# Fragmentation of *Escherichia coli* Type 1 Fimbriae Exposes Cryptic D-Mannose-Binding Sites

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Cells of the gram-negative bacterium *Escherichia coli* are able to attach to various host cells by means of a mannose-specific adhesin associated with type 1 fimbriae. Here we show that fragmentation of type 1 fimbriae by freezing and thawing results in increased mannose-binding activity as demonstrated by increased hemagglutination, increased stimulation of human lymphocyte proliferation, and increased binding of the mannose-containing enzyme horseradish peroxidase. Increased activity in all three assays was mannose sensitive and was not exhibited by FimH⁻ mutant type 1 fimbriae lacking the adhesin. Scatchard analysis of the data from peroxidase binding assays showed that unfrozen and frozen fimbriae contain binding sites displaying two classes of affinity. Frozen and thawed fimbriae expressed an increase in the number of high-affinity binding sites. These results show that fragmentation of the fimbrial structure exposes cryptic mannose-binding activity associated with type 1 fimbriae, presumably that of internally located adhesin molecules. Our data support earlier observations that adhesin moieties of type 1 fimbriae are located both at the tips and at intervals along the length of the fimbriae. In addition, our data suggest that only the adhesin moieties that are located at the fimbrial tips are functional in binding mannose. Adhesins located along the length of the fimbriae have their mannose-binding activity buried within the fimbrial structure and hence are not functional. We propose an updated model for the structure of type 1 fimbriae that is in agreement with the above observations.

Several members of the family *Enterobactericeae*, including most strains of *Escherichia coli*, express on their surfaces numerous proteinaceous filaments called fimbriae. The fimbriae expressed by *E. coli* can be of one or more types based on the specific mode of recognition or interaction mediated by these organelles in promoting adherence of the bacteria to various host cell surfaces (10, 12, 13, 19). In this respect, among the more common fimbriae expressed by *E. coli* are type 1 fimbriae, which are known to facilitate attachment of the bacteria to mannose-containing receptors exposed on a variety of host cells (4, 17, 18, 22). The mannose-binding activity associated with type 1 fimbriae is commonly demonstrated by specific adhesion assays, including mannose-sensitive agglutination of guinea pig erythrocytes (21). Facilitated adherence of the bacteria to mannose-containing glycoproteins on host cells is believed to be an important prerequisite for successful invasion and colonization in the pathogenesis of several *E. coli* and other enterobacterial infections (2, 3, 7). Recently, we have found that type 1 fimbriae can also stimulate human lymphocyte proliferation, which is mannose sensitive (20).

The structure of type 1 fimbriae was initially described by Brinton as being entirely composed of 17-kDa protein subunits, now called FimA, which are assembled into a right-handed helix 7 nm in diameter and approximately 1 μm in length (5). Abraham et al. later described the existence of two additional minor protein components in type 1 fimbriae with molecular masses of 14 and 29 kDa that were called

FimG and FimH, respectively (1). Recently, Hanson and Brinton reported a third minor protein component, called FimF, which is a 16.5-kDa protein (8). Therefore the currently accepted structure of type 1 fimbriae consists largely of FimA monomers with FimF, FimG, and FimH as minor components. It has also been demonstrated that FimH by itself is the mannose-specific adhesin of type 1 fimbriae (11).

Although it is well established that the mannose-binding activity of type 1 fimbriae is associated with the fimbrial structure, there is to date no conclusive evidence indicating the location of the adhesin moiety on these organelles. Brinton and others have suggested a fimbrial tip location for the adhesin based upon the observation that purified aggregates of type 1 fimbriae attach to erythrocyte membranes in an end-on fashion (5, 8). We have proposed a combination of both tip and integrated locations for the adhesin, based upon data from immunoelectron microscopy performed on purified fimbriae exposed to antibodies made against a synthetic peptide from FimH (1).

In this paper, we report observations that are in agreement with both of the above hypotheses for the location of the adhesin on type 1 fimbriae. We show that freezing and thawing of type 1 fimbriae leads to fragmentation of the fimbrial structure, resulting in an increase in mannose-binding activity as demonstrated by increased hemagglutination and increased stimulation of human lymphocyte proliferation. The increased mannose-binding activity of the fragmented fimbriae is also demonstrated by a novel peroxidase-binding assay. With the peroxidase-binding assay, we have also been able to show through Scatchard analysis that there are two classes of binding affinities present in both unfrozen and frozen fimbrial preparations, with an increase in the number of high-affinity mannose-binding sites in the frozen and thawed preparations. We also propose an updated model of type 1 fimbriae in which adhesin moieties are

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located at the tip and at intervals along the length of the fimbriae but in which only the adhesin moieties that are located at the tips are functional in binding mannose.

## MATERIALS AND METHODS

**Bacterial strains and culture conditions.** *E. coli* ORN103 (pSH2) and *E. coli* ORN103(pUT2002) were the bacterial strains used for the preparation of wild-type and FimH$^-$ mutant type 1 fimbriae, respectively. The only difference between these strains is that the *fimH* gene has been deleted from the pUT2002 strain, thereby making it nonadhesive. The genotypes of these two strains have been described previously (20). Both strains were cultured in brain heart infusion broth under static conditions for 20 h at 37°C.

**Isolation and purification of type 1 fimbriae.** The isolation and purification of type 1 fimbriae were performed by the method of Dodd and Eisenstein (6). The purity of our fimbrial preparations was determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and electron microscopy as described previously (20).

