

ANTIADHESIVE PROPERTIES OF A QUATERNARY
STRUCTURE-SPECIFIC HYBRIDOMA ANTIBODY AGAINST
TYPE 1 FIMBRIAE OF *ESCHERICHIA COLI**

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Type 1 fimbriae (or pili) on the surfaces of various *Enterobacteriaceae* have been shown to mediate the attachment of these microorganisms to mannose-containing receptors on eucaryotic cells (1, 2). Purified type 1 fimbriae agglutinate guinea pig erythrocytes and mannose-containing yeast cells and bind to several other eucaryotic cell types (3–5). Binding of type 1 fimbriae to each of these cell types is inhibited by the addition of various derivatives of D-mannose (3–5) or by the addition of a mannose-specific lectin, such as concanavalin A (6). Immune sera directed against the fimbriae have been shown to prevent attachment of *Escherichia coli* to mammalian cells in vitro (3, 7, 8) and to prevent colonization and infection in vivo in an experimental rat model of *E. coli*-induced pyelonephritis (7, 8).

Brinton (9) has shown that type 1 fimbriae are composed of 17,000–mol wt subunits assembled into a right-handed helix 7 nm in diameter. Each turn of the helix consists of 3 1/8 subunits. The quaternary structure is highly resistant to disruption but can be dissociated into its subunits by being boiled in acid (10) or treated with saturated solutions of guanidine-HCl (11). The dissociated subunits have been shown to reassemble in vitro in the presence of MgCl₂ into fimbrial structures (11). In order to study the relationship between fimbrial structure and biological function, we have produced a set of monoclonal antibodies directed at specific structural conformations of type 1 fimbriae. In this paper we use these monoclonal antibodies to show that at least one antibody is quaternary structure-specific, recognizing only higher polymers (≥6 subunits), whereas others are subunit-specific and are directed toward determinants “buried” in the quaternary structural conformation of fully assembled fimbriae. We show that dissociation of the fimbriae into its subunits exposes the inaccessible determinants but eradicates the quaternary structural determinant(s). Conversely, reassembly of the subunits in vitro restores the quaternary structural epitope but masks the subunit epitopes. We show that the quaternary structure-specific monoclonal

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antibody inhibits the adhesive properties of the fimbriae in vitro, whereas the subunit-specific antibodies lack antiadhesive properties.

Materials and Methods

Bacterial Strain and Culture Conditions. *E. coli* strain CSH50 is a Cold Spring Harbor K-12-derived strain of *E. coli* (kindly provided by Dr. Barry I. Eisenstein, University of Texas, San Antonio). The bacterial cells were cultured in brain-heart infusion broth (BHI, BBL Microbiology Systems, Becton, Dickinson & Co., Cockeysville, MD) under static conditions at 37°C for 48 h.

Purification of Type 1 Fimbriae. CSH50 fimbriae were purified according to the method of Dodd and Eisenstein (10). Briefly, bacteria were washed with 0.5% NaCl and resuspended in 5 mM Tris buffer, pH 7.8. Fimbriae were removed by mechanical agitation at high speed in an Osterizer blender with five 2-min bursts. The defimbriated cells were sedimented at 27,000 *g* for 30 min, and the supernatant was then subjected to ultracentrifugation at 227,000 *g* for 2 h. The pellet of semi-pure fimbriae was resuspended in a small volume of 5 M urea, 5 mM Tris, pH 7.0, and incubated for several hours at 37°C. The urea buffer mixture was subsequently diluted to 1 M by addition of an appropriate volume of Tris buffer. This suspension was layered on top of an equal volume of 1 M sucrose-5 mM Tris, and pure fimbriae were obtained by centrifuging for 16 h at 200,000 *g*. The purity of the fimbrial preparation was verified by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE)¹ and by electron microscopy.

Fimbrial Dissociation and Reassembly. Fimbrial dissociation was achieved by incubating fimbriae in saturated (about 8.6 M) guanidine hydrochloride at 37°C for 5 h (11). Any undissociated fimbriae were removed by filtration through an Amicon filter (YM 30). To remove guanidine HCl, fimbriae were dialyzed against Tris-HCl buffer, pH 8, containing 10 mM ethylene diamine tetraacetic acid (EDTA), at 4°C for 18 h. The fimbrial subunits were prevented from reassembling by the presence of EDTA. Reassembly of fimbriae was carried out by dialysis against Tris buffer, pH 8, containing 10 mM MgCl₂ for 18 h at 20°C (11). Partial dissociation of fimbriae to fimbrial fragments was obtained by incubating fimbriae in 5.6 M guanidine HCl for 2 h followed by dialysis against Tris HCl buffer, pH 8, containing 10 mM EDTA for 18 h at 4°C.

Immunization of Mice. Purified fimbriae (50 µg) were suspended in 0.1 ml 0.02 M phosphate/0.15 M NaCl, pH 7.4 (PBS), emulsified with an equal volume Freund's complete adjuvant, and injected subcutaneously into female BALB/c mice (15 g). The same dose emulsified in Freund's incomplete adjuvant was repeated 2 wk later. 1 mo later, mice were injected intravenously with 50 µg of fimbriae in PBS via the tail vein, and the animals were sacrificed 3 d later.

Production of Hybridoma Antibodies. The protocol followed was similar to that described previously (12). Briefly, 1.5 × 10⁷ nonsecreting myeloma cells, SP2/0-Ag14 (SP2 cells), were combined with 8 × 10⁷ spleen cells obtained from immunized animals. The cells were fused using polyethylene glycol, suspended in complete growth medium containing 0.8% methylcellulose, hypoxanthine, aminopterin, and thymidine, and seeded into 30-mm tissue culture dishes. The cultures were incubated in a humidified atmosphere of 5% CO₂ and 95% air at 37°C until clones developed to an appropriate size. Clones were picked up and transplanted into 96-well plates containing growth medium with hypoxanthine and thymidine, and 4 × 10⁵ normal splenocytes were added as feeder cells. Culture medium was assayed within 1 wk. Antibody-producing cells were expanded for production of ascites fluid, recloning, or freezing. All hybridomas described in this report have been cloned a minimum of two times.

