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Characterization and Identification of a Porcine Small Intestine Mucus Receptor for the K88ab Fimbrial Adhesin

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The ability of *Escherichia coli* K-12(K88ab) to adhere to immobilized porcine small intestine mucus was examined. *E. coli* K-12(K88ab) but not the isogenic *E. coli* K-12 strain was found to adhere readily to immobilized crude mucus but not to bovine serum albumin. The adhesion of *E. coli* K-12(K88ab) was inhibited in a specific fashion by anti-K88 antiserum. Adhesion was also inhibited by pretreatment of receptor-containing crude mucus preparations with sodium metaperiodate or proteolytic enzymes. Removal of glycolipids from crude mucus by chloroform-methanol extraction did not affect the ability of *E. coli* K-12(K88ab) to bind to mucus preparations. Adsorption of crude mucus preparations with K88ab fimbriae but not type 1 fimbriae resulted in the removal of K88-specific receptors. Analysis of the pelleted fimbriae-receptor complex by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, together with gel filtration chromatography of crude mucus preparations, suggest that the K88-specific receptor present in procine small intestine mucus is a 40- to 42-kDa glycoprotein.

Enterotoxigenic *Escherichia coli* strains bearing the K88 antigen have been associated with outbreaks of diarrhea in pigs (10, 13). The K88 antigen represents a virulence factor for these microorganisms that is contained in a fimbrial structure and acts as an adhesin, thus allowing the bacteria to adhere to the mucosal surface of the porcine small intestine. Such adhesion promotes colonization of the small intestine and is believed to be a prerequisite for infection (10, 11, 13, 15, 16). Indeed, the K88 adhesion system has frequently served as a model illustrating the relationship between adhesion and the ability to cause disease (31). There are several antigenic variants of the K88 fimbrial adhesin; three major subtypes (K88ab, K88ac, and K88ad) have been identified (12). It appears that these antigenic variants have adhesion specificities to brush borders isolated from pigs of different phenotypes (2, 12, 29).

The genetic organization and the specific characteristics of K88 fimbriae subunits have been analyzed by a number of investigators (for reviews, see references 16, 23, and 26). The K88 fimbrial adhesins have also been extensively investigated by using a variety of in vitro adhesion systems (for reviews, see references 6 and 14), but relatively little information is available regarding the nature of the porcine mucosal components that act as receptors for the K88 adhesin in vivo. Recently, it has been shown that a K88-specific receptor is present in porcine ileal mucus and that the amount of the receptor is age dependent (3, 4).

In the present study, the nature of K88ab fimbrial adhesin-specific receptors in porcine small intestinal mucus has been investigated. *E. coli* K-12 bearing K88ab fimbriae bind specifically to porcine small intestinal mucus, and such binding is reduced by pretreatment of the mucus with proteolytic enzymes or sodium metaperiodate. Data are also presented which suggest that the K88-specific receptor is a 40- to 42-kDa glycoprotein.

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MATERIALS AND METHODS

Bacterial strains. *E. coli* K-12 (Gyles) and *E. coli* K-12 (Gyles) (K88ab) were kindly provided by R. Wilson, *E. coli* Reference Center, Department of Veterinary Science, The Pennsylvania State University. *E. coli* K-12 (Gyles) (K88ab) was derived from *E. coli* K-12 (Gyles) by the addition of the K88ab plasmid; hereafter these strains will be referred to as *E. coli* K-12(K88ab) and *E. coli* K-12, respectively. The *E. coli* 2699 strain, described by Eshdat et al. (9), was used for purification of type 1 fimbriae.

Labeling of bacteria. *E. coli* K-12 and *E. coli* K-12(K88ab) were labeled as described previously by Laux et al. (20). Briefly, bacteria were grown overnight at 37°C on plates of brain heart infusion medium (BHI; Scott Laboratories, Fiskeville, R.I.) containing 10 μCi of [^{3}H]acetate per ml. Cells were harvested in *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid–Hanks buffer (HH buffer, pH 7.4), washed, and adjusted to an optical density at 600 nm of 1.0, corresponding to a cell density of about 10^9 CFU/ml.

