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Oligomannoside-Type Glycopeptides Inhibiting Adhesion of *Escherichia coli* Strains Mediated by Type 1 Pili: Preparation of Potent Inhibitors from Plant Glycoproteins

JEAN-RICHARD NEESER,* BRIGITTE KOELLREUTTER, AND PIERRE WUERSCH
Nestlé Research Department, Nestec Ltd., CH-1800 Vevey, Switzerland

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Various structurally defined glycopeptides of natural origin were tested as inhibitors of guinea pig erythrocyte agglutination by enteropathogenic *Escherichia coli* strains expressing type 1 pili. Besides hybrid-type glycoasparagines from ovalbumin which were not active, large oligomannoside-type carbohydrate chains from legume storage glycoproteins moderately inhibited hemagglutinations, whereas the short oligomannoside-type glycoasparagine from ovalbumin Manα(1→6)[Manα(1→3)]Manα(1→6)[Manα(1→3)]Manβ(1→4)GlcNAcβ(1→4)GlcNAcβ(1→N)Asn exhibited a potent activity. These results strongly suggested that the nonsubstitution of the α(1→3)-linked mannosyl residue from the N-linked glycopeptide core structure is the key determinant in the minimal structural requirement specific to this fimbrial lectin. Such $Man_5GlcNAc_2$-containing glycopeptides were obtained from larger N-linked carbohydrate chains, occurring abundantly in natural sources. The ability of jack bean α-mannosidase to cleave the α(1→2)-linked mannoses more rapidly than the others allowed the controlled digestion of large oligomannoside-type glycopeptides from legume storage glycoproteins. Such shortened glycopeptides of plant origin were prepared which strongly inhibited guinea pig erythrocyte agglutinations as well as bacterial adhesion on human buccal cells, thus confirming their similarity (if not identity) with the receptor of type 1 pili on mammalian cells. The importance of this preparation of a receptorlike compound that inhibits bacterial adhesion with regard to the research on the role of type 1 pili in *E. coli* pathogenicity is discussed.

The specific attachment of bacteria to mucosal surfaces of animal tissues is gaining increasing attention, as it is considered to be a prerequisite to colonization of the host in the pathogenesis of bacterial infections (33). The adhesion is generally mediated by pili (fimbriae) which recognize cell surface carbohydrate structures on the epithelial cells (21). For *Escherichia coli* strains of various origins, only some of the epithelial receptors are now known at the molecular level, and the concerned adhesins can be placed in three distinct groups with regard to their carbohydrate specificities. The first group includes adhesins that recognize digalactoside moieties like the P fimbriae of human pyelonephritic *E. coli* strains specific for the P blood group antigens [containing the structural element Galα(1→4)Galβ] (22, 28) and the K88 pili of *E. coli* strains which are enterotoxigenic for piglets and specific for the glycolipids of the pig small intestine [containing the structural element Galα(1→3)Gal] (G. Nilsson, S. Svensson, and A. A. Lindberg, Proceedings of the 7th International Symposium on Glycoconjugates, Lund, Sweden, p. 637, 1983). The second group comprises adhesins recognizing sialylated glycoconjugates and includes the M fimbriae of human pyelonephritic *E. coli* strains specific for the blood group M determinant of glycophorin A (20, 43); the S fimbriae of various human *E. coli* strains which bind to sialyl α(2→3)galactosyl structures on human erythrocytes (34); and probably the K99 pili of enterotoxigenic *E. coli* strains pathogenic for calves, lambs, and piglets, binding apparently to glycophorin A on human erythrocytes (D. E. Brooks, Biophys. J., abstract, vol. 45, p. 198A, 1984; M. Lindhal, R. Brossmer, and T. Wadström, Abstracts Interlec 6, Poznan, Poland, p. 110, 1984) and to NeuGcα(2→3)lactosylceramide on equine erythrocytes (39). The third group includes type 1 fimbriae that are expressed by many strains of nonpathogenic and pathogenic *E. coli* and which are isolated, for instance, from patients with diarrheal diseases or urinary tract infections or from animals with diarrhea. When mediated by type 1 pili, the bacterial adherence of *E. coli* strains is specifically inhibited by D-mannose (Man) and methyl-α-D-mannoside, and is thus called mannose specific or mannose sensitive (10, 11). It has been shown that type 1 pili bind to guinea pig erythrocytes (12), as well as to human buccal cells (32) and yeast cells (12).

