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Analysis of genes coding for the sialic acid-binding adhesin and two other minor fimbrial subunits of the S-fimbrial adhesin determinant of *Escherichia coli*

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Summary

The S fimbrial adhesin (Sfa) enables *Escherichia coli* to attach to sialic acid-containing receptor molecules of eukaryotic cells. As previously reported, the genetic determinant coding for the Sfa of an *E. coli* O6 strain was cloned, the gene coding for the major fimbrial subunit was identified and sequenced and the S specific adhesin was detected. Here we present evidence that in addition to the major subunit protein SfaA three other minor subunit proteins, SfaG (17 kD), SfaS (14 kD) and SfaH (31 kD) can be isolated from the S-specific fimbrial adhesin complex. The genes coding for these minor subunits were identified, mutagenized separately and sequenced. Using haemagglutination tests, electron-microscopy and quantitative ELISA assays with monoclonal anti-SfaA and anti-SfaS antibodies the functions of the minor subunits were determined. It was determined that SfaS is identical to the S-specific adhesin, which also plays a role in determination of the degree of fimbriation of the cell. The minor subunit SfaH also had some influence on the level of fimbriation of the cell, while SfaG is necessary for full expression of S-specific binding. It was further shown that the amino-terminal protein sequence of the isolated SfaS protein was identical to the protein sequence calculated from the DNA sequence of the *sfaS* gene locus.

Introduction

Adhesins represent structures of the cell envelope of bacteria which mediate attachment to organic and inorganic surfaces (Savage and Fletcher, 1985). They may play a role in the interaction of saprophytic and symbiotic bacteria with plant cell surfaces (Dazzo *et al.*, 1986; Korhonen *et al.*, 1988). Bacterial adhesins also enable pathogenic isolates of Gram-positive and Gram-negative species to attach to receptor molecules of animal or human cells (Mirelman, 1986; Ott *et al.*, 1986; Meyer, 1987; Jann and Jann, 1989). Pathogenic bacteria may produce adhesins which are very often associated with fimbrial structures and are able to attach to erythrocytes. A single fimbria forms an appendage of the bacterial cell wall that is up to 2 μm in length and is 2.8–7 nm in diameter (Klemm, 1985).

Escherichia coli isolated from extraintestinal sources may cause urinary-tract infections (UTI), newborn meningitis (NBM) and sepsis (Orskov and Orskov, 1985). Fimbrial adhesins produced by extraintestinal *E. coli* can be distinguished by their receptor specificity. Common type 1 fimbrial adhesins recognize α-mannose in glycoproteins (Orskov and Orskov, 1983), and P fimbriae of uropathogenic *E. coli* interact with glycolipids containing α-gal-(1-4)-β-gal (Källenius *et al.*, 1980). S-fimbrial adhesins expressed by *E. coli* causing UTI or NBM attach to glycoproteins terminating with α-sialic acid-(2-3)-β-gal (Korhonen *et al.*, 1984; 1985; Parkkinen *et al.*, 1986). For F1C fimbriae, which are genetically related to S fimbriae, the receptor specificity has not yet been determined (van Die *et al.*, 1985; Virkola *et al.*, 1988).

Genetic and biochemical studies of type 1 and P fimbriae have shown that the major fimbrillin proteins which form the fimbrial structure of the cells and the adhesin proteins represent two different molecules encoded by different genes of the fimbrial adhesin determinants (Lindberg *et al.*, 1984; Maurer and Orndorff, 1987; Minion *et al.*, 1986). It was also shown that genes located at the immediate distal regions of the determinants code for the adhesins. In both cases the adhesins are proteins of about 30–35 kiloDaltons (kD) (Klemm and Christiansen, 1987; Lund *et al.*, 1988a,b; Hanson and Brinton, 1988; Hoschützky *et al.*, 1989). Recently the genetic determinant coding for the S-fimbrial adhesin (Sfa) of an *E. coli* O6 strain was cloned and the DNA sequence of the major subunit gene was established. It was also demonstrated that the subunit gene is not identical to the adhesin coding locus (Hacker *et al.*, 1985; Schmoll *et al.*, 1987; Ott *et al.*, 1987). The adhesin of the S-specific fimbrial adhesin complex (FAC) was isolated and monoclonal antibodies specific for this protein of 12–14 kD

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(depending on the gel system used) were selected (Moch *et al.*, 1987).

In this paper we show that the *sfa* determinant codes for the major subunit SfaA and three minor fimbrial subunit proteins, one of which is identical to the S adhesin. It is further demonstrated that the adhesin-specific gene locus *sfaS* is located at the distal region but not at the immediate 3′ end of the determinant. The DNA sequences of *sfaS* and the other two minor subunit-specific genes are determined, the *N*-terminal amino acid sequence of SfaS is established, and the functions of the corresponding proteins are analysed.

Results

Detection of minor subunits in the S-fimbrial adhesin complex

The fimbria adhesin complex (FAC) of S fimbriae was isolated from recombinant clones harbouring the *sfa*-specific plasmid, pANN801-13, and the subunits were separated by heating the FAC in the presence of Zwittergent 3-16. As already shown previously, one protein of 16 kD, which represents the major fimbrial subunit of the S-fimbria adhesin complex (see Schmoll *et al.*, 1987), was detected. Another three minor fimbrial subunits with molecular weights of 14 kD, 17 kD and 31 kD were also found after separation using SDS-PAGE (see Fig. 1, lane C).

