

Identification of a *Streptococcus gordonii* SspB Domain That Mediates Adhesion to *Porphyromonas gingivalis*

WANDA BROOKS,¹ DONALD R. DEMUTH,¹ SUCHEOL GIL,² AND RICHARD J. LAMONT^{2*}

Department of Biochemistry, School of Dental Medicine, University of Pennsylvania, Philadelphia, Pennsylvania 19104,¹ and Department of Oral Biology, University of Washington, Seattle, Washington 98195²

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Porphyromonas gingivalis, a primary pathogen in adult periodontitis, may establish itself in the oral cavity by adhering to early plaque bacteria such as *Streptococcus gordonii*. Our previous studies (R. J. Lamont et al., *Microbiology* 140:867–872, 1994) suggested that this interaction is mediated by the SspB polypeptide, a member of the antigen I/II family of streptococcal surface proteins. *S. gordonii* was recently shown to express a second Ssp polypeptide (SspA) that resembles SspB and the structurally homologous antigen I/II polypeptide (Pac) of *Streptococcus mutans*. To determine if all of these related antigen I/II proteins interacted with *P. gingivalis*, SspA, SspB, and Pac were tested for adhesion to *P. gingivalis* cells. Both of the *S. gordonii* Ssp proteins bound labeled target cells, whereas the *S. mutans* Pac polypeptide did not, suggesting that antigen I/II-mediated binding of *P. gingivalis* by streptococci may be species specific. To investigate the molecular basis for this functional difference, the *P. gingivalis* binding domain of SspB was mapped. The binding properties of a family of truncated SspB polypeptides lacking C-terminal sequences were determined. In addition, the lack of binding activity exhibited by the Pac protein was exploited to construct and analyze chimeric SspB-Pac polypeptides. Both approaches revealed that the region defined by residues 1167 to 1250 of SspB was essential for *P. gingivalis* binding. This region of SspA and SspB is entirely conserved, consistent with the binding properties determined for these proteins. However, the corresponding region of Pac differs in both the primary sequence and predicted secondary structure, suggesting that the overall structure of this domain may define its functional activity.

Periodontal diseases are a group of chronic inflammatory infections that can cause tissue destruction and loss of the alveolar bone, leading to exfoliation of teeth (17, 27). Although a variety of bacteria are associated with the initiation and progression of these diseases, *Porphyromonas gingivalis*, a gram-negative anaerobic rod, is considered a primary pathogen in severe forms of adult periodontitis (19, 27). *P. gingivalis* possesses a vast array of potential virulence factors that can impinge upon host tissue integrity and immune function (1a). In order to first colonize the oral cavity, *P. gingivalis* may adhere to a variety of oral surfaces, including epithelial cells, the salivary pellicle coating the tooth surfaces, or antecedent bacteria that comprise dental plaque (1a, 14, 19). For example, in vitro studies have demonstrated that *P. gingivalis* adheres to *Actinomyces naeslundii* and oral streptococci of the sanguis group (e.g., *Streptococcus gordonii*) (7, 14). Both of these organisms are major constituents of early dental plaque (20). In addition, in vivo studies documented preferential colonization of preformed early plaque by *P. gingivalis* after introduction of *P. gingivalis* cells into the mouths of human volunteers (26), suggesting that this interaction is important for the establishment of the *P. gingivalis* population in the oral cavity.

The interaction between *P. gingivalis* and *S. gordonii* is multimodal and may involve several polypeptides on the *P. gingivalis* surface, including the fimbrial subunit, FimA, and an outer membrane protein of 35 kDa (12, 15). In addition, other studies have suggested that proteases expressed by *P. gingivalis* may play a role in the colonization process (16, 22). With regard to the streptococcal adhesins, Lamont et al. (13) identified two *S. gordonii* polypeptides of 62 and 45 kDa that bound

to *P. gingivalis* cells. These polypeptides were subsequently shown to be derived from a 200-kDa precursor, the SspB protein of *S. gordonii* (13). Expression of the *sspB* gene in *Enterococcus faecalis* resulted in a transformed cell that was capable of binding to *P. gingivalis* and which produced the 62-, 45-, and full-length 200-kDa polypeptides (13).

