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Regulation and Binding Properties of S Fimbriae Cloned from E. coli Strains Causing Urinary Tract Infection and Meningitis

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With 2 Figures

Summary

S fimbriae are able to recognize receptor molecules containing sialic acid and are produced by pathogenic *E. coli* strains causing urinary tract infection and menigitis. In order to characterize the corresponding genetic determinant, termed S fimbrial adhesin *(sfa)* gene cluster, we have cloned the S-specific genes from a urinary pathogen and from a meningitis isolate. Nine genes are involved in the production of S fimbriae, two of these, *sfa*B and *sfa*C code for regulatory proteins being necessary for the expression of S fimbriae. Two promoters, P_B and P_C, are located in front of these genes. Transcription of the *sfa* determinant is influenced by activation of the promoters via SfaB and SfaC, the action of the H-NS protein and an RNaseE-specific mRNA processing. In addition, a third promoter, P_A, located in front of the major subunit gene *sfa*A, can be activated under special circumstances. Four genes of the *sfa* determinant code for the subunit-specific proteins, SfaA (16 kda), SfaG (17 kda), SfaS (14 kda) and SfaH (29 kda). It was demonstrated that the protein SfaA is the major subunit protein while SfaS is identical to the sialic-acid-specific adhesin of S fimbriae. The introduction of specific mutations into *sfa*S revealed that a region of six amino acids of the adhesin which includes two lysine and one arginine residues is involved in the receptor specific interaction of S fimbriae. Additionally, it has been shown that SfaS is necessary for the induction of fimbriation while SfaH plays a role in the stringency of binding of S fimbriae to erythrocytes.

Zusammenfassung

S-Fimbrien können Sialinsäure-haltige Rezeptoren erkennen. Sie werden von pathogenen *E. coli*-Isolaten gebildet, die Harnwegsinfektionen oder Meningitiden auslösen können. Um die für S-Fimbrien kodierenden Gene zu bestimmen und zu charakterisieren, haben wir die

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sfa („S fimbrial adhesin")-Determinanten eines uropathogenen Stammes und eines Meningitis-Erregers kloniert. Neun Gene sind an der Produktion der S-Fimbrien beteiligt, zwei von diesen, *sfa*B und *sfa*C, kodieren für Regulatorproteine, die für die Expression der S-Fimbrien notwendig sind. Zwei Promotoren P_C und P_B sind vor diesen Genen lokalisiert. Die Transkription der sfa Determinante wird durch die SfaB- und SfaC-spezifische Aktivierung der Promotoren, durch die Wirkung des H-NS Proteins und durch eine RNaseE abhängige mRNA-Prozessierung beeinflußt. Zusätzlich kann unter bestimmten Umständen ein dritter Promotor, P_A, der vor dem Hauptstrukturgen *sfa*A lokalisiert ist, aktiviert werden. Weiterhin kodieren vier Gene für die Proteinuntereinheiten SfaA (16 kda), SfaS (14 kda), SfaG (17 kda) und SfaH (29 kda). Es konnte gezeigt werden, daß das Protein SfaA identisch mit der Hauptstrukturuntereinheit der Fimbrien ist, während SfaS das Sialinsäurespezifische Adhäsin darstellt. Durch die Einführung von site-spezifischen Mutationen konnte eine aus sechs Aminosäuren bestehende Region von SfaS, die zwei Lysin- und einen Argininrest beinhaltet, für die Rezeptor-spezifische Interaktion von S-Fimbrien verantwortlich gemacht werden. Weiterhin wurde evident, daß SfaS an der Induktion der Fimbrienbildung beteiligt ist, während SfaH einen Einfluß auf die Stabilität der Bindung von S-Fimbrien an Erythrocyten hat.

S Fimbrial Adhesins: Distribution and Role in Infectious Diseases

S fimbriae and P fimbriae (also termed Pap – *p*ili *a*ssociated with *p*yelonephritis) represent the main adherence factors of extraintestinal *E. coli* strains (16, 29). The majority of strains isolated from urinary tract infections express P fimbriae (52, 53). S-fimbrial adhesins are produced by a minority of uropathogenic *E. coli* strains and by 80% of isolates causing meningitis in newborns (NBM) (18, 28, 31). S fimbriae bind to glycoproteins which terminate with the sequence α-sialic acid-(2-3)-β-lactose (17, 35). Recent evidence exists that S fimbriae also recognize carbohydrate structures of glycolipids (*Kim*, personal communication).

