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Isolation and Characterization of a Minor Fimbria from *Porphyromonas gingivalis*

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We have discovered two distinctly different fimbriae expressed by the same *Porphyromonas gingivalis* strain. The construction of a *fimA* mutant of *P. gingivalis* ATCC 33277 has previously been reported by N. Hamada et al. (Infect. Immun. 62:1696–1704, 1994). Expression of fimbriae on the surface of the *fimA* mutant and the wild-type strain, ATCC 33277, were investigated by electron microscopy. The wild-type strain produced long fimbrial structures extending from the cell surface, whereas those structures were not observed on the *fimA* mutant. However, short fimbrial structures were seen on the surface of the *fimA* mutant. The short fimbrial protein was purified from the *fimA* mutant by selective protein precipitation and chromatography on DEAE Sepharose CL-6B. We have found that the second fimbrial structure of *P. gingivalis* ATCC 33277 is distinct from the 41-kDa (43-kDa) major fimbrial protein (FimA). We provisionally call this protein minor fimbriae. The molecular mass of the minor fimbriae is 67 kDa as estimated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis under reducing conditions after boiling at 100°C. The component shows a ladder-like pattern at 80°C under nonreducing conditions, suggesting a tendency to aggregate or polymerize. In immunoblotting analysis, anti-minor fimbria serum reacted with both the 100°C- and the 80°C-treated minor fimbriae. The anti-minor fimbria serum also reacts with the same-molecular-size fimbrial preparation from the wild-type strain. Immunogold electron microscopy showed that the anti-minor fimbria serum bound to the minor fimbria on the cell surface of the wild-type strain. This is the first report on the identification of the minor fimbria produced by *P. gingivalis*. These results suggest that the minor fimbriae appearing on the *fimA* mutant strain are produced together with numerous long major fimbriae on the wild-type strain. Moreover, the minor fimbriae are different in size and antigenicity from the earlier-reported FimA, a major 41-kDa fimbrial component of *P. gingivalis*.

Periodontal diseases are infectious in nature and induce inflammatory responses in the gingival and underlying supportive periodontal tissues as a response to bacterial accumulation in the subgingival crevice. This process is complex both microbiologically and immunologically (4).

Porphyromonas gingivalis is an obligately anaerobic gram-negative coccobacillus that has been associated with periodontal destruction in humans (29, 30, 32, 33, 36, 37, 41). *P. gingivalis* is thought to be one of the most prominent periodontopathogens, possessing several characteristics of an overt pathogenic organism. Adherence is an essential step in the pathology leading to periodontitis (31, 33). This adherence capacity is thought to be mediated by various surface proteins. *P. gingivalis* adheres to salivary components (5), epithelial cells (1, 26, 31), erythrocytes (12, 27), fibronectin-collagen complexes (23), and other bacteria (6). Fimbriae in particular have been suggested to play an important role in facilitating the initial interaction between the bacteria and the host (13). Moreover, immunization with major fimbrial proteins of *P. gingivalis* provides protection from periodontal tissue destruction in experimental animals (3).

Early electron-microscopic observations of *P. gingivalis* and related black-pigmented anaerobic rods revealed that these organisms are peritrichous, exhibiting fine fibrillar appendages (fimbriae) emerging from the cell surface (9, 18, 31). A method for purifying major fimbrial proteins from a highly fimbriated strain, 381, was first developed by Yoshimura et al. (39), who demonstrated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) that the major subunit (fimbrillin) was a 43-kDa (41-kDa) protein (FimA). By using a synthetic oligonucleotide probe designed on the basis of the amino acid sequence near the N-terminal domain of the protein, Dickinson et al. (2) cloned and sequenced a gene encoding fimbrillin on a 2.5-kb *Sac*I segment. It has been shown that the majority of *P. gingivalis* isolates are fimbriated and possess the *fimA* gene (19, 35). Fimbriae of *P. gingivalis* act as a strong antigen in host immune responses (8), strongly suggesting that the FimA protein plays a crucial role in bacterium-host interactions in periodontal diseases.

A *fimA* mutant of *P. gingivalis* ATCC 33277 was constructed (7), and it was found that the *fimA*-inactivated mutant strain MPG1 was incapable of producing the 41-kDa FimA protein or adhering to tissue-cultured cells, demonstrating that the *fimA* gene is an essential determinant for the adhesive ability of *P. gingivalis*. The *fimA* mutant MPG1 (7) constructed from *P. gingivalis* strain 381 (20) failed to express long fimbriae from the bacterial surface. Electron microscopy studies of *fimA* mutant strain MPG1 have led to the discovery of short fimbria-like appendages, and the wild-type strain also has the same appendages. We found that two distinctive fimbrial types are expressed by the wild-type strain. In this study, we have shown the presence of the different kinds of fimbriae on the *P. gingivalis* cell surface and have purified and characterized the minor

