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Analysis of P Fimbriae on *Escherichia coli* O2, O4, and O6 Strains by Immunoprecipitation

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P fimbriae on *Escherichia coli* O2, O4, and O6 strains were analyzed by immunoprecipitation. Fimbrial extracts were prepared from a total of 35 strains and tested for precipitation with four anti-P-fimbria sera. The overall fimbrial composition of the strains was related to the O:K:H serotype, and two to three P fimbrial variants per strain were found on most of the O4 and some of the O6 strains. The O2 strains, in contrast, showed only one antigenic variant of P fimbriae per strain, which was serologically unrelated to those of the O4 and O6 strains. The results stress the multiplicity and serological complexity of *E. coli* P fimbriae.

Several properties of uropathogenic *Escherichia coli* strains have been recognized as possible virulence factors in human childhood pyelonephritis. They include certain O and K antigens, hemolytic activity, and ability to adhere to human uroepithelial cells (2, 7, 22, 30, 33, 36). Of these, adherence to the uroepithelium seems to be of major importance (33). This adherence is mediated by P fimbriae (12), which recognize the P blood group-specific glycosphingolipids on human epithelial cells and erythrocytes (8). P fimbriae are thought to increase bacterial virulence by helping the bacteria to resist the cleansing action of urine flow, thus aiding the ascending infection in the urinary tract.

Recognition of P fimbriae as virulence factors has focused much interest on their use as a vaccine against human pyelonephritis. Antibodies and Fab fragments against purified P fimbriae prevent bacterial adhesion to human urinary tract cells (24), and vaccination of monkeys (29) or mice (20) with purified P fimbriae has given protection against experimental pyelonephritis. P fimbriae thus seem to be promising candidates for a vaccine.

A problem encountered in the purification and serological analysis of P fimbriae is the multiplicity of fimbrial antigens on pathogenic *E. coli* strains. Most pyelonephritis-associated *E. coli* strains have, in addition to P fimbriae, mannose-binding type 1 fimbriae, and many of the strains possess other fimbrial or fimbria-like adhesins, of which only some have been characterized (32). The latter include S (13) and type 1C (9, 23, 24) fimbriae, M (6) and O75X (31) adhesins, and a variety of so-called X adhesins (32, 33). Moreover, a strain may have more than one variant of P fimbriae with differing chemical and perhaps serological properties (12, 21, 24, 25). This has made the purification of specific P-fimbrial antigens difficult. The approaches used to overcome this problem have included cloning of the structural genes of individual P fimbriae (18, 25, 35) and immunofractionation of fimbriae (28) or fimbriate cells (19, 26).

Using crossed-immunoelectrophoresis, Ørskov and Ørskov (21) have demonstrated a number of serological variants of fimbriae on uropathogenic *E. coli* strains. The fimbriae were designated as F antigens, and it is apparent that some of them correspond to P fimbriae, but so far no comparison of serological and chemical properties of the P fimbriae on *E. coli* strains of different serogroups has been done. This, however, is important in the design of P-fimbrial vaccines against human urinary tract infection. We have recently developed a simple immunoprecipitation assay for the serological analysis of adhesins on pathogenic *E. coli* strains (13, 23, 31). We now report an application of this method to P fimbriae on *E. coli* strains of serogroups O2, O4, and O6, which belong to the common P-fimbriate serogroups associated with childhood pyelonephritis (33).

MATERIALS AND METHODS

Bacteria. A total of 37 *E. coli* strains (see Table 2) were used in the precipitation assays; two of them were nonfimbriate O4 strains used as controls and were not included in Table 2. The strains and their fimbriation have been characterized in detail (33). The P-fimbriate strains (n = 35) have been isolated from the urine of young girls (n = 23) or boys (n = 5) with pyelonephritis, from urine of girls with cystitis (n = 2), asymptomatic bacteriuria (n = 1), or unclassified urinary tract infection (n = 1), or from the feces of healthy children (n = 3). The two control strains have been isolated from cases of asymptomatic bacteriuria. The P-fimbriate strains were of serogroups O2 (n = 7), O4 (n = 15), or O6 (n = 15) (See Table 2). *E. coli* strain KS71 (serogroup O4:K12) and its fimbriae, as well as the type 1 fimbriate strain 2131, have been described previously (12, 24, 28). For the extraction of P fimbriae, the strains were grown on colonization factor antigen agar (4) as described before (13).

Agglutination tests. Although the agglutination reactions of the strains were known from previous work (33), they were always tested before performing immunoprecipitations. Bacterial agglutination with yeast cells or human OP_1 and $O\bar{p}$ erythrocytes was performed as described previously (10).

Purification of fimbriae and preparation of fimbrial extracts. KS71ABC, KS71A, KS71B, KS71C (type 1C), and KS71D (type 1) fimbriae were those described previously (12, 25, 28). Also, P fimbriae were purified from two test strains, IH11024 (O6:K^-) and IH11902 (O2:K^-), by using deoxycholate and concentrated urea (11).

