INFECTION AND IMMUNITY, Feb. 1989, p. 446–451
0019-9567/89/020446-06$02.00/0
Copyright © 1989, American Society for Microbiology
Vol. 57, No. 2

Molecular Analysis and Epidemiology of the Dr Hemagglutinin of Uropathogenic *Escherichia coli*

BOGDAN NOWICKI,[1]* CATHARINA SVANBORG-EDÉN,[2] RICHARD HULL,[1] AND SHEILA HULL[1]

Department of Microbiology and Immunology, Baylor College of Medicine, Texas Medical Center, Houston, Texas 77030,[1] and Department of Clinical Immunology, University of Göteborg, Göteborg S-41346, Sweden[2]

Received 9 August 1988/Accepted 18 October 1988

The genetic organization and epidemiology of Dr hemagglutinin was studied. Plasmids derived from pBJN406 and carrying transposon inserts were analyzed for their abilities to confer the mannose-resistant hemagglutination phenotype and expression of plasmid-encoded proteins. The 6.6-kilobase DNA fragment expressed five polypeptides with molecular masses of 15.5, 5, 18, 90, and 32 kilodaltons encoded by the *draA*, *draB*, *draC*, *draD*, and *draE* genes, respectively. Four genes, *draA*, *draC*, *draD*, and *draE*, were required for full mannose-resistant hemagglutination expression. Mutation in the *draA* gene, previously identified as encoding fimbrillin, resulted in loss of the adherence phenotype. We screened 658 strains isolated from patients with urinary tract infections (UTI) or from fecal samples for the presence of DNA sequences homologous to the *draD* gene. A significantly higher frequency of *draD*-related sequences was found among *Escherichia coli* strains from patients with cystitis than among strains from patients with other clinical forms of UTI. Association of *draD*-related sequences with O75 and other serotypes was observed. A possible role of Dr hemagglutinin as a virulence factor in lower UTI is discussed.

Bacterial adhesion to tissues of mammalian hosts is important in the initiation of various infectious diseases (6, 7). This adhesion may be mediated by bacterial proteins generally called adhesins or hemagglutinins which may or may not be associated with fimbriae (7). A uropathogenic strain of *Escherichia coli* may produce several adhesins at the same time (8, 9, 11). They differ in hemagglutination capacity, serological properties, and tissue tropism associated with clinical conditions (6, 7, 11, 26, 31, 36). Different adhesins occur mostly on separate cells and show a rapid phase variation both in vitro and in vivo, creating a number of ways to increase the pathogenic potential of a strain (15, 28–31, 35, 36). Uropathogenic *E. coli* strains carrying P fimbriae, a major virulence factor in pyelonephritis, may express other adhesins of unknown receptor specificity (6, 33, 40). These adhesins have been termed X and occur on about 20% of non-P-fimbriated pyelonephritis-associated *E. coli* strains (39). Some X adhesins might be potential virulence factors by themselves or in association with other adhesins; for example, they might supply an *E. coli* strain with additional tissue tropism at different stages of the pathogenic process. In a recent study by Nowicki et al., differential kidney tropism for *E. coli* strains carrying different fimbriae was shown (26). P-fimbriated *E. coli* showed adherence to the lumen of proximal and distal tubules, whereas Dr (O75X)-positive *E. coli* showed adherence to the renal interstitium and Bowman's capsule. To study the contribution of Dr hemagglutinin to virulence, the genes that encode the Dr adhesin that occurs on uropathogenic *E. coli* strains of the O75:K5:H$^-$ serotype have been cloned (25). A recombinant strain, *E. coli* BN406 carrying plasmid pBJN406, was found to express mannose-resistant hemagglutination (MRHA) and specific adherence to the basement membrane and Bowman's capsule. The Dr adhesin-coding region was estimated to be about 6 kilobases (kb) and found to encode multiple proteins, including a 15-kilodalton (kDa) fimbrial subunit protein. Further study showed that the O75X adhesin expressed by the recombinant strain *E. coli* BN406 recognized the Dr blood group antigen (27).

The receptor structure on the Dr blood group antigen recognized by Dr hemagglutinin was suggested to be a tyrosine-containing molecule. The attachment of Dr hemagglutinin to human erythrocytes could be inhibited by chloramphenicol or a modified tyrosine, which show similarity between their chemical structures. High-density sites of the Dr receptor have been shown in various types of human tissues, including colonic glands and different parts of the urinary tract. These receptor sites were postulated to facilitate ascending colonization and infection of the urinary tract (30a). However, the frequency and role of Dr hemagglutinin-positive *E. coli* strains in causing urinary tract infection (UTI) and the genetics of its synthesis are not well understood. In this communication, we report the organization of the genes that encode Dr hemagglutinin and the frequency of Dr-related DNA sequences among *E. coli* and other *Enterobacteriaceae* strains isolated from patients with UTIs.

MATERIALS AND METHODS

Bacteria and media. *E. coli* BN406 is a recombinant strain carrying plasmid pBJN406 and exhibits MRHA of Dr-positive erythrocytes and adherence to the basement membrane and Bowman's capsule. The other *E. coli* K-12 strains used in this study are listed in Table 1. The UTI and fecal *E. coli* isolates used are listed in Table 2. Bacteria were grown on Luria agar or Luria broth supplemented with ampicillin (100 μg/ml), chloramphenicol (20 μg/ml), or kanamycin (20 μg/ml) if required.