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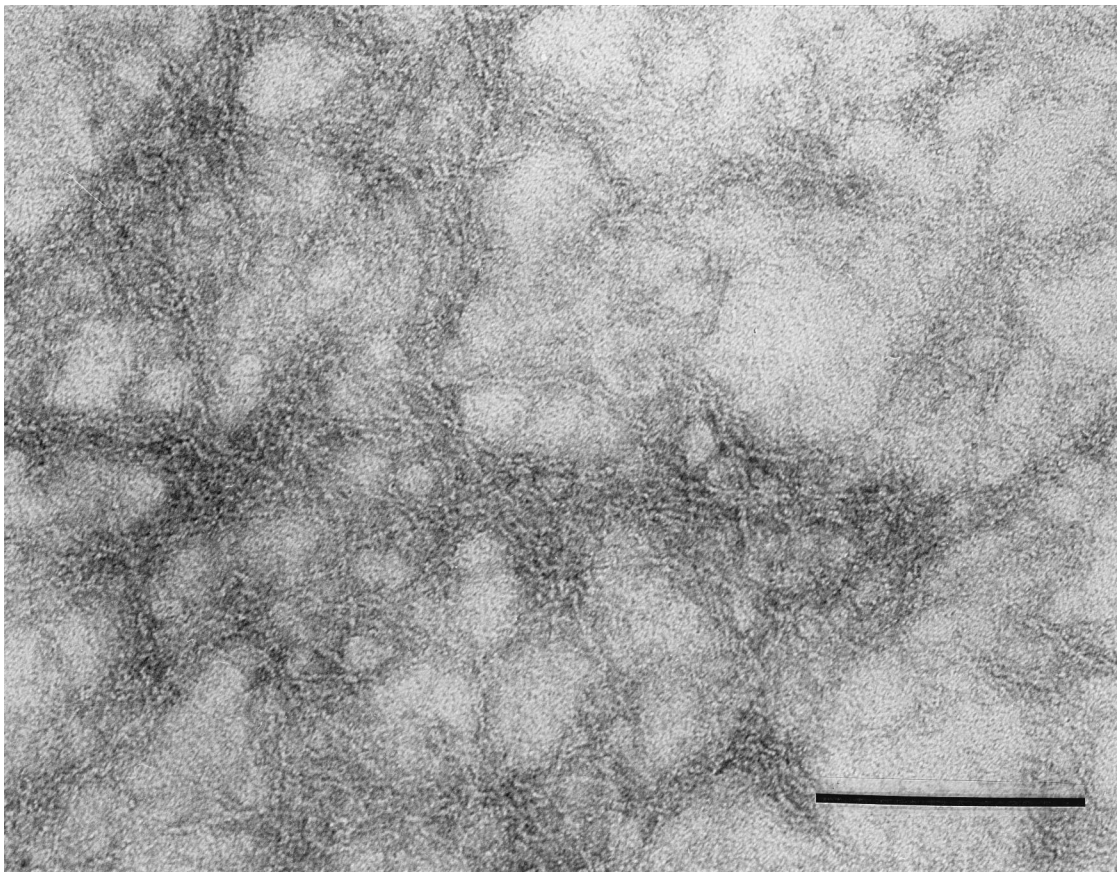

FIG. 1. Electron micrograph of purified minor fimbriae from *P. gingivalis* MPG1. The fimbria preparation purified from the crude fimbrial extract was negatively stained with 2% uranyl acetate. Bar, 0.1 μm.

fimbrial protein from the *fimA* mutant of *P. gingivalis* ATCC 33277.

MATERIALS AND METHODS

Bacterial strains and growth conditions. The bacterial strains used were *P. gingivalis* ATCC 33277 and a *fimA* mutant, MPG1 (7). *P. gingivalis* strains were grown at 37°C for 18 h in an anaerobic chamber (Forma Scientific, Marietta, Ohio) with an atmosphere of 85% N_2, 10% H_2, and 5% CO_2 in brain heart infusion broth (Difco) supplemented with 5 mg of yeast extract, 5 μg of hemin, and 0.2 μg of menadione per ml. For maintenance of the *fimA* mutant strain, MPG1, antibiotic was added to the media: erythromycin at 10 μg/ml.