Fimbrial extracts were obtained as described recently (13). Briefly, bacterial growth from 10 to 20 petri dishes was suspended in 5 to 10 ml of phosphate-buffered saline, pH 7.1, and treated for 2 min in an ice bath with an Ultra-Turrax homogenizer (Janke & Kunkel KG, Breisgau, Federal Republic of Germany). The cells were removed by centrifugation, and sodium deoxycholate (E. Merck AG, Darmstadt, Federal Republic of Germany) was added to the suspension to a concentration of 0.1% (wt/vol). The suspension was kept

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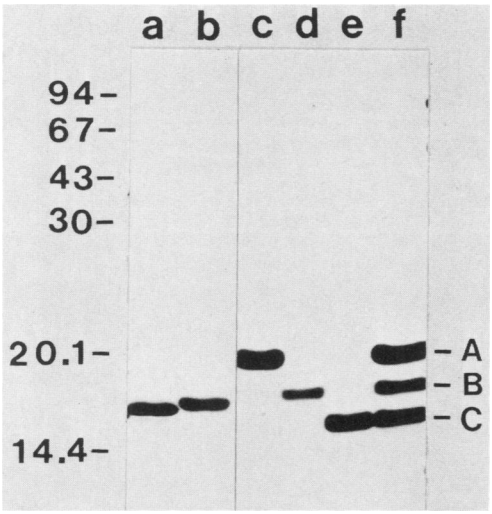

FIG. 1. SDS-PAGE analysis of fimbriae used for antiserum production. IH11024 (lane a) and IH11902 (lane b) fimbriae are P specific. KS71A (lane c), KS71B (lane d), and KS71C (a type 1C fimbria, lane e) were purified from recombinant strains that had received the corresponding structural genes. Lane f shows the total fimbrial fraction (KS71ABC) from agar-grown cells of KS71. KS71A and KS71B are P fimbriae and serologically cross-reactive. The serotypes of the strains are as follows: KS71, O4:K12; IH11024, O6:K$^-$; IH11902, O2:K$^-$. The positions of KS71A, KS71B, and KS71C fimbrillins are indicated on the right and those of standard proteins (in kilodaltons) are given on the left.

at 4°C overnight, centrifuged for 10 min (10,000 × *g*, 4°C), and diluted with deoxycholate–phosphate-buffered saline to give a protein concentration of 200 μg/ml. The extracts were stored at 4°C.

Protein determination. Protein was estimated by a modified Lowry procedure (16), with bovine serum albumin as a standard.

SDS-PAGE. Polyacrylamide gel electrophoresis in sodium dodecyl sulfate (SDS-PAGE) was performed in slab gels (gel concentration, 15%; 1 mm thick) by the system of Laemmli (15). Peptide bands were stained with Coomassie brilliant blue R250. A low-molecular-weight electrophoresis calibration kit (Pharmacia Fine Chemicals, Uppsala, Sweden) was used as a standard. For denaturation of the type 1 fimbriae, the fimbrial preparations were boiled at low pH (17) before the SDS treatment.

Immunological methods. Rabbit antisera to P fimbriae purified from IH11024 and IH11902 were produced in rabbits as described previously (11). Antisera against the KS71ABC, KS71A, KS71B, and KS71C fimbriae and against the type 1 fimbriae of *E. coli* 2131 were available from previous work (12, 24). An enzyme-linked immunosorbent assay (ELISA; 3) was performed essentially as described before (12).

Immunoprecipitation (13, 23, 28, 31) was performed by adding 25 μl of an antiserum to 1 ml of the fimbrial extracts in centrifuge tubes (Eppendorf, Hamburg, Federal Republic of Germany). The suspensions were kept at 37°C for 3 h and then overnight at 4°C. Immunoprecipitates were pelleted (7 min, 4°C) in a Hemifuge (Heraeus Christ, Oesterboede, Federal Republic of Germany), washed three times with phosphate-buffered saline, and suspended in 50 μl of the SDS-PAGE solubilization mixture. In each case, 25 μl of the solution was analyzed by SDS-PAGE. With each strain and antiserum the precipitation was done at least twice and in many cases, thrice. We always used the same fimbrial extract in precipitation assays with the various antisera; in most cases, however, at least two extracts were analyzed. Occasionally, serial precipitations with different antisera (28) were applied to confirm the identity or nonidentity of fimbrial antigens.

RESULTS

Characterization of the fimbriae used in immunization. SDS-PAGE analysis of the purified fimbrial antigens used in immunization is shown in Fig. 1. The KS71ABC fimbriae (lane f) denote the total fimbrial fraction of *E. coli* KS71 grown on agar plates (12). Purity of the individual KS71A (apparent molecular weight, 22,000), KS71B (19,500), and KS71C (17,300) fimbriae, which were isolated from recombinant strains that had received the structural genes for only one of the antigens (25), is shown in lanes c to e.

