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Outer Membrane Proteins of *Pseudomonas aeruginosa* Serotype Strains

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The basis of differentiation of *Pseudomonas aeruginosa* into the 17 serotypes of the International Antigenic Typing Scheme is differences in an outer membrane glycolipid, lipopolysaccharide (LPS). This observation, together with the high toxicity and pyrogenicity of LPS, has led to the search for alternative "common" antigens for use as vaccines. The relation between the major outer membrane proteins of serotype strains was studied in three ways. By demonstrating conservation of outer membrane protein receptors for bacteriophages, a high similarity of outer membrane protein patterns on sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) gels, and antigenic cross-reactivity of major outer membrane proteins, it was shown that the major outer membrane proteins were closely related. Radioiodinated antibodies to outer membrane proteins interacted with outer membrane proteins after SDS-PAGE separation and electrophoretic blotting of the separated outer membrane proteins onto nitrocellulose paper. This demonstrated that major outer membrane proteins F, H2, and I were antigenically related in all serotype strains.

Pseudomonas aeruginosa is an opportunistic gram-negative pathogen which has become a major cause of nosocomial infections. It owes its prominence, in part, to its high intrinsic resistance to antibiotics. Treatment with antibiotics of patients with *Pseudomonas* bacteremia is difficult and often unsuccessful [1]. Thus, there is considerable interest in the development of immune therapy through either passive or active immunization. A number of vaccines have been produced [2] but they have generally been of moderate to high toxicity (since they rely substantially on LPSs as immunogens) and induce immunity to only a limited range of serotypes of *P. aeruginosa*. Therefore, it seems worthwhile to investigate the potential of alternative vaccines, including outer membrane proteins, which have been studied recently in other organisms [3-6]. We have undertaken a comprehensive study of the outer membranes of *P. aeruginosa* type PAO1. Hancock and Carey [7] have demonstrated the existence of between six and nine major outer membrane proteins (depending on the growth conditions) and developed a set of guidelines for nomenclature and electrophoretic analysis of these proteins. In other studies our laboratory has characterized the roles in outer membrane function and structure of five of these polypeptides [8-11]. In this paper we demonstrate, using a variety of techniques, that outer membrane proteins are highly conserved in all 17 serotypes of *P. aeruginosa* and provide evidence that specific major outer membrane proteins from strains of different serotypes cross-react antigenically.

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Materials and Methods

Bacterial strains and growth conditions. *P. aeruginosa* PAO1 (strain H103) (Dr. A. Kropinski, Queen's University, Kingston, Ontario, Canada) was used as a standard strain for the isolation of major outer membrane proteins and for the production of antisera to outer membranes. A set of 17 serotype-specific strains were obtained from Dr. P. Liu, University of Louisville, Louisville. These strains were representatives of the International Antigenic Typing Scheme (IATS; commercially marketed by Difco Laboratories, Detroit), which contains as subsets the type strains from all other commonly used *P. aeruginosa* serotyping systems. Relations between the various systems have been described [12, 13]. The strains were named as follows: type 1 (ATCC 33348), type 2 (ATCC 33349),

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type 3 (ATCC 33350), type 4 (ATCC 33351), type 5 (ATCC 33352), type 6 (ATCC 33354), type 7 (ATCC 33353), type 8 (ATCC 33355), type 9 (ATCC 33356), type 10 (ATCC 33357), type 11 (ATCC 33358), type 12 (ATCC 33359), type 13 (ATCC 33360), type 14 (ATCC 33361), type 15 (ATCC 33362), type 16 (ATCC 33363), and type 17 (ATCC 33364). In addition, a mucoid isolate from a cystic fibrosis patient, P1, and a spontaneous nonmucoid revertant were obtained from Dr. D. Speert, Children's Hospital, Vancouver, British Columbia, Canada.

Cultures were maintained on 1% (wt/vol) proteose peptone (no. 2; Difco) agar and used after growth in proteose peptone broth to an OD at 600 nm of ~0.5–0.8.

Bacteriophage studies. All methods used in the handling of bacteriophages have been described [14]. Phages were characterized using a pilus-deficient derivative of *P. aeruginosa* PAO1, AK1144, and an LPS-altered (rough) strain, AK43, both obtained from Dr. Kropinski.