**Freezing and thawing of fimbrial preparations.** Purified preparations of wild-type and FimH$^-$ mutant type 1 fimbriae were divided into 2-ml aliquots and subjected to multiple cycles of freezing and thawing at a concentration of 1 mg/ml. Each cycle consisted of freezing the fimbrial suspension at −20°C for 2 h and then thawing at 37°C for 5 min. All experiments for the detection of increased activity of the frozen fimbriae were initially performed with samples that had been subjected to various numbers of freeze-thaw cycles to study the effects of extensively treating fimbriae in this manner. However, the maximum activities in all of the assays were already achieved with just one cycle of freezing and thawing of the fimbrial preparations (data not shown). Furthermore, the average lengths of fragments in samples subjected to 1 cycle of freezing and thawing were not significantly different from those in samples subjected to 11 cycles. As a result, we have reported our experimental data simply as a comparison of unfrozen and frozen fimbriae.

**Electron microscopy.** For rotary shadow casting, purified preparations of fimbriae were diluted in water and 1 to 3 μl was sandwiched between two 1-cm pieces of freshly cleaved mica. The mica sheets were then separated and placed on the stage of a Blazers apparatus and dried for 15 to 20 min at approximately 10$^{-5}$ torr (ca. 133 × 10$^{-5}$ Pa). The adsorbed fimbriae were shadowed with approximately 2 nm of platinum at an angle of 7° while rotating at 50 to 60 rpm and then coated with carbon at an angle of 90°. Replicas were removed from the mica by floating onto water, and small pieces were picked up on uncoated grids. Electron micrographs were made with a JEOL 1200 EX electron microscope at an initial magnification of ×20,000 and enlarged to ×60,000 for measurements.

**Measurement of fimbrial lengths.** Electron micrographs taken of random fields from each sample were used for the measurement of fimbrial fragment length. To avoid any ambiguity, the measurements were performed only on isolated individual fragments that were not in contact with surrounding fragments. Length measurements were made with Macmeasure 3.04 software (W. Rasband, National Institutes of Health) and a Summagraphics Bit Pad Plus graphics tablet.

**Hemagglutination assay.** Unfrozen and frozen samples of wild-type and FimH$^-$ mutant type 1 fimbriae were diluted to a concentration of 0.5 mg/ml in phosphate-buffered saline (PBS; pH 7.2). Aliquots of each sample (100 μl) were then placed into the first row of wells of a round-bottom 96-well microtiter plate. The samples were then serially diluted twofold, and 25 μl of a 2 to 3% suspension of guinea pig erythrocytes washed in PBS was added to each well and mixed gently. The plates were incubated overnight at 4°C, and the hemagglutination titer was recorded as the highest dilution of fimbriae that caused agglutination of the erythrocytes. Hemagglutination assays were performed in the absence and presence of 10 mg of α-methyl-mannose per ml.

**Human lymphocyte proliferation assay.** Human peripheral blood mononuclear cells that had been depleted of adherent cells were stimulated with frozen and unfrozen samples of wild-type and FimH$^-$ mutant type 1 fimbriae in a lymphocyte proliferation assay as described in detail elsewhere (20). Briefly, 4 × 10$^5$ peripheral blood mononuclear cells were stimulated with purified fimbriae at a concentration of 100 μg/ml in 96-well microtiter plates. The cultures were incubated at 37°C with 5% CO$_2$ for 3 days, and proliferation was measured as incorporation of [$^3$H]thymidine added 18 h before the cells were harvested.

**Peroxidase binding assay.** Samples of unfrozen and frozen preparations of wild-type and FimH$^-$ mutant type 1 fimbriae were diluted in PBS at a concentration of 100 μg/ml. Then 100-μl aliquots of the diluted samples were added to 96-well microtiter plates and incubated overnight at 4°C. The plates were washed three times with PBS, 200 μl of PBS containing 3% bovine serum albumin (PBS-BSA) was added to each well, and the plates were incubated at 37°C for 1 h. The plates were again washed three times with PBS-BSA, and then 100 μl of horseradish peroxidase (type VI-A; Sigma), diluted in PBS at a concentration of 100 μg/ml, was added to each well in the presence and absence of α-methyl-mannose. The plates were incubated overnight at 4°C, washed three times with PBS containing 0.05% Tween-20, and finally developed with the peroxidase substrate *o*-phenylenediamine. After 1 h of incubation at room temperature, the optical density at 450 nm was read with a Biotek EL310 enzyme-linked immunosorbent assay (ELISA) reader. The assay was done in the presence and absence of α-methyl-mannose. In experiments to test for sugar inhibition, 25 μl of a 100-mg/ml solution of α-methyl-mannose, β-methyl-D-galactose, or *N*-acetyl-D-glucosamine in PBS-BSA was added to each well before the peroxidase enzyme was added. A protein microassay (Micro-BCA, Pierce, Rockford, Ill.) indicated that the amounts of fimbriae bound to the plate were 1.0 ± 0.1 μg per well for unfrozen fimbriae and 1.1 ± 0.1 μg per well for frozen fimbriae.