Antisera were produced also against the P fimbriae purified from strains IH11024 (17,500; Fig. 1, lane a) and IH11902 (18,500; Fig. 1, lane b; see also Table 2). These strains were chosen for antibody production because the fimbrial preparations (Fig. 1) or extracts (see lane o in Fig. 3) showed only one peptide which could not be fractionated in serial precipitation tests with any of the anti-non-type 1 fimbria antisera used in this study (not shown). This suggested that the two strains had only one type of P fimbria. However, the IH11902 fimbriae gave rise to significant amount of anti-type 1 fimbria antibodies (see below), and the strain showed a weak, and variable, agglutination of yeast cells (see Table 2). It was concluded that the 18,000-dalton (18K) peptide in the IH11902 preparation actually was comprised of a type 1 and a P fimbria. This was confirmed by more intense staining of the 18K peptide in SDS-PAGE analysis after denaturation of the preparation at low pH (17).

The serological cross-reactivity of the purified fimbriae was tested by ELISA (Table 1). In accordance with our previous results (25, 28), KS71A and KS71B fimbriae were highly cross-reactive (level of cross-reactivity, 4 to 40% of the homologous reactions in ELISA), whereas KS71C (type 1C) fimbriae cross-reacted with KS71D (type 1) fimbriae only (to 0.16% of the homologous reaction). The IH11024 fimbriae cross-reacted (2.5 to 25%) with KS71A and KS71B (both P) fimbriae but not with the IH11902 fimbriae, which showed a strong cross-reaction (5 to 12.6%) with KS71D (type 1) fimbriae only. This indicated that the P fimbriae of IH11902 were serologically different from those of KS71 and IH11024.

Fimbrial extracts. We prepared fimbrial extracts from 37 *E. coli* strains, including the two nonfimbriate strains used as controls. The extracts from the O4 and O6 strains are shown

TABLE 1. Cross-reactions of *E. coli* fimbriae

Antiserum to *E. coli* fimbriae	Antibody titer to fimbriae[a]						
	KS71ABC	KS71A	KS71B	KS71C	KS71D	IH11024	IH11902
KS71ABC	4.5	4.4	4.4	4.1	<2.0	3.1	<2.0
KS71A	3.9	3.9	3.5	<2.0	<2.0	2.4	2.0
KS71B	3.9	2.4	3.8	<2.0	<2.0	3.5	<2.0
KS71C	3.7	<2.0	<2.0	3.9	<2.0	<2.0	<2.0
Type 1	<2.0	<2.0	<2.0	2.0	4.8	<2.0	3.9
IH11024	2.9	2.8	3.8	<2.0	<2.0	4.4	<2.0
IH11902	2.7	2.3	<2.0	<2.0	3.7	<2.0	5.0

[a] Antibody titer, as tested by the ELISA, is given as the logarithm of the reciprocal of the highest dilution of the antiserum giving an A_{405} twice that given by the preimmune serum.

