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Glycerol-Induced Unraveling of the Tight Helical Conformation of *Escherichia coli* Type 1 Fimbriae

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Glycerol was found to unravel the helical conformation of *Escherichia coli* type 1 fimbriae without appreciable depolymerization. The linearized fimbrial polymers have a diameter of 2 nm, react strongly with a monoclonal antibody directed at an inaccessible epitope on native fimbriae, and display greater mannose-binding activity and trypsin sensitivity than native fimbriae. Removal of glycerol by dialysis results in spontaneous reassembly of the linear polymers into structures morphologically, antigenically, and functionally indistinguishable from native fimbriae.

A single 17-kDa structural protein, FimA, comprises over 95% of the subunits in type 1 fimbrial filaments of *Escherichia coli* (9). In addition, there are at least three minor proteins associated with the fimbriae, including FimH, a 29-kDa protein which mediates the mannose-binding properties of type 1 fimbriae (3, 9, 12). The quaternary structure of type 1 fimbriae is extremely stable and is resistant to the action of most dissociating agents (6, 13). Saturated guanidine hydrochloride can completely dissociate type 1 fimbriae (6), and the removal of this denaturant will result in the formation of structures morphologically (8) and antigenically (4) indistinguishable from native type 1 fimbriae.

We recently discovered that in the presence of glycerol, type 1 fimbriae lost their rigidity and became flexuous filaments. Type 1 fimbriae suspended in 0.1 M NH_4CO_3 buffer (pH 8.6) were mixed with 50% glycerol for 4 h at 37°C. The fimbrial preparations were then rotary shadowed with platinum and carbon (14). Electron microscopy revealed that the loss of fimbrial rigidity associated with glycerol treatment was due to unraveling of the tight helical conformation of fimbrial subunits into flexible and linear structures. Figure 1A shows untreated fimbriae (control); Fig. 1B and C show fimbriae in 50% glycerol, revealing various degrees of unraveling. The diameter of the unraveled fimbriae varied depending on the degree of linearization. In completely linearized regions of the fimbriae, the diameter was approximately 2 nm (Fig. 1C), which is considerably thinner than the 7-nm diameter of the native fimbrial structure. Unraveling appeared to be initiated at the tips as well as at several apparently random points along the length of the fimbriae (Fig. 1B). Interestingly, removal of glycerol by overnight dialysis against 5 mM Tris (pH 8.0) at 22°C resulted in spontaneous reassembly into structures of uniform diameter that were morphologically indistinguishable from native type 1 fimbriae (Fig. 1D). The ability of the linearized or unraveled fimbriae to reassemble into the native conformation suggests that the action of glycerol on type 1 fimbriae was nondenaturing and reversible.

If type 1 fimbriae are unraveled by glycerol, as suggested by electron microscopy, then antigenic epitopes previously inaccessible to antibody on the intact fimbriae should become exposed. To determine if this was the case, the unraveled fimbriae were probed with monoclonal antibody AE1. This immunoglobulin G2a (IgG2a) antibody is a FimA-specific monoclonal antibody which reacts poorly with native fimbriae in an enzyme-linked immunosorbent assay (ELISA) but reacts strongly with the isolated FimA molecules on an immunoblot (4). Thus, the epitope on FimA recognized by the AE1 monoclonal antibody is inaccessible on the intact fimbrial filament (4). To determine whether AE1 monoclonal antibody would demonstrate greater reactivity with glycerol-treated than native fimbriae, an ELISA was performed in which the reactivity of AE1 antibody with glycerol and untreated type 1 fimbriae adsorbed to microtiter wells was examined. As shown in Fig. 2, AE1 antibody exhibited poor reactivity with native fimbriae. In contrast, glycerol-treated fimbriae showed strong reactivity with the antibody. Reassembled fimbriae showed the same lack of reactivity with AE1 as the native fimbriae (Fig. 2). Thus, the increased reactivity of AE1 antibody with glycerol-treated fimbriae indicates that glycerol has induced a significant alteration in the antigenicity of the fimbriae. More specifically, this finding demonstrates that in the presence of glycerol, an epitope previously inaccessible to antibody in the intact quaternary conformation was now exposed, which is in agreement with the electron microscopic data that show that glycerol induces unraveling of the helical conformation of the fimbriae. The lack of reactivity of AE1 antibody with the reassociated fimbriae supports the idea that the fimbriae reassumed their highly ordered conformation once the glycerol was removed.