The SspB polypeptide is a major surface protein of *S. gordonii* and is a member of the antigen I/II (24) family of cell surface proteins that are expressed by virtually all streptococci that inhabit the human oral cavity (18). The SspB protein is 1,500 residues in length and contains the seven structural domains that are conserved in all members of the antigen I/II polypeptide family (10). They are, from the N terminus, (i) signal peptide (residues 1 to 38), (ii) N-terminal region (residues 39 to 163), (iii) HR (helical repeat) domain (residues 164 to 471), (iv) divergent central region (residues 472 to 770), (v) proline-rich repeats (residues 771 to 887), (vi) C-terminal region (residues 888 to 1413), and (vii) cell wall anchoring sequences (residues 1414 to 1500). Several studies suggest that SspB is a multifunctional polypeptide. Demuth et al. (2, 4) showed that SspB binds to a mucin-like salivary glycoprotein (SAG) in a calcium-dependent reaction. This interaction has been suggested to be important in the adherence of *S. gordonii* cells to oral tissue surfaces. Duan et al. (6) subsequently showed that the SspB polypeptide itself binds calcium with high affinity. In addition, mutant *S. gordonii* cells, in which the *sspB* gene was insertionally inactivated, exhibit significantly reduced adherence to *A. naeslundii* (3), suggesting that the SspB protein may also participate in the multimodal interbacterial interactions that occur between *S. gordonii* and *A. naeslundii*. Recently, *S. gordonii* has been shown to express a second Ssp protein, SspA, that is highly similar to SspB with respect to both structure and function (3). Thus, the SspA and SspB polypeptides appear to play a role in many of the adherence properties of *S. gordonii*. However, the functional do-

* Corresponding author. Mailing address: Department of Oral Biology, University of Washington, Box 357132, Seattle, WA 98195. Phone: (206) 543-5477. Fax: (206) 685-3162. E-mail: lamont@u.washington.edu.