Large quantities of ascites fluid were obtained by injecting 5 × 10⁶ antibody-producing cells into the peritoneal cavities of pristane-primed BALB/c mice. Ascites fluids were collected and clarified by centrifugation, and 0.02% sodium azide was added to inhibit

¹ Abbreviations used in this paper: BSA, bovine serum albumin; EDTA, ethylene diamine tetraacetic acid; ELISA, enzyme-linked immunosorbent assay; PBS, 0.02 M phosphate/0.15 M NaCl, pH 7.4; SDS-PAGE, sodium dodecyl sulphate-polyacrylamide gel electrophoresis.

contamination. The IgG isotype of each hybridoma antibody was determined by double immunodiffusion in agar gel against mouse IgG subclass-specific antisera (Miles Laboratories Inc., Elkhart, IN).

Assays of Antibody Reactivity. Detection of antibody-secreting hybridomas and titration of ascites fluids were performed using an enzyme-linked immunosorbent assay (ELISA) of the culture medium supernatants or ascites fluids. The assay was performed as previously described (13) except that purified fimbriae (5 $\mu\text{g}/\text{ml}$) were adsorbed to ELISA trays as the solid phase antigen. In order to determine which antibodies were able to recognize fimbrial immunodeterminants in their native state on the surface of *E. coli*, ELISAs were performed as described previously (12) using whole stationary phase *E. coli* as particle antigens.

ELISA inhibition experiments were performed by making serial, twofold dilutions of inhibitor in 0.5 ml PBS to which was added 0.5 ml of a constant dilution of ascites fluid. The mixture was incubated at 37°C for 30 min and then added to the antigen-coated trays. Following this incubation, standard ELISA procedures were carried out.

SDS-PAGE. SDS-PAGE was performed in 1.5-mm thick 12% slab gels by the system of Laemmli (14). Samples of fimbriae were dissociated before electrophoresis by being heated in acid (10) or treated with saturated guanidine HCl (11). In some cases, samples were applied to the gels without dissociation. Molecular weight standards were obtained from Bio-Rad Laboratories (Richmond, CA).

Immunoelectroblots of Fimbriae. After SDS-PAGE, the bands of fimbrial protein were electrophoretically transferred onto nitro-cellulose paper using an electroblot apparatus (E-C Apparatus Corp., St. Petersburg, FL) at 5 W for 3 h in 24 mM Tris/192 mM glycine/20% methanol, pH 8.3 (15). After transfer, the nitro-cellulose paper strips were placed in 1% bovine serum albumin (BSA) in PBS for 1 h and then incubated in appropriate dilutions of ascites fluid in 1% BSA buffer with 0.05% Tween 20 for 3 h at 20°C. The strips were washed extensively with 0.9% NaCl-0.05% Tween 20 and then treated with [¹²⁵I]labeled goat anti-mouse IgG (specific activity 103.9 $\mu\text{Ci}/\text{ml}$; New England Nuclear, Boston, MA) diluted in BSA buffer with 0.05% Tween 20 to 200,000 cpm/ml. The strips were incubated for 2 h at 37°C, washed extensively, and then air-dried and exposed to XAR film (Eastman Kodak, Rochester, NY) for 8 h at 20°C.

Electron Microscopy. Samples of bacteria or fimbriae were applied to Formvar-coated copper grids and negatively stained with 0.5% phosphotungstic acid (pH 4.0). For immunoelectronmicroscopy, bacteria- or fimbriae-coated grids were placed on a drop of ascites fluid diluted 1:10,000 for 20 min and then washed several times with deionized water. The grids were finally stained with phosphotungstic acid. Electron micrographs were made using either an AEI EM 6B, or Philips EM 201 electron microscope. When necessary, micrograph magnifications were determined using a grating replica standard. Stereo pairs were made by tilting the specimen $\pm 6^\circ$ between micrographs.

Bacterial Adherence to Human Buccal Epithelial Cells. The adhesion assays were performed as previously described (2). Test mixtures consisted of 10^5 epithelial cells mixed with an equal volume of 10^8 *E. coli* CSH50 suspended in ascites fluid at its lowest nonagglutinating titer. Ascites fluids that did not agglutinate the *E. coli* cells were used at a fixed titer of 1:200. The mixture was rotated at ambient temperature for 30 min, and unattached bacteria were removed by differential centrifugation. The number of adherent bacteria was determined microscopically on stained smears of the epithelial cells.

Assays for Inhibition of E. coli-induced Guinea Pig Erythrocyte and Yeast Cell Agglutination. The number of bacterial cells to be added to the test system was initially determined by titrating the bacteria for erythrocyte and yeast cell agglutinating activity. The lowest concentration of bacteria that produced a strong agglutination reaction was used for the inhibition test. Twofold dilutions (25 μl) of each ascites fluid in PBS were combined with an equal volume of 0.5% guinea pig erythrocytes or 1% yeast cell suspension. The mixtures of bacteria, ascites fluid, and guinea pig or yeast cells were incubated at 37°C for 60 min. The agglutination inhibition titers are expressed as the reciprocal of that dilution of ascites fluid which completely inhibited yeast cell or guinea pig erythrocyte agglutination. In some cases, purified fimbriae were substituted for intact *E. coli* cells in

the inhibition assays.

Direct Antibody-induced Bacterial Agglutination. Small drops of bacterial suspension were mixed with appropriate dilutions of ascites fluid on glass slides. Agglutination was assessed visually over a 1-min period.

Results

BALB/c mice were hyperimmunized with purified preparations of type 1 fimbriae isolated from *E. coli* CSH50. Cells from the immune mouse spleens were fused with nonsecreting SP2 myeloma cells 3 d after the final intravenous booster injection. The fused cells were then plated in methylcellulose and thus the initial colonies that developed were already cloned. The supernatants of clones picked and inoculated into wells of microtiter plates were screened by ELISA using purified fimbriae as antigen. Antibody-producing cultures were recloned in methylcellulose medium, expanded by culture in vitro and injected into the peritoneal cavities of pristane-primed BALB/c mice. Three different hybridoma clones were selected for the following studies.