Type 1 fimbriae are characterized by their ability to mediate mannose-sensitive adhesive and agglutination reactions. The yeast agglutination properties of type 1 fimbriated *E. coli* ORN103(pSH2) exposed to 50% glycerol for 4 h at 37°C were therefore compared with an equal concentration (A_{550} of 0.1) of untreated control and glycerol-treated but washed *E. coli* ORN103(pSH2). The morphology of type 1 fimbriae expressed on the surface of bacteria after exposure

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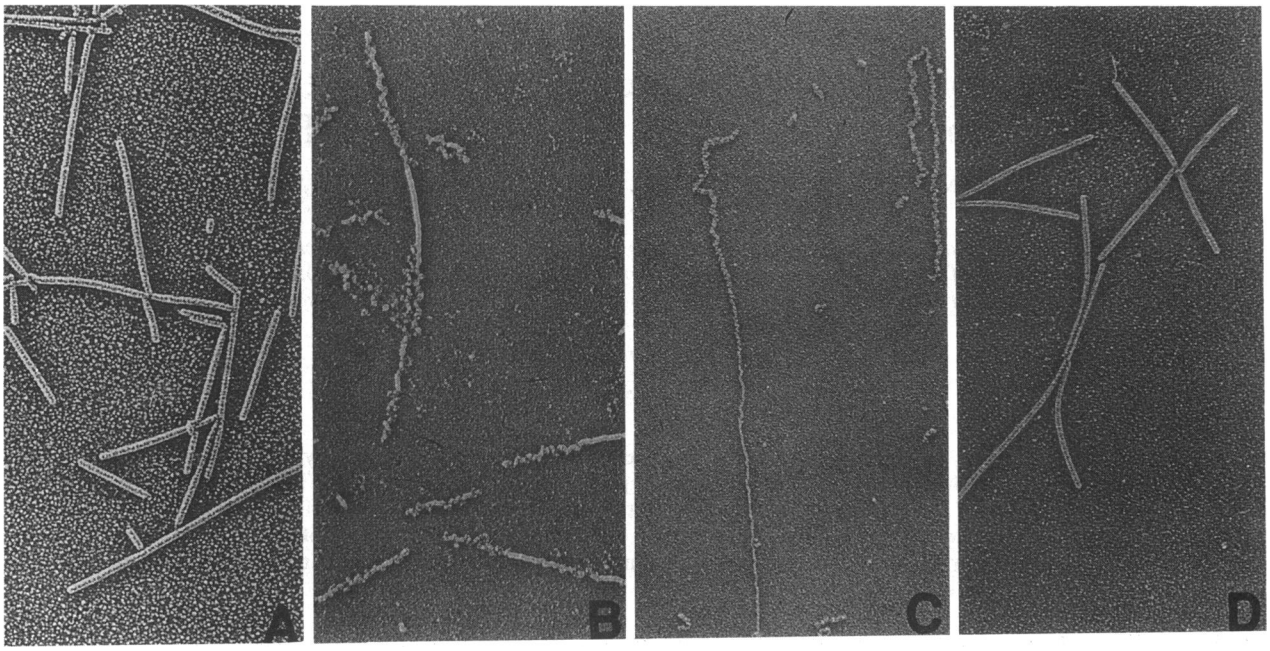

FIG. 1. Electron micrographs of rotary-shadowed preparations showing native type 1 fimbriae after various treatments. (A) Control (untreated); (B) 50% glycerol treated, showing partial unraveling (note that the unraveling appears to be initiated at the tips as well as at apparently random sites along the fimbrial length); (C) 50% glycerol treated, showing unraveled and stretched fimbriae; (D) after removal of glycerol by dialysis, showing reassembled fimbriae.

to glycerol was similar to that observed with isolated type 1 fimbriae when examined by electron microscopy. The agglutination reactions, based on a procedure described previously (11), revealed that in the presence of glycerol, *E. coli* (with unraveled fimbriae) exhibited a yeast agglutination titer of 1:64. In contrast, the titer of untreated control bacteria (with native fimbriae) or glycerol-treated but washed bacteria (with reassembled fimbriae) was only 1:16. Thus, glycerol induced a fourfold increase in the agglutination activity of type 1 fimbriated bacteria. The glycerol-induced alteration in type 1 fimbriated bacterial adherence correlated with morphological and antigenic changes induced by glycerol on isolated type 1 fimbriae. All the bacterially induced yeast agglutinating reactions were completely inhibitable by 1% α-methyl-D-mannopyranoside, confirming that the adhesive interactions were mediated by FimH, the mannose-binding adhesin of type 1 fimbriae.

