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Comparative Biochemical Studies on F and EDP208 Conjugative Pili

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EDP208 pili are encoded by a derepressed derivative of a naturally occurring *lac* plasmid, F_0*lac* (incompatibility group FV), originally isolated from *Salmonella typhi*. EDP208 pili are serologically unrelated to F pili and do not promote infection by F-specific ribonucleic acid bacteriophages. However, they do confer sensitivity to the F-specific filamentous deoxyribonucleic acid phages. EDP208-containing cells are multi-piliated and contain approximately 20 pili per cell. These pili contain a single polypeptide subunit of 11,500 daltons. EDP208-specific RNA phages were readily isolated from local sewage. These phages were somewhat smaller in diameter than the F-specific ribonucleic acid phages and absorbed relatively weakly to EDP208 pili. Comparing EDP208 pilin to F, it was found that both contain the equivalent of two to three hexose units per subunit as well as blocked N-termini. EDP208 pilin contains one covalently linked phosphate residue per subunit, whereas the F pilin subunit contains two such residues. Although notable differences were found in the case of three or four amino acids, the overall amino acid compositions of F and EDP208 were very similar. Moreover, the tryptic peptide maps of the two proteins contained seven peptides with similar mobilities, suggesting considerable homology in their amino acid sequences. Substantial similarities were also noted in the secondary structures of F and EDP208 pilin on the basis of circular dichroism studies. The α-helix content of both proteins was calculated to be 65 to 70%. X-ray fiber diffraction studies have indicated that the arrangements of subunits in F and EDP208 pili are also similar. It was concluded that F and EDP208 pili are closely related structures.

Pili are a diverse group of nonflagellar filamentous appendages found on many strains of bacteria. Because the characterization of these structures is still at a preliminary stage, it is not yet possible to classify them in a systematic manner. However, two broad groups of pili may be distinguished. The first, conjugative pili, are encoded by self-transmissible plasmids (1). These pili promote infection by certain pilus-specific bacteriophages and play an important role in the process of bacterial conjugation. The second group, common pili, is of more general occurrence. These pili are apparently unrelated to donor function in bacterial mating, but have been associated with many other bacterial properties, e.g., adhesiveness (12, 29), surface translocation (20), growth enhancement at limiting oxygen concentrations (5), genetic transformation competence (2), and sensitivity to phage infection (4).

The present communication describes comparative studies on two types of conjugative pili, F and EDP208. F pili have already been partially characterized (6, 9, 19). EDP208 is a derepressed derivative of the naturally occurring *lac* plasmid F_0*lac* (incompatibility group FV) originally isolated from *Salmonella typhi* (14). The derepressed EDP208 plasmid has been shown by Bradley and Meynell (3) to produce pili which are identical with the pili encoded by the original *S. typhi* (F_0*lac*) strain described by Falkow and Baron (14). However, EDP208 pili are serologically unrelated to F pili (3). Moreover, cells carrying EDP208 are resistant to F-specific RNA phages (3). Interestingly, the filamentous DNA phage fl is plated with equal efficiency on F- and EDP208-carrying cells (10, 14), suggesting possible structural similarities at the tips of these pili. In what follows, compositional studies and comparative studies on the primary and secondary structures of these two types of pili are described. These studies show that F and EDP208 pili share many features of a structural and compositional nature.

MATERIALS AND METHODS

Bacterial strains and plasmids. F pili were prepared from strain ED2692, which carried the *traD8* mutant of the F*lac* plasmid JCFLO (1) in WP1000 (*Escherichia coli* B/r Fla$^-$ Fim$^-$). The *traD8* mutation affects the number of pili per cell but not the composition of the F pili (unpublished results). ED3873 is

JC6256 (*E. coli* K-12 F^- *trp lac*) containing the plasmid EDP208. The EDP208 plasmid was constructed from F_0*lac* by N. S. Willetts, Department of Molecular Biology, University of Edinburgh.

Purification of pili. ED2692 and ED3873 bacteria were grown on solid media in aluminum trays as previously described (18). The cells were scraped off the surface of the agar and resuspended in 1.0 liter of SSC (pH 7.2; 0.15 M sodium chloride, 0.015 M sodium citrate) at 4°C. The cells were gently stirred for 2 h and were removed by low-speed centrifugation. The cells were washed with a second 1-liter portion of SSC, and the resultant supernatants were combined and adjusted to 2% polyethylene glycol 6000 and 0.5 M NaCl. The precipitate was collected by centrifugation at 8,000 × *g* for 20 min and was resuspended in 120 ml of distilled water. This solution was layered on six preformed CsCl step gradients (1.1 to 1.5 g/cm^3), and the pili were banded in an SW27.1 rotor at 20,000 rpm for 18 h at 5°C. The pili were removed with a syringe and dialyzed extensively against distilled water. The pili were judged pure when sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoretic examination of the preparation showed a single protein band.

