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Purification and Characterization of Fimbriae Isolated from *Bordetella pertussis*

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Fimbriae were detached from *Bordetella pertussis* by mechanical shearing and purified by successive precipitations with ammonium sulfate, phosphate buffer (pH 6.0), and magnesium chloride. In each of these purification steps, the fimbriae aggregated into bundles as seen by electron microscopy. These aggregates could be disaggregated at pH 9.5. By electron microscopy, the purified fimbriae appeared as long filaments with a diameter of 5 nm. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of the purified fimbriae showed a single protein subunit with a molecular weight of 22,000. The purified fimbriae did not have hemagglutinating activity when assayed with several types of erythrocytes, and they were antigenically, chemically, and structurally distinct from the filamentous hemagglutinin of *B. pertussis*. The purified fimbriae were also identified as serotype 2 agglutinogens, since antibody to the purified fimbriae agglutinated *B. pertussis* strains serotyped as 1.2.4, 1.2.3, or 1.2.3.6 but did not agglutinate those serotyped as 1.3.6.

Bacterial fimbriae (pili) are filamentous, nonflagellar surface appendages that are composed of identical protein subunits. The presence of these structures is often associated with the ability of bacteria to agglutinate erythrocytes (13, 25, 27), to bind to other eucaryotic cells (4, 13, 25), and to cause disease (4). Since *Bordetella pertussis* has fimbriae (3, 5, 19, 29), it is possible that they are involved in the pathogenicity of this bacterium. *B. pertussis* is a human pathogen that causes disease (pertussis or whooping cough) after attachment and noninvasive colonization of the lumenal surface of respiratory epithelial cells (18, 34).

Since first proposed by Sato et al. (29), the filamentous hemagglutinin (FHA), which when purified appears as rod-shaped structures (2 to 3 by 40 to 100 nm) by electron microscopy (1, 5, 20, 29, 30), has been considered to be a fimbrial protein of *B. pertussis* (4, 5, 12). However, recent data do not support the fimbrial origin of FHA (3). Ashworth et al. (3) found that the amount of cell-associated FHA did not correlate with the amount of fimbriae present, and they also observed that fimbriae projecting from the cell surface could not be labeled with antibody to FHA but could be labeled with antibody to serotype 2 agglutinogen.

To better understand the relationship of FHA to fimbriae and to establish the role(s) of fimbriae in the biology of *B. pertussis*, the purification and characterization of fimbriae from *B. pertussis* was undertaken. The results described in this paper show that fimbriae of *B. pertussis* contribute to serotype 2 reactivity and are independent of FHA.

MATERIALS AND METHODS

Bacterial strains. *B. pertussis* 325, obtained from Alison A. Weiss, Stanford University, is a spontaneous mutant of strain Tohama phase I and was selected based on its more clear zones of hemolysis on Bordet-Gengou blood agar plates. Stationary culture of strain 325 in liquid Cohen-Wheeler medium (28) showed growth and lymphocytosis-promoting factor (LPF) production similar to those of Tohama I, but unlike Tohama I, no FHA was detected in the culture supernatant when assayed by enzyme-linked immunosorbent assay (ELISA) (28). *B. pertussis* 325 did not agglutinate goose erythrocytes, and extraction of the cells with 50 mM Tris hydrochloride buffer (pH 8.0) containing 0.5 M NaCl detected FHA by ELISA; however, the amount was less than 1% of the FHA extracted from an equivalent amount of Tohama I. Strain 325 was typed as an agglutinogen serotype 1.2.4, which is the same as Tohama I. Other strains of *B. pertussis*, used in the antibody agglutination assay, were from our culture collection at the Office of Biologics Research and Review.