Firon et al. (11, 12) have recently observed that the site of combination of the *E. coli* type 1 fimbrial lectin fitted best the structure Manα(1→3)Manβ(1→4)GlcNAc. The close analogy between oligosaccharide structures that bind to the type 1 fimbriae and the oligomannoside-type units of glycoproteins strongly suggests that such *N*-glycosidic carbohydrate chains of mammalian cell surface glycoproteins act as receptors for these pili (12). However, the receptor for type 1 adhesins has not yet been isolated, and oligomannoside-type glycopeptides (as they occur naturally in glycoproteins) have never been used to determine the specificity of the type 1 fimbrial lectin. Therefore, the first aim of this study was to test various structurally defined, naturally occurring glycopeptides (including large and short oligomannoside-type as well as hybrid-type carbohydrate chains) as inhibitors of guinea pig erythrocyte agglutination to establish the most probable structure for the natural receptor of the lectin on mammalian cells.

Because of the possible role of the type 1 pilus in the pathogenicity of *E. coli* strains (see below), there is now an urgent need for the preparation of specific and potent inhibitors of bacterial adhesion mediated by these pili. In this regard, receptorlike complex carbohydrates could be an

* Corresponding author.

important tool for further studies. Firon et al. (11, 12) have tested synthetic oligosaccharides as well as carbohydrates isolated from the urine of patients with mannosidosis or GMl gangliosidosis, but these compounds are not routinely available and cannot be easily prepared. Therefore, our second aim was to develop a simple preparation of specific inhibitors of bacterial adherence mediated by type 1 pili. Starting with abundant natural sources, oligomannoside-type glycopeptides were processed to obtain the minimal active structure.

MATERIALS AND METHODS

High-performance thin-layer chromatographic (HPTLC) precoated plates (Silicagel 60) and pronase E were purchased from E. Merck AG (Darmstadt, Federal Republic of Germany). Endo-β-*N*-acetylglucosaminidase H was obtained from Seikagaku Kogyo (Tokyo, Japan); jack bean α-mannosidase and chicken egg albumin (ovalbumin, grade V) were obtained from Sigma Chemical Co. (St. Louis, Mo.). Known procedures were used for the isolation of 7S soybean glycoprotein from defatted soy flour (27, 36) and of kidney bean glycoprotein II from milled white beans (35).

Colorimetric neutral sugar determination (9); gas-liquid chromatographic quantification of neutral and amino sugars (31); and HPTLC of oligosaccharides (42), including spot visualization and scanning (14), were performed.

Naturally occurring glycopeptides and oligosaccharides. The oligomannoside-type glycopeptide mixture containing $Man_{6-8}GlcNAc_2$ chains was prepared from 7S soybean glycoprotein by a partial pronase digestion following a previously described procedure (44). Briefly, 25 g of protein was digested with two successive portions of pronase E (500 mg followed by 250 mg). After filtration, passage through a Dowex column (50W-X8; H^+ form), and neutralization with Amberlite IRA 400 (CO_3^{2-} form), the freeze-dried residue was further purified on Sephadex G-25 by eluting with 0.1 M acetic acid. The oligomannoside-type glycopeptide mixture containing $Man_{6-9}GlcNAc_2$ chains was prepared from kidney bean glycoprotein II, as described above. The hybrid-type and short oligomannoside-type glycopeptides of ovalbumin were obtained as glycoasparagine units after extensive pronase digestion, and the mixture was further separated into five fractions (GP-I to GP-V) by ion-exchange chromatography, exactly as described previously (16, 41).

Oligomannoside-type oligosaccharides were obtained from the 7S soybean and kidney bean II glycopeptides by treatment with endo-β-*N*-acetylglucosaminidase H. Samples containing 7.5 mg of Man were incubated with 0.1 U of enzyme for 24 h at 37°C in a total volume of 10.5 ml containing 10 ml of 3.3 mM citric acid–11.7 mM Na_2HPO_4 buffer (pH 6.0) and 0.5 ml of toluene. After boiling for 3 min, the mixtures were treated with Amberlite MB-3 and freeze-dried.