DNA sequence of the genes coding for the minor fimbrial subunits of Sfa

It was shown that the genes coding for the minor fimbrial subunits must be located at the distal part of the *sfa* determinant (Hacker *et al.*, 1985; Moch *et al.*, 1987). In order to characterize these genes more accurately we have sequenced the corresponding region of 2.3 kilobases (kb) of DNA (Fig. 2). As indicated in Fig. 2, three open reading frames (ORFs) were found. One ORF starts at an ATG codon at position 61 to 63 and the gene ends at a TAA at position 586 to 588. The second ORF extends from position 610 to 1101 and the third gene is located between positions 1163 and 2038. The three open reading frames represent three genes which have been designated *sfaG*, *sfaS* and *sfaH*. The three genes code for three mature proteins with calculated molecular masses of 14.9 kD, 16.8 kD and 29.1 kD. The molecular weights of the three putative proteins calculated from the DNA sequences are similar to those of the minor subunits isolated from the fimbria adhesin complex (see Fig. 1).

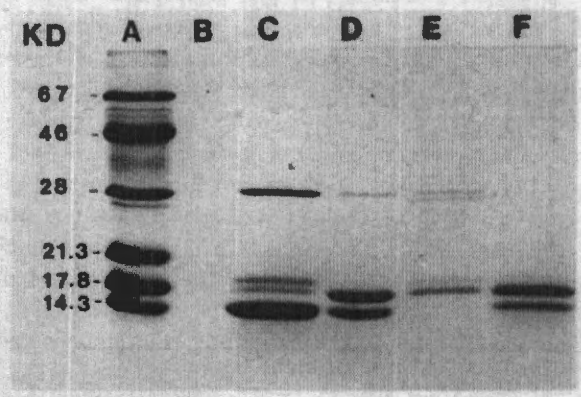

Fig. 1. SDS-PAGE analysis of major and minor subunit proteins of the S-fimbrial adhesin complexes encoded by *sfa* wild-type and mutant gene clusters. The following clones are indicated: HB101(pBR322), lane B; HB101(pANN801-13, *sfa*$^+$), lane C; HB101(pANN801-1320, *sfaG*$^-$), lane D; HB101(pANN801-1321, *sfaS*$^-$), lane E; HB101(pANN801-13/Tn*5*-033, *sfaH*$^-$), lane F. Lane A: size markers.

Mutations in the genes sfaG, sfaS *and* sfaH

The recombinant DNAs pANN801-1320 and pANN801-1321 were constructed to carry frameshift mutations in the genes *sfaG* and *sfaS* (see the *Experimental procedures* and Fig. 2). In addition, three Tn*5* mutants of the *sfa*-specific recombinant plasmid pANN801-13 were analysed. It was shown by DNA sequence studies that the Tn*5* mutants pANN801-13/Tn*5*-033 and pANN801-13/Tn*5*-026 carry the transposon inserted in the gene *sfaH* at positions 1248 and 1543, respectively (see Fig. 2). In Mutant pANN801-13/Tn*5*-020, a Tn*5* transposon was inserted into the gene *sfaS* at map position 622. Because transposon Tn*5* causes polar effects (Berg and Berg, 1983), *sfaH* was eliminated together with *sfaS* in the latter mutant.

From the HB101(pANN801-1320), HB101(pANN801-1321) and HB101(pANN801-13/Tn*5*-033) mutant clones the major and minor subunit proteins were isolated and separated using SDS-PAGE. As demonstrated in Fig. 1 (lanes D–F), the *sfaG*-negative mutant, HB101(pANN801-1320) had lost the 17 kD subunit protein whereas the *sfaS*-negative clone HB101(pANN801-1321) did not produce the 14 kD minor component. In the *sfaH*-negative strain, HB101(pANN801-13/Tn*5*-033), the 31 kD protein was eliminated. In all mutant clones tested, the major subunit protein of 16 kD was still present. These results clearly demonstrate that the genes *sfaG*, *sfaS*, and *sfaH* code for minor fimbrial subunit proteins of 17 kD (SfaG), 14 kD (SfaS) and 31 kD (SfaH) in size.

Further characterization of clones carrying mutations in the genes sfaG, sfaS *and* sfaH

In order to analyse the functions of the minor subunit proteins of the S-fimbrial adhesin complex the mutant clones mentioned above, the wild-type *sfa* determinant

