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Cloning and Characterization of the S Fimbrial Adhesin II Complex of an *Escherichia coli* O18:K1 Meningitis Isolate

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S fimbrial adhesins (Sfa), which are able to recognize sialic acid-containing receptors on eukaryotic cells, are produced by *Escherichia coli* strains causing urinary tract infections or newborn meningitis. We recently described the cloning and molecular characterization of a determinant, termed *sfaI*, from the chromosome of an *E. coli* urinary tract infection strain. Here we present data concerning a S fimbria-specific gene cluster, designated *sfaII*, of an *E. coli* newborn meningitis strain. Like the SfaI complex, SfaII consists of the major subunit protein SfaA (16 kDa) and the minor subunit proteins SfaG (17 kDa), SfaS (15 kDa), and SfaH (29 kDa). The genes encoding the subunit proteins of SfaII were identified and sequenced. Their protein sequences were calculated from the DNA sequences and compared with those of the SfaI complex subunits. Although the sequences of the two major SfaA subunits differed markedly, the sequences of the minor subunits showed only a few amino acid exchanges (SfaG, SfaH) or were completely identical (SfaS). The introduction of a site-specific mutation into the gene *sfaSII* and subsequent analysis of an SfaS-negative clone indicated that *sfaSII* codes for the sialic acid-specific adhesin of the meninigitis isolate. These data were confirmed by the isolation and characterization of the SfaSII protein and the determination of its N-terminal amino acid sequence. The identity between the sialic acid-specific adhesins of SfaI and SfaII revealed that differences between the two Sfa complexes with respect to their capacities to agglutinate erythrocytes must result from sequence alterations of subunit proteins other than SfaS.

Extraintestinal *Escherichia coli* isolates are the causative agents of urinary tract infections (UTI) (23, 37) and severe cases of sepsis or newborn meningitis (NBM) (17, 23). The majority of the pathogens causing NBM belong to the O-serogroups O1, O2, O18, and O83 (23). In addition, the strains possess important virulence factors such as the capsule antigen K1 (17), the iron uptake complex aerobactin (22), and the S fimbrial adhesins (Sfa) (17, 24, 28). Such adhesins are able to bind to eukaryotic glycoproteins terminating with α-sialic acid 2,3-β-Gal (29). It was recently demonstrated that S fimbriae bind to the extracellular matrix protein laminin (40a) and to plasminogen, inducing the formation of plasmin with fibrinolytic capacity (27). It is therefore suggested that S fimbriae play a role in the process of penetration of *E. coli* across the basement membrane.

In contrast to NBM isolates, extraintestinal *E. coli* strains that cause UTI frequently produce as virulence factors P-fimbrial adhesins, which interact with glycolipids containing α-Gal-1,4-β-Gal (12, 37), F1C fimbriae (40), and cytolytic proteins termed hemolysins (7). Uropathogenic strains of the O6 type, however, are also able to express Sfa (3, 6). We recently cloned and characterized the Sfa gene cluster (*sfa*) of a uropathogenic *E. coli* strain of serotype O6:K15 (8, 20, 21, 33-35). These Sfa are composed of a major subunit protein of 16 kDa (SfaA; 33) and three minor subunit proteins of 15 kDa (SfaS), 17 kDa (SfaG), and 29 kDa (SfaH) (20, 34), which together form the Sfa complex (11). The minor subunit protein SfaS was identified as the sialic acid-binding adhesin (21, 33). DNA-DNA hybridization data demonstrated that the cloned *sfa* determinant of the UTI strain and the *sfa* gene clusters located on the chromosomes of NBM isolates were very similar (24). Immunological studies, however, revealed differences between the SfaA proteins expressed by UTI and NBM *E. coli* pathogens (24, 25, 31).

In this paper we describe the molecular cloning and characterization of a *sfa* gene cluster from a NBM isolate of serotype O18:K1 termed *sfaII*. We demonstrate that the deduced amino acid sequences of three subunit proteins of the SfaII complex differ from those of SfaI, whereas the sialic acid-specific adhesins SfaS of the UTI and the NBM isolate are identical.

MATERIALS AND METHODS

Media, enzymes, and chemicals. Bacteria were grown in enriched nutrient broth or in L broth. For the isolation of fimbrial subunits, the clones were grown on solid media containing 1% Bacto-Peptone, 1% yeast extract, 0.25% glycerin, 50 mM sodium phosphate buffer (pH 7), and 1.5% agar. Radiochemicals were purchased from New England Nuclear Corp., Boston, Mass. Antibiotics were from Bayer, Leverkusen, Germany. All other chemicals were obtained from E. Merck AG, Darmstadt, Germany. Restriction enzymes, T4 polynucleotide kinase, and T4 ligase were purchased from Bio-Rad Laboratories, Richmond, Calif. DNA polymerase I was obtained from Boehringer, Mannheim, Germany.

