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F7 and Type 1-Like Fimbriae from Three *Escherichia coli* Strains Isolated from Urinary Tract Infections: Protein Chemical and Immunological Aspects

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Fimbriae from three *Escherichia coli* strains, C1212, C1214, and C1023, isolated from urinary tract infections, have been purified and characterized by determination of the N-terminal sequences, amino acid composition, and molecular weights of their respective subunits. Furthermore, their immunological interrelationships have been investigated. The three strains all harbored more than one fimbrial species each. Immunologically different type 1-like fimbriae, termed 1A, 1B, and 1C, with highly homologous N-terminal sequences were isolated, of which strain C1212 possessed 1A and 1C, strain C1214 possessed 1A and 1B, and strain C1023 possessed 1A and 1C. Type 1A is known to cause a mannose-sensitive hemagglutination similar to that described for type 1 fimbriae, whereas the functions of types 1B and 1C are not yet known. Strain C1212, in addition, harbored the F7 fimbrial antigen which causes mannose-resistant hemagglutination and adherence to urinary epithelial cells. The N-terminal structure of this antigen seems to indicate a possible evolutionary kinship to the type 1-like fimbriae, although they are immunologically unrelated. Our results indicate that fimbriation of pathogenic wild-type strains can be of an intricate variety.

The ability to attach to host epithelium plays a significant role in the pathogenicity of *Escherichia coli*. Such an adhesion can be mediated by fimbriae, which are thin threadlike surface structures found in many *E. coli* strains. A single fimbria consists of approximately 100 identical protein subunits.

In *E. coli*, 50 to 70% of all strains possess the chromosomally determined type 1 fimbriae (I. Ørskov, F. Ørskov, A. Birch-Andersen, P. Klemm, and C. Svanborg-Edén, Semin. Infect. Dis., in press) which cause mannose-sensitive hemagglutination and adhesion to uromucoid, namely, Tamm-Horsfall glycoprotein (14). The expression of type 1 fimbriae is suppressed to a large extent by cultivation on solid medium (5).

In human urinary tract infections fimbriated *E. coli* strains are frequently isolated from cases of cystitis and pyelonephritis (8, 17), and a clear correlation has been shown to exist between adherence to urinary tract epithelial cells and presence of non-type 1 fimbriae on the bacteria (15, 16).

Recently, two urinary tract infection strains, C1212 and C1214, showing mannose-resistant and mannose-sensitive hemagglutination, respectively, were investigated (15). C1212 adheres to urinary epithelial cells, whereas C1214 attaches to uromucoid. The presence of fimbriae in strain C1212, grown on solid medium, was demonstrated by immunoelectron microscopy. These fimbriae were antigenically termed F7, although two distinctly different lines were formed in crossed immunoelectrophoresis (CIE). One of these was later shown to be caused by the mannose-resistant hemagglutination F7 fimbriae, and the other was shown to originate from a distinct fimbrial antigen which is also found in strains not having F7, e.g. C1023, a urinary tract infection strain not adhering to urinary epithelial cells. This fimbrial species has provisionally been called pseudo-type 1 (Ørskov et al., Semin. Infect. Dis., in press) but is in this study called 1C because of similarities to type 1 in the N-terminal sequence. Furthermore, it does not cause agglutination of the commonly used erythrocytes (I. Ørskov, A. Birch-Andersen, M. Kanamori, and C. Svanborg-Edén, Scand. J. Infect. Dis., in press). The presence of different fimbriae, some of which were mannose-sensitive hemagglutination type 1 fimbriae and termed 1A and others of which were termed 1B because of sequence similarity with type 1 fimbriae (Ørskov et al., Semin. Infect. Dis., in press), was demonstrated in the C1214 strain.

In this study we present the isolation and protein chemical characterization, i.e., N-terminal sequences and amino acid composition, of fimbriae from strains C1212 and C1214, as well as fimbriae, notably type 1C, from a third urinary tract infection strain, C1023.

MATERIALS AND METHODS

Bacterial strains. The three *E. coli* strains studied (Table 1) were all isolated from cases of urinary tract infection. Strains C1212 and 1214, both of serotype O6:K2:H1, have been described previously (15). Strain C1023, of serotype O83:K24:H31, was received from C. Svanborg-Edén, University of Gothenborg, Gothenborg, Sweden. C1023, which was isolated from a case of cystitis, has been shown not to adhere to urinary epithelial cells or to agglutinate human erythrocytes but to be able to agglutinate guinea pig erythrocytes in an mannose-sensitive manner (C. Svanborg-Edén, personal communication) and to attach to uromucoid (I. Ørskov, unpublished data).