Purification of plasmid DNA fragments. Plasmid DNA was digested with restriction endonucleases. Reaction mixtures were diluted with several volumes of stop mix (5% Ficoll 400, 1.8% sodium dodecyl sulfate), and fragments were separated by electrophoresis through a 0.7% agarose gel with a Tris-borate-EDTA buffer system. Desired fragment bands were cut from the gel, and the DNA was recovered with an Elutrap electroseparation system (Schleicher & Schuell, Inc., Keene, N.H.). The purification process was

* Corresponding author.

TABLE 1. *E. coli* strains used

Strain	Relevant genotype	Source or reference
EC901	As AB1133, but *hsdR4 recA*	24
HU736	F_{ts}::Tn*5 lac trp*	Laboratory collection
HU679	F_{ts}::Tn*3 lac trp*	Plasmid from R. Gill
CC118	*phoA20 recA1*	From S. Highlander
BN406	EC901(pBJN406)	24
BN409	EC901(pBJN409)	24
BN410	EC901(pBJN410)	24
BN1	EC901(pBJN1::Tn*phoA*)	This study
BN2	EC901(pBJN2::Tn*phoA*)	This study
BN4	EC901(pBJN4::Tn*phoA*)	This study
BN6	EC901(pBJN6::Tn*3*)	This study
BN7	EC901(pBJN7::Tn*phoA*)	This study
BN04	EC901(pBJN04::Tn*phoA*)	This study
BN10	EC901(pBJN10::Tn*phoA*)	This study
BN11	EC901(pBJN11::Tn*phoA*)	This study
BN14	EC901(pBJN14::Tn*3*)	This study
BN16	EC901(pBJN16::Tn*phoA*)	This study
BN17	EC901(pBJN17::Tn*phoA*)	This study
BN21	EC901(pBJN21::Tn*phoA*)	This study
BN26	EC901(pBJN26::Tn*phoA*)	This study
BN29	EC901(pBJN29::Tn*phoA*)	This study
BN34	EC901(pBJN34::Tn*phoA*)	This study
BN36	EC901(pBJN36::Tn*phoA*)	This study
BN412	EC901(pBJN412::Tn*5*)	24
BN413	EC901(pBJN413::Tn*5*)	24
BN414	EC901(pBJN414::Tn*5*)	24
BN415	EC901(pBJN415::Tn*5*)	24
BN416	EC901(pBJN416::Tn*5*)	24
BN417	EC901(pBJN417::Tn*5*)	24
BN418	EC901(pBJN418::Tn*5*)	24
BN517	EC901(pBJN517::Tn*phoA*)	This study

repeated with GTG agarose (SeaKem; FMC Corp., Marine Colloids Div., Rockland, Maine), and the DNA was alcohol precipitated and suspended in TE buffer (10 mM Tris hydrochloride, 1 mM EDTA, pH 8.0).

Preparation of probe DNA and dot blot hybridization. Radiolabeling of purified DNA fragments and hybridizations were performed as previously described (10).

In vitro translation of plasmid-encoded proteins. Circular or restriction endonuclease-digested plasmid DNA (1 to 4 μg) was transcribed and translated in vitro in the presence of L-[^{35}S]methionine for 45 min with a commercial procaryotic DNA-directed translation kit (Amersham Corp., Arlington Heights, Ill.), followed by a 5-min methionine chase. Translated proteins (10^5 trichloroacetic acid-precipitable cpm per lane) were solubilized at 100°C for 5 min in sodium dodecyl sulfate-gel sample buffer and electrophoresed on sodium dodecyl sulfate–15% polyacrylamide gels by the method of Laemmli (19). After electrophoresis, gels were fixed in 30%

TABLE 2. Frequency of *draD*-related sequences among *E. coli* strains isolated from patients with different clinical forms of UTI

Source of isolates	No. of strains	No. (%) *draD* positive
Patients with asymptomatic bacteriuria[a]	70	4 (5.7)
Patients with cystitis[a]	70	18 (25.7)
Patients with pyelonephritis[a]	66	4 (6.0)
Patients with symptomatic UTI[b]	279	77 (27.6)
Feces of healthy persons[b]	68	10 (14.7)

[a] Each urinary isolate was cultured from Swedish children experiencing their first episode of UTI.
[b] Isolates from the United States.

methanol–10% glacial acetic acid, dried onto filter paper, and autoradiographed with XAR-5 film (Eastman Kodak Co., Rochester, N.Y.). Chloramphenicol acetyltransferase encoded by the vector pACYC184 was strongly expressed in vitro, and therefore plasmid DNA was digested to completion with *Eco*RI to abolish synthesis of the chloramphenicol acetyltransferase polypeptide, resulting in better visualization of Dr hemagglutinin-coding region polypeptides.

