

Nucleotide sequence of the genes coding for minor fimbrial subunits of the F1C fimbriae of *Escherichia coli*

I. van Die⁽¹⁾(*), C. Kramer⁽¹⁾, J. Hacker⁽²⁾, H. Bergmans⁽¹⁾,
W. Jongen⁽¹⁾ and W. Hoekstra⁽¹⁾

⁽¹⁾ Dept. of Molecular Cell Biology, University of Utrecht,
Padualaan 8, CH 3584 Utrecht (the Netherlands) and

⁽²⁾ Institut für Genetic und Mikrobiologie, Universität Würzburg,
Röntgenring 11, D-8700 Würzburg (Germany)

SUMMARY

F1C fimbriae allow uropathogenic *Escherichia coli* to adhere to specific epithelial surfaces. This adhesive property is probably due to the presence of minor fimbrial components in F1C fimbriae. The *foc* gene cluster encoding F1C fimbriae has been cloned, as described previously. Here we present the nucleotide sequence (2081 bp) coding for the F1C minor fimbrial subunits. The structural genes code for polypeptides of 175 (FocF), 166 (FocG), and 300 (FocH) amino acids.

The deduced amino acids of the F1C minor subunits were compared with the reported sequences of the minor subunits of other types of fimbriae. The data show that the Foc minor subunits are highly homologous to the corresponding Sfa proteins, whereas homology to the minor subunits of type 1 and P fimbriae is much lower.

Key-words: Pilus, *Escherichia coli*, Adherence, Urinary tract; Foc protein, Minor subunits, Sequencing, Homology.

INTRODUCTION

Virulence of uropathogenic *Escherichia coli* strains has been related to their ability to adhere to uroepithelial cells. Adherence is mediated by adhesive proteins (minor subunits) that are often located at the tip of fimbriae (Klemm, 1985). In uropathogenic *E. coli*, various fimbrial adhesins

(e.g. type 1, P, S, and F1C) are found that are distinguished by their receptor specificities (Orskov and Orskov, 1983; Klemm, 1985; Hacker, 1989). It has been shown that F1C fimbriae contribute to the adhesive properties of uropathogenic *E. coli*. Virkola *et al.* (1988) showed that F1C fimbriae mediate adherence to the collecting ducts and the distal tubules of the human kidney. Recently, Marre *et al.* (1990)

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(*) Corresponding author. Present address: Dept. of Medical Chemistry, Vrije Universiteit, Van der Boechorststraat 7, 1081 BT Amsterdam (the Netherlands).

showed the adherence of F1C fimbriae to cultured renal tubulus cells.

The gene cluster encoding F1C fimbriae has been cloned and analysed in detail (Van Die *et al.*, 1985; Riegman *et al.*, 1990). Genetically, F1C fimbriae have been shown to be very closely related to S fimbriae (Ott *et al.*, 1987, 1988; Riegman *et al.*, 1990). In both gene clusters, the distal part encodes minor fimbrial subunits (Hacker, 1989; Schmoll *et al.*, 1989; Riegman *et al.*, 1990). It is expected that (one of) the minor subunits will confer the adhesive properties to the F1C fimbriae, as has been shown for other fimbrial types (Lindberg *et al.*, 1986; Klemm and Christiansen, 1987; Morschhäuser *et al.*, 1990). For the S fimbriae, the 15-kDa protein SfaS has been determined as the component that binds to carbohydrate chains terminating with sialyl- α 2 \rightarrow 3Gal- β 1 \rightarrow R (Parkinen *et al.*, 1986; Moch *et al.*, 1987; Morschhäuser *et al.*, 1990). In this paper, we present the nucleotide sequence of the region coding for minor fimbrial components of the F1C fimbriae. This sequence is compared with the published nucleotide sequence of the corresponding region of the S-fimbrial gene cluster.

MATERIALS AND METHODS

Bacterial strains and phages

The *E. coli* K12 strain JM101 was used to propagate phages M13 mp8, mp9 and clones derived from these strains (Messing and Vieira, 1982).