Culture and crude extracts of fimbriae. Bacteria were grown on solid ox heart infusion broth medium prepared as described earlier (15). Cells from confluent growth on 50 large (14-cm) agar plates were suspended in 300 ml of 0.1 M sodium phosphate, pH 7.0. The suspension was heated at 60°C for 20 min and sheared in an Ultra Turrax dispension apparatus (Janke and Kunkel, Staufen, West Germany) for 2 × 1 min to detach the fimbriae. Bacteria and large debris were removed by centrifugation at 27,000 × *g* for 15 min, and the supernatant was filtered through a 0.80-μm-pore size filter (Millipore Corp., Bedford, Mass.).

TABLE 1. Characteristics of the three *E. coli* strains

Bacterial strain	Type of fimbriae	Adhesion of strain to urinary epithelial cells or uromucoid and to human or guinea pig erythrocytes[a]
C1212	F7 1C[b] (1A)[c]	Mannose-resistant adherence to epithelial cells and to human erythrocytes
C1214[d]	1A 1B	Mannose-sensitive adherence to uromucoid (Tamm Horsfall protein) and to guinea pig erythrocytes
C1023[d]	1A 1C	Mannose-sensitive adherence to uromucoid (Tamm Horsfall protein) and to guinea pig erythrocytes

[a] Tested after growth on solid medium.

[b] Type 1C fimbriae are not included in the adherence results given (see text).

[c] Type 1A is in parentheses because only a minor amount was found. The strain showed no mannose-sensitive agglutination of guinea pig erythrocytes (the test indicating presence of type 1A) unless it has been grown in fluid medium.

[d] Apart from the lines formed in CIE by type 1A, 1B, or 1C fimbriae, strains C1214 and C1023 each formed an additional line of unknown origin.

Gel filtration. Gel chromatography of crude extracts were carried out on a Sepharose 2B column, and the fimbrial proteins were further purified on Sepharose CL 6B or G75 columns (Pharmacia, Uppsala, Sweden) in guanidinium chloride-containing buffers.

Polyacrylamide gel electrophoresis. The purity and molecular weight of fimbrial subunits were assessed by electrophoresis in polyacrylamide slab gels in the presence of 0.1% sodium dodecyl sulfate (13). Samples were boiled in 1% sodium dodecyl sulfate for 15 min and treated with fluorescamine (Hoffmann-LaRoche, Basel, Switzerland) before electrophoresis (4).

Amino acid analysis. Amino acid analysis was performed on a Durrum D500 analyzer. Samples in duplicate were hydrolyzed for 24, 48, and 72 h at 110°C in 6 N HCl containing 0.1% phenol in evacuated ampoules. Furthermore, samples of performic acid-oxidized proteins were hydrolyzed to determine cysteine as cysteic acid. For serine and threonine, values extrapolated to zero time were used, whereas the values of 72-h hydrolysis were used in the cases of valine and isoleucine.

Sequence analysis. The amino-terminal sequences of the fimbrial proteins were examined by manual Edman degradation in the presence of sodium dodecyl sulfate (11), and identification of the phenyl thiohydantoin derivatives and parent amino acids was performed as described earlier (10).

Digestion with carboxypeptidase Y. The carboxy-terminal composition of the fimbrial proteins was determined by hydrolysis with carboxypeptidase Y in the presence of sodium dodecyl sulfate (6).

CIE. For CIE, the method of Weeke (18, 19) was used, as reported previously (15). Glass plates (5 by 5 cm) were covered by a 1.5-mm layer of agarose Litex (HSA) in Tris-barbital buffer at pH 8.6 (ionic strength, 0.05). A 15-μl extract, prepared as described previously (15), of strain C1212, C1214, or C1023 was placed in the well in the lower gel. The first-dimension electrophoresis was run at 5 V/cm for 60 min. Then the corresponding antiserum prepared with whole cell culture and diluted 1:8 was incorporated in the top gel (30 μl/cm^2), either unabsorbed or made fimbria specific by absorption (15). For examination of content of antigens or of relatedness between antigens (absorption in situ [1]), samples originating from gel filtrations (1 mg/ml) or extracts of the strains, antisera (undiluted), or phosphate-buffered saline, included for comparison, were used in the intermediate gel (32 μl/cm^2). Application of antigen extract in the intermediate gel will cause elevation of the line formed by a related antigen present in the extract in the lower gel well, whereas the same line will be formed closer to the bottom if antiserum instead of antigen extract is incorporated in the intermediate gel. The second-dimension electrophoresis was run at 2 V/cm overnight. The dried gels were stained with Coomassie blue in acetic acid-ethanol-water (1:4.5:4.5, by volume).