Specific antisera. The antisera used, kindly provided by R. Wilson, were prepared in rabbits against *E. coli* K-12(K88ab) and *E. coli* K-12(K99) strains. Each antiserum was exhaustively adsorbed with *E. coli* K-12 before use. When employed in adhesion assays, all antisera were present at a final dilution of 1/150.

Purification of *E. coli* K-12 K88ab and type 1 fimbriae. Purification of K88ab fimbriae was performed as described by Stirm et al. (33). Routinely, bacteria were harvested, after overnight growth, from 12 150-mm plates containing BHI agar. Bacteria were pelleted and washed, and the fimbriae were sheared off. Bacterial and membrane debris were removed by centrifugation, and the fimbriae were recovered from the supernatant by precipitation. Acetic acid was used to adjust the pH to 5.3, which is close to the isoelectric point of K88ab fimbriae. Pelleted fimbrial preparations were then suspended in 0.01 M phosphate buffer (pH 7.4), and the fimbriae were further purified by ultracentrifugation (42,000

× g, 18 h). The resultant pelleted fimbriae were suspended in HH buffer (pH 7.4)–0.2% sodium azide and stored at 4°C.

Type 1 fimbriae were purified by $MgCl_2$ precipitations as described by Eshdat et al. (8). Briefly, bacterial cells were grown overnight in Luria broth, harvested, and washed, and the fimbriae were sheared off by blending. Pure type 1 fimbriae were obtained after several salt precipitations.

SDS-PAGE. Protein analysis was performed by 12.5% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) by the method of Laemmli (19). After electrophoretic separation, the gels were stained with Coomassie blue.

Porcine small intestinal mucus isolation. All porcine small intestine mucus used in this study was generously provided by Paul Runnels of the Animal Disease Center in Ames, Iowa. Mucus was isolated from 2- to 7-day-old outbred piglets. For isolation of mucus, the caudal one-fourth of the small intestine was separated and flushed with HH buffer (pH 7.4), until the buffer was clear. Next, the gut was cut into 10-cm-long sections and immersed into HH buffer. The sections were then split along the mesenteric border. Next, the opened section of the gut was swirled in the buffer to remove any debris, transferred to and rinsed in HH buffer, and finally transferred to 20 ml of fresh HH buffer. A rubber spatula was then used to gently scrape the mucosal surface into the buffer. This was repeated for each 10-cm section. The mucosal scraps from each section were then pooled and centrifuged at 27,000 × g at 4°C for 15 min to remove solids. The supernatant, containing crude intestinal mucus, was stored at −70°C. Before use in fimbrial adsorption experiments (see Results), mucus samples were centrifuged at 27,000 × g at 4°C for 9 h.

Determination of antibody present in crude mucosal preparations. Crude porcine mucus was assayed for the presence of specific anti-K88ab antibodies by an enzyme-linked immunosorbent assay with the ABTS Microwell Peroxidase Substrate System (Kirkegaard and Perry Laboratories, Gaithersburg, Md.). Purified K88ab fimbriae were immobilized on polystyrene microtiter plates. Twofold serial dilutions of each mucus preparation were then added, and the plates were incubated for 1 h at room temperature. After washing, peroxidase-labeled goat anti-swine immunoglobulin G, M, or A was added, and the plates were incubated for 1 h at room temperature. All plates were then washed, and substrate was added. As a control, rabbit anti-K88 antiserum was diluted in bovine serum albumin (BSA) (1 mg/ml) or procine small intestine mucus (1 mg of protein per ml) and assayed for reactivity with goat anti-rabbit immunoglobulin G as the second antibody. Both antiserum diluted in BSA and antiserum diluted in mucus had titers of 10,240.

Adhesion assay. The adhesion of *E. coli* K-12(K88ab) to porcine mucosal receptors was measured by using the in vitro assay of Laux et al. (20). All adhesion assays were performed in triplicate in multiwell polystyrene tissue culture plates (Linbro, flat bottom, 1.6 cm; Flow Laboratories, McLean, Va.). Crude porcine mucus was immobilized overnight at 4°C in wells at a concentration of 1 mg of protein per ml. Control wells were immobilized with BSA at a concentration of 1 mg of protein per ml. After immobilization, wells were washed twice, 0.2 ml of [^{3}H]acetate-labeled *E. coli* was added to each well, and the plates were incubated for 1 h at 37°C. Wells were then washed twice with HH buffer. Adherent bacteria were recovered by adding 0.5 ml of 0.5% SDS. After 3 h at 37°C, samples were collected and the level of radioactivity was determined.

