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Molecular Cloning and Expression of a *Streptococcus mutans* Major Surface Protein Antigen, P1 (I/II), in *Escherichia coli*

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Antigen P1, also called I/II, is one of the most abundant cell wall proteins of the mutans streptococci. It has been suggested that P1 may be involved in cell adherence to tooth surfaces and in sucrose-induced cell aggregation. As a first step toward fully understanding its biological functions, the P1 gene, which has been designated *spaP1*, from *Streptococcus mutans* NG5 (serotype c) has been cloned into *Escherichia coli* JM109 by a shotgun procedure with pUC18 as the vector. The recombinant strain expressing P1 carries a 5.2-kilobase DNA insert whose restriction map has been determined. This map is completely different from that of *spaA* of *Streptococcus sobrinus* (serotype g), even though P1 and SpaA are antigenically related. Southern hybridization revealed that DNA sequences closely homologous to *spaP1* were present in serotypes c, e, and f, and similar sequences also existed in strains of serotypes a and d. The expression of the cloned *spaP1* was found to be independent of the *lac* inducer and the orientation of the DNA insert, suggesting that it carries its own promoter. Western blotting (immunoblotting) revealed at least 20 bands reacting with a mixture of three anti-P1 monoclonal antibodies. The highest-molecular-weight reactive band was comparable in size to the parent P1 (185 kilodaltons [kDa]); however, the major reactive bands were smaller (~160 kDa). Expression of cloned P1 in *E. coli* LC137 (*htpR lonR9*) resulted in the increased prominence of the 185-kDa protein reactive band. Ouchterlony immunodiffusion showed partial identity between the parent and cloned P1. In *E. coli*, P1 was detected primarily in the periplasm and extracellular fluid.

A number of cell wall-associated proteins from *Streptococcus mutans* Ingbritt (serotype c) have been identified serologically by several laboratories. These include antigens I, I/II, III, and IV, reported by Russell and Lehner (25), and antigens A, B, C, and D, reported by Russell et al. (28). Forester et al. (6) demonstrated that P1, a 185-kilodalton (kDa) protein isolated from the same organism, was biochemically and immunologically identical to antigens I/II, B, and IF (9). The presence of antigens cross-reacting with P1 was evident in *S. mutans* serotypes a, d, e, f, and g as well as strains of *Streptococcus sanguis* (26). In serotype g (*Streptococcus sobrinus*), the gene coding for the P1-like protein, *spaA*, has been cloned in *Escherichia coli* (8). Antiserum raised against the SpaA protein purified from the recombinant *E. coli* reacted with antigens from serotypes a, c, d, e, and f (8). DNA sequences homologous to *spaA* were detected in serotypes d and g but not in other serotypes, suggesting that there are fundamental differences within this group of proteins (3).

The potential importance of P1 was indicated in immunization studies. The administration of purified P1 in monkeys (27) and local, passive immunization of humans with anti-P1 monoclonal antibodies (MAbs) (15) were found to confer significant protection against dental caries. The use of P1 as an anticaries vaccine, however, should be treated cautiously, since immuno-cross-reactivity between this antigen and human heart tissue has been repeatedly observed (6, 9). Smith et al. (29) failed to detect cross-reactivity between P1 and human heart tissue using anti-P1 MAbs. Similar results have been obtained recently in this laboratory (1).

The biological function of P1 remains very much unclear. Ayakawa et al. (1) demonstrated that P1 is associated with the fibrillar fuzzy layer on the cell surface of *S. mutans* Ingbritt. Douglas and Russell (5) showed that anti-P1 sera inhibited adherence of *S. mutans* to saliva-coated hydroxyapatite. Curtiss et al. (4) found that anti-SpaA sera inhibited sucrose-induced aggregation of *S. sobrinus*. As a first step toward fully understanding the biological functions of P1, we have isolated the P1 gene (designated *spaP1*) from *S. mutans* NG5 (serotype c). The preliminary characterization of the cloned *spaP1* and its product are described in this paper.