RESULTS

Table 1 shows the fimbrial antigens and the adherence abilities of the three strains examined. After growth on solid medium, fimbrial antigens F7 and 1C could be demonstrated by CIE in strain C1212 and antigens 1A and 1B

could be shown in strain C1214 (Ørskov et al., Semin. Infect. Dis., in press). CIE with extract of strain C1023 against unabsorbed, homologous antiserum showed the presence of several lines, two of which were of unquestionable fimbrial origin relevant for the present study (Fig. 1a); one of these two lines was caused by the type 1 fimbrial antigen, termed 1A. This antigen is related but not identical to 1A of strain C1214, as demonstrated by CIE. The other fimbrial line formed by C1013 was identical to the 1C line of strain C1212.

In the initial purification step, i.e., chromatography on Sepharose 2B, the large size of intact fimbriae was exploited and virtually all fimbrial material was eluted in the void volume (Fig. 2). In all three strains part of this fimbrial material proved much more resistant to disruption by guanidinium chloride than the remaining material. This fact was exploited in the second purification step on Sepharose CL6B (Fig. 3), where the sturdy material appeared early in the elution profiles, (e.g., pool B in Fig. 3), whereas the other fimbrial material present seemed to depolymerize much more readily and therefore was eluted later, apparently as monomers (e.g., pool C in Fig. 3).

The N-terminal sequence (Fig. 4) of the early eluted sturdy material proved in all three strains to be identical with previously reported results for type 1 fimbriae (7), and in CIE it corresponded to the fimbrial line termed 1A (regard-

