INFECTION AND IMMUNITY, Apr. 1990, p. 868–873
0019-9567/90/040868-06$02.00/0

Vol. 58, No. 4

Role of the Putative "Link" Glycopeptide of Intestinal Mucin in Binding of Piliated *Escherichia coli* Serotype O157:H7 Strain CL-49

S. UMADEVI SAJJAN[1] AND JANET F. FORSTNER[1,2]*

Research Institute, Hospital for Sick Children,[1] and Department of Biochemistry, University of Toronto,[2] Toronto, Ontario M5G 1X8, Canada

Received 20 July 1989/Accepted 18 December 1989

Purified rat intestinal mucin was used to identify mucin-binding sites for type 1-piliated *Escherichia coli* O157:H7 strain CL-49 isolated from a patient with hemorrhagic colitis and hemolytic uremic syndrome. Optimum binding of bacteria in a microtiter binding assay occurred with a mucin coating concentration of 15 μg (protein)/150 μl. In hapten inhibition studies, several nonmucin glycoproteins bearing exposed mannosyl residues in N-linked oligosaccharides were effective inhibitors, as was rat mucin. The same glycoproteins caused bacterial aggregation. High-molecular-mass glycoproteins of the mucin were separated from its 118-kilodalton "link" glycopeptide fraction, and the latter was shown to be the mucin-binding component for *E. coli* CL-49 and its purified type 1 pili. This was confirmed in hemagglutination inhibition studies. Treatment of the link glycopeptide with jack bean α-mannosidase or endo-β-*N*-acetylglucosaminidase H destroyed bacterial binding activity. Chemical or enzymatic modifications of intact rat mucin were undertaken to evaluate the normal accessibility of the link glycopeptide receptors to *E. coli* CL-49. Deglycosylation with trifluoromethane-sulfonic acid abolished binding, whereas pronase digestion had no effect. Reduction and alkylation as well as lipid extraction enhanced bacterial binding by the mucin, presumably by causing greater exposure of receptor sites. In summary, our binding studies revealed, for the first time, that intestinal mucin bears oligomannosyl receptors for type 1 pili and that these receptors are located on N-linked oligosaccharides of the 118-kilodalton link glycopeptide region of the mucin. Our experiments suggest the receptors are normally partly "covered" by noncovalently bound lipid. In addition, release of the link component from the rest of the mucin by disulfide bond reduction causes greater exposure of specific bacterium-binding sites.

In the preceding paper (18), we showed that a model mucin from rat intestinal goblet cells (and several other gastrointestinal mucins) was capable of binding one strain (CL-49) of *Escherichia coli* O157:H7, a verotoxin-producing *E. coli* strain isolated from a patient who had hemorrhagic colitis and hemolytic uremic syndrome. Binding to mucin was observed only after the bacteria were grown to express type 1 (mannose-sensitive) fimbriae (pili). Other *E. coli* strains that expressed type 1 pili did not bind to mucin. The unique characteristic of *E. coli* CL-49 was its high degree of hydrophobicity, suggesting that strong hydrophobic interactions between CL-49 pili and mucin are important in stabilizing the mannose-specific adherence.

Because mannose and, particularly, α-mannosyl di- and trisaccharides are effective hapten inhibitors and because mannose is present only within the "link" glycopeptide of mucin macromolecules (5, 13, 17), we speculated that the link glycopeptide serves as the specific receptor for piliated *E. coli* CL-49.

Virtually nothing is known about the linkage of mannose residues within intestinal mucin oligosaccharides or about the factors that control the accessibility of these residues to bacterial pili. It is not clear, for example, whether mannose is present in hybrid, complex, or high-mannose N-linked oligosaccharides. It is also not known how the link glycopeptide fits within the three-dimensional structure of mucin polymers.

The experiments described here were designed to confirm that exposed oligomannosyl residues of the 118-kilodalton (kDa) link glycopeptide serve as the receptor for the type 1 pili of *E. coli* CL-49. In addition, we wished to discover whether experimental modifications of mucin samples would provide new information about the accessibility of the mannosyl ligand, hydrophobic interaction sites, or both. Modifications of mucin included delipidation, reduction and alkylation, proteolytic degradation, and deglycosylation.