IgG Class, ELISA Titers, Agglutinating and Immunoprecipitation Activities of Hybridoma Antibodies. Two of the antibodies (CD3 and AA8) were of the IgG1 isotype and one (GG1) was of the IgG 2a isotype. ELISA titers against type 1 fimbriae isolated from *E. coli* CSH50 were 409,600 for CD3 and GG1, and 819,200 for AA8 (Table I). Only CD3 antibodies agglutinated fimbriated *E. coli* CSH50 or precipitated the isolated CSH50 fimbriae in agar gel immunodiffusion tests (Table I and Fig. 1). These results suggested that the antibody produced by CD3 is directed against a repeating antigenic determinant exposed on intact fimbriae, whereas antibodies AA8 and GG1 appear to be directed against determinants that are inaccessible on the surface of intact fimbriated *E. coli*. Moreover, the determinant recognized by CD3 has limited distribution among type 1 fimbriated strains, since it reacted with only one of nine clinical isolates of *E. coli* bearing type 1 fimbriae.

Electron Microscopy of the Reaction of Monoclonal Antibodies with Native Fimbriae on Intact Cells of E. coli. To visually examine the interaction of CD3 antibody with intact fimbriae, whole cells of *E. coli* CSH50 were attached to Formvar-coated grids, incubated with each of the monoclonal antibodies, and stained with phosphotungstic acid. Electron microscopic examination showed that CD3 anti-

TABLE I
Immunological Properties of Hybridoma Antibodies Against Type 1 Fimbriae

Hybridoma antibodies	IgG class	Agglutination of fimbriated <i>E. coli</i>	Immunoprecipitation of fimbriae*	ELISA titers against isolated fimbriae [‡]
CD3	1	+	+	409,600
AA8	1	—	—	819,200
GG1	2a	—	—	409,600
IIG1 (Control) [§]	1	—	—	<200

* Immunoprecipitation tests were performed by double diffusion in agar gel.

[‡] ELISA were performed using purified fimbriae (5 µg/ml) to coat the antigen wells.

[§] IIG1, ascites fluid containing monoclonal antibodies against an unrelated antigen (human fibronectin).

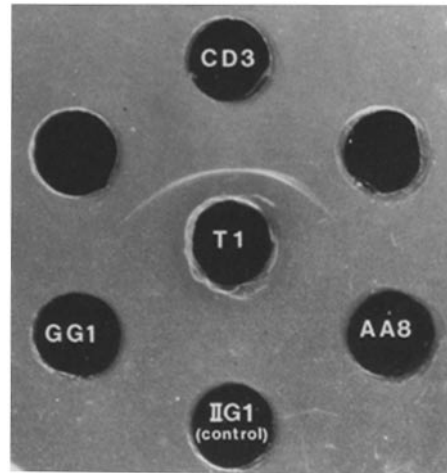


FIGURE 1. Immunodiffusion reactions of purified type 1 fimbriae (*T1*) with different ascites fluids from clones *CD3*, *AA8*, and *GG1* containing antibodies raised against type 1 fimbriae. An ascites fluid (*IG1*) with monoclonal antibodies against an unrelated antigen (human fibronectin) was added as a control.

body bound in a highly discrete and periodic manner (spacing $\sim 25.3 \pm 4.5$ nm; $n = 100$) along the length of each of the fimbriae (Fig. 2, *A* and *B*). In numerous instances the antibodies appeared to decorate the fimbriae in a spiral pattern along the fimbria. This is best illustrated by stereo pairs of electronmicrographs (Fig. 3). In contrast, hybridoma antibodies *AA8* and *GG1* were not detected either on the surface of the fimbriae or on the organisms themselves (Fig. 2, *C*). The periodic binding of *CD3* is consistent with the immunoprecipitating properties of this antibody. The lack of reactivity of *AA8* and *GG1* antibodies is consistent with a masked location for the fimbrial determinant recognized by these monoclonal antibodies.

Inhibition of the Adhesion of Type 1 Fimbriae and Cells of E. coli to Eucaryotic Cells by Monoclonal Antibody. To determine whether the hybridoma antibodies would inhibit the interaction of type 1 fimbriae with eucaryotic cells, the effects of the three antibodies on the adhesion of *E. coli* to oral epithelial cells and on *E. coli*- or type 1 fimbriae-induced agglutination of guinea pig erythrocytes or yeast cells was investigated. As can be seen in Table II, *CD3*, but not *AA8* or *GG1*, antibodies prevented the interaction of *E. coli* and type 1 fimbriae with the eucaryotic cells. These results indicate that the fimbriae-reactive monoclonal antibody produced by clone *CD3* interferes with the adhesive properties of type 1 fimbriae and are consistent with its immunoprecipitating and *E. coli* agglutinating properties.

Reaction of Monoclonal Antibodies with Native, Fragmented, Dissociated, or Reassembled Fimbriae. Since clones *AA8* and *GG1* produced antibodies that interacted with isolated fimbriae but failed to agglutinate type 1 fimbriated *E. coli* or to inhibit the adhesive properties of these organelles, we examined the accessibility in various type 1 fimbriae preparations of the antigenic determinants against which each of these antibodies were directed in greater detail. In the first