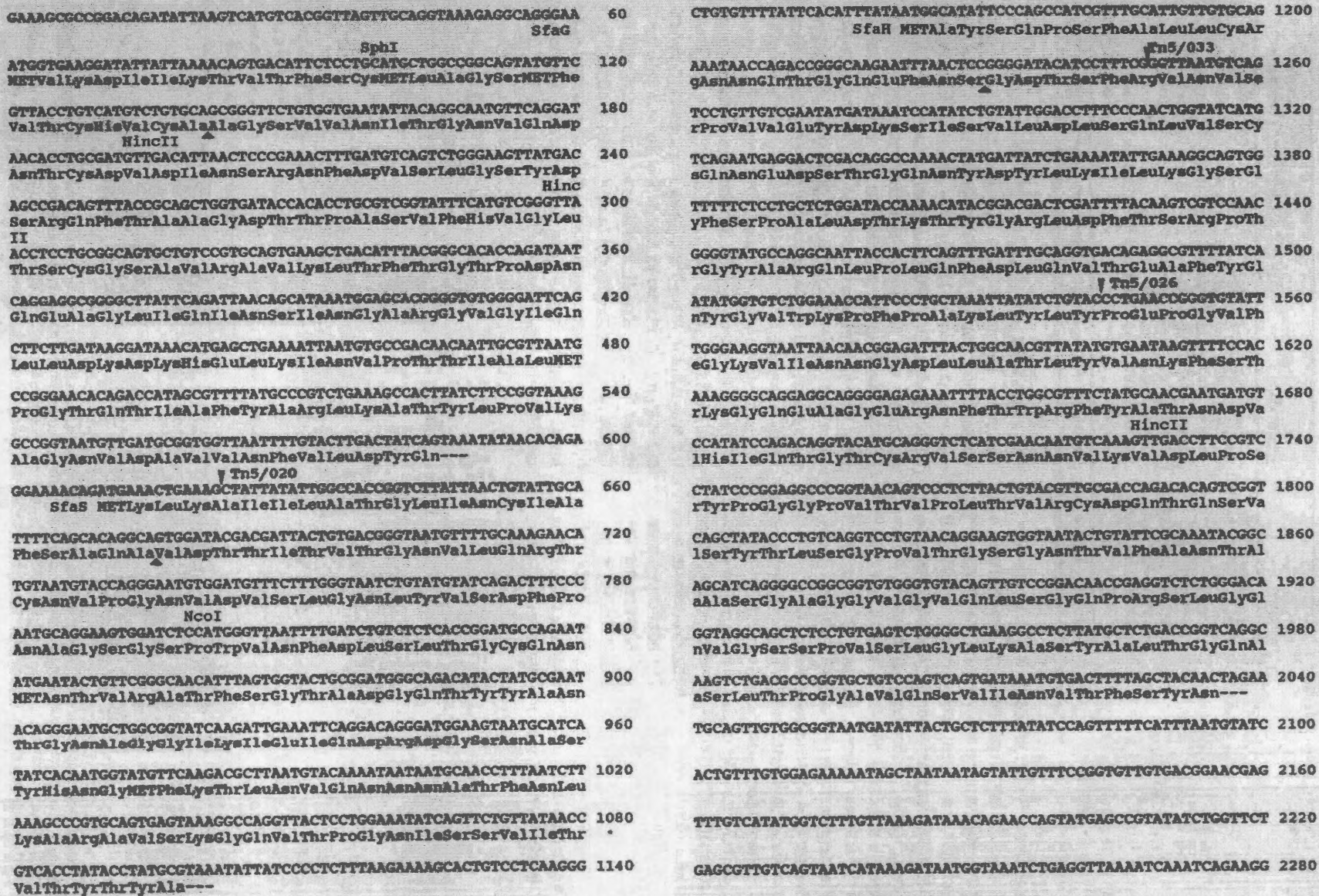

Fig. 2. Nucleotide- and protein sequence of the distal region of the *sfa* determinant coding for the minor fimbrial subunits SfaG, SfaS and SfaH. The positions of integration of *Tn5* transposons are indicated by arrows. The cleavage site of SfaS and the potential processing positions of SfaG and SfaH are indicated by triangles. Important restriction sites are shown. These sequence data will appear in the EMBL/GenBank/DDBJ Nucleotide Sequence Databases under the accession number X16664.

Table 1. Characterization of *E. coli* K12 clones carrying *sfa*-specific DNAs.

Plasmid	Genotype	HA-titre[a]	Degree of fimbriation[b]
pANN801-13	*sfaA*$^+$, *sfaG*$^+$, *sfaS*$^+$, *sfaH*$^+$	32	Heavy
pANN801-1	*sfaA*$^-$, *sfaG*$^+$, *sfaS*$^+$, *sfaH*$^+$	8	Non-fimbriated
pANN801-1320	*sfaA*$^+$, *sfaG*$^-$, *sfaS*$^+$, *sfaH*$^+$	16	Heavy
pANN801-1321	*sfaA*$^+$, *sfaG*$^+$, *sfaS*$^-$, *sfaH*$^+$	0	Low
pANN801-13/Tn*5*-033	*sfaA*$^+$, *sfaG*$^+$, *sfaS*$^+$, *sfaH*$^-$	8	Low
pANN801-13/Tn*5*-026	*sfaA*$^+$, *sfaG*$^+$, *sfaS*$^+$, *sfaH*$^-$	8	Low
pANN801-13/Tn*5*-020	*sfaA*$^+$, *sfaG*$^+$, *sfaS*$^-$, *sfaH*$^-$	0	Low
pBR322	Control	0	Non-fimbriated

a. Haemagglutination was carried out in microtitre plates as described by Jones and Rutter (1972).
b. The degree of fimbriation was evaluated using electron microscopy (see also Fig. 4).

encoded by clone HB101(pANN801-13) and the non-fimbriated subclone HB101(pANN801-1), which shows a deletion of the major fimbrial subunit gene *sfaA* (Hacker *et al.*, 1985; Schmoll *et al.*, 1987), were further characterized in a quantitative haemagglutination test and in quantitative ELISA assays using monoclonal antibodies (Mabs) directed against the major fimbrial subunit (MabF1) and the S-specific adhesin (MabA1) (Table 1 and Fig. 3). In addition, the degree of fimbriation of these clones was evaluated by electron microscopy (see Fig. 4).