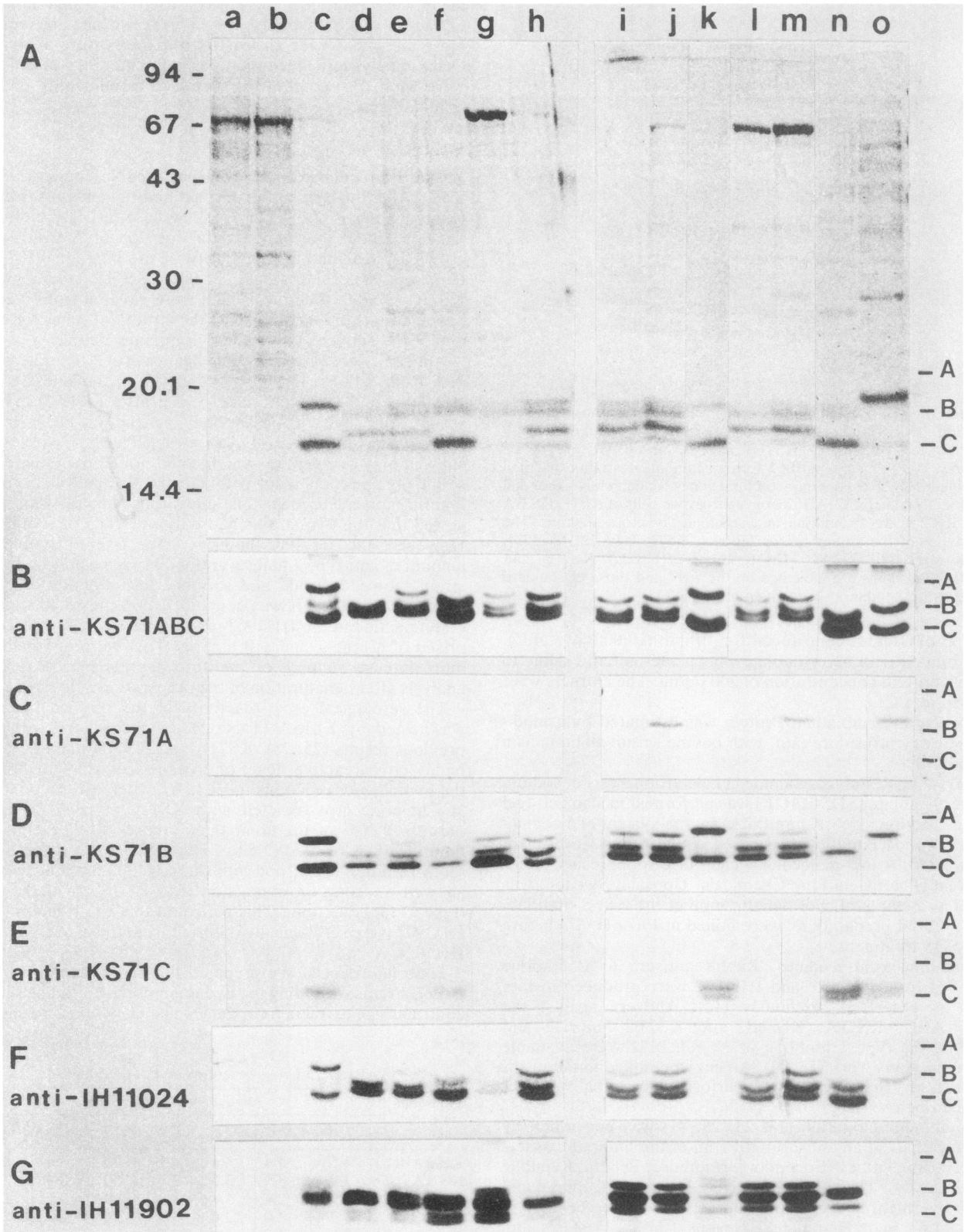

FIG. 2. SDS-PAGE analyses. (A) SDS-PAGE of fimbrial extracts from O4 strains. Two nonfimbriate strains were included as controls (lanes a and b). (B to G). SDS-PAGE analysis of immunoprecipitates obtained from the fimbrial extracts with anti-KS71ABC (B), anti-KS71A (C), anti-KS71B (D), anti-KS71C (E), anti-IH11024 (F), and anti-IH11902 (G) fimbria sera. The strains in lanes c to o are in the same order as in Table 2. The positions of the KS71A, KS71B, and KS71C fimbrillins are indicated on the right and those of the standard proteins (in kilodaltons) are given on the left.