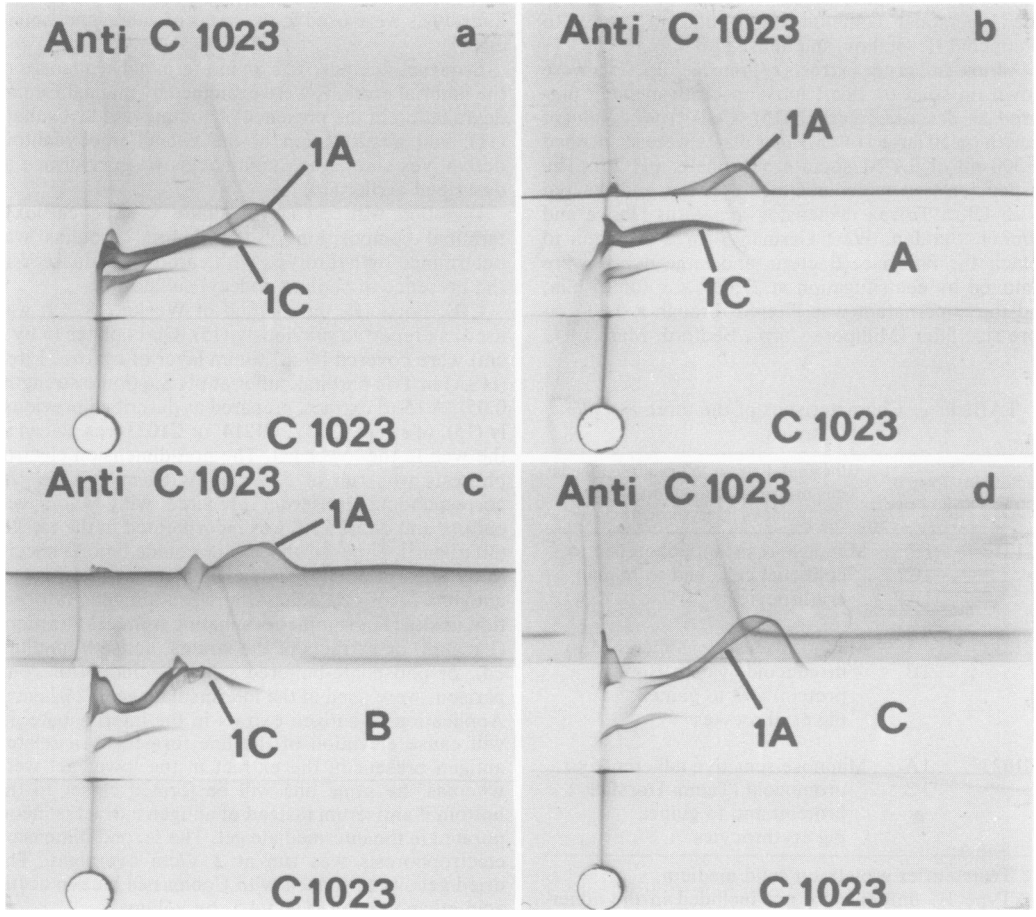

FIG. 1. CIE showing the content of fimbrial antigens 1A and 1C in pooled fractions of C1023 after fractionation by means of absorption in situ. Lower gel wells (a, b, c, and d): antigen extract C1023; upper gels (a, b, c, and d): antiserum against C1023. Intermediate gel (a): buffered saline; (b, c, and d): pooled fractions A, B, and C, respectively, of C1023 referring to Fig. 3. (c) shows that the main content of pooled fraction B is 1A, since the 1A line is much more elevated than the 1C line compared with (a). Conversely, pooled fraction C (d) has a much higher content of substance 1C than of 1A, since the 1C line has been elevated to such an extent that it has disappeared, whereas the 1A line is only somewhat elevated. None of the fractions was thus completely pure. The anode is to the right in the first dimension and at the top in the second dimension.

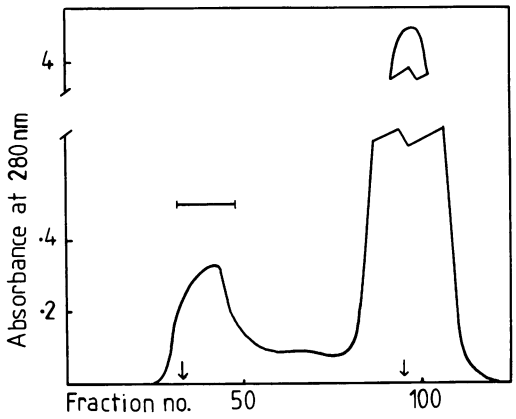

FIG. 2. Fractionation of a crude extract of fimbriae from *E. coli* strain C1212 on a Sephadex 2B column (5 by 85 cm). A 300-ml suspension was applied to the column and eluted with 0.1 M NH_4HCO_3 at a flow rate of 65 ml/h. Fractions of 18.5 ml were collected. Arrows indicate V_0 (left) and V_t (right). Bar indicates fractions that were used for further purification.

ing C1023, see Fig. 1c). The presence of type 1A fimbriae, albeit in small amounts, in strain C1212 after growth on solid medium was surprising, since this strain agglutinates guinea pig erythrocytes only after cultivation in fluid medium.

Figure 4 further shows the N-terminal sequence of type 1C fimbriae, isolated from pool C in Fig. 3. The preparation contained minute quantities of type 1A material (Fig. 1d), but in

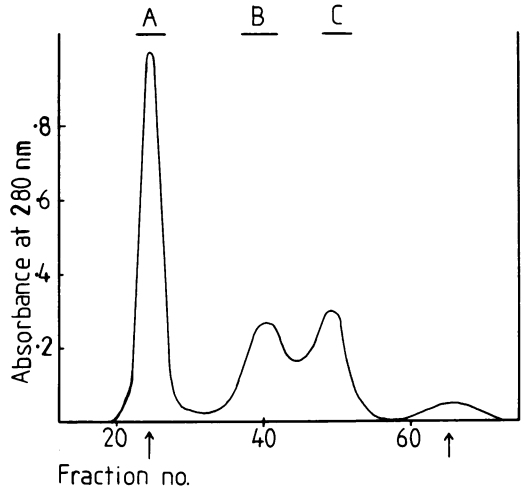

FIG. 3. Further fractionation of fimbriae from *E. coli* strain C1023 on a Sepharose CL 6B column (1.5 by 90 cm). A 3.5-ml portion of material dissolved in 6 M guanidinium chloride in 0.1 M NH_4HCO_3, pH 8, was applied and eluted with the same buffer. Arrows indicate V_0 (left) and V_t (right). Bars and letters indicate pooled fractions.