Bacterial strains and plasmids. The *sfa* determinants were cloned from the chromosomes of the uropathogenic strain 536 of serotype O6:K15 (*sfaI*) (8) and of the meningitis

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TABLE 1. Characterization of Sfa-specific *E. coli* strains and isolated SfaSII protein

Strain or protein	*sfa* genotype	HA of erythrocytes[a]		Degree of fimbriation[b]	ELISA results[c] (A_{490})	
		Human	Bovine		A1	F1
536 (wild type, O6:K15)	*sfaAI sfaGI sfaSI sfaHI*	1:2	1:4	+++	0.30	0.40
IHE3034 (wild type, O18:K1)	*sfaAII sfaGII sfaSII sfaHII*	1:16	1:8	++	0.30	0.03
HB101(pANN801-13)	*sfaAI sfaGI sfaSI sfaHI*	1:16	1:16	++++	0.78	1.38
HB101(pAZZ50)	*sfaAII sfaGII sfaSII sfaHII*	1:32	1:32	++++	0.95	0.02
HB101(pAZZ50-67)	*sfaAII sfaGII sfaSII⁻ sfaHII*	–	–	++	0.02	0.03
SfaSII[d]		+++	+++	NT[e]	+++	–

[a] Determined by a quantitative hemagglutination (HA) assay (13).
[b] Determined by electron microscopy.
[c] Monoclonal antibody A1 is specific for sfaSI and sfaSII, and monoclonal antibody F1 is specific for sfaAI.
[d] Only qualitative test systems are used for hemagglutination, binding of monoclonal antibodies was tested by Western blotting.
[e] NT, not testable.

isolate IHE3034 of serotype O18:K1 (*sfaII*) (17). The *sfa*-specific recombinant DNAs are listed in Table 1.

Recombinant DNA techniques. Plasmid DNA was isolated as described previously (2, 15). 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 (32). After agarose gel electrophoresis, DNA fragments were isolated 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. *E. coli* K-12 strains were transformed by the $CaCl_2$ method (18).

Construction of a genomic library of *E. coli* IHE3034 (O18:K1). Chromosomal DNA of strain IHE3034 was isolated and digested with *Sau*3A. After ligation of 20- to 22-kb DNA fragments into the *Bam*HI site of vector pLAFR2 (16) and in vitro packaging (10), about 1,200 recombinant *E. coli* K-12 clones were selected. The *E. coli* K-12 strain HB101 is S fimbriae negative. To select for *sfaII*-specific plasmids, the recombinant clones were tested by hemagglutination (8, 13).

Construction of a clone carrying a mutation in the gene *sfaS*. To mutagenize the gene *sfaS* of the *sfaII* determinant, the 5.3-kb *Eco*RI-*Bam*HI fragment of plasmid pAZZ50 (Fig. 1) was cloned into the vector pBR322. The recombinant plasmid pAZZ50-4 was cleaved with the restriction enzyme *Nco*I. An *Nco*I site is located in the gene *sfaS* at position 799 (see Fig. 4a). The sticky ends were filled by a Klenow reaction. To control the reaction, DNA was isolated from 20 putative SfaS⁻ mutants and cleaved with *Nco*I. One recombinant plasmid (pAZZ50-4) with a destroyed recognition site was sequenced around the former *Nco*I site by using a DNA plasmid sequencing kit from Boehringer. The sequences gave clear evidence that a frameshift mutation was introduced into the gene *sfaS*. To restore the *sfaII* determinant,

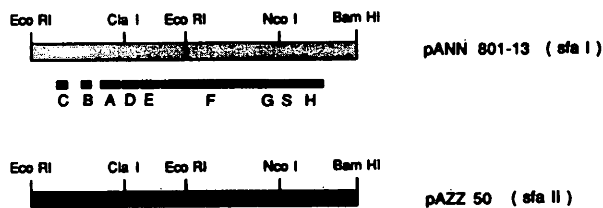

FIG. 1. Genetic organization of the *sfaI* and *sfaII* determinants. The black boxes represent *sfa*-specific genes. The main direction of transcription of the determinants is from left to right; relevant restriction sites are indicated.

the 4.7-kb *Eco*RI fragment of pAZZ50, which represents the proximal part of the *sfaII* determinant, was ligated into the *Eco*RI site of the plasmid pAZZ50-4. The clones were screened by DNA-DNA colony dot blotting (32). DNA cleavage data indicated that the construct pAZZ50-67 was identical to the wild-type plasmid pAZZ50 with the exception of the frameshift mutation in *sfaS*.