Only a limited number of *E. coli* isolates are able to express S fimbriae: Strains with O antigen O6 and isolates of the serotypes O2:K1, O18:K1 and O83:K1 (3, 34, 53). The uropathogenic O6 strains are more frequently involved in infections of the bladder (cystitis) than those of the kidney (pyelonephritis; 49, 53). In contrast, K1 isolates are the main infectious agents causing cases of meningitis in newborns. These strains, however, have also been isolated from cases of pyelonephritis (18, 52).

S fimbriae together with F1C fimbriae and Sfr fimbriae form a special family of adherence factors, which are strongly related on the genetic level (10, 32, 33, 39, 41). F1C and Sfr fimbriae, however, do not express an α-sialic acid-(2-3)-β-lactose binding specificity and in contrast to S fimbriae, they do not bind to erythrocytes (50, 51). While only limited information exists on the distribution of Sfr fimbriae, F1C fimbriae are expressed by about 30% of UTI strains of various serotypes including O18:K5, O6:K5 and O75:K5 (40).

There is no doubt that fimbrial adhesins play a key role in the initial colonization of bacteria on eukaryotic host tissues (4, 5). S fimbriae are involved in the binding of bacteria to uroepithelial cells and to brain tissues (19, 23, 36). Despite the fact that S fimbriae exhibit a strong affinity to kidney cells (20) and are involved in urinary tract infection in the experimental rat model (21, 22), their incidence among UTI strains is rather low in comparison to that of P fimbriae (52). This may be a consequence of S-specific binding to the Tamm-Horsfall glycoprotein which may act as a trapping factor to eliminate S-fimbriated bacteria from urine (37). In addition, S fimbriae are able to bind to endothelial cells and to a constituent of the extracellular matrix, laminin

(*Virkola* et al., submitted). In addition, this particular attachment factor is involved in the activation of plasminogen to form plasmin which leads to fibrinolytic processes on tissues (38). The latter mechanism seems to be an important step in the pathogenesis of meningitis in newborns. Interestingly, human milk contains factors that specifically bind to S-fimbriated bacteria with the consequence of a protection of newborns against these potentially dangerous *E. coli* bacteria (46–48).

Overall Composition of the S-Fimbrial Adhesin (*sfa*) Determinant

The *sfa* determinants were cloned from the chromosomes of two pathogenic *E. coli* isolates, the uropathogenic *E. coli* strain 536 (O6:K15) and the meningitis isolate IHE3034 (O18:K1) (9, 11, 13). The determinant of strain 536 termed *sfa*I was fully sequenced and it became evident that nine genes were necessary for the production of S fimbriae (45). As indicated in Fig. 1a, four genes, *sfa*A, *sfa*G, *sfa*S and *sfa*H code for the fimbrial subunits, being proteins of 16 kda, 17 kda, 14 kda, and 29 kda which compose the fimbrial rod (42). While the protein SfaA represents the major subunit protein of S fimbriae (42), the proteins SfaG, SfaS and SfaH are minor subunit proteins. In both cases (the *sfa*I determinant and the *sfa*II gene cluster), the proteins SfaS are identical to the sialic-acid-specific adhesins (17, 24, 43). In contrast to the SfaA and the SfaH proteins of the SfaI and SfaII complexes which show several amino acid exchanges in relation to each other, the SfaS proteins of both types of S fimbriae are completely identical (12, 13).

Two genes, *sfa*B and *sfa*C, are located at the 5′ end of the gene cluster and are necessary for the regulation of expression of the *sfa* determinant (see below). In addition, three genes, *sfa*D, *sfa*E, and *sfa*F which code for proteins of 18 kda, 24 kda and 90 kda are part of the *sfa* gene cluster. While a mutation in the gene *sfa*D does not affect fimbriation and hemagglutination of the corresponding clones, SfaE and SfaF are involved in the transport of the subunit proteins across the outer membrane (*Morschhäuser* et al., in preparation). Interestingly, SfaE exhibits homology to chaperone-like proteins of other fimbrial adhesin complexes while SfaF seems to be an outer membrane protein (4, 13).