TABLE 2. Amino acid composition and molecular weights of fimbrial antigens 1A, 1B, 1C, and F7

Antigen (bacterial strain)	Amino acid composition (no. of residues)																Mol wt[a]
	Asp	Thr	Ser	Glu	Pro	Gly	Ala	Cys	Val	Met	Ile	Leu	Tyr	Phe	His	Lys	Arg	
1A (C1214)	22	16	12	14	4	17	19	2	13	1	7	10	4	6	1	8	3	17,000
1B (C1214)	23	19	13	15	4	18	20	2	14	1	7	11	4	5	1	8	3	17,500
1C (C1023)	20	19	11	13	4	17	20	2	19	0	6	8	3	7	2	7	3	17,000
1C (C1212)	22	18	12	14	4	17	18	2	18	0	6	8	3	7	2	7	3	17,000
F7 (C1212)	24	22	15	19	6	22	21	2	22	1	10	11	3	11	2	13	3	22,000

[a] Molecular weights are apparent values, as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

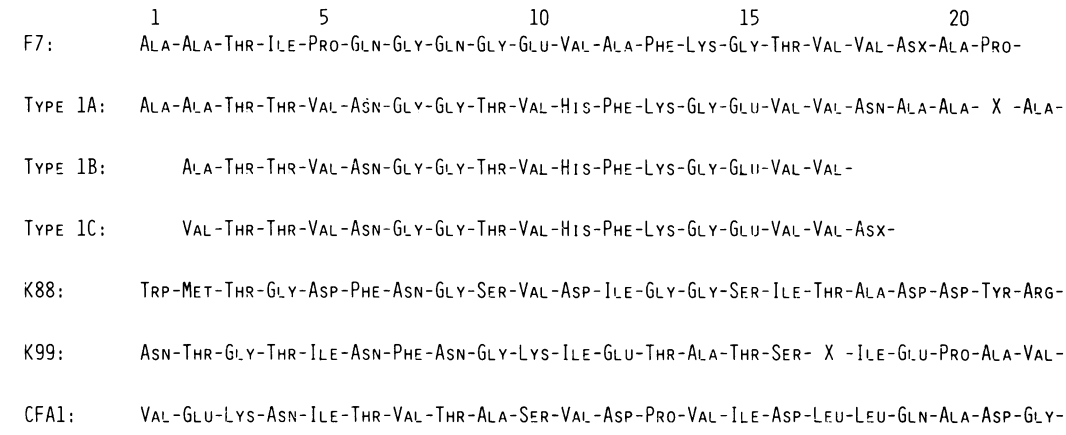

FIG. 4. N-terminal amino acid sequences of F7 and type 1A, 1B, and 1C fimbrial proteins of *E. coli* strains C1212, C1214, and C1023. The N-terminal sequences of the K88, K99, and CFA1 fimbrial subunits of *E. coli* have been included for comparison. X indicates unidentified residues.

such small amounts that both sequence analysis and amino acid analysis could be performed with confidence. In a similar manner, type 1B fimbrial protein from strain C1214 was isolated and characterized (Fig. 4; Table 2). In the case of strain C1212, which harbored more than two different fimbriae, an additional purification step, using chromatography on Sephadex G75 in 6 M guanidinium chloride, was used to obtain sufficiently pure material. The subunits shown to constitute the F7 and type 1C fimbriae were thus separated from each other to more than 90% purity, as estimated by sequence analysis. In this analysis type 1C of C1212 was identical to 1C of C1023. From Fig. 4, in which the N-terminal structures of the K88, K99, and CFA1 fimbriae from *E. coli* have been included for comparison (2, 9, 10), it is seen that the N-terminal structures of types 1B and 1C closely resemble the type 1A sequence. The 1B and 1C sequences are one residue shorter and, furthermore, an alanine has been replaced by a valine residue in the case of type 1C. The sequence of the 21 N-terminal amino acid residues of the F7 subunit reported here might indicate a relationship with the type 1 sequences. Thus, the three N-terminal residues are identical to the first three residues in the type 1A sequence. Furthermore, the sequence that encompasses residues 13 to 20 shows seven of eight residues identical to the corresponding type 1 sequence.