Phages were obtained from the following sources: 2, 7, 21, 44, 68, 109, 352, 1214, C21, F7, F8, F10, 119X, and M6 from T. L. Pitt (Public Health Laboratory, London); G101, F116, D3c⁺1⁺, and D3c⁻1⁺ from T. Iijima (Institute for Fermentation, Osaka, Japan); PLS27 and E79 from A. Kropinski; PB1 and B39 from D. E. Bradley (Memorial University, St. John's, Newfoundland, Canada); S1 from R. Warren (University of British Columbia, Vancouver); and 176p from J. D. Piguet (Institute of Hygiene, Geneva). These phages were purified from single plaques using H103 as a host strain. All other phages were isolated in our laboratory as host range mutants of phages which plated poorly on pilus- or LPS-deficient strains. The phages that were selected for ability to form plaques on AK1144 (pilus-deficient) cultures were B6B, B6C, and B6D (independent isolates derived from 352), B9F (from M6), B5A (from 119X), C7B (from 176p), B1A (from F7), C3A (from C21), and A8A (from 68). B7A was derived from 1214 and selected on AK43 (an LPS-altered rough strain).

Phages M6, B39, and 119X were characterized as pilus-specific by their inability to form plaques on pilus-deficient strains. Phages 44, 109, F8, E79, 1214, PB1, S1, 362, and C3A were characterized as *P. aeruginosa* type PAO1 smooth LPS-specific because they failed to form plaques on AK43 and other LPS-altered strains and could be shown to adsorb to purified LPS. Phage PLS27 has been characterized by Jarrell and Kropinski [15] as specific for PAO rough core and does not form plaques on smooth strains. Phages 7, 21, 68, F10, C21, F116, G101, B6B, B6C, B9F, D3c⁻1⁺, B1A, and A8A formed plaques well on LPS-altered and pilus-deficient strains and did not adsorb to LPS; thus, they appear to have protein receptors. Phages 2, 119X, D3c⁺1⁺, B5A, B7A, and C7B formed plaques well on pilus-deficient and wild-type strains but poorly on LPS-altered strains and they did not adsorb to LPS; thus, they may have LPS or LPS-associated protein receptors.

Outer membrane isolation and protein purification. Outer membranes were isolated by the one-step procedure described [7]. The method of isolation of protein F was exactly as detailed [9]. Purified protein F contained no other major outer membrane proteins as judged by SDS-PAGE [9] and titration against specific monoclonal antibodies to protein H2 and specific polyclonal antisera to proteins I and H1, and it contained <0.07 mg of LPS/mg of protein [7].

Production of antisera. New Zealand white rabbits (University of British Columbia Animal Care Center, Vancouver) were injected four times at intervals of two weeks with 50 μg of strain H103 outer membranes in Freund's incomplete adjuvant to produce antiserum to outer membrane. For the immunologic detection of proteins on electrophoretic blots, this antiserum to outer membrane was purified by adsorption to a protein A–Sepharose affinity column (Sigma Chemical Co., St. Louis) followed by elution with 0.1 M glycine (pH 2.5). The eluate was then passed across an LPS-linked Sepharose 4B (Sigma) affinity column [16] to remove antibodies to LPS, followed by radioiodination of the unadsorbed antibodies to outer membrane protein using the chloramine T method (Amersham Corp., Arlington Heights, Ill.). Antisera to purified outer membrane proteins were produced by two to four injections, at intervals of one week, of 10 μg of purified outer membrane protein in 0.1 ml of Freund's incomplete adjuvant into BALB/c mice (Jackson Laboratories, Bar Harbor, Me.). The isolation of monoclonal antiserum MA1-8 to *P. aeruginosa* PAO1 O antigen has been described [17].

Titration of antibodies. Antisera were analyzed by an enzyme-linked immunosorbent assay (ELISA) [18], after coating of wells of polystyrene micro-

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titer plates with 50–100 μg of outer membranes, 20 μg of purified outer membrane proteins, or 50 μg of LPS.[1] Control experiments indicated that the amount of antigen was not limiting and that normal rabbit or mouse sera as sources of first antibodies, or heterologous outer membrane proteins as antigens (for example, *Edwardsiella tarda* outer membranes) produced only background responses.

Electrophoretic blotting procedure and immunologic detection of proteins. The outer membrane proteins of the 17 *P. aeruginosa* serotype strains, strain H103, and strain P1 were separated by SDS-PAGE, using a 14% acrylamide running gel containing 0.07 M NaCl after solubilization at 88 C for 10 min in solubilization-reduction mix containing 20 mM EDTA, 2% (wt/vol) sodium dodecyl sulfate, 5% (vol/vol) 2-mercaptoethanol, 10% (vol/vol) glycerol, and 62.5 mM Tris-HCl (pH 6.8) as previously described [7]. The proteins were then transferred to nitrocellulose paper by the Western blotting procedure as described by Towbin et al. [19] for SDS-containing gels. The blotting was done overnight at room temperature (∼23 C) using constant current of 1 A.