Treatments with jack bean α-mannosidase. The conditions for the control of the enzymatic digestion were investigated as follows. One milligram samples of the oligomannoside-type oligosaccharides described above were incubated with 0.5 U of enzyme at room temperature in 0.1 ml of 5.4 mM citric acid–4.6 mM sodium citrate buffer (pH 4.5). Fractions of 10 μl were withdrawn after 0.5, 1, 2, 3, 4, 5, 6, and 22 h; mixed with 10 μl of ethanol; and subjected to HPTLC analysis.

Short oligomannoside-type glycopeptides containing mainly the $Man_5GlcNAc_2$ chain were prepared from the 7S soybean and kidney bean II glycopeptides as follows: samples containing 350 mg of Man were incubated with 190 U of enzyme for 1 h at room temperature in 75 ml of the citrate buffer described above. After heat denaturation of the enzyme, the solution was concentrated and freeze-dried, and the resulting mixture was further purified on Sephadex G-25 by eluting with 0.1 M acetic acid.

Agglutination of guinea pig erythrocytes, adhesion on human buccal cells, and inhibition studies. Wild-type enteropathogenic *E. coli* strains that were isolated from infants with gastroenteritis and that expressed type 1 pili were used in this study. They were *E. coli* O119:K69 (L74-30), O86:K61 (B74-10), and O111:K58 (B75-44) and noncharacterized *E. coli* 16375 (clinical isolate from Universität klinik für Kinderheilkunde, Innsbruck, Austria). The bacteria were subcultured into brain heart infusion broth for 24 h at 37°C to favor type 1 pilus production and again subcultured for 24 h.

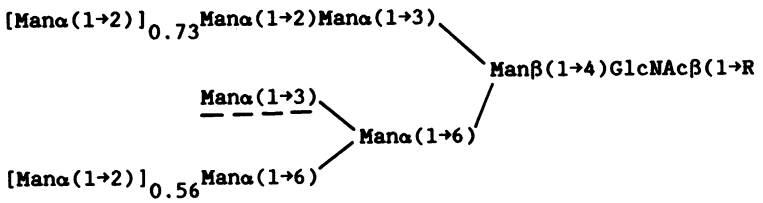
For hemagglutination and inhibition studies, the bacteria were washed with a 0.9% NaCl solution, and the suspensions were adjusted to 10^9 bacteria per ml by optical density measurement. The guinea pig erythrocytes (freshly drawn) were suspended to 1% in a 0.9% NaCl solution. Hemagglutination and inhibition tests were performed by mixing 25 μl of the bacterial suspension, 25 μl of a 0.9% NaCl solution without or with inhibitor, respectively, and 50 μl of the erythrocyte suspension. When inhibitors were present, serial twofold dilutions were prepared and tested. The results were recorded after 2 h at 4°C.

For adhesion on human buccal cells and inhibition studies, cells were collected from one of us (B.K.), washed 4 times with a phosphate-buffered saline solution (10 mM phosphate in 0.15 M NaCl solution), and suspended in the same buffer to give 2×10^9 bacteria per ml. Adhesion and inhibition tests were performed by mixing 500 μl of the cell suspension, 250 μl of the bacterial suspension, and 250 μl of the phosphate-buffered saline solution described above without or with inhibitor, respectively. In the latter case, three different concentrations were prepared and tested for each inhibitor. The suspensions were gently mixed by slow rotation for 30 min at room temperature and then washed 4 times with the phosphate-buffered saline solution (5 ml) and colored with Gram stain. Counts of adherent bacteria to 50 cells per sample were made by optical microscopy.