Figure 5 shows the results from an analysis of the carboxy-terminal structures of type 1B and 1C fimbriae. Both of these proteins proved to possess identical C-terminal structures not resembling those of hitherto reported C-terminal structures of fimbriae, which have been included for comparison (2, 9; P. Klemm, unpublished data). The technique used did not permit distinction between asparagine and glutamine. The preparations of F7 and type 1A fimbrial proteins were not considered to be of sufficient purity to allow analysis of their C-terminal structures.

The amino acid composition of fimbrial subunits and their molecular weights, as estimated by polyacrylamide gel electrophoresis, are presented in Table 2. The amino acid compositions of type 1A fimbriae originated from strain C1214, whereas those from strains C1023 and C1212 were not pure enough to give meaningful results in these examinations, although they were pure enough for sequencing, in which case a 10% impurity is normally not a problem.

As previously reported (15; Ørskov et al.,

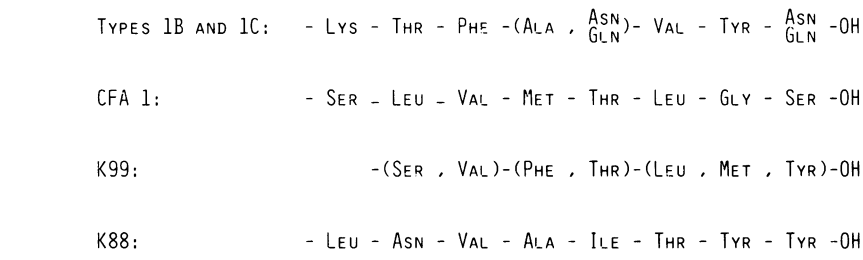

FIG. 5. C-terminal structures of the type 1B and 1C fimbrial proteins from *E. coli* strains C1212, C1214, and C1023. C-terminal sequences of the K88, K99, and CFA1 fimbrial proteins have been included for comparison.

Semin. Infect. Dis., in press), fimbriae of types 1A, 1B, 1C, and F7 are immunologically distinct. However, some relationship can be demonstrated between types 1B and 1C. Figure 6 shows that line 1B of C1214 can be displaced by application of antiserum against C1023 containing 1C antibodies, but not by application of the C1023 extract in the intermediate gel (Fig. 6b). The relationship between the type 1A fimbrial antigens of the two strains can primarily be visualized by the elevation of the type 1A precipitation line in Fig. 6b (see legend to Fig. 6).

DISCUSSION

In a recent report (Ørskov et al., Scand. J. Infect. Dis., in press) it was shown that the ability of strain C1212 to attach to urinary epithelial cells was due to the presence of F7 and not type 1C fimbriae. F7 is evidently a completely distinct fimbrial species. It shows no or only minor immunological connection with other fimbrial proteins. The available data indicate a partial sequence homology with type 1 fimbriae, but the different sizes of the respective subunits,

