fragment is indicated. The proposed Rho-independent terminator is indicated by arrows centered at 2641. GenBank accession number is listed in the text.

TABLE 1. Amino acid compositions of the 20-kDa, SsaB, and type 1 fimbrial proteins

Residue	No. of residues in protein[a]				
	20-kDa		SsaB		Type 1 fimbrial protein (deduced)[b]
	Deduced	Determined	Deduced	Determined	
Ala	16	19	22	26	27
Cys	2	2	3	1	2
Asp	15	27[c]	22	52[c]	17
Glu	7	15[d]	27	35[d]	25
Asn	9	—	21	—	18
Gln	5	—	6	—	9
Phe	8	9	9	8	11
Gly	8	10	15	19	19
His	3	3	4	4	3
Ile	8	9	23	20	22
Lys	8	8	39	31	40
Leu	16	19	25	23	26
Met	2	2	5	5	5
Pro	4	5	12	16	12
Arg	4	5	3	6	2
Ser	10	12	22	23	20
Thr	16	18	16	15	15
Val	15	17	18	20	19
Trp	2	2	3	—	4
Tyr	5	5	14	14	13

[a] —, Not determined.
[b] Composition deduced from sequence in reference 6.
[c] Total amino acid compositions for both Asp and Asn.
[d] Total amino acid compositions for both Glu and Gln.

The 2.9-kb DNA sequence was compared with other DNA sequences in the GenBank and EMBL DNA data base libraries. The only DNA sequence which showed a high degree of homology was the 1.6-kb DNA sequence containing the type 1 fimbrial gene from *S. sanguis* FW213 (6). Comparison of the two sequences was made by using the BestFit program of the University of Wisconsin Genetics Computer Group software package (Fig. 2). The corresponding regions of the 1.6-kb fragment of *S. sanguis* FW213 and the 2.9-kb fragment from *S. sanguis* 12 are 73% identical. The DNA sequence of the type 1 fimbrial gene shows differences at the 5′- and 3′-flanking regions when compared with the corresponding *ssaB* gene from *S. sanguis* 12. These differences are designated by circled numbers from 1 to 4 (Fig. 2). Region 1 is 12 nucleotides and is present in the 5′ region of the type 1 fimbrial gene. Regions 2, 3, and 4 are located near the 3′ end of the type 1 fimbrial gene, are 26, 42, and 7 nucleotides long, respectively, and are located coincidentally near the 5′ end of the gene coding for the 20-kDa protein. DNA homology is also seen in the region coding for the 20-kDa protein, although the DNA sequence reported by Fenno et al. (6) does not include the complete gene for the 20-kDa peptide (Fig. 2).

Amino acid sequence comparison between SsaB and the type 1 fimbrial protein shows that they are 87% identical (Fig. 3). The major difference between *ssaB* and the type 1 fimbrial gene lies in the signal sequence. The molecular weight of the unprocessed type 1 fimbrial peptide is almost identical (M_r, 34,349) to that of the unprocessed SsaB protein (M_r, 34,684). A comparison between the deduced amino acid composition of type 1 fimbrial protein and that of the SsaB protein is shown in Table 1. These data indicate that these two molecules are very similar.

Antigenic relationship between SsaB adhesin and a coaggregation-mediating adhesin. A 38,000-M_r streptococcal surface protein was identified by Kolenbrander and Andersen (16) and was proposed to mediate coaggregation between *S. gordonii* PK488 and *A. naeslundii* PK606. Since the molecular weight of this adhesin was similar to the molecular weight of the SsaB protein involved in binding to saliva, a Western blot analysis was done to see whether these two proteins were immunologically cross-reactive (Fig. 4). Sonic lysates of *S. gordonii* PK488 and its coaggregation-defective mutant PK1804, which does not coaggregate with *A. naeslundii* PK606, were compared with sonic lysates of *S. sanguis* 12 and its non-saliva-aggregating variant 12na. Proteins separated by SDS-PAGE were Western blotted and probed with IgG prepared from either anti-SsaB serum or anti-PK488 serum absorbed with PK1804 cells. The Western blots clearly show that SsaB from *S. sanguis* 12 (Fig. 4B and C, lanes 4) and the 38,000-M_r protein from *S. gordonii* PK488 (Fig. 4B and C, lanes 2) are indistinguishable in size and antigenicity.