The electrophoretic blots were soaked in 3% fetal calf serum in saline (0.9% NaCl with 10 mM Tris-HCl; pH 7.4) for 2 hr at 37 C to saturate nonspecific protein binding sites. The blots were then rinsed in saline and incubated for 3 hr at 37 C in 10 ml of the above-mentioned ^{125}I-labeled rabbit antiserum to strain H103 outer membrane protein diluted in saline containing 3% fetal calf serum (final specific activity, 68 cpm/μg; antibody concentration, 11.8 mg/ml). The electrophoretic blots were subsequently washed with several changes of saline overnight. Blots obtained in the same way were stained with amido black (0.1% in 45% methanol with 10% acetic acid) for 5 min to identify the location of the various major outer membrane protein bands. The blots were dried and exposed to x-ray film (XAR-5; Eastman Kodak, Rochester, N.Y.) for five to seven days at −70 C.

Results

Phage susceptibility of P. aeruginosa type strains. It has been shown that most phages use outer membrane proteins [20, 21], LPS [14, 15], or pili [22] as their cellular receptors. Resistant strains to these phages usually have an alteration or loss of these cellular receptors. Thus, testing bacteriophage resistance patterns is a useful diagnostic procedure for demonstrating the presence or absence of such receptors on the cell surface. Our methods for partial characterization of the receptors for the 31 phages used in this study allowed separation of these phages into five major groups (table 1).

None of the bacteria studied appeared rough as tested either with a bacteriophage which used rough but not smooth LPS as its receptor, or by examination of gross colony morphology. With the exception of the type 11 strains, all typing strains were sensitive to one or more smooth LPS–specific phages (table 1). Indeed, nine of the 17 serotypes were sensitive to seven or more of the nine smooth LPS–specific phages. This indicated that there is some homology (that is, of the receptor site) in the O antigens of different serotyping strains despite the prior observation of significant chemical variations [23]. We further observed that only three of the 17 strains were sensitive to phages specific for PAO1 pili. This is in agreement with the finding that pili exhibit antigenic and chemical variations among strains, as demonstrated for *P. aeruginosa* and other bacteria [24].

Thirteen of the phages studied apparently had protein receptors, as judged by their inability to be neutralized by purified LPS and their ability to form plaques on LPS-altered (rough) and pilus-deficient mutants of *P. aeruginosa* PAO1. Similar criteria have been previously used to group protein-specific phages of *P. aeruginosa* [25] and *Escherichia coli* [14]. Although we did not identify the specific outer membrane receptor proteins for these phages, characterization of sensitivity to such phages allows general conclusions to be made about the distribution of their receptor proteins, because phages are thought to interact with chemically unique sites on specific cell surface molecules [20]. It should be noted, however, that lack of sensitivity to a bacteriophage does not necessarily imply the loss of the receptor protein, because single amino acid substitutions in such proteins have been shown to result in resistance for phages that normally use this protein as a receptor (for example, *E. coli* CR63 [20]). The results with the 13 phages that had uncharacterized protein re-

[1] R. P. Darveau and R. E. W. Hancock, "A New Procedure for the Isolation of Bacterial Lipopolysaccharides," manuscript submitted for publication.

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Table 1. Bacteriophage susceptibility of serotype-specific strains of *Pseudomonas aeruginosa* as determined using phages that were propagated on and characterized with *P. aeruginosa* type PAO1 strains.

Strain of *P. aeruginosa*	No. of phages to which strain is susceptible					
	Smooth LPS-specific phages (nine tested)	Rough LPS-specific phage (one tested)	Pilus-specific phages (three tested)	Possible smooth LPS receptor phages (six tested)	Possible protein receptor phages (13 tested)	Combined susceptibility (%)
H103	9	0	3	6	13	100
Pilus-deficient	9	0	0	5	13	90
LPS-altered	0	1	3	0	13	50
O serotype						
1	9	0	0	4	12	83
2	9	0	0	4	12	83
3	9	0	0	5	11	83
4	9	0	0	5	12	87
5	9	0	0	3	13	83
6	7	0	3	2	7	60
7	7	0	3	3	6	60
8	3	0	0	0	4	23
9	1	0	0	0	1	7
10	1	0	0	3	4	27
11	0	0	0	0	2	7
12	1	0	0	0	5	20
13	3	0	0	2	6	37
14	7	0	0	1	8	53
15	3	0	0	1	5	30
16	8	0	3	2	6	60
17	1	0	0	2	4	23
No. of phages reacting with 45% of the serotype strains	9	0	0	2	11	73

NOTE. Only phages forming visible plaques on cultures of the various strains have been included. LPS = lipopolysaccharide.

ceptors indicated that these receptor sites were quite well conserved. For example, phage 7 formed plaques with all 17 *P. aeruginosa* serotyping strains tested, whereas phages B9F and F116 formed plaques with 16 and 11 strains, respectively.

