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ESCHERICHIA COLI STRAINS BINDING NEURAMINYL α2-3 GALACTOSIDES

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Summary. A total of 46 E. coli strains showing mannose-resistant, P-blood-group independent hemagglutination of human erythrocytes were tested for binding to neuraminic acid. Nine of the strains completely lost their hemagglutination activity after the erythrocytes were treated with neuraminidase. To charaterize the receptor structure, different neuraminic acid containing glycoproteins, their desialylated derivatives and neuraminyl oligosaccharides were tested for hemagglutination inhibition. These studies showed that the nine strains had binding specificity for α2-3 linked neuraminic acid.

Bacterial adhesion to host epithelial cells is an important virulence factor for many bacteria (1). Bacterial adhesion to epithelium is receptor-specific, although only few adhesion systems are at present understood at a molecular level. Binding to α-D-mannosyl residues on epithelial cells and erythrocytes is a common property among enteric bacteria (2,3). This binding is mediated by type I fimbriae (4). Escherichia coli strains associated with human pyelonephritis carry another type of fimbriae, which recognize blood group P specific glycosphingolipids on human erythrocytes and urinary tract epithelial cells (5-7). We have recently shown that about 10 % of pyelonephritogenic E. coli strains recognize other receptors than mannosides or P antigens on human erythrocytes (6). One of these "X-specific" strains recognizes glycophorin A and shows blood group M specificity (8). We now describe a novel binding specificity present in other X-specific strains. This interaction is based on the binding to terminal neuraminic acid units and shows linkage specificity for neuraminyl α2-3 galactosides.

MATERIALS AND METHODS

Bacteria. All E. coli strains were human isolates showing X-specific hemagglutination. 27 of the strains were from the urine of patients

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Abbreviations: PBS, 10 mM sodium phosphate, 0.15 M NaCl, pH 7.1

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with different levels of urinary tract infection, four strains were fecal isolates, nine were from newborn meningitis and for eight strains the clinical diagnosis was not known. O serotyping and isolation of most of the strains has been described elsewhere (6,9). The presence of K 1 capsular antigen was tested by latex agglutination (10). The strains belonged to O serotypes 1 (three strains), 2 (seven strains), 4 (two strains), 6 (one strain), 7 (three strains), 18 (seven strains), 75 (ten strains) or 86 (one strain). Five of the strains were rough and seven were nontypable by the available antisera. The strains were preserved in nutrient agar at room temperature for 1 to 5 years until the time of testing. For hemagglutination assays they were subcultured twice on colonization factor antigen agar plates (11).

Glycoproteins and oligosaccharides. Orosomucoid was kindly supplied by Dr. G. Myllylä (Finnish Red Cross Blood Transfusion Service, Helsinki). Fetuin, bovine submaxillary mucin, colominic acid and dextran sulphate were purchased from Sigma Chemical Co. Glycophorin glycopeptides were obtained by trypsin treatment of human erythrocytes (12) followed by pronase digestion and purification on DEAE-Sephadex (13). The monosaccharide compositions were analyzed by gas-liquid chromatography (14). Desialylated glycoproteins and glycopeptides (less than 5 % of original neuraminic acid) were obtained by treatment with 0.1 M HCl at 80 ^{O}C for 1 h followed by dialysis against PBS or gel filtration on Bio-Gel P-2 eluted with PBS. A crude neuraminyl oligosaccharide fraction was obtained from bovine colostrum as described by Öhman (15). Mononeuraminyl and dineuraminyl compounds were separated by ion exchange chromatography on DEAE-Sephadex (13) and the individual mononeuraminyl oligosaccharides, NeuAc(α2-3)Gal-(β1-4)Glc, NeuAc(α2-6)Gal(β1-4)Glc and NeuAc(α2-6)Gal(β1-4)GlcNAc (16), were obtained by a modification of the method of Schneir and Rafelson (17). NeuAc(α2-8)NeuAc(α2-3)Gal(β1-4)Glc (16) was purified by preparative paper chromatography with pyridine-ethyl acetate-water-acetic acid 5:5:3:1 (v/v). NeuAc(α2-3)Gal(β1-4)GlcNAc was purified from human urine as described elsewhere (J. Parkkinen and J. Finne, manuscript in preparation). All inhibitor solutions were adjusted to pH 7.1.