Isolation and purification of fimbriae from MPG1. Fimbriae were prepared according to a modification of the method described by Yoshimura et al. (39). Briefly, *P. gingivalis* MPG1 from 4.0 liters of brain heart infusion broth culture was harvested by centrifugation at 8,000 × *g* for 25 min. The cell pellet was suspended in 20 mM Tris buffer, pH 8.0, containing 0.15 M NaCl and 10 mM $MgCl_2$ and subjected to ultrasonication with a 3-mm microtip at 20-W output on pulse setting with 50% duty cycle for 5 min in an ice bath. The supernatant was obtained by centrifugation at 10,000 × *g* for 30 min at 4°C. Ammonium sulfate was added to the supernatant to make a 40% saturated solution at 4°C. The precipitated proteins were collected by centrifugation at 10,000 × *g* for 30 min at 4°C, suspended in a small volume of 20 mM Tris buffer, pH 8.0, and dialyzed against the same buffer. The dialysate sample containing most of the fimbriae was subjected to further purification on a DEAE Sepharose CL-6B column (1.5 by 20 cm) equilibrated with 20 mM Tris buffer, pH 8.0. The column was washed with 20 mM Tris buffer and then eluted with a linear gradient of 0 to 0.3 M NaCl. The protein content of the fractions was measured by UV light adsorption at 280 nm, and the presence of fimbrial structures was determined by electron microscopy. The fractions were analyzed by SDS–12.5% PAGE, and those containing fimbriae were pooled and concentrated by ammonium sulfate precipitation and dialyzed against 5 mM Tris buffer, pH 8.0. This concentrated pool was termed minor fimbriae. The 41-kDa fimbriae were purified by the same procedure from the wild-type *P. gingivalis* ATCC 33277.

Antibodies. Polyclonal antibodies (PAbs) were raised in rabbits against the purified 67-kDa minor fimbriae and the 41-kDa major fimbriae. Purified native fimbriae (50 μg) were mixed with Freund's complete adjuvant (Difco), and the mixture was injected subcutaneously at multiple sites into New Zealand White rabbits (3 kg). After 2 weeks, the rabbits were injected subcutaneously weekly for 4 weeks with the respective immunogens in Freund's incomplete adjuvant (Difco). Each rabbit was bled after the last booster injection, and the antibodies were tested against the corresponding antigen by Western blotting. After the appropriate antibody titer was obtained, the rabbits were bled by cardiac puncture and the sera were prepared and stored at −20°C.

SDS-PAGE. SDS-PAGE was performed in a minigel system (Mini-PROTEIN II Cell; Bio-Rad) by the method of Laemmli (15). Protein samples were prepared either under denaturing conditions at 100°C for 5 min in the presence of 5% β-mercaptoethanol or under partially denaturing conditions at 80°C for 5 min. Samples were electrophoresed at a 30-mA constant current for 1.5 h. The proteins were stained with Coomassie brilliant blue R-250 or silver nitrate. For molecular weight calibration, a low-molecular-weight marker kit (Bio-Rad) was used.

Isoelectric point determination. Isoelectric focusing was carried out with the Multiphor II electrophoresis system (Pharmacia) using an IsoGel agarose isoelectric focusing plate (pH 3 to 10; FMC BioProducts). The purified minor fimbrial protein was loaded on the gel and electrophoresed at 100 V for 1 h and then at 300 V for 3 h. The gel was fixed and stained with Coomassie brilliant blue R-250. For determining the isoelectric point (pI) of the protein, isoelectric focusing 3.6-9.3 (Sigma) was used.

Western blotting. For immunoblot analysis, the proteins separated by SDS–10% PAGE were transferred to a polyvinylidene difluoride membrane (Immobilon-P; Millipore) at 200 mA for 1 h. The membranes were then treated with Tris-buffered saline (20 mM Tris-HCl and 0.5 M NaCl [pH 7.5]) containing 1% (vol/vol) bovine serum albumin (BSA) to block unoccupied protein binding sites. The membranes were incubated with the PAbs at 4°C for 16 h, washed with Tris-buffered saline–Tween, and incubated for 1 h with 1:500-diluted goat anti-rabbit immunoglobulin G conjugated with horseradish peroxidase (Bio-Rad). The membranes were then immersed into a 4-chloro-1-naphthol (Bio-Rad) solution to develop the color. The reaction was stopped by immersing the membranes in distilled water, and the membrane was then dried.

Amino acid analysis. The amino acid composition of the purified minor fimbriae was determined as follows. Samples for amino acid analysis were hydrolyzed in 6 N HCl at 110°C for 22 h in evacuated sealed tubes. The samples were

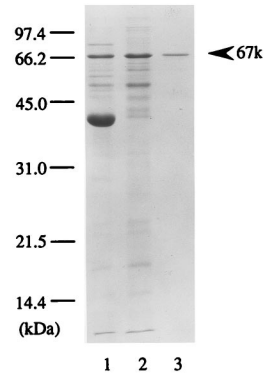

FIG. 2. SDS-PAGE analysis of crude fimbriae and the purified minor fimbriae isolated from *P. gingivalis*. The crude fimbrial extract was precipitated with 40% saturated ammonium sulfate, and then the precipitate was suspended in 20 mM Tris-HCl (pH 8.0). The suspension was dialyzed, and the dialysate was applied to a DEAE-Sepharose CL-6B ion-exchange column. The protein was treated at 100°C for 5 min in the presence of β-mercaptoethanol to obtain the fimbrillin monomers of completely dissociated fimbriae. The fimbriae were then electrophoresed on an SDS–12.5% polyacrylamide gel and stained with Coomassie brilliant blue R-250. Lanes: 1, crude fimbriae of *P. gingivalis* ATCC 33277 (wild-type strain); 2, crude fimbriae of MPG1 (*fimA* mutant); 3, purified 67-kDa minor fimbriae. The position of the purified protein is indicated.

dried in a vacuum, and the hydrolysates were analyzed with the Beckman 121MB amino acid analyzer.