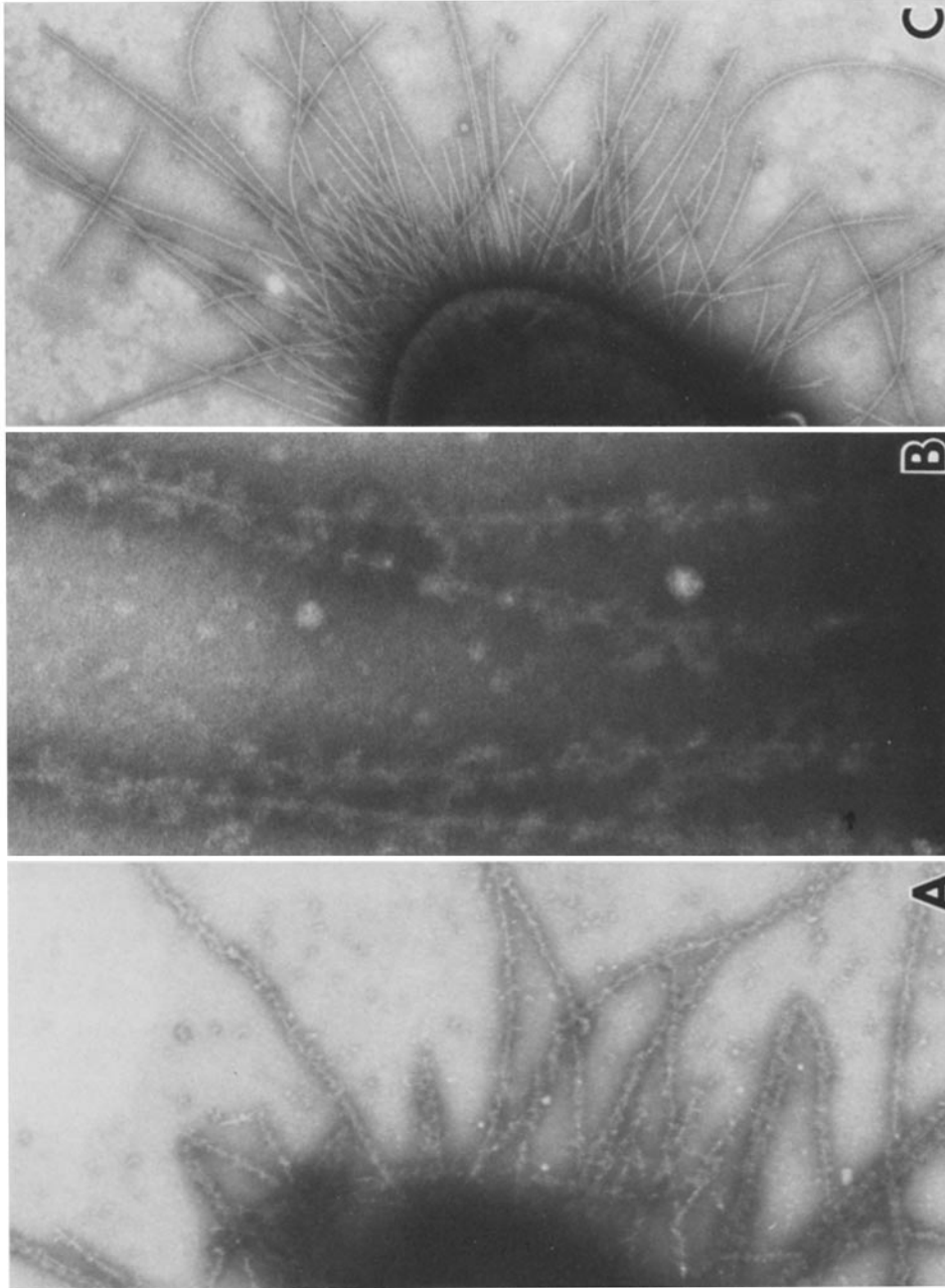


FIGURE 2. Electron micrographs of negatively stained *E. coli* CSH50: (A) type 1 fimbriae; (C) *E. coli* CSH50 cells incubated with 1:100 dilution of AA8 after incubation in a 1:10,000 dilution of CD3 ascites fluid ($\times 45,000$); (B) ascites fluid ($\times 48,000$). Results similar to that obtained with AA8 were obtained with GG1 and with control ascites fluid IIG1.

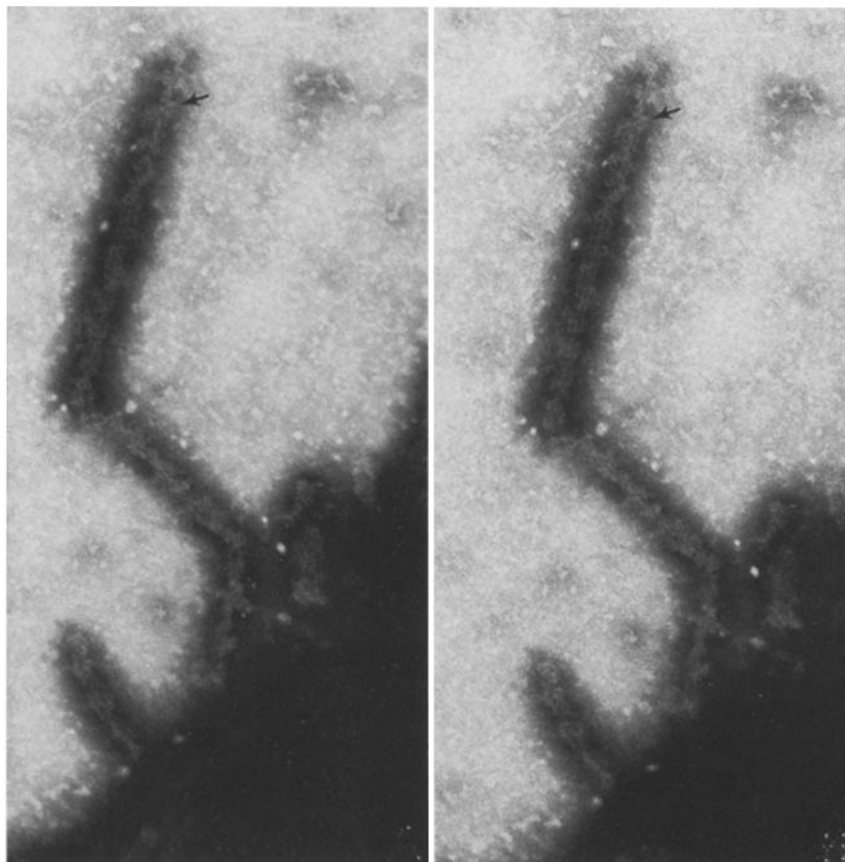


FIGURE 3. Stereo pair of electron photomicrographs demonstrating the spiral binding of monoclonal antibody CD3 (arrow) with type 1 fimbriae of *E. coli*. $\times 90,000$.

experiment, we utilized whole *E. coli* with intact fimbriae as the solid phase antigen in the ELISA. The ELISA titer of antibody CD3 was 12,800, whereas the titers of antibodies AA8 and GG1 were <800 (Table III). This was in contrast to the interaction with isolated fimbriae in which the titers of all three antibodies was high. This suggested the possibility that the partial fragmentation that occurs during isolation of the fimbriae may have exposed previously inaccessible antigenic determinants. Therefore, in the second experiment, purified fimbriae were further fragmented by partial dissociation with 5.6 M guanidine HCl (11) and then used as the ELISA antigen. The titers of all three antibodies, especially AA8 and GG1, were much higher against the more highly fragmented fimbriae than against the original preparation of isolated fimbriae (Table III).