Taken overall, the results suggested that the surfaces of *P. aeruginosa* cells were moderately well conserved; nine of the 17 strains plated ≥53% of the phages screened.

Outer membrane protein patterns. We previously isolated outer membranes from *P. aeruginosa* PAO1 and characterized the outer membrane protein patterns [7]. Five to eight polypeptides were found to be "major" proteins (that is, present in >5,000 copies per cell) according to the growth conditions. We confirmed that our previously published outer membrane isolation methods [7] were successful for all serotyping strains and characterized the outer membrane protein patterns of these strains. To aid in identification of the polypeptides, we used our previous observations that the apparent mobility of outer membrane proteins on SDS-PAGE gels varied according to the solubilization conditions [7]. Thus, all strains studied had polypeptide bands that coelectrophoresed under all solubilization conditions with proteins E, F, H1, H2, and I, although the relative levels varied somewhat (figure 1). For example, the type 1, 5, and 7 strains had low but detectable levels of H2, whereas the type 6 and 7 strains were reduced in protein H1 and the type 5 and 7 strains had reduced protein I (data not shown). Protein G was observed in all strains except types 7, 14, and 16, whereas D2 was only missing (or altered) in the outer membranes of the type 5, 12, 15, and 17 strains. As we have studied only a single representative of each serotype, we are unable to say that outer membrane protein patterns in *P. aeruginosa* have serotype-related differences. However, our results suggest that the major outer membrane patterns are substantially similar throughout the 17 serotypes of *P. aeruginosa.*

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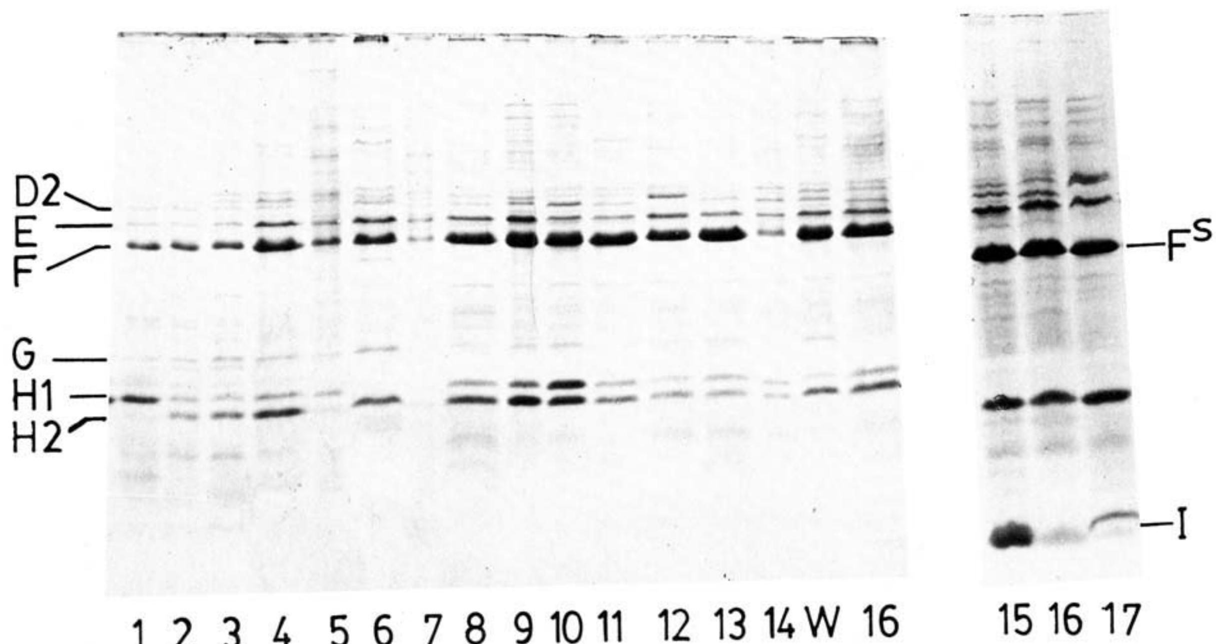