As demonstrated in Table 1 and Fig. 4, only mutants with the mutagenized gene *sfaS* (pANN801-1321; pANN801-13/Tn*5*-020) were unable to recognize erythrocytes in a haemagglutination assay, although they were still fimbriated. In addition, the *sfaS*-negative clones also failed to react with the anti-adhesin monoclonal antibodies (see Fig. 3). It was also demonstrated that the *sfaS*-negative mutants showed reduced values in the quantitative ELISA with the fimbrial-specific MabF1. In addition, the degree of fimbriation of these clones was decreased in comparison with the *sfa* wild-type clone HB101(pANN801-13) (Figs 3 and 4). These data show that the minor subunit SfaS is identical to the S-specific adhesin. In contrast to the *sfaS*-negative clones, the *sfaH*-negative clones HB101(pANN801-13/Tn*5*-033) and HB101(pANN-13/Tn*5*-026) showed haemagglutination and were able to bind the anti-adhesin-specific antibodies. The binding capacities of the strains, however, were strongly reduced relative to that of the wild-type clone HB101(pANN801-13). As indicated by electron microscopy and ELISA tests with the anti-SfaA antibody MabF1, the degree of fimbriation was also decreased in the two *sfaH*-negative clones. The clone HB101(pANN801-1320), which carries a frameshift mutation in the gene *sfaG* also gave a low value in the ELISA assay with the anti-adhesin MabA1 and gave a decreased haemagglutination titre relative to that of the *sfa* wild-type gene cluster (Fig. 3, Table 1). The level of fimbriation and the ELISA value with the fimbrial-specific antibody (MabF1) of this clone, however, were comparable to what has been found for the wild-type gene cluster located on plasmid pANN801-13 (Figs 3 and 4).

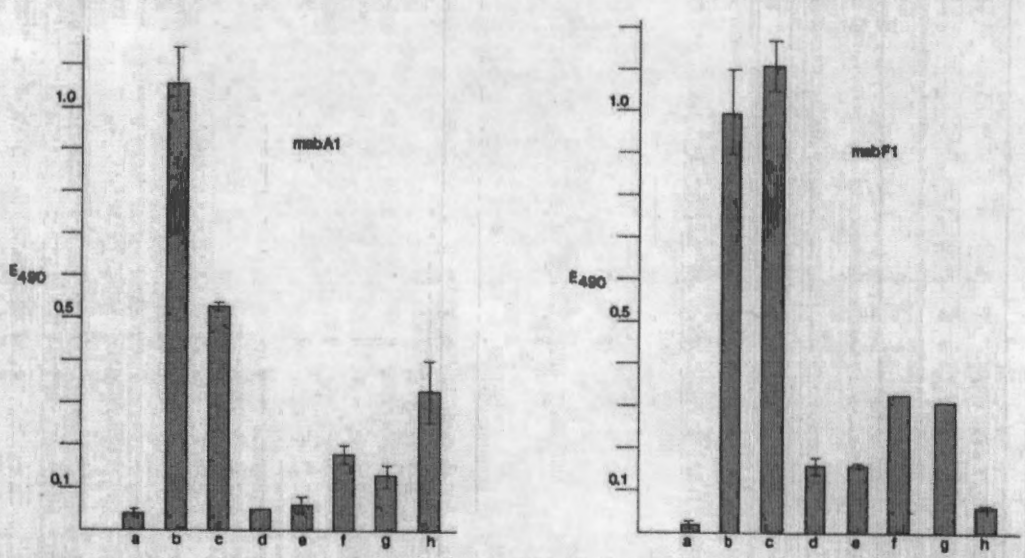

Fig. 3. Quantitative whole-cell ELISA tests of the *sfa* wild-type and mutant clones using monoclonal antibodies specific for the S-specific adhesin SfaS (MabA1) and the major subunit protein SfaA (MabF1). The following clones are indicated: HB101(pBR322), columns a; HB101(pANN801-13, *sfa*$^+$), columns b; HB101(pANN801-1320, *sfaG*$^-$), columns c; HB101(pANN801-1321, *sfaS*$^-$), columns d; HB101(pANN801-13/Tn*5*-020, *sfaS*$^-$, *sfaH*$^-$), columns e; HB101(pANN801-13/Tn*5*-026, *sfaH*$^-$), columns f; HB101(pANN801-13/Tn*5*-033, *sfaH*$^-$), columns g; HB101(pANN801-1, *sfaA*$^-$), columns h.