Proteolytic enzyme treatment of pig mucus. Trypsin or pronase (800 μg/ml) was added directly to wells containing immobilized mucus, and the plates were incubated for 2 h at 37°C and overnight at 4°C. The plates were then washed, and the adhesion assay was carried out. Controls were performed by treating the mucus with BSA (800 μg/ml) rather than enzyme.

TABLE 1. Effect of specific antisera on adhesion of *E. coli* K-12(K88ab) to porcine small intestine mucus

Prepn immobilized	Treatment[a]	Adhesion (cpm ± SE)[b]
Mucus	Anti-K88	323 ± 25
Mucus	Anti-K99	2,370 ± 153
Mucus	Normal serum	2,084 ± 147
BSA	None	340 ± 49

[a] All sera were obtained from rabbits. Sera were added to wells at the same time as the bacteria. The final dilution for all sera was 1/150.

[b] In this experiment, the specific activity of the bacteria was 2.9×10^{-4} cpm/CFU. These data are means from triplicate samples.

Sodium metaperiodate oxidation of pig mucus. Mucus immobilized on tissue culture wells was treated with sodium metaperiodate or sodium iodate (0.01 M) in 0.2 M sodium acetate buffer (pH 4.5), incubated in the dark for 3 h at 4°C, washed twice with HH buffer, and assayed for bacterial adhesion.

Glycolipid extraction. Isolated porcine mucus was extracted twice with 10 volumes of chloroform-methanol-water (4:8:3, vol/vol/vol) (34) and then centrifuged at 10,000 × g for 30 min. Delipidated mucus was dried under nitrogen and stored at 4°C. When used for adhesions assays, delipidated mucus was suspended in its original volume of HH buffer and immobilized as described above.

Gel filtration chromatography. Crude porcine intestinal mucus was fractionated on a Bio-Rad agarose gel A.5 column (1.5 by 35 cm) equilibrated with HH buffer. Mucus samples containing a total of 10 mg of protein were applied to the column and eluted with HH buffer. Eluted protein was detected by reading the optical density at 280 nm. Individual fractions were immobilized and assayed for adhesion to *E. coli* K-12(K88ab) as described above.

RESULTS

The ability of *E. coli* K-12(K88ab) to adhere to immobilized crude porcine small intestine mucus was assessed. First, the ability of *E. coli* K-12(K88ab) to adhere to crude porcine small intestine mucus was compared with that of the isogenic *E. coli* K-12 strain, which lacks the plasmid that codes for the production of K88ab fimbriae. *E. coli* K-12(K88ab) was found to adhere readily to immobilized porcine small intestine mucus (4,164 ± 199 cpm) but not to BSA-treated control wells (501 ± 120 cpm), whereas the isogenic *E. coli* K-12 strain failed to adhere to the immobilized mucus preparation (323 ± 18 cpm) or to BSA (209 ± 19 cpm). [Data are means ± standard errors of triplicate samples. The specific activities of *E. coli* K-12 and K-12(K88ab) were 2.1×10^{-4} and 4.4×10^{-4} cpm/CFU, respectively.]

Binding of *E. coli* K-12(K88ab) to immobilized porcine small intestine mucus was inhibited in the presence of anti-K88 antiserum but not in the presence of normal serum or anti-K99 antiserum (Table 1). To ensure that the binding of the K88ab fimbriae to the porcine mucus was not due to the presence of anti-K88ab antibodies in the mucus preparations, all mucus preparations were assayed for the pres-

TABLE 2. Effect of proteolytic enzymes and sodium metaperiodate on adhesion of *E. coli* K-12(K88ab) to immobilized crude porcine small intestine mucus

