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Direct Evidence that the FimH Protein Is the Mannose-Specific Adhesin of *Escherichia coli* Type 1 Fimbriae

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Type 1 fimbriae of *Escherichia coli* are surface organelles which mediate binding to D-mannose-containing structures. By direct binding of FimH to D-mannose attached to a carrier protein, we demonstrated that this protein was uniquely responsible for the receptor specificity. Furthermore, we show by receptor immunoelectron microscopy that the FimH protein is located laterally in the structure of the type 1 fimbriae.

Most strains of *Escherichia coli* are able to express type 1 fimbriae, which are threadlike appendages consisting of about 1,000 subunits of the FimA protein as well as a few percent minor components. These fimbriae mediate binding to D-mannose-containing structures and thereby enable the bacteria to colonize various host tissues (5, 6, 21). Inhibition of binding of type 1-fimbriated bacteria as well as purified type 1 fimbriae to various cell types, such as erythrocytes and buccal and intestinal epithelial cells, has been extensively studied. In all cases it was found that D-mannose itself and most derivatives of this sugar were very potent inhibitors of type 1 fimbria-mediated adhesion, whereas all saccharides not containing D-mannose showed no inhibitory effect (7, 20, 22, 25). Ørskov et al. (23) have proposed that the biological role of type 1 fimbriae is to provide bacterial adhesion to mucus in the large intestine, which is the natural habitat of *E. coli*. Furthermore, several lines of evidence point to a role of type 1 fimbriae as possible virulence factors in uropathogenic *E. coli* strains (8, 10). It has been shown that one or more of the minor fimbrial components FimF, FimG, and FimH are involved in receptor binding (11, 14, 17). Recombinant bacteria expressing fimbriae consisting of FimA only or FimA, FimF, and FimG but not FimH do not exhibit any binding ability (14). Several lines of investigation, with genetic and immunological techniques, have pointed to the FimH protein as the adhesin (2, 3, 11, 14, 19). We decided to use a more straightforward approach, using the biological receptor molecular, to show this.

Escherichia coli K-12 strain HB101 (4) is nonfimbriated and is unable to bind to guinea pig erythrocytes or to other D-mannoside-containing surfaces (14). When the *fim* gene cluster from *E. coli* K-12 strain PC31 was introduced into HB101 in the form of plasmid pPKL4 (Fig. 1), D-mannose-binding fimbriae were produced, as judged from the ability of the recombinant strain to agglutinate guinea pig erythrocytes and to specifically adhere to Sepharose beads coated with D-mannose (16; P. Klemm, K. A. Krogfelt, L. Hedegaard, and G. Christiansen, Mol. Microbiol. in press). Such fimbriae contained four components, FimA, FimF, FimG, and FimH (17). Introduction of plasmid pPKL5, a deletion mutant of pPKL4 which lacks the *fimF*, *fimG*, and *fimH* genes, resulted in the production of pure FimA fimbriae which were devoid of mannose recognition specificity (14, 16). We have also described an HB101 host containing two compatible plasmids, pPKL5 (*fimA*$^+$) and pPKL53 (*fimF*$^+$ *fimG*$^+$), which produces fimbriae consisting of the FimA, FimF, and FimG components and is unable to agglutinate guinea pig erythrocytes or to bind to D-mannose-coated Sepharose beads (14). Similar results have been reported by Maurer and Orndorff (19).

Fimbriae were purified from HB101 cells containing either plasmid pPKL4, plasmid pPKL5, or plasmids pPKL5 plus pPKL53, and purity was assessed by electrophoresis in sodium dodecyl sulfate (SDS) reported previously (17) (Fig. 2A). The FimH protein migrated with an apparent molecular weight of 30,000, whereas FimA and the two minor components, FimF and FimG, migrated with molecular weights in the 16,000 to 18,000 range (17).