DNA-DNA colony dot blot. For rapid detection of the presence of specific insert DNA fragments in plasmids, colony dot hybridization was performed as described by Sambrook et al. (32).

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 previously (11, 20).

Agglutination and adhesion tests. S-specific adhesion was determined after mixing the bacterial cells and the adhesin preparations with human or bovine erythrocytes with and without 2% mannose and with erythrocytes treated with neuraminidase (8, 29). To quantify hemagglutination, the test was performed in microtiter plates as described previously (13). The hemagglutination tests were carried out at pH 7.

Electron microscopy. Bacteria were transferred to copper grids coated with polyvinyl-Formvar. After sedimentation of the bacteria and removal of remaining fluid, the samples were shadowed with platinum-palladium and examined with a Zeiss 10 A transmission electron microscope.

Preparation of monoclonal antibodies. The preparation and characterization of the monoclonal antibodies were as described previously (20).

ELISA. A quantitative enzyme-linked immunosorbent assay (ELISA) was carried out with whole bacteria by the method of Boylan et al. (4).

Oligonucleotide synthesis. Oligonucleotides were synthesized with an Applied Biosystems 380A DNA synthesizer by the phosphoramidite method of Beaucage and Caruthers (1a). Oligonucleotides were purified on a polyacrylamide gel.

DNA sequencing. The sequences of the DNA regions representing the genes *sfaA*, *sfaG*, *sfaS*, and *sfaH* of strain IHE3034 were determined by using a sequencing kit from Boehringer Mannheim (Germany) as described by the manufacturer.

Protein sequencing. The protein was subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis on 12% polyacrylamide gels and electroblotted onto a siliconized glass fiber sheet (glassy bond; Biometra) essentially as described by Eckerskorn et al. (5). The SfaSII-containing

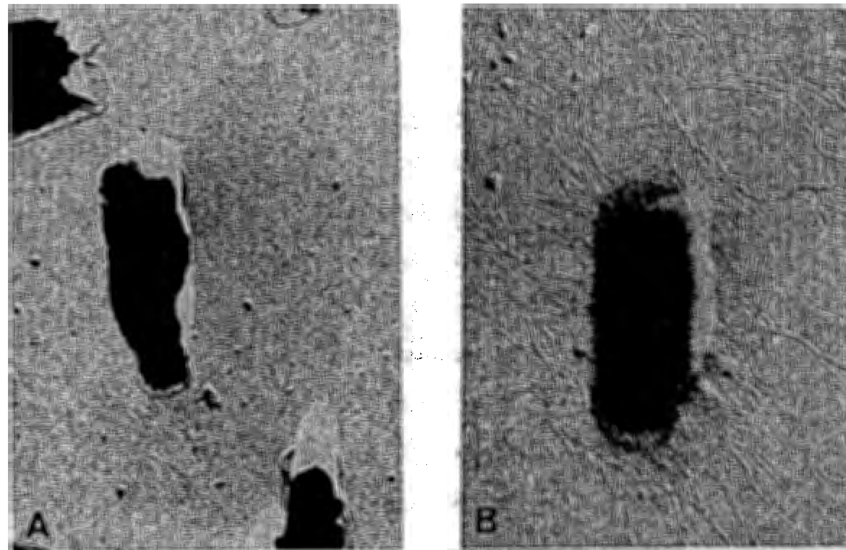

FIG. 2. Electron micrographs of clones HB101(pBR322) (A) and HB101(pAZZ50) (B).

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 (University of Wisconsin Genetics Computer Group).

RESULTS

S fimbriae of *E. coli* 536 (O6:K15) and IHE3034 (O18:K1). *E. coli* producing Sfa agglutinate human and bovine erythrocytes in a mannose-resistant, neuraminidase-sensitive manner (29). The S-fimbriated wild-type strains 536 (O6: K15), which was isolated from a case of UTI, and IHE3034 (O18:K1), an NBM isolate, show differences in their hemagglutination patterns. Whereas IHE3034 gives a strong agglutination of human and bovine erythrocytes, 536 shows a lower hemagglutination titer (Table 1). The Sfa of strain 536 was termed SfaI, and that of IHE3034 was designated SfaII.

