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

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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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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 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-α2→3Gal-β1→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 manufacturer. Primers used were the M13 primer supplied with the kit, or alternatively 18-mer oligodeoxynucleotides 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 *foc*F, *foc*G and *foc*H genes

The approximate localization of the *foc*F, *foc*G and *foc*H 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-

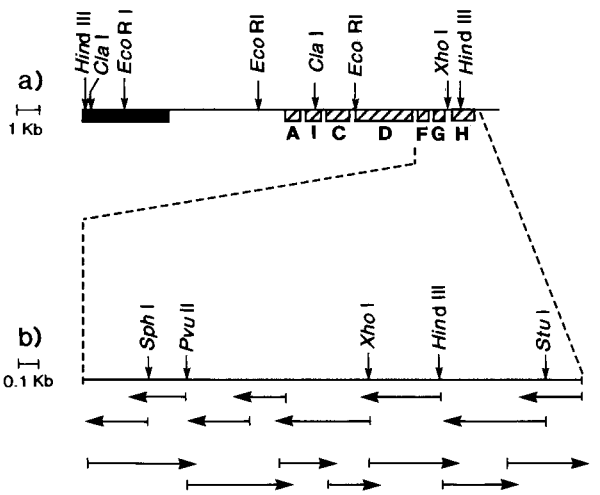

Fig. 1. Physical and genetic map of the *foc* gene cluster and sequencing strategy.

(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 (*foc*F) 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 *foc*F product with the abundant 16-kDa FocA protein.

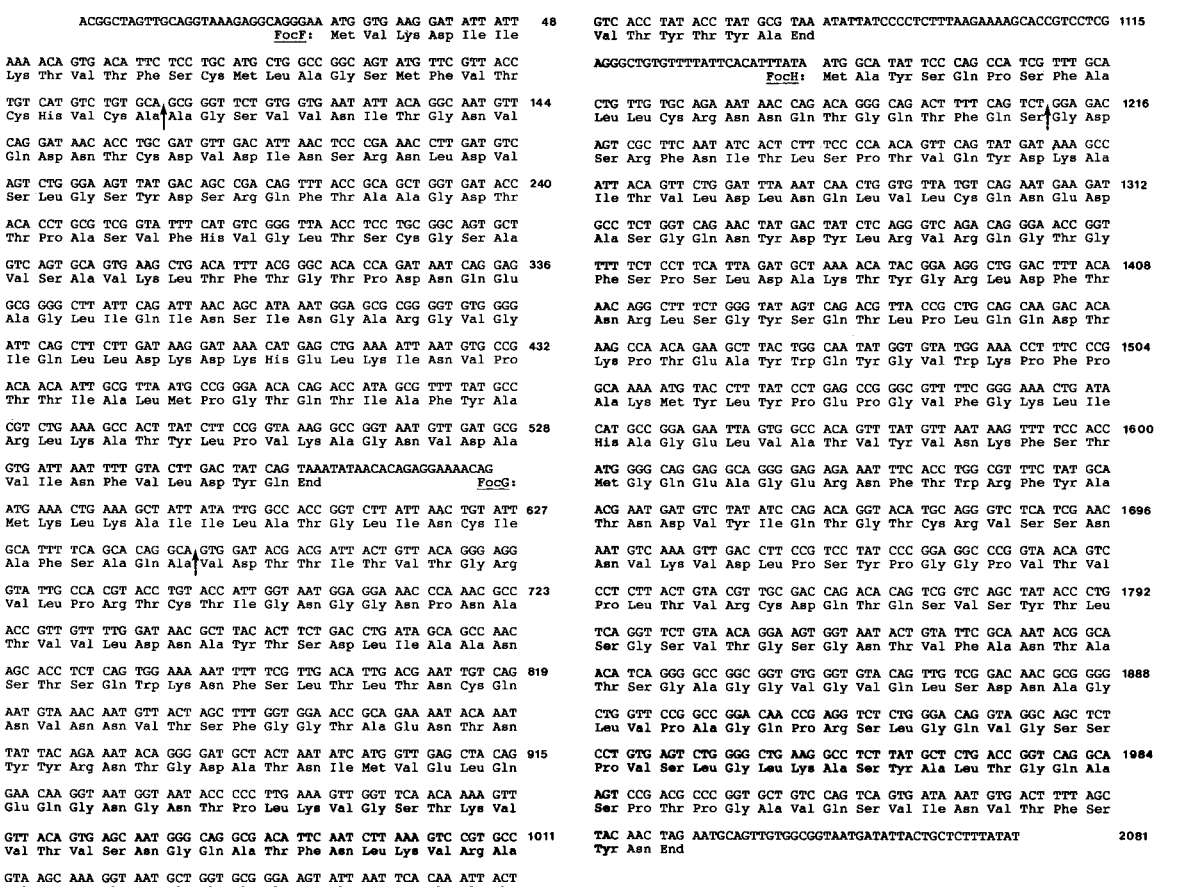

Fig. 2. Nucleotide sequence and deduced amino acid sequence of the *foc*F, *foc*G, and *foc*H gene.

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

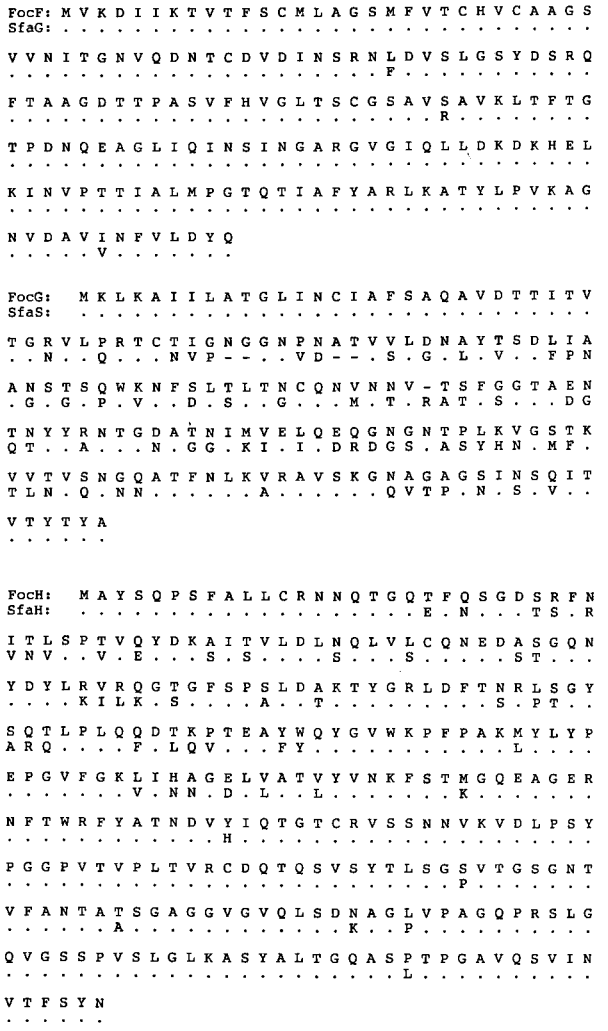

Fig. 3. Comparison of the deduced amino acid sequences (given in standard one-letter code) of FocF, FocG and FocH with the SfaG, SfaS and SfaH minor subunit proteins.

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 $F7_1$) 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 comparés 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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