Growth of cells for isolation of fimbriae. *B. pertussis* 325 was held as a freeze-dried culture until grown at 35°C on plates of Bordet-Gengou agar medium (Difco Laboratories, Detroit, Mich.) containing 15% defribinated rabbit blood. The cultures were grown for 48 to 72 h and subcultured on plates of Bordet-Gengou blood agar for 20 h. Cells were transferred to 500-ml Erlenmeyer flasks containing 200 ml of Cohen-Wheeler medium (7), and the flasks were incubated for 22 h with shaking (150 rpm) at 35 to 37°C. This growth was used to inoculate 1.3 liters of Cohen-Wheeler medium in 2.8-liter Fernbach flasks to a final absorbance at 650 nm of 0.07. Final growth was for 24 to 48 h at 35 to 37°C with gyratory shaking (120 rpm). Cells were harvested when cultures reached an absorbance at 650 nm of about 2.

Isolation and purification of fimbriae. All steps were conducted at 4 to 8°C unless stated otherwise. Bacteria were collected by centrifugation for 30 min at 5,000 × *g*. The bacterial pellet was resuspended to 20% (wt/vol) in 0.01 M sodium phosphate-buffered saline (PBS; pH 7.4) and blended at a speed setting of 6 in a Sorvall Omni-mixer in an ice bath for three 5-min periods, with 5-min cooling intervals in between. Cells were pelleted twice by centrifugation for 20 min at 10,000 × *g*. The supernatant was precipitated over 30 min with 2.4 g of ammonium sulfate per 10 ml. After centrifugation (27,000 × *g* for 20 min), the pellet, was

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dissolved in 50 mM Tris hydrochloride buffer (pH 9.5) with 1/10 of the volume of the supernatant after blending and dialyzed for 16 h against 400 volumes of the same buffer. All subsequent centrifugations were done at 27,000 × *g* for 20 min. Insoluble material was removed by centrifugation, and the fimbriae in the supernatant were precipitated by dialysis for 16 h against 500 volumes of 50 mM sodium phosphate buffer (pH 6.0). After centrifugation, the precipitate was dissolved in the above Tris hydrochloride buffer with 1/20 of the volume of the original supernatant. After adjustment of the pH to 7.4 to 8.0, fimbriae were precipitated over 16 h with 0.1 M $MgCl_2$. The precipitate was collected by centrifugation, dissolved in 50 mM Tris hydrochloride (pH 9.5) with one-half of the volume of the previous step, and stored at −70°C in 50 mM Tris hydrochloride buffer (pH 8.0) containing 0.8% (wt/vol) NaCl.

Electrophoresis. Sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis was done by using 1.5-mm-thick slab gels by the method of Laemmli (15). Protein samples containing 2% SDS and 2% mercaptoethanol were heated for 5 min in a boiling water bath and electrophoresed at 15 mA per gel for 7 to 8 h. Gels were fixed in methanol-acetic acid and stained with Coomassie brilliant blue R250.

Protein estimation. Protein was routinely determined by the method of Lowry et al. (16) with bovine serum albumin as the standard. Amino acid analysis of the purified fimbriae indicated that the Lowry procedure underestimated the amount of fimbrial protein by a factor of 1.7. Therefore, protein estimates measured with the Folin reagent were multiplied by 1.7 to obtain the accurate value.

Amino acid analysis. After dialysis against water and drying in a vacuum centrifuge (Model SVC-100; Savant Instruments, Inc., Hicksville, N.Y.), the protein was hydrolyzed in 4 N methanesulfonic acid containing 0.2% tryptamine (32) or oxidized with performic acid as described (23). After hydrolysis, the amino acid composition was determined with a Beckman 121 M analyzer (Beckman Instruments, Inc., Fullerton, Calif.) (23). Calculations were made on a mole percent basis of the individual amino acids as detected by the analyzer without corrections for losses during hydrolysis.

Detection of FHA and LPF. The ELISA for the detection of LPF and FHA was as described (28). The wells of microtiter plates were coated with goat anti-LPF or anti-FHA antibody made monospecific by affinity chromatography. The same antibody conjugated with alkaline phosphatase was used to detect bound antigen. Purified LPF and FHA were used as standards (28).