**Binding affinity studies.** Microtiter ELISA plates were coated with frozen and unfrozen type 1 fimbriae and incubated overnight with 0.1 ml of peroxidase in the range of 0.1 to 100 μg/ml in the presence and absence of 10 mg of α-methyl-mannose per ml. The plates were washed and developed with the peroxidase substrate, and the optical density at 450 nm was measured. A standard curve for peroxidase was obtained by titrating peroxidase concentrations in the range of 1 to 1,000 pg/ml. The amount of peroxidase bound to the fimbriae was determined by taking the optical density measurement and applying linear regression to the standard curve. Specific binding was obtained by subtracting the amount of enzyme bound in the presence of mannose from that bound in the absence of mannose. Experiments were performed in duplicate. The data were analyzed by using the computer program LIGAND (16). Data were entered into the program as picograms of peroxidase per well rather than the usual disintegrations per minute per well, substituting the value for picograms of

peroxidase per picomole (40,000) for the specific activity called for by the program.

## RESULTS

**Effect of freezing and thawing on type 1 fimbriae.** The effect of freezing upon the structure of type 1 fimbriae was determined by using electron microscopy after rotary shadow casting was performed on the samples. The fimbrial fragments were shorter in the frozen sample than in the unfrozen sample (Fig. 1). A quantitative estimate of the fragmentation in these samples was obtained by measuring the lengths of the fragments from a series of micrographs for each sample. Figure 2 shows the distribution of fimbrial lengths in both the treated and untreated samples for the wild-type and FimH⁻ mutant type 1 fimbriae. The mean length of the unfrozen wild-type fimbriae was somewhat larger than that of the unfrozen FimH⁻ mutant fimbriae. We do not attribute any significance to this observation, because the average length of fimbrial fragments can vary noticeably from batch to batch within the same strain of *E. coli*. A comparison of the net change between unfrozen and frozen fimbriae from each strain shows that the wild-type fimbriae underwent a greater reduction in mean fragment length than did the FimH⁻ mutant fimbriae. This observation suggests that the presence of FimH within the fimbrial structure may result in weak spots, causing the wild-type fimbriae to be more fragile than the FimH⁻ mutant fimbriae.

The data in Fig. 2 can also be used to determine the relative number of fimbrial fragment tips in each preparation. The number of tips per unit of mass is proportional to the number of fragments per unit of length. Dividing the number of fragments counted by the total length of all those fragments and expressing the result as the number of fragments per 1,000 nm of length gives the following results: unfrozen wild type, 3.5; frozen wild type, 13.4; unfrozen FimH⁻ mutant, 5.0; and frozen FimH⁻ mutant, 9.6. Thus the number of fragments per 1,000 nm of length is increased almost fourfold in the wild type and less than twofold in the FimH⁻ mutant by freezing.

**Hemagglutinating activity of frozen type 1 fimbriae.** Since the frozen fimbrial samples appeared to be extensively fragmented, we wanted to study what effects, if any, the fragmentation had on the mannose-binding activity associated with type 1 fimbriae. Therefore, we tested the agglutination of guinea pig erythrocytes by unfrozen and frozen samples of wild-type and FimH⁻ mutant type 1 fimbriae in the presence and absence of α-methyl-mannose. Unfrozen wild-type fimbriae only exhibited hemagglutination at 0.5 mg/ml, whereas frozen wild-type fimbriae agglutinated the erythrocytes at a 16-fold lower concentration. Hemagglutination by both samples was completely inhibited by the addition of α-methyl-mannose. No hemagglutination was observed with mutant fimbriae at any concentration. Multiple cycles of freezing and thawing did not further increase hemagglutination titers of the wild-type fimbriae. The observation that this increased hemagglutinating activity was totally inhibited by the presence of α-methyl-mannose indicates that FimH plays a role in the increased mannose-binding activity in the frozen samples. Further evidence to support this conclusion is the fact that similarly treated FimH⁻ mutant type 1 fimbriae, which lacked the adhesin moiety but were otherwise identical to wild-type fimbriae and which were also extensively fragmented, did not exhibit any hemagglutinating activity in the absence or presence of α-methyl-mannose.

**Stimulation of human lymphocyte proliferation by frozen type 1 fimbriae.** We previously reported that the adhesin of type 1 fimbriae causes mannose-sensitive stimulation of human B-lymphocyte proliferation at 3 days (20). The type 1 fimbrial samples used in the reported experiments were frozen preparations. In our current studies, based upon the observation that the frozen fimbrial suspension had increased hemagglutinating activity, we were interested in comparing the abilities of unfrozen and frozen fimbriae to stimulate human lymphocyte proliferation. Cultures of human peripheral blood lymphocytes were stimulated with unfrozen and frozen wild-type and FimH⁻ mutant type 1 fimbrial preparations in the presence and absence of α-methyl-mannose. The 3-day proliferative responses of human lymphocytes stimulated in this manner are shown in Fig. 3. The proliferative response of the cultures stimulated with the frozen wild-type fimbrial preparation was indeed significantly higher than the response obtained with unfrozen wild-type fimbriae. Once again, the fact that this increased response was inhibited in the presence of α-methyl-mannose and was not observed with FimH⁻ mutant fimbria-stimulated cultures is an indication of increased mannose-binding activity in the frozen wild-type sample.