RESULTS

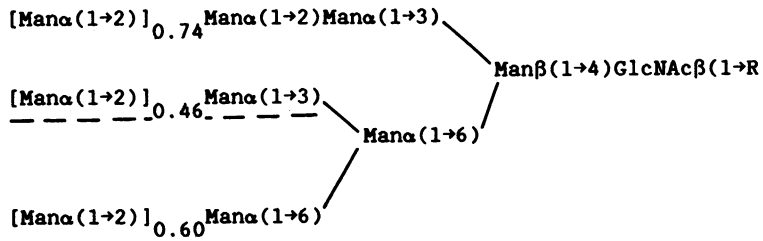
Oligomannoside-type carbohydrate chains from both animal and plant origins exhibit the same pattern of branching (8, 30a). We then selected glycopeptides and oligosaccharides from 7S soybean glycoprotein (a mixture containing $Man_{6-8}GlcNAc_2$ units, with $Man_8GlcNAc_2$ being the major oligomer [30a]) and similar compounds from kidney bean glycoprotein II (a mixture containing $Man_{6-9}GlcNAc_2$ units, with $Man_9GlcNAc_2$ being the major oligomer [30a]) as sources of large oligomannoside-type carbohydrates to be tested as potential inhibitors of *E. coli* bacterial adherence mediated by type 1 pili. The treatment of 7S soybean glycoprotein by partial pronase digestion led to the isolation of a glycopeptide mixture (molar ratio of Man/2-acetamido-2-deoxy-D-glucose [GlcNAc], 7.3:2.0) containing 65% Man with an 88% yield (based on Man recovery from the starting storage protein). A similar mixture was obtained by the same treatment of kidney bean glycoprotein II (molar ratio of Man/GlcNAc, 7.8:2.0). Because each of the glycopeptides was fully digestible by endo-β-*N*-acetylglucosaminidase H, the corresponding oligosaccharide mixture was obtained in almost quantitative yield. Based on our detailed structural

7S soybean carbohydrate chains

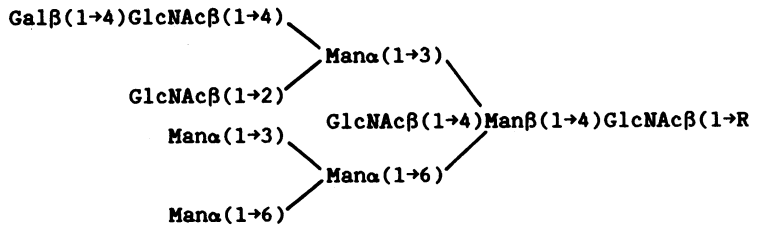


1 R = 4)GlcNAcβ(1→N)Asn - polypeptide
2 R = H

Kidney bean II carbohydrate chains



3 R = 4)GlcNAcβ(1→N)Asn - polypeptide or R = H



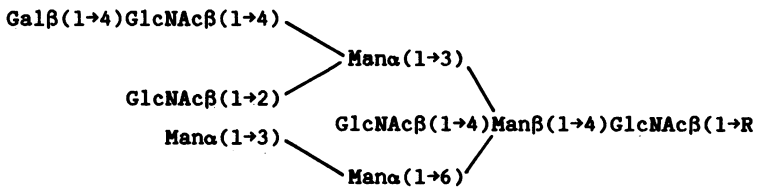
4 R = 4)GlcNAcβ(1→N)Asn: ovalbumin GPI glycoasparagine

FIG. 1. Formula and numbering of structures of the compounds tested in this study.

assignments (30a), carbohydrate chains can be depicted as shown in Fig. 1 (structures 1, 2, and 3). The important difference between structures 1 and 2 and structure 3 is that all of the 7S soybean oligosaccharides lacked the α(1→2)-linked Man on the medium antenna (dashed underlined elements in structures 1, 2, and 3, Fig. 1), whereas half of the kidney bean II oligosaccharides contained this residue.

Ovalbumin glycopeptides are well known as being a mixture of short oligomannoside-type and hybrid-type structures (41). Their separation as glycoasparagine units after extensive pronase digestion led to the isolation of the five previously described fractions (41) that contain structures 4 to 8 (Fig. 1). It should be noted that only GP-I (structure 4, hybrid type) and GP-IV and GP-V (structures 7 and 8, respectively, oligomannoside type) are pure compounds, whereas GP-II (structure 5) and GP-III (structure 6) are mixtures of carbohydrate chains.

The glycopeptides and oligosaccharides described above were tested as inhibitors of guinea pig erythrocyte agglutination by two enteropathogenic *E. coli* strains, and the



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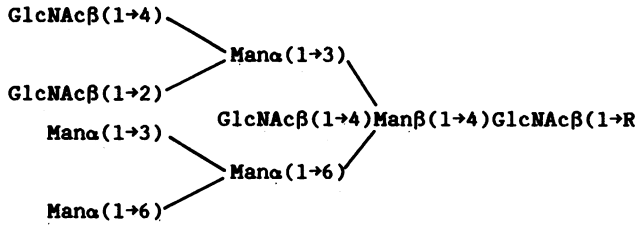
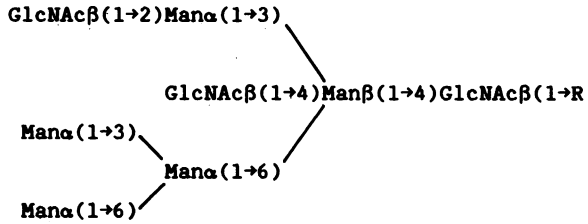