Detection of endotoxin. Purified fimbriae were assayed for endotoxin content by the *Limulus* amoebocyte lysate assay, which was able to detect the U.S. reference endotoxin EC-4 at 0.125 ng/ml (11). Protein samples were diluted with pyrogen-free water and heated at 90°C for 15 min before assay. Percent concentrations are given as weight percent.

Preparation of goat antibody. For the preparation of goat anti-fimbria antibody, 240 μg of fimbriae was emulsified with complete Freund adjuvant (Difco) and injected intradermally. After 4 weeks, a second dose of 190 μg emulsified with incomplete Freund adjuvant (Difco) was injected intradermally, and the animals were bled 2 weeks later. The gamma globulin fraction of the sera was prepared by ammonium sulfate precipitation and dialysis (10).

Preparation and assay of mouse antibody. Mouse antibody to purified fimbriae, FHA, or glutaraldehyde-inactivated (21) LPF was prepared by the subcutaneous injection of 10 μg of protein emulsified with complete Freund adjuvant into 4- to 5-week-old N:NIH(S) mice (National Institutes of Health, Bethesda, Md.). A second subcutaneous injection of 10 μg was given 4 weeks later with incomplete Freund adjuvant. Mice were bled two weeks after the last injection. Mice that were used to obtain control serum received two injections of PBS (pH 7.4) without adjuvant.

Indirect ELISA was used to assay the mouse antibodies to the appropriate antigens as described (22). The wells of microtiter plates were coated with purified antigen at 5 μg of protein per ml, and the conjugate used to detect antibody bound to antigen was goat anti-mouse immunoglobulin G (Cappel Laboratories, Cochranville, Pa.) coupled to alkaline phosphatase.

Hemagglutination assay. The various animal erythrocytes were obtained in Alsevier solution (Truslow Farms, Chestertown, Md.). Human type O erythrocytes were from Biological Corp. of America, West Chester, Pa. All erythrocytes were washed three times with PBS (pH 7.4) and resuspended to 0.7% (vol/vol) in PBS. The assay was as described (28). Test samples were diluted in 0.05-ml volumes of PBS (pH 7.4) in microtiter plates. After the addition of an equal volume of the erythrocyte suspension, the plates were shaken for 1 min on a Micro-Shaker II (Dynatech Laboratories, Inc., Alexandria, Va.) and incubated at 23°C for 1 h. Units of hemagglutinin in the undiluted sample were expressed as the reciprocal of the final dilution after the addition of erythrocytes that caused complete agglutination.

Agglutinating antibody assay. A standard assay (17) for the microagglutination of *B. pertussis* was used to detect agglutinating antibody. Briefly, test samples were diluted in 0.05-ml volumes of PBS (pH 7.4) in microtiter plates. After the addition of an equal volume of bacterial suspension, the plates were shaken, sealed, and incubated at 35°C overnight. Activity was expressed as the reciprocal of the highest dilution that gave complete agglutination.

Electron microscopy. *B. pertussis* 325 and purified fimbriae were prepared for electron microscopy by negative staining. Cell suspensions were applied to 300-mesh copper grids coated with a Parlodion film (Mallinkrodt, Inc., St. Louis, Mo.). The grids were fixed with 2% glutaraldehyde for 2 min, washed twice with distilled water, and stained for 15 to 30 s with 1% phosphotungstic acid (pH 6.0). Microscopy of whole cells was done with a Hitachi H-500 transmission electron microscope at 75 kV.

Purified fimbriae (10 μl) were applied to a carbon film supported by a film of nitrocellulose on the electron microscope grid. The specimen was washed twice with double-distilled water and stained for 10 s with 1% aqueous uranyl acetate; excess liquid was removed with filter paper, and the grid was allowed to air-dry. These grids were observed in a Philips EM-400T transmission electron microscope operated at 80 kV with a liquid nitrogen anticontamination device in routine use (33). Micrographs of purified fimbriae were recorded at nominal magnifications of ×46,000 and calibrated according to the 2.49-nm periodicity of Olive-T crystallites (14).