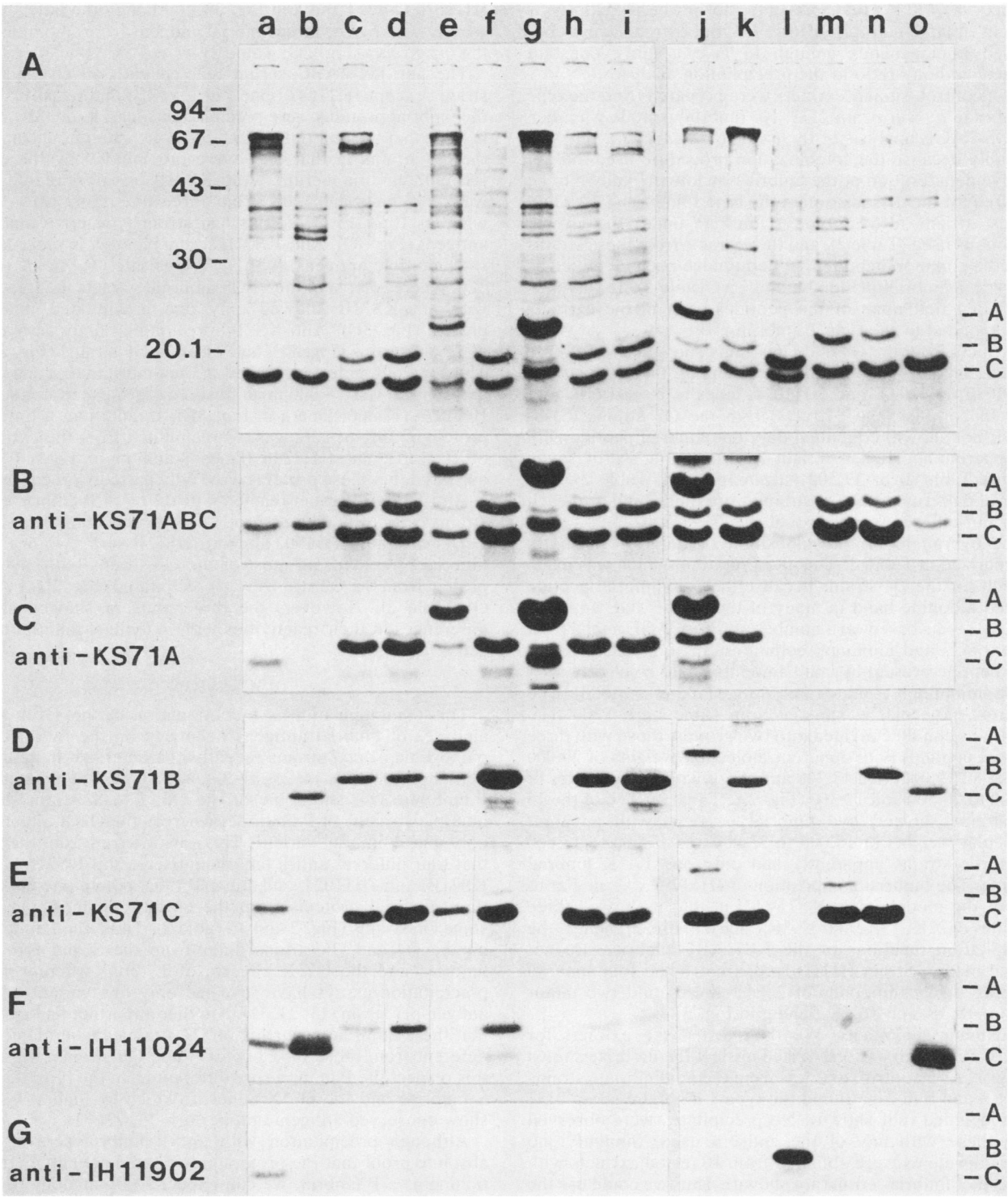

FIG. 3. SDS-PAGE analyses. (A) SDS-PAGE of fimbrial extracts from O6 strains. (B to G) SDS-PAGE analysis of immunoprecipitates obtained from the fimbrial extracts with anti-KS71ABC (B), anti-KS71A (C), anti-KS71B (D), anti-KS71C (E), anti-IH11024 (F), and anti-IH11902 (G) fimbria sera. The strains in lanes a to o are in the same order as in Table 2. The positions of the KS71A, KS71B, and KS71C fimbrillins are indicated on the right and those of standard proteins (in kilodaltons) are given on the left.

in Fig. 2A and 3A. Most extracts showed multiple peptide bands with apparent molecular weights of 17,000 to 22,000. These peptides were considered to be fimbrillins because they were lacking in the extracts from the two nonfimbriate strains (lanes a and b in Fig. 2) and could be precipitated by the anti-fimbria sera used (see below). Fimbrial peptides were the dominant proteins in the extracts, which contained only small amounts of outer membrane proteins. In many

extracts, a 70K peptide, presumed to be the flagellin, was present in large amounts. However, the extracts were considered homogeneous enough to give a fairly constant antigen/antibody ratio in the precipitation analyses.

As a control, similar extracts were prepared from the type 1 fimbriate *E. coli* strain 2131. No fimbrial peptide was seen in SDS-PAGE analysis of the extract (not shown); this was probably because the solubilization procedure used did not involve denaturation of the fimbriae by low pH known to be required for the dissociation of the type 1 fimbriae (17). Only 11 test strains formed type 1 fimbriae under the growth conditions used (Table 2), and the extracts from these strains lacked a common peptide that could have been identified as the type 1 fimbrillin (Fig. 2A and 3A; Table 2). It was thus concluded that none of the peptides seen in the extracts corresponded to the type 1 fimbrillin.

Extracts from the O4 and O6 strains showed two to three peptide bands (Fig. 2A and 3A) excepting three O6 strains (IH11070, IH11022, and IH11024; lanes a, b, and o in Fig. 3A). In contrast, the extracts from the O2 strains (SDS-PAGE not shown) contained only one fimbrial peptide with an apparent molecular weight of 18,500 (in six of seven strains; Table 2) or 17,500 (strain IH11918; Table 2). The fimbrial patterns of the O4 strains were quite similar to each other but differed from those of the O6 strains.

The overall fimbrial composition of the test strains is summarized in Table 2. The interpretation, which was made difficult for the O4 strains because the 20K fimbrial peptide formed a double band in many of the SDS-PAGE analyses (Fig. 2A), was based on a number of SDS-PAGE analyses of the extracts and immunoprecipitates. In some cases a fimbrial peptide present in small amounts could be clearly seen in the immunoprecipitates only (e.g., lane e in Fig. 3). The apparent molecular weights given in Table 2 are ±200. The O4 strains could be divided into two groups: those with three fimbrial peptides with apparent molecular weights of 20,000 to 20,500, 19,000, and 17,300 and those with two peptides of 19,000 to 20,500 and 17,300 (Fig. 2A; Table 2). Ten of the 15 O6 strains showed two fimbrial peptides with apparent molecular weights of 18,000 to 22,000 and 17,300 to 18,000 and three strains apparently had only one 17.5K fimbrial peptide. The fimbrial composition of IH11097 was similar to that of the model strain KS71 (12) in that it showed three peptides of 22K, 19K, and 17.3K (lane e in Fig. 3); hence, the precipitation reactions of the KS71ABC fimbriae are not shown below. Strain IH11361 apparently had four fimbrial proteins, two major ones of 22K and 18K and two minor ones of 18.3K and 16.5K (lane g in Fig. 3).

Immunoprecipitations. We then tested the extracts for precipitation with antisera raised against the fimbriae shown in Fig. 1. As a control, extracts from the nonfimbriate strains (lanes a and b in Fig. 2) and the type 1 fimbriate strain 2131 were included (not shown). No precipitates were observed from these with any of the antisera used. Similarly, no fimbrial proteins were obtained from 10 tested extracts with anti-type 1 fimbria serum (not shown). Thus we could use the anti-IH11902 fimbria serum in the analysis of P fimbriae, although it contained a considerable amount of anti-type 1 fimbria antibodies (Table 1).