In the third experiment, the fimbriae were completely dissociated with saturated guanidine HCl and electrophoresed in SDS gels. Nitrocellulose electroblots of the SDS-gels were then treated with CD3, AA8, or GG1 ascites fluids followed by radiolabeled goat anti-mouse IgG. As can be seen in Fig. 4, transblots of gels purposely overloaded in order that the various polymers of the 17,000-dalton subunit could be visualized, show that both AA8 and GG1 antibodies recognized

TABLE II
*Inhibition of the Attachment of E. coli and Isolated Type 1 Fimbriae to Epithelial Cells, Erythrocytes, and Yeast Cells by Hybridoma Antibody CD3**

Hybridoma antibody added to test mixture of eucaryotic cells and:	Attachment of <i>E. coli</i> or fimbriae to:		
	Epithelial cells (% of control)	Erythrocytes (agglutination)	Yeast cells (agglutination)
<i>E. coli</i>			
None	100	+	+
CD3	10	0	0
AA8	100	+	+
GG1	100	+	+
Type 1 fimbriae			
CD3	ND	0	0
AA8	ND	+	+
GG1	ND	+	+

* Subagglutinating concentrations of hybridoma antibodies were added to the test mixtures of *E. coli* cells (10^8 /ml) or purified type 1 fimbriae (50 μ g/ml) and human oral epithelial cells (10^5 /ml), guinea pig erythrocytes (0.5% suspension), or yeast cells (1.0% suspension).

TABLE III
ELISA Titers of Hybridoma Antibodies Against Type 1 Fimbriae

Ascites fluid	ELISA titers against:		
	Intact fimbriae on <i>E. coli</i> cells	Isolated fimbriae*	Fragmented fimbriae [†]
CD3	12,800	409,600	3,276,800
AA8	<800	819,200	6,553,600
GG1	<800	409,600	6,553,600
IIG1 (Control) [§]	<800	<200	<200

* Data from Table I are shown for comparison.

[†] Isolated fimbriae were partially dissociated (fragmented) by treatment with 5.6 M guanidine HCl at pH 8.0 for 2 h at 37°C. The fragmented fimbriae were then dialyzed against 5.0 mM EDTA in 5.0 mM Tris HCl at pH 8.0 and then adsorbed to microtiter plates for ELISA.

[§] See footnote, Table I.

bands in the region consistent with the fimbrial monomer (mol wt 17,000). CD3 antibody, however, reacted with polymer \geq mol wt 102,000 and did not react with the band of mol wt 17,000. Fig. 4 also shows the result of a similar experiment in which the gels were loaded with less protein in order to show that the molecular weight of the proteins with which GG1 (and AA8, data not shown) reacts is 17,000 mol wt. The observation that CD3 antibody recognized only polymers of mol wt 102,000 or greater was consistent with the fimbriae immunoprecipitating and *E. coli* agglutinating properties of the antibody.

In the fourth experiment, the comparative abilities of dissociated and reassembled fimbriae to inhibit the reaction of CD3 antibody with isolated fimbriae or the reactions of AA8 and GG1 antibodies with dissociated fimbrial subunits were studied in the ELISA. Dissociated fimbriae (Fig. 5, B) had no effect on the binding of CD3 antibody to isolated fimbriae (Fig. 6). In contrast, reassembled fimbriae (Fig. 5, C) regained inhibitory activity equal to that of the original

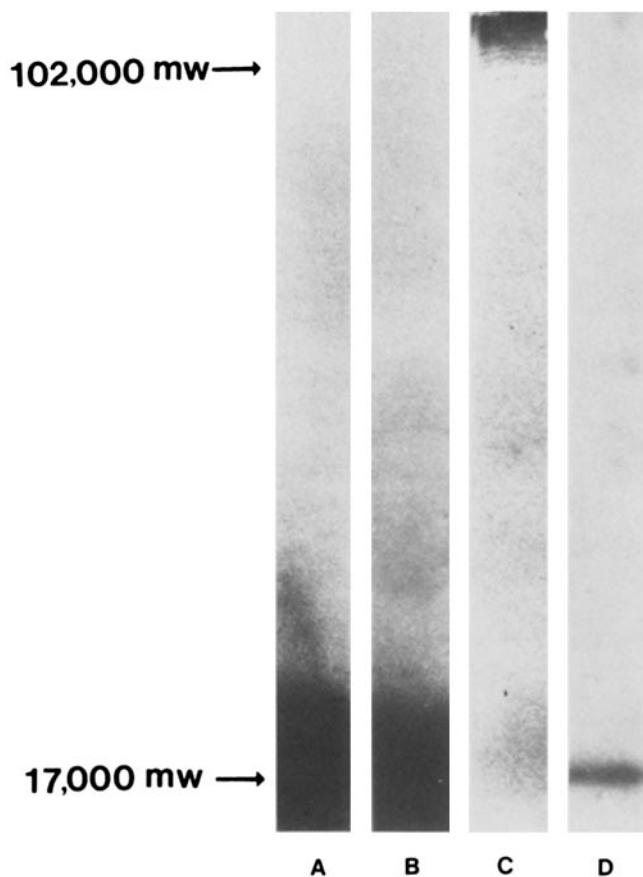


FIGURE 4. Immunoelectroblots of type 1 fimbrial preparations incubated with different ascites fluids. Lane A, GG1; lane B, AA8; lane C, CD3; lane D, GG1. Lanes A-C were heavily overloaded with fimbrial protein so that fimbrial fragments other than the monomer could be detected.

isolated fimbrial preparation (Fig. 6, A and C). Furthermore, CD3 antibodies bound to the reassembled fimbriae in a periodic pattern (Fig. 5, D). Conversely, the dissociated fimbrial subunits (Fig. 7, B) but not the reassembled fimbriae (Fig. 7, A and C) inhibited the binding of GG1 and AA8 antibodies to dissociated fimbrial subunits. Dissociation of the reassembled fimbriae once again exposed determinants recognized by the latter antibodies (Fig. 7, D). These results further support the idea that AA8 and GG1 antibodies are directed toward antigenic determinants that are inaccessible in the assembled fimbriae, whereas CD3 antibody recognizes a quaternary structural determinant.