RESULTS

Isolation and purification of fimbriae. The fimbriae were removed from the bacteria by homogenization, and after precipitation of the proteins in the supernatant with ammonium sulfate, the fimbriae increased in purity (Fig. 1, lane C). Precipitation of the fimbriae at pH 6.0 and ensuing precipitation with 0.1 M $MgCl_2$ yielded highly purified fimbriae that gave one major protein band after SDS-polyacrylamide gel electrophoresis (Fig. 1, lanes D and E). The yield of purified

fimbriae was about 1.5 mg per 23 g wet weight of cells (10 liters of culture).

SDS-polyacrylamide gel electrophoresis of the purified fimbriae at gel concentrations of 10, 12, and 14% also showed a single protein. A Ferguson Plot (35) of the relative mobility of this subunit versus the different gel concentrations gave a slope which, at a gel concentration approaching zero, intersected the slopes of the similarly analyzed standards listed in Fig. 1. This indicated that the fimbrial subunit and the standards were binding the same amount of SDS per unit weight (35). The molecular weight of the fimbrial subunit was calculated relative to the above standards by using relative mobilities in 14% gels. The molecular weight was estimated to be 22,000, and the amino acid composition of the fimbriae was determined based on a subunit molecular weight of 22,000 (Table 1).

Electron microscopy. *B. pertussis* 325 contained fimbriae with a diameter of about 5 nm projecting from the bacterial surface (Fig. 2). Electron microscopic analysis of the purified fimbriae revealed long filamentous structures with a diameter of about 5 nm (Fig. 3a), which formed bundles of longitudinally aggregated fimbriae when precipitated at pH 6.0 (Fig. 3b). The fimbriae also formed these bundles when precipitated with ammonium sulfate and $MgCl_2$ (data not shown).

Electron micrographs indicated that the purified fimbrial samples contained very small amounts of membrane material. This is consistent with assays for endotoxin by *Limulus* amoebocyte lysate (11). When eight individual lots of purified fimbriae were assayed at 85 μg of protein per ml, endotoxin was detected at 0.15 to 1.5% with a mean of 0.74 ± 0.64% (standard deviation).

Hemagglutinating activity. The purified fimbriae did not agglutinate several different types of erythrocytes (Table 2) when assayed at 170 μg of fimbrial protein per ml (the highest concentration tested). With these same erythrocytes, purified FHA caused agglutination at protein concentrations of 0.012 to 0.10 μg/ml, and the LPF of *B. pertussis* caused hemagglutination at 0.07 to 1.1 μg of protein per ml (Table 2).

TABLE 1. Amino acid composition of purified fimbriae from *B. pertussis* 325

Amino acid	Content (mol%)[a]	Assumed no. of residues per fimbrial subunit[b]
Aspartic acid	11.1	21
Threonine	11.3	25
Serine	7.4	19
Glutamic acid	8.9	15
Proline	6.1	14
Glycine	11.5	44
Alanine	9.7	30
Cysteine[c]	1.0	2
Valine	6.8	15
Methionine	1.9	3
Isoleucine	4.1	8
Leucine	4.5	9
Tyrosine	3.6	5
Phenylalanine	2.1	3
Lysine	6.5	11
Histidine	0.8	1
Arginine	3.0	4
Tryptophan	Not detected	0

[a] Average of three determinations.
[b] Total no., 229; molecular weight, 21,933; calculated based on a molecular weight of 22,000.
[c] Determined as cysteic acid after performic acid oxidation.