MATERIALS AND METHODS

Bacteria and growth conditions. The mutans streptococci used in this study were *S. cricetus* E49 (serotype a); *S. rattus* BHT (serotype b); *S. mutans* NG5 (serotype c), V100 (serotype e), and OMZ 175 (serotype f); and *S. sobrinus* SL-1 (serotype d), K1R (serotype g), and ATCC 33748 (serotype h). The sources for these strains are listed elsewhere (1). *S. sanguis* Challis e and *S. salivarius* ATCC 25975 were obtained from our departmental culture collection. *E. coli* JM109 [*recA1 endA1 gyrA96 thi hsdR17 supE44 relA1* Δ(*lac-proAB*) (F *traD36 proAB lacIZ*ΔM15)] (32) and LC137 [*htpR*(AmTs) *lonR9*(Ts) *lac*(Am) *trp*(Am) *pho*(Am) *rpsL supC*(Ts) *mal*(Am) txs::Tn*10*] (2, 7) were obtained from International Biotechnologies, Inc., New Haven, Conn., and from A. L. Goldberg, Harvard University, respectively. Strains of streptococci were grown aerobically in the chemically defined medium of Terleckyj et al. (30) at 37°C without agitation. *E. coli* was grown in LB medium (1% tryptone, 0.5% yeast extract, and 1% NaCl, wt/vol) at 37°C, unless otherwise specified.

Isolation of chromosomal DNA. Streptococcal cells from late-exponential-phase cultures (200 ml) were harvested by centrifugation at 10,000 × *g* (4°C, 10 min) and washed twice with 2 M NaCl and cold distilled water. The cells were suspended in 20% glucose and 20 mM Tris buffer, pH 6.9, and treated with 0.1 mg of mutanolysin per ml (Miles Laboratories, Inc., Naperville, Ill.) for 1 h at 37°C with gentle mixing. The DNA from these streptococcal sphero-

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plasts and late-exponential-phase *E. coli* cells was isolated by the method of Marmur (18).

Isolation of plasmid DNA. Plasmids were isolated by the method of Ish-Horowicz and Burke (10).

Endonuclease restriction. All restriction endonucleases used in this study except *Sca*I were purchased from International Biotechnologies, Inc. *Sca*I was obtained from Bethesda Research Laboratories, Inc., Gaithersburg, Md. The digestion of DNA was conducted under the conditions suggested by the manufacturers. Analysis of digested DNA was routinely performed by horizontal agarose gel (0.7%, wt/vol) electrophoresis in TAE buffer, pH 8.6 (40 mM Tris acetate, 1 mM EDTA) at 15 V per cm of gel.

Cloning. *S. mutans* NG5 chromosomal DNA partially digested with *Hin*dIII was mixed with restricted and dephosphorylated pUC18 at a ratio of 2:1 (wt/wt). Ligation was performed with T4 DNA ligase (International Biotechnologies, Inc.) at 4°C for 18 to 24 h. $CaCl_2$-treated *E. coli* JM109 was transformed with the ligated DNA by the method described by Maniatis et al. (17). Recombinant strains were selected on LB-ampicillin (50 μg/ml) agar plates which had been spread with 100 μl of 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (10 mg/ml) (International Biotechnologies, Inc.) and 10 μl of 100 mM isopropyl-thiogalactoside (IPTG; Sigma Chemical Co., St. Louis, Mo.). White colonies, presumably carrying streptococcal DNA inserts, were picked after 24 h of growth.

Colony blotting. Recombinant (white) colonies were grown on LB-ampicillin-IPTG agar medium at 37°C. After 5 to 18 h of incubation, the colonies were exposed to chloroform vapor for 15 min and overlaid with nitrocellulose membranes (Bio-Rad Laboratories, Richmond, Calif.) for another 15 min at room temperature. The membranes were then carefully peeled off and saturated with 1% (wt/vol) bovine serum albumin in phosphate-buffered saline, pH 7.4, for 2 h. Cell debris was washed away with two rinses of water. The membranes were probed with a mixture of three anti-P1 MAbs at a dilution of 1:7,000 in phosphate-buffered saline. These MAbs (designated 3-3B, 4-10A, and 6-11A [1]) were used in the form of mouse ascites fluids. After 1 h of incubation at room temperature, the membranes were rinsed five times with water or phosphate-buffered saline and reacted with a solution of affinity-purified peroxidase-conjugated goat anti-mouse immunoglobulins (1:1,000; Organon Teknika, Malvern, Pa.) for 1 h. The blots were then washed five times and developed with 3.5 mM 4-chloro-1-naphthol (Sigma) in Tris-buffered saline, pH 7.4, in the presence of 0.3% H_2O_2. Reactions were terminated by rinsing with water (twice) after 30 min.