Antisera preparation. Antisera were prepared by injecting purified pili into rabbits intravenously. Approximately 100 μg of protein in 1 ml of 0.15 M NaCl was injected at 3- to 4-day intervals for 15 days. The rabbits were bled from an ear vein by using gentle suction 4 to 7 days after the final injection. The blood was allowed to clot at room temperature for 4 to 6 h, and then the serum was removed and stored at −20°C after the addition of 0.1% sodium azide.

Agglutination tests. Cells were harvested from petri dishes and resuspended in 0.15 M NaCl to 5 × 10^9 cells per ml. To each 0.2-ml portion of serially diluted antiserum, 0.2 ml of cell suspension was added, and the mixtures were incubated at 37°C for 1 h before scoring.

Phosphate analysis. To assay for total organic phosphate, separate 1- and 2-mg samples of pure pili were hydrolyzed in 6 N HCl for 48 h, dried over NaOH pellets, and assayed for total phosphate according to the method of Chen et al. (7).

Amino acid analysis. Amino acid analyses were performed by the method of Moore (25) using a Durham D-500 automated amino acid analyzer. The values reported are average values for 24-, 48-, and 72-h hydrolysis periods, in 6 N HCl–0.1% phenol at 110°C in evacuated sealed tubes. The values for serine and threonine were estimated by extrapolating to zero time. The value for tryptophan was determined by hydrolyzing the protein in 3 N *p*-toluene sulfonic acid–0.2% 3-(2-aminoethyl-indole) as described by Liu and Chang (23). The value for cysteine was estimated as cysteic acid after oxidizing the protein with performic acid by the method of Hirs (22). Methionine content was estimated as methionine sulfone in the oxidized protein.

N-terminal sequencing. Automated Edman degradations were performed on 150 nmol of pilin with a Beckman 890B Sequencer, utilizing the standard 1 M Quadrol procedure of Edman and Begg (13).

Tryptic peptide maps. Performic acid oxidation was not used in this procedure since it did not significantly alter the peptide map. The pili were first dissociated into subunits by dissolving 10 nmol of pure pili in 0.2 ml of 100% formic acid and lyophilizing to dryness. The pili were then dissolved in 0.2 ml of 0.1 M NH_4HCO_3 (pH 8.0) containing 0.1% SDS, after which they were diluted 10-fold to 2.0 ml with 0.1 M NH_4HCO_3 (pH 8.0). Tolylsulfonyl phenylalanyl chloromethyl ketone-trypsin (Worthington Biochemical Corp.) (1 mg/ml) was added to give an enzyme/substrate ratio of 1:20, and the mixture was incubated for 6 h at 37°C. A second addition of an equal amount of tolylsulfonyl phenylalanyl chloromethyl ketone-trypsin was added to the digestion mixture, and the incubation was continued for an additional 18 h. The digestion mixture was lyophilized, then redissolved in water and relyophilized three times. The residue was resuspended in 20 μl of chromatography solvent (*n*-butanol-pyridine-water-acetic acid, 5:4:4:1, vol/vol), and 5 μl was used for each peptide map.

Peptide mapping was performed on plastic sheets (10 by 20 cm) coated with 0.1 mm of microcrystalline cellulose CEL400 (Brinkmann). The sample was spotted 6 cm from one end of the sheet (anode) and 2 cm from the edge of the sheet. The first dimension was ascending chromatography in the solvent mentioned above, to within 1 cm of the top of the plastic sheet. Electrophoresis in the second dimension was carried out at 500 V for 45 min using 8% formic acid–2% acetic acid (pH 1.8) in the electrode chambers. Under these conditions, all peptides migrated towards the cathode. Staining of peptides was with the cadmium-ninhydrin reagent (11). Detection of [^{3}H]lysine was by autoradiography (30).

