Characterization of O-glycan moieties of the 210 and 240 kDa pig intestinal receptors for *Escherichia coli* K88ac fimbriae

Didier Seignolé,¹ Philippe Grange,¹ Yvonne Duval-Iflah² and Michèle Mouricout¹

Author for correspondence: Michèle Mouricout. Tel: +33 55 45 76 61. Fax: +33 55 45 76 53.

The porcine brush border glycoproteins of 210 and 240 kDa, recognized by *Escherichia coli* K88ac fimbriae, contained O-linked oligosaccharides. The carbohydrate moieties were analysed by deglycosylation, lectin-binding and agglutination assays. Neuraminidase susceptibility of the 210 kDa receptor suggested that a sialoglycoprotein may act as receptor for the K88ac fimbriae. In contrast, K88ac-binding to the 210 and 240 kDa glycoproteins totally disappeared after fucosidase treatment, indicating the critical role of fucosyl residues at the receptor sites. Among the oligosaccharides extracted from these O-glycoproteins, K88ac fimbriae showed affinity for neutral sugar chains while sialylated species were not recognized. Our data suggest a possible role of the polypeptide backbone in the definition of receptor sites. Specific agglutination by K88ac-fimbriated *E. coli* of the erythrocytes of the hamster *Mesocricetus auratus* was inhibited by the anti-T peanut lectin and the lectins of *Datura stramonium, Aleuria aurantia* and *Maackia amurensis*. Hence, we propose that Galβ1-3GalNAc- and Fucα1-2Galβ1-3/4GlcNAc- are the main sequences mediating K88ac fimbrial binding. These structures were not detected in the non-adhesive piglet brush borders characterized by a high carbohydrate content. Additional oligosaccharides probably masked the underlying receptor structures.

**Keywords:** pig intestinal receptors, O-glycan moieties, *E. coli* K88ac fimbriae, lectin binding

INTRODUCTION

Among the enterotoxigenic *Escherichia coli* expressing one of the K88 fimbrial variants ab, ac or ad, strains possessing the K88ac variant are dominant in piglets older than one week in different geographic areas (Söderlind *et al.*, 1988; Westerman *et al.*, 1988; Osek & Svennerholm, 1991). It has been found that K88ac-positive *E. coli* bind only to the brush border of enterocytes and not to undifferentiated cryptic cells or incomplete microvillar borders (Cox *et al.*, 1988). Two glycoproteins of 210 and 240 kDa that bind to K88ac fimbriae have been identified (Erickson *et al.*, 1992). Their presence in adhesive brush borders may be the basis for the susceptibility of piglets to K88ac-positive *E. coli* infection.

We have previously identified intestinal glycoproteins recognized by the K88 (ab, ac and ad) fimbriae, and we noticed that binding patterns depended on the piglet brush border adhesion phenotype (Mouricout *et al.*, 1990). In fact, to establish a relationship between K88-binding and brush border phenotypes, it was necessary to determine the oligosaccharide moieties of intestinal glycoproteins involved in the bacterial recognition. Most of the studies have been concerned with the characterization of receptors of K88ab fimbriae (Laux *et al.*, 1986; Conway *et al.*, 1990; Metcalfe *et al.*, 1991; Willemsen & de Graaf, 1992; Blomberg *et al.*, 1993); no detailed study is available for K88ac fimbriae. Therefore, piglet brush borders of non-adhesive phenotype I [corresponding to K88ab(−), ac(−) and ad(−)] and of adhesive phenotypes III

---

**Abbreviations:** Fuc, fucose; Gal, galactose; Man, mannose; GalNAc, N-acetylgalactosamine; GlcNAc, N-acetylglucosamine; SA, sialic acids; NeuAc, N-acetylneuraminic acid; HPTLC, high-performance thin-layer chromatography; AAA, DSA, GNA, MAA, PNA, SNA, digoxigenin-labelled lectins from *Aleuria aurantia*, *Datura stramonium*, *Galanthus nivalis*, *Maackia amurensis*, *Arachis hypogaea* and *Sambucus nigra*. 

© 1994 SGM 0001-8657 2467
[K88ab(+), ac(+), and ad(-)] and IV [K88ab(+), ac(-) and ad(+)] (Rapacz & Hasler-Rapacz, 1986) were analysed.