Cloning and subcloning of the *sfaII* determinant of *E. coli* IHE3034. To clone the *sfaII* gene cluster of the *E. coli* meninigitis isolate IHE3034 (17), a cosmid library was constructed (10). The 1,200 recombinant *E. coli* K-12 clones were tested by S-specific hemagglutination. Two *E. coli* K-12 cosmid clones exhibited S-fimbria production; one of these, HB101(pAZZ37), was further characterized. The DNA of the recombinant cosmid was cleaved with the restriction enzyme *Eco*RI and with both *Bam*HI and *Eco*RI. As expected, an *Eco*RI fragment of 4.7 kb and a 5.3-kb *Bam*HI-*Eco*RI fragment were observed. These fragments gave strong signals after DNA-DNA hybridization with gene probes containing DNA regions of the *sfaI* determinant, indicating that these fragments contain the *sfaII*-specific sequences (data not shown). The *Eco*RI-*Bam*HI fragment was further subcloned into the vector molecule pBR322 to obtain the plasmid pAZZ50-4. After cleavage of pAZZ37 with *Eco*RI, the 4.7-kb *Eco*RI fragment was ligated into the *Eco*RI site of pAZZ50-4. The resulting plasmid, pAZZ50, contained the whole *sfaII* determinant of strain IHE3034, as indicated by suitable restriction cleavages, hemagglutination, and examination of fimbriation of clone HB101 (pAZZ50) (Fig. 1).

Characterization of the cloned SfaII complex. It was demonstrated by electron microscopy that clone HB101 (pAZZ50) was strongly fimbriated (Fig. 2). The SfaII complex was isolated, and the subunits were separated (Fig. 3, lane C). As shown for the SfaI complex (Fig. 3, lane B), a 16-kDa protein that represents the major fimbrial subunit was detected (33). Three other minor fimbrial subunits with molecular masses of 15, 17, and 29 kDa were detected after separation by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Although the degrees of fimbriation in HB101(pAZZ50) and HB101(pANN801-13) were similar (data not shown), the SfaII-specific clone exhibited a hemagglutination titer that was higher than that of the SfaI-positive clone (Table 1). It is interesting to note that monoclonal antibody A1, specific for the S adhesin of the SfaI complex (20), also recognized SfaII, whereas monoclonal antibody F1, specific for S fimbriae of SfaI, did not bind to SfaAII protein.

DNA sequences of the genes coding for the major and minor fimbrial subunits of SfaII. It was shown previously that the *sfa* determinants of different *E. coli* strains exhibit a high degree of homology (24). Gene probes representing the