MATERIALS AND METHODS

Binding of bacteria to mucin. A microtiter binding assay for *E. coli* O157:H7 strain CL-49 was carried out as described in the previous report (18). For hapten inhibition studies with mucin, mucin subfractions, or other glycoproteins, prospective haptens were preincubated for 1 h at 37°C with ^{3}H-labeled *E. coli* CL-49; and the mixture then added to mucin- or bovine serum albumin (BSA)-coated wells. Specific binding was calculated after subtraction of BSA-binding data. Each binding experiment was performed in triplicate.

Mucin purification. Intestinal mucins were purified by using CsCl density gradient ultracentrifugation and Sepharose CL-2B chromatography, as described earlier (5, 12, 13, 17, 22). Purity was established by compositional analyses (amino acid and carbohydrate) as well as by confirming the lack of contaminating low-molecular-mass (<200 kDa) bands after sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) (9, 14). In addition, gelatin Sepharose CL-4B chromatography and immunoassay (1) gave no indication of contamination of mucin samples by fibronectin.

Compositional analyses. Amino acid analyses were carried out on mucin samples with the Picotag system (Waters Associates, Mississauga, Ontario, Canada) (7) after hydrolysis of samples in 6 M HCl at 110°C for 22 h. Carbohydrates were analyzed by gas-liquid chromatography by the method of Zanetta et al. (23).

Bacterial aggregation. Mucin and other glycoprotein sam-

* Corresponding author.

ples containing 50 μg (as protein) were added to a suspension of *E. coli* CL-49 (3×10^9 bacteria) in phosphate-buffered saline (PBS; pH 7.2) in a final volume of 1 ml. After 1 h at 37°C, aggregation was detected by examining mixtures under oil immersion in a microscope (Leitz Wetzler) equipped with a camera (Wild MPS45 Photoautomat) (magnification, ×265). Nonmucin glycoproteins included thyroglobulin, ovalbumin, RNase B, orosomucoid, and fetuin (Sigma Chemical Co., St. Louis, Mo.).

Treatment of rat intestinal mucin prior to hapten inhibition studies. (i) Reduction and alkylation. Mucin (0.5 mg of protein) was reduced with β-mercaptoethanol (0.2 M) and alkylated with iodoacetamide (0.4 M) as described earlier (4, 5). The final product was dialyzed against distilled H_2O prior to use as a hapten inhibitor.

(ii) Delipidation. Mucin (0.5 mg of protein) was subjected to five sequential extractions in chloroform-methanol by the method of Slomiany et al. (19). After dialysis against distilled H_2O, the extracted mucin was used in hapten inhibition studies.

(iii) Deglycosylation. Mucin (1 mg of protein) was treated for 2.5 h at 0°C with trifluoromethanesulfonic acid (TFMS) as described by Edge et al. (3). The resulting deglycosylated product was dialyzed overnight against distilled H_2O in a dialysis bag with a molecular mass cutoff of 1,000 Da and was used in subsequent hapten inhibition studies.

(iv) Proteolytic degradation. Mucin (0.5 mg of protein) was incubated for 24 h at 37°C with 1 mg of pronase (protease type X1V; Sigma) in PBS (pH 7.5) as described earlier (12). The reaction was stopped by the addition of 1 mM phenylmethylsulfonyl fluoride and dialyzed against PBS (molecular mass cutoff, 1,000 Da).

(v) Separation of mucin into high-molecular-mass glycoproteins and the 118-kDa link glycopeptide. After reduction and alkylation of purified mucin as described above, the reduced mucin (2 mg of protein) was subjected to CsCl density gradient ultracentrifugation for 48 h at 150,000 × *g*. Each of the 20 fractions from the gradient was dialyzed and assayed for carbohydrate (11) and for its mobility on SDS-PAGE (9, 14). The high-density glycoprotein fraction (buoyant density, 1.41 to 1.52 g/ml) was collected separately from the 118-kDa link glycopeptide fraction (density, 1.32 to 1.37 g/ml), and each was used in hapten inhibition or direct bacterium-binding assays.

Purification of pili and hemagglutination assays. Purification of type 1 pili from three strains of *E. coli* was carried out by the method of Dodd and Eisenstein (2). Hemagglutination experiments were performed on glass microscope slides (18) by using guinea pig erythrocytes. Inhibition of hemagglutination was tested by using 1% D-mannose, native (untreated) mucin, and mucin subfractions as described in the Results section.