Although strain 12na specifically has lost the ability to bind to neuramindase-treated S-HA, it maintains the ability to bind to pH-sensitive receptors on S-HA, mediated by the SsaB adhesin (28), it coaggregates with *A. naeslundii* PK606, and it has the SsaB protein (Fig. 4B and C, lanes 5). The apparent lower immunoreactivity of the SsaB protein in lanes 5 (Fig. 4B and C) may be due to a smaller amount of protein applied (Fig. 4A, lane 5). Strain PK1804 (Fig. 4, lanes 3) is unable to coaggregate with *A. naeslundii* PK606 but appears to have a small amount of immunoreactive protein (Fig. 4B and C, lanes 3). The mutant may be producing small amounts of nonfunctional (in coaggregation), immunoreactive protein. Alternatively, the mutant could be producing a small amount of functionally active protein that remains cytoplasmic.

Coaggregation assays were done to compare the coaggregation properties of *S. sanguis* 12 and 12na with those of *S. gordonii* PK488, whose only actinomyces coaggregation partner is *A. naeslundii* PK606 (16). Strains 12 and 12na coaggregated only with *A. naeslundii* strains PK947 and PK606, which represent actinomyces coaggregation groups C and D, respectively (15). Therefore, *S. sanguis* 12 and 12na exhibit coaggregation properties similar to those of *S. gordonii* PK488, which is the reference strain of streptococcal coaggregation group 6.

Further evidence that these two proteins are similar was obtained by treating sonic lysates of strains 12 and PK488 with trypsin. The digested material was separated by SDS-PAGE, and the proteins were Western blotted and probed with IgG prepared from either anti-SsaB serum or anti-PK488 serum absorbed with PK1804 cells. Results obtained indicate that the trypsin-treated proteins from both streptococci (Fig. 5B and C, lanes 2 and 4) migrated slightly faster than untreated proteins (Fig. 5B and C, lanes 3 and 5). Thus, trypsin cleavage results in the formation of polypeptides of similar molecular weight which are reactive with both antisera (Fig. 5). These data suggest that the SsaB protein and the 38,000-M_r protein, in addition to their immunological similarity, exhibit a structural relatedness.

DISCUSSION

The nucleotide sequence of *ssaB* shows a striking homology with that of a functionally similar fimbrial gene from *S. sanguis* FW213 (6). Both proteins bind to a component present in whole saliva, although the nature of this receptor is not yet known. The homology of the SsaB protein and the type 1 fimbrial protein raises the question of whether the