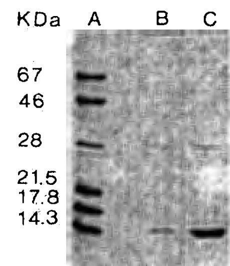

FIG. 3. Electrophoretic analysis of major and minor subunit proteins of Sfa complexes encoded by *sfaI* and *sfaII*. Lanes: A, size markers; B, HB101(pANN801-13); C, HB101 (pAZZ50).

```
A
GAAAGCGCCGGACAGATATTAAGTCATGTCACGGTTAGTTGCAGGTAAAGAGGCAGGGAA   60
                                                        SfaG
                          Sph I
ATGGTGAAGGATATTATTAAAACAGTGACATTCTCCTGCATGCTGGCCGGCAGTATGTTC  120
METValLysAspIleIleLysThrValThrPheSerCysMETLeuAlaGlySerMETPhe

GTTACCTGTCATGTCTGTGCAGCGGGTTCTGTGGTGAATATTACAGGCAATGTTCAGGAT  180
ValThrCysHisValCysAlaAlaGlySerValValAsnIleThrGlyAsnValGlnAsp
             Hinc II ▲
AACACCTGCGATGTTGACATTAACTCCCGAAACTTTGATGTCAGTCTGGGAAGTTATGAC  240
AsnThrCysAspValAspIleAsnSerArgAsnPheAspValSerLeuGlySerTyrAsp
                                                        Hinc
AGCCGACAGTTTACCGCAGCTGGTGATATCACACCTGCGTCGGTATTTCATGTCGGGTTA  300
SerArgGlnPheThrAlaAlaGlyAspIleThrProAlaSerValPheHisValGlyLeu
II
ACCTCCTGCGGCAGTGCTGTCCGTGCAGTGAAGCTGACATTTACGGGCACACCAGATAAT  360
ThrSerCysGlySerAlaValArgAlaValLysLeuThrPheThrGlyThrProAspAsn

CAGGAGGCGGGGCTTATTCAGATTAACAGCATAAATGGAGCACGGGGTGTGGGGATTCAG  420
GlnGluAlaGlyLeuIleGlnIleAsnSerIleAsnGlyAlaArgGlyValGlyIleGln

CTTCTTGATAAGGATAAACATGAGCTGAAAATTAATGTGCCGACAACAATTGCGTTAATG  480
LeuLeuAspLysAspLysHisGluLeuLysIleAsnValProThrThrIleAlaLeuMET

CCGGGAACACAGACCATAGCGTTTTATGCCCGTCTGAAAGCCACTTATCTTCCGGTAAAG  540
ProGlyThrGlnThrIleAlaPheTyrAlaArgLeuLysAlaThrTyrLeuProValLys

GCCGGTAATGTTGATGCGGTGGTTAATTTTGTACTTGACTATCAGTAATATAACACAGAG  600
AlaGlyAsnValAspAlaValValAsnPheValLeuAspTyrGln---

GAAAACAGATGAAACTGAAAGCTATTATATTGGCCACCGGTCTTATTAACTGTATTGTAT  660
   SfaS METLysLeuLysAlaIleIleLeuAlaThrGlyLeuIleAsnCysIleValP

TTTCAGCACAGGCAGTGGATACGACGATTACTGTGACGGGTAATGTTTTGCAAAGAACAT  720
heSerAlaGlnAlaValAspThrThrIleThrValThrGlyAsnValLeuGlnArgThrC
              ▲
GTAATGTACCAGGGAATGTGGATGTTTCTTTGGGTAATCTGTATGTATCAGACTTTCCCA  780
ysAsnValProGlyAsnValAspValSerLeuGlyAsnLeuTyrValSerAspPheProA
                Nco I
ATGCAGGAAGTGGATCTCCATGGGTTAATTTTGATCTGTCTCTCACCGGATGCCAGAATA  840
snAlaGlySerGlySerProTrpValAsnPheAspLeuSerLeuThrGlyCysGlnAsnM

TGAATACTGTTCGGGCAACATTTAGTGGTACTGCGGATGGGCAGACATACTATGCGAATA  900
ETAsnThrValArgAlaThrPheSerGlyThrAlaAspGlyGlnThrTyrTyrAlaAsnT

CAGGGAATGCTGGCGGTATCAAGATTGAAATTCAGGACAGGGATGGAAGTAATGCATCAT  960
hrGlyAsnAlaGlyGlyIleLysIleGluIleGlnAspArgAspGlySerAsnAlaSerT

ATCACAATGGTATGTTCAAGACGCTTAATGTACAAAATAATAATGCAACCTTTAATCTTA 1020
yrHisAsnGlyMETPheLysThrLeuAsnValGlnAsnAsnAsnAlaThrPheAsnLeuL

AAGCCCGTGCAGTGAGTAAAGGCCAGGTTACTCCTGGAAATATCAGTTCTGTTATAACCG 1080
ysAlaArgAlaValSerLysGlyGlnValThrProGlyAsnIleSerSerValIleThrV

TCACCTATACCTATGCGTAAATATTATCCCCTCTTTAAGAAAAGCACCGTCCTTAAGGGC 1140
alThrTyrThrTyrAla---

CGGTGTTTTATTCACACTTATAATGGCATATTCCCAGCCATCGTTTGCACTGTTGTGCAG 1200
                      SfaH  METAlaTyrSerGlnProSerPheAlaLeuLeuCysAr

AAATAACCAGACCGGGCAAGTATTTAACTCCGGGGATACATCCTTTCGGGTTAATGTCAG 1260
gAsnAsnGlnThrGlyGlnValPheAsnSerGlyAspThrSerPheArgValAsnValSe
                                 ▲
TCCTGTTGTGCAATATGATAAATCCATATCTGTATTGGACCTTTCCCAACTGGTATCATG 1320
rProValValGlnTyrAspLysSerIleSerValLeuAspLeuSerGlnLeuValSerCy

TCAGAATGAGGACTCGACAGGCCAAAACTATGATTATCTGAAAATATTGAAAGGCAGTGG 1380
sGlnAsnGluAspSerThrGlyGlnAsnTyrAspTyrLeuLysIleLeuLysGlySerGl

TTTTTCTCCTGCTCTGGATACCAAAACATACGGACGACTCGATTTTACAAGTCGTCCAAC 1440
yPheSerProAlaLeuAspThrLysThrTyrGlyArgLeuAspPheThrSerArgProTh

GGGGTATGCCAGGCAATTACCACTTCAGTTTGATTTGCAGGTGACAGAGGCGTTTTATCA 1500
rGlyTyrAlaArgGlnLeuProLeuGlnPheAspLeuGlnValThrGluAlaPheTyrGl

ATATGGTGTCTGGAAACCATTCCCTGCTAAATTATATCTGTACCCTGCACCGGGTGTATT 1560
nTyrGlyValTrpLysProPheProAlaLysLeuTyrLeuTyrProAlaProGlyValPh