Enzymatic treatment of the 118-kDa link glycopeptide. The purified link component (30 μg of protein) was incubated at 37°C with or without 0.05 U of endo-β-*N*-acetylglucosaminidase H (endo H; Boehringer GmbH, Mannheim, Federal Republic of Germany) in 0.02 M acetate buffer (pH 5.5) containing 1 mM phenylmethylsulfonyl fluoride and 0.02% NaN_3. After 24 h, the digestion mixture was dialyzed against PBS (pH 6.8) in 0.02% NaN_3 to remove free oligosaccharides and was subsequently used in hapten inhibition assays.

In separate experiments, the link glycopeptide (30 μg of protein) was incubated at 37°C with or without 2 U of jack bean α-mannosidase (Sigma) in 0.05 M acetate buffer (pH 4.5) containing 1 mM phenylmethylsulfonyl fluoride and 0.02% NaN_3. After 24 h, the mixture was dialyzed against PBS (pH 6.8) in 0.02% NaN_3 to remove free mannose, concentrated, and used in hapten inhibition assays.

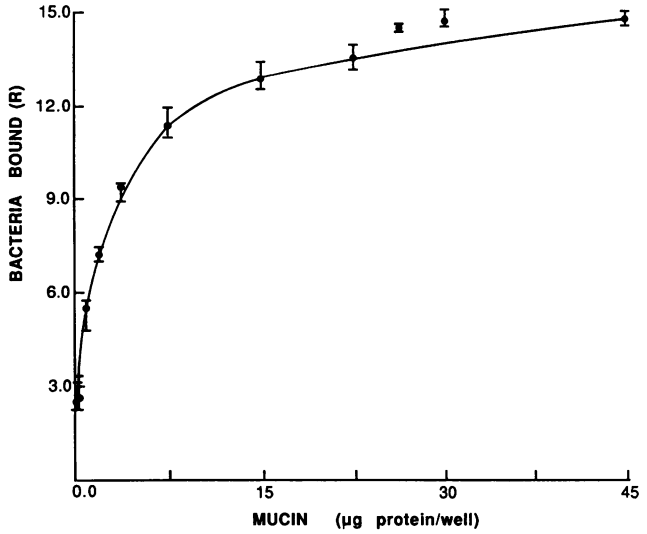

FIG. 1. Binding of *E. coli* CL-49 to rat intestinal mucin. ^{3}H-labeled bacteria (3.4×10^5 dpm) were added to wells coated with various concentrations of mucin (0.5 to 45 μg per well). Binding was expressed as a ratio (*R*) of the binding to mucin (disintegrations per minute) to binding to albumin (disintegrations per minute). Each value is the average of triplicate analyses, and vertical bars represent the total range of values obtained.

RESULTS

Purified rat intestinal mucin was used as a model mucin throughout these experiments because of its relative ease of preparation and accessibility, although many other intestinal mucins have also been noted to bind to type 1-piliated *E. coli* O157:H7 strain CL-49 (18). The rat mucin had a buoyant density ranging from 1.424 to 1.555 g/ml and consisted of 80% carbohydrate, 16% protein, and 4% noncovalently bound lipid (dry weight basis) (R. E. F. Fahim, Ph.D. thesis, University of Toronto, Toronto, Ontario, Canada, 1985). No low-molecular-mass contaminants were detected by SDS-PAGE (7.5%), and there was no detectable DNA, mannose, uronic acid, or glucose. Evidence of purity of this mucin has been published earlier (4, 5). Gelatin-Sepharose chromatography (1) also failed to indicate contamination by fibronectin.

The optimum concentration of mucin for the binding of ^{3}H-labeled *E. coli* was established by adding increasing amounts of mucin to coat the microtiter wells (Fig. 1). After the wells were blocked with 10% BSA, approximately 1×10^8 bacteria (3.4×10^5 dpm) were added to each well and incubated in PBS (pH 6.8) for 2 h at 37°C, the wells were washed six times, and the individual wells were counted. Saturation of mucin sites by bacteria was achieved with 15 μg of mucin (protein) coating. Therefore, for subsequent experiments coating of wells was carried out with 15 μg of mucin unless otherwise specified. Control wells were coated with 10% BSA.