Prepn immobilized	Pretreatment	Adhesion (cpm ± SE)[a]
Proteolytic enzymes		
Mucus	HH buffer	2,491 ± 153
Mucus	Pronase	432 ± 62
Mucus	Trypsin	1,244 ± 102
Mucus	BSA	2,199 ± 198
BSA	HH buffer	579 ± 56
BSA	Pronase	580 ± 37
BSA	Trypsin	679 ± 78
BSA	BSA	774 ± 100
Sodium meteriodate		
Mucus	Sodium acetate	2,224 ± 218
Mucus	Sodium iodate	1,976 ± 73
Mucus	Sodium metaperiodate	1,108 ± 245
BSA	Sodium acetate	497 ± 91

[a] These data are represented as means from triplicate wells. The specific activity of the bacteria was 2×10^{-4} cpm/CFU.

ence of anti-K88 antibodies. None of the 24 mucus preparations tested had detectable amounts of antibodies against K88ab fimbriae (data not shown), although 23 of the 24 mucus preparations were positive when immobilized and assayed for *E. coli* K-12(K88ab) adhesion.

Effect of proteases and sodium metaperiodate on adhesion. Previous studies have shown that adhesion of *E. coli* K-12(K88ab) to crude mouse small intestine mucus could be inhibited by treatment of the immobilized mucus with proteolytic enzymes or treatment with sodium metaperiodate (21). Pretreatment of crude porcine small intestine mucus with proteolytic enzymes or sodium metaperiodate produces a similar pattern of inhibition (Table 2). Pronase treatment reduced the level of *E. coli* K-12(K88ab) adhesion by about 80%, whereas trypsin treatment reduced the level of adhesion by about 50%. Similarly, pretreatment with sodium metaperiodate reduced the level of adhesion to crude mucus by approximately 50%. Treatment of immobilized mucus with sodium iodate or sodium acetate buffer had little effect on the level of adhesion (Table 2). When considered together, the above data indicate that porcine small intestine mucus contains receptors for the K88ab adhesin and suggest that the receptors involved may be glycoproteins.

Adhesion to glycolipids extracted from crude porcine mucus and glycolipid-free crude porcine mucus. Glycolipids extracted from the mucus were dissolved in chloroform-methanol (1:1, vol/vol) and subjected to high-performance thin-layer chromatography (17). Although crude porcine mucus contained substantial amounts of glycolipids, binding of radiolabeled *E. coli* K-12(K88ab) to mucus glycolipids separated on thin-layer chromatograms was not observed (data not shown). However, after extraction with chloroform-methanol, 10 independent lipid-free porcine mucus preparations were recovered and immobilized on polystyrene, and adhesion of *E. coli* K-12(K88ab) was assessed. Removal of glycolipids from the mucus had no effect on the ability of K88ab fimbriae to bind to mucus preparations. For the mucus, lipid-free mucus, and BSA preparations, the adhesion values were 3,202 ± 33, 3,300 ± 11, and 317 ± 57 cpm, respectively (means ± standard errors for triplicate wells). The specific activity of the bacteria was 1.4×10^{-4} cpm/CFU.

Identification of the receptor to K88ab fimbriae in crude porcine mucus. To identify the mucosal receptors interacting with K88ab fimbriae, crude porcine mucus was adsorbed with purified K88ab fimbriae as follows. Fimbriae (1 mg/ml) were added to crude porcine mucus (1 mg/ml) and incubated for 1 h at room temperature. The complex consisting of fimbriae and receptor(s) was then pelleted overnight by centrifugation (27,000 × *g* at 4°C). Pellets were suspended in a small amount of buffer, and both the pelleted material and supernatants were subjected to SDS-PAGE (Fig. 1). As a control, mucus was adsorbed with purified type 1 fimbriae and treated in an identical manner (Fig. 2). In addition, after adsorption, the supernatants were immobilized, and adhesion to *E. coli* K-12(K88ab) was assessed. Representative