Circular dichroism spectra. Circular dichroism studies were performed on a Cary model 60 recording spectropolarimeter with a Cary 6001 CD attachment. Samples of purified pili (0.5 mg/ml; 1.0 mg) were prepared in 0.01 M phosphate buffer (pH 7.2) containing 8 M urea, 4 M guanidine-hydrochloride, 10 mM deoxycholate (native), or 1.0% SDS. These were subsequently examined over a wavelength range of 200 to 250 nm. At a given wavelength, λ, the mean residue ellipticity, $[\Theta]_\lambda$, was determined by the equation: $[\Theta]_\lambda = \Theta_{obs}M/10\,lc$, where Θ_{obs} is the observed ellipticity in millidegrees at that wavelength, M is the mean residue molecular weight (taken as 115), l is the path length in centimeters (0.0501 cm), and c is the protein concentration in milligrams per milliliter (determined by amino acid analysis). $[\Theta]_\lambda$ is therefore expressed in degrees per square centimeter per decimole. The apparent α-helical content of each sample was calculated by using parameters given in the literature (8).

RESULTS

Preliminary studies on the EDP208 pili producer strain. Initial electron microscopic examination of an EDP208-containing *E. coli* K-12 strain (ED3873) showed the cells to be highly piliated (Fig. 1A). Moreover, the pili were found to be readily purifiable by procedures in use in this laboratory. SDS-gel electrophoretic analysis of the pure pili preparations revealed that they contain a single protein component of molecular