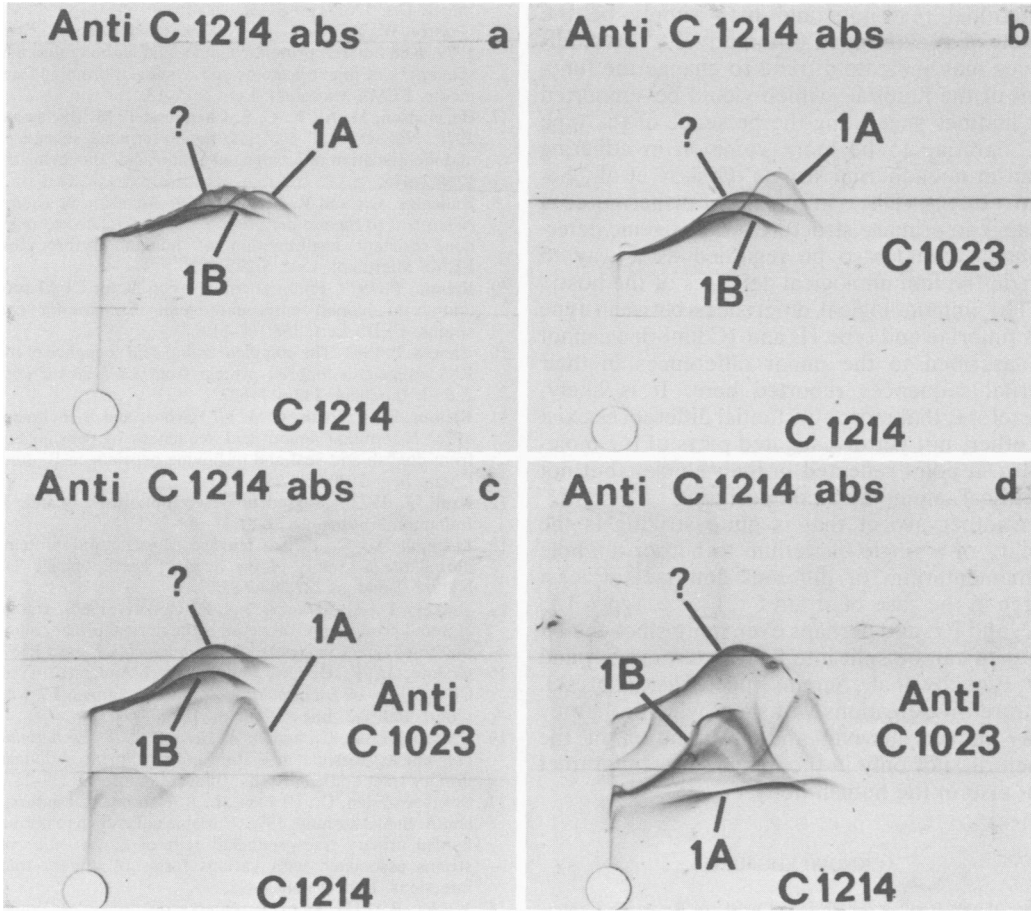

FIG. 6. CIE showing relationship between fimbrial antigens 1B and 1C. Lower gel wells (a, b, c, and d): antigen extract C1214. Upper gels (a, b, c, and d): absorbed antiserum against C1214 (anti-1A and -1B). Intermediate gel (a): buffered saline; (b) antigen extract C1023; (c and d) antiserum against C1023 (anti-1A and -1C) from the same rabbit, but the one in (d) originates from a later bleeding in the immunization procedure than that in (c) and thus is the most hyperimmune of the two antisera. A relationship between 1A of C1214 and C1023 is seen in (b), where the 1A peak is a little enlarged compared with (a), and in (c) and particularly in (d), where the 1A lines have been formed closer to the bottom. It is known that application of antisera in the intermediate gel is a more sensitive method for demonstration of cross-reactions than application of antigen extracts, but it does not define the degree of structural relatedness between antigens. The presence of antibodies in C1023 antiserum reacting with 1B antigen of C1214 is shown in (d), where the 1B line is formed at a lower position than in (a), (b), and (c). This relationship between 1B and 1C was confirmed by crosswise examinations in all three reference systems: C1212, C1214, and C1023.

i.e., 22,000 versus 17,000, and their different amino acid compositions suggest that these structures are not closely related. The N-terminal sequence of F7 does not reveal any special features, such as high hydrophobicity, that could point to a possible function of this part of the molecule.

The function of type 1B and 1C fimbriae, found on strains described in this work and on other *E. coli* strains (Ørskov et al., Semin. Infect. Dis., in press) is currently being investigated. No strain has yet been found with only type 1B or 1C. The differences in structure of these type 1-like fimbriae might not impose functional restraints and may simply be the result of genetic drift. Alternatively, the differences may indicate a trend to change the function of the fimbriae, which would be supported by findings suggesting the presence of the type 1C fimbriae to be more common in adhering than in nonadhering strains (Ørskov et al., Semin. Infect. Dis., in press). Furthermore, a change in primary structure and antigenic determinants could also be regarded as a way to evade the immunological defenses of the host.

The immunological differences between type 1A fimbriae and type 1B and 1C fimbriae cannot be ascribed to the minor differences in their partial sequences reported here. It is likely, therefore, that more substantial differences exist in other, not yet investigated parts of the molecules, a point reflected in their similar, but not identical, amino acid compositions.

Another aspect that is quite striking is the ability of a single bacterium to harbor a whole armamentarium of different fimbriae, at least three in the case of strain C1212, i.e. types 1A, 1C, and F7, and perhaps even more since the F7 antigen can be split into factors termed $F7_1$ and $F7_2$ (Ørskov et al., Semin. Infect. Dis., in press). Future investigations will show which role they play in the survival and proliferation of the bacteria, not only in the human urogenital tract but also in the human body.

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