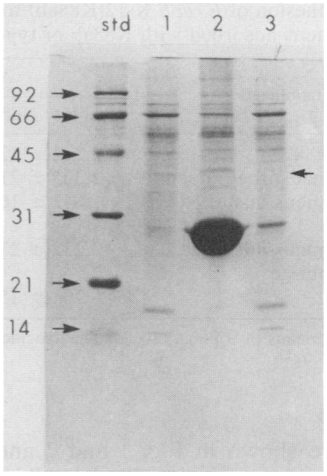

FIG. 1. Adsorption of porcine small intestinal mucus by purified K88ab fimbriae. Lanes: std, size standards; 1, small intestinal mucus; 2, pelleted intestinal mucus after adsorption with K88ab fimbriae; 3, mucus supernatant after adsorption with K88ab fimbriae. The arrow on the right indicates the 40- to 42-kDa protein.

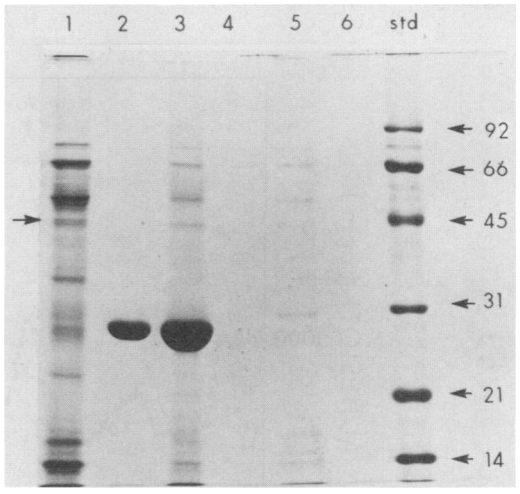

FIG. 2. Adsorption of porcine small intestinal mucus by purified fimbriae. Lanes: 1, small intestinal mucus; 2, purified K88ab fimbriae; 3, pelleted mucus after adsorption with K88ab fimbriae; 4, pelleted unadsorbed mucus; 5, mucus adsorbed with purified type 1 fimbriae; 6, purified type 1 fimbriae; std, size standards. Purified type 1 fimbriae do not enter the polyacrylamide gel, since they are not dissociated. The arrow on the left indicates the 40- to 42-kDa protein (lane 3).

TABLE 3. Adhesion of *E. coli* K-12(K88ab) to porcine small intestine mucus adsorbed with K88ab or type 1 fimbriae

Prepn immobilized	Adhesion	
	cpm ± SE[a]	% of control
Untreated mucus	2,372 ± 12	100
Supernatant of unadsorbed mucus	2,305 ± 21	97
Supernatant of mucus adsorbed with K88ab	842 ± 16	35
Supernatant of mucus adsorbed with type 1 fimbriae	2,232 ± 27	94
BSA	706 ± 15	29

[a] These data are means of triplicate wells. The specific activity was 2.5×10^{-4} cpm/CFU.

experiments are shown in Fig. 1 and 2 and Table 3. Ten mucus preparations were examined by this procedure; all produced similar results.

Whereas adsorption of mucus with K88 fimbriae resulted in the removal of all receptor activity, adsorption of the mucus with type 1 fimbriae did not remove the mucus receptor specific to the K88ab fimbriae. The mucus supernatant after adsorption with type 1 retained the ability of binding K88ab fimbriated *E. coli* when compared with unadsorbed mucus (Table 3).

Examination of the pelleted complex of K88ab fimbriae and receptor(s) by means of SDS-PAGE revealed that a number of proteins were present in the pelleted material. All but one of the proteins, a 40- to 42-kDa protein, were present in both the pelleted fimbrae-receptor complex and the receptor-free supernatant (Fig. 1, lanes 2 and 3, respectively). The 40- to 42-kDa protein was clearly enriched in the K88ab pelleted material and had been removed from the supernatant. Similarly, when the protein patterns obtained from two different fimbrial adsorptions are compared, i.e., K88ab and type 1 fimbriae (Fig. 2, lanes 3 and 5, respectively), the 40- to 42-kDa component is clearly present in the K88ab pelleted complex but not in the type 1 pelleted material; whereas the remaining major components are present in both samples. Attempts were made to stain the pelleted K88 fimbria-receptor complex with periodic acid-Schiff stain to detect glycosylated proteins; however, these proved to be negative. This may have been due to the small amount of protein present. Figure 2 also reveals the presence of two minor bands (22 and 16 kDa) in the K88ab pelleted material. These may represent breakdown products of the higher-molecular-weight protein. This hypothesis is supported by the observation that as mucus proteins degraded over time in crude mucus preparations, the smaller proteins appeared to increase in concentration, whereas the high-molecular-weight band appeared to decrease (data not shown). Figure 2 also reveals the presence of a 29-kDa protein in the material which pelleted with the type 1 fimbriae (lane 5) but did not appear to pellet with the K88 fimbriae (lane 3). It may be, however, that the 29-kDa protein is present in lane 3 but obscured by the large K88 fimbrial subunit band.