**Increased peroxidase-binding activity of frozen type 1 fimbriae.** Both the increased hemagglutination titers and the enhanced mitogenic activities exhibited by the frozen samples of type 1 fimbriae indicated an increased exposure of mannose-binding activity in these fimbrial samples. To obtain a more direct quantitation of the increased mannose-binding activity, we tested the abilities of unfrozen and frozen type 1 fimbrial preparations to bind the highly mannosylated protein horseradish peroxidase. This was achieved by adding the peroxidase enzyme to microtiter plates that had been coated with unfrozen and frozen wild-type and FimH⁻ mutant type 1 fimbria samples and then washing away the unbound enzyme. The amount of bound peroxidase was then measured by standard ELISA techniques. The results of such an experiment done in the absence and presence of α-methyl-mannose are shown in Table 1. The amount of fimbriae bound to the plate for each of the samples had been determined to be equivalent based upon a protein microassay. Peroxidase binding to wells coated with wild-type fimbriae was mannose sensitive and increased greatly after freezing and thawing. We also found that other sugars (β-methyl-D-galactose and *N*-acetyl-D-glucosamine) failed to block the peroxidase-binding activity, whereas concanavalin A, a known mannose-binding protein, did significantly block the binding of peroxidase to the frozen type 1 fimbriae (data not shown).

**Scatchard analysis of mannose-specific adhesins of frozen and unfrozen type 1 fimbriae.** Although the above results indicated that the type 1 fimbrial fragments resulting from freezing and thawing exhibited an increase in mannose-binding activity, they did not distinguish between an increase in affinity versus an increase in the number of binding sites. To resolve this question, we applied Scatchard analysis to the peroxidase binding assay as described in Materials and Methods. The mannose-specific binding to both frozen and unfrozen fimbriae was saturable, and the maximal peroxidase binding increased after fimbriae were frozen (Fig. 4). The binding of horseradish peroxidase to both preparations of fimbriae was determined to be a reversible reaction (data not shown). Figure 5 shows Scatchard plots of the peroxidase binding to unfrozen and frozen samples of wild-type fimbriae. These plots were generated from computer analysis of the data by using the program LIGAND; similar plots
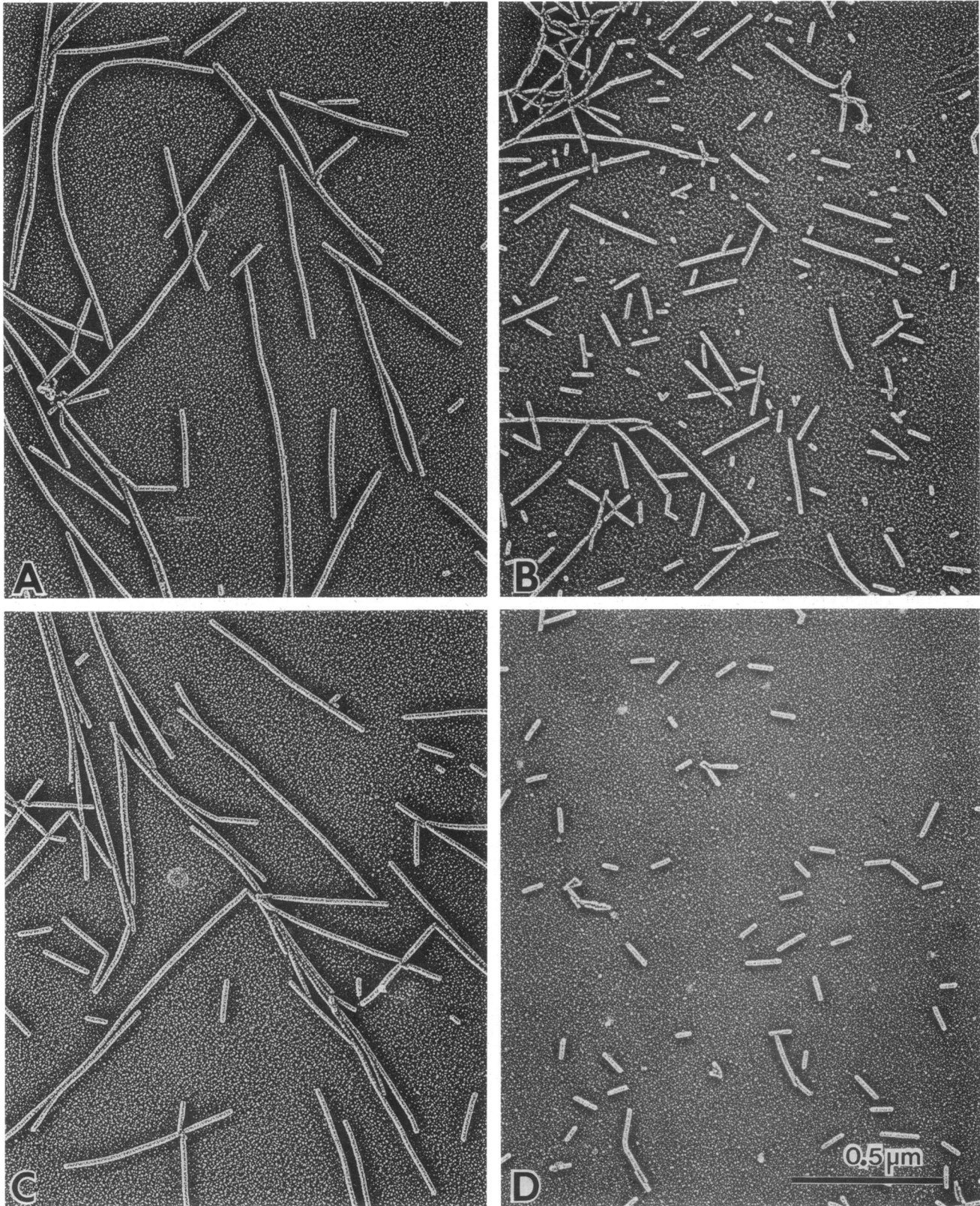
FIG. 1. Electron micrographs of rotary shadowed preparations of unfrozen (A) and frozen (B) wild-type type 1 fimbriae and unfrozen (C) and frozen (D) FimH⁻ mutant type 1 fimbriae.
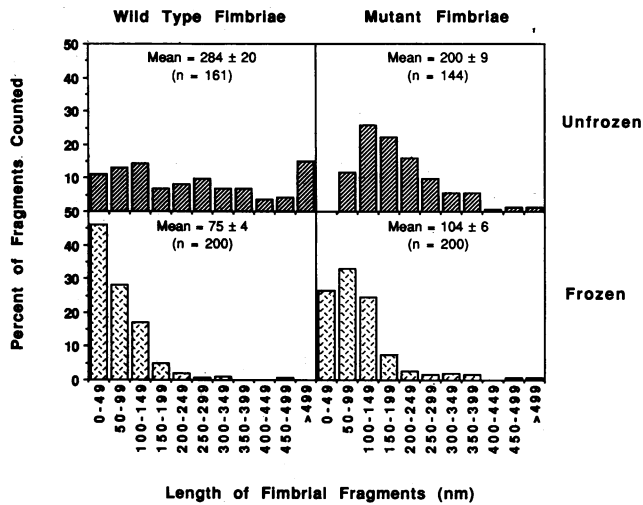
**Length of Fimbrial Fragments (nm)**