Electron microscopy. The fimbriae of *P. gingivalis* cells were examined with a transmission electron microscope. Bacterial cells from an 18-h anaerobic culture were collected by centrifugation (10,000 × *g* for 1 min), washed, and resuspended (5×10^8/ml) in phosphate-buffered saline (PBS, pH 7.4). Ten microliters of the cell suspension or purified fimbriae was applied on a copper grid coated with a thin Formvar film and air dried. The samples were then negatively stained with 2% (wt/vol) uranyl acetate for 1 min, air dried, examined, and photographed with a Hitachi H-600 electron microscope operating at 75 kV.

Immunogold labeling. *P. gingivalis* cells were washed with PBS, resuspended, transferred to nickel grids coated with Formvar film, and air dried as described above. The cells were then incubated with 10 μl of PAbs to the minor fimbriae prepared in a rabbit (1:1,000 in PBS containing 1% BSA) at 37°C for 1 h. After five washes with PBS, the cells were incubated for 30 min with sheep anti-rabbit immunoglobulin G conjugated with 5-nm gold particles (1:20; AuroProbe EM; Amersham) at 37°C for 30 min. The cells were rinsed five times with PBS and negatively stained with 2% (wt/vol) uranyl acetate for 1 min. The fixed and stained cells were examined and photographed with a Hitachi H-600 electron microscope operating at 75 kV.

Hemagglutination assay. Hemagglutinating activity was determined in a round-bottom microtiter plate by a modification of the method of Inoshita et al. (12). Samples were diluted in a twofold series with PBS (pH 7.4). A 50-μl aliquot of this dilution of test solution and an equal volume of a 2% suspension of sheep erythrocytes were mixed and incubated for 90 min at room temperature.

RESULTS

Purification of *P. gingivalis* minor fimbriae. Minor fimbriae were separated from the crude fimbriae by anion-exchange chromatography. The minor fimbrial protein was detected as a major component of the main peak of a DEAE-Sepharose CL-6B column. The minor fimbriae were eluted at 0.15 M NaCl. Each fraction was examined by electron microscopy. Negative staining of the enriched fimbrial preparation revealed a dense network of fimbria-like structures (Fig. 1). The fimbrial structure-rich fraction contained mainly the 67-kDa component when assessed by SDS-PAGE at 100°C. The minor fimbrial protein appeared to be in a highly polymerized form, suggesting that the fimbrial protein was not completely dissociated into monomeric fimbrillin in the SDS-polyacrylamide gel unless subjected to 100°C and 5% β-mercaptoethanol. The purified minor fimbrial protein from *fimA* mutant strain MPG1 had an apparent molecular mass of 67 kDa under these conditions (Fig. 2, lane 3). The purified protein showed a single band of 67 kDa in SDS-PAGE under reducing conditions when stained either with Coomassie brilliant blue R-250 or by the more sensitive silver staining method, suggesting that this is the monomeric form of the short or minor fimbriae. Lipopolysaccharide was not detected on silver-stained gels of the same preparation (data not shown).

The crude fimbria proteins of the *P. gingivalis* strains were analyzed by SDS-PAGE (Fig. 2, lanes 1 and 2). Antigens for the immunoblot analysis were prepared by heating the crude fimbrial preparations either at 100°C in the sample buffer to obtain the monomeric (completely dissociated) fimbrillin or at 80°C in the sample buffer to obtain the oligomeric (partially dissociated) fimbrial forms. When the purified minor fimbrial protein was treated at room temperature, 40, 60, and 80°C under nonreducing conditions instead of 100°C, we observed a ladder-like pattern in the gel, suggesting that under these conditions it exists as the oligomeric fimbrial forms (data not shown).

To confirm the absence of the 41-kDa major fimbrial protein (FimA) product in MPG1, crude fimbriae prepared from the wild-type strain and MPG1 were analyzed by SDS-PAGE followed by Coomassie brilliant blue R-250 staining (Fig. 2, lanes 1 and 2, respectively). It can be seen in Fig. 2 that the 67-kDa minor fimbrial component was in both strains while the major 41-kDa band was absent in the preparation from mutant strain MPG1.

Isoelectric focusing was performed to estimate the pI values of the minor fimbrial protein. The isoelectric point of the minor fimbriae was estimated to be 4.7 in the agarose isoelectric focusing gel (data not shown).

Amino acid composition. The amino acid composition of the minor fimbriae is shown in Table 1. The minor fimbrial protein is composed of 39.4% hydrophobic amino acids. The amino acid composition of the 67-kDa minor fimbrial protein and FimA protein are similar. Aspartic acid, alanine, and glutamic acid were present at high molar concentrations in both types of fimbriae. Slight differences between the major and minor fimbriae were observed in the proline, glycine, threonine, and tyrosine contents.