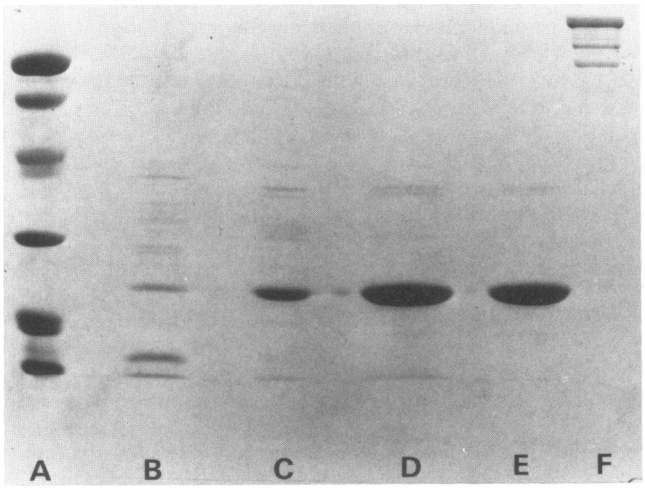

FIG. 1. SDS-polyacrylamide gel electrophoresis. Electrophoresis was conducted from the top (cathode) to the bottom (anode) in 10% polyacrylamide gels. Lanes: A, 10 μg each of the molecular weight standards (Bio-Rad Laboratories, Richmond, Calif.) phosphorylase B (92,500), bovine serum albumin (66,200), ovalbumin (45,000), carbonic anhydrase (31,000), soybean trypsin inhibitor (21,500), and lysozyme (14,400); B, 20 μg of protein of the supernatant after blending *B. pertussis*; C, 20 μg of ammonium sulfate precipitate; D, 20 μg of precipitate after dialysis at pH 6.0; E, 20 μg of protein (34 μg based on amino acid analysis) of $MgCl_2$-precipitated fimbriae; F, 20 μg of FHA purified as described previously (28).

The lack of hemagglutinating activity with the purified fimbriae could mean that the fimbriae were not contaminated with FHA or LPF. This was more rigorously tested by ELISA (28). When assayed with anti-FHA, FHA was not detected at a sensitivity of 6 ng of FHA per ml in four separate purified fimbrial samples assayed at 180 to 240 μg of protein per ml. Therefore, the fimbriae contained <0.003% FHA. Not only did the purified fimbriae not react with affinity-purified goat anti-FHA antibody bound to microtiter plates, but it was also found by ELISA that mouse anti-fimbria antibody did not react with wells coated with FHA, and that mouse anti-FHA antibody did not react with wells coated with fimbriae (Table 3). These results showed that FHA and the purified fimbriae are antigenically distinct, which is consistent with the distinct nature of the fimbriae and FHA as revealed by SDS gel analysis. (Fig. 1, lanes E and F). Assay of the purified fimbriae (190 μg) with anti-LPF antibody by ELISA (28) found less than 0.005% LPF in some fimbrial preparations and from 0.007 to 0.015% LPF in other samples of purified fimbriae.

Relationship of fimbriae to serotype agglutinogens. The immunization of mice or goats with purified fimbriae stimulated the production of the antibody that agglutinated *B. pertussis* (Tables 3 and 4), whereas the immunization of mice with FHA or inactivated LPF did not (Table 3). *B. pertussis* strains containing agglutinogen serotype 2 (serotypes 1.2.4, 1.2.3, or 1.2.3.6) were agglutinated by goat or mouse anti-fimbria antibody at relatively high dilutions of the antisera (Table 4). However, *B. pertussis* strains serotyped as 1.3.6 were not agglutinated by the mouse anti-fimbria antibody or were agglutinated only at high concentrations of goat anti-fimbria antibody.