Transposon mutagenesis. Transposons Tn*5*, Tn*5* IS*50*$_L$::*phoA* (Tn*phoA*), and Tn*3* were used for insertional inactivation mapping. For Tn*5* or Tn*3* insertional mutagenesis, *E. coli* BN406 was mixed with *E. coli* HU736 or HU679, respectively, at an initial concentration of 10^7 CFU of each per ml in 20 ml of L broth without antibiotics and incubated overnight at 30°C without shaking. Transconjugants were selected on L agar containing chloramphenicol and kanamycin. Plates with about 500 well-isolated transconjugant colonies were harvested into 1 liter of L broth with kanamycin and chloramphenicol, and cultures were grown to an optical density at 600 nm of 0.4. Plasmids were amplified overnight in the presence of spectinomycin (300 μg/ml) and isolated by the method of So et al. (37). A purified plasmid was used to transform *E. coli* EC901 to kanamycin and chloramphenicol resistance. Plasmid DNA was screened for Tn*5* insertion by observing its migration in 0.7% agarose gels relative to that of nonmutagenized pBJN406. Transformants were tested for hemagglutination and agglutination with specific anti-Dr hemagglutinin rabbit antibodies. The site of Tn*5* insertion was determined by examining restriction fragments generated during single digest of plasmid DNA with *Hin*dIII and *Cla*I. For Tn*phoA* insertional mutagenesis, *E. coli* CC118(pBJN406) was grown in L broth supplemented with 10 mM $MgSO_4$ and chloramphenicol to the early stationary phase. Bacteriophage λ Tn*phoA* (provided by S. Highlander) was added to the culture at a multiplicity of infection of about 1. Several separate cultures from each infection were grown for 15 h at 30°C with aeration. Transductants were grown on L agar containing kanamycin (300 μg/ml) and chloramphenicol. Colonies were harvested and suspended in L broth containing selective antibiotics. After 18 h, plasmids were isolated, purified, and used to transform *E. coli* EC901, as described for Tn*5*. The site and orientation of the Tn*phoA* insertion was determined by digestion of plasmid DNA with *Cla*I, *Sal*I, *Ava*I, and *Sst*I.

RESULTS

Identification and location of genes required for Dr hemagglutinin expression. The pBJN406 plasmid is the smallest in vitro-constructed recombinant molecule that expresses MRHA (25). To determine precisely the DNA region required for expression of Dr hemagglutination, pBJN406 derivatives carrying Tn*5*, Tn*3*, and Tn*phoA* insertions were isolated and analyzed for MRHA in the host strain *E. coli* EC901. Of 24 insertions selected, 7 derivatives had entirely lost the capacity for hemagglutination, whereas 17 still expressed MRHA (Fig. 1). These results show that a 6.6-kb region on pBJN406, from transposon *26* to transposon *2*, is required for expression of MRHA. However, Tn*phoA* inserts in the 1.1-kb *Sst*I-*Sst*I DNA fragment within this region showed no effect on the MRHA phenotype of the host strain.

Plasmid pBJN406 and its transposon insertion derivatives were examined for protein synthesis by the in vitro transcription-translation method in the presence of L-[^{35}S]methionine. Five genes, designated *draA*, *draB*, *draC*, *draD*, and *draE*, encoding polypeptides of 15.6, 5, 18, and 90 kDa and

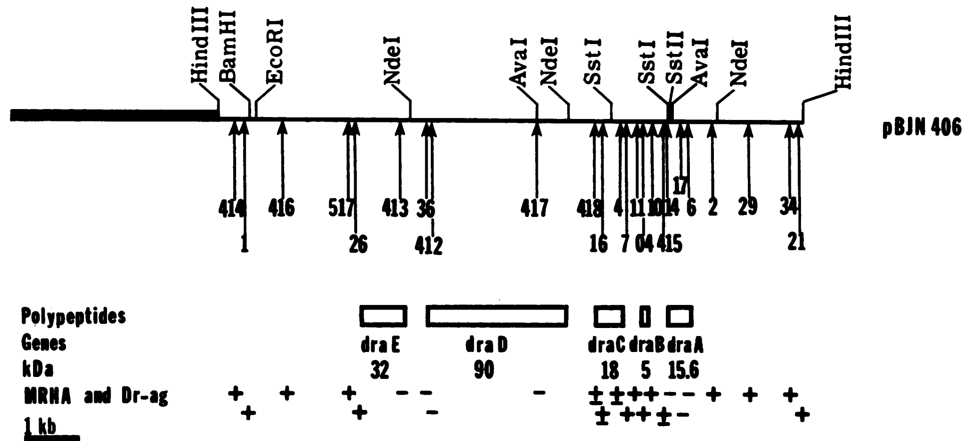

FIG. 1. Relative positions of insertion mutations of pBJN406 and proposed genetic organization of the Dr hemagglutinin-coding region. Proposed protein map positions relative to restriction endonuclease recognition sites on pBJN406 are presented. The presence of MRHA and Dr fimbrillin is marked by + (strong reaction), ± (weak reaction), or − (no reaction). Since the reactions of MRHA and Dr fimbrillin for the same insertion derivative were similar, a common designation for both features is presented. ↑, Transposon insertion mutation; Dr-ag, fimbrial antigen, tested by anti-Dr antibody.