Figure 1. Sodium dodecyl sulfate-polyacrylamide gel electrophoretograms of outer membranes of *Pseudomonas aeruginosa* strain H103 and typing strains. The 17 lanes on the left-hand side were run after solubilization at 88 C for 10 min in solubilization-reduction mix (20 mM EDTA, 2% [wt/vol] sodium dodecyl sulfate, 5% [vol/vol] 2-mercaptoethanol, 10% [vol/vol] glycerol, and 62.5 mM Tris-HCl [pH 6.8]) [7]. Under these conditions protein I is not visible while the other major proteins migrate to their heat-modified positions [7]. The three lanes on the right-hand side were run after solubilization at 88 C for 10 min in the absence of 2-mercaptoethanol (causing protein F to move with a higher mobility to position F^S) and in the presence of 0.1 M $MgCl_2$ (causing protein I to appear in the gel and proteins G and H1 to shift to their unmodified positions [7], where they stain poorly). The positions of proteins D2, E, and H2 are also indicated. The numbers below the lanes refer to the serotypes, with the exception of our wild type H103 strain, which is labeled W. The molecular weights and characterizations of the indicated polypeptides have been described [7].

Titration of antisera to outer membranes. The reaction of antiserum to PAO1 outer membrane against the outer membranes of typing strains was one to three orders of magnitude lower against these heterologous outer membranes than against the homologous PAO1 outer membranes (table 2). Because this serum had quite a low titer (10^4) of antibodies to LPS, as judged using purified PAO1 LPS as an antigen (table 2), it seems that at least some of the differences in the ELISA titers of the serotype strains must be due to antibodies directed against non-LPS (that is, protein) antigens. For example, an antiserum to protein F reacted to widely varying extents with the outer membranes of the serotype strains (table 2), although it is of some significance that this antiserum was able to interact with all of the serotypes. Similar results were obtained with antisera to other purified and partially purified outer membrane proteins (data not shown). Although antiserum to protein F contained some antibody to PAO1 LPS, indicating that LPS was a minor contaminant in the immunizing antigen, it is clear, from the low titers (<10) of antiserum to LPS against heterologous outer membranes, that antibody to LPS could not account for the ELISA titers of those outer membranes with antiserum to protein F. In contrast, our smooth LPS-specific monoclonal antibodies interacted only with type 5 (strongly) and type 17 (weakly) outer membranes. Interestingly, we could find no difference in major outer membrane protein patterns (data not shown) or in interaction with antiserum to outer membrane (table 2) of a mucoid isolate (P1) from a patient with cystic fibrosis and its spontaneous nonmucoid revertant.

Antigenic relationship of outer membrane proteins. To obtain more specific information about the immunologic similarities of major outer membrane proteins from the serotype strains, the proteins were transferred from SDS-PAGE gels to

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Table 2. Cross reactions of antisera to outer membranes and purified outer membrane components with outer membranes from various serotype strains of *Pseudomonas aeruginosa*.

Outer membrane antigen	Log ELISA titers using antiserum to		
	PAO1 outer membranes	Protein F	LPS O antigen
PAO1	8	3	3
Serotype			
1	6	2	<1
2	6	2	<1
3	5	1	<1
4	7	1	<1
5	7	3	3
6	5	4	<1
7	6	2	<1
8	6	1	<1
9	6	3	<1
10	6	3	<1
11	6	2	<1
12	6	3	<1
13	6	1	<1
14	6	2	<1
15	6	1	<1
16	7	3	<1
17	6	3	1
P1 mucoid	5	2	<1
P1 revertant	5	2	<1
PAO1 LPS	4	1	4
Protein F	6	4	1

NOTE. *P. aeruginosa* PAO1 (strain H103) was used as a standard strain for the isolation of major outer membrane antigens. Seventeen serotype-specific strains, a mucoid isolate from a patient with cystic fibrosis (P1 mucoid), and a nonmucoid spontaneous mutant (P1 revertant) were also tested. Antisera to PAO1 outer membranes and protein F were produced in BALB/c mice. Monoclonal antiserum to serotype 5 lipopolysaccharide (LPS) O antigen was isolated as described [17]. ELISA = enzyme-linked immunosorbent assay.