Transcription of the *sfa* Genes

Using lamda-*plac*Mu fusion, three promotors, pA, pB and pC, could be identified in the *sfa* determinant in front of the genes *sfa*A, *sfa*B and *sfa*C, respectively (8, 45). Transcriptional fusions with *phoA* and translational fusions with *lacZ* showed that the *sfa* genes were mainly transcribed from the two divergently oriented promotors, pB and pC, and to a low degree, from pA. To analyse the transcription of the *sfa* gene cluster in detail, we performed Northern blotting experiments with RNA from strains harbouring the cloned *sfa* genes (26). One transcript of 500 bases encoding *sfa*C could be identified using an *sfa*C-specific probe. Another abundant transcript of 700 bases that codes for the main fimbrial subunit was detected using an *sfa*A-specific probe. In addition, an *sfa*BA bicistronic transcript of 1400 bases was seen in low amounts with both an *sfa*A-specific and an *sfa*B-specific probe (see Fig. 1b).

To localize the respective promotors exactly, we undertook primer extension studies with primers complementary to the *sfa*A, *sfa*B and *sfa*C 5′ coding regions. The mRNA

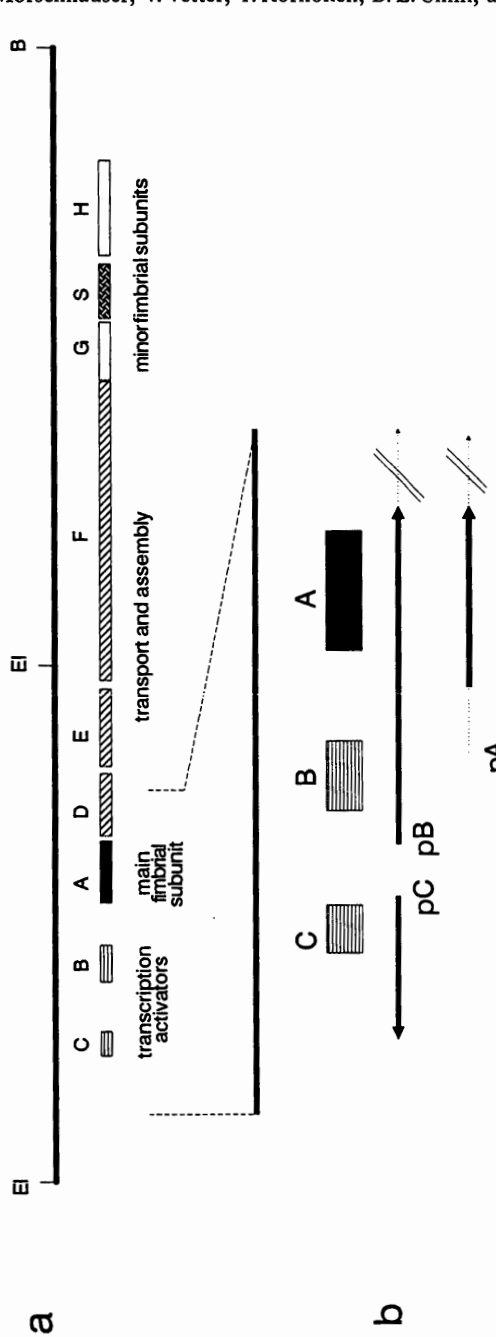

Fig. 1a. Genetic organization of the *sfa* determinant. Boxes represent *sfa*-specific genes. Functions of the proteins are indicated.