TABLE 1. Amino acid composition of *P. gingivalis* 67-kDa minor fimbriae

Amino acid	No. of residues/100 residues	
	67 kDa[a]	FimA[b]
Asp	12.6	13.1
Thr	6.5	8.6
Ser	5.3	3.6
Glu	11.1	9.5
Pro	6.6	3.9
Gly	10.3	7.7
Ala	9.8	12.8
Cys/2	0.6	0.8
Val	8.4	7.4
Met	1.3	1.8
Ile	4.5	5.0
Leu	6.0	7.1
Tyr	2.8	5.6
Phe	2.8	2.1
His	2.0	1.5
Lys	6.9	6.8
Arg	2.5	1.5
Trp	ND[c]	1.2

[a] Purified protein was hydrolyzed with 6 N HCl at 110°C for 22 h.
[b] Calculated from the translated DNA sequence of Dickinson et al. (2).
[c] ND, not determined.

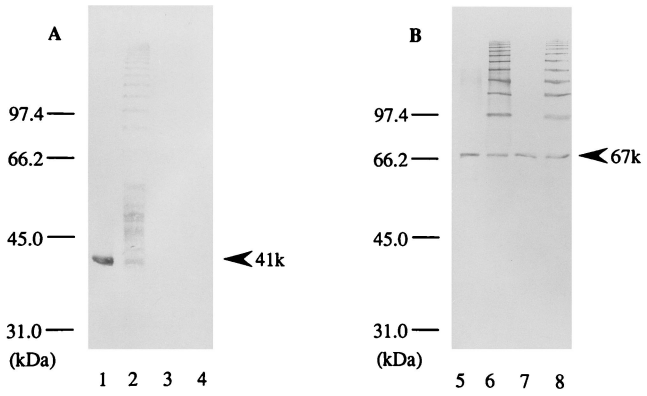

FIG. 3. Immunoblot analysis of *P. gingivalis* fimbriae. The SDS–10% polyacrylamide gel was electrophoretically transferred to a polyvinylidene difluoride membrane and incubated with the 41-kDa fimbria PAbs (A) or 67-kDa minor fimbria PAbs (B). The second antibody was goat anti-rabbit immunoglobulin G conjugated with horseradish peroxidase. Lanes: 1 and 5, crude fimbriae of *P. gingivalis* ATCC 33277 (0.5 μg of protein loaded, 100°C for 5 min); 2 and 6, crude fimbriae of *P. gingivalis* ATCC 33277 (2.0 μg of protein loaded, 80°C for 5 min); 3 and 7, crude fimbriae of MPG1 (0.5 μg of protein loaded, 100°C for 5 min); 4 and 8, crude fimbriae of MPG1 (2.0 μg of protein loaded, 80°C for 5 min).

Immunologic reactions. Immunoblotting of the fimbrial proteins was carried out with PAbs directed to purified 41-kDa major fimbriae and to the 67-kDa minor fimbriae. The protein band of the wild type was shown to react with the 41-kDa-fimbria PAbs (Fig. 3A, lane 1). However, no reaction was seen in the protein band of MPG1 (Fig. 3A, lane 3). This indicated that the 41-kDa protein is not expressed by MPG1. This is consistent with the lack of a protein band in the 41-kDa region seen for wild-type but not MPG1 (Fig. 2). At 80°C under nonreducing conditions, the ladder-like pattern of the 41-kDa major fimbrial protein is revealed, suggesting that the antiserum made to the 41-kDa component reacts with determinants on the oligomeric forms of the major fimbriae as well as the monomeric forms (Fig. 3A, lane 2). Both the wild type and MPG1 expressed the 67-kDa protein (Fig. 3B, lanes 5 and 7). Furthermore, the 67-kDa fimbria from either strain shows a ladder-like pattern under nonreducing conditions, suggesting that the oligomeric forms share a determinant with the monomeric forms (Fig. 3B, lanes 6 and 8). The PAbs specific for the 67-kDa minor fimbriae were found to react strongly with crude fimbria preparations of the *fimA* mutant and the wild-type strain. These results indicated that the minor fimbria is different in both molecular weight and antigenicity from the 41-kDa major fimbriae of *P. gingivalis*. Moreover, the oligomers of the 41-kDa protein and 67-kDa protein are of different molecular sizes (Fig. 3, lanes 2 and 8).

Electron microscopy and immunogold labeling. Examination of fimbriae on the surface of the wild-type strain and MPG1 after negative staining revealed two distinct types of fimbriae. The wild-type strain has numerous long fimbriae (5 nm in width and 0.5 to 3.0 μm in length) and a small number of short fimbriae (Fig. 4A). On the other hand, MPG1 has a small number of short fimbriae (3 nm in width and 0.1 to 0.5 μm in length) (Fig. 4B).