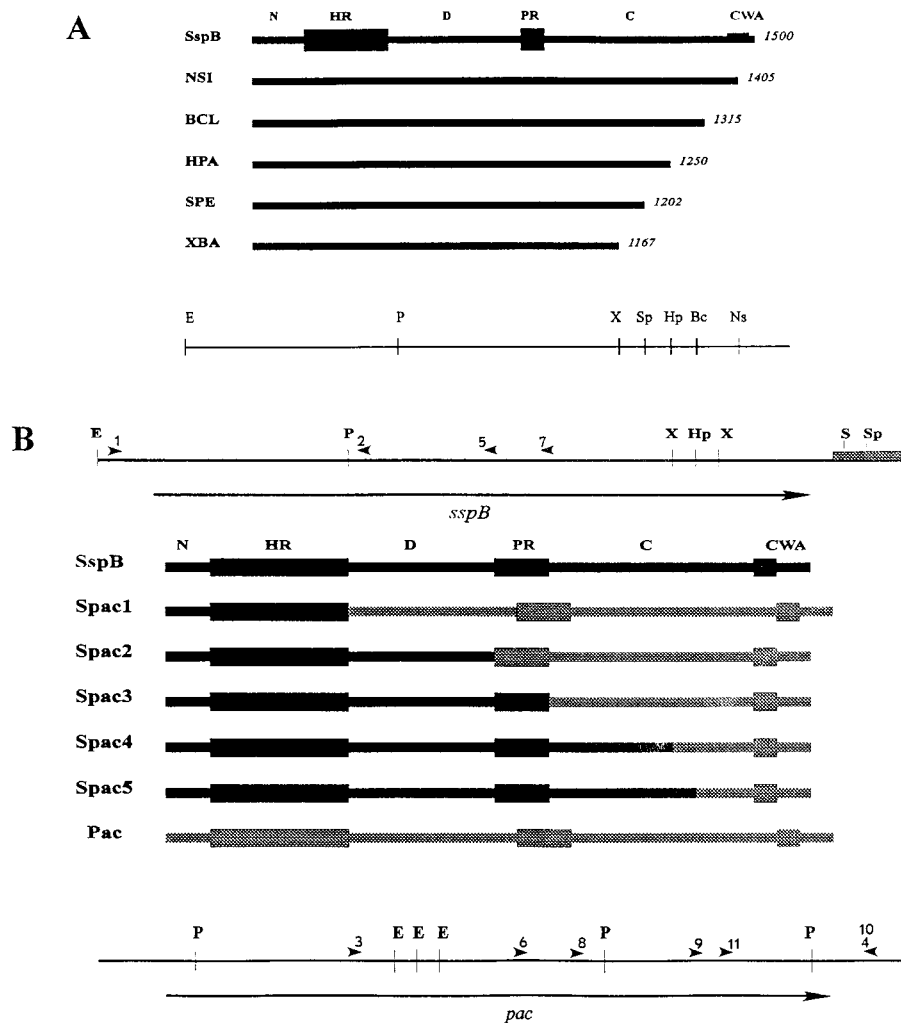


FIG. 1. (A) Schematic representation of the truncated SspB polypeptides. A family of truncated SspB polypeptides was constructed by digestion of the *sspB* gene at the unique restriction sites shown. The resulting constructs were expressed in *E. coli*, and the truncated proteins were purified as described in Materials and Methods. The six structural domains of SspB are indicated for the full-length protein: N, N terminus; HR, helical repeat; D, divergent region; PR, proline-rich repeat; C, C terminus; and CWA, cell wall anchoring sequences. The number of amino acid residues in each of the truncated polypeptides is indicated on the right. Abbreviations for restriction enzymes: Bc, *BclI*; E, *EcoRI*; Hp, *HpaI*; Ns, *NsiI*; P, *PstI*; Sp, *SphI*; X, *XbaI*. (B) Construction of chimeric Spac proteins. SspB protein sequence is shown in black; Pac protein sequence is shown in grey. The six structural domains of SspB are as described above and are conserved in the Pac polypeptide. Chimeric genes encoding the Spac polypeptides were constructed by ligating PCR-amplified *sspB* and *pac* gene fragments encoding the appropriate domains of the corresponding proteins (see text). The annealing sites of the oligonucleotide primers 1 to 11 used for the PCR amplifications are shown on the restriction maps of the *sspB* and *pac* genes. Oligonucleotides 4 and 10 anneal to the same sequence of *pac* but specify different restriction enzyme sites used for cloning the amplified fragments (see text). Abbreviations for restriction enzymes: E, *EcoRI*; Hp, *HpaI*; P, *PstI*; S, *SalI*; Sp, *SphI*; X, *XbaI*. The *SalI* and *SphI* sites are located in the vector sequence (shown by the cross-hatched area in the *sspB* restriction map) of plasmid pEB5 (2).

mains of these polypeptides that mediate their interactions with SAG and with other bacteria have not yet been precisely mapped.

In this study, we show that both SspA and SspB bind to *P. gingivalis*, but the related Pac polypeptide from *Streptococcus mutans* does not. In addition, analysis of a series of C-terminal deletion mutants of SspB and chimeric polypeptides, comprised of defined regions of SspB fused to Pac sequences, showed that the region of SspB spanning residues 1167 to 1250 is important for its interaction with *P. gingivalis*. Consistent with their binding activities, this region of SspB is completely conserved in the SspA protein but differs in primary sequence and predicted secondary structure from the corresponding region of the Pac polypeptide. These results suggest that the antigen I/II-mediated interactions of oral streptococci with *P.*

gingivalis may be species specific and that the properties and structure of the 1167–1250 region may contribute to this specificity.

MATERIALS AND METHODS

Bacteria and culture conditions. *Escherichia coli* cultures were grown from frozen stocks in Luria-Bertani broth (10 g of tryptone, 5 g of yeast extract, and 5 g of sodium chloride per liter) at 37°C with shaking. When necessary, the broth was supplemented with 100 µg of ampicillin (Sigma) per ml. *P. gingivalis* 33277 was grown from frozen stocks in Trypticase soy broth supplemented with 1 g of yeast extract, 5 mg of hemin, and 1 mg of menadione per liter. *P. gingivalis* was incubated at 37°C under anaerobic conditions of 85% N₂, 10% H₂, and 5% CO₂, and bacterial numbers were determined with a Klett-Summerson photometer. When necessary, *P. gingivalis* was metabolically labeled by including [³H]thymidine (10 µCi/ml) in the culture medium.

Construction of SspB C-terminal deletion mutants. A family of SspB deletion mutants (Fig. 1A) was previously constructed for the analysis of the calcium

binding properties of the SspB polypeptide (6). For the present study, an additional deletion construct was synthesized by digesting plasmid pEB5 (2) with *EcoRI* and *SpeI*. This digestion produced a 4,142-bp band which was purified from low-melting-point agarose (Sigma) by standard procedures (25), extracted once with phenol and once with chloroform, and precipitated with ethanol. The purified fragment was ligated to *EcoRI/SpeI*-digested pUC19 and transformed into *E. coli* DH5 α (8). This clone encoded a 1,212-residue polypeptide designated SPE. Proteins encoded by all of the SspB deletion clones were secreted into the periplasmic space of *E. coli*.