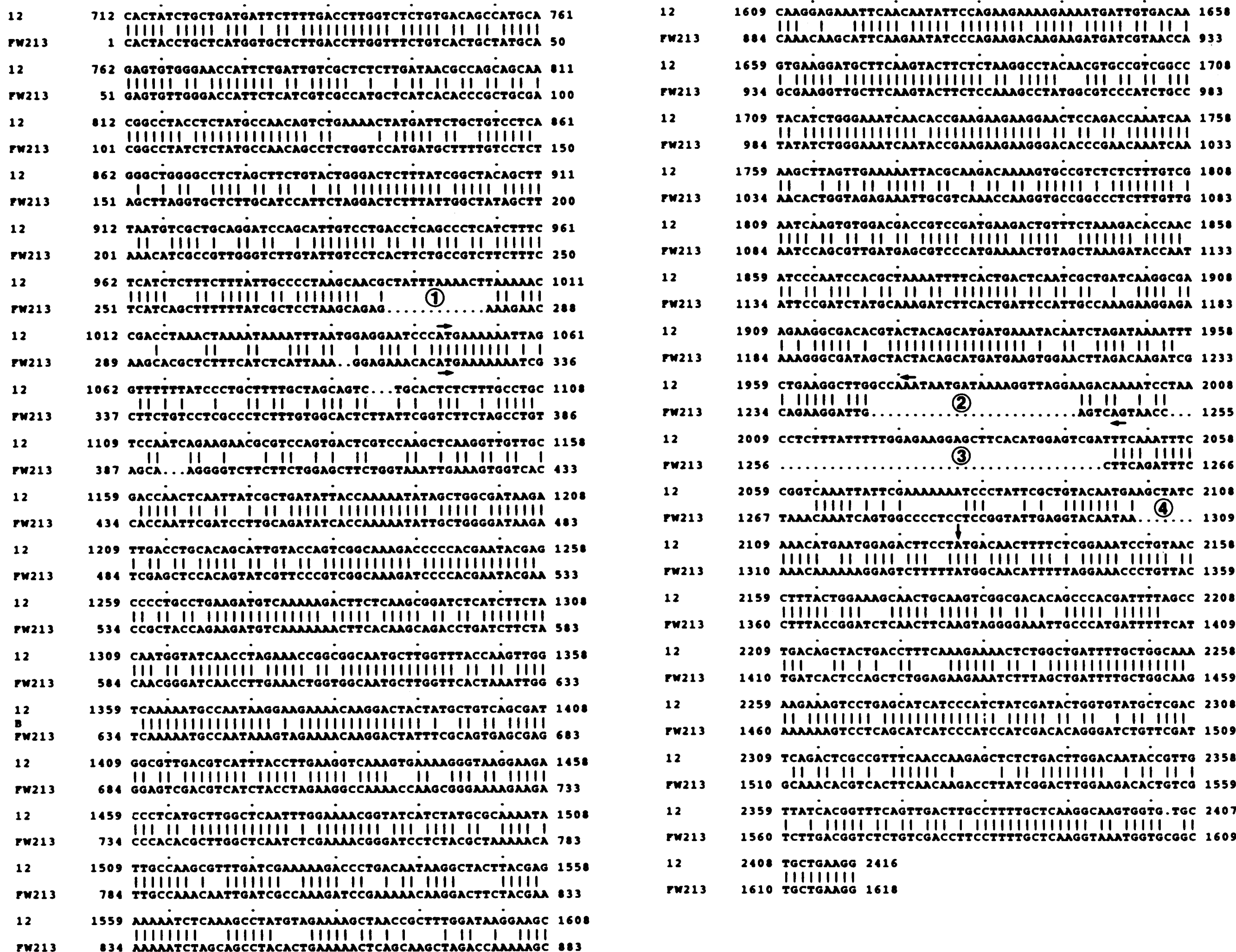

FIG. 2. DNA sequence homology of the type 1 fimbrial gene from *S. sanguis* FW213 and *S. sanguis* 12 DNA containing the *ssaB* gene. DNA sequences were aligned by using the program BestFit (University of Wisconsin Genetics Computer Group) (gap weight, 5; gap length weight, 0.3). Identical (|) nucleotide bases are indicated. The four regions discussed in the text are indicated by numbers within circles. The DNA sequences coding for SsaB and type 1 fimbrial protein are indicated by horizontal arrows → ←. The vertical arrow (↓) indicates the translation initiation of the 20-kDa protein in *S. sanguis* 12.

SsaB protein is a fimbrial component. Antisera raised against SsaB and fibrils (surface appendages without a measurable width) from *S. sanguis* 12 do not cross-react with fibrils or SsaB, respectively (9), which suggests that the SsaB protein is not part of fibrils. We have examined the arrangement of the SsaB protein on the surface of *S. sanguis* 12. Chemical cross-linking studies have shown that SsaB only forms dimers and trimers, indicating that SsaB is not part of a larger, organized, structural unit such as fibrils (8a).

The *S. sanguis* FW213 type 1 fimbrial gene product was identified by using absorbed (with an adhesion-negative mutant) adhesion-blocking fimbriae-specific antiserum (8), as well as polyclonal antisera raised against *S. sanguis* FW213 (6). It is possible that these antisera may have identified the adhesin rather than the fimbrial monomer. In gram-negative bacteria, adhesins are usually minor components distinct from the fimbrial subunit (19, 23). Therefore, the published DNA sequence of *S. sanguis* FW213 type 1 fimbrial gene may not be that of a fimbrial subunit monomer but in fact that of an adhesin. This notion is supported by the observations that neither streptococcal type 1 fimbrial gene nor *ssaB* has a hydrophobic membrane anchor sequence on its carboxy-terminal region, as seen with other fimbrial subunits (35, 36).