nitrocellulose by the Western blotting procedure and allowed to interact with ^{125}I-labeled rabbit antiserum to strain H103 outer membrane proteins that had been purified on affinity columns to remove antibodies to LPS and all serum proteins except IgG. As judged from stained blots of outer membrane proteins in control experiments and from the Coomassie brilliant blue staining of the polyacrylamide gels after blotting (figure 2, right), transfer of outer membrane proteins to nitrocellulose was quite efficient. Of the major outer membrane proteins, I, H2, H1, G, and E were quantitatively transferred, whereas F and D2 were transferred with somewhat lower efficiency (⩾80%). Autoradiography (figure 2, left) demonstrated

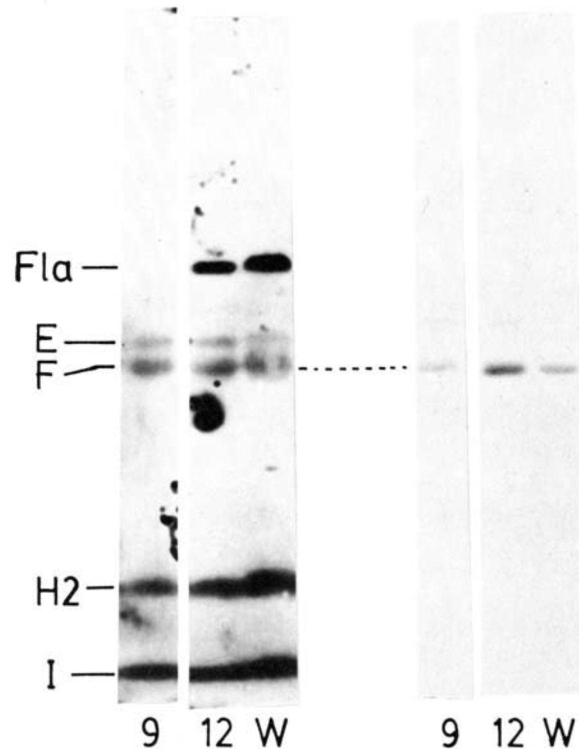

Figure 2. *Left*, autoradiogram of an electrophoretic blot of outer membrane proteins of *Pseudomonas aeruginosa* after treatment with radioiodinated antibodies to outer membrane protein. The blot was made by electrophoretic transfer of separated outer membrane proteins from a sodium dodecyl sulfate-polyacrylamide gel electrophoretogram, similar to that in figure 1, left, to nitrocellulose paper. The numbers below the lanes refer to the serotypes; W is strain H103. The indicated polypeptides (proteins E, F, H2, and I and flagellin [Fla]) were identified by amido black staining of the electrophoretic blots. The band labeled "Fla" was identified as flagellin on the basis of coelectrophoresis with purified flagellin. *Right*, sodium dodecyl sulfate-polyacrylamide gel electrophoretogram after electrophoretic transfer of outer membrane proteins to nitrocellulose. All proteins not appearing on this electrophoretogram were transferred to nitrocellulose. Note that only partial (80%) transfer of protein F was achieved.

that specific major outer membrane proteins (E, F, H2, and I) were capable of interacting with the radiolabeled antibodies. By densitometer scanning of the autoradiographs (figure 3) we were able to obtain an estimate of the amounts of radiolabeled antibodies bound to major outer membrane proteins. The results strongly suggested that proteins F, H2, and I were antigenically related in all strains, whereas only the serotype 4 strain failed to bind antibodies to protein E. Antibodies to outer