Immunogold labeling demonstrated that the short fimbriae of MPG1 were labeled with PAbs against the 67-kDa minor fimbrial protein (Fig. 5A). The long fimbriae of the wild-type strain were not labeled with the same antibody; however, the short fimbriae of the wild-type strain were specifically labeled with the 67-kDa-fimbria PAbs (Fig. 5B). These results strongly

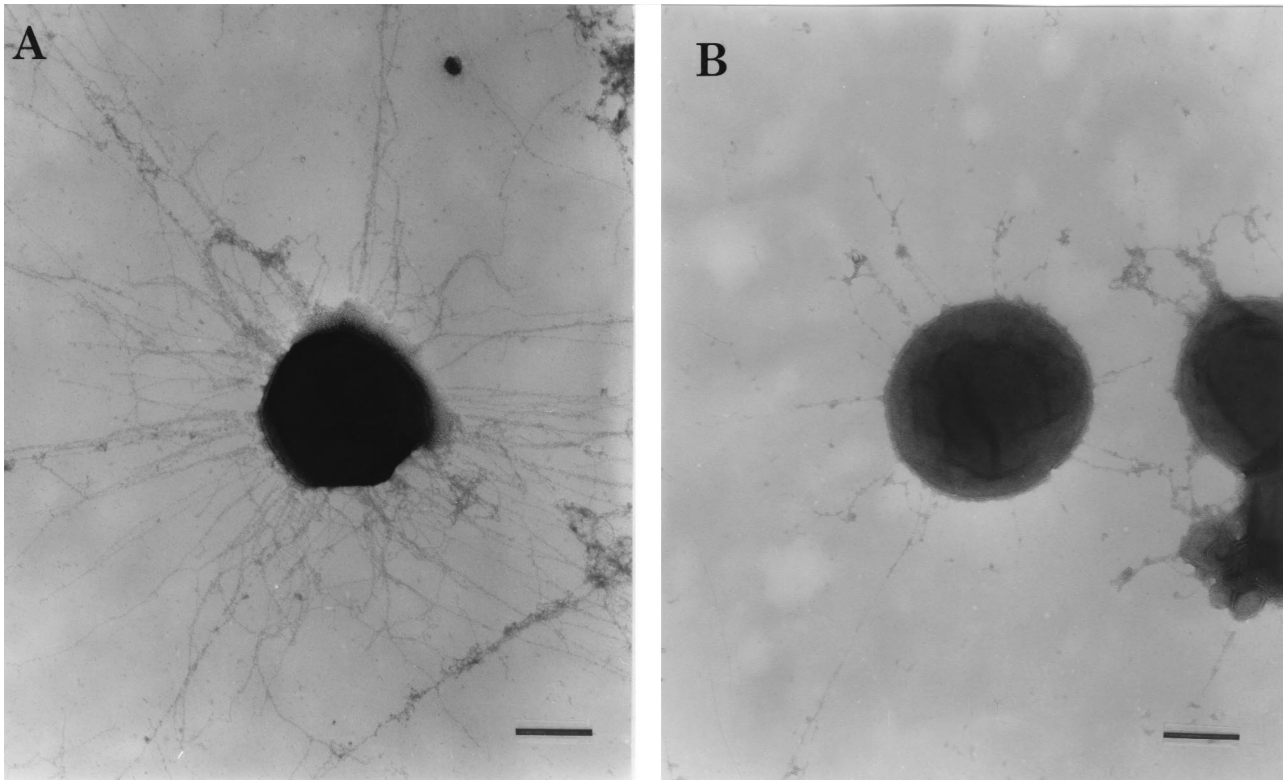

FIG. 4. Electron micrographs of negatively stained *P. gingivalis* ATCC 33277 and MPG1. Cells were cultured at 37°C anaerobically and negatively stained with 2% uranyl acetate. *P. gingivalis* ATCC 33277 possessed numerous long fimbriae on its surface (A), whereas MPG1 possessed thin, short fimbrial structures (B). Bars, 0.5 μm.