Because of the interference with the mannose-sensitive binding properties of fimbriae by monoclonal antibody CD3, we attempted to inhibit binding of CD3 antibody to type 1 fimbriae with the monosaccharides α -D-mannose and α -D-methyl mannoside. We were unable to block CD3 antibody-induced agglutination of *E. coli* or the binding of the antibody to isolated fimbriae in ELISA with 2.5% solutions of these monosaccharides, suggesting that CD3 antibody is directed

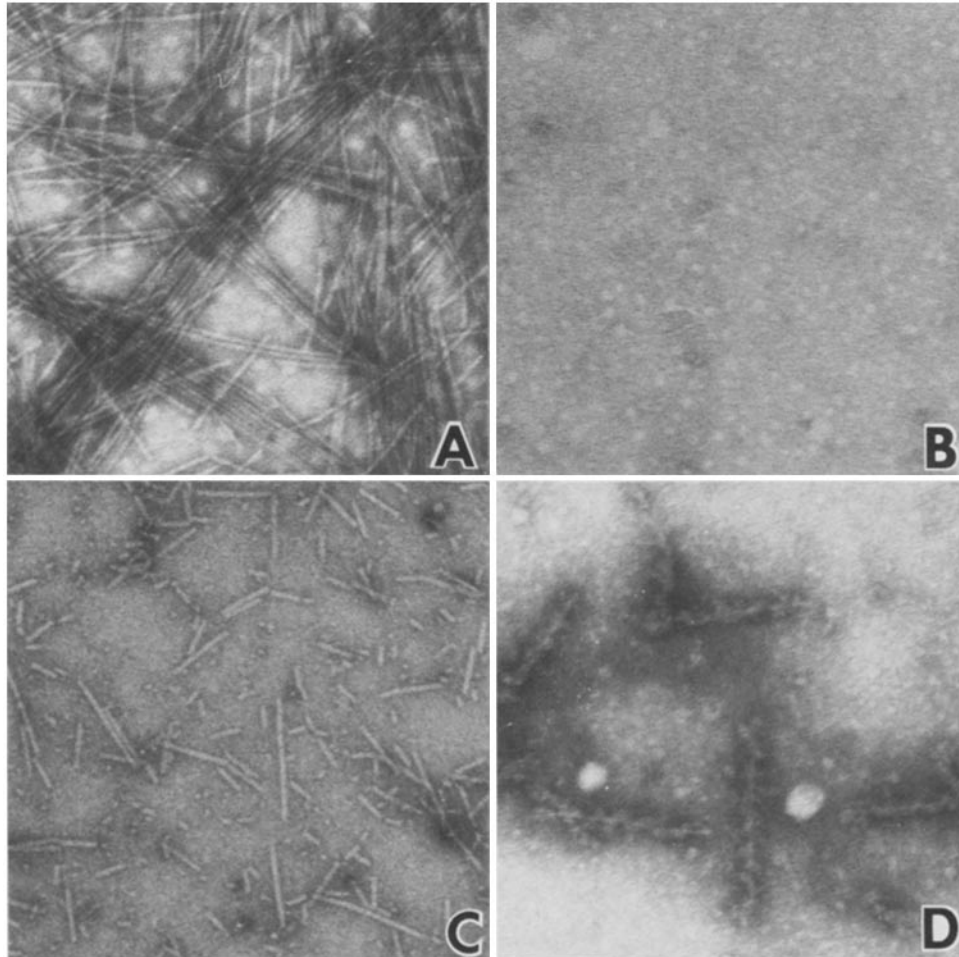


FIGURE 5. Electron micrographs of negatively stained preparations of type I fimbriae: (A) isolated fimbriae; (B) fimbrial monomers; (C) reassembled fimbriae; (D) reassembled fimbriae labeled with CD3. Magnification of each, $\times 72,000$.

against sites other than the mannose-specific binding site.

Discussion

In these studies, we have demonstrated the use of hybridoma technology in studies of structure as it relates to biological function of bacterial surface appendages. Using a set of monoclonal antibodies, we were able to show that at least one of the antigenic determinants of type I fimbriae of *E. coli* resides in a quaternary structural conformation, whereas other determinants reside in fimbrial subunits that become inaccessible for antibody binding in fully assembled fimbriae. Thus, one of the monoclonal antibodies (CD3) reacted only with fully assembled fimbriae, whereas the other two (AA8 and GG1) reacted only with dissociated subunits. When reassembled into fimbrial polymers, the subunits regained reactivity with CD3 antibody but lost reactivity with AA8 and GG1

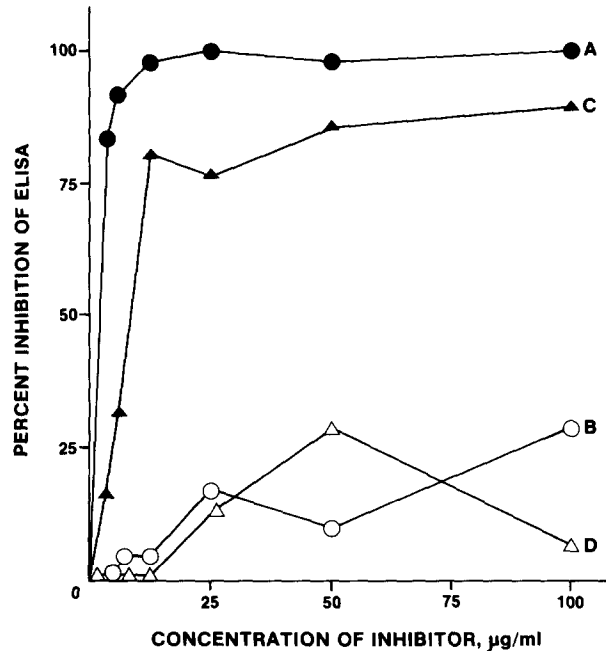


FIGURE 6. ELISA inhibition assays of CD3 with isolated fimbriae using as inhibitor: (A) isolated fimbriae; (B) fimbrial monomers; (C) reassembled fimbriae; and (D) fimbrial monomers obtained after dissociation of reassembled fimbriae.

antibodies. An analogy can be drawn between these findings of the structural constraints of type 1 fimbriae with those reported for PAK fimbriae of *Pseudomonas aeruginosa*. The binding site on these fimbriae for the bacteriophage PO4 is lost when the fimbriae are dissociated into individual subunits (16), indicating that a quaternary structural site is necessary for binding.