To establish that the FimH protein, apart from being a component of type 1 fimbriae, is uniquely responsible for the binding to D-mannose, we made use of a modified Western immunoblot (Fig. 2). This technique utilized D-mannose coupled to bovine serum albumin (BSA) as the primary reagent. Highly purified type 1 fimbrial preparations were depolymerized by boiling in dilute hydrochloric acid, neutralized, run on a 15% acrylamide-SDS gel, and transferred to a nitrocellulose filter (18). Lanes A_1, A_2, and A_3 were visualized by specific anti-type 1 antiserum. Lanes B_1, B_2, and B_3 and C_1, C_2, and C_3 were treated as follows: excessive binding sites on the filter were blocked with 2% Tween 20, followed by incubation with either D-mannose–BSA (Carbohydrate International, Stockholm, Sweden) (panel B) or with BSA (panel C). In order to visualize the mannose-specific binding, the filter was incubated with anti-BSA rabbit immunoglobulins and finally incubated with horseradish peroxidase-conjugated anti-rabbit immunoglobulin G (IgG). Tetramethylbenzidine (TMB) was used as the substrate.

Only the FimH protein reacted with the D-mannose–BSA conjugate (Fig. 2, lane B_1). Therefore, this protein is at least partially able to renature to regain its biological activity. However, this is in no way uncommon and has previously been reported for many other proteins (9). As controls we

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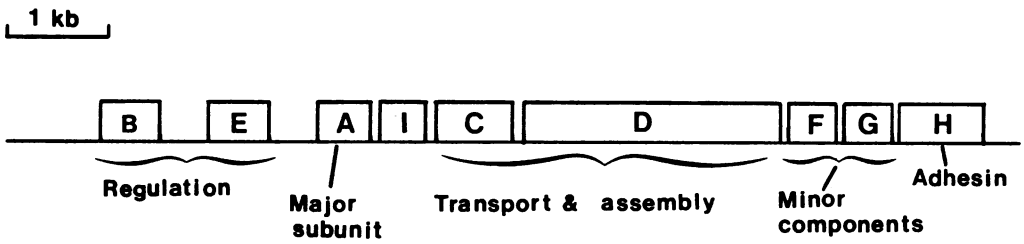

FIG. 1. The *fim* gene cluster on plasmid pPKL4, and a brief description of the gene products. The *fimA* gene encodes the major subunit protein (12). The products of the *fimB* and *fimE* genes are involved in regulation of fimbriae expression by controlling the configuration of the phase switch in front of the *fimA* gene (13, 24). The FimC and FimD proteins are required for transport and surface localization of the fimbriae (15, 16). The products of the *fimF* and *fimG* genes are minor constituents of the fimbriae (14, 17), and the FimH protein is the adhesin as shown in this work. The *fimI* gene encodes a protein homologous to FimA (P. Klemm, unpublished data).

used pure FimA fimbriae from HB101 (pPKL5) and pure FimA, FimF, and FimG fimbriae from HB101(pPKL5, pPKL53), neither of which contains the FimH protein. As a further control, BSA without covalently linked D-mannose was tested. Neither of the controls showed any reaction. Furthermore, in order to see whether free D-mannose could inhibit the binding of FimH to the D-mannose–BSA conjugate, an additional experiment was performed. The nitrocellulose filters were washed with 50 mM D-mannose before incubation with the conjugate (data not shown). No reaction was observed, confirming the specific receptor affinity of FimH. As a control, we washed another set of filters with 50 mM D-glucose. In this case no inhibition of binding of the FimH protein to the D-mannose–BSA conjugate was seen.

Since the D-mannose–BSA conjugate was specifically recognized by the FimH adhesin, we also tried to localize the position of this protein in intact type 1 fimbriae. HB101 cells harboring plasmid pPKL4 were incubated with D-mannose–BSA, followed by anti-BSA rabbit serum and colloidal gold-labeled protein A. The cells were inspected by electron microscopy (Fig. 3). It is evident from these results that the FimH adhesin is laterally positioned at intervals along the fimbriae.