In the present paper, we characterized the oligosaccharide moieties of the receptive glycoproteins compared with those of non-receptive ones by chemical and lectin-binding analyses and investigated the effects of glycidosides on the receptivity of K88ac receptors.

**METHODS**

*Escherichia coli* strains. Strains P2200 (O149:K91: K88ac, ST', LT'), P423.1 (O08:K2:K88ad, ST', LT') and CS148 (O117:K88ab, ST', LT') were grown on Minca agar for 18 h at 37 °C, harvested, washed and resuspended in PBS, pH 7.2, to a concentration of 10⁶ cells ml⁻¹ (Seignole et al., 1991). Strains were radiolabelled with sodium acetate (2 GBq mol⁻¹). Fimbrination was assessed by haemagglutination of guinea-pig erythrocytes in presence of 0.4 M mannose and by chromatography on Phenyl-Sepharose (Lindahl et al., 1987). Bacterial suspensions expressing four haemagglutination units (1 HU) was the highest dilution at which agglutination occurred.

Animal tissue samples. Nineteen Large White pigs (Institut National de la Recherche Agronomique, La Miniere, France) were killed at the age of 15–21 d and brush borders from the jejunum were prepared (Seignole et al., 1991). Animals were phenotyped in vitro for the ability of the brush borders to bind K88-fimbriated *E. coli* with CS148 (K88ab), P2200 (K88ac) and P423.1 (K88ad) strains (Duval-Iflah & Chappuis, 1984).

Membrane proteins were extracted with 1% (w/v) sodium deoxycholate, 1 mM phenylmethylsulfonyl fluoride in 10 mM Tris/HCl buffer, pH 8 (Juliano & Li, 1978). After incubation at 4 °C for 1 h with stirring, the mixture was centrifuged (86 500 g for 1 h), the supernatant fractions were collected and lyophilized.

Neutral and acidic oligosaccharides were separated by HPLC (HPTLC, Silica Gel 60, Merck) using ethanoll:n-butanol/pyridine/acidic acid/water (100:10:10:3:30, v/v). Anion-exchange chromatography on pulsed liquid chromatography (HPLC, Silica Gel 60, Merck) using ethanoll:n-butanol/pyridine/acidic acid/water (100:10:10:3:30, v/v). 2-Dimensional gel electrophoresis was performed on 6–30% gradient ampholytes. Densitometry was performed on 4800 unit, 10⁸ cells ml⁻¹) was added to the incubation mixture and the minimum inhibitory concentration was estimated.

**Analytical methods.** Proteins were separated by 7.5% SDS-PAGE, (SE 250 Mighty Small II unit, Hoeffer). Samples (50 μg) were treated at 100 °C for 5 min in buffer containing 600 mM β-mercaptoethanol. Molecular mass markers (carbonic anhydrase, ovalbumin, BSA, phosphorylase, β-galactosidase and myosin) were purchased from Sigma. Proteins, visualized with Coomassie brilliant blue, and glycoproteins, revealed by the periodic acid–Schiff method, were recorded by densitometric scanning. Sialylated and neutral sugars were assayed according to Svenneholm (1957) and Rao & Patrabiraman (1989), respectively.

Monosaccharides were analysed by gas chromatography (Staley & Wilson, 1983). Carbohydrates after methanolysis were reacetylated in pyridine/acidic anhydride (1:1, v/v) for 24 h and derivatized by bis(trimethylsilyl)trifluoroacetamide in pyridine for 2 h. Trimethylsilyl monosaccharides were separated on a DB-1 column (30 m × 0.33 mm, J & W Scientific) and quantified with methyl-1,1-trimethylsilane as standard.

**Enzymatic treatments of glycoproteins.** Glycoproteins (1 mg) were incubated at 37 °C overnight with *Vibrio cholerae* stialidase (0.012 U) in 50 mM sodium acetate, 10 mM calcium chloride, 100 mM sodium chloride, pH 5.5. The glycoproteins were also treated with bovine kidney α-L-fucosidase, (400 U) in 100 mM sodium citrate/phosphate buffer, pH 6, for 48 h at 37 °C. This treatment has been shown to lead to 100% desialylation of Fucα1-2Galβ1-4GlcNAcβ-oligosaccharides without affecting Galβ1-4[Fucα1-3]GlcNAcβ1-3Galβ1-1 (Leα antigen) and Fucα1-6GlcNAc linkages (Monteau et al., 1986).