TGGGAAGGTAATTAACAACGGAGATTTACTGGCAACGTTATATGTGAATAAGTTTTCCAC 1620
eGlyLysValIleAsnAsnGlyAspLeuLeuAlaThrLeuTyrValAsnLysPheSerTh

AAAGGGGCAGGAGGCAGGGGAGAGAAATTTCACCTGGCGTTTCTATGCAACGAATGATGT 1680
rLysGlyGlnGluAlaGlyGluArgAsnPheThrTrpArgPheTyrAlaThrAsnAspVa
                                                Hinc II
CCATATCCAGACAGGTACATGCAGGGTCTCATCGAACAATGTCAAAGTTGACCTTCCGTC 1740
lHisIleGlnThrGlyThrCysArgValSerSerAsnAsnValLysValAspLeuProSe

CTATCCCGGAGGGCCGGTAACAGTCCCTCTTACTGTACGTTGCGACCAGACACAGTCGGT 1800
rTyrProGlyGlyProValThrValProLeuThrValArgCysAspGlnThrGlnSerVa

CAGCTATACCCTGTCAGGTCCTGTAACAGGAAGTGGTAATACTGTATTCGCAAATACGGC 1860
lSerTyrThrLeuSerGlyProValThrGlySerGlyAsnThrValPheAlaAsnThrAl

AGCATCAGGGTCTGGCGGTGTGGGTATACAGTTGTCGGATAACGTGGGGCCGGTTCCGGC 1920
aAlaSerGlySerGlyGlyValGlyIleGlnLeuSerAspAsnValGlyProValProAl

CGGACAACCGAGGTCTCTGGGACAGGTAGGCAGCTCTCCTGTGAGTCTGGGGCTGAAGGC 1980
aGlyGlnProArgSerLeuGlyGlnValGlySerSerProValSerLeuGlyLeuLysAl

CTCTTATGCTCTGACCGGTCAGGCAAGTCCGACGCCCGGTGCTGTCCAGTCAGTGATAAA 2040
aSerTyrAlaLeuThrGlyGlnAlaSerProThrProGlyAlaValGlnSerValIleAs

TGTGACTTTTAGCTACAACTAGAATGCAGTTGTGGCGGTAATGATATTACTGCTCTTTAT 2100
nValThrPheSerTyrAsn
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B
TTGTATCTGTTGTGATGACAGATGCGGTGTGCGTAGTTCGATTAAAAACAGGAATTAAAT   60
                    Nco I                                   SfaA
ATGAAGTTAAAATTCATCTCCATGGCTGTATTTTCAGCCCTGACCCTGGGTGTTGCGACA  120
METLysLeuLysPheIleSerMETAlaValPheSerAlaLeuThrLeuGlyValAlaThr

AGTGCGTCTGCCGCACCTGCTACTGTCAATGGTGGCACTGTTCATTTTAAGGGGGAAGTG  180
SerAlaSerAlaAlaProAlaThrValAsnGlyGlyThrValHisPheLysGlyGluVal
            ▲
GTTAATGCTGCATGTGCTGTAAATATGAACTCAGTAGATCAGACAGTTTTGCTCGGTCAA  240
ValAsnAlaAlaCysAlaValAsnMetAsnSerValAspGlnThrValLeuLeuGlyGln

GTTCGTACGAAAAAGTTAGCTAATCTTGATGATGTTAGTGGACCGGTTGGATTTAATATT  300
ValArgThrLysLysLeuAlaAsnLeuAspAspValSerGlyProValGlyPheAsnIle

CAATTAGATGAGTGTGATTCCACAACATCTGGATCTGTGAAGATTCTTTTTTCTGGCACA  360
GlnLeuAspGluCysAspSerThrThrSerGlySerValLysIleLeuPheSerGlyThr

CCTGTTGCTGGAAAAAATAACGCCTTGGCTATACAAAGCTCGGCATCTGGAGCTGCAACA  420
ProValAlaGlyLysAsnAsnAlaLeuAlaIleGlnSerSerAlaSerGlyAlaAlaThr

AATGTTGGGATTCAGATACTTGACTCCTCAGGGAATCCTGTGACATTAAATTCGGATCAA  480
AsnValGlyIleGlnIleLeuAspSerSerGlyAsnProValThrLeuAsnSerAspGln

AGCGCAGTATATACATTGACAGACGGAACTAATAATATTCCGTTCCAGGCTCGCTATATT  540
SerAlaValTyrThrLeuThrAspGlyThrAsnAsnIleProPheGlnAlaArgTyrIle

GCTACTGGTCAATCAACAGCTGGTACAGCCAACGCCGACGCCACCTTTAAAGTTCAGTAC  600
AlaThrGlyGlnSerThrAlaGlyThrAlaAsnAlaAspAlaThrPheLysValGlnTyr

CAGTAATATCAGAACAGTGTAACGGATATATACCCGGCCAGGGAGGGCTGTTTTTATCCA  660
Gln---
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FIG. 4. Nucleotide and protein sequences of the *sfaA* gene (B) and the loci *sfaG*, *sfaS*, and *sfaH* of the *sfaII* gene cluster (A). The putative cleavage sites of the corresponding proteins are indicated by triangles. Important restriction sites are shown.

genes responsible for the fimbrial subunits of SfaI (25) were used to determine the exact locations of the corresponding loci of the *sfaII* determinant (Fig. 1). To characterize these genes more accurately, we sequenced the corresponding regions of 2.8-kb DNA (Fig. 4). One open reading frame (ORF), which represents the gene *sfaA* of the *sfaII* determinant starts, at an ATG codon at positions 61 through 63 and ends with TAA at position 604. The mature SfaA protein, which represents the major subunit of the SfaII complex of the O18:K1 strain IHE3034, has a calculated molecular mass of 16.0 kDa, and its amino acid sequence shows 62% identity to the sequence of the SfaA protein from the SfaI complex of strain 536 (Table 2; Fig. 5).