The most striking difference in the immunoprecipitation reactions of the strains was that the fimbriae from the O2 strains were precipitated by the anti-IH11902 serum only (Table 2). This is in accordance with the lack of cross-reaction between the IH11902 and the other non-type 1 fimbriae observed in the ELISA (Table 1). However, the anti-IH11902 serum precipitated a fimbria from IH11547 and IH11070 (lanes l and a in Fig. 3G), although the latter quite weakly, and two or three fimbrial antigens from all but one of the O4 strains (Fig. 2G; Table 2).

The anti-KS71ABC serum reacted with all O4 and O6 strains except IH11547 (lane l in Fig. 3B) and usually all of the fimbrial proteins were precipitated (Fig. 2B and 3B). It is notable that the fimbriae of IH11547 were exceptional among the O6 strains in that they were precipitated by the anti-IH11902 fimbria serum. Anti-KS71A serum precipitated weakly a fimbria of 20K from three 04 strains (Fig. 2C), whereas 10 of 15 O6 strains had strongly reactive fimbrial antigens (Fig. 3C). Anti-KS71B serum reacted, in most cases with multiple antigens, with all P-fimbriate O4 strains (Fig. 2D), whereas most of the 11 O6 fimbrial extracts that reacted with anti-KS71B showed only one precipitated fimbrial protein (Fig. 3D). Anti-KS71C precipitated fimbriae from 5 of 15 O4 strains (Fig. 2E) and 11 of 15 O6 strains (Fig. 3E). The apparent molecular weight of the fimbrillin reacting with anti-KS71C was 17,300 in all positive cases except for strain IH11070 (17,500; lane a in Fig. 3E). In addition, a fimbrial protein of 19K to 22K was coprecipitated from the extracts of IH11402 and IH11238 (lanes j and m in Fig. 3E). It appeared that these proteins were only partially precipitated.

Anti-IH11024 serum reacted with 12 of 15 P-fimbriate O4 strains (Fig. 2F) and with 7 of 15 O6 strains (Fig. 3F; Table 2). As with anti-IH11902 and anti-KS71B sera, two or three fimbrial proteins from most of the O4 strains but only one protein from the O6 strains was precipitated (Fig. 2D, F, and G; Table 2). However, the three antisera showed slight differences in their reactivities with individual fimbrial antigens.

DISCUSSION

This communication is a continuation of the serological analyses of fimbrial antigens occurring on the two sets of pathogenic *E. coli* strains recently characterized in detail in our laboratory (13, 14, 23, 31–33). We found that most of the P-fimbriate O4, and some of the O6, *E. coli* strains have multiple variants of P fimbriae, whereas the O2 strains have only one P-fimbrial variant. This was inferred from the fact that four different anti-P fimbria antisera, anti-KS71A, anti-KS71B, anti-IH11024, and anti-IH11902, precipitated two or three fimbrial proteins from the extracts of most O4 and some O6 strains (Fig. 2 and 3; Table 2). Thus, the P fimbriae on the O4 and O6 strains differ from the S and type 1C fimbriae and the O75X adhesin of *E. coli*, where similar precipitation assays have revealed only one variant of the antigen per strain (13, 23, 31). Another important finding was that the P fimbriae occurring on O2 strains are serologically different from those on *E. coli* O4 and O6 strains. Also, in this respect the P fimbriae are different from the type 1C and S fimbriae and the O75X adhesin, which in similar assays show conserved antigenic properties (13, 23, 31).

Although precipitation with anti-P fimbria sera is not absolute proof that the protein precipitated actually is functioning as a P fimbria, we can expect so by analogy to our model strain, *E. coli* KS71. Agar-grown cells of KS71 have three different fimbriae, namely, KS71A, KS71B, and KS71C (12; Fig. 1), which have been separately cloned into HB101 (25). Recombinants that received either the KS71A or the KS71B fimbrial genes hemagglutinate in a P-specific manner (25), which indicates that they both function as P fimbriae. The KS71A and KS71B fimbriae cross-react strongly and are coprecipitated by a number of anti-P fimbria sera (12, 24, 25, 28; Table 1). A similar fimbrial content has been found on *E. coli* strain AD110 (serotype O6), the model