**Preparation of O-glycans from glycoproteins by elimination and separation of oligosaccharides.** Glycoproteins (25 mg ml⁻¹) were incubated in 50 mM sodium hydroxide/1 M sodium borohydride at 45 °C for 16 h and excess borohydride was neutralized. After evaporation, the residue was dissolved in water and O-glycans were separated from O-deglycosylated proteins on a Sephadex G50 column (Corelli et al., 1991). Neutral and acidic oligosaccharides were separated by HPLC (MicroPAK AX10, Spectra Physics), on a potassium phosphate gradient (0–200 mM, flow rate 1 ml min⁻¹, pH 4; Green & Baenziger, 1986). Oligosaccharides were detected by absorption at 205 nm and calibrated with GlcNAc and NeuAc. They were separated by high-performance thin-layer chromatography (HPTLC, Silica Gel 60, Merck) using ethanol/n-butanol/pyridine/acidic acid/water (100:10:10:3:30, v/v). 2-Dimensional gel electrophoresis was performed on 4800 unit, 10⁸ cells ml⁻¹. Neutral and sialylated glycans were detected by the 80% (v/v) resorcinol/10% (v/v) HCl reagent.

**Characterization of the 210 and 240 kDa glycoprotein oligosaccharide chains by plant lectins.** Glycoproteins (15 μg) were electrotransfered onto nitrocellulose membranes (Schleicher & Schuell) after 7.5% SDS-PAGE. Glycosylation was analysed with digoxigenin-labelled lectins (1 mg ml⁻¹; from Boehringer) *Alteoria aurantia* (AAA), *Datura stramonium* (DSA), *Galanthus nivalis* (GNA), *Maackia amurensis* (MMA), *Arabis hypogaea* (PNA) and *Sambucus nigra* (SNA) according to Haselbeck et al. (1990). Carboxypeptidase Y, transferrin, fetuin and asialofetuin were used as controls with the appropriate lectins. Specificities of the lectins are as follows. PNA, the anti-T lectin (Loton et al., 1975), is specific to the non-sialylated core unit of O-glycans, Galβ1-3GalNAc. AAA recognizes Fucα1-6GlcNAc, Fucα1-2Galβ1-3GlcNAcβ1-3Galβ1-1 (H antigen), Galβ1-3[Fucα1-4GlcNAcβ1-3Galβ1-1 and Fucα1-2Galβ1-3Fucα1-4GlcNAcβ1-3Galβ1-1. DSA binds to Galβ1-4GlcNAc and GlcNAc/Ser-Tr. GNA recognizes Manα1-2(or 3 or 6)Man. MAA recognizes Saα2-3Gal or GalNAc, Saα2-3Galα1-4GlcNAcβ1-1 or Galβ1-3GlcNAcβ1-1. SNA reacts with Saα2-6Gal, Saα2-6[Galβ1-4 GlcNAcβ1-1 or Galβ1-3GlcNAcβ1-1] (Goldstein & Poretz, 1986).

**Mannose resistant haemagglutination (MRHA) and its inhibition.** A suspension of *E. coli* strain P2200 (75 μl, 10⁶ cells ml⁻¹) was incubated with an identical volume of hamster (*Mesocricetus auratus*) erythrocytes (3%, v/w) in PBS in the presence of α-methyl o-nanopynosanide for 2 h at 4 °C. For inhibition assays, haemagglutination was carried out with 2 HU bacterial cells for 2 h at 4 °C. The assays contained either serially twofold diluted lectins AAA, DSA, GNA, MAA, PNA or SNA (10 μl, 1 mg ml⁻¹) or oligosaccharides of O-glycoproteins extracted from adhesive brush borders, or N,N′,N′′,N″-tetraacetylchitotetraose, siaulactose or disialylallo-N-tetraose (50 μg ml⁻¹). Incubations were performed for 2 h with shaking and the minimum inhibitory concentration was estimated.

**E. coli** binding assays to blotted glycoproteins and O-glycans. After SDS-PAGE, proteins were electrod other onto nitrocellulose sheets. Membranes were coated with 1% (w/v) BSA in 10 mM PBS, pH 7.2, at 4 °C overnight (Conway et al., 1990). ¹⁴C-labelled K88-positive or K88-negative *E. coli* suspension (1 OD₅₅₀ unit, 10⁹ cells ml⁻¹, 14–30 μBq per bacterium) was added and incubated for 2 h at 20 °C. Membranes were washed in PBS containing 0.02% Tween 20.