a set of three polypeptides of 32, 29, and 27 kDa, respectively, were localized (Fig. 1). Introduction of the Tn*5*, Tn*3*, and Tn*phoA* sequences into pBJN406 led to the addition of several transposon-specific polypeptides. The mutant plasmid with a insertion in the *draE* gene, pBJN413, did not express the 29- and 27-kDa polypeptides, whereas the 32-kDa polypeptide seemed to be truncated to a 30-kDa form (Fig. 2, lane 2). The other polypeptides remained unchanged in size; however, the amount of the 90-kDa polypeptide was strongly reduced. Insertions in the *draD* gene, plasmids pBJN412 and pBJN417, resulted in the disappearance or premature termination, respectively, of the 90-kDa polypeptide (Fig. 2, lanes 3 and 4). These insertions did not reduce the synthesis of other polypeptides. Insertions in the *draC* gene, plasmids pBJN16 and pBJN4, caused premature termination or disappearance, respectively, of the 18-kDa polypeptide (Fig. 2, lanes 8 and 9). Hemagglutination by strains harboring these mutant plasmids was strongly reduced but not totally negative. An insert in the *draB* gene, plasmid pBJN04, resulted in disappearance of the 5-kDa polypeptide. This plasmid also expressed reduced amounts of the *draA* gene product, but hemagglutination was not affected. The transposon inserts in the *draA* gene, in plasmids pBJN17, pBJN6, and pBJN14, resulted in premature termination or reduced expression of the 15.6-kDa polypeptide. A strong reduction in MRHA was observed for pBJN415 and

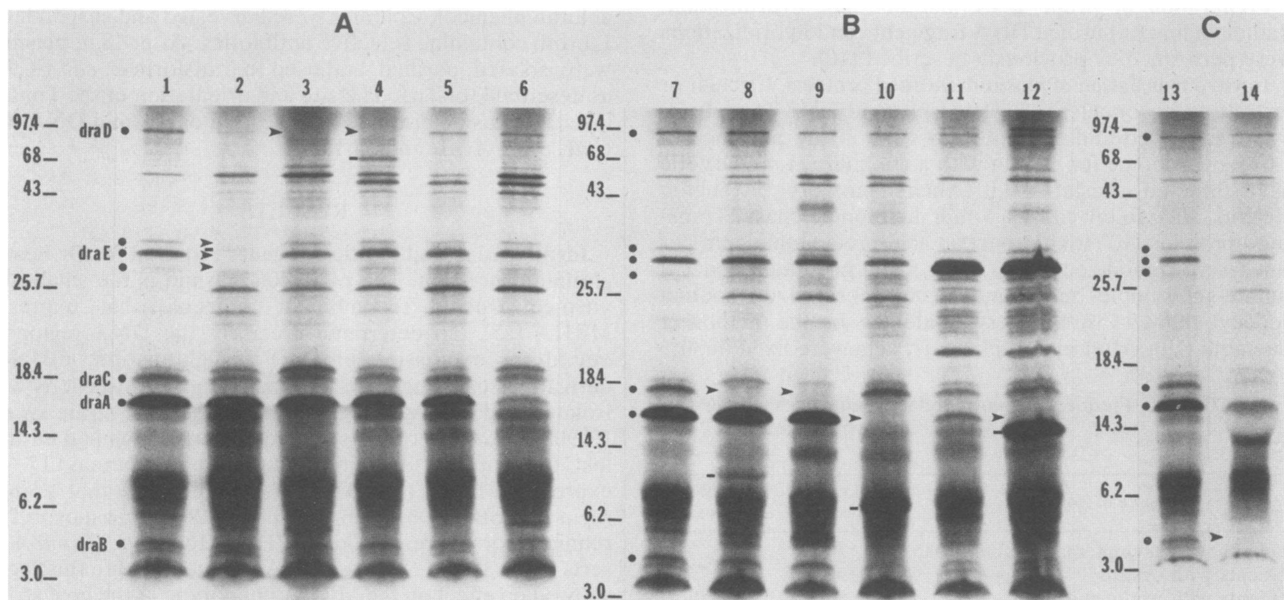

FIG. 2. Autoradiograph of [^{35}S]methionine-labeled proteins encoded on pBJN406 and its derivatives. Plasmid DNA was translated in vitro by a coupled transcription-translation procedure. Symbols: ●, proteins expressed by *dra* coding region; ▶; corresponding mutated protein; —, corresponding truncated protein. Lanes: 1, pBJN406; 2, pBJN413; 3, pBJN412; 4, pBJN417; 5, pBJN418; 6, pBJN415; 7, pBJN406; 8, pBJN16; 9, pBJN4; 10, pBJN17; 11, pBJN14; 12, pBJN6; 13, pBJN406; 14, pBJN04. The positions of molecular size standards are given in kilodaltons.

pBJN418, but only for pBJN415 was the polypeptide profile changed, i.e., a reduced amount of the 15.6-kDa polypeptide. Apparently, the insert in plasmid pBJN418 is very near the C terminus of the *draC* gene product. Plasmid pBJN409, a deletion mutant that lacks the 1.1-kb *Sst*I-*Sst*I fragment from pBJN406, did not express the 15.6-, 5-, and 18-kDa polypeptides (data not shown), confirming the locations of the *draA*, *draB*, and *draC* genes. No disappearance or modification of the pBJN406 polypeptides was observed for three of the Dr MRHA-positive insertion mutants, pBJN414, pBJN416, and pBJN2 (data not shown).