Western blotting (immunoblotting). Proteins were separated by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis on 7.5% gels by using the discontinuous buffer system of Laemmli (11). The proteins were transferred onto nitrocellulose membranes at 200 mA for 1 h in the buffer of Towbin et al. (31). The blots were probed with antibodies as described above. Tween 20 (Fisher Scientific Co., Fair Lawn, N.J.) at a concentration of 0.01% (vol/vol) was added to the phosphate-buffered saline to minimize nonspecific binding.

Protein estimation. Proteins were measured by the modified method of Lowry et al. (14) by using the BCA reagent (Pierce Chemical Co., Rockford, Ill.) in place of the Folin reagent. Bovine serum albumin was used as the standard.

Ouchterlony double diffusion. The double-diffusion test was done in 1% (wt/vol) agarose in 50 mM Tris buffer, pH 7.5, containing 3% (wt/vol) polyethylene glycol (molecular weight, 4,000; Sigma) (21). The nonprecipitated proteins were removed by the pressing procedure of Laurell (12), and the precipitated proteins were stained with Coomassie brilliant blue R-250. The antibody used was a rabbit anti-P1 serum kindly given by R. R. B. Russell, Royal College of Surgeons, England.

DNA hybridization. Complete DNA digests, after electrophoresis on 0.7% agarose in 1× TAE buffer, pH 8.6, were denatured in 0.2 M NaOH and 0.4 M NaCl (30 min at room temperature). Excess alkali and salt were removed by soaking the gel in four changes of 0.1 × TAE buffer over 30 min. The denatured DNA was transferred to nylon membranes (Zeta-Probe; Bio-Rad) by blotting at 200 mA for 1 h in 0.1× TAE buffer. The blots, after being air dried and baked at 80°C for 2 h under vacuum, were prehybridized for 12 to 16 h at 42°C in 50% (vol/vol) formamide containing 6× SSC (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate), 5× Denhardt solution (0.1% [wt/vol] each of bovine serum albumin, Ficoll, and polyvinylpyrolidone), 0.1% SDS, and 100 μg of denatured salmon sperm DNA (Sigma) per ml. The blots were then hybridized with [α-^{32}P]CTP-labeled probes, prepared by nick translation (23), for 20 to 24 h in the buffer and conditions described above. Unhybridized probes were removed by washing the blots in two changes of 2× SSC containing 0.1% SDS at room temperature for 15 min each. The washings were repeated with 0.1× SSC containing 0.1% SDS. Autoradiography was carried out at −70°C with K-Omat R film (Eastman Kodak Co., Rochester, N.Y.).

Reversal of the insert orientation. pSM2949 was partially restricted with *Hin*dIII, and the products were resolved by electrophoresis on 0.7% (wt/vol) agarose gels. DNA fragments of 5.2 kilobases (kb) were isolated from the gel by electroelution (17) and purified with an Elutip-d column (Schleicher & Schuell, Inc., Keene, N.H.) before phenol and chloroform extractions were performed. The DNA was then ligated into the *Hin*dIII cloning site of pUC19, and the recombinant plasmid was transformed into *E. coli* JM109. White clones were tested for the expression of P1 antigen by colony blotting. The orientation of the inserts was determined after double digestions with restriction endonucleases *Eco*RI and *Sca*I.

Localization of cloned gene product. Recombinant *E. coli* SM2949 was grown in LB broth containing 50 μg of ampicillin per ml with vigorous shaking until the late exponential phase of growth was obtained. The cells were sedimented by centrifugation at 4°C for 10 min at 10,000 × *g* and washed twice with cold 50 mM Tris buffer, pH 7.5. The culture supernatant fluid and washes were collected and concentrated by ultrafiltration through a YM10 membrane (Amicon Corp., Danvers, Mass.) and designated as the extracellular fraction. The cells were suspended in Tris buffer, and the periplasmic content was expelled by cold osmotic shock (20). After being washed in Tris buffer, the shocked cells were broken by sonication (four 15-s blasts). The cytoplasmic content was separated from cell debris by centrifugation at 35,000 × *g* (4°C, 60 min). The cell debris was boiled with 0.1% SDS in 50 mM Tris for 10 min and designated as the solubilized membrane fraction. Alkaline phosphatase (16) and glucose-6-phosphate dehydrogenase (13) were assayed as periplasmic and cytoplasmic markers, respectively.