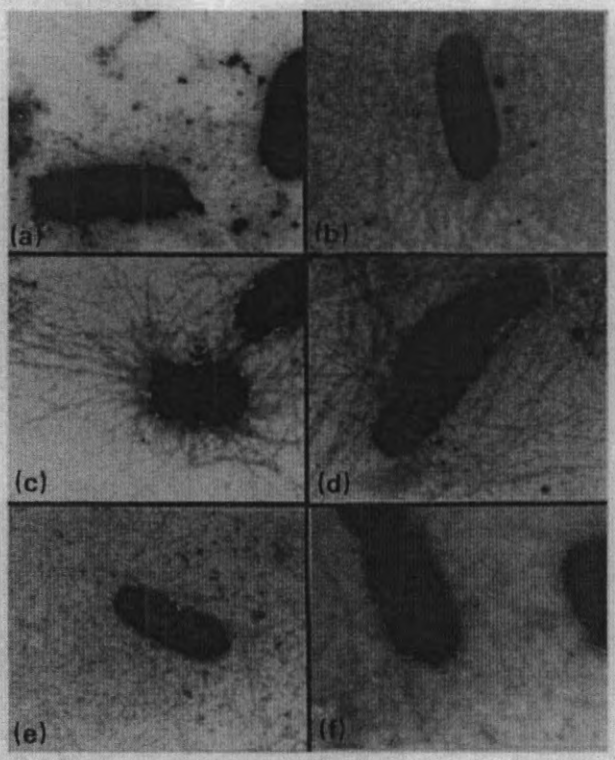

Fig. 4. Electron micrographs of the *sfa* wild-type and mutant clones. The following strains are indicated: a, HB101(pBR322); b, HB101(pANN801-13, *sfa*$^+$); c, HB101(pANN801-1320, *sfaG*$^-$); d, HB101(pANN801-1321, *sfaS*$^-$); e, HB101(pANN801-13/Tn*5*-033, *sfaH*$^-$); f, HB101(pANN801-13/Tn*5*-020, *sfaS*$^-$, *sfaH*$^-$).

Protein sequence analysis of the N*-terminal part of SfaS and the primary sequence of the three minor Sfa subunit proteins*

The *N*-terminal sequence of the mature protein SfaS, which is able to bind to human and bovine erythrocytes after purification (see Moch *et al.*, 1987), was determined. It was shown that the first amino acid residues of the mature protein were valine, aspartic acid and threonine (see Figs 2 and 5). Therefore it is clear that the first 22-amino-acid residues of the precursor protein were cleaved following transport. All the 40-amino-acid residues of the *N* terminus of the mature SfaS protein determined were identical to those of the protein sequence deduced from the DNA sequence of *sfaS*, as indicated in Fig. 2.

It is evident from Figs 2 and 5 that the three proteins SfaG, SfaS and SfaH carry tyrosine residues in the second last position of the sequences. In addition, two (or, in the case of the 31 kD protein SfaH, four) cysteine residues are located at well-defined positions which may form Cys-Cys bridges in native proteins (Jann *et al.*, 1981). The presence of the tyrosine residues in the *C* termini and the cysteine residues are characteristic of the primary sequences of fimbrial subunit proteins (Hacker, 1989).

Discussion

In this paper we demonstrate that the fimbrial adhesin complexes of *E. coli* S-fimbrial adhesins consist of four components: the major subunit SfaA, a protein of 16 kD and three minor fimbrillins designated SfaG, SfaS and SfaH (proteins of 17 kD, 14 kD and 31 kD, respectively). It is also shown that mutations in *sfaS*, but not in *sfaG*, *sfaH* or *sfaA*, completely eliminated S-specific binding, indicating that SfaS represents the S-specific adhesin (see Table 1). In addition, SfaS and also SfaH-negative clones showed a significant reduction in fimbriation of the cells (Figs 3 and 4). It is therefore concluded that SfaS and SfaH also play a role in the determination of S-specific fimbriation. A mutation in *sfaG* did not influence fimbriation of the cells but reduced the binding capacity of the clones. It is suggested that SfaG acts as a linker molecule for the adhesin which could bind the SfaS protein to the fimbriae.

The minor subunits of P fimbriae of serotypes F13 (*pap*), $F7_1$ (*fso*), $F7_2$ (*fst*) and of type 1 fimbriae (*fim*), which are indicated in Fig. 6, represent proteins of molecular weights equivalent to those of SfaG, SfaS and SfaH. These minor fimbrillins also play a role in the determination of the degree of fimbriation of the cells and in anchoring the adhesins to the fibre-like structures. The F protein of the *fst* gene cluster (equivalent to SfaS) and the G proteins of *fso*, *fst* and *pap* determinants (equivalent to SfaH) are necessary for heavy fimbriation of cells (Lindberg *et al.*, 1987; Riegman *et al.*, 1988). In the case of type 1 fimbriae, the proteins FimF and FimG, which are equivalent to SfaG and SfaH, are involved in modulation of fimbriation (Klemm and Christiansen, 1987; Krogfelt and Klemm, 1988).

It is also shown in Fig. 6 that the genes coding for the minor subunits of P fimbriae of different serotypes, of type 1 fimbriae and of S fimbriae are located at the 3′ ends of the determinants. The minor subunits PapG and FimH, proteins of 35 kD and 32 kD, respectively and equivalent to the SfaH protein represent the P (F13) and type 1-specific adhesins (Lindberg *et al.*, 1987; Lund *et al.*, 1987; Hoschützky *et al.*, 1989; Klemm and Christiansen, 1987; Hanson and Brinton, 1988). Adhesins of similar molecular weights are also encoded by P-fimbrial gene clusters of other serotypes (Lund *et al.*, 1988a,b; Riegman *et al.*, 1988). While the largest protein of the minor P- and type 1 subunits represents the adhesin of these attachment factors, the S-specific adhesin is identical to the smallest subunit of the S-fimbrial adhesin complex. The gene *sfaS* is located at equivalent positions to the F gene loci of P-specific determinants and to the gene *fimG* of the type 1 gene cluster (Fig. 6). Thus in contrast to P and type 1 determinants, the S-specific adhesin locus is not located at the immediate distal part of the *sfa* gene cluster. Summarizing these data, it seems that a 'functional shift'