Construction of *spac* genes. A family of chimeric *sspB-pac* genes, designated *spac* genes, was constructed to express hybrid SspB-Pac (Spac) polypeptides (Fig. 1B) in order to further define the SspB domains involved in binding *P. gingivalis*. The general strategy for construction of each of the *spac* genes is described below for *spac1*. Gene fragments encoding the desired portions of the SspB and Pac proteins were amplified by PCR. The amplification reaction conditions consisted of 30 cycles of 94°C denaturation for 1 min, 55°C annealing for 3 min, and 72°C extension for 3 min, followed by a final 72°C extension for 9 min. For the construction of *spac1*, an *sspB* gene fragment containing the *sspB* promoter and encoding the signal peptide, N terminus, and HR repeat domain of the SspB polypeptide (residues 1 to 482) was amplified from *S. gordonii* genomic DNA by using primers 1 (CCGAATTCTAGTAGGCATA) and 2 (CCGGTACCATCTTGTTTCT). The resulting fragment of 1,963 bp was cleaved at the *EcoRI* and *KpnI* sites specified by the primers (underlined) and ligated into *EcoRI/KpnI*-cleaved pUC19 to generate clone pUCsp1. Subsequently, a gene fragment encoding the appropriate C-terminal portion of the Pac protein (residues 501 to 1565) was amplified from *S. mutans* KPSK2 genomic DNA by using primers 3 (CCGGTACCGAACCATTCTGCTC) and 4 (CCGTCGACAAGGCAGTGCGAAGT). The resulting fragment of 3,455 bp was cleaved with *KpnI* and *SalI* (underlined), ligated into *KpnI/SalI*-cleaved pUCsp1, and transformed into *E. coli* DH5 α . Expression of the chimeric polypeptide was verified by Western blotting of periplasmic extracts (see below). The *spac2* and *spac3* genes were constructed in a similar fashion. The *sspB* gene fragment used in the construction of *spac2* contained the *sspB* promoter and encoded residues 1 to 775 of the SspB polypeptide. It was amplified from *S. gordonii* M5 genomic DNA with primer 1 and primer 5 (CCGGTACCCCAATTGCATTAATGT). The corresponding region of *pac* (residues 828 to 1565) contained in *spac2* was amplified by using primer 4 and primer 6 (CCGGTACCTAAAGTTACTAAGG). The discrepancy in the numbering of the SspB and Pac peptide fragments arises from the larger divergent domain found in the Pac polypeptide (4) (Fig. 1B).

The *sspB* gene fragment used in the construction of *spac3* contained the *sspB* promoter and encoded residues 1 to 935 of the SspB polypeptide. It was amplified from *S. gordonii* M5 genomic DNA with primer 1 and primer 7 (CCGGTACCATCCTCATTCTT). The corresponding region of *pac* (residues 1014 to 1565) contained in *spac3* was amplified by using primer 4 and primer 8 (CCGGTACCAATATTGACAGAAGT). The stepwise construction of *spac2* and *spac3* was carried out as described for the construction of *spac1*.

The *spac4* and *spac5* genes were constructed by using a different strategy. The *sspB* gene fragments contained in *spac4* and *spac5* were generated by digesting plasmid pEB5 (2) with *XbaI* and *SphI* for *spac4* or with *HpaI* and *SalI* for *spac5* (Fig. 1B). These digests remove the 3' end of *sspB*, generating truncated *sspB* genes contained in pUC19 that code for residues 1 to 1168 (for *XbaI/SphI*) or residues 1 to 1250 (for *HpaI/SalI*) of the SspB protein. The gene fragments encoding the appropriate C-terminal Pac polypeptide sequences, i.e., residues 1241 to 1565 for *spac4* and residues 1406 to 1565 for *spac5*, were amplified by using oligonucleotide pairs 9 (CTCTAGAACGAGCCCTC)-10 (CCGCATGCAAGGCAGTGCGAAGT) and 4-11 (CCGATATCAATAATGTTCTT AAG), respectively. The amplified *pac* fragments were cleaved with the appropriate restriction enzymes at the sites contained in the primers (underlined in all sequences given above) and ligated to the truncated *sspB* gene fragments described above. Transformation into *E. coli* and verification of the expressed polypeptide products were as described above.

Purification of SspB deletion and Spac proteins. All polypeptides were expressed under the control of the *sspB* gene promoter and secreted into the *E. coli* periplasm. Crude periplasmic preparations were generated by osmotically shocking washed *E. coli* cells as described by Heppel (9). Further purification of the SspB polypeptides was carried out by chromatographing the crude periplasmic protein samples on Sepharose 6B (Pharmacia) and DEAE-Sephadex (Sigma Chemical Co.) as described by Demuth et al. (2). Purity was assessed by sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis.

Immunoblotting. Periplasmic preparations containing both the SspB deletion series and the Spac hybrid proteins were examined by immunoblotting to confirm the presence of an Ssp-derived protein of the appropriate size. Briefly, 2- μ g protein samples, as determined by a Micro BCA Protein Assay Reagent kit (Pierce, Rockford, Ill.), were separated by SDS-polyacrylamide gel electrophoresis and electroblotted onto nitrocellulose. Filters were blocked with phosphate-buffered saline (PBS; 0.15 M, pH 7.2) containing 0.1% Tween 20 (PBS-Tween) as previously described (13) and reacted at room temperature for 1 h with rabbit anti-SspB antibody (1:5,000 dilution) followed by goat anti-rabbit horseradish peroxidase conjugate (1:3,000 dilution; Bio-Rad). The antigen-antibody complexes were visualized with the chromogenic substrate 3,3'-diaminobenzidine tetrahydrochloride.