Fig. 1b. Transcriptional organization of the proximal part of the *sfa* determinant. Promotors are indicated. EI = *Eco*RI, B = *Bam*HI.

starting points identified in front of the genes *sfa*B and *sfa*C were in the correct position with respect to the predicted −10 and −35 regions of *E. coli* consensus promotors. The mRNA starting point in front of *sfa*A, however, did not reveal such homology (26). Further analysis showed that the *sfa*A mRNA starting point had not been a result of transcription initiation at this site but was produced by posttranscriptional cleavage of mRNAs starting at pB and pA (*Morschhäuser* et al., in preparation). The precursor transcripts were cleaved at two sites to yield a rapidly degraded *sfaB* encoding part and a very stable *sfa*A-specific mRNA. As was recently shown also for the *pap* determinant (1, 2, 27), this processing of the *sfa* transcript was dependent on the *rne* locus encoding or regulating RNaseE. In the RNaseE-negative mutant strain N3431, production of the 700 bases *sfa*A transcript was strongly inhibited in comparison to the isogenic *rne*$^+$ strain N3433.

The rest of the *sfa* genes was also transcribed mainly from the proximal promotor pB. These transcripts, when produced from the cloned wild type gene cluster, were too rapidly degraded to be detected by Northern hybridization. However, when transcriptional terminators stabilizing the mRNA's against nucleolytic attack by 3′ exonucleases were inserted into the *sfa* determinant, longer transcripts starting at the second processing site in front of *sfa*A demonstrated that the genes *sfa*B to *sfa*H were presumably transcribed as a long polycistronic transcript, but most of the transcripts ended behind *sfa*A (*Morschhäuser*, unpublished).

Regulation of *sfa* Expression by *trans* Acting Factors

Fimbrial adhesin determinants are not expressed constitutively, rather, they are regulated by environmental signals (6, 30, 44). In order to determine factors which influence the expression of the *sfa* determinant, several mutations were introduced into the proximal region of the gene cluster to mutagenize the putative regulators, SfaB and SfaC (see 26, 45). Mutations in *sfa*B or *sfa*C demonstrated that both gene products were indispensable for effective *sfa* expression from the wild type gene cluster. The mutations could be complemented by providing the genes on a compatible plasmid, indicating the *trans* activating role of SfaB and SfaC (26). A deletion of part of the intercistronic region between the two genes in plasmid pANN 81–5 resulted in *sfa* expression that was to some degree independent of the activators. These results led us to the hypothesis that an unknown repressor might bind to the DNA region between *sfa*B and *sfa*C, thus inhibiting *sfa* expression in the absence of the activators. Examination of *sfa* transcripts in the mutant strains by Northern hybridization showed that these regulatory effects were at the mRNA level (26).

An activator-independent *sfa* transcription could also be observed when different mutant *sfa* derivatives were introduced into strain HMG 5 which has a mutation in *drd*X, the gene encoding the histone-like protein H-NS. The H-NS protein plays a role in the expression of several virulence factors (7). In the *drd*X$^-$ strain, expression of the *sfa* genes was achieved even when both activators and the promoters pC and pB had been deleted, indicating an activation of the otherwise almost inactive promotor pA (plasmid pANN 81–2 in Table 1). These results suggest that H-NS acts as a negative regulator of *sfa* expression by inhibiting transcription from the *sfa* promoters and that SfaB and SfaC counteract this activity.

Table 1. Effects of *sfa* mutations on hemagglutination capacity in *drd*X$^+$ and *drd*X$^-$ strains

Strain	Genotype	Hemagglutination[1]
MC 1029 (pANN 801–13)	(*drd*X$^+$, *sfa*C$^+$, B$^+$, A–H$^+$)	1:16
MC 1029 (pANN 81–2)	(*drd*X$^+$, *sfa*C$^-$, B$^-$, A–H$^+$)	0
MC 1029 (pANN 81–3)	(*drd*X$^+$, *sfa*C$^+$, B1, A–H$^+$)	0
MC 1029 (pANN 81–5)	(*drd*X$^+$, *sfa*C$^-$, B1, A–H$^+$)	1:4
HMG 5 (pANN 801–13)	(*drd*X$^-$, *sfa*C$^+$, B$^+$, A–H$^+$)	1:64
HMG 5 (pANN 81–2)	(*drd*X$^-$, *sfa*C$^-$, B$^-$, A–H$^+$)	1:32
HMG 5 (pANN 81–3)	(*drd*X$^-$, *sfa*C$^+$, B1, A–H$^+$)	1:32
HMG 5 (pANN 81–5)	(*drd*X$^-$, *sfa*C$^-$, B1, A–H$^+$)	1:64

[1] Determined by a quantitative hemagglutination assay.