Screening for *draD*-related sequences among urinary tract isolates. We screened 658 strains by dot blot DNA hybridization for the presence of sequences homologous to the *draD* gene. These consisted of 452 strains collected in the United States and isolated from persons with symptomatic UTI (384 strains) and random individuals (68 fecal strains) and 206 strains collected in Sweden and isolated from patients with pyelonephritis (n = 66), cystitis (n = 70), or asymptomatic bacteriuria (n = 70). The probe used was a 3-kb *Nde*I-*Nde*I DNA fragment encoding the *draD* gene. Hybridization was observed with 27.6% of the U.S. *E. coli* UTI strains and none of 105 strains belonging to other species of *Enterobacteriaceae*, including *Klebsiella* sp. (n = 55), *Enterobacter* sp. (n = 17), *Citrobacter* sp. (n = 12), *Proteus* sp. (n = 16), *Providencia* sp. (n = 2), *Serratia* sp. (n = 2), and *Morganella* sp. (n = 1), isolated from patients with symptomatic UTI. The proportion of hybridizing strains among fecal isolates was 14.7% (Table 2). The probe did not hybridize with two control strains, *E. coli* SH1 and GR12, both carrying type 1 and P fimbriae, or *E. coli* HU1151, carrying S fimbriae. Analysis of the Swedish *E. coli* strains isolated from patients with different clinical forms of UTI showed that the Dr probe hybridized with 5.6 and 6.0% of strains from patients with asymptomatic bacteriuria and pyelonephritis, respectively, but with 27.6% of strains from patients with cystitis. The greater frequency of $draD^+$ bacteria among cystitis isolates was statistically significant ($P <$ 0.01).

Characteristics of *E. coli* strains harboring *draD*-related sequences. The frequency of *draD*-related sequences among *E. coli* strains of different O serotypes is presented in Table 3. Strains that hybridized with the Dr probe were observed predominantly among uropathogenic O serotypes. The highest proportion of *draD*-positive *E. coli* strains was found among two serotypes, O75 and O12, and the lowest was in serotype O6. The *draD*-positive O75 strains were present in both the U.S. and Swedish collections, whereas *draD*-positive O12 strains were found only in the U.S. set. The frequency of *pap*- and *hly*-related sequences among *draD*-positive *E. coli* strains was also estimated; the probes used were the 4-kb *Hin*dIII fragment for *pap* and the 2-kb *Pvu*II fragment for *hly* (10, 40). The frequencies of hybridization with the *draD* probe among strains previously recognized as positive and negative for *pap* were the same, indicating a lack of sequence homology between these two adhesins. The frequency of *hly*-positive isolates among *draD*-positive strains was 46%.

TABLE 3. Frequency of *draD*-related sequences among *E. coli* strains of different O serotypes

O serotype	No. of strains	No. (%) *draD* positive
O1	7	1 (14)
O2	26	7 (27)
O4	24	3 (12)
O6	43	3 (7)
O11	1	1 (100)
O12	8	6 (75)
O15	2	1 (50)
O17	1	1 (100)
O18	11	1 (9)
O21	2	2 (100)
O75	20	9 (45)
O78	1	1 (100)
O133	2	2 (100)
O157	1	1 (100)
O7, O16, O25	29	0 (0)
R[a]	22	3 (14)

[a] R, Rough strains.

DISCUSSION

Molecular characterization of *E. coli* adhesins cloned from diarrheal and extraintestinal infections indicated that multiple proteins are involved in their biogenesis. A degree of similarity between both the organizations and the sizes of the gene products of fimbrial coding regions has been observed (6). Molecular analysis of the P fimbria-encoding genes have shown that at least eight proteins compose the functional units required for the biogenesis of P fimbriae (1, 3, 4, 8, 34, 36, 37, 40). We found five genes in the Dr hemagglutinin-coding region; among those, four, *draA*, *draC*, *draD*, and *draE*, were required for full expression of the MRHA phenotype. The *draA* gene encodes a 15.6-kDa polypeptide in vitro thought to be a 15.0-kDa fimbrial subunit, as identified in recent studies (25). Insertion of transposons into *draA* resulted in abolition of both MRHA and agglutination by the anti-Dr antibody. These results are in contrast to those of similar experiments done with the *papA* fimbrial gene (24, 38). In the *pap* studies, nonpolar insertion mutations in the *papA* gene, especially near the beginning of the gene, resulted in loss of antigen expression but not loss of adherence. It is unlikely that insertions in the *draA* gene exert polarity effects upon another separate adhesin gene, because on the basis of the size of the prematurely terminated *draA* gene products shown in Fig. 2, lanes 6 and 10 to 12, one can conclude that translation of *draA* proceeds left to right (Fig. 1); there are no genes required for adherence immediately to the right of *draA*. In addition, 53 insertion mutations with Tn*3*, Tn*5*, and Tn*phoA* were screened for expression of both MRHA and the fimbrial antigen; for all of the strains tested, expression of adherence correlated with expression of the fimbrial antigen. These results are consistent with the hypothesis that the *draA* product is both fimbrillin and adhesin. However, until it can be shown whether the purified *draA* product displays specific adherence, the possibility of an unidentified adhesin gene should not be excluded. Although hemagglutinin and fimbrillin have been found to be encoded by separate genes in several fimbrial operons, fimbrillin and adhesin seem to be encoded by one structural gene in the K99 operon (5, 6, 23, 32).