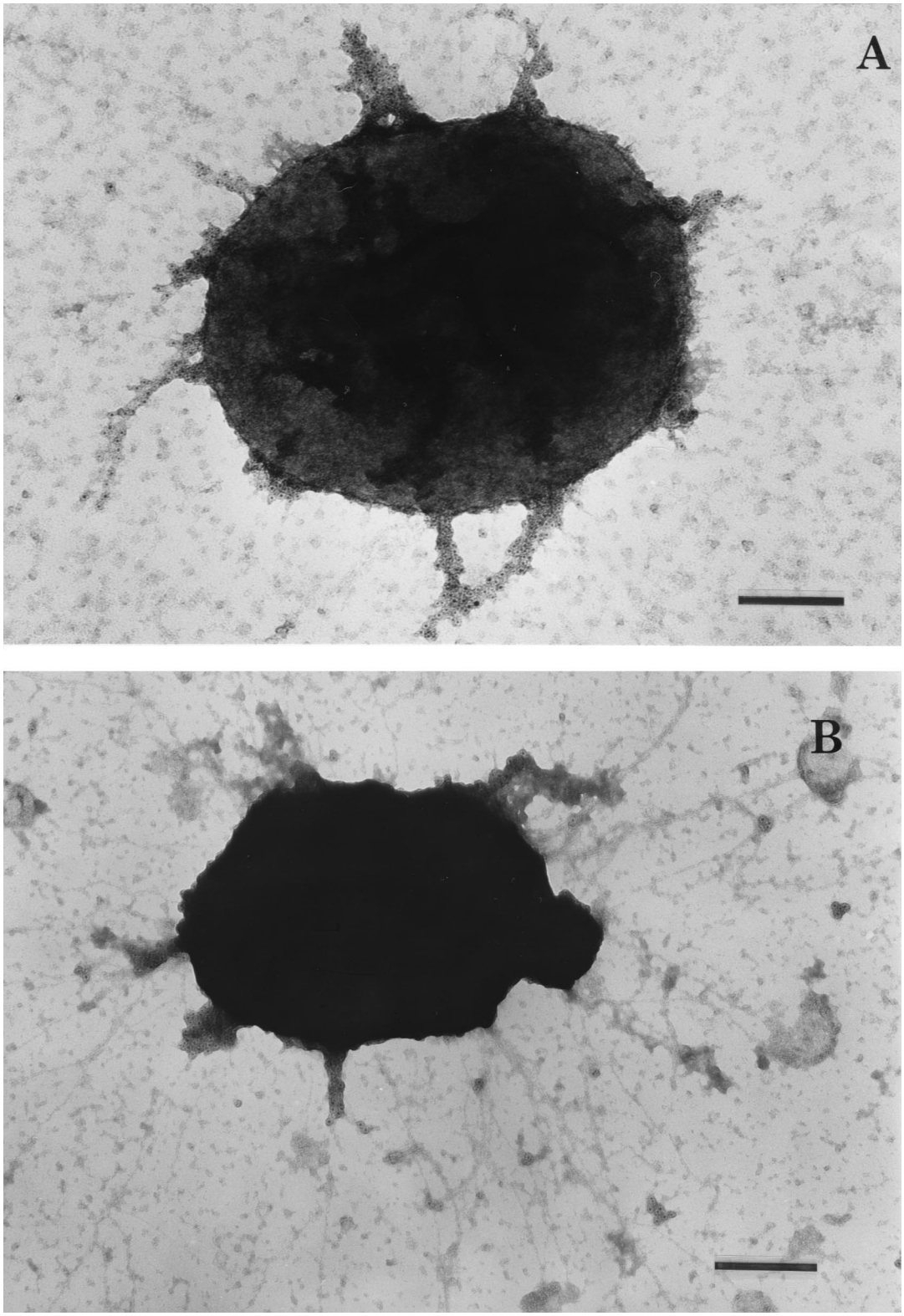

FIG. 5. Localization of the 67-kDa minor fimbriae on a whole cell by immunogold electron microscopy. *P. gingivalis* MPG1 (A) and ATCC 33277 (B) cells were incubated with the 67-kDa PAbs and then with 5 nm of colloidal gold-labeled goat anti-rabbit serum. Samples were prepared by negative staining with 2% uranyl acetate. The minor fimbriae on the surface of MPG1 were labeled with the 67-kDa PAbs (A). The long major fimbriae were not labeled; however, short minor fimbriae were specifically labeled with the 67-kDa PAbs (B). Bars, 0.2 μm.

indicate that both short and long fimbriae were present on the wild-type strain surface and that the short and long fimbriae are distinct antigenically.

Heat stability. Treatment of the 41-kDa fimbriae at 100°C for 5 min showed a 41-kDa band, and heating at 70°C did not cause full dissociation. After being heated at 80°C, the fimbriae were partially dissociated to monomers and various-size oligomers, creating a ladder-like pattern in SDS-PAGE. Moreover, even treatment of the 67-kDa protein at room temperature gave a ladder-like pattern (data not shown). We presume that this is due to the effect of molecular shape and incomplete binding of SDS. Thus, the two proteins differ in the extent of temperature-dependent dissociability.

Hemagglutinating activity of the minor fimbriae. *P. gingivalis* has hemagglutinating activity as well as the ability to adhere to other cells; therefore, we examined the hemagglutinating activity of the 67-kDa protein using sheep erythrocytes. The purified minor fimbriae failed to agglutinate sheep erythrocytes up to the concentration of 100 μg/ml (data not shown).

DISCUSSION

We have shown the presence of two different kinds of fimbriae on the *P. gingivalis* cell surface. This study, which characterizes the purification of minor fimbriae of *P. gingivalis*, establishes the existence of a second fimbria in *P. gingivalis* ATCC 33277 strain.