RESULTS

Cloning and recombinant screening. A genomic library of *S. mutans* NG5 was constructed in *E. coli* JM109 with *Hin*dIII partial DNA digests (1 to 20 kb) and pUC18 as the

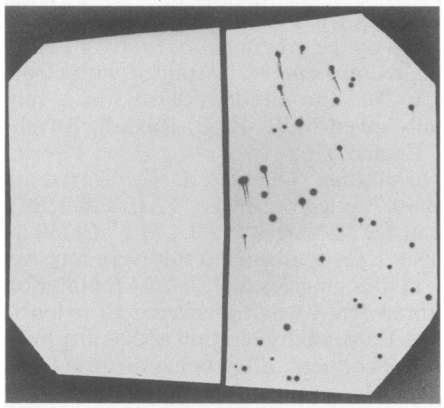

FIG. 1. Colony immunoblots of recombinant SM2949 (right) and *E. coli* JM109 carrying pUC18 (left). Cultures were grown on LB-ampicillin medium for 5 h at 37°C in the absence of IPTG. Immunoblotting was performed as described in Materials and Methods by using a cocktail of three anti-P1 MAbs.

vector. This library, which consists of 4,500 clones with DNA inserts of an average size of 3.4 kb, is believed to be a comprehensive representation of the entire *S. mutans* genome. Five transformants were identified by colony immunoblotting as putative P1-expressing recombinant clones. One of these clones, designated SM2949, reacted strongly with the anti-P1 MAbs in the absence of IPTG (Fig. 1). The other four required IPTG for the expression of anti-P1 reacting activity.

Characterization of *spaP1*. Recombinant SM2949 was found to harbor a 7.9-kb plasmid. Complete digestion of this plasmid, pSM2949, with *Hin*dIII yielded two DNA fragments of 2.6 kb and the pUC18 vector (2.7 kb). The insert in pSM2949, therefore, was 5.2 kb. This insert was assumed to carry the gene encoding the surface protein antigen P1 and is designated *spaP1*. The abbreviated restriction map of pSM2949 is shown in Fig. 2A. The detailed restriction map of *spaP1* is shown in Fig. 2B. There were no *Bam*HI, *Kpn*I, *Sal*I, *Xba*I, or *Sca*I sites detected in *spaP1*. The restriction map of *spaA* of *S. sobrinus* is shown in Fig. 2B for comparison.

To determine whether the cloned *spaP1* gene carried its own promoter, the 5.2-kb DNA insert was excised from pSM2949 with *Hin*dIII and ligated to pUC19. Clone SR14 was found to carry a 7.9-kb plasmid. Double restriction of pSR14 (Fig. 2C) with *Sca*I and *Eco*RI resulted in four DNA fragments of 3.9, 3.0, 0.8, and 0.2 kb, while restriction of pSM2949 with the same enzymes yielded DNA fragments of 3.0, 2.8, 1.9, and 0.2 kb. These digestion patterns indicate that the *spaP1* in pSR14 was ligated in a reversed orientation with respect to the *lac* promoter of the plasmid. Continued expression of anti-P1 reacting activity by clone SR14 in the absence of IPTG was observed, further evidence that the insert DNA contains the *spaP1* promoter.

Southern hybridization. To test the origin of *spaP1* and the occurrence of homologous DNA sequences in other oral streptococci, *S. mutans* NG5 DNA, pUC18, and pSM2949 were nick translated and used as probes for Southern hybridization. Labeled NG5 DNA hybridized to *Eco*RI-digested pSM2949 but not to *E. coli* JM109 DNA or pUC18 (data not shown). Labeled pUC18 hybridized very strongly with the 4.7-kb fragment of pSM2949 which contained pUC18 sequences but only weakly with the 3.0-kb fragment containing only the insert, and it did not hybridize with *S.*