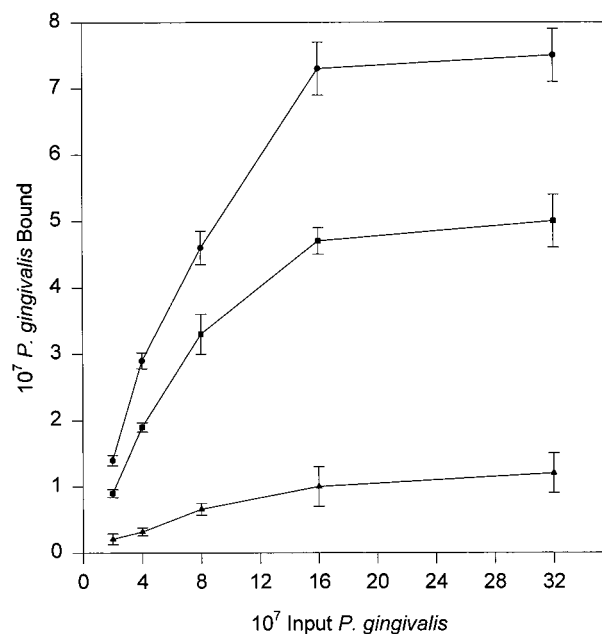


FIG. 2. Binding of *P. gingivalis* cells to SspA (●), SspB (■), and Pac (▲) polypeptides. Samples of purified SspA, SspB, and Pac protein preparations were immobilized onto nitrocellulose paper and incubated with ³H-labeled *P. gingivalis* cells for 1 h. After washing, bound cells were quantitated by scintillation spectroscopy. Error bars represent standard deviation ($n = 3$).

Biotinylation of *P. gingivalis* cells. *P. gingivalis* cells were surface labeled with biotin as described previously (15). Briefly, washed *P. gingivalis* cells were suspended in 0.1 M NaHCO₃ (pH 8.1) and reacted with *N*-hydroxysuccinimidobiotin (3 mg/10¹⁰ cells) for 3 h at room temperature. Bacteria were recovered by centrifugation (10,000 × *g*, 10 min), washed twice, and suspended in PBS.

Binding of *P. gingivalis* to SspB polypeptides. Adherence of *P. gingivalis* to the SspB C-terminal deletions or the Spac hybrid polypeptides was examined by two procedures: a quantitative dot blot assay and an overlay blot assay. In the dot blot assay, periplasmic protein preparations were deposited under vacuum onto nitrocellulose paper in a dot blot apparatus. The membrane was subsequently blocked with PBS-Tween for 1 h and reacted with [³H]thymidine-labeled *P. gingivalis* cells (10⁸) at room temperature for 2 h with shaking. The blot was washed four times with PBS-Tween and dried, and binding of *P. gingivalis* was quantitated by scintillation spectroscopy (Packard Tri-Carb 2200CA). Controls of *P. gingivalis* with periplasmic proteins from nontransformed *E. coli* were included in all experiments. All assays were run in triplicate. In the overlay blot procedure, periplasmic protein preparations were electrophoresed on SDS-10% polyacrylamide gels and transferred to nitrocellulose. Following electroblotting, the nitrocellulose membrane was washed once with PBS containing 0.2% Triton X-100 and once with PBS-Tween for 1 h at room temperature to facilitate renaturation of the proteins and to block excess protein binding capacity of the membrane. Biotinylated whole *P. gingivalis* cells (10⁸) in PBS-Tween were reacted with the blotted proteins for 1 h at room temperature. The blots were then washed four times with PBS-Tween. *P. gingivalis* binding was detected by using streptavidin-horseradish peroxidase (1:30,000 dilution; Bio-Rad) with 3,3'-diaminobenzidine tetrahydrochloride as the substrate.

RESULTS

Interaction of *P. gingivalis* with *S. gordonii* SspA and SspB and *S. mutans* Pac polypeptides. To determine the adhesion of SspA and the antigen I/II polypeptide of *S. mutans* to *P. gingivalis*, we compared the binding properties of the SspA, SspB, and Pac polypeptides. As shown in Fig. 2, both the SspA and SspB proteins bound *P. gingivalis* cells, whereas significantly lower binding was detected with the Pac protein of *S. mutans*. Similar results were obtained in binding studies using intact *S. gordonii* and *S. mutans* cells. Intact *S. gordonii* readily interacted with *P. gingivalis*, whereas the *S. mutans* strains that were tested exhibited 5- to 10-fold-lower binding than *S. gordonii* (not shown). It was observed that an equivalent amount of

SspA bound a greater number of *P. gingivalis* cells than did SspB. However, the K_{diss} values for SspA and SspB, calculated by using a single-site binding isotherm and data fit via nonlinear least squares, were virtually identical (9.8 and 9.3 free *P. gingivalis* cells/ml). To ensure that equivalent amounts of SspA and SspB protein were being tested, the protein concentrations in solution were equalized before immobilization of the polypeptides onto nitrocellulose. In addition, the amounts of SspA and SspB that were bound to the nitrocellulose filter were compared by staining the filter with India ink. The protein spots were then quantified by scanning densitometry. No significant difference was found in the amounts of SspA and SspB polypeptide on the filter paper (not shown). These results indicate that the interaction of streptococcal antigen I/II proteins with *P. gingivalis* may be species specific. The nature of the quantitative differences between SspA and B is currently under investigation.