Of particular interest was our finding that CD3, but not AA8 or GG1, antibody blocked the adhesive properties of *E. coli* CSH50 and of a clinical isolate of *E. coli* bearing type 1 fimbriae that reacted with the same antibodies. The quaternary structure-specific antibody blocked the adhesion of the organisms to oral epithelial cells, guinea pig erythrocytes, and mannan-containing yeast cells, whereas the subunit-specific antibodies had no effect.

The antiadhesive properties of CD3 antibody suggested the possibility that it might be directed against the mannose-specific binding site. In preliminary studies using 2.5% solutions of the monosaccharides α -D-mannose or α -D-methylmannoside, however, we were unable to block the CD3 antibody-mediated agglutination of *E. coli* or the binding of CD3 antibody to isolated fimbriae in ELISA. Further studies utilizing trimannosides and higher membrane sugars as described by Firon et al. (17) are needed to determine whether or not CD3 antibody binds to any part of the extended sugar binding region (18) of type 1 fimbriae. Indeed, the data showing that CD3 antibody reacts only with a limited number of other type 1 fimbriated *E. coli* suggest that the antibody reacts with another site rather than the mannose-specific binding site itself. Thus, monoclonal antibody CD3 differs from the monoclonal antibodies against type 1

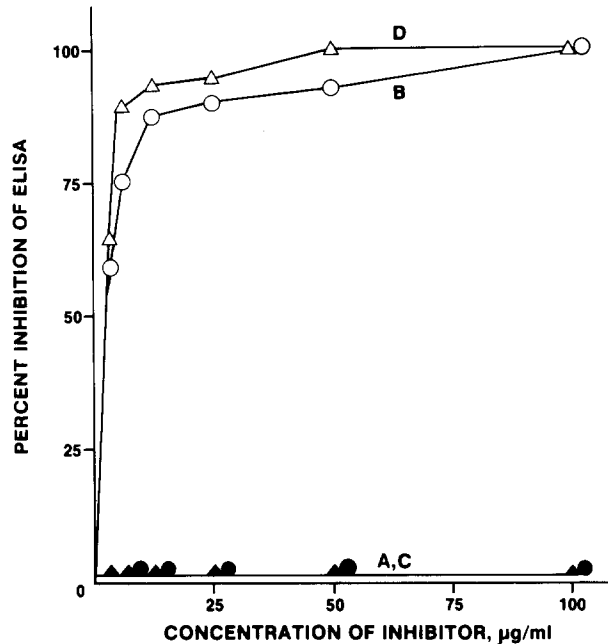


FIGURE 7. ELISA inhibition assays of GG1 with fimbrial monomers using as inhibitor: (A) isolated fimbriae; (B) fimbrial monomers; (C) reassembled fimbriae; and (D) fimbrial monomers obtained after dissociation of reassembled fimbriae.

fimbriae produced by Soderstrom et al. (19), which appeared to exhibit broad cross-reactivity with type 1 fimbriae of various strains of *E. coli*. Our findings further support antigenic heterogeneity among type 1 fimbriae of *E. coli*, and are in agreement with the findings of Fader et al. (20), which showed that type 1 fimbriae of different strains of *Enterobacteriaceae* lacked immunological cross-reactivity even though the amino acid sequence homologies among the different type 1 fimbriae was as high as 79%.

One of the most intriguing findings in the present study was the high degree of periodicity of the binding of monoclonal antibody CD3 along the length of intact and reassembled fimbriae; hybridoma antibodies AA8 and GG1 were not detected either on the surfaces or the tips of the fimbriae, or on the cells of *E. coli* themselves. Type 1 fimbriae have been shown to be composed of 17,000 mol wt subunits arranged in a right-handed helical array of $3\frac{1}{8}$ subunits per turn with a pitch of 2.3 nm (9). Due to this regular pattern of subunit polymerization, a regular pattern of epitope presentation would be predicted that would spiral around the fimbria in three left-handed helices separated by ~ 6.5 nm (Fig. 8). Each epitope in one of these left-handed helices should then be separated by 2.3 nm. Also, due to this highly ordered subunit packing, one would predict that a total of eight turns of the right-handed fimbrial helix would be required to reproduce the spatial presentation of an epitope in any particular longitudinal plane. From these assumptions based upon Brinton's model of fimbrial structure (9), we can calculate that antibodies bound in the lateral plane on either side of the fimbriae (i.e. those that would be most prominent in negatively stained

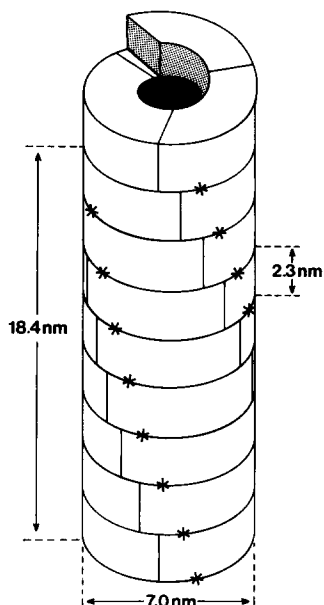


FIGURE 8. Diagrammatic representation of the structure of type 1 fimbriae. The indicated site (*) is a proposed binding region for CD3.

preparations) would repeat at intervals of eight turns, or 18.4 nm. Our actual measurements of the periodicity of antibody binding gave a somewhat higher value of 25.3 ± 5.6 nm. Any inconsistencies in measurements such as these, as well as in the often observed spiral pattern to the binding of CD3 antibodies to fimbriae, are not easily explained by the limited numbers of experiments we have done to date. At least some variation in antibody binding patterns is probably due to the size of antibody molecules relative to fimbrial epitope spacing resulting in steric hindrance sufficient to inhibit antibodies from binding at each possible epitope. Such an interpretation is consistent with the observation that the ELISA titer of CD3 antibody increased upon fragmentation of the fimbriae (see Table III). Nevertheless, our observations are consistent with the helical fimbrial model proposed by Brinton (9) and show the value of quaternary structure-specific monoclonal antibodies in studies of fimbrial structure.