FIG. 2. Histogram showing the numbers of fimbriae in the various fimbrial length classes for unfrozen and frozen preparations of wild-type and FimH⁻ mutant type 1 fimbriae. The mean length, standard error, and number of fragments counted are shown for each preparation.

TABLE 1. Peroxidase binding activity of unfrozen and frozen preparations of wild-type and FimH⁻ mutant type 1 fimbriae[a]

| Fimbria sample | α-Methyl-mannose | Optical density (450 nm) | |
| --- | --- | --- | --- |
| | | Unfrozen | Frozen |
| Wild type | − | 0.60 ± 0.03 | 1.33 ± 0.07 |
| Wild type | + | 0.09 ± 0.03 | 0.02 ± 0.01 |
| FimH⁻ mutant | − | 0.05 ± 0.01 | 0.03 ± 0.01 |
| FimH⁻ mutant | + | 0.05 ± 0.02 | 0.03 ± 0.01 |

[a] Plates were coated with samples of unfrozen and frozen fimbrial preparations, blocked with BSA, and incubated overnight with perioxidase in the presence and absence of α-methyl-mannose. The plates were then developed with the substrate, and the optical density was measured at 450 nm. Binding of peroxidase to wells not coated with fimbriae but blocked with PBS-BSA gave an optical density of 0.10 and has been subtracted from the values shown.

were obtained for three separate experiments with two batches of fimbrial preparations. Analysis of the data with LIGAND showed that both unfrozen and frozen samples of fimbriae contained sites displaying two classes of binding affinities (Table 2). The numbers of low-affinity binding sites were nearly equal in the unfrozen and frozen fimbrial samples. In contrast the number of high-affinity binding sites increased by about threefold in the frozen fimbrial preparations. Assuming a molecular mass of 17 kDa for the fimbrial protein, since FimA constitutes >99% of the fimbrial structure, we obtained average ratios of 1 high-affinity peroxidase-binding site per 53,000 fimbrial subunits and 1 peroxidase-binding site of either affinity per 16,000 subunits in frozen preparations. Structural data provided previously by Brinton indicate that fimbrial fragments that are 75 nm in length (as in our frozen wild-type fimbriae) should be composed of approximately 100 subunits each (5). Thus, 1 binding site per 16,000 subunits suggests that our frozen wild-type preparations contained approximately 1 binding site per 160 fragments. The ratio of binding sites to fimbrial
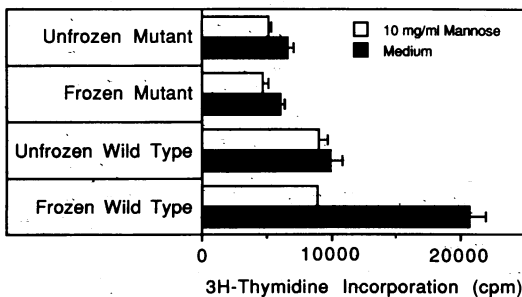
subunits is lower than we might have expected, based on previously reported polyacrylamide gel analysis (8). The low level of active mannose-binding sites suggests that other FimH molecules may exist in a completely inactive form, even after fragmentation, and also emphasizes the potency of the active sites.

## DISCUSSION

In this report, we have investigated the effects of freezing and thawing upon type 1 fimbrial structure and function. We have observed by means of electron microscopy that type 1 fimbriae are extensively fragmented by freezing and thawing. This observation was made for both wild-type and FimH⁻ mutant type 1 fimbriae, which possess and lack the mannose-binding activity, respectively, although the degree of fragmentation was greater for wild-type fimbriae. Three assays were used to show that mannose-binding activity increased as a result of freezing and thawing the wild-type fimbriae. Frozen preparations displayed increased hemagglutinating activity, increased ability to stimulate lymphocyte proliferation, and increased activity in a novel peroxidase-binding assay. In all three types of experiment, the increase in activity was inhibited in the presence of mannose