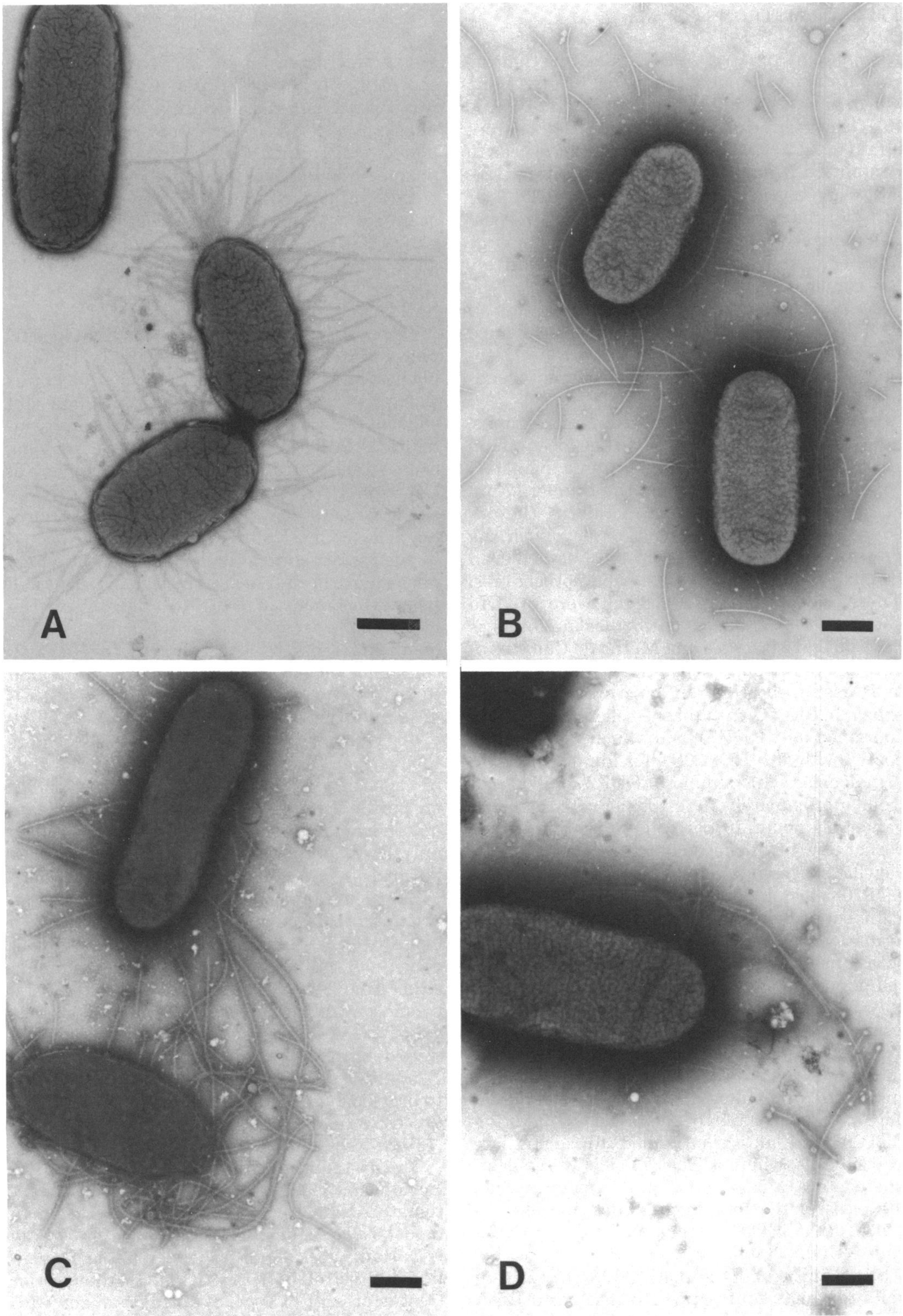

FIG. 1. *Electron micrographs of negatively stained E. coli cells. (A) ED3873 cells (EDP208/JC6256). (B) JC6256 (F^-) cells. (C) ED3873 cells treated with anti-EDP208 pilus antiserum. (D) Occasional cell in ED3873 culture which contained type 1 pili. Bar, 0.5 μm.*

weight approximately 11,000, indicating that the preparation was not contaminated by type 1 pili, which have a subunit molecular weight of 17,000 (5). However, examination of the F^- parent strain of ED3873 (JC6256) showed these cells to contain both flagella and type 1 pili (Fig. 1B). This suggested that ED3873 cells (EDP208/JC6256) may not be multipiliated with respect to conjugative pili, but rather that the large number of pili per cell may include relatively few conjugative pili among predominantly type 1 common pili. The successful purification of EDP208 pili free of type 1 pili could be explained by the fact that conjugative pili are preferentially removed from cell surfaces by mild blending procedures (26).

Careful examination of electron micrographs, however, led to the conclusion that most pili on ED3873 cells are of the thicker (9-nm diameter) conjugative type rather than the thinner (7-nm diameter) type 1 variety. Nonetheless, the unequivocal identification of EDP208 pili in the presence of type 1 pili could only be achieved through the use of specific labeling procedures. Therefore, purified EDP208 pili were used to elicit anti-EDP208 pilus antibodies in rabbits as described in Materials and Methods. Cultures of ED3873 were then treated with the antiserum and examined electron microscopically. As shown in Fig. 1C, most of the cells in the culture contained only EDP208 pili, which were heavily coated with anti-EDP208 pilus immunoglobulin G molecules. The number of EDP208 pili varied from approximately 10 to 100 per cell; the mean value was about 20 per cell. As we had noted earlier, few of the cells contained type 1 pili or flagella, suggesting that the introduction of the EDP208 plasmid into JC6256 cells causes significant suppression of flagella and type 1 pilus formation. Figure 1D shows an example of an occasional cell in the ED3873 (EDP208/JC6256) cell population which did contain type 1 pili. It may be seen that antibody-coated EDP208 pili were easily distinguished from type 1 pili, which did not bind antibody molecules. When the parent strain, JC6256, was treated with EDP208 pili antiserum, no binding of antibodies to common pili or flagella could be detected. In addition EDP208 pili antiserum failed to agglutinate JC6256 cells, whereas ED3873 cells were agglutinated to a 1/5,000 dilution of antiserum. On the basis of the foregoing studies, it was concluded that ED3873 cells produce predominantly EDP208 pili.

Specificity of F- and EDP208-containing cells towards male-specific RNA and DNA phages. Since EDP208 pili do not absorb F-specific RNA phages such as R17 (3, 28), a search was made for other RNA phages with specificity towards EDP208 pili. Phage UA-6 was chosen as a representative of several such phages isolated from local sewage. This phage plated only on EDP208-containing cells, and plaque formation was prevented by the presence of 25 μg of pancreatic RNase per ml in the top agar (Table 1). As expected, the F-specific RNA phage R17 plated only on *E. coli* cells carrying an F plasmid, whereas the filamentous DNA phage M13 plated equally well on F- and EDP208-containing cells.