Recombinant DNA techniques

Restriction endonucleases and ligase (Pharmacia, Sweden) were used according to the manufacturers' specifications. Isolation of plasmid or phage RF DNA was carried out by the mini-lysate method, essentially as described by Holmes and Quigley (1981). Isolation of single-stranded M13 DNA was performed essentially according to Heidecker *et al.* (1980). Nucleotide sequencing was carried out by the chain termination method of Sanger *et al.* (1977). With the aid of a T7-sequence kit (Pharmacia), recombinant M13 or plasmid DNA was sequenced according to the protocol supplied by the manufac-

turer. Primers used were the M13 primer supplied with the kit, or alternatively 18-mer oligodeoxy-nucleotides synthesized on a "Biosearch 8600" DNA synthesizer.

Transformation was carried out essentially as described by Mandel and Higa (1970).

RESULTS AND DISCUSSION

Nucleotide sequence of the *focF*, *focG* and *focH* genes

The approximate localization of the *focF*, *focG* and *focH* genes, encoding minor fimbrial subunits, in the F1C gene cluster has been described previously (Riegman *et al.*, 1990). The strategy for the determination of the nucleotide sequence is outlined in figure 1. Restriction frag-

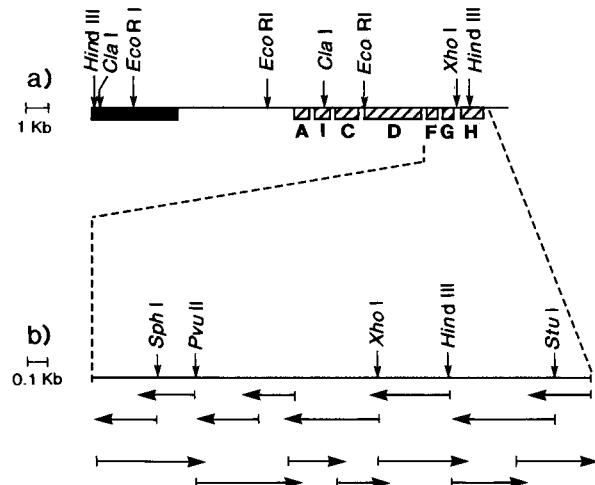


Fig. 1. Physical and genetic map of the *foc* gene cluster and sequencing strategy.

(a) Physical and genetic map of pPIL110-51 (Van Die *et al.*, 1985; Riegman *et al.*, 1990); the hatched boxes represent genes A-I, involved in the expression of F1C fimbriae; the black box represents pACYC184 (Chang and Cohen, 1978) vector DNA.

(b) Strategy of nucleotide sequence analysis. The length of the sequenced DNA fragments and the direction of sequencing is shown by arrows.

ments of pPIL110-51 were cloned in M13 mp8 or mp9 and sequenced. A nucleotide sequence of 2081 bp was determined by sequencing both strands of the entire region, and is shown in figure 2. Since the amino acid sequences of the F1C minor subunit proteins have not been elucidated, it is not possible to precisely locate the N termini. However, three open-reading frames (ORF) became apparent from the nucleotide sequence. The first ORF (*focF*) starts at an ATG codon at position 31, the second and third ORF at positions 579 and 1142, respectively. The first part of the sequences show the characteristics of prokaryotic signal sequences, with potential cleavage sites as indicated in

figure 2. The molecular weights, calculated on the basis of the sequence data, of the proteins encoded by these genes are 15, 17 and 30 kDa, respectively. Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis of F1C fimbriae showed, next to the 16-kDa FocA major subunit, two subunits with apparent molecular weight of 14 and 32 kDa, which correspond well with the calculated masses for FocG and FocH (Riegman *et al.*, 1990). No 17-kDa protein has been observed by SDS-PAGE analysis of F1C fimbriae. This might be explained by comigration of the putative *focF* product with the abundant 16-kDa FocA protein.