Analysis of the Dr hemagglutinin-coding region showed an organization similar to that of the *pap* and other fimbrial coding regions. The *papA* gene, coding fimbrillin, and *papC* and *papD*, both involved in export and assembly of P fimbriae, could correspond to genes *draA*, *draD*, and *draE*, respectively. No insertions to the left of *draE* (Fig. 1) abolished the MRHA phenotype. The 0.7-kb *Hin*dIII-*Eco*RI fragment located in that region was not required for expression of the MRHA phenotype (25). The corresponding DNA segment in *pap* contains *papE*, *papF*, and *papG* genes,

which encode the adhesin and may differ from the *dra* operon. There is also similarity with the K99 operon (5, 6). The recently reported organization of the afimbrial adhesin genes (AFA-I) seems to be similar to that of the *dra* gene cluster. However, *afa*-positive *E. coli* strains were infrequent and did not correlate with clinical forms of UTI (16–18).

Analysis of O serotypes among *draD*-positive strains indicates that *draD* sequences are not restricted to the O75 serotype only. It is possible that Dr^+ O75 strains create a worldwide clone, whereas the Dr^+ O12 serotype could be a regional U.S. clone. Association with more than one O serotype indicates that the name Dr hemagglutinin is more appropriate than the O75X fimbria-like adhesin. Hybridization studies have shown that several *E. coli* virulence-associated factors are encoded by genes that occur on the same DNA cluster (2, 10, 13, 14, 22). The frequency of *hly* and *pap* sequences among *draD*-positive strains suggests no clear association between these properties. In contrast, *afa* exhibited a negative correlation with hemolysin.

Since uropathogenic strains may express several adhesins that recognize different receptors on the same erythrocyte, identification of an adhesin on the basis of hemagglutination or agglutination with antibodies may be inconclusive. For that reason, the use of DNA-DNA hybridization would be more accurate. In the present study, we used the *draD* gene as a probe and analyzed the frequency of *draD*-related sequences among strains isolated from patients with different clinical forms of UTI. *E. coli* strains isolated from patients with cystitis showed a significantly higher frequency of *draD*-related sequences than did strains from patients with asymptomatic bacteriuria or pyelonephritis. Association of bacterial properties and the clinical form of infection led to the discovery of P fimbriae as a virulence factor in upper UTI (6, 12, 20, 40). Despite several efforts, a similar association has not been described for lower UTI (16, 21). Our study indicates that the Dr hemagglutinin may be a virulence factor in lower UTI. This may occur because of Dr hemagglutinin-mediated attachment to the transitional cells that line the lower urinary tract. Previous studies have shown a high density of the Dr receptor in this region (30a). The relatively high frequency of *draD*-positive bacterial strains among fecal isolates may be due to a very high Dr receptor density in colonic glands, a natural factor possibly increasing the rate of colonization of the colon by Dr^+ *E. coli* (35). Studies regarding a role of Dr^+ *E. coli* in UTI are in progress.

ACKNOWLEDGMENTS

This work was supported by Public Health Service grants AI18462 and AI 21009 from the National Institutes of Health.