Interaction of *P. gingivalis* with C-terminal truncated SspB polypeptides. The SspB polypeptide was used to map the *P. gingivalis* binding site, since it has been more thoroughly characterized and a family of C-terminal truncated SspB proteins, the shortest of which comprised 1,167 amino acid residues (shown schematically in Fig. 1A), was available from our previous studies to map the calcium binding domain (6). For this study, an additional truncated peptide (SPE, 1,202 amino acids) was constructed and analyzed. To quantitate the binding of *P. gingivalis* to the truncated SspB polypeptides, 5- and 10- μg samples of the purified SspB proteins were immobilized onto nitrocellulose and reacted with ^3H -labeled *P. gingivalis* cells. As shown in Fig. 3, the intact SspB and the truncated polypeptides NSI, BCL, and HPA exhibited similar levels of binding, whereas the SPE protein showed at least 40% reduced binding. The XBA polypeptide did not bind to *P. gingivalis* cells at levels greater than those seen for a crude periplasmic protein preparation derived from *E. coli* DH5 α . These results suggest that the region of SspB residing between residues 1167

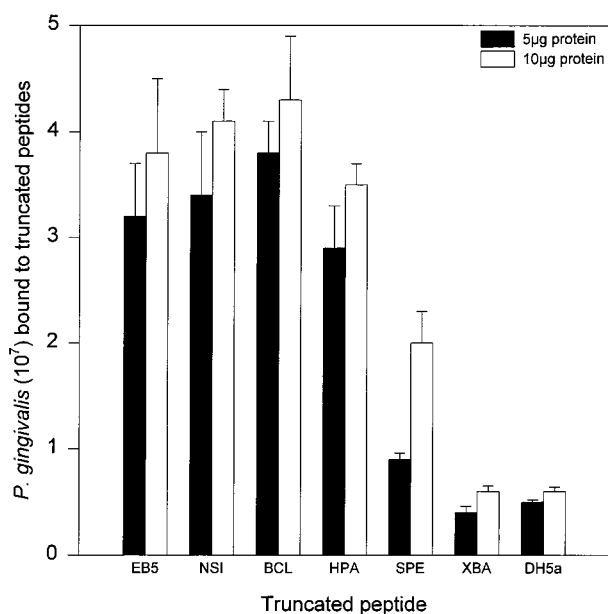


FIG. 3. Binding of *P. gingivalis* cells to truncated SspB polypeptides. ^3H -labeled *P. gingivalis* cells were incubated with 5- and 10- μg samples of the intact SspB protein (EB5) and the truncation constructs (NSI, BCL, HPA, SPE, and XBA). A crude periplasmic protein preparation derived from *E. coli* DH5 α served as the negative control. Error bars represent standard deviation ($n = 3$).

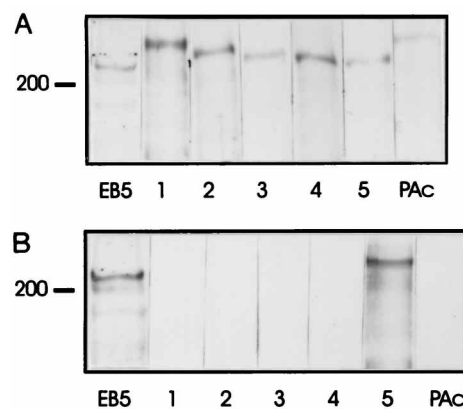


FIG. 4. (A) Western blot of SspB, Pac, and chimeric Spac polypeptides. Periplasmic protein preparations were derived from transformed *E. coli* cells expressing the intact *spsB* gene in plasmid pEB5 (EB5) or the *spac* chimeric gene constructs. Protein samples were electrophoresed in SDS-10% polyacrylamide gels, transferred to nitrocellulose, and reacted with polyclonal anti-SspB antibodies. Lanes: 1, Spac1; 2, Spac2; 3, Spac3; 4, Spac4; 5, Spac5. (B) Binding of biotin-labeled *P. gingivalis* cells to SspB, Pac, and the Spac polypeptides. Protein samples were prepared and electrophoresed as described above and incubated for 1 h with biotin-labeled *P. gingivalis* cells. Bound cells were detected by using streptavidin-horseradish peroxidase conjugate. Lane assignments are as for panel A.

and 1250 is important for the interaction of SspB with *P. gingivalis*.