ACGGCTAGTTGCAGGTAAAGAGGCAGGGAA	ATG GTG AAG GAT ATT ATT	48
<i>FocF:</i> Met Val Lys Asp Ile Ile		
AAA ACA GTG ACA TTC TCC TGC ATG CTG GCC GGC AGT ATG TTC GTT ACC		
Lys Thr Val Thr Phe Ser Cys Met Leu Ala Gly Ser Met Phe Val Thr		
TGT CAT GTC TGT GCA GCG GGT TCT GTG GTG AAT ATT ACA GGG AAT GTT	144	
Cys His Val Cys Ala Ala Gly Ser Val Val Asn Ile Thr Gly Asn Val		
CAG GAT AAC ACC TGC GAT GTT GAC ATT AAC TCC CGA AAC CTT GAT GTC		
Gln Asp Asn Thr Cys Asp Val Asp Ile Asn Ser Asn Leu Asp Val		
AGT CTG GGA AGT PAT GAC AGC CGA CAG TTT ACC GCA GCT GTT GAT ACC	240	
Ser Leu Gly Ser Tyr Asp Ser Arg Gln Phe Thr Ala Ala Gly Asp Thr		
ACA CCT GCG TCG GTA TTT CAT GTC GGG TTA ACC TCC TGC GGC AGT GCT		
Thr Pro Asn Val Phe His Val Gly Leu Thr Ser Cys Gly Ser Ala		
GTC AGT GCA GTG AAG CTG ACA TTT ACG GGC ACA CCA GAT ATT CAG GAG	336	
Val Ser Ala Val Lys Leu Thr Phe Thr Gly Thr Pro Asp Asn Gln Glu		
GCG GGG CTT ATT CAG ATT AAC AGC ATTA AAT CGG CCG CCG GGT GTG GGG		
Ala Gly Leu Ile Glu Ile Asn Ser Ile Asn Gly Ala Arg Phe Val Val Gly		
ATT CAG CTT CTT GAT AAG GAT AAA CAT GAG CTG AAA ATT ATT GTG CCG	432	
Ile Gln Leu Leu Asp Lys Asp Lys His Glu Leu Lys Ile Asn Val Pro		
ACA ACA ATT GCG TTG ATT ACG CCG GGA ACA CAG ACC ATA GCG TTT ATT GCT		
Thr Thre Ile Ala Leu Met Pro Gly Thr Gln Thr Ile Ala Phe Tyr Ala		
CGT CTG AAA GCC ACT TAT CTT CCG GTC AAG GCC GGT ATT GTT GAT GCG	528	
Arg Leu Lys Ala Thr Tyr Leu Pro Val Lys Ala Gly Asn Val Asp Ala		
GTG ATT ATT TTT GTC CTT GAC TAT CAG TAAATAAACACAGAGGAAAAACAG		
Val Ile Leu Phe Val Leu Asp Tyr Lys End		
<i>FocG:</i>		
ATG AAA CTG AAA GCT ATT ATA TTG GCC ACC GGT CTT ATT AAC TGT ATT	627	
Met Lys Leu Lys Ala Ile Ile Leu Ala Thr Gly Leu Ile Asn Cys Ile		
GCA TTT TCA GCA CAG GCA GGT GAT ACG ACC GGT ATT ACT GTT ACA GGG AGG		
Ala Phe Ser Ala Gln Ala Val Asp Thr Thr Ile Thr Val Thr Gly Arg		