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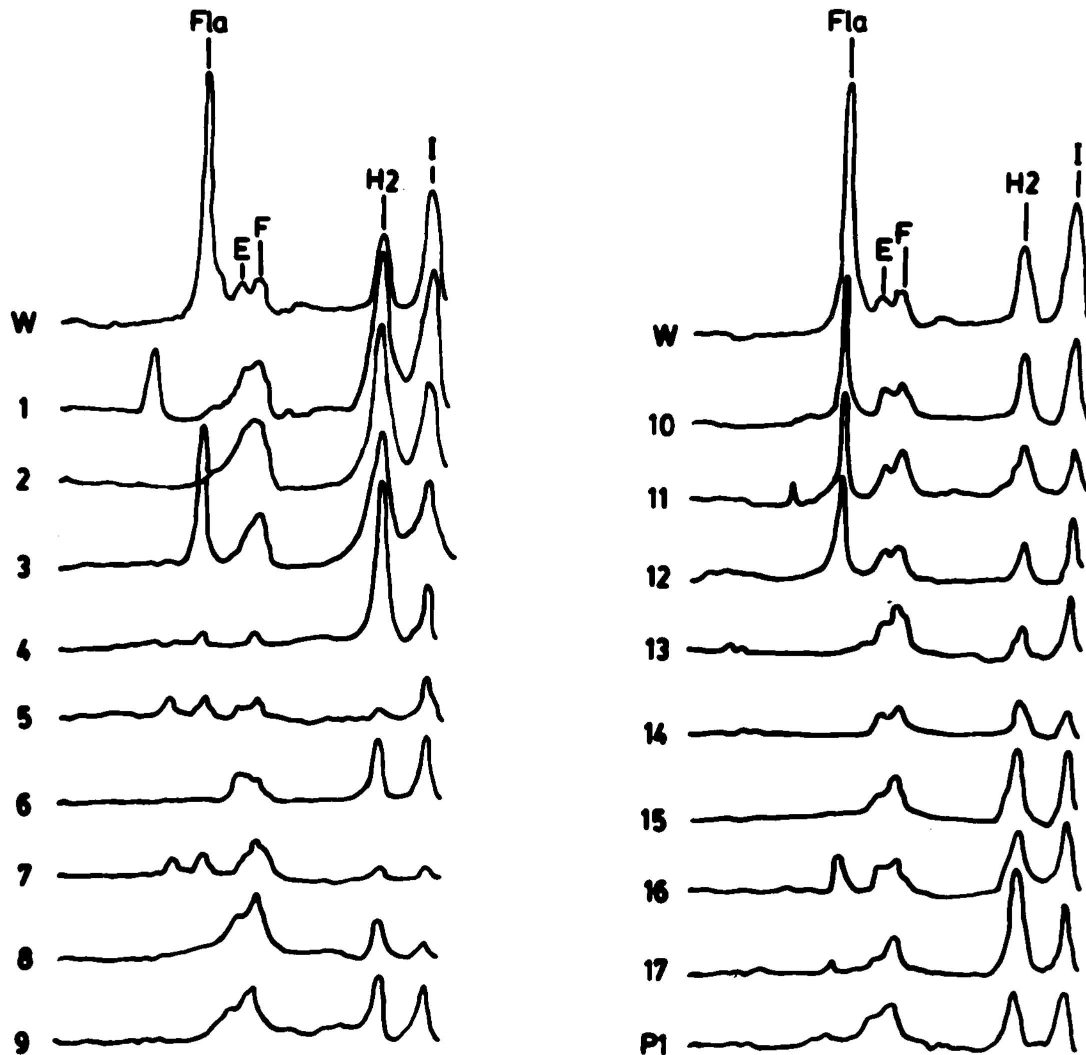

Figure 3. Densitometer tracings of autoradiograms of separated outer membrane proteins from *Pseudomonas aeruginosa* strain H103 (W), serotype 1–17 strains, and isolate P1 from a patient with cystic fibrosis, after treatment with radioiodinated antibodies to outer membrane protein. Exposed x-ray films similar to those shown in figure 2 were analyzed by densitometer scanning. The indicated polypeptides (proteins E, F, H2, and I and flagellin [Fla]) were identified by amido black staining of the electrophoretic blots (see figure 2 for examples).

membrane proteins D2, H1, and G were not detected. It was further observed that a protein with a molecular mass of 53,000 daltons in the outer membrane of strain H103 bound a large amount of radioactive antibody (figure 2, left). This protein was identified as flagellin on the basis of molecular weight [26] and coelectrophoresis with purified flagellin obtained from Dr. R. Ansorg (Hygiene-Institut der Universität Krezberging, Göttingen, Federal Republic of Germany). Presumably the large amount of antibody to flagellin present in our rabbit antiserum to outer membrane reflected the relatively high immunogenicity of flagellin, because it was a minor component of outer membranes. Only the outer membranes of strains representing serotypes 3 and 10–12 showed large peaks of antibody binding at the position of flagellin, whereas the strains of serotypes 4, 5, 7, 16, and 17 and strain P1 all demonstrated weak antibody binding at this position.

Discussion

The present study provides three independent lines of evidence that outer membrane proteins are highly conserved in *P. aeruginosa*. This evidence

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includes conservation of receptors for bacteriophages (table 1), highly similar outer membrane protein patterns (figure 1), and antigenic cross-reaction of specific major outer membrane proteins (figures 2 and 3 and table 2). Furthermore, the data presented here agree with observations that the LPSs (table 2) [12, 13, 23] and flagella (figure 3) [26] of *P. aeruginosa* strains vary markedly from strain to strain.