The genes coding for the minor subunits of the SfaII complex are located at the distal part of the *sfaII* determinant (Fig. 1). The corresponding region of 2.1-kb DNA shows three ORFs that represent the genes *sfaG*, *sfaS*, and *sfaH* of the *sfaII* gene cluster (Fig. 4b). The first ORF (*sfaG*) starts at position 61 and ends at position 586, the second ORF (*sfaS*) extends from position 609 to position 1098, and

TABLE 2. Degree of identity and similarity of amino acid sequences of mature major and minor subunit proteins of the SfaII complex compared with those of the proteins of the SfaI and Foc complexes[a]

Proteins	% Identity (similarity) of SfaII-specific proteins			
	SfaA	SfaG	SfaS	SfaH
SfaI homologous	62 (73)	99 (99)	100 (100)	97 (98)
Foc homologous	65 (75)	97 (98)	54 (64)	83 (87)

[a] See references 33, 34, 39, and 40.

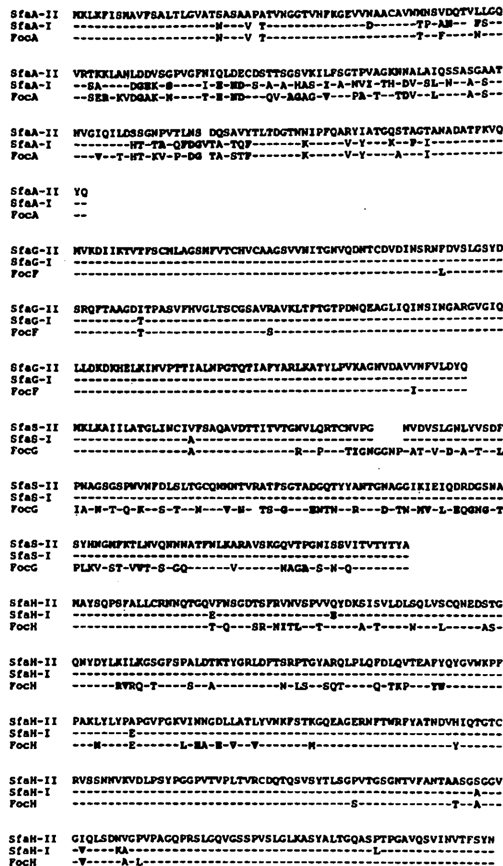

FIG. 5. Comparison of the primary amino acid sequences of the subunit proteins SfaA, SfaG, SfaS, and SfaH encoded by the *sfaII* determinant with the SfaI and Foc (F1C) fimbrial adhesins (33, 34, 39, 40). Identical amino acid residues are indicated by gaps, and amino acid residues that are functionally similar are in boldface type. Dashes represent gaps introduced for optimal alignment.

the third ORF (*sfaH*) is located between positions 1163 and 2059. These three genes code for mature proteins with calculated molecular masses of 15.7 kDa (SfaG), 14.9 kDa (SfaS), and 29.4 kDa (SfaH). Although the proteins SfaS show identical amino acid sequences, the two mature proteins SfaG and SfaH differ in one (SfaG) and six (SfaH) amino acid residues (Fig. 5; Table 2).

Mutation in the gene *sfaS* of the *sfaII* determinant. To determine whether SfaS is identical to the sialic acid-specific adhesin of the SfaII complex, recombinant pAZZ50-67 DNA was constructed to carry a frameshift mutation in the gene *sfaS* of the *sfaII* gene cluster. The mutant clone was unable to recognize erythrocytes in a hemagglutination assay and was negative in the ELISA with the adhesin-specific monoclonal antibody A1 (Table 1), indicating that the minor subunit SfaS of the SfaII complex and the sialic acid-specific adhesin are identical.

Isolation of the S-specific adhesin and protein sequence analysis of the N-terminal part of SfaSII. To confirm the identity between SfaSII and the sialic acid-specific adhesin of the meningitis isolate IHE3034, the protein was isolated as described previously (11, 20). As demonstrated for the SfaSI protein, SfaSII was able to agglutinate human and bovine erythrocytes and reacted with the monoclonal antibody A1 (Table 1). Furthermore the N-terminal sequence of the mature protein SfaSII was determined. It was shown that the first amino acids of the mature protein were valine, aspartic acid, and threonine (Fig. 4 and 5). Therefore, it is clear that the first 22 amino acid residues were cleaved after transport. All the 20 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 *sfaSII* (Fig. 4).

DISCUSSION

Sfa are produced by *E. coli* strains causing sepsis and NBM or UTI (3, 17). Although the *sfa* determinant of an UTI isolate was described previously in great detail (6, 8, 9, 21, 33–35), there was limited information on the *sfa* gene cluster of NBM or sepsis strains. The data presented here concerning the molecular cloning and characterization of an *sfa* gene cluster from the NBM *E. coli* isolate IHE3034 (O18:K1) confirm our previous observations (24, 25, 26, 30) that S fimbriae of UTI and NBM strains and F1C (Foc) and S/F1C-related (Sfr) fimbriae form one particular family of fimbrial adhesins produced by extraintestinal *E. coli*. The coding sequences of the *sfa*, *foc*, and *sfr* determinants and the flanking DNA regions exhibited a high degree of relatedness (9, 25, 30, 39).