GTC TTG CCA CGT ACC TGT ACC ATT GGT ATT CGG GGA AAC CCA AAC GCC	723	
Val Leu Pro Arg Thr Cys Thr Ile Gly Asn Gly Asn Pro Asn Ala		
ACC GTT TTG TTG GAT AAC GCT TAC ACT TGT GAC CTG ATA GCA GCC AAC		
Thr Val Val Leu Asp Asn Ala Tyr Thr Ser Asp Leu Ile Ala Asn Asn		
AGC ACC TCT CAG TGG AAA ATT TTT TCG TTG ACA TTG ACG ATT TGT CAG	819	
B19 Ser Thr Ser Gln Trp Lys Asn Phe Ser Leu Thr Asn Cys Glu		
ATT GTC AAC ATT GTT ACT AGC TTT GGT GGA AAC GCA GAA ATT ACA ATT		
Asn Val Asn Asn Val Thr Ser Phe Gly Gly Thr Ala Glu Asn Thr Asn		
TAT TCT AGA AAC ATT GGT GAT GCT ACT ATT ATC ATT GTT GAG CTA CAG	915	
Tyr Tyr Arg Asn Thr Gly Asp Ala Thr Asn Met Val Gln Leu Gln Ala		
GAA CAA GGT AAT GGT ATT ACC CCC TTG AAA GTT GGT TCA ACA AAA GTT		
Glu Gln Gly Asn Gly Asn Thr Pro Leu Lys Val Gly Ser Thr Lys Val		
GTT ACA GTG AGC ATT GGG CAG GGC ACA TTC ATT CTT AAA GTC COT GCC	1011	
Val Thr Val Ser Asn Gln Ala Ile Thr Phe Asn Leu Lys Val Arg Ala		
GTA AGC AAA GGT AAT GCT GGT GCG GGA AGT ATT ATT TCA CAA ATT ACT		
Val Ser Lys Gly Asn Ala Gly Ser Ile Asn Ser Gln Ile Thr		
<i>FocH:</i>		
GTC ACC TAT ACC TAT GCG TAA ATATTATCCCCTCTTTAAGAAAAGCACCGTCCTCG	1115	
Val Thr Tyr Thr Tyr Ala End		
AGGGCTGTGTTTATTACATTTATA		
ATG GCA TAT TCC CAG CCA TCG TTT GCA		
FocH: Met Ala Tyr Ser Gln Pro Ser Phe Ala		
CTG TTG TGC AGA ATT AAC CAA ACA GGG CAG ACT TTT CAG TCT CGA GAC	1216	
Leu Leu Cys Arg Asn Asn Gln Thr Gly Gln Thr Phe Gln Ser Gly Asp		
AGT CGC TTC AAT ATC ACT CTT TCC CCA ACA GTT CAG TAT GAT AAA GCC		
Ser Arg Phe Asn Ile Thr Leu Ser Pro Thr Val Gln Tyr Asp Lys Ala		
ATT ACA GTT CTG GAT TTA ATT CAA CTG GNG TTA TGT CAG ATT GAA GAT	1312	
Ile Thr Val Leu Asp Leu Asn Gln Leu Val Leu Cys Gln Asn Glu Asp		
GCC TCT GGT CAG AAC TAT GAC TAT CTC AGG GTC AGA CAG GGA ACC GGT		
Ala Ser Gly Gln Asn Tyr Asp Tyr Leu Arg Val Arg Gln Gly Thr Gly		
TTC TCT CCT TCA TTA GAT GCT AAA ACA TAC GGA AGG CTG GAC TTT ACA	1408	