Analysis of chimeric Spac polypeptides. Since it is possible that the truncated polypeptides are unable to assume the native conformation(s) required for binding *P. gingivalis*, we constructed a series of chimeric Spac polypeptides in order to determine the functional role of the 1167–1250 domain in the context of full-length proteins. The primary amino acid sequences and the overall structural organization of the SspB and Pac polypeptides are highly conserved (4), yet Pac interacts weakly, if at all, with *P. gingivalis* (see above). Five chimeric polypeptides (shown schematically in Fig. 1B) were expressed in *E. coli* and analyzed. As shown in Fig. 4A, Western blots of crude periplasmic protein preparations confirmed that full-length proteins were expressed from the chimeric genes. When the crude periplasmic preparations were incubated with biotin-labeled *P. gingivalis* cells, only SspB and the Spac5 hybrid polypeptide bound to *P. gingivalis* (Fig. 4B). No binding was seen with the chimeric polypeptides Spac1 to -4 or the Pac protein. As shown in Fig. 5, similar results were obtained in the dot blot assay. Binding of *P. gingivalis* cells by the hybrid constructs did not occur unless the chimeric protein contained the 1167–1250 region of the SspB polypeptide sequence (e.g., Spac5). Clearly, no binding activity was present in Spac4, which contained 1,166 residues of SspB fused to the appropriate C-terminal sequences of Pac. These results are consistent with those obtained with the truncated SspB polypeptides and support the previous conclusion that the region of SspB between residues 1167 and 1250 encodes a determinant that is essential for its interaction with *P. gingivalis*. Within this region, the primary amino acid sequences of the SspB and SspA polypeptides of *S. gordonii* are identical, consistent with their binding activities previously determined. In contrast, the SspB and *S. mutans* Pac sequences exhibit only 59% identity. In addition, comparison of predicted secondary structures (23) of the 1167–1250 region indicated that structural differences may exist within this region of SspB and Pac. For example, a predicted amphipathic α helix extending from residues 1245 to 1259 in Pac is terminated by a β turn at residues 1260 to 1265. How-

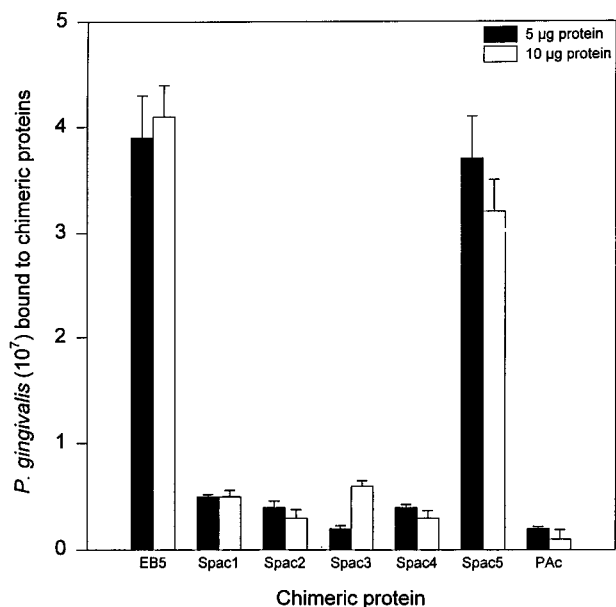


FIG. 5. Binding of *P. gingivalis* cells to chimeric Spac polypeptides. ³H-labeled *P. gingivalis* cells were incubated with the purified Spac proteins or SspB (EB5) and Pac as described in the text. Bound cells were quantitated by scintillation spectroscopy. Error bars represent standard deviation ($n = 3$).

ever, two of the three α -helix-breaking residues in this predicted turn (Gly¹²⁶⁰ and Pro¹²⁶³) are not conserved in SspB. As a result, no β turn is predicted to occur at this position in the SspB sequence. In addition, the predicted α helix of Pac exhibits a net charge of -1 , whereas the corresponding α helix of SspB (residues 1167 to 1181) exhibits a net charge of $+2$. This finding suggests that both the primary amino acid sequence and possibly the higher-order structure of this region may strongly influence its role in the interaction with *P. gingivalis* cells.

DISCUSSION

Adult periodontitis is a progressive, episodic disease that is associated with the overgrowth of *P. gingivalis* at the diseased site in the oral cavity (17, 27). One of the mechanisms that pathogenic organisms such as *P. gingivalis* may utilize to colonize oral tissues is to adhere to the preexisting biofilm of commensal organisms that exists on many oral surfaces (7, 14, 26). Indeed, *P. gingivalis* binds efficiently to *S. gordonii*, a non-pathogenic organism that is a major component of supragingival plaque, and the Ssp polypeptides appear to mediate this association (13).