LITERATURE CITED

1. **Båga, M., M. Norgren, and S. Normark.** 1987. Biogenesis of *E. coli* pap pili: papH, a minor pilin subunit involved in cell anchoring and length modulation. Cell **49**:241–251.
2. **Berger, H., J. Hacker, A. Juarez, C. Hughes, and W. Goebel.** 1982. Cloning of the chromosomal determinants encoding hemolysin production and mannose-resistant hemagglutination in *Escherichia coli*. J. Bacteriol. **152**:1241–1247.
3. **Clegg, S.** 1982. Cloning of genes determining the production of mannose-resistant fimbriae in a uropathogenic strain of *Escherichia coli* belonging to serogroup O6. Infect. Immun. **38**: 739–744.
4. **Clegg, S., and J. K. Pierce.** 1983. Organization of genes responsible for the production of mannose-resistant fimbriae of a uropathogenic *Escherichia coli* isolate. Infect. Immun. **42**:900–906.
5. **De Graaf, F. K., B. E. Krenn, and P. Klaasen.** 1984. Organization and expression of genes involved in the biosynthesis of K99 fimbriae. Infect. Immun. **43**:508–514.
6. **De Graaf, F. K., and F. R. Mooi.** 1986. The fimbrial adhesins of *Escherichia coli*. Adv. Microb. Physiol. **28**:74–143.
7. **Duguid, J. P., and D. C. Old.** 1980. Adhesive properties of *Enterobacteriaceae*, p. 185–217. *In* E. H. Beachey (ed.), Receptors and recognition, series B, vol. 6: bacterial adherence. Chapman & Hall, Ltd., London.
8. **Hull, R. A., R. E. Gill, P. Hsu, B. H. Minshew, and S. Falkow.** 1981. Construction and expression of recombinant plasmids encoding type 1 or D-mannose-resistant pili from a urinary tract infection *Escherichia coli* isolate. Infect. Immun. **33**:933–938.
9. **Hull, S., S. Clegg, C. Svanborg-Edén, and R. Hull.** 1985. Multiple forms of genes in pyelonephritogenic *Escherichia coli* encoding adhesins binding globoseries glycolipid receptors. Infect. Immun. **47**:80–83.
10. **Hull, S. I., S. Bieler, and R. A. Hull.** 1988. Restriction fragment length polymorphism and multiple copies of DNA sequences homologous with probes for P-fimbriae and hemolysin genes among uropathogenic *Escherichia coli*. Can. J. Microbiol. **34**: 307–311.
11. **Jann, K., B. Jann, and G. Schmidt.** 1981. SDS polyacrylamide gel electrophoresis and serological analysis of pili from *Escherichia coli* of different pathogenic origin. FEMS Microbiol. Lett. **11**:21–25.
12. **Kallenius, G., R. Mollby, S. B. Svenson, J. Windberg, A. Lundblad, S. Svenson, and B. Cedergren.** 1980. The p^k antigen as receptor for the haemagglutination of pyelonephritic *Escherichia coli*. FEMS Microbiol. Lett. **7**:297–302.
13. **Knapp, S., J. Hacker, T. Jarchau, and W. Goebel.** 1986. Large, unstable inserts in the chromosome affect virulence properties of uropathogenic *Escherichia coli* O6 strain 536. J. Bacteriol. **168**:22–30.
14. **Knapp, S., J. Hacker, I. Then, D. Muller, and W. Goebel.** 1984. Multiple copies of hemolysin genes and associated sequences in the chromosome of uropathogenic *Escherichia coli* strains. J. Bacteriol. **159**:1027–1033.
15. **Korhonen, T. K., B. Nowicki, M. Rhen, V. Väisänen-Rhen, A. Pere, R. Virkola, and J. Vuopia-Varkila.** 1986. Fimbrial phase variation in *Escherichia coli*: a mechanism of bacterial virulence, p. 245–251. *In* D. Lark, S. Normark, H. Wolf-Waltz, and B. E. Uhlin (ed.), Protein-carbohydrate interactions in biological systems. Academic Press, Inc. (London), Ltd., London.
16. **Labigne-Roussel, A., and S. Falkow.** 1988. Distribution and degree of heterogeneity of the afimbrial-adhesin-encoding operon (*afa*) among uropathogenic *Escherichia coli* isolates. Infect. Immun. **56**:640–648.
17. **Labigne-Roussel, A. F., D. Lark, G. S. Schoolnik, and S. Falkow.** 1984. Cloning and expression of an afimbrial adhesin (AFA-I) responsible for P blood group-independent, mannose-resistant hemagglutination from a pyelonephritic *Escherichia coli* strain. Infect. Immun. **46**:251–259.
18. **Labigne-Roussel, A. F., M. A. Schmidt, W. Walz, and S. Falkow.** 1985. Genetic organization of the *afa* operon and nucleotide sequence from a uropathogenic *Escherichia coli* gene encoding an afimbrial adhesin (AFA-I). J. Bacteriol. **162**:1285–1292.
19. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
20. **Leffler, H., and C. Svanborg-Edén.** 1981. Glycolipid receptors for uropathogenic *Escherichia coli* on human erythrocytes and uroepithelial cells. Infect. Immun. **34**:920–929.
21. **Lomberg, H., M. Hellstrom, U. Jodal, H. Leffler, K. Lincoln, and C. Svanborg-Edén.** 1984. Virulence-associated traits in *Escherichia coli* causing first and recurrent episodes of urinary tract infection in children with or without vesicoureteral reflux. J. Infect. Dis. **150**:561–569.
22. **Low, D. V., D. Lark, G. Schoolnik, and S. Falkow.** 1984. Gene clusters governing the production of hemolysin and mannose-resistant hemagglutination are closely linked in *Escherichia coli* serotype O4 and O6 isolates from urinary tract infections. Infect. Immun. **43**:353–358.