We found that the fimbriae reassembled from subunits *in vitro* not only regained their binding of CD3 antibody, but the binding exhibited the same periodicity as with the original fimbriae. These results indicate that the fimbrial subunits reassemble themselves in a highly ordered fashion *in vitro*. Thus, the harsh treatment required to dissociate the fimbriae failed to irreversibly alter the conformational potential of the subunits. The site of binding of the subunit-specific monoclonal antibodies AA8 and GG1 has not been established. We know, however, that the antigenic sites become inaccessible after reassembly. Whether or not the binding site is an inaccessible site in the axial hole, or another site that is lost due to conformational rearrangement of each subunit as it undergoes assembly, requires further studies of primary, tertiary, and quaternary structural conformations recognized by the host immune system.

Summary

The relationship between the structure and biological function of type 1 fimbriae of *Escherichia coli* was investigated using a set of monoclonal antibodies directed against conformation-specific antigenic determinants. Of three monoclonal antibodies tested, only one (clone CD3) prevented adhesion of the vaccine strain to epithelial cells or guinea pig erythrocytes. The antibody produced by CD3, but not that produced by the other two hybridoma clones (AA8 and GG1), precipitated isolated fimbriae by double diffusion in agar gel and was shown to bind in a highly discrete, periodic manner along the length of each of the fimbriae by immunoelectron microscopy. Immunoelectroblots of type 1 fimbrial subunits and polymers electrophoresed in SDS-gels indicated that the antibodies in AA8 and GG1 reacted only with fimbrial monomers (mol wt 17,000), whereas the antibody in CD3 reacted only with polymers of mol wt 102,000 (hexamers) or higher. ELISA inhibition assays demonstrated that dissociated fimbrial subunits lost their reactivity with antibody CD3 but gained reactivity with antibodies AA8 and GG1. Conversely, when allowed to reassemble *in vitro* in the presence of 5 mM MgCl₂, the reassembled fimbriae lost their reactivity with antibodies AA8 and GG1 but regained reactivity with antibody CD3. These results demonstrated that certain antigenic epitopes are dependent on quaternary structural determinants, whereas others are independent of quaternary fimbrial structure and also are inaccessible for antibody binding in fimbriae once they have been assembled. These monoclonal antibodies should prove useful in studies of the structural determinants of the biological function of type 1 fimbriae as well as in studies of fimbrial synthesis, transport, and assembly.

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References

1. Duguid, J. P., and D. C. Old. 1980. Adhesive properties of Enterobacteriaceae *In* Bacterial Adherence. E. H. Beachey, editor. Chapman and Hall, London. 187-217.
2. Ofek, I., and E. H. Beachey. 1978. Mannose binding and epithelial cell adherence of *Escherichia coli*. *Infect. Immun.* 22:247.
3. Salit, I. E., and E. C. Gotschlich. 1977. Hemagglutination by purified type 1 *Escherichia coli* pili. *J. Exp. Med.* 146:1169.
4. Salit, I. E., and E. C. Gotschlich. 1977. Type 1 *Escherichia coli* pili: characterization of binding to monkey kidney cells. *J. Exp. Med.* 146:1182.
5. Korhonen, T. K. 1979. Yeast cell agglutination by purified enterobacterial pili. *FEMS (Fed. Eur. Microbiol. Soc.) Microbiol. Lett.* 6:421.
6. Ofek, I., E. H. Beachey, and N. Sharon. 1978. Surface sugars of animal cells as determinants of recognition in bacterial adherence. *Trends Biochem. Sci.* 3:159.
7. Silverblatt, F. J., and L. S. Cohen. 1979. Antipili antibodies affords protection against experimental ascending pyelonephritis. *J. Clin. Invest.* 64:333.
8. Silverblatt, F. J., R. Weinstein, and P. Rene. 1982. Protection against experimental pyelonephritis by antibody to pili. *Scand. J. Infect. Dis. Suppl.* 33:79.
9. Brinton, C. C. 1965. The structure, function, synthesis and genetic control of bacterial

- pili and a molecular model for DNA and RNA transport in gram negative bacteria. *Trans. N. Y. Acad. Sci.* 27:1003.
10. Dodd, D. C., and B. I. Eisenstein. 1982. Antigenic quantitation of type 1 fimbriae on the surface of *Escherichia coli* cells by an enzyme-linked immunosorbent inhibition assay. *Infect. Immun.* 38:764.
 11. Eshdat, Y., F. J. Silverblatt, and N. Sharon. 1981. Dissociation and reassembly of *Escherichia coli* type 1 pili. *J. Bacteriol.* 148:308.
 12. Hastly, D. L., E. H. Beachey, W. A. Simpson, and J. B. Dale. 1982. Hybridoma antibodies against protective and nonprotective antigenic determinants of a structurally defined polypeptide fragment of streptococcal M protein. *J. Exp. Med.* 155:1010.
 13. Dale, J. B., I. Ofek, and E. H. Beachey. 1980. Heterogeneity of type-specific and cross-reactive antigenic determinants with a single M protein of group A streptococci. *J. Exp. Med.* 151:1026.
 14. Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T₄. *Nature (Lond.)* 117:680.
 15. Towbin, H., T. Staehelin, and J. Gordon. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and applications. *Proc. Natl. Acad. Sci. USA* 76:4350.
 16. Watts, T. H., D. G. Scraba, and W. Paranchych. 1982. Formation of 9-nm filaments from pilin monomers obtained by octyl-glucoside dissociation of *Pseudomonas aeruginosa* pili. *J. Bacteriol.* 151:1508.
 17. Firon, N., I. Ofek, and N. Sharon. 1982. Interaction of mannose-containing oligosaccharides with the fimbrial lectin of *Escherichia coli*. *Biochem. Biophys. Res. Commun.* 105:1426.
 18. Kabat, E. A. 1978. Dimensions and specificities of recognition sites on lectins and antibodies. *J. Supramol. Struct.* 8:79.
 19. Soderstrom, T., K. Stein, C. C. Brinton, S. Hosea, C. Burch, H. A. Hansson, A. Karpas, R. Schneerson, A. Sutton, W. I. Vann, and L. A. Hanson. 1983. Serological and functional properties of monoclonal antibodies to *Escherichia coli* type 1 pilus and capsular antigens. *Prog. Allergy.* 33:259.
 20. Fader, R. C., L. D. Duffy, C. P. Davies, and A. Kurosky. 1982. Purification and chemical characterization of type 1 pili isolated from *Klebsiella pneumoniae*. *J. Biol. Chem.* 257:3301.