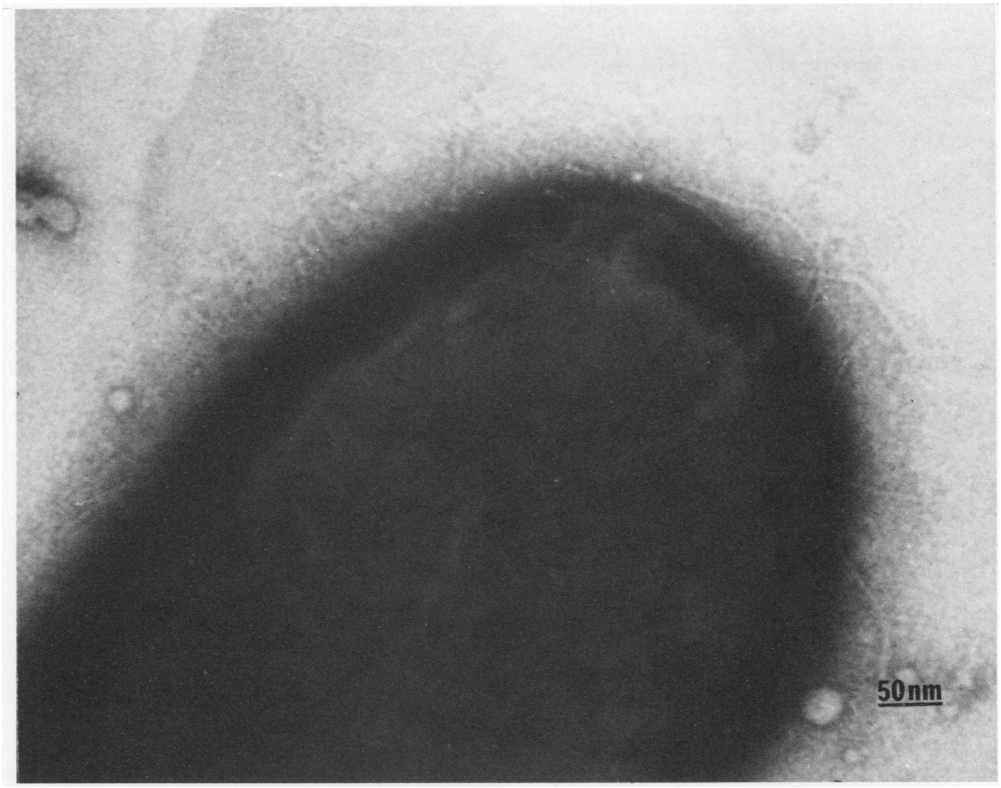

FIG. 2. Electron micrograph of *B. pertussis* 325. Cells were grown for 22 h in 200 ml of Cohen-Wheeler medium as described in the text. The diameter of each fimbria projecting from the cell surface was about 5 nm. Magnification, ×168,000.

DISCUSSION

A relatively simple procedure for obtaining highly purified fimbriae from *B. pertussis* is described. The purified fimbriae were characterized as nonhemagglutinating serotype 2 agglutinogens with a subunit molecular weight of about 22,000. The purification procedure relied on the aggregation of the fimbriae by ammonium sulfate, by phosphate buffer (pH 6.0), and by magnesium chloride. Repeated precipitations with either ammonium sulfate or magnesium chloride have