Phe Ser Pro Ser Leu Asp Ala Lys Thr Tyr Gly Arg Leu Asp Phe Thr		
AAC AGG CTT TCT GGG TAT AGT CAG ACC TTA CCG CTG CAG CAA GAC GCA		
Asn Asn Ser Gly Gln Thr Leu Pro Leu Gln Gln Asp Thr		
AAG CCA ACA GAA GCT TAC TGG CAA TAT GGT GTC TGG AAA CCTT TTC CGG	1504	
Lys Pro Thr Glu Ala Tyr Trp Gln Tyr Val Trp Lys Pro Phe Pro		
GCA AAA ATG TAC CCT TAT CCT GAG CCC GGC GTT TTC GGG AAA CTG ATA		
Ala Lys Met Tyr Leu Tyr Pro Glu Pro Gly Val Phe Gly Lys Leu Ile		
CAT GCG GGA GAA TTA GTG GCC ACA GTT TAT GTT ATT AAT TTT TCC ACC	1600	
His Ala Gly Glu Leu Val Val Ala Thr Val Tyr Val Asn Lys Phe Ser Thr		
ATG GGG CAG GAC GCA GGG GAG AGA ATT TTC ACC TGG CGT TTC TAT GCA		
Met Gly Gln Gln Ala Gly Glu Arg Asn Phe Thr Trp Arg Phe Tyr Ala		
ACG AAT GAT GTC TAT ATC CAG ACA GGT ACA TGC AGG GTC TCA TCG AAC	1696	
Thr Asn Asp Val Tyr Ile Gln Thr Gly Thr Cys Arg Val Ser Ser Asn		
ATG GTC AAA GTT GAC CTT CGG TCC TAT CCT GGA GGC CGG GTC ACA GTC		
Asn Asp Val Lys Val Asp Leu Pro Ser Tyr Pro Gly Gly Pro Val Thr Val		
CCT CTT ACT GTC CGT TGC GAC CAG ACA CAG TCG GTC AGC TAT ACC CTG	1792	
Pro Leu Leu Val Arg Cys Asp Gln Thr Gln Ser Val Ser Tyr Thr Leu		
TCA GGT TCT GTC ACA GGA AGT GGT ATT ACT GTC TTC GCA ATT ACG GCA		
Ser Gly Ser Val Thr Gly Ser Gly Asn Thr Val Phe Ala Asn Thr Ala		
ACA TCA GGG GCC GGC GGT GTG GGT GTC CAG TTG TCG GAC AAC CGG GGG	1888	
Thr Ser Gln Ala Gly Val Gln Leu Ser Asp Asn Ala Gly		
CTG GTT CGG GCC CAA CGG AGG CCT TCT CTG GGA CAG GTC GGC AGC TCT		
Leu Val Pro Ala Gly Gln Pro Arg Ser Leu Gly Gln Val Gly Ser Ser		
CCT GTG AGT CTG CGG CTG AAG GCC TCT TAT GCT CTG ACC GGT CAG GCA	1984	
Pro Val Ser Leu Gly Leu Lys Ala Ser Tyr Ala Leu Thr Gly Gln Ala		
AGT CCG ACC CCC GGT GCT GTC CAG TCA GTG ATA ATT GTG ACT TTT AGC		
Ser Pro Thr Pro Gly Ala Val Gln Ser Val Ile Asn Val Thr Phe Ser		
TAC AAC TAG AATGCCAGTTGCGCGGAAATGATATTACTGCTCTTTATAT	2081	
Tyr Asn End		