5 R = 4)GlcNAcβ(1→N)Asn: ovalbumin GP II glycoasparagines



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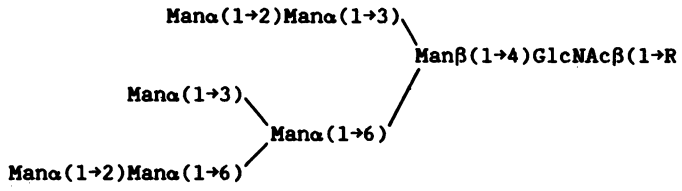

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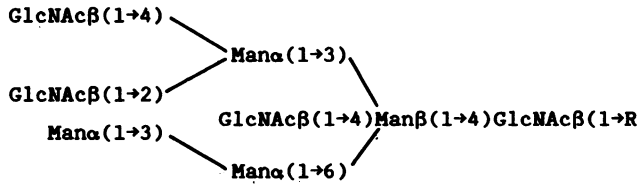
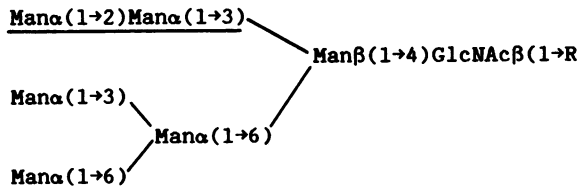
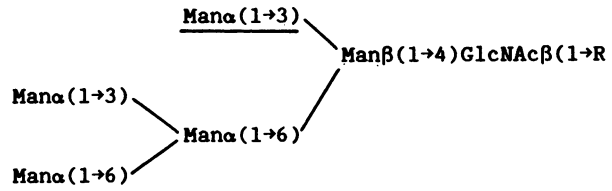


6 R = 4)GlcNAcβ(1→N)Asn: ovalbumin GP III glycoasparagines



7 R = 4)GlcNAcβ(1→N)Asn: ovalbumin GP IV glycoasparagine



8 R = 4)GlcNAcβ(1→N)Asn: ovalbumin GP V glycoasparagine

9 R = 4)GlcNacβ(1→N)Asn - polypeptide: As main compound of a controlled α-mannosidase digestion from 7S soybean glycopeptides

10 R = H: As main compound of a controlled α-mannosidase digestion from 7S soybean oligosaccharides.

11 R = 4)GlcNAcβ(1→N)Asn - polypeptide or R = H: As main compounds of controlled α-mannosidase digestions from kidney bean II glycopeptides or oligosaccharides.

results of their behavior are described in Table 1. It first appeared that large oligomannoside-type structures (1, 2, and 3) exhibited an activity similar to that of methyl-α-D-mannoside, when expressed in micrograms of Man per ml. This activity was 5.5 to 8.5 times that of the latter compound when expressed as a molar ratio, with each of the α-linked Man of the complex structures apparently behaving as a single unit. This result could be explained by postulating that a clustering of these residues is favorable for lectin recognition. In addition, the oligosaccharides produced by endo-β-*N*-acetylglucosaminidase H digestion behaved similarly to the corresponding native glycopeptides, suggesting a minor role for the internal GlcNAc residue that is N-linked to asparagine at the site recognized by the fimbrial lectin. However, the inhibitory activity of these large oligomannoside-type chains was still weak, as compared with that exhibited by the trisaccharide Manα(1→3)Manβ(1→4)GlcNAc.