If the structural organization of FW213 fimbriae resembles that of *E. coli* fimbriae, then the gene encoding the fimbrial subunit could be part of a fimbrial operon (14, 30). Therefore, some of the gene(s) involved in fimbrial synthesis or assembly could be present in the 6-kb fragment cloned by Fives-Taylor et al. (8), and it is also possible that the 20-kDa protein identified in *S. sanguis* 12 may be involved in one of these yet undetermined functions.

Fenno et al. (6) found that the level of expression of the type 1 fimbriae in *E. coli* was low. The difference observed in the 5′ region of the type 1 fimbrial gene when compared with the *S. sanguis* 12 *ssaB* gene may very well account for this poor expression in *E. coli*. The reasons for this difference are unknown. These differences could have been the result of

```
                 .         .         .         .         .
  1 MKKLG.FLSLLLLAVCTLFACSNQKNASSDSSKLKVVATNSIIADITKNI SsaB
    |||:: .|.|::  : .|:|||.. ..|:.|:|||||.||||:|||||||
  1 MKKIASVLALFVALLFGLLACSKG.SSSGASGKLKVVTTNSILADITKNI type 1

                 .         .         .         .         .
 50 AGDKIDLHSIVPVGKDPHEYEPLPEDVKKTSQADLIFYNGINLETGGNAW SsaB
    |||||:||||||||||||||||||||||||||||||||||||||||||||
 50 AGDKIELHSIVPVGKDPHEYEPLPEDVKKTSQADLIFYNGINLETGGNAW type 1

                 .         .         .         .         .
100 FTKLVKNANKEENKDYYAVSDGVDVIYLEGQSEKGKEDPHAWLNLENGII SsaB
    |||||||||| |||||:|||:||||||||||.: |||||||||||||||:
100 FTKLVKNANKVENKDYFAVSEGVDVIYLEGQNQAGKEDPHAWLNLENGIL type 1

                 .         .         .         .         .
150 YAQNIAKRLIEKDPDNKATYEKNLKAYVEKLTALDKEAKEKFNNIPEEKK SsaB
    ||.||||.||.|||.||. ||||| ||.|||. ||..||: |.||||:||
150 YAKNIAKQLIAKDPKNKDFYEKNLAAYTEKLSKLDQKAKQAFKNIPEDKK type 1

                 .         .         .         .         .
200 MIVTSEGCFKYFSKAYNVPSAYIWEINTEEEGTPDQIKSLVEKLRKTKVP SsaB
    ||||||||||||||||.|||||||||||||||||:|||.||||||.||||
200 MIVTSEGCFKYFSKAYGVPSAYIWEINTEEEGTPEQIKTLVEKLRQTKVP type 1

                 .         .         .         .         .
250 SLFVESSVDDRPMKTVSKDTNIPIHAKIFTDSIADQGEEGDTYYSMMKYN SsaB
    .||||||||:||||||.|||||||.|||||||||.:||.||.||||||:|
250 ALFVESSVDERPMKTVAKDTNIPIYAKIFTDSIAKEGEKGDSYYSMMKWN type 1

                 .
300 LDKISEGLAK SsaB
    ||||.|||..
300 LDKIAEGLSQ type 1
```

FIG. 3. Amino acid homology between the SsaB protein (upper sequence) and the type 1 fimbrial protein (lower sequence). The predicted amino acid sequences of the two proteins were aligned by using the program BestFit (University of Wisconsin Genetics Computer Group) (gap weight, 3.0; gap length, 0.1). Identical (|) and conserved (:) amino acid residues are indicated.

either deletions in the FW213 genome or insertions in the strain 12 chromosome. It would be interesting to compare the nucleotide sequences in the flanking regions in other related streptococcal strains such as *S. gordonii* PK488, which show antigenically similar, similar-molecular-weight proteins.