23. **Moch, T., H. Hoschutzky, J. Hacker, K. D. Kroncke, and K. Jann.** 1987. Isolation and characterization of the α-sialyl-β-2,3-galactosyl-specific adhesin from fimbriated *Escherichia coli*. Proc. Natl. Acad. Sci. USA **84**:3462–3466.
24. **Norgren, M., S. Normark, D. Lark, P. O'Hanley, G. Schoolnik, S. Falkow, C. Svanborg-Edén, M. Baga, and B. E. Uhlin.** 1984. Mutations in *E. coli* cistrons affecting adhesion to human cells do not abolish Pap pili fiber formation. EMBO J. **3**:1159–1165.
25. **Nowicki, B., J. P. Barrish, T. Korhonen, R. A. Hull, and S. I. Hull.** 1987. Molecular cloning of the *Escherichia coli* O75X adhesin. Infect. Immun. **55**:3168–3173.
26. **Nowicki, B., H. Hölthofer, T. Saraneva, M. Rhen, V. Väisanen-Rhen, and T. K. Korhonen.** 1986. Location of adhesion sites for P-fimbriated and for O75X-positive *Escherichia coli* in the human kidney. Microb. Pathog. **1**:169–180.
27. **Nowicki, B., J. Moulds, R. Hull, and S. Hull.** 1988. A hemagglutinin of uropathogenic *Escherichia coli* recognizes the Dr blood group antigen. Infect. Immun. **56**:1057–1060.
28. **Nowicki, B., M. Rhen, V. Väisänen-Rhen, A. Pere, and T. K. Korhonen.** 1984. Immunofluorescence study of fimbrial phase variation in *Escherichia coli* KS71. J. Bacteriol. **160**:691–695.
29. **Nowicki, B., M. Rhen, V. Väisänen-Rhen, A. Pere, and T. K. Korhonen.** 1985. Kinetics of phase variation between S and type-1 fimbriae of *Escherichia coli*. FEMS Microbiol. Lett. **28**:237–242.
30. **Nowicki, B., M. Rhen, V. Väisänen-Rhen, A. Pere, and T. K. Korhonen.** 1985. Organization of fimbriate cells in colonies of *Escherichia coli* strain 3040. J. Gen. Microbiol. **131**:1263–1266.
30a. **Nowicki, B., L. Truong, J. Moulds, and R. Hull.** 1988. Presence of the Dr receptor in normal human tissues and its possible role in the pathogenesis of ascending urinary tract infection. Am. J. Pathol. **133**:1–4.
31. **Nowicki, B., J. Vuopio-Varkila, P. Viljanen, T. K. Korhonen, and P. H. Mäkelä.** 1986. Fimbrial phase variation and systemic *Escherichia coli* infection studied in the mouse peritonitis model. Microb. Pathog. **1**:335–347.
32. **Orndoff, P. E., and S. Falkow.** 1984. Organization and expression of genes responsible for type 1 piliation in *Escherichia coli*. J. Bacteriol. **159**:736–744.
33. **Rhen, M., P. Klemm, and T. K. Korhonen.** 1986. Identification of two new hemagglutinins of *Escherichia coli*, *N*-acetyl-D-glucosamine-specific fimbriae and a blood group M-specific agglutinin, by cloning the corresponding genes in *Escherichia coli* K-12. J. Bacteriol. **168**:1234–1242.
34. **Rhen, M., J. Knowles, M. E. Penttila, M. Sarvas, and T. K. Korhonen.** 1983. P-fimbriae of *Escherichia coli*: molecular cloning of DNA fragment containing the structural genes. FEMS Microbiol. Lett. **19**:119–123.
35. **Rhen, M., P. H. Mäkelä, and T. K. Korhonen.** 1983. P fimbriae of *Escherichia coli* are subject to phase variation. FEMS Microbiol. Lett. **19**:267–271.
36. **Saukkonen, K. M. J., B. Nowicki, and M. Leinonen.** 1988. Role of type 1 and S fimbriae in the pathogenesis of *Escherichia coli* O18:K1 bactermia and meningitis in the infant rat. Infect. Immun. **56**:892–897.
37. **So, M., W. S. Dallas, and S. Falkow.** 1978. Characterization of an *Escherichia coli* plasmid encoding for synthesis of heat-labile toxin: molecular cloning of the toxin determinant. Infect. Immun. **21**:405–411.
38. **Uhlin, B. E., M. Norgren, M. Båga, and S. Normark.** 1985. Adhesion to human cells by *Escherichia coli* lacking the major subunit of a digalactoside-specific pilus adhesin. Proc. Natl. Acad. Sci. USA **82**:1800–1804.
39. **Väisänen-Rhen, V.** 1984. Fimbria-like hemagglutinin of *Escherichia coli* O75 strains. Infect. Immun. **46**:401–407.
40. **Väisänen, V., J. Elo, L. G. Tallgren, A. Siitonen, P. H. Mäkelä, C. Svanborg-Edén, G. Kallenius, S. B. Svenson, H. Hultberg, and T. K. Korhonen.** 1981. Mannose-resistant hemagglutination and P antigen recognition are characteristics of *Escherichia coli* causing primary pyelonephritis. Lancet **ii**:1366–1369.
41. **van Die, I., I. van Megen, W. Hoekstra, and H. Bergmans.** 1984. Molecular organization of the genes involved in the production of $F7_2$ fimbriae, causing mannose resistant hemagglutination, of a uropathogenic *Escherichia coli* O6:K2:H1:F7 strain. Mol. Gen. Genet. **194**:528–533.
42. **Welch, R. A., R. Hull, and S. Falkow.** 1983. Molecular cloning and physical characterization of a chromosomal hemolysin from *Escherichia coli*. Infect. Immun. **42**:178–186.