That UA-6 was probably a pilus-specific phage was suggested by its inability to form plaques on F^- cells. Further confirmation of this was provided by the observation (data not shown) that anti-EDP208 pilus antiserum inhibited UA-6 infection of EDP208-containing cells. Attempts to visualize the attachment of UA-6 phage to free EDP208 pili directly by electron microscopy were only partially successful, since at most 6 to 10 phage particles could be seen attached to a single pilus even at a high phage particle-to-free pilus ratio. This suggested poor attachment of UA-6 to EDP208 pili, an idea which was supported by subsequent studies on the rate of UA-6 attachment to EDP208-containing cells. UA-6 phage was found to bind to its host much less efficiently ($K = 1.7 \times 10^{-10}$ ml min^{-1}) than R17 phage to its host ($K = 3.0 \times 10^{-9}$ ml min^{-1}) (Fig. 2). Figure 2 also shows that phage UA-6 did not bind at all to F^- cells or cells carrying an F plasmid. Further studies on purified preparations of UA-6 phage (data not shown) revealed that the phage contained only RNA as the nucleic acid moiety and that purified preparations consisted of small spherical particles approximately 20 nm in diameter.

Buoyant density of F and EDP208 pili in cesium chloride. To determine the buoyant density of pili in CsCl, gradients containing either EDP208 or F pili were subjected to isopycnic centrifugation in CsCl as described previously (18). Buoyant density values (at 5°C) were determined to be 1.232 for EDP208 pili and 1.256 for F pili. These values may be compared to previously reported values of 1.197 (32), 1.257 (6), 1.223 (9), and 1.20 (19) for F pili and 1.295 (18) for PAK pili.

The N-terminus of F and EDP208 pilin. Both F and EDP208 pili were subjected to automated Edman degradations in a Beckman model 890B Sequenator as described previously (27). Both types of pili suspended in the conventional Quadrol buffer with and without prior treatment with 1% SDS failed to react with phenylisothiocyanate, indicating the existence of a blocked amino group on the N-terminal amino

TABLE 1. *Specificity of F- and EDP208-containing E. coli cells towards male-specific RNA and DNA phages*

Phage used in spot test[a]	EDP208/JC6256		JCFLO[b]/JC6256		JC6256 (F⁻)	
	−RNase	+RNase[c]	−RNase	+RNase	−RNase	+RNase
UA6	++[d]	−	−	−	−	−
R17	−	−	++	−	−	−
M13	++	++	++	++	−	−

[a] Approximately 10^7 plaque-forming units of phage in 0.05 ml of L-broth was spotted onto a freshly seeded lawn of the appropriate indicator strain. The petri dishes were examined for evidence of lysis after incubation for 12 h at 37°C.
[b] JCFLO is the F′ plasmid F*lac* used by Achtman et al. (1).
[c] Pancreatic RNase (25 μg/ml in top agar).
[d] ++, clear lysis; −, no lysis.

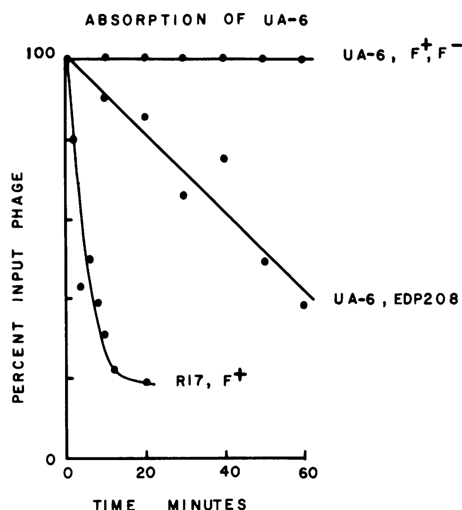

FIG. 2. *Rate of adsorption of UA-6 and R17 phage to JC6256 cells carrying F and EDP208 plasmids. Phage attachments were performed in L-broth at 37°C. UA-6 phage was added to 9 × 10^7 bacteria per ml at a multiplicity of 0.06. R17 phage was added to 3 × 10^7 bacteria per ml at a multiplicity of 0.07. Samples were removed at intervals and centrifuged for 3 min in a Beckman microfuge to remove infected cells, and the unadsorbed phage in the supernatants was titrated by plaque assay.*

acid residue of these proteins. Unfortunately, we have not yet identified the blocked N-termini of F and EDP208 pilin.

Compositional analysis of F and EDP208 pili. Purified preparations of F and EDP208 pili were subjected to amino acid, phosphate, and sugar analyses as described in Materials and Methods. The results of these studies are summarized in Table 2. It may be seen that both types of pili lack histidine and proline; the absence of the latter is indicative of a possible high α-helical content. F pilin was also found to lack arginine and cysteine, whereas EDP208 pilin lacked tryptophan. Both proteins were found to contain 1 to 2 mol of covalently linked phosphate per mol of protein and 2 to 3 mol of covalently linked sugar.