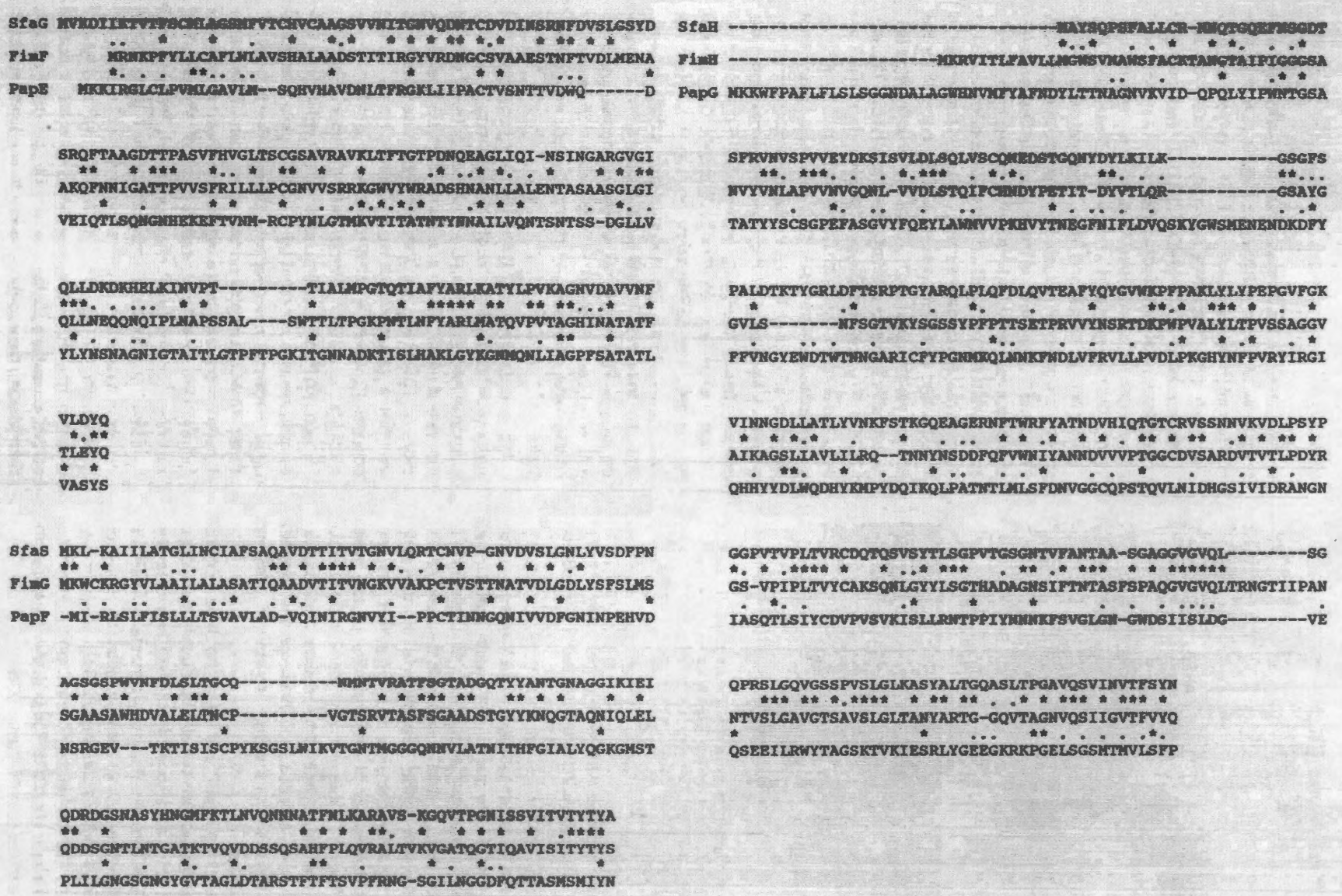

Fig. 5. Comparison of the primary amino acids sequences of the minor subunit proteins SfaG, SfaS and SfaH with those of the Pap (P) and Fim (type 1) fimbrial adhesins (data from Klemm and Christiansen, 1987; Lund *et al.*, 1988a,b). Identical amino acid residues are indicated by stars, and amino acid residues that are functionally similar are indicated by dots. Dashes represent gaps introduced for optimal alignment.