The *fimA* gene encodes the major subunit protein of *P. gingivalis* fimbriae (fimbrillin) (2). The *fimA* mutant, MPG1, was found to lack the long fimbriae; however, we observed that the *fimA* mutant strain, MPG1, has the short fimbria-like structure (7). Thus, we purified and characterized the minor fimbriae from MPG1. We have demonstrated that the minor fimbria is a distinct fimbria of *P. gingivalis* different from the previously reported *P. gingivalis* major fimbriae (FimA protein). The size and antigenic variation of the FimA protein has been reported for different fimbriated strains of *P. gingivalis* (16, 25, 34). All strains previously tested, except for strains W50, W83, and AJW 5, show a fimbrial protein band in the 41- to 49-kDa range, and the PAbs to fimbrillin monomers purified from *P. gingivalis* 2561 react with these protein bands (16). None of these molecular forms of FimA protein are identified as the minor fimbriae.

The minor fimbrial protein purified by using a DEAE Sepharose CL-6B column is a single band of 67 kDa in SDS-PAGE, and its antigenicity is different from the previously reported 41-kDa major fimbrial protein (Fig. 2 and 3). While the amino acid composition is similar to the FimA protein (Table 1), the isoelectric points differ. The isoelectric point of the FimA protein is 6.0 (40), while the more acidic 67-kDa fimbrial protein is estimated to have an isoelectric point of 4.7. Both PAbs produced against native fimbriae recognized epitopes present both on the oligomeric forms and on the monomeric forms as demonstrated by the immunoblot analysis. The 41-kDa major fimbrial protein was expressed only by the wild-type strain; however, the 67-kDa minor fimbrial protein was expressed by both strains (Fig. 3). These findings suggested that the PAbs react with unique determinants on FimA protein and minor fimbrial protein. Expression of fimbriae on the surface of MPG1 and the wild-type strain, ATCC 33277, was investigated by electron microscopy.

Hamada et al. (7) showed that a monoclonal antibody specific to the 41-kDa fimbriae bound only to the long fimbria-like projections of the wild-type strain. In contrast, no binding of the immunogold to the surface of MPG1 was observed. The immunoelectron-microscopic studies as well as immunoblot analysis show that the antigenicity of minor fimbriae is distinct from the 41-kDa major fimbriae.

The presence of two or more antigenically and morphologically distinct fimbriae other than type 1 fimbriae on the same *Escherichia coli* strain has been reported previously (14, 17, 21). Since the minor fimbriae appearing on MPG1 are produced together with numerous long fimbriae on the wild-type strain, it is tempting to speculate that the expression of the minor fimbriae is under the control of a gene regulatory system different from that for the long major fimbriae (FimA protein). Several genes are involved in the biosynthesis and expression of functional *E. coli* P and type 1 pili. These genes reside in clusters at different sites in the *E. coli* chromosome (11). The assembly and surface localization of pili requires multiple genes which are clustered together on the bacterial chromosome. For example, 11 genes clustered in an operon structure are involved in the biogenesis and expression of functional *E. coli* P pili (10). Recently, Yoshimura et al. (38) reported that the flanking regions of the *fimA-Pst*I fragment carrying the gene were cloned and examined for expression of genes on a fragment in the bacteriophage T7 RNA polymerase/promoter expression system. The *fimA* gene is located in a 10.4-kb *Pst*I segment of the *P. gingivalis* chromosome, in which several other genes, encoding 20-, 50-, 63-, and 80-kDa polypeptides have been assigned as the putative fimbria-associated genes. However, the association of these proteins with the *P. gingivalis* fimbriae is unclear.

P. gingivalis possesses at least two adhesins, the fimbriae and hemagglutinin. Both have been shown to be involved in bacterial attachment to human cells, autoaggregation and aggregation to other organisms (6). The *fimA* mutant retained a level of hemagglutinating activity similar to that of the wild-type strain, indicating that the *fimA* function does not directly affect the expression of hemagglutinating activity of *P. gingivalis* (7, 20). These results indicate that fimbriae are not required for binding to the erythrocyte surface, in agreement with other observations (39). The fimbriae (FimA) and hemagglutinin reported to date are considered to be physically distinct (22, 24). It has been known that the fimbriae of various species of microorganisms participate in hemagglutination (28). Therefore, we attempted to determine whether or not the minor fimbria has hemagglutinating activity; however, the 67-kDa minor fimbriae also failed to agglutinate sheep erythrocytes (data not shown). The function of the minor fimbria is not clear; however, it is a surface antigen and may play a role in host-bacterium interaction in periodontal disease.

The fimbriae of *P. gingivalis* have been studied extensively as a potential virulence factor and immunogen (6, 8, 13, 16, 39). In addition to the possibility that the minor fimbrial protein may be important in bacterial pathogenesis, the minor fimbrial structure on the surface of the *P. gingivalis* cell may be a virulence factor. Further investigation of the molecular events occurring during the interaction between *P. gingivalis* and host cells will be needed to elucidate the exact role of the minor fimbriae in the pathogenesis of periodontal disease.

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