TABLE 2. *Compositional analysis of F and EDP208 pili*

Amino acid	No. of residues per pilin subunit	
	F	EDP208
Lys	9	8
His	0	0
Arg	0	2
Asx	8	9
Thr	7	10
Ser	11	2
Glx	4	3
Pro	0	0
Gly	13	11
Ala	14	10
1/2 Cys	0	3
Val	20	12
Met	8	5
Ile	4	9
Leu	9	13
Tyr	2	2
Phe	6	6
Trp	2	0
Blocked N-term	1	1
Hexose	3	2–3
Phosphate	2	1
Total amino acid residues per subunit	118	106
Subunit mol wt based on amino acid composition	12,100	11,500

Detection of carbohydrate residues in F and EDP208 pilin. Colorimetric assays using the anthrone and phenol sulfuric acid procedures indicated that both F and EDP208 pili contain carbohydrate residues. Assuming the residues to be hexoses, the pili were found to contain 2 to 3 mol of sugar per mol of protein.

Identification and characterization of the pilus-associated sugar residues is presently under way in this laboratory and will be reported separately in a future communication.

Circular dichroism studies on F and EDP208 pili. Circular dichroism spectra of intact F and EDP208 pili were measured in 0.01 M phosphate buffer (pH 7.2) in the presence of 10 mM deoxycholate. Under these conditions, light scattering was reduced significantly because there is less tendency for whole F pili to aggregate into bundles as judged by electron microscopy. Figure 3 shows molar ellipticities for both F and EDP208 pili in the wavelength range of 205 to 235 nm. The bottom curve in each profile is representative of a protein that is 100% α-helix.

From the circular dichroism spectrum obtained with pili, in the presence of 10 mM deoxycholate, the apparent α-helical content of both F and EDP208 pilin was calculated to be 65 to 70% by the method of Chen et al. (8). This relatively high value is in agreement with that reported for F pili by Date et al. (9).

Figure 3 also demonstrates rather marked changes in the secondary structure of the two proteins when they are exposed to 8 M urea or 4 M guanidine-hydrochloride. It may be seen that urea and guanidine-hydrochloride caused a marked decrease of helicity in both proteins, although it would appear that the α-helical structure in F pilin is more sensitive to these reagents than is EDP208. Despite these striking changes in secondary structure, urea and guanidine-hydrochloride apparently exert little or no effect on subunit-subunit interactions in these pili. When examined by electron microscopy, both F and EDP208 pili were found to retain apparently normal morphology after treatment with 8 M urea or 4 M guanidine-hydrochloride. On the other hand, treatment of pili with 1% SDS resulted in the complete dissociation of pili into subunit monomers. This treatment caused only slight alterations in the α-helicity of the two proteins: a slight decrease in the case of F pilin and a slight increase in the case of EDP208 pilin.

Tryptic peptide maps of F and EDP208 pilin. To compare the primary sequences of F and EDP208 pilin, purified pili were digested with trypsin and analyzed by two-dimensional chromatography-electrophoresis as described in Materials and Methods. The resulting peptide maps of the two proteins are shown in Fig. 4. In general, the number of peptide spots detected agrees well with the amino acid compositions shown in Table 2. F pilin, which contains a total of nine potential cleavage sites (9 Lys + 0 Arg), yielded 10 peptides, including one that comigrated with free lysine (note that no assignment was made for peptide no. 7 in the F pilin map). EDP208 pilin, which contains a total of 10 po-