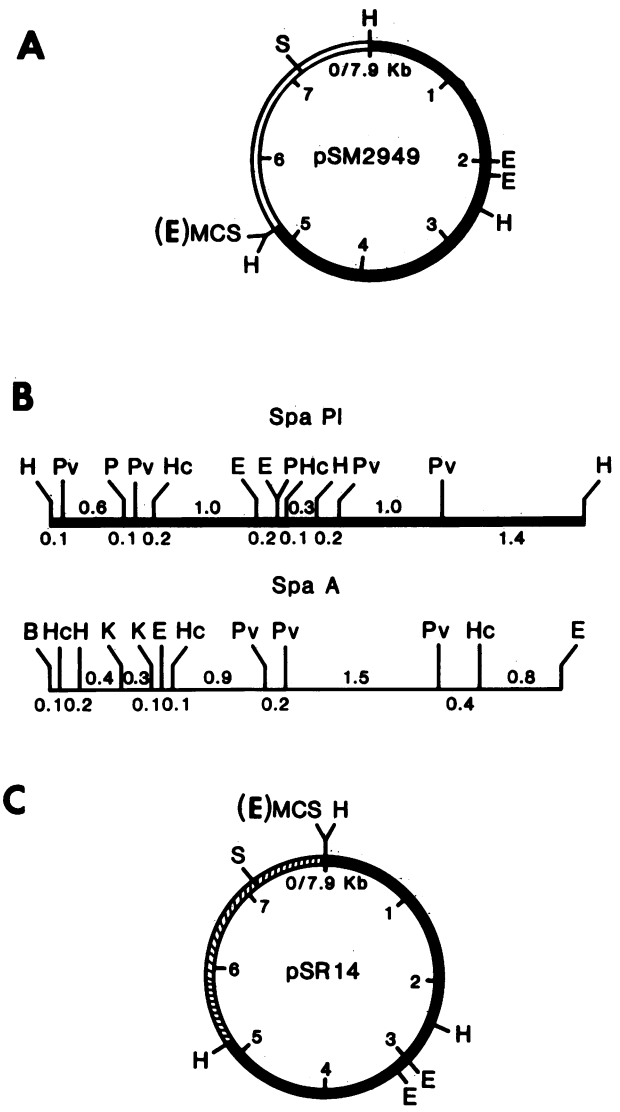

FIG. 2. Restriction maps of recombinant plasmids and cloned inserts. (A) pSM2949; (B) *spaP1* of *S. mutans* NG5 (top) and *spaA* of *S. sobrinus* 6715 (bottom); (C) pSR14. Symbols: ▬, NG5 DNA inset; ▭, pUC18 DNA; ▨, pUC19 DNA. MCS, multiple cloning site from pUC18 or pUC19; B, *Bam*HI; E, *Eco*RI; H, *Hin*dIII; Hc, *Hin*cII; K, *Kpn*I; P, *Pst*I; Pv, *Pvu*II; S, *Sca*I. The *spaA* map is adapted from the work of Curtiss et al. (3).

mutans NG5 DNA (data not shown). Labeled pSM2949, in addition to hybridizing very strongly to pUC18 and pSM2949 (Fig. 3A), hybridized with a 2.6-kb fragment from the *Hin*dIII complete digest of NG5 DNA (Fig. 3B, lane 3). Homologous DNA fragments were also detected in serotypes a, d, e, and f. The fragments containing homologous sequences in these serotypes were estimated to be 5.4 kb (for serotype a), 3.2 kb (serotype d), 2.9 and 2.6 kb (serotype e), and 2.6 kb (serotype f). No homology was detected with DNA from serotypes b, g, and h (Fig. 3B); *S. sanguis*; *S. salivarius*; or *E. coli* JM109 (data not shown).

Western blotting of the cloned product. When the total soluble proteins from the sonicated recombinant SM2949 were analyzed by Western blotting, at least 20 immunoreactive bands were observed (Fig. 4A, lane 6). The reaction,