It should be noted that lane 5 in Fig. 2 does not show the presence of type 1 fimbrial structural proteins because the sample was not subjected to acid hydrolysis. As a result the type 1 fimbriae are not disassociated and do not enter the gel. When the fimbriae are subjected to such treatment, a prominent 18-kDa type 1 fimbrial subunit band is obtained.

Fractionation of crude mucus by gel filtration. Crude porcine mucus was subjected to gel filtration chromatography. Fractions were collected, and the A_{280} of each fraction was determined. Individual fractions were then immobilized, and the ability of *E. coli* K-12(K88ab) to adhere to individual fractions was assessed. Adhesion was primarily associated with fractions representing components in the range of 40 to 60 kDa, whereas the majority of components present were of lower molecular mass (Fig. 3). Individual fractions were also subjected to SDS-PAGE. A 40-42 kDa protein, together with a large number of other proteins, was observed in the fractions where specific adhesion to K88ab fimbriae was detected (fractions 75 through 79 in Fig. 3; data not shown). This protein was not observed in any of the fractions that were negative in the adhesion assay. The presence of a 40- to 42-kDal component in the higher-molecular-mass fractions

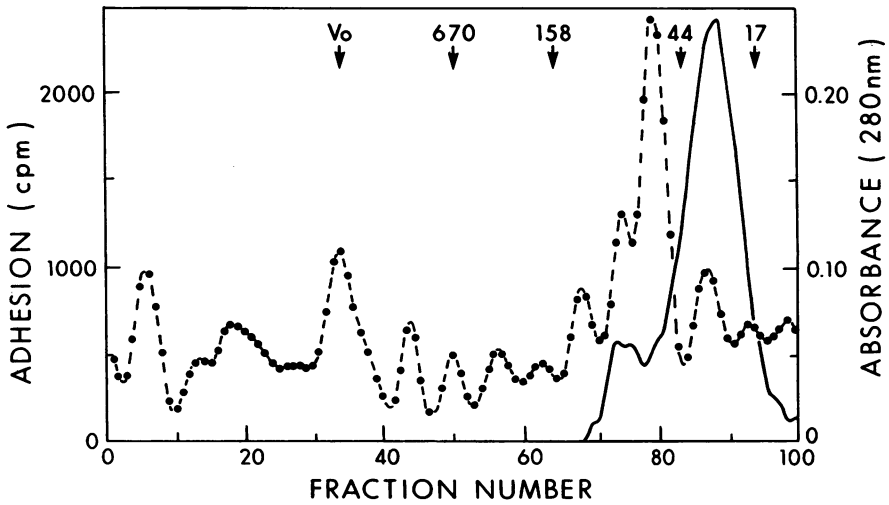

FIG. 3. Adhesion of *E. coli* K-12(K88ab) to fractions of porcine mucus obtained by gel filtration using Bio-Gel A.5. A total of 10 mg protein was applied to the column, which was equilibrated with HH buffer. Fractions of 2.5 ml were collected. Symbols: (——) A_{280}; (●----●) adhesion of *E. coli* K-12(K88ab) to immobilized fractions. Vo, Void volume. Numbers and arrows at the top represent approximate location of molecular size standards (kilodaltons).

suggests that this component may be present as an oligomer or that it may be associated with other mucus components.