Type 1 pili are known to bind to short oligomannosyl receptors in cell membrane glycoproteins (15; see accompanying report [18] for other references). These receptor structures have not been described in mucin macromolecules, yet they are known to be present in various commercially available glycoproteins. To confirm that the type 1 pili

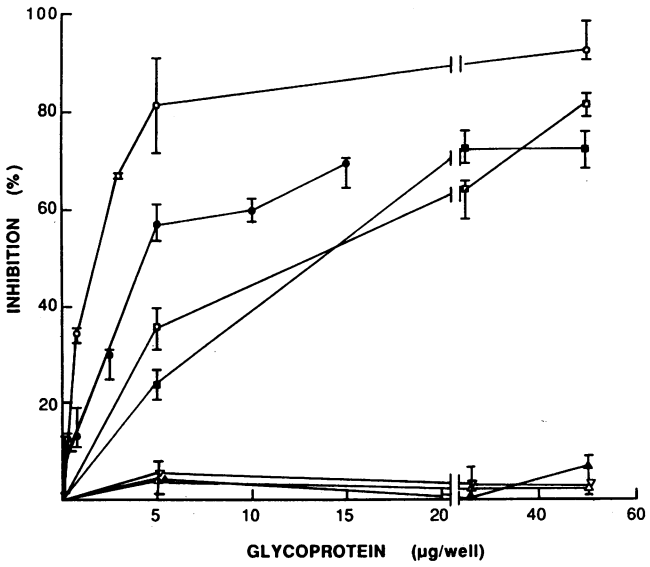

FIG. 2. Hapten inhibition of *E. coli* CL-49 binding to rat intestinal mucin. ^{3}H-labeled bacteria (3×10^5 dpm) were preincubated in PBS (pH 7.0) with various glycoproteins (0 to 50 μg) for 1 h at 37°C and then tested for their ability to bind to mucin. Inhibition of 0% was equivalent to the binding of 22,401 dpm. Symbols: ●, mucin; ○, thyroglobulin; □, RNase; ■, ovalbumin; △, fetuin; ▽, orosomucoid; ▲, BSA. Each value is the average of triplicate assays, and vertical bars represent the total range of values obtained.

of *E. coli* CL-49 behaved with the expected specificity for receptor sites, several nonmucin glycoproteins (and rat mucin itself) were used in hapten inhibition studies. Figure 2 indicates that all glycoproteins bearing exposed mannosyl residues in either high-mannose or hybrid N-linked oligosaccharides (i.e., thyroglobulin, ovalbumin, and RNase B) (20) bound *E. coli* CL-49. The rat mucin had an intermediate inhibitor potency. Fetuin and orosomucoid do not contain exposed oligomannosides (6, 8) and did not bind to *E. coli* CL-49.

Functional effects of binding were noted in separate studies of bacterial agglutination (data not shown). The same glycoconjugates that bound *E. coli* CL-49 also caused mannose-sensitive bacterial agglutination in a fashion that correlated qualitatively with their potencies as hapten inhibitors. That is, fetuin and orosomucoid were ineffective; thyroglobulin was the most potent; and mucin, RNase B, and ovalbumin were intermediate in their agglutination potencies.

Taken together, these experiments provided circumstantial evidence that exposed mannosyl residues are required for the binding of *E. coli* CL-49 and that glycoproteins bearing these receptor sites can cross-link and aggregate the bacteria.

Since the only component of intestinal mucins known to contain mannose is the link glycopeptide of 118 kDa, we suspected strongly that this component was responsible for binding piliated *E. coli* CL-49. However, the link glycopeptide is a minor mucin component making up only about 10% of the total carbohydrate and only about 25% of the total protein of purified intestinal mucin (13; Fahim, Ph.D. thesis). We were interested, therefore, in knowing the degree of accessibility (to bacteria) of the link component within the native mucin. Several experiments were thus performed on mucin treated in various ways to alter the tertiary structure, the exposure of the link glycopeptide, or both. Treatments

TABLE 1. Effectiveness of modified mucins as hapten inhibitors[a]

Hapten	Protein (μg/well)	% Inhibition	% Range
None		0	
Intact mucin	2.5	29.9	26.1–32.1
	5.0	58.3	52.0–70.7
Pronase-digested mucin	2.5	29.3	26.4–32.2
	5.0	59.4	46.3–72.5
TFMS-treated mucin	2.5	7.6	2.6–12.3
	5.0	14.4	12.0–17.4
Reduced, alkylated mucin	2.5	92.3	89.7–95.4
	5.0	95.8	90.9–99.9
Delipidated mucin	2.5	83.1	78.3–86.2

[a] ^{3}H-labeled *E. coli* CL-49 (3.5×10^5 dpm) was preincubated with mucin samples (haptens) for 1 h at 37°C, and the mixture (150 μl) was then added to mucin-coated wells. In the absence of a hapten, binding to mucin was 19,784 dpm after subtraction of nonspecific binding to BSA. Each value is the average of triplicate assays, and the total range of values is provided.

included thiol reduction, which causes separation of the link component from its attachment to high-molecular-mass glycoproteins (4), proteolytic degradation of naked (nonglycosylated) regions of the mucin backbone (12), partial deglycosylation (with TFMS), and extraction of noncovalently bound lipid contaminants (19). None of these treatments (with the exception of pronase treatment) altered the amino acid composition of the mucin (data not shown). Changes induced by pronase have been reported earlier (22).