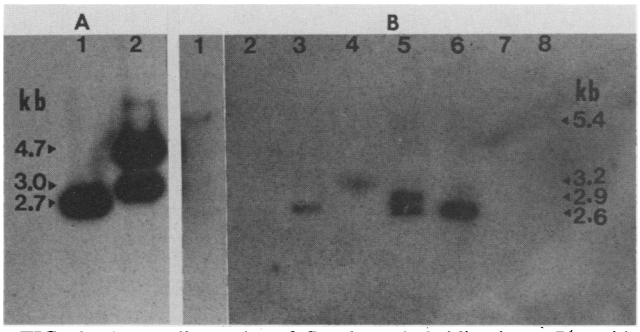

FIG. 3. Autoradiographs of Southern hybridizations. Plasmids (0.2 μg) and chromosomal DNA (2 μg) were digested to completion with *Eco*RI and *Hin*dIII, respectively. After electrophoresis, the DNA was blotted onto nylon membranes and hybridized with α-^{32}P-labeled pSM2949 prepared by nick translation. (A) Lanes: 1, pUC18; 2, pSM2949. (B) Lanes 1 to 8, Chromosomal DNA from mutans streptococci serotypes a to h.

however, was specific as indicated by the lack of reactivity for nonrecombinant *E. coli* strains (Fig. 4A, lanes 1 and 2) as well as other randomly selected transformants (data not shown). The highest-molecular-weight reactive band was comparable in size (~185 kDa) to the native *S. mutans* P1 molecule (Fig. 4A, lane 7). However, the most prominent reactive bands were near 155 to 165 kDa. When SM2949 was cultivated at 30°C, however, the lower-molecular-weight (<155 kDa) reacting bands were absent (Fig. 4A, lane 5). Similar results were obtained with the sonicate of *E. coli* HB101 carrying pSM2949 (Fig. 4A, lane 4). For the sonicate of the transformant SMLC (*E. coli* LC137, a *lon* and *htpR* defective mutant which harbors pSM2949), multiple immunoreactive bands were again observed. The 185-kDa band, however, appeared as a more prominent species (Fig. 4A, lane 3) than in SM2949 transformants (lanes 5 and 6).

Ouchterlony double diffusion. Both the sonicates of SM2949 and those of SMLC produced a single precipitin band with the polyclonal rabbit anti-P1 serum (Fig. 5). Almost complete identity, indicated by a slight spur formation, was observed between the cloned products (Fig. 5, wells 2 and 6) and the parent P1 (well 1).

Localization of cloned P1 in recombinant *E. coli*. Because P1 is known to be localized to the cell surface of *S. mutans*, it was of interest to determine whether the cloned P1 is translocated across the cytoplasmic membrane in *E. coli*. The recombinant SM2949 was separated into extracellular, periplasmic, cytoplasmic, and solubilized membrane fractions. In the periplasmic and cytoplasmic fractions, 93% of the alkaline phosphatase and 87% of the glucose-6-phosphate dehydrogenase activities were detected, respectively. The cloned gene product was found mainly in the periplasmic and extracellular contents (Fig. 6).

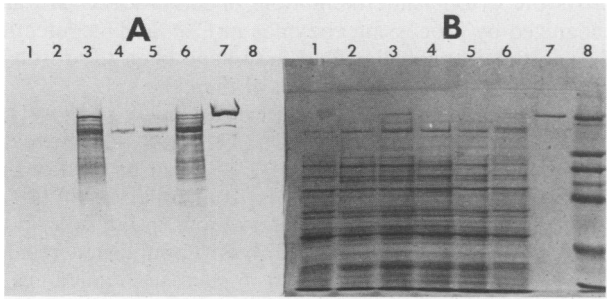

FIG. 4. Western immunoblot (A) and SDS-polyacrylamide gel electrophoresis (B) of sonicates from *E. coli* and recombinants. Lanes: 1, *E. coli* LC137 (grown at 30°C); 2, *E. coli* JM109 (39°C); 3, SMLC (30°C); 4, *E. coli* HB101 harboring pSM2949 (30°C); 5, SM2949 (30°C); 6, SM2949 (39°C); 7, *S. mutans* NG5 culture supernatant fluid (3 μg of protein); 8, protein markers. The amount of proteins applied was 11 μg for each sonicate. Protein markers are (from top) myosin (200 kDa), β-galactosidase (116 kDa), phosphorylase *b* (97 kDa), bovine serum albumin (66 kDa), ovalbumin (45 kDa), and carbonic anhydrase (29 kDa).