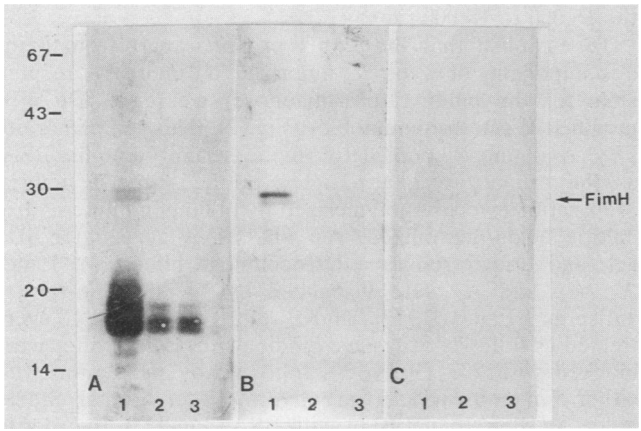

FIG. 2. Analysis of specific receptor binding of type 1 fimbriae proteins from (lanes 1) HB101(pPKL4) (wild-type fimbrriae), (lanes 2) HB101(pPKL5, pPKL53) (fimbriae constituted of FimA, FimF, and FimG), and (lanes 3) HB101(pPKL5) (FimA only fimbriae). (A) Western blots of fimbria preparations. The reaction with anti-type 1 fimbriae serum followed by horseradish peroxidase-labeled antibodies and visualization with TMB. (B) Incubation with D-mannose–BSA. The D-mannose–BSA conjugate reacts uniquely with a 30,000-M_r protein, FimH, as indicated by the arrow. (C) Incubation with BSA. No reaction was observed. Molecular weights (10^3) are shown to the left.

As controls, two experiments were carried out. HB101(pPKL4) cells were incubated with BSA without covalently attached D-mannose, and HB101 cells harboring plasmid pPKL5, and therefore not producing the FimH protein, were incubated with the D-mannose–BSA conjugate. In neither of these control experiments was labeling of the fimbriae seen (data not shown).

By using direct binding of the natural receptor, D-mannose, for type 1 fimbriae, we proved that the FimH protein is the D-mannose-specific adhesin and that this adhesin is laterally located on the fimbriae at long intervals. In order to integrate the FimH protein in the fimbriae, the two other minor components, FimF and FimG, are needed (14). We have previously proposed a model, based on stoichiometric data, in which the adhesive complex is integrated in the fimbria on the average once every 100 to 200 FimA subunits (17). This would correspond to one complex on the average per 100 to 150 nm, which is roughly in agreement with the present observations.

Recent electron microscopic evidence has indicated that the FimH protein is located both laterally and at the tip of the type 1 fimbriae. These findings have relied on the use of antisera against the FimH protein and not on receptor binding (1, 3). We have not observed the receptor bound at the tip of the fimbriae. This might be due to the different experimental approaches, i.e., immunological versus lectin binding. However, we are confident that monitoring of binding to the D-mannose receptor molecule must be the best approximation to the natural conditions under which this bacterial adhesin functions.

The D-mannose–BSA complex is not a naturally occurring receptor for type 1 fimbriae. One could therefore argue that fimbrial components other than FimH could be involved in adhesion to such receptors. However, D-mannose has been found to inhibit the binding of type 1-fimbriated *E. coli* to all hitherto-described receptor substances. In light of this, we think that our data point very strongly to D-mannose as being, if not the only, then the major ingredient of any naturally occurring receptor for type 1 fimbriae.