**3H-Thymidine Incorporation (cpm)**

FIG. 3. Proliferation of human lymphocytes stimulated with preparations of unfrozen and frozen wild-type and FimH⁻ mutant type 1 fimbriae (100 μg/ml) for 3 days in the presence or absence of 10 mg of α-methyl-mannose per ml. The counts of medium control cultures have been subtracted from the values shown. Each bar represents the mean of triplicate cultures ± the standard deviation.
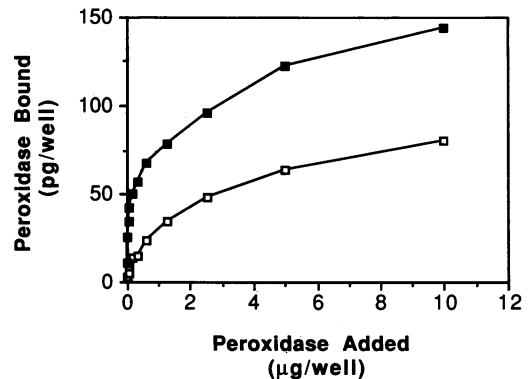


**Peroxidase Added (μg/well)**

FIG. 4. Saturation curve of peroxidase binding by unfrozen and frozen type 1 fimbriae. Microtiter ELISA plates were coated with frozen and unfrozen type 1 fimbriae as described in Materials and Methods. Specific binding was obtained by subtracting the amount bound in the presence of mannose from that in the absence of mannose for unfrozen (□) and frozen (■) samples of wild-type fimbriae. The amount of bound peroxidase was calculated from a standard curve by linear regression.
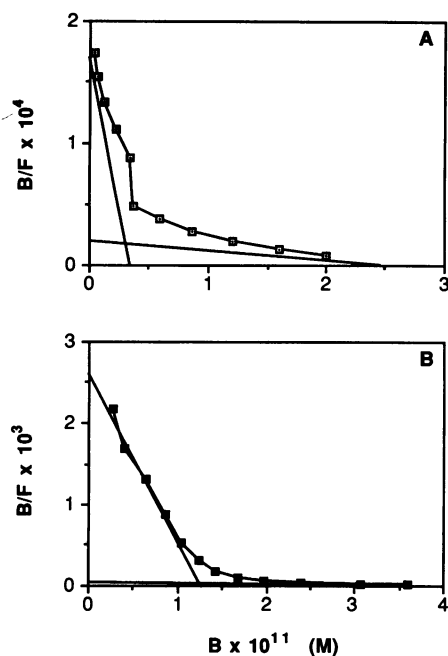
FIG. 5. Scatchard analysis of peroxidase binding by unfrozen and frozen type 1 fimbriae. Data from peroxidase binding assays like the one represented in Fig. 4 were analyzed with the computer program LIGAND to obtain the binding affinity and number of sites for unfrozen (A) and frozen (B) type 1 fimbriae.

and was not detected for similarly treated FimH$^-$ mutant type 1 fimbriae. To resolve the question of increased affinity versus increased number of binding sites in the frozen fragments, we performed Scatchard analysis on the data from the peroxidase-binding assay. Both unfrozen and frozen fimbriae were found to display sites with two binding affinities. A population of low-affinity sites exhibited $K_a$

values in the range of $0.8 \times 10^6$ to $3.8 \times 10^6$ M$^{-1}$, and a population of high-affinity sites had $K_a$ values in the range of $0.5 \times 10^8$ to $2.0 \times 10^8$ M$^{-1}$. Although the number of low-affinity binding sites remained unchanged, the number of high-affinity binding sites was increased by two- to four-fold in the frozen compared with unfrozen samples, thereby supporting our conclusion that cryptic mannose-binding sites become exposed upon fragmentation of type 1 fimbriae by freezing and thawing. Unfortunately, a quantitative comparison between the degree of fimbrial fragmentation, or even the number of high-affinity binding sites, and the biological activities in each of the assays cannot reasonably be made because of the fact that the responses in all of the assays are not linearly proportional to the doses of the inciting agent.

It is conceivable that the fracturing of the fimbriae may occur preferentially at locations weakened by insertions of the minor fimbrial proteins. A correlation between the occurrence of these minor protein components and an increased fragility of type 1 fimbrial structures has been reported (9). Our preparations of wild-type and FimH$^-$ mutant fimbriae have similar compositions, except for the presence or absence of the 29-kDa FimH protein (data not shown). At present we are unable to conclude whether the fractures we observe occur preferentially at the points of insertion of the minor protein components or are random in nature. Either possibility would be expected to expose an increased number of adhesin molecules with a fimbrial tip location. Since our data show that wild-type fimbriae are more extensively fragmented than are FimH$^-$ mutant fimbriae, we would suggest that sites of FimH insertion may become fracture points during freezing. The 2- to 4-fold increase in high-affinity binding sites coincidentally agreed with the 3.8-fold increase in the number of fragments per 1,000 nm of length, although this observation cannot be taken to indicate that all fracture points occurred at sites of FimH insertion, because fragmentation of the mutant fimbriae was also observed.