The only other streptococcal adhesin of similar molecular weight identified so far is the 38,000-M_r protein from *S. gordonii* PK488 (16). The observation that the SsaB adhesin is also antigenically related to the *S. gordonii* PK488 adhesin was unexpected. Their immunological cross-reactivity raises the possibility that the adhesin may be a bifunctional protein and thus mediates coaggregation with *A. naeslundii* PK606 as well as recognizes salivary receptors. This proposed bifunctional property of the adhesins may be a unique mechanism for bacterial adherence. For example, these adhesins may be useful in recognizing salivary molecules in the acquired pellicle on a freshly cleaned tooth surface and may also be used as a mechanism for accretion of actinomyces in the developing plaque.

The nature of the receptors for these adhesins on the surface of actinomyces or in saliva is unknown. The salivary receptor may be derived from the host. Alternatively, since saliva-binding studies are conducted with clarified whole saliva and since it is known that whole saliva contains some bacterial debris, the receptor may be a bacterial component shed into the saliva. Purification of the receptors from saliva and *A. naeslundii* will be required to test the potential bifunctionality of the SsaB protein and the 38,000-Da protein from *S. gordonii* PK488.

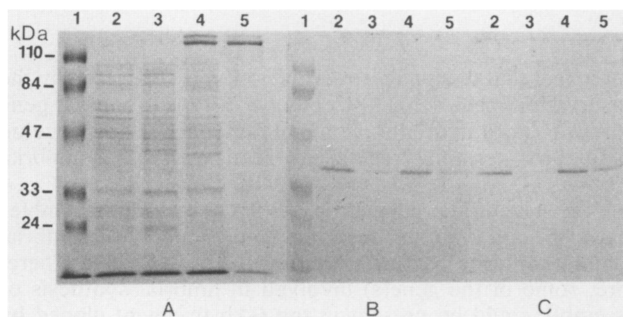

FIG. 4. Western blots of sonic cell lysates of *S. gordonii* and *S. sanguis* were probed with IgG prepared from either anti-SsaB serum or anti-PK488 serum absorbed with PK1804 cells. (A) Gel stained with Coomassie brilliant blue; (B) Western blot of gel reacted with anti-SsaB IgG; (C) Western blot of gel reacted with anti-PK488 absorbed IgG. Lanes 1, Molecular mass markers; lanes 2, *S. gordonii* PK488; lanes 3, *S. gordonii* PK1804 (coaggregation-defective mutant of strain PK488); lanes 4, *S. sanguis* 12; and lanes 5, *S. sanguis* 12na (non-saliva-aggregating variant of strain 12). Prestained molecular mass markers are indicated at the left.

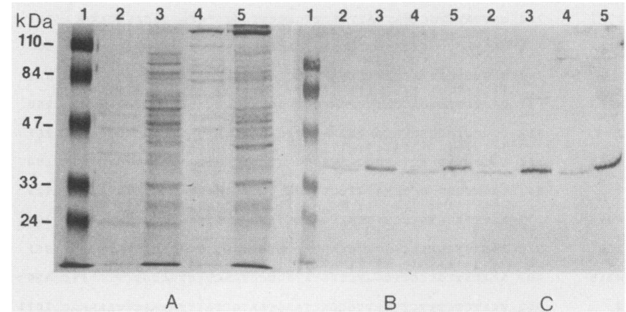

FIG. 5. Western blots of trypsin-digested sonic cell lysates of *S. gordonii* and *S. sanguis* were probed with IgG prepared from either anti-SsaB serum or anti-PK488 serum absorbed with PK1804 cells. (A) Gel stained with Coomassie brilliant blue; (B) Western blot of gel reacted with anti-SsaB IgG; (C) Western blot of gel reacted with anti-PK488 absorbed IgG. Lanes 1, Molecular weight markers; lanes 2, *S. gordonii* PK488 digested with trypsin; lanes 3, *S. gordonii* PK488 (undigested); lanes 4, *S. sanguis* 12 digested with trypsin; and lanes 5, *S. sanguis* 12 (undigested). Prestained molecular mass markers are indicated at the left.

ACKNOWLEDGMENTS

We thank Jacob Donkersloot, Christopher Hughes, Douglas Brown, and Jack London for critical review of the manuscript. Technical assistance of Roxanna Andersen, Carl-Alfred Alpert, and Kirsten Vadheim is gratefully appreciated.

This work was supported in part by a grant from the Medical Research Council of Canada.

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