Hemagglutination tests. Hemagglutination tests were carried out on glass slides over crushed ice as described previously (4,6). All hemagglutinations were performed in the presence of 0.1 M α-methyl-D-mannoside to measure only mannose-resistant hemagglutination. Human blood group $\bar{p}$ erythrocytes supplied by Dr. Anna Pirkola (Finnish Red Cross Blood Transfusion Service, Helsinki, Finland) were used to exclude the P-specific hemagglutination present in some bacterial strains. Neuraminidase treatment of the erythrocytes were carried out as previously described (18). For hemagglutination inhibition studies the bacterial suspensions were titrated with the erythrocytes, and bacterial concentrations corresponding to twice the lowest concentration giving complete hemagglutination were used (about 10^{10} bacteria/ml in most cases).

RESULTS

Nine of the 46 *E. coli* strains showing X-specific hemagglutination completely lost their hemagglutinating activity when the erythrocytes were treated with neuraminidase (Table 1). In contrast neuraminidase treatment of the bacteria had no effect on the hemagglutination. The strains showing neuraminidase-sensitive hemagglutination were of O serotypes 2 and 18, and one of the strains was nontypable. To characterize the receptor structure, different neuraminic acid containing glycoproteins and their desialylated derivatives were tested for hemagglutination inhibition. As shown in Table 2, orosomucoid and fetuin completely inhibited hemagglutination with

Table 1 Properties of the E. coli strains showing neuraminidase-sensitive, mannose-resistant hemagglutination (MR-HA).

Strain	Serotype	Origin[a]	MR-HA of human p erythrocytes	
			untreated	neuraminidase-treated
IH11005	Ont	ABU	+	-
IH11054	O2K1	PN	+	-
IH11934	O2	F	+	-
A159	O2K1	UTI	+	-
108	O18K1	UTI	+	-
113	O18K1	UTI	+	-
A468	O18acK1H7	NBM	+	-
101	O18acK1H7	NBM	+	-
102	O18acK1H-	F	+	-

[a]ABU, asymptomatic bacteriuria; PN, pyelonephritis; F, faecal; UTI, urinary tract infection (unclassified); NBM, newborn meningitis

all the nine strains. Bovine submaxillary mucin was less effective, and glycophorin glycopeptides induced only partial inhibition with six of the strains. None of the corresponding desialylated derivatives showed any inhibition. When the glycoproteins were tested in tenfold dilutions, orosomucoid induced complete inhibition at 0.01 to 0.1 mM concentrations (calculated as neuraminic acid), whereas the corresponding concentrations for fetuin and bovine submaxillary mucin were 0.1 to 1.0 mM. Colominic

Table 2 Hemagglutination inhibition by glycoproteins and polysaccharides

Inhibitor tested[a]	Inhibition of hemagglutination by E. coli strain[b]								
	IH11005	IH11054	IH11934	A159	108	113	A468	101	102
Orosomucoid	+	+	+	+	+	+	+	+	+
Desialized orosomucoid	-	-	-	-	-	-	-	-	-
Fetuin	+	+	+	+	+	+	+	+	+
Desialized fetuin	-	-	-	-	-	-	-	-	-
BSM	+	(+)	(+)	+	+	+	+	+	(+)
Desialized BSM	-	-	-	-	-	-	-	-	-
Glycophorin GP	(+)	(+)	(+)	-	-	(+)	(+)	(+)	-
Desialized glycoph. GP	-	-	-	-	-	-	-	-	-
Colominic acid	-	-	-	-	-	-	-	-	-
Dextran sulphate	-	-	-	-	-	-	-	-	-

[a]The final concentration of the native glycoproteins was 1 mM (as neuraminic acid) and the desialized derivatives were used at the corresponding protein concentration. The concentration of colomic acid was 5 mM (as neuraminic acid) and that of dextran sulphate 3 mg/ml. BSM, bovine submaxillary mucin; GP, glycopeptides.

[b]+, complete inhibition; (+), partial inhibition; -, no inhibition.

Table 3 Hemagglutination inhibition by oligosaccharides.