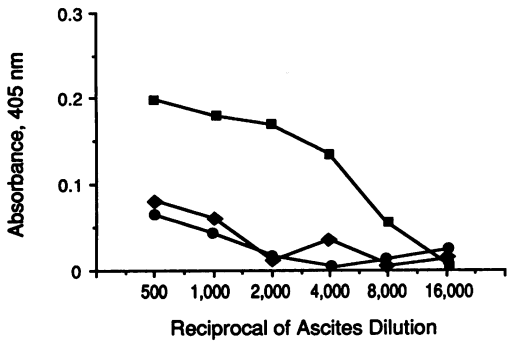

FIG. 2. Binding of fimbrial subunit-specific monoclonal antibody AE1 to native, unraveled, and reassembled type 1 fimbriae. Each of the fimbrial preparations was immobilized on microtiter trays incubated at 60°C for 4 h (a protein assay was initially performed [Bio-Rad protein microassay] on a similarly treated microtiter tray to ensure that the amount of fimbrial protein bound in the presence of glycerol was comparable to the amount bound in the absence of glycerol). The trays were washed and then incubated with serial twofold dilutions of the AE1 ascites for 2 h at 37°C as described previously (1, 4). The binding of the antibody was detected by the addition of a second peroxidase-conjugated antibody directed at mouse IgG, followed by the appropriate chromophore. Symbols: ●, reaction with untreated fimbriae; ■, reaction with glycerol-treated type 1 fimbriae; ◆, reaction with reassembled fimbriae after glycerol has been removed.

Type 1 fimbrial filaments have been noted for their ability to resist the action of a variety of dissociating agents and proteolytic enzymes (6, 7, 13). To determine whether unraveled fimbriae were sensitive to tryptic digestion, we coated microtiter dishes with equal concentrations of native, unraveled, and reassembled fimbriae and then exposed these wells to 25 μg of trypsin or phosphate-buffered saline (PBS, controls) per ml. At the end of the 60-min incubation, the wells were thoroughly washed and the amount of residual, presumably undegraded, fimbriae in the microtiter wells was monitored by ELISA with a polyclonal antibody against type 1 fimbriae. As shown in Fig. 3A, the reactivities of the antiserum with trypsin-treated and untreated native type 1 fimbriae were very similar. In contrast, the reactivity with trypsin-treated unraveled fimbriae was considerably less than with untreated unraveled fimbriae (Fig. 3B). The reactivities with trypsin-treated and untreated reassembled fimbriae were similar (Fig. 3C). These findings suggest that, unlike the native conformation, the unraveled fimbrial conformation is susceptible to trypsin digestion. To examine the action of trypsin on FimH specifically, we exposed unraveled and native type 1 fimbriae to trypsin or PBS (control) and then compared their reactivity with FimH-specific antibody on an immunoblot (1, 2). As shown in Fig. 4, lane 1, the

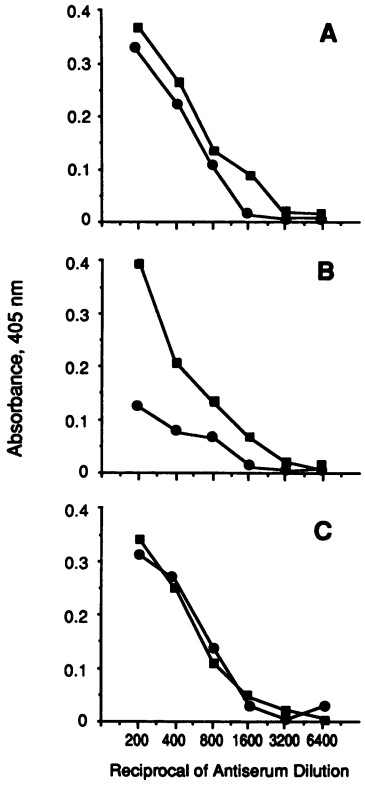

FIG. 3. Effect of trypsin treatment on native (A), unraveled (B), and reassembled (C) fimbriae. Equal amounts of native, unraveled, and reassembled fimbriae were immobilized on microtiter dishes and incubated with 100 μl of 25-μg/ml trypsin (●) or PBS (■) for 60 min. At the end of incubation, the wells were thoroughly washed and reacted with antiserum to type 1 fimbriae as described in the text. The susceptibility of each of the fimbrial preparations to degradation by trypsin was assessed by comparing the reactivity of the antiserum with fimbrial proteins remaining on the tray after exposure to trypsin.

FimH moiety in the unraveled fimbriae appeared to be degraded, whereas the same moiety in native fimbriae remained virtually intact. Neither preparation showed any signs of degradation in the absence of trypsin (Fig. 4, lanes 3 and 4). When reassembled fimbriae were exposed to trypsin, the results were identical to those obtained with native type 1 fimbriae (data not shown). Both experiments suggest that type 1 fimbriae as well as their adhesin molecules are susceptible to degradation by trypsin once the fimbrial structure is destabilized by glycerol.