Neutral and sialylated oligosaccharides (2–3 μg) were chromatographed on silica gel G 60 thin-layer plates in solvent A. Plates were treated with 0.5% (w/v) poly(isobutyl methacrylate)
Table 1. Carbohydrate analyses of glycoproteins extracted from E. coli K88ac-adhesive and E. coli K88ac-non-adhesive brush border membranes from piglet intestines

Trimethylsilylmonosaccharides were separated by gas chromatography and quantified with meso-inositol as internal standard.

<table>
<thead>
<tr>
<th>Carbohydrate</th>
<th>Sugar proportion (% w/w)</th>
<th>K88ac-adhesive brush borders</th>
<th>K88ac-non-adhesive brush borders</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>N &amp; O-Glycans*</td>
<td>O-Glycans†</td>
</tr>
<tr>
<td>Fucose</td>
<td>2.3 ± 1.7</td>
<td>8.11</td>
<td>7.3 ± 3.3</td>
</tr>
<tr>
<td>Glucose</td>
<td>9.4 ± 2.3</td>
<td>9.11</td>
<td>10.5 ± 4.8</td>
</tr>
<tr>
<td>Galactose</td>
<td>24.3 ± 3.8</td>
<td>41.50</td>
<td>31.0 ± 4.0</td>
</tr>
<tr>
<td>Mannose</td>
<td>150 ± 3.5</td>
<td>—</td>
<td>180 ± 2.4</td>
</tr>
<tr>
<td>N-Acetylglactosamine</td>
<td>161 ± 5.8</td>
<td>5.6</td>
<td>145 ± 1.9</td>
</tr>
<tr>
<td>N-Acetylglucosamine</td>
<td>261 ± 3.5</td>
<td>15.30</td>
<td>152 ± 4.5</td>
</tr>
<tr>
<td>Sialic acid</td>
<td>60 ± 15</td>
<td>5.8</td>
<td>38 ± 2.1</td>
</tr>
</tbody>
</table>

* Total glycans linked to N and O-glycoproteins were determined. Mean values ± SD were determined by analysis of brush border membranes from four 14-20-d-old piglets.
† Oligosaccharides were isolated from O-glycoproteins by β-elimination in the presence of 1 M NaBH₄.
‡ Results were obtained from two individuals with adhesive brush borders (phenotypes III and IV) and two with non-adhesive brush borders.

Table 2. Characteristics of the 210 and 240 kDa glycoprotein receptors for E. coli K88ac fimbriae

<table>
<thead>
<tr>
<th>Pig phenotype</th>
<th>Intensity of adhesion*</th>
<th>Protein or carbohydrate proportion (% w/w)‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>210 kDa receptor</td>
<td>240 kDa receptor</td>
</tr>
<tr>
<td></td>
<td>Protein</td>
<td>Carbohydrate</td>
</tr>
<tr>
<td>IV</td>
<td>++++</td>
<td>++++</td>
</tr>
<tr>
<td>IV</td>
<td>+++</td>
<td>++++</td>
</tr>
<tr>
<td>III</td>
<td>+++</td>
<td>++++</td>
</tr>
<tr>
<td>III</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td></td>
<td>tr</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>0.3</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td>tr</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>6 ± 1</td>
<td>16 ± 1</td>
</tr>
</tbody>
</table>

* Adhesion of K88ac-fimbriated E. coli strain P2200 (10⁹ c.f.u. ml⁻¹) was assayed on brush borders isolated from proximal jejunum of piglets by in vitro adhesion tests as indicated in Methods. Results were scored as follows: ++++, all brush borders adhesive; ++, 75% and +, 50% of brush borders with 6–8 bacteria attached; –, all brush borders free of bacteria. In control experiments, the non-fimbriated strain did not bind to any component of adhesive brush borders and the fimbriated strain did not bind to any component of non-adhesive ones.
† Proteins were stained by the Coomassie brilliant blue reagent and glycoproteins by periodic acid-Schiff assay. Quantification of the protein backbone and carbohydrate moieties was performed by integration after densitometric scanning. tr, trace amounts. Mean carbohydrate proportions ± SD are given at the bottom of the respective columns.