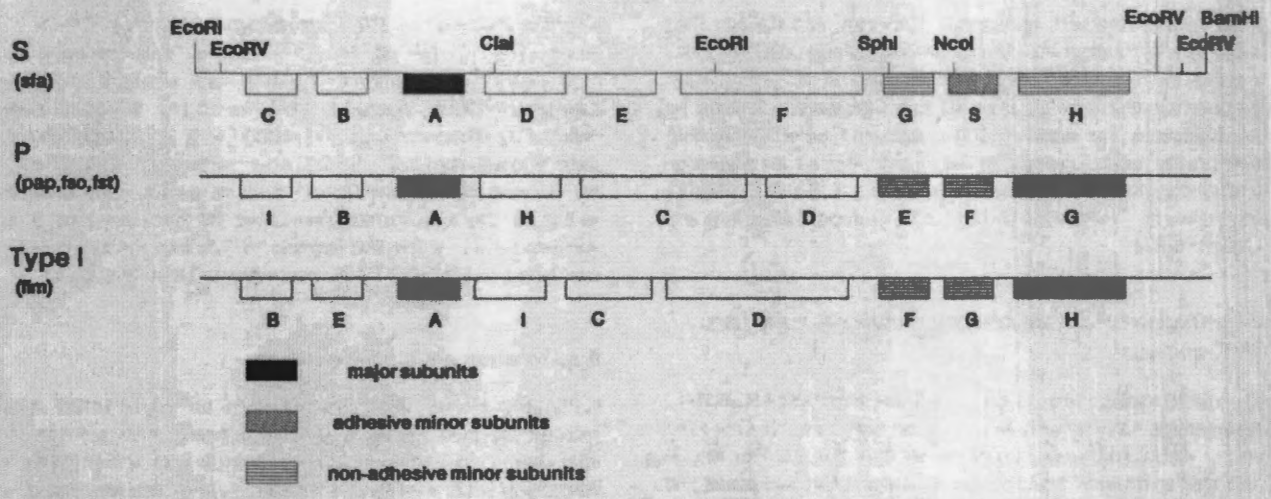

Fig. 6. Comparison of the genetic organization of the S-specific *sfa* determinant (Hacker, 1989) and the gene clusters coding for P- and type 1-fimbriae. The gene loci are indicated by boxes. The letters below the P-specific determinant mark the genes of *pap*, *fst* and *fso* determinants (Lund *et al.*, 1988a,b; Riegman *et al.*, 1988). The letters below the type 1-specific determinant mark the genes of the *fim* determinant (Klemm and Christiansen, 1987; H. Bergmans and H. Klemm, personal communication). The boxes specific for major and minor subunit genes are indicated by specific patterns. The open boxes represent genes involved in regulation (the boxes of the left site) and in transport and assembly processes. The main directions of transcription are from left to right. Important restriction sites of the *sfa* determinant are indicated.

had occurred among the minor subunit genes of P and type 1 fimbriae on one hand and S-fimbrial adhesins on the other hand because different minor subunits represent the corresponding adhesins.

The functional differences established among the minor subunits of P, S and type 1 fimbrial adhesins are also reflected in the primary protein sequences (see Fig. 5). After comparison of the amino acid sequences of the equivalent subunits it is evident that the adhesin SfaS shows a smaller degree of homology to the corresponding proteins FimG (44.3%) and PapF (32.6%) compared with SfaG and SfaH, which are more homologous to their P- and type 1-specific counterparts. In general, the relatedness among the minor subunits is more pronounced between the *sfa*- and the *fim*-encoded proteins compared with *sfa*- and *pap*-specific fimbrillins. These results are in line with sequence data for the major subunit proteins. The major subunit proteins SfaA and FimA are more related to each other than SfaA and PapA fimbrillins (Schmoll *et al.*, 1987). From an evolutionary point of view, it can be speculated that the genes specific for major and minor fimbrial subunits of S and type 1 fimbriae may have been evolved from a common ancestral gene cluster. It is interesting to note that the *N*- and *C*-terminal portions of the proteins show the highest degree of relatedness (see Fig. 5). These regions may play a role in the interaction of subunits during fimbria formation (Klemm, 1985) and are therefore more conserved among the different subunit proteins than the other regions of the different fimbrillin proteins.

Experimental procedures

Media, enzymes and chemicals

Bacteria were grown in enriched nutrient broth or in L-broth. For isolation of fimbrial subunits, the clones were grown in a medium containing 1% bactopepton, 1% yeast extract, 0.25% glycerine and 50 mM sodium phosphate buffer (pH 7). Radiochemicals were purchased from New England Nuclear Corp. (Boston, MA), and antibiotics were a gift from Bayer (Leverkusen, FRG). All other chemicals were obtained from E. Merck AG (Darmstadt, FRG). Restriction enzymes and T4 ligase were purchased from Bio-Rad Laboratories (Richmond, CA). DNA polymerase I was obtained from Boehringer (Mannheim, FRG).

Bacterial strains and plasmids

All recombinant plasmids were transformed into the *E. coli* K12 strain HB101. The different S-fimbrial adhesin (*sfa*)-specific recombinant DNAs were derived from the plasmid pANN801-13 (*sfa*$^+$, Ap^R; Hacker *et al.*, 1985). For the construction of frameshift pANN801-12 and pANN801-11 were used. pANN801-12 (Tc^R) carries a 4.7 kb *Eco*RI fragment which represents the proximal part of the *sfa* determinant and pANN801-11 (Ap^R) harbours a 5.3 kb *Bam*HI/*Eco*RI fragment specific for the distal part of the *sfa* gene cluster (Hacker *et al.*, 1985; Ott *et al.*, 1988; see also Fig. 6). The Tn*5* insertion mutants pANN801-13/Tn*5*-033, pANN801-13/Tn*5*-026 and pANN801-13/Tn*5*-020 have been described previously (Hacker *et al.*, 1985).

Recombinant DNA techniques

Plasmid DNA was isolated as described earlier (Grinstedt *et al.*, 1978; Birnboim and Doly, 1979). For restriction enzyme analysis,

DNA was treated with appropriate enzymes, and the resulting fragments were separated by gel electrophoresis on 0.7 to 1.0% agarose gels as described before (Knapp *et al.*, 1984). DNA fragments were isolated after agarose gel electrophoresis by electroelution. For cloning, DNA fragments were ligated into suitable vector molecules after heat-inactivation of the restriction endonucleases at 65°C for 6 min (Maniatis *et al.*, 1982). *E. coli* K12 strains were transformed by the $CaCl_2$ method (Lederberg and Cohen, 1974).