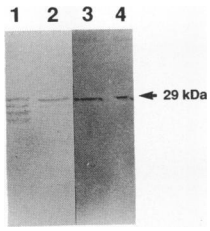

FIG. 4. Effect of trypsin treatment on FimH, the mannose-binding adhesin on native and unraveled type 1 fimbriae. Unraveled (glycerol-treated) and native (PBS-treated) fimbriae were incubated for 60 min at 37°C with an equal volume of 25-μg/ml trypsin or PBS. The samples were boiled in sodium dodecyl sulfate, electrophoresed, and reacted on an immunoblot with antibodies specific to FimH. Lane 1, trypsin digest of unraveled fimbriae; lane 2, trypsin digest of native type 1 fimbriae; lane 3, unraveled fimbriae in PBS; lane 4, native type 1 fimbriae in PBS.

There is little information on the nature of the intermolecular forces that hold together the quaternary conformation of the fimbriae. The observation that linearization can be achieved without significant depolymerization suggests that the intermolecular forces involved in forming the helical arrangement are weaker than the forces involved in forming the linear polymer. Alternatively, these latter forces may be different in nature and not sensitive to the action of glycerol. At this time, we are unable to distinguish between these two possibilities. Also not clear is why *E. coli* expressing type 1 fimbriae in their unraveled conformation displays stronger binding activity than *E. coli* expressing the native fimbrial form. We have recently reported that the FimH molecules are intercalated at intervals along the type 1 fimbrial length (2, 3, 5), but with the exception of those exposed at the fimbrial tips, these adhesins are essentially inactive (14). One explanation for why FimH buried along the longitudinal length was functionally cryptic is that the putative mannose-binding domains of FimH were inaccessible (14). Possibly, the unraveled fimbrial conformation makes the FimH molecules more accessible and therefore more functional than in the native fimbriae.

The finding that unraveled but not native fimbriae are susceptible to digestion by the pancreatic enzyme trypsin suggests that the tight helical conformation of the fimbriae provides not only rigidity to the fimbrial filament but also resistance to proteolytic degradation. Until now, the tight helical conformation of type 1 fimbriae has been a major impediment for structure-function studies. The linearized conformation induced by glycerol may make these structures more amenable to study.

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REFERENCES

1. **Abraham, S. N., and E. H. Beachey.** 1987. Assembly of a chemically synthesized peptide of *Escherichia coli* type 1 fimbriae into fimbria-like antigenic structures. J. Bacteriol. **169:** 2460–2465.
2. **Abraham, S. N., J. D. Goguen, and E. H. Beachey.** 1988. Hyperadhesive deletion mutant of type 1 fimbriated *Escherichia coli* associated with formation of FimH organelles (fimbriosomes). Infect. Immun. **169:**1023–1029.
3. **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:**5530–5535.
4. **Abraham, S. N., D. L. Hasty, W. A. Simpson, and E. H. Beachey.** 1983. Antiadhesive properties of a quaternary structure-specific hybridoma antibody against type 1 fimbriae of *Escherichia coli*. J. Exp. Med. **158:**1128–1144.
5. **Abraham, S. N., D. Sun, J. B. Dale, and E. H. Beachey.** 1988. Conservation of the mannose-adhesin protein among type 1 fimbriated members of the family *Enterobactericeae*. Nature (London) **336:**682–684.
6. **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:**1003–1165.
7. **Dodd, D. C., and B. I. Eisenstein.** 1982. Antigenic quantitation of

type 1 fimbriae on the surface of *Escherichia coli* by an enzyme-linked immunosorbent inhibition assay. Infect. Immun. **38:**764–768.

8. **Eshdat, Y., F. J. Silverblatt, and N. Sharon.** 1982. Dissociation and reassembly of *Escherichia coli* type 1 pili. J. Bacteriol. **148:**308–314.
9. **Hanson, M. S., and C. C. Brinton.** 1988. Identification and characterization of *Escherichia coli* type 1 pilus tip adhesion protein. Nature (London) **332:**265–268.
10. **Klemm, P., and F. 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.
11. **Korhonen, T.** 1979. Yeast-cell agglutination by purified enterobacterial pili. FEMS Microbiol. Lett. **6:**421–425.
12. **Krogfelt, K. A., H. Bermans, and P. Klemm.** 1990. Direct evidence that the FimH protein is the mannose-specific adhesion of *Escherichia coli* type 1 fimbriae. Infect. Immun. **58:** 1995–1998.
13. **McMichael, J. C., and J. T. Ou.** 1979. Structure of common pili from *Escherichia coli*. J. Bacteriol. **138:**969–975.
14. **Ponniah, S., R. O. Endres, D. L. Hasty, and S. N. Abraham.** 1991. Fragmentation of *Escherichia coli* type 1 fimbriae exposes cryptic mannose binding sites. J. Bacteriol. **173:**4195–4202.