Other organisms do not show the high degree of similarity in the outer membrane protein patterns demonstrated by *P. aeruginosa* strains. For example, a variety of outer membrane protein patterns have been observed for *Haemophilus influenzae* (eight to nine subtypes based on outer membrane protein patterns [27]), *Neisseria gonorrhoeae* (nine subtypes [28]), *Neisseria meningitidis* (15 subtypes [29]), *E. coli* (36 subtypes [30]), *Bacteroides fragilis* (four subtypes [31]), *Vibrio cholerae* (two subtypes [32]), and *Vibrio anguillarium* (three subtypes [33]). Indeed in *H. influenzae*, *N. gonorrhoeae*, and *N. meningitidis*, outer membrane protein patterns and antisera to outer membrane proteins have been proposed as the bases for subtyping these organisms. In contrast, despite the many outer membrane protein patterns observed in *E. coli* and among other enteric organisms, there is evidence that a number of outer membrane polypeptides are antigenically related among the Enterobacteriaceae [34]. Thus, it would seem that outer membrane protein patterns provide a far more sensitive measure for differentiating strains than antigenic relations between given outer membrane proteins. It is therefore highly significant that all *P. aeruginosa* strains studied in our laboratory to date have had similar outer membrane protein patterns (figure 1 and authors' unpublished observations). Two observations in the literature are consistent with this finding. Sadoff and Artenstein [35], studying the "native complex" of the seven Fisher immunotype strains, observed similar protein patterns, whereas Mizuno and Kageyama [36] made a similar observation for the outer membrane proteins of five different *P. aeruginosa* strains. Conservation of outer membrane protein patterns has also been observed in our laboratory for the eel pathogen *P. anguilliseptica* [33].

Electrophoretic transfer of *P. aeruginosa* major outer membrane proteins to nitrocellulose paper and interaction of these electrophoretic blots with radiolabeled antibodies demonstrated that proteins F, H2, and I possess common antigenic determinants in *P. aeruginosa* outer membranes and that protein E is apparently closely related in 16 of the 17 serotype strains (figure 3). The results were obtained with polyclonal serum and do not imply that single antigenic sites within given outer membrane proteins are conserved. However, we have recently demonstrated conservation of two separate single antigenic sites in the outer membranes of the serotype strains of *P. aeruginosa* using two monoclonal antibodies, one of which was directed against protein H2 [17].

Despite the similarity of outer membrane protein patterns, not all serotype strains showed identical antibody binding to their outer membrane proteins. There are at least two possible explanations for this observation: (*1*) individual antigenic sites on given outer membrane proteins could have differing affinities for the test antibodies (as demonstrated by experiments using a monoclonal antibody to protein H2 [17]), or (*2*) the amounts of the given protein in the various outer membranes could differ markedly. Thus, the observed small amounts of protein H2 in the strains of serotypes 5 and 7 (figure 1) correlated with the small amount of antibody bound to protein H2 of these strains (figure 3). Similarly, the serotype 7 strain had a very small amount of protein I and very little antibody was bound to protein I from this strain. However, no similar correlations could be made for protein E. The different amounts of antibody to protein E bound to these strains might be related to antibody-antigen affinity. It should be noted that since protein F was not quantitatively transferred to nitrocellulose by our procedure, only qualitative statements could be made about the antigenicity of this protein.

An important implication of these data is that major outer membrane proteins may well provide "common" antigens for use in vaccines. Previous animal trials using outer membrane proteins of *N. gonorrhoeae* [3, 4], *Shigella* species [5], and *Salmonella typhimurium* [6] as antigens have shown the potential efficacy of such vaccines. We believe that other *P. aeruginosa* vaccines which have been shown to demonstrate cross protection, such as ribosomal [37], slime glycolipoprotein [38], and original endotoxic protein [39] vaccines, might well be contaminated with outer membrane proteins. With regard to this latter vaccine, original endotoxic protein, our preliminary observations are that certain of the >50 polypeptides pres-

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ent in original endotoxic protein coelectrophorese with major outer membrane proteins, whereas our antisera to purified outer membrane proteins react strongly with original endotoxic protein (L.M.M., unpublished observations).

There is some evidence that human patients are capable of developing antibodies to outer membrane proteins during infection, because Fernandes et al. [40] were able to immunoprecipitate antibodies to cell envelope proteins of 37,500 daltons, possibly porin protein F, and 58,500 daltons, possibly flagellin, from the sera of patients with cystic fibrosis. Høiby and Axelson [41] demonstrated, in another group of patients with cystic fibrosis, precipitating antibodies to 173 different antigens of *P. aeruginosa*, some of which would almost certainly be outer membrane cell surface proteins. Thus, there is considerable potential for outer membrane proteins as nontoxic, nonpyrogenic vaccine components.

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