Genetic Studies on *sfa*S Coding for the S-Specific Adhesin

The minor subunit protein SfaS is able to recognize sialic acid containing receptor substances. In order to elucidate the S-specific binding properties of SfaS, a *trans*-complementation system with plasmids containing different origins of replication was developed. As demonstrated in Fig. 2, the introduction of the plasmid pMWW50 carrying the gene *sfa*S under the control of an inducible promotor is able to restore the S-specific binding properties of SfaS-negative mutant clones. Interestingly, SfaS is able to mediate binding in clones expressing the major subunit SfaA and the minor subunits SfaG and SfaH and it also converts the non hemagglutinating SfaA$^+$, SfaG$^-$, SfaS$^-$, SfaH$^-$ clone HB101 (pMWW107) to a hemagglutination-positive strain.

The introduction of seven site-directed mutations in the *sfa*S sequence made it possible to identify an epitope involved in binding of the sialic-acid-specific adhesin. Table 2 indicates the phenotypes of the mutant clones. Only mutations introduced into the C-terminal part of the protein between amino acids 116 to 122 failed to agglutinate bovine red blood cells while amino acid exchanges affecting other regions of the

Table 2. Characterization of *E. coli* clones carrying site-specific mutations in the adhesin gene *sfa*S

Plasmid	Amino acid exchange in *Sfa*S	Hemagglutination[1]	Fimbriation[2]
pANN 801–13	wildtype	1:32	++++
pMWW 100/50	wildtype	1:32	++++
pANN 801–116	Lys116 – Ser116	0	++++
pANN 801–118	Arg118 – Ser118	0	++++
pANN 801–122	Lys122 – Thr122	1:32	++++
pANN 801–89	Arg89 – Ile89	1:16	++++
pMWW 100/pMWW51	Lys83 – Thr83	1:16	++
pMWW 100/pMWW52	Trp43 – Leu43	1:32	++++
pMWW 100/pMWW53	Ala117 – Val117	1:16	++

[1] Determined by a quantitative hemagglutination assay.
[2] Determined by electron microscopy.