DISCUSSION

The gene coding for the surface protein antigen P1 of *S. mutans* serotype c has been cloned in *E. coli*. This gene, which has been designated *spaP1*, was found to be located within a 5.2-kb *Hin*dIII DNA fragment (Fig. 2). The cloned *spaP1* very probably carries its own promoter as indicated by its nondependence on both the *lac* inducer and insertional orientation for expression. Detailed restriction analysis of *spaP1* reveals its lack of resemblance to the *spaA* gene of *S. sobrinus*. Curtiss et al. (3) failed to detect hybridization of *spaA* with DNA from serotype c *S. mutans*. The lack of homology between *spaP1* and serotype g DNA in the present study confirms that *spaP1* and *spaA* are indeed different (Fig. 3B).

It is, however, rather interesting that DNA sequences homologous to *spaP1* were detected in mutans streptococcus serotypes a, d, e, and f (Fig. 3B). This observation demonstrates, for the first time, the molecular basis for the observed immuno-cross-reactivity between P1 and related surface antigens from these serotypes (1, 26). The differences in the sizes of the homologous fragments suggest that there are subtle differences among the P1-like proteins of mutans streptococci. The possession of common homologous (2.6 kb) *Hin*dIII fragments by serotypes c, e, and f suggests that the P1 antigens in these serotypes are more closely related to each other than to the analogous surface proteins (SpaA, I/II) found on other mutans streptococcal cell surfaces.

Western immunoblotting showed that the *E. coli* recombinants SM2949 and SMLC produced proteins reacting specifically with the anti-P1 MAbs (Fig. 4A). The highest-molecular-weight reactive band was comparable in size to the parent P1, suggesting that the entire *spaP1* may have been cloned. The cloned DNA fragment (5.2 kb) is large enough to encode the entire P1, since the coding requirement for the structural P1 (185 kDa) is approximately 4.8 kb. The Ouchterlony double-diffusion test reveals almost complete identity between the cloned and native P1 (Fig. 5). The missing antigenic determinant in the cloned product may be a carbohydrate, since the native P1 is known to be a glycoprotein (24).

The multiple banding patterns observed in the Western blots may have been due to extensive proteolysis of P1 in *E. coli*. The use of *E. coli* LC137, a strain which has mutations in two genes (*lon* and *htpR*) whose products are responsible for the degradation of abnormal or foreign proteins (19), did not resolve the multiple-banding problem (Fig. 4A). Nonetheless, the 185-kDa band appears as a more prominent species. P1 has been noted to be very unstable even when isolated from *S. mutans* (unpublished observations). Hence, the multiple-banding pattern may be partially due to an inherent instability of this protein. It is worth noting, however, that when SM2949 was cultivated at 30°C instead of 39°C, the lower-molecular-weight (<155 kDa) immunoreactive bands were not detected. This suggests a catalytic (or autocatalytic) process that results in P1 fragmentation.