In this study, we show that both the *S. gordonii* SspA and SspB proteins bind to *P. gingivalis*, whereas the highly related antigen I/II polypeptide (Pac) of *S. mutans* KPSK2 does not. The lack of binding activity exhibited by Pac (and by *S. mutans* cells) suggests that the binding of *P. gingivalis* by oral streptococci may be species specific, even though SspB and Pac exhibit a relatively high level of primary sequence similarity and the overall structural organization of these proteins is well conserved (4). However, functional specificity has previously been demonstrated for the SspB and Pac proteins in their interaction with SAG, a mucin-like human salivary glycoprotein (5). For example, both *S. gordonii* cells and purified SspB interact with sialic acid containing constituents of SAG, whereas the *S. mutans* (or Pac) interaction occurs indepen-

dently of sialic acid. The species specificity of these interactions, whether with *P. gingivalis* or SAG, is potentially very important in the development of the biofilm that exists on oral tissue surfaces and in the establishment of pathogenic organisms. For example, our results suggest that *P. gingivalis* may have a greater propensity to colonize dental plaque consisting mostly of streptococci expressing SspB-like polypeptides (e.g., *S. gordonii* and *S. sanguis*) rather than plaque comprised of streptococci expressing Pac-like proteins (*S. mutans*). Although numerous factors are involved in the onset of oral bacterial diseases, this observation may explain, in part, the clinical observation that the adult periodontal diseases caused by *P. gingivalis* do not usually occur simultaneously with coronal dental caries caused by *S. mutans* (11).

Functional analyses of a family of truncated SspB proteins and chimeric Spac polypeptides identified a discrete region of the SspB protein, delineated by residues 1167 and 1250, that is essential for its interaction with intact *P. gingivalis* cells. Consistent with the observed binding activities of SspA, SspB, and Pac, this region is entirely conserved within the SspB and SspA proteins of *S. gordonii* but exhibits only 59% sequence identity between SspB and the Pac polypeptide. Therefore, it is possible that the differences within the SspB and Pac primary amino acid sequences influence the overall structure and charge profile of this region (as described in Results), which in turn may explain why Pac does not interact with *P. gingivalis*. It is tempting to speculate that differences in both the physical properties and the overall structure of this region may influence its function in SspB and Pac. More detailed analyses of this region are being carried out to identify the specific secondary structures and residues that are required for this interaction.

SspB is a multifunctional polypeptide that binds calcium (6), interacts with SAG (2), and also binds to several other oral bacteria (3). The putative *P. gingivalis* binding region of SspB resides just downstream from sequences that are involved in its interaction with SAG (10) and overlaps with a region of SspB previously identified as being important for calcium binding (6). Thus, many of the functional domains of SspB appear to be clustered within a relatively small region of the SspB protein, between residues 900 and 1250. However, the interactions of SspB with *P. gingivalis* and SAG appear to be independent, since truncated SspB polypeptides that are nonreactive toward *P. gingivalis* (e.g., the XBA polypeptide) still bind SAG (1). In contrast, deletion of the 1167–1250 region results in the loss of both the *P. gingivalis* and calcium binding activities of SspB. The functional relationship of the *P. gingivalis* and calcium binding activities of SspB remains to be determined. One possible explanation for the loss of both activities in SspB is that the 1167–1250 region contains independent binding sites for *P. gingivalis* (not conserved in the Pac polypeptide) and calcium (conserved in Pac). This would explain our observations that both Spac4 (which contains 1,166 residues of SspB fused to the C terminus of Pac) and Pac itself bind calcium yet do not interact with *P. gingivalis*. Alternatively, calcium may be an integral part of the *P. gingivalis* binding site of SspB which cannot be restored by substitution of the equivalent region of Pac. In this case, the interaction of SspB with *P. gingivalis* would exhibit a strict dependency for calcium. Although we have noticed that binding of whole cells of *P. gingivalis* to *S. gordonii* proceeds more efficiently in the presence of 1 mM CaCl₂ (unpublished observations), it is difficult to determine with certainty the precise role of calcium in SspB-mediated adherence. Whole-cell adhesion is multimodal and involves molecules distinct from SspB. In addition, results of studies with calcium-chelating agents such as EDTA are not definitive, as chelating agents extract material from the *P. gingivalis* outer

membrane. Experiments are under way to identify the *P. gingivalis* receptor for SspB in order to determine directly if the interaction of the two purified components requires calcium. Finally, we cannot exclude the possibility that the structures of the truncated and/or chimeric peptides differ from that of native SspB, resulting in altered function. However, we feel that this is unlikely, since SAG binding is largely unaffected by deletion of the C terminus (residues 1167 to 1500) of SspB and all of the truncated SspB polypeptides react with a monoclonal anti-SspB antibody (4) that recognizes a conformational epitope. This finding suggests that these proteins are similar in gross structure to native SspB.

In summary, both of the Ssp polypeptides expressed by *S. gordonii* (SspA and SspB) were shown to bind to *P. gingivalis* cells, whereas a highly related surface polypeptide of *S. mutans* did not. A specific region of the SspB polypeptide (amino acids 1167 to 1250) was shown to be essential for its interaction with *P. gingivalis*. This sequence is completely conserved in SspA and SspB but differs in both primary and predicted secondary structure between SspB and the Pac polypeptide of *S. mutans*. Thus, the structure of this region is consistent with the activity profiles determined for these streptococcal polypeptides.

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