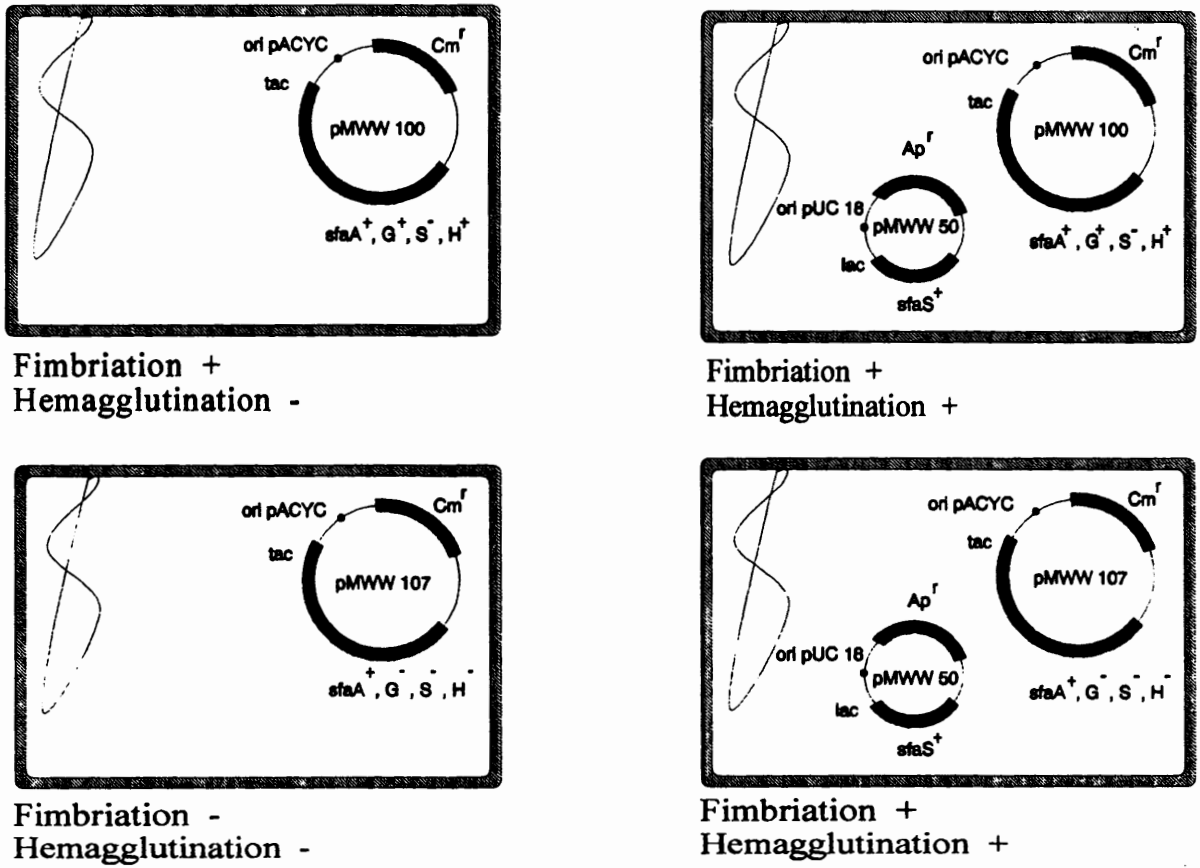

Fig. 2. Complementation studies with plasmids carrying *sfa*-specific genes.

adhesin molecule had no influence on receptor-specific binding (see also 25). Interestingly, the region Lys^{116}-Ala-Arg-Ala-Val-Ser-Lys^{122} revealed extensive homologies to epitopes located in other sialic-acid-specific proteins like K99 adhesin or the B subunits of the cholera toxin and the LT enterotoxin (15, 25).

Functional Analysis of the Minor Subunit Proteins

In previously published reports, *sfa*S-negative and *sfa*H-negative clones showed a definite reduction in fimbriation of recombinant clones (23, 25). It was therefore speculated that the SfaS and SfaH proteins played a role in the determination of S-specific fimbriation. In contrast, an *sfa*G-negative mutant did not influence fimbriation of the cell but reduced the binding capacity of the corresponding clones. SfaG was therefore termed an „alternative" binding protein (23). In order to prove whether or not the adhesin SfaS induced fimbriae formation, trans complementational tests with pMWW50 (*sfa*S^+) were carried out (see Fig. 2). Following introduction of this plasmid into clones specific for $SfaA^+$, $SfaG^+$, $SfaS^-$, and $SfaH^+$, the degree of fimbriation of the bacteria increased dramatically. To our surprise, SfaS was also able to induce fimbriation following introduction into the non-fimbriated $SfaA^+$, $SfaG^-$, $SfaS^-$, and $SfaH^-$ clones carrying plasmid pMWW107. From these data it can be concluded that the adhesive molecule, SfaS, plays an additional role in the biogenesis of fimbriae.

New data about SfaH strongly indicate that this molecule influences binding of the *sfa* complex to sialic-acid-containing receptor structures (13, *Vetter*, unpublished data). It was observed previously, that the UTI strain 536 exhibited a weaker binding to erythrocytes than the meningitis isolate IHE3034 (9, 18). The same observation was made for the two *sfa* gene clusters, *sfa*I and *sfa*II, cloned from the chromosomes of the two strains. While the amino acid sequences of the two SfaS adhesins of SfaI and SfaII were identical (see above), the SfaH proteins showed seven amino acid exchanges. These alterations seem to control the differences in the binding capacity.

Table 3. Characterization of *E. coli* clones expressing different S fimbrial adhesin (*Sfa*) complexes

Strain/clone	*sfa* genotype	Hemagglutination[1]	Fimbriation[2]
HB101 (pANN 801–13)	*sfa*A-I, *sfa*G-I *sfa*S-I, *sfa*H-I	1:16	++++
HB101 (pAZZ 50)	*sfa*A-II, *sfa*G-II *sfa*S-II, *sfa*H-II	1:64	++++
HB101 (pAZZ 50–67)	*sfa*A-II, *sfa*G-II *sfa*S-II^-, *sfa*H-II	–	++

[1] Determined by a quantitative hemagglutination assay.
[2] Determined by electron microscopy.

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