TABLE 2. Affinities and numbers of mannose-specific binding sites on unfrozen and frozen wild-type type 1 fimbriae[a]

| Expt | Sample | $K_a$ (M$^{-1}$) | Number of binding sites (mol, 10$^{-11}$) |
|---|---|---|---|
| 1 | Unfrozen (low affinity) | $1.1 \times 10^6$ | 1.7 |
|   | Unfrozen (high affinity) | $1.0 \times 10^8$ | 0.3 |
|   | Frozen (low affinity) | $3.8 \times 10^6$ | 1.3 |
|   | Frozen (high affinity) | $2.0 \times 10^8$ | 0.8 |
| 2 | Unfrozen (low affinity) | $1.0 \times 10^6$ | 3.0 |
|   | Unfrozen (high affinity) | $0.3 \times 10^8$ | 0.4 |
|   | Frozen (low affinity) | $1.8 \times 10^6$ | 3.0 |
|   | Frozen (high affinity) | $1.4 \times 10^8$ | 1.3 |
| 3 | Unfrozen (low affinity) | $0.8 \times 10^6$ | 2.5 |
|   | Unfrozen (high affinity) | $0.5 \times 10^8$ | 0.4 |
|   | Frozen (low affinity) | $0.9 \times 10^6$ | 3.3 |
|   | Frozen (high affinity) | $2.1 \times 10^8$ | 1.3 |
| Mean ± SE | Unfrozen (low affinity) | $1.0 \times 10^6 \pm 0.1 \times 10^6$ | 2.4 ± 0.4 |
|   | Unfrozen (high affinity) | $0.6 \times 10^6 \pm 0.2 \times 10^8$ | 0.3 ± 0.0 |
|   | Frozen (low affinity) | $2.2 \times 10^6 \pm 0.9 \times 10^6$ | 2.5 ± 0.6 |
|   | Frozen (high affinity) | $1.8 \times 10^6 \pm 0.2 \times 10^8$ | 1.1 ± 0.2 |

[a] Microtiter plates coated with fimbriae and blocked with BSA were incubated overnight with dilutions of peroxidase in the presence and absence of α-methyl-mannose. Specific binding was obtained by subtracting the amount bound in the presence of mannose from that in the absence of mannose. The amount of bound peroxidase was calculated from a standard curve by linear regression. The data were then analyzed by using the computer program LIGAND to obtain the binding affinities and numbers of binding sites.
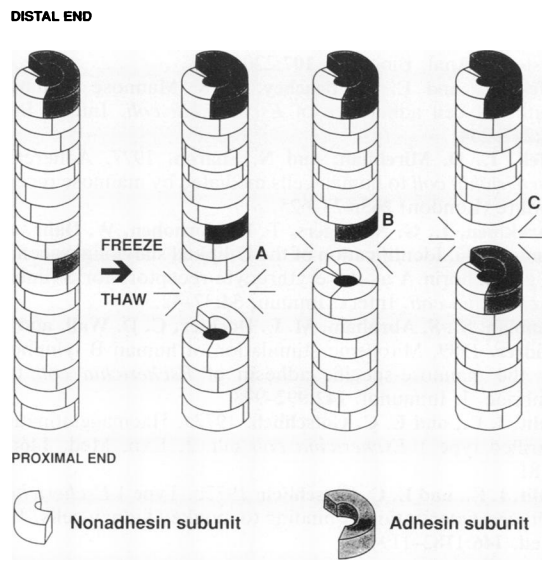
**DISTAL END**



FIG. 6. Model of the structure of type 1 fimbriae, showing locations of proposed cryptic and active adhesin moieties. Fracturing of the fimbriae structure could occur at one or more of three sites relative to an adhesin moiety: remote (A), immediately proximal (B), or immediately distal (C). Two observations suggest that case C, or the distal side of an adhesin moiety, may be a preferential site for the fimbrial structure to break. First, wild-type fimbriae seem to be more fragile than adhesin-negative FimH⁻ mutant fimbriae. Second, new mannose-binding sites appear when wild-type fimbriae are fragmented. This model does not address whether an adhesin moiety is composed of FimH alone or FimH combined with one of the other fimbrial components, but the observation of two binding affinities suggests that the affinity of FimH may be influenced by its association with different fimbrial components.

The mannose-binding activity expressed by type 1 fimbriated organisms has been clearly shown to be associated with the fimbrial structures (21). The exact location of functional adhesin molecules on the type 1 fimbrial structure has, however, been a controversial issue. Brinton has reported that isolated fimbriae attach to polystyrene latex or erythrocyte membranes in an end-on fashion, leading to the conclusion that the adhesin moiety is situated only at the tips of the cells (5, 8). In contrast, observations by Salit and Gotschlich have led them to propose the occurrence of lateral sites for the adhesin moiety on the fimbrial structure (21). Work from our laboratory with immunoelectron microscopy has indicated that the FimH molecule, which is considered an integral part of the adhesin if not the entire adhesin moiety itself, resides both at the tip and at long intervals along the length of the fimbriae (1). These studies did not address the ability of those moieties to bind mannose. Based on our current results, we propose a revised model of type 1 fimbriae that takes into account both the location of the adhesin moiety and its functional activity (Fig. 6). This model retains the earlier suggestion that adhesin moieties are located both at the tips and at intervals along the fimbrial structure; however, we now further propose that only the adhesin moieties located at the fimbrial tips are functional in binding mannosylated glycoproteins. Adhesins located along the length of the fimbriae have their mannose-binding activity buried within the fimbrial structure. These internally located adhesins become active mannose-binding moieties when they acquire a distal tip location upon fragmentation of

the fimbriae, as in case C of Fig. 6. If fragmentation occurred on the proximal side of an adhesin subunit, its active site might remain buried, as in case B of Fig. 6.

The occurrence of two binding affinities displayed by both the unfrozen and frozen fragments is an interesting observation that requires further investigation and also raises the question of the nature of the adhesin in the physiological state. Preliminary data from our laboratory have shown that whole bacteria expressing type 1 fimbriae are capable of agglutinating guinea pig erythrocytes and stimulating human lymphocyte proliferation in a mannose-sensitive manner. Since these activities correlate with the numbers of high-affinity binding sites in purified fimbriae, the data suggest that the high-affinity sites are present on intact bacteria. Apparently many of the high-affinity sites are lost or are converted into low-affinity sites during purification of the fimbriae.