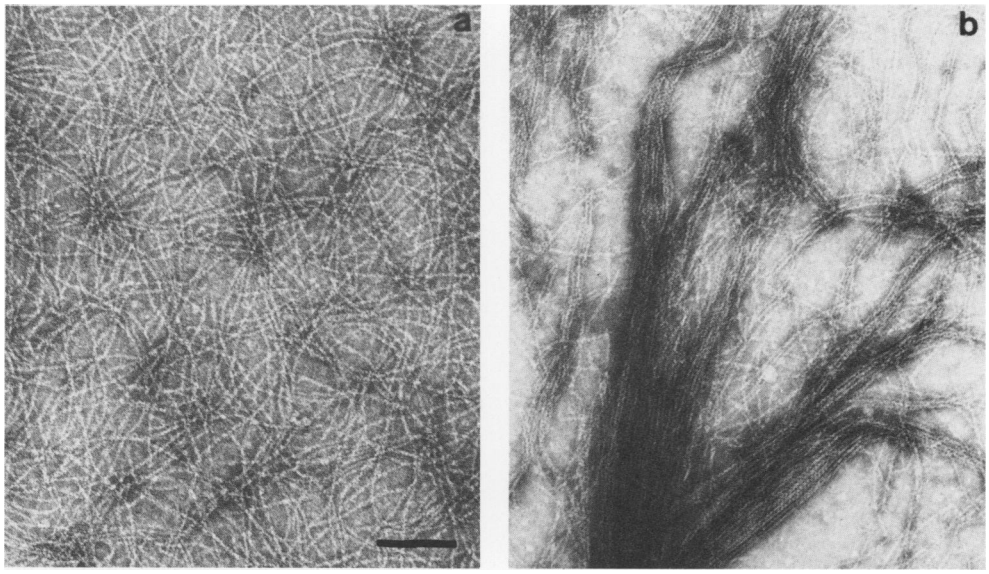

FIG. 3. Electron micrographs of purified fimbriae of *B. pertussis* 325. The same preparation of purified fimbriae at 0.2 mg of protein per ml was observed (a) in 50 mM Tris hydrochloride buffer (pH 8.0) and (b) after dialysis against 50 mM sodium phosphate buffer (pH 6.0), which produced paracrystalline bundles of fimbriae. Bar, 100 nm; magnification, ×100,000.

TABLE 2. Absence of hemagglutinating activity with purified fimbriae

Erythrocytes	Minimum concn for hemagglutination[a] (μg of protein per ml)		
	Fimbriae	FHA[b]	LPF[b]
Goose	>170	0.012	0.07
Pigeon	>170	0.025	0.07
Chicken	>170	0.025	0.28
Chick	>170	0.025	0.56
Turkey	>170	0.050	0.56
Human, type O	>170	0.050	1.12
Sheep	>170	0.100	1.12

[a] Hemagglutination was assayed as described in the text.
[b] FHA and LPF of *B. pertussis* were purified as described before (28).

been used successfully by others to obtain purified fimbriae of *Escherichia coli* (6, 27, 31). Under the conditions we used, the fimbriae were not quantitatively precipitated by dialysis at pH 6.0 or by magnesium chloride, and methods to improve the yield of purified fimbriae are being explored. However, the goal of isolating purified fimbriae in sufficient quantity for further study was accomplished. It should also be mentioned that the purification procedure was used successfully to obtain purified fimbriae from *B. pertussis* Tohama I (serotype 1.2.4) grown in stationary conditions (28; data not shown). The yield obtained was similar to that obtained from strain 325 grown in shake culture.

Ashworth et al. (3) were the first to suggest that fimbriae of *B. pertussis* were serotype-specific agglutinogens. They observed by electron microscopy that antibody to purified agglutinogen 2 attached quite well to fimbriae of *B. pertussis* serotypes 1.2 and 1.2.3 but reacted to a much lesser extent with fimbriae of serotype 1.3 cells. They also reported that in SDS-polyacrylamide gel electrophoresis, purified agglutinogen 2 gave a single band with a molecular weight of 22,000.

Blom et al. (5) suggested that, based on electron microscopy data, subunits of fimbriae of *B. pertussis* are connected by fragile regions that give rise to the short, filamentous structures observed in preparations of FHA (1, 5, 21, 29, 30). Our biochemical data do not support this concept but do support other data suggesting that fimbriae of *B. pertussis* are distinct from the filamentous hemagglutinin (3). Purified FHA had hemagglutinating activity with a variety of erythrocytes, whereas the purified fimbriae lacked hemagglutinating activity (Table 2). Antibody specific for FHA did not react with purified fimbriae in an ELISA assay, and mouse anti-fimbria antibody did not react with FHA. In addition, the SDS-polyacrylamide gel pattern of FHA was quite distinct from that of the purified fimbriae (Fig. 1). The amino acid composition of the purified fimbriae (Table 1) was also different from that of FHA (28). Thus, the purified agglutinogen 2 fimbriae were distinct from purified FHA on the basis of biological activity, antigenicity, structure, and chemistry.