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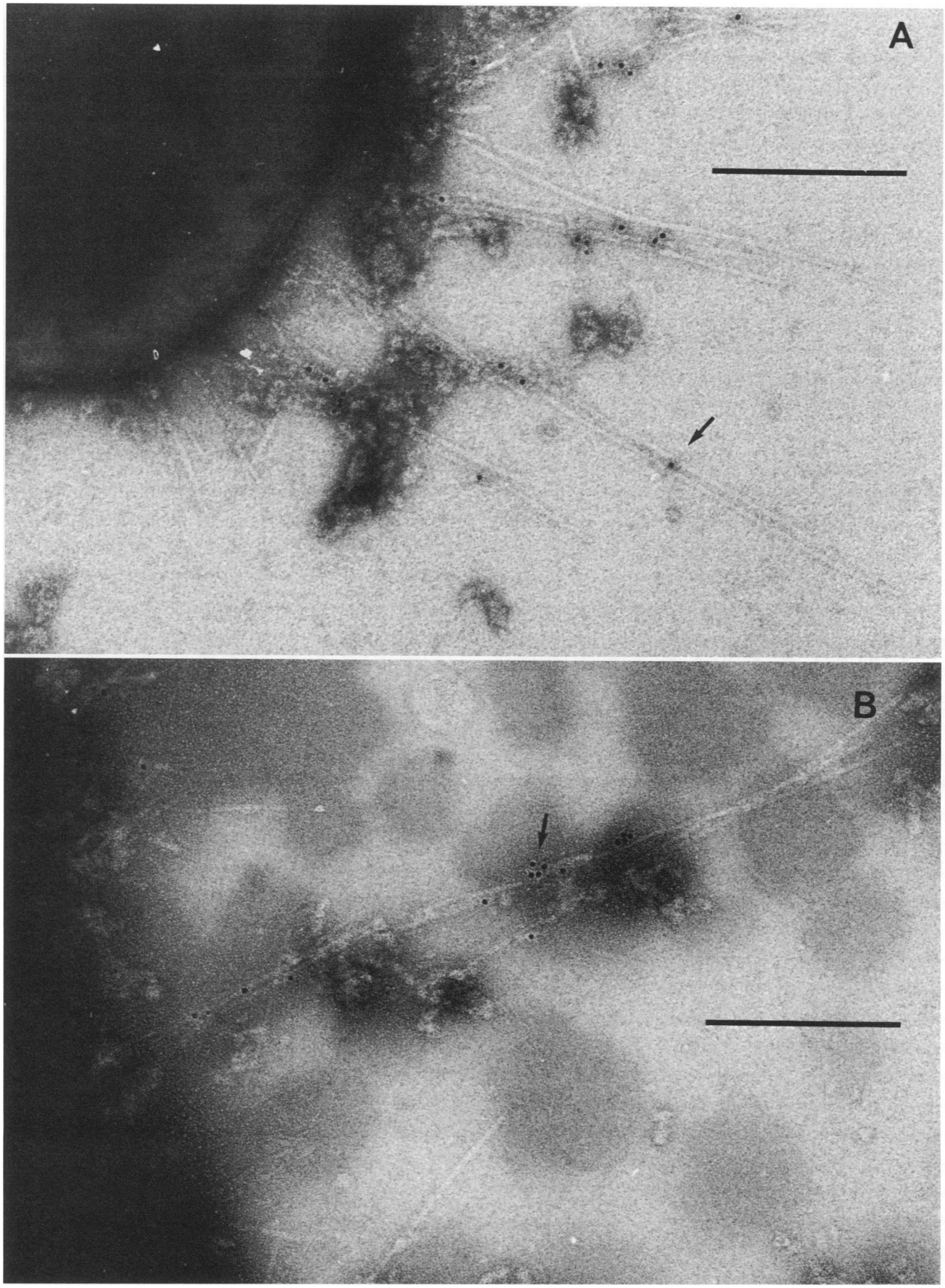

FIG. 3. Electron micrographs of *E. coli* K-12 strain HB101(pPKL4) producing type 1 fimbriae after incubation with D-mannose–BSA. Binding was visualized by incubation with rabbit anti-BSA, followed in incubation with gold-labeled protein A. FimH is indicated by the arrows. Bars 0.25 μm.

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