The localization of adhesion proteins associated with two other types of *E. coli* fimbriae have been reported without regard to their functional capabilities. Like the adhesin of type 1 fimbriae, the adhesin of the S fimbriae of *E. coli* was shown to be located both at the tip and at intervals along the length of the fimbriae (15). In contrast, the adhesin of Pap fimbriae has only been localized to the tips of these structures (14). It would be interesting to see how preparations of these two types of fimbriae behave upon freezing and thawing, since our model of type 1 fimbriae structure may also apply to S fimbriae but not to Pap fimbriae.

## ACKNOWLEDGMENTS

## REFERENCES

1. **Abraham, S. N., J. D. Goguen, D. Sun, P. Klemm, and E. H. Beachey.** 1987. Identification of two ancillary subunits of *Escherichia coli* type 1 fimbriae by using antibodies against synthetic oligopeptides of *fim* gene products. J. Bacteriol. **169:**5530–5536.
2. **Andrade, J. R. C.** 1980. Role of fimbrial adhesiveness in experimental guinea pig keratoconjunctivity by *Shigella flexneri.* Rev. Microl. **11:**117–125.
3. **Aronson, M., O. Medalia, L. Schori, D. Mirelman, N. Sharon, and I. Ofek.** 1979. Prevention of colonization of the urinary tract of mice with *Escherichia coli* by blocking of bacterial adherence with methyl-α-D-mannopyranoside. J. Infect. Dis. **139:**329–332.
4. **Bar-Shavit, Z., I. Ofek, R. Goldman, D. Mirelman, and N. Sharon.** 1977. Mannose residues on phagocytes as receptors for the attachment of *Escherichia coli* and *Salmonella typhi.* Biochem. Biophys. Res. Commun. **78:**455–460.
5. **Brinton, C. C., Jr.** 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:**1003–1054.
6. **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:**764–773.
7. **Fader, R. C., and C. P. Davis.** 1980. Effect of piliation on *Klebsiella pneumoniae* infection in rat bladders. Infect. Immun. **30:**554–561.
8. **Hanson, M. S., and C. C. Brinton, Jr.** 1988. Identification and characterization of *Escherichia coli* type-1 pilus tip adhesion protein. Nature (London) **332:**265–268.
9. **Klemm, P., and G. Christiansen.** 1987. Three fim genes required for the regulation of length and mediation of adhesion of

*Escherichia coli* type 1 fimbriae. Mol. Gen. Genet. **208:**439–445.

10. **Korhonen, T. K., V. Vaisinen-Rhen, V. Rhen, M. Pere, A. Parkkinen, and J. Finne.** 1984. *Escherichia coli* fimbriae recognizing sialyl galactosides. J. Bacteriol. **159:**762–766.

11. **Krogfelt, K. A., H. Bergmans, and P. Klemm.** 1990. Direct evidence that the FimH protein is the mannose-specific adhesin of *Escherichia coli* type 1 fimbriae. Infect. Immun. **58:**1995–1998.

12. **Leffler, H., and C. Svanborg-Eden.** 1980. Chemical identification of a glycosphingolipid receptor for *Escherichia coli* attaching to human urinary tract epithelial cells and agglutinating human erythrocytes. FEMS Microbiol. Lett. **8:**127–134.

13. **Leffler, H., and C. Svanborg-Eden.** 1986. Glycolipids as receptors for *Escherichia coli* lectins or adhesins, p. 83–112. *In* D. Mirelmann (ed.), Microbial lectins and agglutinins: properties and biological activity. John Wiley & Sons, Inc., New York.

14. **Lindberg, F., B. Lund, L. Johansson, and S. Normark.** 1987. Location of the receptor-binding protein adhesin at the tip of the bacterial pilus. Nature (London) **328:**84–87.

15. **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. **84:**3462–3466.

16. **Munson, P. J., and D. Robbard.** 1980. LIGAND: a versatile computerized approach for characterization of ligand-binding systems. Anal. Biochem. **107:**220–239.

17. **Ofek, I., and E. H. Beachey.** 1978. Mannose binding and epithelial cell adherence of *Escherichia coli*. Infect. Immun. **22:**247–254.

18. **Ofek, I., D. Mirelman, and N. Sharon.** 1977. Adherence of *Escherichia coli* to human cells mediated by mannose receptors. Nature (London) **265:**623–625.

19. **Parkkinen, J., G. N. Rogers, T. K. Korhonen, W. Dahr, and J. Finne.** 1986. Identification of the O-linked sialyloligosaccharides of glycophorin A as the erythrocyte receptors for S-fimbriated *Escherichia coli*. Infect. Immun. **54:**37–42.

20. **Ponniah, S., S. Abraham, M. E. Dockter, C. D. Wall, and R. O. Endres.** 1989. Mitogenic stimulation of human B lymphocytes by the mannose-specific adhesin of *Escherichia coli* type 1 fimbriae. J. Immunol. **142:**992–998.

21. **Salit, I. E., and E. C. Gotschlich.** 1977a. Haemagglutination by purified type 1 *Escherichia coli* pili. J. Exp. Med. **146:**1169–1181.

22. **Salit, I. E., and E. C. Gotschlich.** 1977b. Type 1 *Escherichia coli* pili: characterization of binding to monkey kidney cells. J. Exp. Med. **146:**1182–1194.