Construction of clones carrying mutations in the genes sfaG *and* sfaS

In order to mutagenize the gene *sfaG*, the plasmid pANN801-11 was partially cleaved with the restriction enzyme *Sph*I. One *Sph*I site is located in *sfaG* at position 98 (see Fig. 2). The single-stranded ends were digested with the help of T4 polymerase and the blunt ends were ligated. For mutagenization of *sfaS*, pANN801-11 was cleaved with the restriction enzyme *Nco*I. A *Nco*I site is located in the gene *sfaS* at position 799 (Fig. 2). The single-stranded ends were filled by a Klenow reaction. In order to control the reactions, DNA was isolated from 20 putative *sfaG*-negative and 20 *sfaS*-negative mutants and cleaved with *Sph*I and *Nco*I, respectively. Two recombinant plasmids (pANN801-13201 and pANN801-13211) with destroyed recognition sites were sequenced around the former *Sph*I and *Nco*I sites using the DNA plasmid sequencing kit of Boehringer. The sequences gave clear-cut evidence that frameshift mutations had been introduced into the genes *sfaG* and *sfaS*. In order to restore the *sfa* determinant, the 4.7 kb *Eco*RI fragment of pANN801-12, which represents the proximal part of the *sfa* determinant, was ligated into the *Eco*RI site of plasmids pANN801-13201 and pANN801-13211, respectively. The clones were screened using a DNA-DNA colony dot blot (Maniatis *et al.*, 1982). It was shown by DNA cleavage that constructs pANN801-1320 and pANN801-1321 were identical to the wild-type plasmid pANN801-13, with the exception of the two frameshift mutations.

Determination of the insertion points of the Tn5 mutants

In order to determine the exact position of Tn*5* insertion into the pANN801-13 derivatives pANN801-13/Tn*5*-026, pANN801-13/Tn*5*-020 and pANN801-13/Tn*5*-033, the DNAs were cleaved with the restriction enzyme *Hpa*I and suitable DNA fragments carrying 195 Tn*5*-specific bp together with some *sfa*-specific regions were ligated into the vectors pUC18 and pUC19. The DNA sequences of the insertion points were determined by the plasmid sequence technique with the help of the DNA sequencing kit from Boehringer.

Isolation of the fimbria adhesin complex and separation of major and minor subunits

The isolation of the fimbria adhesin complexes and the separation of the subunits were carried out as described recently by Moch *et al.* (1987) and Hoschützky *et al.* (1989) with the following modifications: agar-grown bacteria were suspended in phosphate-buffered saline (PBS) and agitated twice for 5 min with an omnimixer. Bacteria were removed by centrifugation (20000 × g, 40 min) and the fimbriae were concentrated by ammonium sulphate precipitation (20% saturation) followed by dialysis to 10 mM Tris-HCl (pH 8). Insoluble material was removed by centrifugation. The fimbriae solution was adjusted to 0.5% Zwittergent-16 and heated to 50°C for 30 min. Fimbriae were pelleted by ultracentrifugation (140000 × g, 2 h), suspended in water and adjusted to 0.5% SDS. After heating to 70°C for 30 min, the fimbriae were pelleted by ammonium sulphate precipitation as before. The supernatant (containing the minor subunits) was dialysed against water and lyophilized. The minor subunits were analysed by 15% SDS-PAGE according to Laemmli (1979).

Agglutination and adhesion tests

Fimbriated clones were characterized by agglutination with antisera on glass slides, S-specific adhesion was determined after mixing the bacterial cells with human and bovine erythrocytes with and without 2% mannose and with erythrocytes treated with neuraminidase (Korhonen *et al.*, 1984; Hacker *et al.*, 1985). In order to quantitate the haemagglutination property the test was done in microtitre plates as described by Jones and Rutter (1972). The haemagglutination (HA) tests were carried out at pH 7.

Electron microscopy

For electron microscopy, the bacteria were transferred to copper grids coated with polyvinyl-Formvar. After soaking in 1% phosphotungstic acid (pH 6.4) for 1 min, the grids were examined under a Zeiss 10A transmission electron microscope.

Preparation of monoclonal antisera

The preparation and characterization of the monoclonal antisera used have been described previously (Moch *et al.*, 1987).

ELISA

The quantitative enzyme-linked immunosorbent assay (ELISA) was performed with whole bacteria according to the method of Boylan *et al.* (1987).

Oligonucleotide synthesis

Oligonucleotides were synthesized in an Applied Biosystems 380A DNA synthesizer using the phosphoramidite method of Beaucage and Caruthers (1981). Oligonucleotides were purified on a polyacrylamide gel.

DNA sequencing

The sequences of the DNA region coding for the genes *sfaG*, *sfaS* and *sfaH* were determined by the Sanger technique with the M13mp18/mp19 system described previously (Hu and Messing, 1982). Sequences of the Tn*5* insertion and the frameshift mutants of plasmid pANN801-13 were determined with the help of a sequence kit from Boehringer, according to a protocol supplied by the manufacturer.

Protein sequencing

The protein was subjected to 12% SDS-PAGE and electroblotted onto a siliconized glassfibre sheet (glassy bond, Biometra) essentially as described by Eckerskorn *et al.* (1988). The SfaS-containing band was excised and sequenced in an Applied Biosystems 477A gas-phase sequencer.

Computer analysis

The programs used for compiling the nucleotide sequence data were from J. Devereux (UWGCG).

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