DISCUSSION

We have examined the ability of an *E. coli* K-12 strain that possesses K88ab fimbriae to adhere to crude small intestine mucus preparations isolated from 2- to 7-day-old piglets. *E. coli* K-12(K88ab), but not *E. coli* K-12, bound specifically to components present in these preparations. Our data suggest that the receptor for the K88ab fimbrial adhesin in porcine small intestinal mucus is glycoprotein in nature, and we present data suggesting that the receptor has a molecular mass of 40 to 42 kDa. Evidence for the molecular mass, however, is indirect and is based on selective removal of both the 40- to 42-kDa component and mucus receptor activity by adsorption with K88 fimbriae, enrichment for and recovery of the 40-42 kDal component in the pelleted K88 fimbria-receptor complex, and the presence of this component in gel filtration fractions with receptor activity.

The glycoprotein nature of the pig intestinal mucus receptor for *E. coli* strains carrying the K88 fimbrial adhesin is supported by previous reports from a number of laboratories. By using in vitro binding assays of K88 fimbriae to isolated porcine intestinal brush borders, Sellwood (27) and Anderson et al. (1) concluded that glycoproteins are involved in the attachment of K88 fimbriae to intestinal epithelial cells. Laux et al. (21) have shown that the receptors to K88 fimbriae present in mouse small intestinal mucus are glycoproteins, which may contain D-galactosamine or D-galactosamine-like residues. In addition, D-galactosamine has been reported to inhibit the binding of K88 fimbriae to brush borders isolated from pig intestines by 40% (28, 32). Furthermore, Staley and Wilson (32) reported that the K88-specific small intestinal receptors in porcine brush borders are 23- and 35-kDa glycoproteins, and it will be of interest to determine the relationship between the 35-kDa brush border glycoprotein receptor and the 40- to 42-kDa mucus glycoprotein receptor reported here.

Several receptors for fimbrial adhesins have been shown to be glycolipids. For example, the K99 fimbrial adhesin receptor was determined to be the glycolipids *N*-glycolyl-GM_3 and *N*-glycolyl sialoparagloboside (18, 25, 30), and the P fimbriae of uropathogenic *E. coli* have been shown to interact with glycolipids containing Gal-α1→4β-Gal (22). Although a number of glycolipids were detected in the crude mucus preparations in this study, no evidence was obtained that would suggest that specific glycolipids play a role in adhesion of K88ab fimbriae to the mucus preparations. Removal of glycolipids did not prevent subsequent adhesion to the remaining lipid-free mucus components, and no adhesion to extracted glycolipids was detected. This does not, of course, rule out a possible role for glycolipid receptors in adhesion to intestinal epithelial cells. Indeed, it has been previously suggested that glycolipids may indeed play a role in the adhesion of K88ab fimbriae to pig intestinal brush borders (13).

Specific receptors for several other adhesins have been identified in intestinal mucus, and it has been suggested that such receptors play an important role in allowing or preventing the attachment of enterotoxigenic *E. coli* to the intestine. Pig small intestinal mucus appears to have specific receptors to 987P fimbriated *E. coli* (5). Calf small intestinal mucus was seen to contain specific receptors for the K99 and F41 fimbriae (21). Specific receptors for the K99 fimbriae were also found in mouse intestinal mucus (24), and rabbit ileal mucus has been shown to contain receptors specific for *E. coli* RDEC-1 AF/R1 fimbriae (7). Although it is clear that receptors for a variety of adhesins are present in intestinal mucus, the role of such receptors in the infectious process is unclear. Similarly, the origin of mucus receptors and their relationship to brush borders remain to be elucidated.

Previously, we utilized a modified Western immunoblot assay (21) for the identification of K88ab-specific glycoprotein receptors in the mouse intestinal mucus. Mucosal proteins were separated by SDS-PAGE, transferred to nitrocellulose, and overlaid with $^{35}SO_4$-labeled *E. coli* K-12(K88ab). In the present study, this procedure was also employed; however, it was not possible to get consistent results. This could be due to the denaturing conditions created by SDS and mercaptoethanol treatments, which altered the conformation of the receptor in such a way as to destroy its binding ability to K88ab fimbriae. Nevertheless, adsorbing the intestinal mucus with purified fimbriae seems promising for further characterization of the K88ab receptor.

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