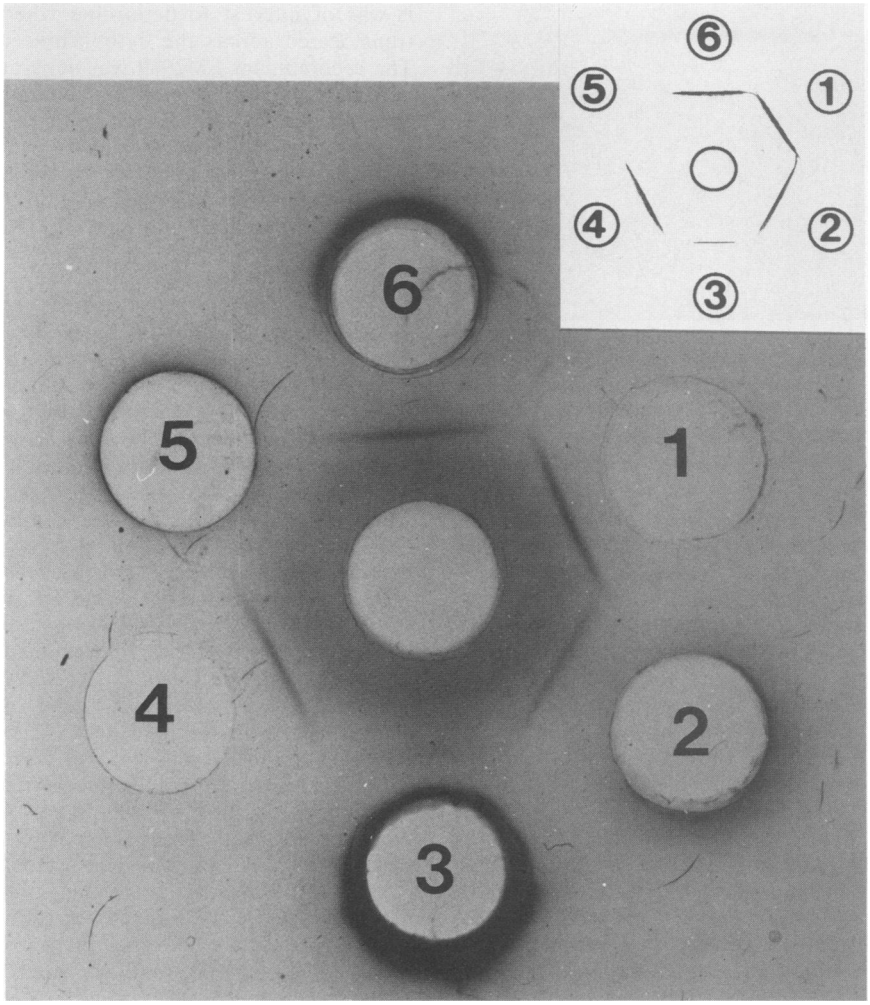

FIG. 5. Ouchterlony double-diffusion test of identity between *S. mutans* NG5 extracellular P1 and the cloned products. Wells: center, rabbit anti-P1 serum; 1 and 4, NG5 extracellular proteins (4 μg); 2, SM2949 (39°C, 40 μg); 3, SM2949 (30°C, 40 μg); 5, *E. coli* JM109 (40 μg); 6, SMLC (30°C, 40 μg). The inset is a diagrammatic representation of the stained Ouchterlony test.

The cloned P1 apparently was able to penetrate the cytoplasmic membrane, since it was found mainly in the periplasm and extracellular fluid (Fig. 6). Holt et al. (8) also indicated that the cloned SpaA was found predominantly in the periplasmic space of *E. coli*. These data suggest that signal sequences required for translocation of proteins across the cytoplasmic membrane of streptococci are also recognized by processing enzymes in *E. coli*. The detection of P1 in the extracellular fluid may have been the result of autolysis of the older population of the cells.

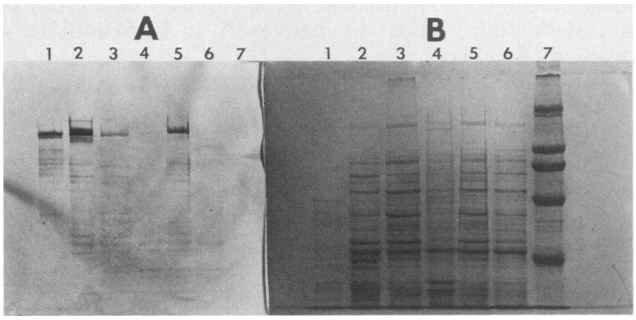

FIG. 6. Localization of cloned product in SM2949 by Western immunoblotting. (A) Immunoblot; (B) SDS-polyacrylamide gel electrophoresis. Lanes: 1, extracellular fraction; 2, periplasm; 3, cytoplasm; 4, solubilized membranes; 5, total soluble proteins; 6, *E. coli* JM109; 7, protein markers. The amount of protein loaded was 11 μg for each sample.

In conclusion, this study describes the cloning of the *spaP1* of *S. mutans* NG5 in *E. coli*. This study also provides a preliminary characterization of *spaP1* and its relationship to *spaA* as well as related genes from other serotypes of mutans streptococci. Recent observations in our laboratory (G. Y. Ayakawa and A. S. Bleiweis, unpublished results) indicate that P1 is not implicated in sucrose-induced aggregation of serotype c *S. mutans*, suggesting that SpaA in *S. sobrinus* and P1 in *S. mutans* are functionally distinct molecules. The isolation of *spaP1* should facilitate the study of the biological function(s) of P1 by genetic approaches. For instance, if *spaP1* can be insertionally inactivated in vitro and transformed back into *S. mutans* (22) to create isogenic mutants, we may be able to ascertain the function of this major surface antigen by comparing the activities of the mutants and the parent strain.

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