TABLE 3. Agglutination and ELISA titers of mouse antibody preparations[a]

Immunogen	Agglutination titer[b]	ELISA titer[c]		
		Fimbriae	FHA	LPF
Fimbriae	3,200–6,400	409,600	<100	NT[d]
FHA	4	<100	128,000	NT[d]
LPF	4	<100	800	256,000
None	4	<100	<100	100

[a] The mouse serum was a pool from ten mice and was mixed before assay for 1 min at 23°C with an equal volume of 25% kaolin (0.1 to 4 μ; Sigma Chemical Co., St. Louis, Mo.) in PBS (pH 7.4) and centrifuged. This reduced nonspecific agglutination but did not affect ELISA titers.
[b] Agglutination of *B. pertussis* was done with strain 460 (serotype 1.2.3.4.6) as described before (17).
[c] ELISA titers are expressed as the reciprocal of the dilution that gave an optical density at 405 nm of two standard deviations above control wells that were coated with the appropriate antigen but did not receive the test antibody.
[d] NT, Not tested.

TABLE 4. Relationship of agglutinating activity of antibody against the purified fimbriae to serotype agglutinogens of *B. pertussis*

B. pertussis strain	Agglutinogen serotype[a]	Agglutination titer		
		Goat anti-fimbria antibody[b]	Mouse anti-fimbria antibody[c]	Rabbit anti-whole *B. pertussis*[d]
Tohama I	1.2.4	25,600	6,400	51,200
Tohama 325	1.2.4	6,400	3,200	12,800
Oklahoma 1	1.2.4	12,800	3,200	25,600
114	1.3.6	400	<200	25,600
432	1.3.6	200	<200	6,400
Oklahoma 2	1.3.6	200	<200	6,400
167	1.2.3.6	6,400	3,200	102,400
104	1.2.3	25,600	3,200	51,200
Tohama III	Not typable[e]	400	<200	400
423 IV	Not typable[e]	400	<200	400

[a] Each strain of *B. pertussis* was typed by the microagglutination assay (17) with typing sera prepared by G. Eldering (9).
[b] Whole serum was used.
[c] Mouse serum was a pool from 10 mice and was mixed with kaolin before assay as described in Table 3, footnote *a*. This reduced the nonspecific agglutination of phase III or IV *B. pertussis* by a factor of 4. Control mouse serum or mouse serum with antibody to FHA had agglutination titers of <20 with all of the above strains of *B. pertussis*.
[d] Rabbit antibody to whole cells of *B. pertussis* was obtained from the Michigan State Department of Health and was concentrated and purified with sodium sulfate (24).
[e] Not agglutinated with typing sera.

Our data also indicate that agglutinogens and hemagglutinins of *B. pertussis* are distinct molecules. The purified fimbriae elicited the production of antibody that agglutinated *B. pertussis*, and thus they were agglutinogens, but they were not hemagglutinins (Tables 2 and 3). However, purified FHA and LPF agglutinated erythrocytes but did not elicit the production of agglutinating antibody. The fact that the purified fimbriae were not hemagglutinins does not necessarily mean that fimbriae on the bacterial surface do not cause agglutination of erythrocytes, since this biological activity of the fimbriae may have been inactivated during the purification process. However, *B. pertussis* Tohama 325, from which type 2 fimbriae were purified, is deficient in the production of FHA and does not cause hemagglutination (data not shown). These data suggest that type 2 fimbriae on the bacterial surface do not function as hemagglutinins.

Both FHA and LPF have been identified as protective antigens in animal models for pertussis (2, 8, 21, 26). The isolation of pure fimbriae from *B. pertussis* should facilitate the study of the role of fimbriae in adhesion, pathogenesis, and immunity, and it should also help in classifying fimbrial serotypes.

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