The inhibitory activity of the ovalbumin glycopeptide mixture was surprising (Table 1), and although most of the concerned saccharide chains were of the hybrid type (and thus anticipated as nonactive [12]), the mixture described above exhibited an important activity. Separate tests involving these glycoasparagines (structures 4 to 8, Fig. 1) provided a solution to the problem. The hybrid-type glycopeptides (structures 4 and 5 and the main component of structure 6) were virtually inactive, whereas the pure $Man_6GlcNAc_2Asn$ (structure 7) exhibited moderate activity which was comparable to that of the large oligomannoside-type saccharides of plant origin. Finally, the potent activity of the pure $Man_5GlcNAc_2Asn$ (structure 8) showed that the crucial requirement for maximal inhibition lies in the occurrence of a nonsubstituted α(1→3)-linked terminal mannosyl residue initiating the first antenna (underlined element of structure 8) on the core structure common to all of these chains. The addition of only one α(1→2)-linked mannose on this antenna (underlined element of structure 7) dramatically decreased this inhibitory activity by a factor of 5 (compare the activities of structures 7 and 8 in Table 1).

The results presented above indicate that the preparation of potent inhibitors of bacterial adhesion mediated by type 1 pili (containing the $Man_5GlcNAc_2$ carbohydrate unit de-

TABLE 1. Inhibition of guinea pig erythrocyte agglutination by two enteropathogenic *E. coli* strains

Inhibitor[a]	Concn (μg/ml) for inhibition of[b]:		Concn (μM) for inhibition of[c]:		Relative activity[d]
	E. coli L74-30	*E. coli* 16375	*E. coli* L74-30	*E. coli* 16375	
Methyl-α-D-mannoside	60	125	335	695	1
Structure no.					
1	65	130	50	100	5.5–8.5
2	75	150	60	120	5.5–8.5
3	60	125	40	90	5.5–8.5
Ovalbumin glycopeptides (mixture)[e]	30	60	30	60	11
Structures no.					
4	No[f]	No			
5	No	No			
6	130	No	180		2.0
7	50	95	45	90	7.5
8	7.5	15	8	17	40
9	7.5	15	8	17	30–40
10	11	18	12	20	30–40
11	7.5	15	8	17	30–40
Manα(1→3)Manβ(1→4)GlcNAc	5	8	14	22	25–30

[a] Structure numbers are those given in Fig. 1.
[b] Minimal concentration necessary for complete inhibition. Expressed in micrograms of Man contained in the tested inhibitor per milliliter of the final suspension.
[c] Minimal concentration necessary for complete inhibition calculated from an average molecular weight when mixtures were tested.
[d] Activity relative to that of methyl-α-D-mannoside.
[e] Mixture of ovalbumin glycopeptides GP-I to GP-V (41).
[f] No inhibitory activity up to 200 μg/ml.

scribed above) could be envisaged by hydrolyzing all the external α(1→2)-linked mannoses from the large oligomannoside-type glycopeptides of plant origin. Since it has been reported that jack bean α-mannosidase cleaves the α(1→2)-linked mannosyl residues more rapidly than the other ones (41), we investigated suitable conditions for such a controlled digestion. Starting from both 7S soybean or kidney bean II oligosaccharides, the products liberated by incubation with jack bean for various time periods were analyzed by HPTLC. In Fig. 2 it is shown that the conversion of the $Man_{6-8}GlcNAc$ or $Man_{6-9}GlcNAc$ mixtures into shorter oligosaccharides (mainly the $Man_5GlcNAc$ unit) could indeed be achieved in 1 h. Longer incubation periods led to structures containing less than five mannoses (Fig. 2). The scaling up procedure based on this experiment allowed us to prepare similar shortened oligomannoside units from both native 7S soybean or kidney bean II glycopeptides (see above).

In Table 1 it is shown that structures 9 to 11 produced by the enzymatic digestions described above exhibited potent biological activity as inhibitors of guinea pig erythrocyte agglutination by two enteropathogenic *E. coli* strains. Structure 9 showed high inhibitory activity on two other well-characterized enteropathogenic *E. coli* strains (also selected for type 1 pilus expression) when compared with that of methyl-α-D-mannoside or native large glycopeptides of plant origin (Table 2). Furthermore, tested as an inhibitor of *E. coli* adhesion on human buccal cells, the $Man_5GlcNAc_2$-containing glycopeptide (structure 9) was again a highly active compound (Table 3). This last result confirmed the anticipated similarity (if not identity) between the lectin receptors on guinea pig erythrocytes and human buccal cells.