Table 1 shows the effectiveness of the treated mucins as hapten inhibitors in *E. coli* CL-49 binding assays. Native mucin was an effective inhibitor, and no differences were observed with mucins that were subjected to prolonged incubation with pronase. It appears, therefore, that the intact state of naked regions in mucin is not a prerequisite for binding. TFMS, on the other hand, destroyed CL-49 binding, presumably because TFMS removed receptor carbohydrates. Disulfide bond reduction of the mucin caused a dramatic increase in haptenic potency, presumably because of the release and increased exposure of the 118-kDa link glycopeptide to the bacteria. Lipid extraction of mucin was almost as effective in exposing receptors for bacterial attachment, which suggests that in the intact mucin, the minor amount of contaminating lipid may normally be located in a position that shields or covers receptor sites. It also suggests that the strong hydrophobic interactions that are necessary to stabilize the binding of the CL-49 pili (18) are not dependent upon the presence of lipid contaminants in the mucin.

Native rat intestinal mucin was reduced and alkylated and then fractionated by CsCl density gradient ultracentrifugation into two major components: the dense high-molecular-mass glycoproteins (fractions 5 to 10; buoyant density, 1.41 to 1.52 g/ml) and the less glycosylated 118-kDa link glycopeptide (fractions 11 to 16; buoyant density, 1.32 to 1.37 g/ml) (Fig. 3). Although the high-molecular-mass glycoproteins were not totally free of the link component (noted as a faint band at an M_r of 118,000 on SDS-PAGE of fraction 10), the separation was judged to be complete enough to warrant comparative studies. Compositional analyses showed that

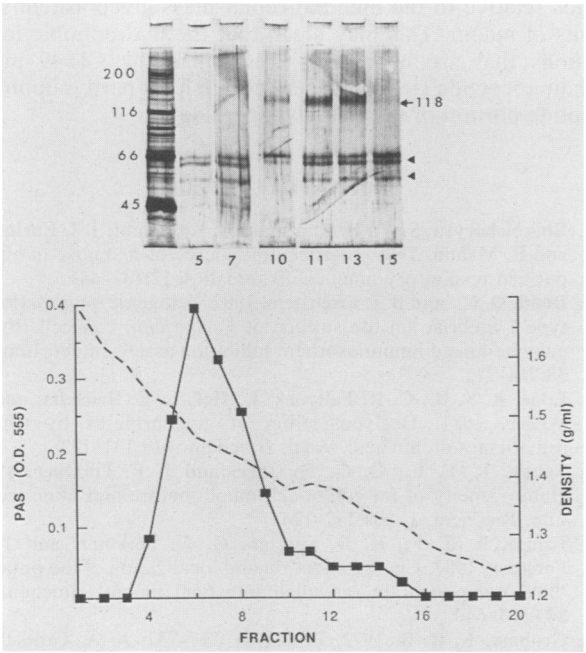

FIG. 3. Separation of mucin into high-molecular-mass glycoproteins and the 118-kDa link glycopeptide. Purified mucin was reduced, alkylated, and then fractionated by CsCl density gradient ultracentrifugation (– – –). Each of the 20 fractions was subjected to the periodic acid-Schiff stain (PAS) assay (■) for carbohydrate and to SDS-PAGE (7.5%) (upper photograph) with staining by silver. Numbers below the gels indicate fractions. Fractions 5 to 10 were pooled as the high-molecular-mass glycoproteins of mucin, and fractions 11 to 16 were pooled as the 118-kDa link glycopeptide. Molecular mass markers (in kilodaltons) are shown on the left of the gels, and arrowheads refer to mercaptoethanol artifacts (17). O.D. 555, Optical density at 555 nm.

the differences between the two were prominent (Table 2). The 118-kDa glycopeptide was enriched in aspartic acid, glutamic acid, and hydrophobic amino acids as well as in *N*-acetylglucosamine and mannose, while the high-molecular-mass glycoproteins were enriched in total carbohydrate, threonine, serine, and proline.