Inhibitor tested[a]	Inhibition of hemagglutination by *E. coli* strain[b]								
	IH11005	IH11054	IH11934	A159	108	113	A468	101	102
NeuAc(α2-3)Gal(β1-4)Glc	+	+	+	+	+	+	+	+	+
NeuAc(α2-6)Gal(β1-4)Glc	-	(+)	(+)	-	-	(+)	-	-	-
NeuAc(α2-3)Gal(β1-4)GlcNAc	+	+	+	+	+	+	+	+	+
NeuAc(α2-6)Gal(β1-4)GlcNAc	-	(+)	(+)	-	-	(+)	-	-	-
NeuAc(α2-8)NeuAc(α2-3)-Gal(β1-4)Glc	-	-	-	-	-	-	-	-	-
Gal(β1-4)Glc[c]	-	-	-	-	-	-	-	-	-
NeuAc[c]	-	-	-	-	-	-	-	-	-

[a] Each inhibitor was tested at 5 mM concentration.

[b] +, complete inhibition; (+) partial inhibition; -, no inhibition.

[c] Not inhibitory even at 33 mM concentration

acid, a polymer of α2-8 linked N-acetylneuraminic acid, and dextran sulphate induced no inhibition, indicating that the inhibitions observed were not due to unspecific charge effects.

Purified neuraminyl oligosaccharides were used to study the linkage specificity of the interaction (Table 3). At 5 mM concentration only the α2-3 isomers of neuraminyl lactose and neuraminyl N-acetyllactosamine produced complete inhibition. This was observed with all strains. The corresponding oligosaccharides containing an α2-6 linked neuraminic acid produced weak or no inhibition. Dineuraminyl lactose, which has an additional α2-8 linked residue bound to an α2-3 linked neuraminic acid, produced no inhibition indicating that a terminal α2-3 linked neuraminic acid is necessary for inhibition. Free neuraminic acid or lactose induced no inhibition even at 33 mM concentration. Different monosaccharides (galactose, glucose, N-acetylgalactosamine, N-acetylglucosamine, and fucose) were noninhibitory at 66 mM concentrations.

DISCUSSION

Recognition of cellular receptors by pathogenic bacteria is a prerequisite for many infectious diseases. About 80 % of *E. coli* strains causing pyelonephritis in children carry P-fimbriae, which enable the bacteria to bind to P blood group antigens on human cells (5-7). However, about 10 % of *E. coli* strains causing urinary tract infections recognize another, non-P receptor on human erythrocytes (6). Our results show that part of these "X-specific" strains, as well as some strains from newborn meningitis and

some fecal strains, recognize neuraminic acid-containing structures on human erythrocytes. This was indicated by two independent lines of evidence: 1) neuraminidase treatment of erythrocytes destroyed the receptor structures, and 2) neuraminic acid containing glycoproteins and oligosaccharides but not their desialylated derivatives abolished hemagglutination.

Orosomucoid, which showed best inhibition among glycoproteins tested (Table 2), contains only N-glycosidic oligosaccharide chains with terminal neuraminyl N-acetyllactosamine sequences (19). Fetuin contains both N-glycosidic and O-glycosidic oligosaccharide chains, whereas bovine submaxillary mucin and glycophorin contain predominantly O-glycosidic oligosaccharide chains (20-22). Our results suggest that neuraminyl N-acetyllactosamine sequences of N--glycosidic oligosaccharide chains are better inhibitors than the sialylated O-glycosidic oligosaccharide chains, which on the erythrocyte occur mainly in glycophorin (22). This is also supported by the finding that trypsin treatment of erythrocytes, which is known to remove the glycosylated part of glycophorin (12,23), did not abolish hemagglutination (unpublished results).

Studies with the purified neuraminyl oligosaccharides indicated that the interaction has a higher affinity for α2-3 linked neuraminic acid than for α2-6 or α2-8 linked residues (Table 3). Taken together, the results suggest that the receptor structures for the bacteria could be neuraminyl α2-3 N-acetyllactosamine or neuraminyl α2-3 lactose units of glycoproteins and glycolipids. Experiments designed to characterize the molecular nature of these receptors are underway.

The clinical significance of the adhesion specificity now described also remains to be established. The strains studied here were from several types of patients but no clear correlation with virulence has so far been established. It is noteworthy that recognition of α2-3 linked neuraminic acid was correlated with O serotypes 2 and 18 and that most of the strains also contained the K 1 capsular polysaccharide.

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