(Phleugum P28, Röhm) in diethyl ether followed by blocking of non-specific sites with 2% (w/v) BSA in PBS. The plates were then incubated with the 14C-labelled K88-positive or K88-negative E. coli suspensions for 2 h at 20 °C. Staining controls were performed with the orcinol/HCl reagent on plates, after removing the plastic by sequential washings in diethyl ether.

Autoradiography was performed using Hyperfilm-MP film (Amerham).

RESULTS

Glycosylation of membrane-bound glycoproteins and K88ac adhesion

Adhesive brush borders contained 0.5 ± 0.2 mmol neutral sugars and 0.08 ± 0.03 mmol sialic acids [(g protein)⁻¹, mean ± SD] whereas non-adhesive ones contained 1.7 ± 0.2 mmol neutral and 0.15 ± 0.05 mmol sialylated
sugars [(g protein)⁻¹, mean ± SD]. In the first group, the ratio of sialic acid to neutral sugars was 0.16; in the second group, this ratio was 0.09. High molecular mass glycopolymers accounted for 22% of total glycopolymers (Fig. 1a, b; lanes 1 and 3). In non-adhesive membranes, their amounts were found to be dependent on the adhesin phenotype (Table 1). Average molar ratios of Fuc: Gal: GalNAc: GlcNAc were 1:3:1:3:0.3 in adhesive brush borders and 1:5:2:1:2:0.05 in non-adhesive types. The two K88ac receptors of molecular masses 210 and 240 kDa represented 0.5–0.6% of total proteins in brush borders of the two adhesive phenotypes III and IV (Fig. 1c, lane 8). These receptors contained, respectively, 65 ± 1.5% and 157 ± 1.3% (mean ± SD) of total carbohydrates (Table 2). β-Elimination of O-glycosidically linked carbohydrate chains from the receptors led to a loss in K88ac-binding (Fig. 1, lanes 5 and 8). Fucosidase destroyed the two receptors (Fig. 1, lane 6) whereas the 210 kDa glycoprotein receptor disappeared after neuraminidase treatment (Fig. 1, lane 7). These two glycoproteins were not recognized by K88ab or K88ad fimbriae (data not shown).

**Table 3. Inhibition of E. coli K88ac-mediated agglutination of M. auratus erythrocytes by lectins and the oligosaccharides isolated from porcine intestinal membrane-bound O-glycoproteins**

Bacteria were titrated with erythrocytes and bacterial densities corresponding to twice the lowest density giving haemagglutination were used for inhibition studies. Agglutination was determined after 2 h at 4 °C, then inhibitors were added to the mixture and haemagglutination followed. Haemagglutination was mannose-resistant.

<table>
<thead>
<tr>
<th>Inhibitors</th>
<th>Minimum concentration (µg ml⁻¹) causing 100% inhibition of agglutination</th>
</tr>
</thead>
<tbody>
<tr>
<td>PNA</td>
<td>0.5</td>
</tr>
<tr>
<td>MAA</td>
<td>4</td>
</tr>
<tr>
<td>DSA</td>
<td>8</td>
</tr>
<tr>
<td>AAA</td>
<td>8</td>
</tr>
<tr>
<td>SNA</td>
<td>64</td>
</tr>
<tr>
<td>GNA*</td>
<td>&gt; 64†</td>
</tr>
<tr>
<td>Neutral O-glycans</td>
<td>1.65‡</td>
</tr>
<tr>
<td>Monosialyl-oligosaccharides</td>
<td>&gt; 100†</td>
</tr>
<tr>
<td>Disialyl-oligosaccharides</td>
<td>&gt; 50†</td>
</tr>
</tbody>
</table>

* GNA which recognizes non-reducing Man α1-2(or 3 or 6)Man was used in control experiments.
† No inhibition occurred at the given concentration.
‡ Lowest concentration tested.

The receptive glycoproteins bound to A3A (Fig. 2, lane 3), DSA (lane 5) and PNA (lane 7) indicating O-glycosylation sites, and the presence of fucose, Galβ1-...
4GlcNac- and Galβ1-3GalNAc- sequences, respectively. Desialylation increased the level of PNA binding (data not shown).