TABLE 2. Properties of *E. coli* fimbriae

Strain	Serotype	Source[a]	Clonal group[b]	Apparent mol wt (10^3) of the fimbrial peptides on agar-grown cells	Apparent mol wt (10^3) of the fimbriae precipitated by antiserum to fimbriae						Possession of type 1 fimbriae	
					KS71ABC	KS71A	KS71B	KS71C	IH11024	IH11902	In liquid culture[c]	On CFA agar[d]
IH11003	O2	PN		18.5						18.5	+	−
IH11156	O2:K$^-$	PN		18.5						18.5	+	−
IH11264	O2:K1	PN		18.5						18.5	+	+
IH11314	O2:K1	PN		18.5						18.5	+	−
IH11902	O2:K$^-$	F		18.5						18.5	+	+
IH11918	O2:K$^-$	F		17.5						17.5	+	−
IH11953	O2:K1	F		18.5						18.5	+	+
IH11130	O4:K$^-$	ABU		20.5; 19; 17.3	20.5; 19; 17.3		20.5; 19; 17.3	17.3	20.5; 17.3	(20.5); 19; (17.3)	+	−
IH11208	O4:K$^-$	C		20; 19; 17.3	20; 19		19; 17.3		19; 17.3	19; (17.3)	−	−
IH11015	O4:K12:H1	PN	III	20; 19; 17.3	20; 19; 17.3		20; 19; 17.3		20; 19; 17.3	(20); 19; (17.3)	+	+
IH11046	O4:K12:H5	PN	IV	20; (19)[e]; 17.3	19; 17.3		19; 17.3	17.3	19; 17.3	19; 17.3	+	−
IH11051	O4:K12:H1	PN	III	20; (19); 17.3	20; 19; 17.3		20; 19; 17.3		19; 17.3	20; 19; 17.3	+	−
IH11086	O4:K12:H1	PN	III	20; 19; 17.3	20; 19; 17.3		20; 19; 17.3		20; 19; 17.3	(20); 19; (17.3)	+	−
IH11125	O4:K12:	PN		20; 19; 17.3	20; 19; 17.3		20; 19; 17.3		20; 19; 17.3	20; 19; 17.3	+	−
IH11248	O4:K12:H5	PN	IV	20; 19; 17.3	20; 19; 17.3	20	20; 19; 17.3		20; 19; 17.3	20; 19; 17.3	+	−
IH11249	O4:H$^-$	PN		20.5; (19); 17.3	20.5; 17.3		20.5; 17.3	17.3	(20.5); (17.3)	20.5; 19; 17.3	+	−
IH11292	O4:K$^-$	PN		20; 19; 17.3	20; 19; 17.3		20; 19; 17.3		20; 19; 17.3	20; 19; 17.3	+	−
IH11167	O4:K12:H1	PN		20; 19; 17.3	20; 19; 17.3	20	20; 19; 17.3		20; 19; 17.3	20; 19; 17.3	+	−
IH11306	O4:K12:H5	PN	IV	20; (19); 17.3	19; 17.3		17.3	17.3	19; 17.3	19; 17.3	+	−
IH11420	O4:K$^-$	PN		20; 17.3	20; 17.3	20	20	17.3	20		+	−
IH11070	O6:K1	C		17.5	17.5	17.5		17.5	17.5	17.5	+	+
IH11022	O6:K53:H1	PN		17.5	17.5		17.5		17.5		+	+
IH11019	O6:K2:H1	PN	V	19; 17.3	19; 17.3	19; (17.3)	19	17.3	19		+	−
IH11020	O6:K2:H1	PN	V	19; 17.3	19; 17.3	19; (17.3)	19	17.3	19		+	−
IH11097	O6:K2:H1	PN	V	22; 19; 17.3	22; 19; 17.3	22; 19	22; 19	17.3			+	−
IH11267	O6:K2:H1	PN	V	19; 17.3	19; 17.3	19; (17.3)	19; (17.3)	17.3	19; (17.3)		+	−
IH11361	O6:K2:H1	PN	V	22; (18.3); 18; (16.5)	22; (18.3); 18; (16.5)	22; (18.3); 18; (16.5)					+	+
IH11416	O6:K2:H1	PN	V	19; 17.3	19; 17.3	19; (17.3)	19; (17.3)	17.3			+	−
IH11431	O6:K2:H1	PN	V	19; 17.3	19; 17.3	19; (17.3)	19; (17.3)	17.3			+	−
IH11402	O6:K2:H1	PN	V	22; 19; 17.3	22; 19; 17.3	22; 19; 17.3	22; 19	22; 17.3			+	+
IH11154	O6:K$^-$	PN		19; 17.3	19; 17.3	19; (17.3)	19	17.3	19		+	−
IH11547	O6:H31	PN		18; 17.3						18	+	+
IH11238	O6:K5:H$^-$	PN		20; 17.3	20; 17.3			20; 17.3	(20)		+	−
IH11280	O6:K5:H1	PN		20; 17.3	20; 17.3		20	17.3			+	+
IH11024	O6	UTI		17.5	17.5		17.5		17.5		+	+

[a] ABU, Asymptomatic bacteriuria; C, cystitis; PN, pyelonephritis; F, feces; UTI, unclassified urinary tract infection (33).
[b] Clonal groups have been constructed on the basis of serotype, fimbriation, outer membrane protein pattern, and hemolytic activity (33).
[c] Taken from reference 33.
[d] CFA, Colonization factor antigen.
[e] Minor fimbrial peptide in the extract or partial precipitation.

strain for the F7 antigens (34), and recent genetic studies have shown that AD110 also has two types of mannose-resistant (probably P) fimbriae designated $F7_1$ and $F7_2$ (27). Our results suggest that the multiplicity of P-fimbrial antigens on a particular *E. coli* strain is not restricted to KS71 and AD110. This conclusion is supported by recent hybridization results showing that many uropathogenic *E. coli* strains contain multiple copies of the genes needed for the synthesis of P fimbriae (5). However, the genetic studies did not reveal whether the P-fimbrial genes encode antigens that are chemically different and whether some of the gene clusters are silent.