DISCUSSION

Our results lead first to the conclusion that type 1 pili recognize preferentially the carbohydrate structure corresponding to structures 8 to 11 (Fig. 1) and that such a short oligomannoside-type glycopeptide is probably identical to the adhesin receptor on both guinea pig erythrocytes and human buccal cells. This $Man_5GlcNAc_2$ unit is usually found in a wide variety of glycoproteins; therefore, it is not surprising that type 1 pili mediate bacterial adherence on different cells of various origin. The question that arises concerns the role of type 1 pili in the pathogenicity of *E. coli* strains, a subject of intensive investigations.

With regard to neonatal diarrhea in pigs, it has been shown that most such *E. coli* isolates (K88, K99, 987P, and F41) express type 1 fimbriae in addition to other known pili (6). In vitro studies have shown that *E. coli* attachment to porcine intestinal epithelium can be blocked by type 1 antiserum (17). In addition, type 1 pili that act as vaccine antigens effectively protected newborn pigs against colibacillosis (19). With regard to diarrheal diseases in humans, three types of pathological interactions of *E. coli* strains with the intestinal mucosa have been described (4). Enterotoxigenic *E. coli* adherence can be promoted by colonization factors CFA-I and CFA-II, as well as by type 1 pili (29). However, the in vitro adhesion to human enterocyte brush borders has been shown to be mediated by CFA-I and CFA-II pili only (7, 24, 25). Conversely, type 1 fimbriae promoted adhesion to human duodenal enterocyte basolateral surfaces (23). Results of an initial study have shown that, when used as a parenteral vaccine in humans, type 1 pili protect vaccinees after challenge with enterotoxigenic *E. coli* (30); but results of a subsequent study did not confirm this result (5). On human enteropathogenic *E. coli* strains, only expression of the type 1 pilus has been demonstrated. Adherence of such organisms on human intestinal tissues in vitro has not been reported, but adhesion to tissue culture cell lines has been described (4). Finally, attachment of enteroinvasive *E. coli* on cultured cells has been shown to involve at least three factors: type 1 fimbriae, bacterial glycocalyx, and mannose-resistant hemagglutinins (26).

In urinary tract infections, many *E. coli* isolates have been reported to express type 1 pili, but bacteria with this adhesin only attached in low numbers to human urinary tract epithelial cells (13). However, by using the mouse as a model, the

inability of mutants that lack the type 1 pilus to adhere on mouse bladder epithelial cells and to infect the animals has been demonstrated (18). Type 1 antiserum (37), methyl-α-D-mannoside (2), and pilus- or Man-specific antibodies (1) have been shown to protect mice against pyelonephritis. Recently, Hagberg (L. Hagberg, Ph.D. thesis, University of Göteborg, Sweden, 1984) demonstrated that the P adhesin provided a significant advantage for bacterial persistence in the kidney, although the type 1 pilus increased the bacterial recovery from the urinary bladder.

With regard to the immunological mechanisms, it has been proposed that type 1 adhesin decreases virulence by enhancing phagocytosis (38, 40). Specific binding of this fimbrial lectin to mouse peritoneal macrophages and human polymorphonuclear leukocytes have been observed; this adhesion was inhibited by Man or methyl-α-D-mannoside (3). Thus, even though it has been established that *E. coli* adherence mediated by type 1 fimbriae occurs in vivo (at least in animal diseases and models), it has not been shown whether these pili are expressed in the human gastrointestinal tract or during the course of human urinary tract infections. It has been proposed that this adhesin may play a crucial role in initiating infections, although the suppression of its phenotypic expression at subsequent stages of the infection may enable *E. coli* to escape phagocytosis (10).