Our studies on the nucleotide sequences of the *sfaI*- and *sfaII*-specific subunit genes together with those of the *foc* and *sfr* determinants revealed that the major subunit genes are the most heterogeneous parts of the subunit specific loci of the *sfa-foc* family. The degree of dissimilarity between the corresponding amino acid sequences of the two SfaA proteins and that of FocA is higher than 30% (Table 2). This is further reflected by the fact that monoclonal antibody F1, specific for SfaA of SfaI, does not recognize the major subunit proteins of SfaII (Table 1), Foc, and Sfr (data not shown). From an evolutionary point of view it might be speculated that the immune system selects for the generation of major subunit variants with divergent serological properties. Observations such as these here presented for the Sfa-F1C adhesin family have also been made for the group of P-fimbrial adhesins and type I fimbriae (1, 14, 36, 38).

As previously demonstrated for the SfaI complex (34), we have further shown that the minor subunit protein SfaS is identical to the sialic acid-specific adhesin of the SfaII complex of the O18:K1 isolate. The fact that the protein sequences of the two mature SfaS molecules investigated are identical (the only amino acid exchange is located in the leader sequence; Fig. 5) underlines the common function of both proteins. The conserved amino acid sequences of the two SfaS proteins are different from the sequences of the equivalent FocG proteins of the F1C fimbrial adhesin complex, which exhibit only 54% identical amino acids (39); the F1C fimbrial adhesin complex does not show sialic acid-specific binding properties (31, 41). Interestingly, the equivalent protein of the Sfr complex, which is also hemagglutination negative, shows 59% sequence homology with SfaS (Schmittroth and Hacker, unpublished). In contrast to SfaS, FocG and SfrS the two SfaG proteins and the equivalent F1C and Sfr specific molecules show identical protein sequences with the exception of the exchange of a few amino acid residues (32a, 39). The conservation of this minor subunit protein among members of the Sfa-F1C family may be due to its function as an additional adhesin that may bind to

tubullus cells as well as to plasminogen independently of the sialyl-lactose residue (19, 26a, 27).

We and others have already shown that S fimbriae expressed by the NBM isolate IHE3034 and the UTI strain 536 differ slightly in their capacities to bind to erythrocytes (8, 17). This observation has now been confirmed by quantitative hemagglutination tests with recombinant *E. coli* K-12 clones harboring the *sfaI* and *sfaII* plasmids (Table 1). The differences between SfaI and SfaII with respect to binding capacities are independent of the amino acid sequences of the two SfaS molecules, which are identical. It is speculated that the sequence alterations between the remaining subunit proteins influence the binding abilities of the sialic acid-specific adhesins. An influence of the copy number of the recombinant plasmids on this phenomenon can be excluded because the DNAs used represent identical constructs containing pBR322 as a cloning vehicle (see Materials and Methods) (Fig. 1). One reason for the differences observed could be different strengths of binding of SfaI and SfaII to identical receptor structures. Second, SfaI and SfaII complexes could also bind to slightly different sialic acid-containing isoreceptor structures as recently shown for Gal-Gal- and GalNac-GalNac-specific adhesins of the P and Prs fimbrial family (14, 35). Preliminary data argue for the occurrence of the second possibility (35a).

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