Fig. 2. Nucleotide sequence and deduced amino acid sequence of the *focF*, *focG*, and *focH* gene.

Numbering of the residues starts at the lefthand side of the DNA fragment sequenced (see fig. 1). Numbers refer to the nucleotide positions. Potential signal sequence cleavage sites are indicated by arrows.

Comparison of the Foc minor subunit proteins with minor proteins of other fimbriae

The amino acid sequences, deduced from the nucleotide sequences, of the FocF, FocG and FocH proteins were compared with the corresponding proteins of S (Sfa), type 1 (Fim), and P (Fst) fimbriae (Klemm and Christiansen, 1987; Lund *et al.*, 1985; Schmoll *et al.*, 1989). The results (fig. 3, table I) show that all three Foc proteins show a high degree of similarity to the corresponding Sfa proteins. Homology to the Fim and Fst minor fimbrial proteins is clearly lower.

FocF and SfaG are nearly identical, with only three amino acid substitutions (homology 98%). Also, FocH and SfaH are very homologous (84%). Interestingly, homology between FocG and SfaS appears to be much lower (59%). SfaS represents the specific adhesin binding to sialic acid (Morschhäuser *et al.*, 1990), whereas F1C fimbriae lack that specific adhesive property. The high degree of homology between the *foc* and *sfa* gene clusters was also found in previous studies (Ott *et al.*, 1987, 1988; Riegman *et al.*, 1990; Schmoll *et al.*, in press). It strongly suggests that these gene clusters represent a family of adhesin determinants, as was observed for the P/Prs and the type 1 fimbrial adhesins; in the P/Prs and type 1 fimbrial systems, adhesive properties have been designated for the large 35-kDa minor subunits (Lindberg *et al.*, 1986; Klemm and Christiansen, 1987; Lund *et al.*, 1988; Riegman *et al.*, 1988). Up until now, it has not been clear which of the F1C subunits is responsible for the observed adhesive properties. It was found that HB101 cells carrying F1C fimbriae lacking the 32-kDa FocH subunit showed

Fig. 3. Comparison of the deduced amino acid sequences (given in standard one-letter code) of FocF, FocG and FocH with the SfaG, SfaS and SfaH minor subunit proteins.

Gaps (-) have been introduced to obtain maximum homology between the proteins. Identical amino acids between Foc and Sfa proteins are indicated as dots in the Sfa sequence.

Table I. Percentage homology between the Foc minor proteins and the corresponding proteins of S (Sfa), type 1 (Fim) and P (Fst) fimbriae (Schmoll *et al.*, 1989; Klemm and Christiansen, 1987; Lund *et al.*, 1985).

	SfaG	SfaS	SfaH	FimF	FimG	FimH	FstE	FstF	FstG
FocF	98	—	—	35	—	—	23	—	—
FocG	—	59	—	—	41	—	—	23	—
FocH	—	—	84	—	—	34	—	—	17

— = not determined.

normal F1C adhesive properties (Van Die and Korhonen, unpublished results). In receptor-binding studies, Marre *et al.* (1990) showed that the inhibition profiles of S and F1C fimbriae, although different in the sialic acid-binding property, resemble each other quite well. In addition, it was shown that the SfaG subunit contributes to binding of S fimbriae to renal tubulus cells. Based on this observation and the very high homology found between FocF and SfaG, the FocF protein most likely mediates the observed F1C adhesion to renal tubulus cells. It might be possible, however, that FocG (or FocH) is also involved in recognition of a still unknown receptor. P fimbriae (type F7₁) have been shown to possess several adhesins: the 35-kDa protein represents the P adhesin (Riegman *et al.*, 1988), whereas the two small minor subunits FsoE and FsoF are responsible for binding to fibronectin (Westerlund *et al.*, submitted for publication).

The latter results suggest that fimbriae can be regarded as very flexible carriers, able to present several different adhesins. Experiments are in progress to construct defined FocA, FocF and FocG mutants, so as to elucidate the possible adhesive properties of the different Foc fimbrial subunits in detail.

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Séquence nucléotidique des gènes codant pour les sous-unités des fimbriae F1C de *Escherichia coli*

Les fimbriae F1C permettent aux souches de *Escherichia coli* uropathogènes d'adhérer aux surfaces épithéliales spécifiques. Cette propriété adhésive est probablement due à la présence de composants mineurs des fimbriae F1C. Le groupe de gènes *foc* codant pour les fimbriae F1C ont été clonés selon des méthodes déjà décrites. Nous présentons ici la séquence nucléotidique (2081 pb) codant pour les sous-unités mineures des fimbriae F1C. Les gènes structuraux codent pour les polypeptides de 175 (FocF), 166 (FocG) et 300 (FocH) acides aminés.

Les séquences d'acides aminés des sous-unités mineures F1C, déduits des séquences nucléotidiques, ont été comparées avec celles connues des séquences des sous-unités mineures d'autres types de fimbriae. Ce travail montre que les sous-unités mineures Foc sont fortement homologues à celles des protéines Sfa, alors que l'homologie avec les sous-unités mineures des fimbriae de type 1 et P est moins importante.

Mots-clés: Pilus, Adhérence, *Escherichia coli*, Tractus urinaire; Sous-unités mineures, Séquençage, Protéine *Foc*, Homologie.

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