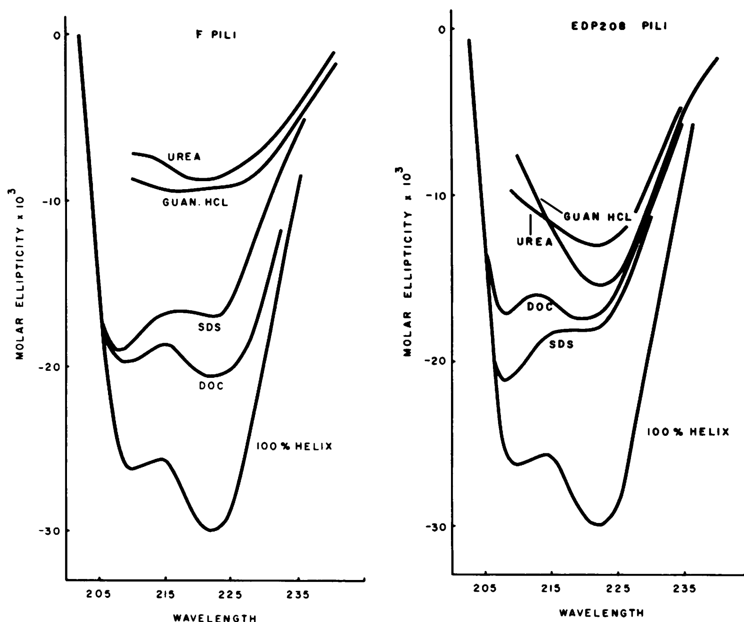

FIG. 3. *Circular dichroism spectra in the far UV of F and EDP208 pili. Purified pili were suspended in 0.01 M phosphate buffer (pH 7.2) plus 10 mM deoxycholate (native pili), 8 M urea, 4 M guanidine-hydrochloride, or 1% SDS. After the addition of denaturants to the pili suspension, the pH was readjusted to 7.2.*

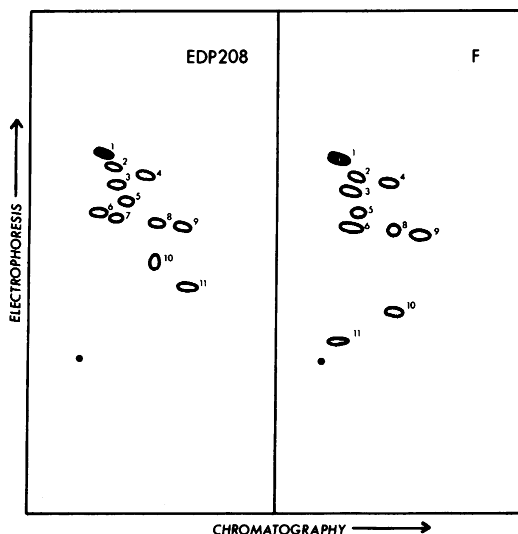

FIG. 4. *Two-dimensional peptide maps of tryptic digests of EDP208 and F pili using chromatography in the first dimension and electrophoresis at pH 1.8 with second dimension. The shaded areas indicate the position of [^{3}H]lysine, which comigrated with spot no. 1 and was detected by autoradiography.*

tential cleavage sites (8 Lys + 2 Arg), yielded a total of 11 peptides, including one that comigrated with free lysine. The free lysine spot suggests that both types of pili may contain at least one cluster of two or more lysine residues.

Despite marked differences in the amounts of certain amino acids in F and EDP208 pilin (see, for example, Ser, Cys), the peptide maps suggest that the two proteins may contain significant amounts of homology in their primary sequences. Peptides 1, 2, 3, 4, 5, 8, and 9 were found to have similar mobilities in the two peptide maps, suggesting closely related sequences. Peptides 6, 10, and 11 occupied different positions in the two maps, whereas peptide 7 was found only in the EDP208 digest. Comparative sequencing studies on F and EDP208 tryptic peptides are in progress and will be reported in a future publication.

DISCUSSION

A number of different sex pili encoded by different sex factors have now been identified (10). Of these, only F pili have been extensively studied biochemically (6, 9, 19). The present report describes biochemical studies on a second type of sex pilus, EDP208, which is morphologically similar to F and confers sensitivity to the F-specific filamentous DNA phages, but not to the F-specific isometric RNA phages (10, 14). Although the EDP208 plasmid apparently shares no significant DNA homology with F (Willetts, personal communication), it has been assigned to the F incompatibility group FV (3, 10).

The level of piliation of EDP208-containing *E. coli* K-12 cells is at least an order of magnitude greater than that normally seen with F and other F-like plasmids. Moreover, whereas type 1 pili and flagella are usually expressed normally by F-containing cells, the expression of these surface filaments appears to be repressed in EDP208-containing *E. coli.*