TABLE 2. Composition of rat small intestinal mucin and its subfractions[a]

Component[b]	Composition (mol%)		
	Intact mucin	118-kDa link glycopeptide	High-molecular-mass glycoproteins
Asp + Glu	12.9	33.5	11.4
Ser + Thr + Pro	54.4	22.2	52.1
Gly + Ala + Val	16.1	17.2	13.7
Ile + Leu + Phe	9.0	16.1	8.9
Cys	2.0	4.0	3
Rest	5.6	7.0	10.9
Fucose	14	10	16
Galactose	27	22	27
GlcNAc	22	30	20
GalNAc	21	22	23
Sialic acid	13	6	13
Mannose	tr	10	tr

[a] For amino acid analyses, mucin samples were hydrolyzed in 6 M HCl at 110°C for 22 h and separated with the Picotag system. Carbohydrates were determined by gas-liquid chromatography of trifluoracetate derivatives. Values represent the averages of two independently hydrolyzed samples.

[b] Abbreviations: GlcNAc, *N*-acetylglucosamine; GalNAc, *N*-acetylgalactosamine.

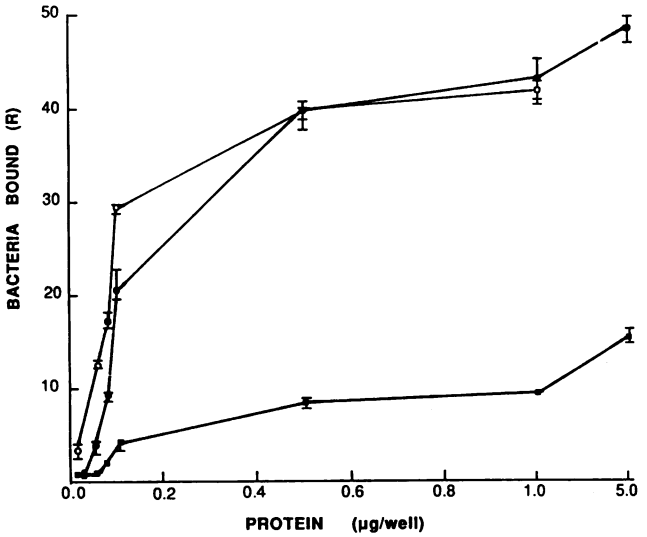

FIG. 4. Binding of *E. coli* CL-49 to mucin and its subfractions. ^{3}H-labeled bacteria (1.5×10^5 dpm) were added to wells coated with mucin or its subfractions (0.0 to 5.0 µg), and binding was expressed as the ratio (*R*) of binding to mucin samples (disintegrations per minute) to binding to BSA (disintegrations per minute). Values represent the average of triplicate assays, and vertical bars represent the total range of values obtained for intact mucin (●), the 118-kDa link glycopeptide (○), and the high-molecular-mass glycoproteins (■).

The isolated components were compared as hapten inhibitors in the binding of *E. coli* CL-49 to mucin-coated wells (data not shown). The link glycopeptide was similar in potency to intact (native) mucin, causing approximately 40% inhibition at a concentration of 3 µg of protein per well. The high-molecular-mass glycoproteins, on the other hand, had no significant effect (data not shown). In direct binding studies in which each component was used separately to coat microtiter wells, the 118-kDa glycopeptide was found to be as effective a ligand as the native mucin (Fig. 4) in binding *E. coli* CL-49, while high-molecular-mass glycoproteins did not cause significant binding.

In the preceding paper (18) we reported that type 1 pili isolated from three strains of *E. coli* caused mannose-sensitive hemagglutination of guinea pig erythrocytes. Intact rat mucin inhibited CL-49 pilus-mediated hemagglutination, but not the hemagglutination caused by the other pili. In the present study, inhibition of CL-49 pilus-mediated hemagglutination by intact mucin and link glycopeptide was compared. The minimum concentrations of link glycopeptide and intact mucin needed to cause inhibition were 0.0058 and 0.039 µg of protein per well, respectively. Thus, the 118-kDa glycopeptide was approximately seven times more potent than the native mucin.