Conversely, the high molecular mass glycoproteins from non-adhesive brush borders or defucosylated glycoproteins extracted from adhesive brush borders did not react with AAA (Fig. 2, lanes 1 and 2), DSA (lane 4) or PNA (lane 6). High molecular mass glycoproteins of non-adhesive and adhesive brush border extracts bound to MAA (Fig. 2, lanes 8 and 9, respectively). α2-6-linked sialic acid was identified only in traces by SNA in brush borders of the two adhesion phenotypes (lanes 10 and 11). PNA completely reversed K88ac-specific binding (Table 3) at low concentration (0.5 μg ml⁻¹), whereas DSA and AAA exerted inhibitory effects only at 8 μg ml⁻¹. MAA bound better than SNA (binding at 4 and 64 μg ml⁻¹, respectively).

**Oligosaccharides extracted from adhesive and non-adhesive O-glycoproteins**

Neutral oligosaccharides were eluted rapidly during HPLC (Fig. 3a, b, peak 1) and sialylated forms were separated in monosialylated (Fig. 3a, peaks 2–3; Fig. 3b, peaks 2–4) and disialylated species (peak 5). Neutral species represented 76 and 70% of carbohydrate from adhesive and non-adhesive O-glycoproteins, respectively.

Neutral oligosaccharides extracted from adhesive brush borders contained respectively, Fuc, Gal, GalNAc and GlcNAc in a molar ratio of 0:5:1:4:1:1:5 with respect to GalNAc.

Monosialylated oligosaccharides represented 23 and 20% in the two adhesion phenotypes, but they were in inverse proportion (Fig. 3a, peaks 2 and 3). Disialylated structures were ten times more abundant in the non-adhesive than in the adhesive phenotype (Fig. 3, peak 5).

Oligosaccharide fractions were then separated by HPTLC. Three neutral species (a, d and e) were found in the adhesive phenotypes (Fig. 4A, lane 1), while a single species (c) was recovered in the non-adhesive one. Neutral oligosaccharides b, f and g (Fig. 4A, B, lane 1) and acidic oligosaccharides (lanes 2 and 3) were present irrespective of the adhesion phenotype.

**Affinity of K88ac fimbriae for the oligosaccharides extracted from O-glycoproteins**

Oligosaccharides were overlaid with radiolabelled K88ac-fimbriated E. coli. The reactive spots corresponded to the neutral oligosaccharides a, b and d (Fig. 4C, lane 1). No adhesion to acidic fractions was detected (lanes 2 and 3). K88ac fimbriae did not react with oligosaccharides extracted from non-adhesive brush borders. No binding...
occurred with \(N,N',N'',N'''\)-tetraacetylchitotetraose (GlcNAc\(\beta\)1-4GlcNAc\(\alpha\)2, sialyllactose (NeuAc\(\alpha\)2-3Gal\(\beta\)1-4Glc) and disialyllacto-N-tetraose (NeuAc\(\alpha\)2-3Gal\(\beta\)1-3\(\alpha\)2-6GlcNAc\(\beta\)1-3Gal\(\beta\)1-4Glc).

Agglutination of \(M. \) auratus erythrocytes induced by K88ac fimbriae was completely reversed by the neutral oligosaccharides isolated from adhesive brush borders (Table 3). They were active to the lowest concentration tested (1.65 \(\mu\)g ml\(^{-1}\), i.e. 16.5 ng). Haemagglutination was not inhibited by mono- and disialylated oligosaccharides, even at the highest concentrations used, namely 100 and 50 \(\mu\)g ml\(^{-1}\), respectively.

**DISCUSSION**

It appears that the structure of the receptor sites should be elucidated with regard to each variant of \(E. \) coli K88 fimbriae. Interestingly, receptors specific for each type seem to exist. K88ab fimbriae bind to porcine, chicken and guinea-pig erythrocytes, whereas K88ac fimbriae weakly agglutinate only guinea-pig erythrocytes (Bijlsma & Frik, 1987). Rabbit erythrocytes are agglutinated by all three fimbrial variants. Erythrocytes of the hamster \(Cricetus cricetus\) are agglutinated by K88ab only. In contrast, erythrocytes of the hamster \(Mesocricetus auratus\) are agglutinated by K88ac fimbriae (Bakker et al., 1992). Our results indicated that K88ac fimbriae exhibit specific binding to the brush border O-glycoproteins of molecular masses 210 kDa and 240 kDa, while K88ab and K88ad fimbriae did not attach to these glycoproteins. Moreover, it is known that K88ab fimbriae bind to receptors of molecular masses 25, 35, 57, 64 and 91 kDa identified in the brush borders and mucus of mouse and pig (Laux et al., 1986; Conway et al., 1990; Metcalfe et al., 1991).