It is of interest that EDP208-specific RNA phages were readily isolated from local sewage. These phages were somewhat smaller in diameter than the F-specific RNA phages (20 nm versus 26 nm in diameter) and absorbed relatively weakly to EDP208 pili. No other F-like pili were found to confer sensitivity to these phages. These findings, together with the observation (3) that F and EDP208 pili are serologically distinct, suggest a significant difference in the structural properties of the lateral surfaces of these two types of pili. On the other hand, the sensitivity of EDP208-containing cells to F-specific filamentous phages suggests that the tips of F and EDP208 pili may be structurally related. However, Bradley and Meynell (3) noted that pili encoded by the plasmid R711b also confer sensitivity to F-specific filamentous phages but not to F-specific RNA phages. R711b pili were found to be serologically distinct from both F and EDP208 pili with respect to lateral surface serology, and no evidence was found to suggest that the tips of F, EDP208, and R711b pili are serologically related. Thus, the structural requirements for the attachment of filamentous phage to pilus tips may not be as stringent as those for the attachment of RNA phages to the sides of sex pili.

Although the foregoing observations point to substantial differences in the biochemical and structural properties of F and EDP208 pili, the comparative biochemical studies reported in the present paper show a surprising number of common features. For example, F and EDP208 pili subunits have similar molecular weights and similar compositions with respect to carbohydrate and phosphate content, and both proteins have blocked N-terminal residues. Although significant differences exist in the content of certain amino acids (Ser, Cys), the overall amino acid compositions share many similarities, including the absence of His and Pro. It is to be noted that the F pilin data shown in Table 2 agree closely with data published previously by Brinton (6) and Date et al. (9), except that no previous reports have made reference to the blocked N-terminus of F pilin. It is also worth mentioning

that Brinton (6) was the first to report the presence of covalently linked glucose in F pilin. Date et al. (9) also reported detecting sugar in F pilin, and Robertson et al. (31) detected one to two hexose groups per pilin subunit in gonococcal pili. No carbohydrate residues were found in polar pili of *Pseudomonas aeruginosa* (18). Recently a study on type 1 pili from *E. coli* (24) reported the presence of carbohydrate; previously, no carbohydrate had been found (5).

The tryptic peptide maps of the two proteins were found to contain seven peptides with similar mobilities, suggesting at least some homology in their primary structures. Striking homologies have already been noted among the amino terminal sequences of common pili isolated from *Moraxella nonliquifaciens* (16), *P. aeruginosa* (27), and *Neisseria gonorrhoeae* (21). Moreover, all three types of pili were found to contain the unusual amino acid *N*-methylphenylalanine at the amino terminus (17, 21, 27). On the other hand, the amino-terminal sequence of type 1 pili of *E. coli* (21) bears no homology to the foregoing three sequences and contains no *N*-methylphenylalanine.

Substantial similarities were also noted in the secondary structures of F and EDP208 pilin. On the basis of circular dichroism studies, it was calculated that both polypeptides contain 65 to 70% α-helix. However, the helicity of F pilin was more extensively disrupted by the denaturing reagents 8 M urea and 4 M guanidine-hydrochloride than was the secondary structure of EDP208 pilin. Moreover, the two polypeptides reacted somewhat differently to treatment with 1% SDS. This detergent caused a slight decrease of α-helical content in F pilin and a slight increase in the helicity of EDP208 pilin. It is of interest that F and EDP208 pili were both extremely sensitive to dissociation by SDS, but neither 8 M urea nor 4 M guanidine-hydrochloride caused any observable disruption of intact pili. It is therefore evident that the subunit-subunit interactions in sex pili are extremely stable, although the nature of the forces holding the subunits together is not presently understood.

Folkhard et al. (15) have recently analyzed F pili by optical and X-ray diffraction. The structure proposed on the basis of this data is that of a hollow cylinder 8.0 nm wide with a central hole of about 2.0 nm. The shell contains a hydrophobic, electron-poor region which may be important for the conformation of the pilus. The subunits are arranged in helices, with four helices per pilus and a helix repeat of 12.8 nm. More recently, Folkhard et al. (W. Folkhard, K. R. Leonard, J. Dubochet, D. A. Marvin, and W. Paranchych, Abstr. 03-3-5116, 11th Int. Congr. Biochem., p. 181, 1979) have reported that the X-ray diffraction pattern of EDP208 pili is quite similar to that of F with regard to layer line spacing and overall distribution of density. This similarity of diffraction patterns indicates that the two structures are similar. However, a meridional reflection at 1.28 nm is observed on patterns of F pili, whereas the corresponding reflection is weak or absent on patterns of EDP208 pili. This meridional reflection may be due to a periodic axial perturbation on the F pili structure which does not exist in the EDP208 structure.

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