The configuration of mannosyl receptors in the 118-kDa link glycopeptide was evaluated by subjecting the isolated link component to enzymatic digestion to remove high-mannosyl chains (with endo H) (21) or α-linked mannosyl residues (with jack bean α-mannosidase) (10). The treated samples were then compared with control (untreated) samples of link glycopeptide as hapten inhibitors in assays of *E. coli* CL-49 binding to rat intestinal mucin. Both endo H and α-mannosidase virtually abolished hapten inhibition by the link glycopeptide. These findings suggest that receptors for

the bacteria are α-linked mannose residues present in high-mannose-type N-linked oligosaccharides of the mucin link glycopeptide.

DISCUSSION

Physical and biochemical studies support a model for mucin macromolecules as one in which long threads are loosely coiled so that they occupy a spherical hydrodynamic realm (16). Large glycoprotein subunits of various sizes make up the threadlike structures, with subunits consisting of variable stretches of glycosylated and nonglycosylated areas. Since disulfide-reducing agents cause a decrease in the molecular weight and viscosity of mucins, it is assumed that the large glycoprotein subunits are linked end-to-end through disulfide bonds.

Studies in our laboratory of purified intestinal mucins have shown that upon reduction, mucins decrease in size and liberate a unique glycopeptide component which bands at a position with an M_r of 118,000 on SDS-polyacrylamide gels (12, 17; Fahim, Ph.D. thesis). The presumed function of this component is to link some of the large glycoprotein subunits together through disulfide bonds. The molar stoichiometry of the link glycopeptide in a typical mucin is unknown, although for human and rat small intestinal mucin we have determined that the peptide of the link component makes up about 25% of the total mucin protein, and the carbohydrate makes up roughly 10% of the total mucin carbohydrate (5, 13). Since the link carbohydrate contributes in such a minor way to the total mucin carbohydrate, it is not surprising that the mannose in the link glycopeptide is undetectable in analyses of the intact (native) mucin (Table 2). Once the link component is purified, however, mannose is seen to be a major constituent in rat and human mucins (10 and 8.9 mol%, respectively [13; Fahim, Ph.D. thesis]), and its presence signals the existence of N-linked oligosaccharides.

Our results suggest that the pili of *E. coli* CL-49 exhibit mannose specificity similar to type 1 pilus adhesins and bind to oligomannosyl residues of the mucin link glycopeptide. Evidence for this interpretation includes the observation that mucin receptors were destroyed by α-mannosidase, which cleaves mannose in α-1,2, α-1,3 and α-1,6 linkages (10), and by endo H, which cleaves between core *N*-acetylglucosamine residues of high-mannose N-linked oligosaccharides (21).

Since intestinal mucin was able to aggregate *E. coli* CL-49, it appears that mucin (and other glycoproteins carrying type 1 pilus receptors) can bridge these bacteria, a process that might be expected to occur with mucins in vivo to facilitate bacterial washout from the intestine or, possibly, to enhance colonization and infection by these pathogens.

Bacterial binding to chemically modified mucin samples was explored in hapten inhibition studies. Our results indicate that the link glycopeptide receptor (in the native mucin) is not fully accessible to *E. coli* CL-49 but that it can be made more accessible by disulfide bond reduction or delipidation. To our knowledge, these experiments constitute the first evidence to suggest that the link glycopeptide may be partly buried within the normal tertiary structure of the mucin and that minor quantities of lipid contaminants may normally cover the link glycopeptide N-linked oligosaccharides. Thus, the binding reaction of *E. coli* CL-49 has proven to be a useful probe of the three-dimensional structure of mucin as well as in the identification of N-linked oligosaccharides in the link component.

The link glycopeptide is enriched in hydrophobic amino acids relative to the high-molecular-mass glycoprotein subunits of mucin. This may mean that the hydrophobic interactions that are necessary to stabilize the CL-49 pilus-binding reaction (18) are dependent, at least partly, upon the peptide portion of the link glycopeptide.