K88ab and K88ac fimbriae each bind to their own set of receptors even if galactosides act as the receptors (Gibbons et al., 1975; Sellwood, 1980). Hence, it is important to note that Gal\(\beta\)1-3GalNAc- and Gal\(\beta\)1-3\(\alpha\)2-4GlcNAc-sequences were an essential part of the receptor sites for K88ac fimbriae, contrary to the Gal\(\alpha\)1-3Gal sequence which was recognized by K88ab fimbriae (Willemansen & de Graaf, 1992). Furthermore, neuraminidase susceptibility of the 210 kDa receptor suggests that a sialylglycoprotein may act as the receptor for the K88ac fimbriae. Nevertheles, we noticed that only neutral oligosaccharides, when they were separated from the protein backbones, bound K88ac fimbriae.

The interactions of the K88ac fimbriae with intestinal brush borders might, in part, be related to the fucosylation of Gal and GlcNAc residues as we characterized Fuc\(\alpha\)1-2Gal\(\beta\)1-4GlcNAc\(\beta\)1 in the 210 and 240 kDa receptors. The presence of Fuc residues in the complex galactoside receptors could be a common feature between the variants ab and ac of K88 fimbriae since the adherence of the K88ab fimbriae to brush borders exposed to a Fuc binding lectin or Fuc residue was significantly reduced (Sellwood, 1980; Laux et al., 1986).

Glycosylation patterns differed between the adhesive and non-adhesive brush borders. Furthermore, we observed that the surface density of oligosaccharides was consistently higher in non-adhesive brush borders than in the adhesive ones. The failure to detect receptor structures in the non-adhesive brush borders might be due to additional oligosaccharide chains shielding the underlying structures. The oligosaccharides of the O-glycoproteins which are associated directly with cell surface membranes contain core structures Gal\(\beta\)1-3GalNAc\(\alpha\)- (core 1), Gal\(\beta\)1-3\(\alpha\)2-6GlcNAc\(\beta\)1-3GalNAc\(\alpha\)- (core 2), GlcNAc\(\alpha\)1-3GalNAc\(\beta\)1-3GalNAc\(\alpha\)- (core 3) and/or GlcNAc\(\alpha\)1-3\(\alpha\)2-6GlcNAc\(\beta\)1-3GalNAc\(\alpha\)- (core 4) (Carraway & Hull, 1989). Cores possess additional backbones identified as Gal\(\beta\)1-3GlcNAc\(\beta\)1- (type 1) and Gal\(\beta\)1-4GlcNAc\(\beta\)1-3- (type 2). The precursor backbones of types 1 and 2 may be extended by A, B, H and Lewis blood group substances. The presence of Gal, GalNAc, Fuc and SA residues in the terminal position has been reported (Strous & Dekker, 1992).

In the pig, blood group substances have been identified from the epithelium of the digestive tract. Despite the high degree of heterogeneity of carbohydrate chains, they have a common oligosaccharide fragment, Gal\(\beta\)1-3GalNAc\(\alpha\)-, linked to the additional fucosylated Gal\(\beta\)1-4GlcNAc sequence (Derevitskava et al., 1978). Although we have not elucidated the complete structures implicated in the recognition of \(E. \) coli K88ac fimbriae, the clear-cut differences between oligosaccharide chains carried by the glycoproteins isolated from K88ac-adhesive and non-adhesive piglet brush borders could be explained by the regulation of the histo-blood group biosynthetic process. The post-natal expression of intestinal histo-blood group antigens A and H have been investigated in pig intestines (King & Kelly, 1991). The expression of the phenotypes depends on the A system with the two antigens A and H (presence of factor A is dominant over the presence of H) and on a second epistatic S system with two alleles S and s (Rasmussen, 1964). This system could be related to susceptibility and resistance, and determine the brush border adhesion phenotypes.

**ACKNOWLEDGEMENTS**

We thank Raymond Julien for helpful discussions and critical reading of the manuscript.

**REFERENCES**