We have previously shown that the P, type 1, type 1C, and S fimbriae and the O75X adhesin of *E. coli* lack, or share only a very low, serological cross-reactivity (12, 13, 23, 24, 31). This is confirmed for the P, type 1, and type 1C fimbriae in this study (Fig. 2 and 3; Table 1). The extracts from strains IH11402 and IH11238 (lanes j and m in Fig. 3) showed a P-fimbrial antigen that was partially precipitated by the anti-KS71C serum. We have previously observed similar weak cross-reactions, which are limited, however, to a few P fimbriae (23, 24).

The anti-KS71A and anti-KS71B antisera were produced against fimbriae purified from recombinant strains having the structural genes for the KS71A or KS71B fimbria only (25). Hence, the multiplicity of fimbrial proteins precipitated by these antisera (Fig. 2 and 3; Table 2) most probably resulted from serological cross-reactivity of a number of P-fimbrial antigens with a single P-fimbrial immunogen. Similarly, multiple fimbrial proteins were precipitated by anti-IH11902 and anti-IH11024 sera (Fig. 2 and 3; Table 2), which were raised against fimbrial preparations showing one peptide band only (Fig. 1). The anti-IH11902 fimbria serum contained antibodies against type 1 fimbriae of *E. coli* (Table 1) but otherwise we have not been able to fractionate the IH11902 or IH11024 fimbrial preparation with any of the anti-fimbrial sera we have at our disposal. This strongly suggests, although it does not necessarily prove, that these strains have only one variant of P fimbria.

Brinton et al. (1) have described asymmetrical serological cross-reactions between fimbriae of *Neisseria gonorrhoeae*. In the present study, a similar asymmetry was observed in the serological properties of P fimbriae of O2 strains. They (including IH11902 fimbriae) were not precipitated by the anti-KS71 (serogroup O4) or anti-IH11024 (O6) fimbria sera (Table 2), but the anti-IH11902 fimbria serum effectively precipitated P fimbriae of O4 strains (Fig. 2G). It thus seems that the P fimbriae differ in their abilities to induce broadly cross-reacting antibodies. It is also apparent that the two P fimbriae of strain KS71 (KS71A and KS71B) differ in their immunogenic properties, as shown by differing precipitations seen with anti-KS71A and anti-KS71B sera (Fig. 2 and 3). Anti-KS71A serum reacted with a number of the O6 strains but was barely able to recognize fimbriae in any of the O4 strains, which is surprising since KS71 is of serotype O4. It appears that in its fimbrial composition KS71 is more related to the O6 than to the O4 strains (cf. lane f in Fig. 1 and Fig. 2A and 3A).

The overall fimbrial composition and the serological properties of the fimbriae were related to the serotype of the strains (Table 2), but within a serotype, especially O6, differing fimbrial patterns could be found (Fig. 2A and 3A). We have previously assigned some of the O4 and O6 strains to certain clonal groups consisting of phenotypically similar strains (Table 2). The grouping has been done on the basis of serotype, outer membrane protein pattern, fimbriation, and hemolytic activity. Our hypothesis was that the patterns of fimbrial antigens would be similar, or identical, within a clonal group. This, however, does not seem to be the case. In the O4 strains, a similar three-protein fimbrial pattern was found on strains belonging to clone III (IH11015 and IH11051) or IV (IH11248) as well as in two of four O4:K^- strains (IH11208 and IH11292; Table 2). The fimbrial composition of the three strains in clone IV was not identical. Similarly, the fimbrial patterns differed among the O6 strains belonging to clone V (Table 2).

For the study of fimbrial serology, the precipitation assay used here is an alternative approach to the crossed-immunoelectrophoresis assay (21) and Western blotting (23, 28). Many fimbria-derived spurs were observed by Ørskov and Ørskov (21) for *E. coli* O4 and O6 strains by crossed-immunoelectrophoresis, and it can be expected that they correspond to the multiple peptides in the immunoprecipitates seen in this study. Since Ørskov and Ørskov did not detail the binding properties of their strains, i.e., whether the strains had P, S, M, or X fimbriae or adhesins, an exact comparison of the results is difficult. It is, however, obvious that the distribution of the KS71A and $F7_1$ fimbriae, which correspond to the same antigen (27), is similar; i.e., they were found on O4 and O6 but not on O2 strains by both methods. The advantage of the immunoprecipitation method over crossed-immunoelectrophoresis is that the apparent molecular weight of the antigens can be obtained and that the method can be used for preparative purposes (28). We have previously shown that Western blotting and immunoprecipitation give similar results in the analysis of *E. coli* fimbriae (23, 28). Immunoprecipitation has the advantage that it is done with intact fimbrial filaments and allows serological analysis under nondenatured conditions.

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