LITERATURE CITED

1. **Bhattacharyya, S. N., B. Kaufman, A. Khorrami, J. I. Enriquez, and B. Manna.** 1988. Fibronectin: source of mannose in highly purified respiratory mucin. Inflammation **12**:433–445.
2. **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.
3. **Edge, A. S. B., C. R. Faltynek, L. Hof, L. E. Reichart, and P. Weber.** 1981. Deglycosylation of glycoproteins by trifluoromethanesulfonic acid. Anal. Biochem. **118**:131–137.
4. **Fahim, R. E. F., G. G. Forstner, and J. F. Forstner.** 1983. Heterogeneity of rat goblet-cell mucin before and after reduction. Biochem. J. **209**:117–124.
5. **Fahim, R. E. F., R. D. Specian, G. G. Forstner, and J. F. Forstner.** 1987. Characterization and localization of the putative 'link' component in rat small intestinal mucin. Biochem. J. **243**:631–640.
6. **Graham, E. R. B.** 1972. Fetuin, p. 717–731. *In* A. Gottschalk (ed.), Glycoproteins, vol. 5, part A. Elsevier Publishing Co., New York.
7. **Heinrikson, R. I., and S. C. Meredith.** 1984. Amino acid analysis by reverse phase high performance liquid chromatography: precolumn derivatization with phenylisothiocyanate. Anal. Biochem. **136**:65–74.
8. **Jeanloz, R. W.** 1972. α-Acid glycoprotein, p. 506–611. *In* A. Gottschalk (ed.), Glycoproteins, vol. 5, part A. Elsevier Publishing Co. New York.
9. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227**:680–685.
10. **Li, Y. T.** 1967. Studies on the glycosidases in jack bean meal. I. Isolation and properties of α-mannosidase. J. Biol. Chem. **242**:5474–5480.
11. **Mantle, M., and A. Allen.** 1978. A colorimetric assay for glycoproteins based on the periodic acid/Schiff stain. Biochem. Soc. Trans. **6**:607–609.
12. **Mantle, M., G. Forstner, and J. F. Forstner.** 1984. Antigenic and structural features of goblet-cell mucin of human small intestine. Biochem. J. **217**:159–167.
13. **Mantle, M., G. G. Forstner, and J. F. Forstner.** 1984. Biochemical characterization of the component parts of intestinal mucin from patients with cystic fibrosis. Biochem. J. **224**:345–354.
14. **Merril, C. R., D. Goldman, S. A. Sedman, and M. H. Ebert.** 1981. Ultrasensitive stain for proteins in polyacrylamide gels shows regional variation in cerebrospinal fluid proteins. Science **211**:1437–1438.
15. **Neeser, J. R., B. Koellreutter, and P. Wuersch.** 1986. Oligomannoside-type glycopeptides inhibiting adhesion of *Escherichia coli* strains mediated by type 1 pili: preparation of potent inhibitors from plant glycoproteins. Infect. Immun. **52**:428–436.
16. **Neutra, M. R., and J. F. Forstner.** 1987. Gastrointestinal mucus: synthesis, secretion, and function, p. 975–1009. *In* L. R. Johnson (ed.), Physiology of the gastrointestinal tract, 2nd ed. Raven Press, New York.
17. **Roberton, A. M., M. Mantle, R. E. F. Fahim, R. D. Specian, A. Bennick, S. Kawagishi, P. Sherman, and J. F. Forstner.** 1989. The putative 'link' glycopeptide associated with mucus glycoproteins. Biochem. J. **261**:637–647.
18. **Sajjan, S. U., and J. F. Forstner.** 1990. Characteristics of binding of *Escherichia coli* serotype O157:H7 strain CL-49 to purified intestinal mucin. Infect. Immun. **58**:860–867.
19. **Slomiany, A., B. L. Slomiany, A. Witas, M. Aono, and L. J. Newman.** 1983. Isolation of fatty acids covalently bound to the gastric mucus glycoprotein of normal and cystic fibrosis pa-

tients. Biochem. Biophys. Res. Commun. **113**:286–293.
20. **Spiro, R. G.** 1973. Glycoproteins, p. 350–466. *In* G. B. Anfinson, J. T. Edsall, and F. M. Richards (ed.), Advances in protein chemistry, vol. 27. Academic Press, Inc., London.
21. **Tarentino, A. L., T. H. Plummer, Jr., and F. Maley.** 1974. The release of intact oligosaccharides from specific glycoproteins by endo-β-*N*-acetylglucosaminidase H. J. Biol. Chem. **249**:811–824.
22. **Wesley, A., M. Mantle, D. Man, R. Qureshi, G. Forstner, and J. Forstner.** 1985. Neutral and acidic species of human intestinal mucin. J. Biol. Chem. **260**:7955–7959.
23. **Zanetta, J. P., W. C. Breckenridge, and G. Vincendon.** 1972. Analysis of monosaccharides by gas liquid chromatography of the *O*-methyl glycosides as trifluoroacetate derivatives. Application to glycoproteins and glycolipids. J. Chromatogr. **69**: 291–304.