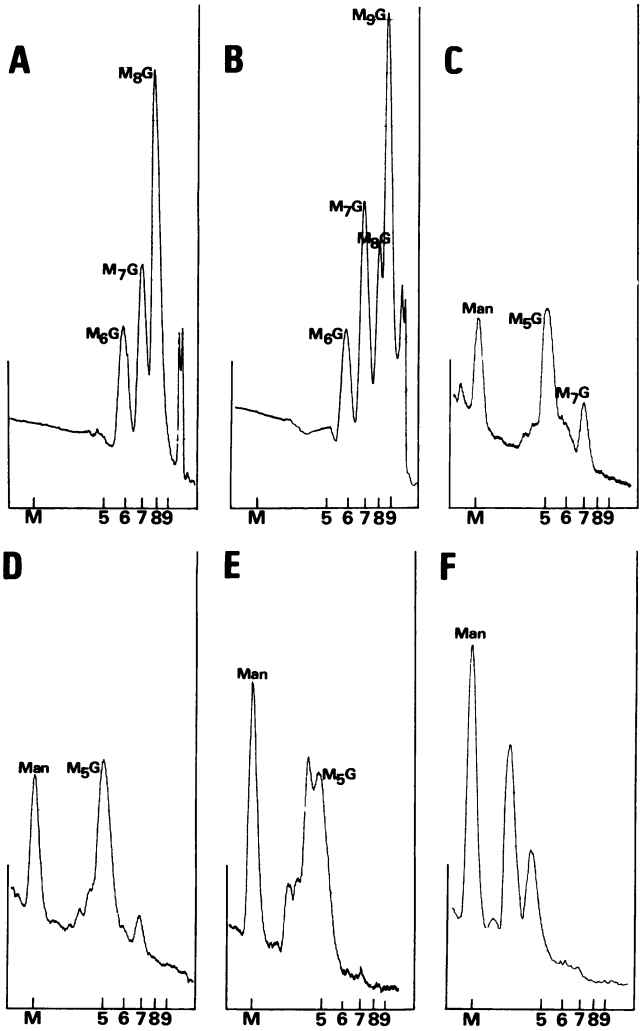


FIG. 2. Scanned patterns of HPTLC spots of oligosaccharides from plant glycopeptides (M_5G to M_9G = $Man_5GlcNAc$ to $Man_9GlcNAc$). (A) 7S soybean oligosaccharides; (B) kidney bean glycoprotein II oligosaccharides; (C through F) incubation of 7S soybean oligosaccharides with jack bean α-mannosidase (conditions were as described in the text). Incubation times were as follows: panel C, 0.5 h; panel D, 1 h; panel E, 4 h; panel F, 22 h.

TABLE 2. Inhibition of guinea pig erythrocyte agglutination by three enteropathogenic *E. coli* strains

Inhibitor[a]	*E. coli* O119:K69 (L74-30)		*E. coli* O86:K61 (B74-10)		*E. coli* O111:K58 (B75-44)	
	Concn (μM)[b]	Relative activity[c]	Concn (μM)[b]	Relative activity[c]	Concn (μM)[b]	Relative activity[c]
D-Mannose	435	0.5	865	0.5	435	0.5
Methyl-α-D-mannoside	215	1	435	1	215	1
Structure 1	35	6	65	7	15	14
Structure 9	5.5	40	5.5	80	3	70

[a] Structure numbers are those given in Fig. 1.
[b] Minimal concentration necessary for complete inhibition.
[c] Activity relative to that of methyl-α-D-mannoside.

Results of this study supply, for the first time, a simple procedure to prepare potent and specific inhibitors of *E. coli* adhesion to mammalian cells mediated by type 1 pili. Because oligomannoside-type oligosaccharides are the only carbohydrate chains in the major glycosylated storage proteins from soybeans and kidney beans, these legume flours represent an abundant source of such pure structures. Along with this natural abundance, the observed ease with which digestion with jack bean α-mannosidase can be controlled makes this procedure very convenient and suitable for preparative purposes. In addition, this enzymatic treatment is a very attractive example of conversion of a microheterogeneous mixture into a homogeneous carbohydrate unit. Finally, unlike methyl-α-D-mannoside, our $Man_5GlcNAc_2$-containing glycopeptide is presumably a nonabsorbable carbohydrate derivative (15) and therefore could be used to study inhibition of bacterial adhesion, especially in the field of gastroenterology. This preparation of short oligomannoside-type glycopeptides should prompt further investigations on the role of type 1 fimbriae in both human and animal *E. coli* pathogenicity, as well as on the feasibility of inhibition of bacterial adhesion by receptorlike carbohydrate structures to prevent infectious diseases.

TABLE 3. Inhibition of *E. coli* 16375 bacterial adhesion on human buccal cells

Inhibitor[a]	Concn (μg/ml)[b]	Adherence (%)
Methyl-α-D-mannoside	100	30
	50	55
	10	65
Structure 1	100	35
	50	70
	10	75
Structure 9	100	10
	25	23
	5	79

[a] Structure numbers are those given in Fig. 1.
[b] Expressed in micrograms of Man contained in the tested inhibitor per milliliter of the final suspension.

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