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Specificity of S Fimbriae on Recombinant *Escherichia coli*: Preferential Binding to Gangliosides Expressing NeuGcα(2-3)Gal and NeuAcα(2-8)NeuAc

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The adhesins of *Escherichia coli* strains HB101(pANN801-13) and HB101(pAZZ50), which express S fimbriae encoded by a recombinant plasmid containing the *sfaI* and *sfaII* gene clusters, respectively, were characterized with regard to the detailed structural requirements of their binding to sialyloligosaccharides on (neo)glycoproteins and (neo)glycolipids. From binding and binding inhibition studies in solid-phase enzyme immunoassays with isolated S fimbriae, several major conclusions can be drawn. S fimbriae bind specifically to sialic acid on gangliosides. The most active structural variant of sialic acid on GM3 ganglioside is *N*-glycolylneuraminic acid (NeuGc). In contrast to previous reports, high binding activities were measured also for b-series gangliosides expressing NeuAcα(2-8)NeuAc. In agreement with earlier studies, the site of sialic acid substitution to subterminal sugars strongly influences the binding to sialyloligosaccharides, i.e., α-6-linked sialic acid is only poorly recognized by the adhesin compared with α-3-linked sialic acid. C-8 and C-9 hydroxyl groups form essential structural elements of sialic acid in the binding event.

Bacterial adhesion to host epithelial cells has long been known to be a prerequisite for the initiation of infection (2). Moreover, species specificity and tissue selectivity of bacterial adhesion and infection had early suggested that the process should be receptor specific. For most bacterial adhesion systems, it has now been established on a molecular level that the recognition process is mediated by lectin-like adhesins on bacterial surfaces that specifically interact with complex carbohydrates on epithelial membranes of host tissues (17). Bacterial adhesins are often carried by hairlike, filamentous fimbriae, which are characterized by their hemagglutination and serological properties and classified according to these into different types (17). Many enteric bacteria possess type I fimbriae with a mannose-sensitive binding characteristic (17). *Escherichia coli* strains associated with human pyelonephritis carry another type of fimbria which recognizes blood group P-specific glycosphingolipids (14). *E. coli* strains that cause sepsis and meningitis in neonatal infants often carry a third type of fimbria with a binding specificity for sialic acid containing glycoconjugates (S fimbriae) (21). The known organ tropism of the hematogenous infection by S-fimbriated *E. coli* to the cerebrospinal fluid and to brain tissue and the temporal restriction of susceptibility to infection to a period of about 4 weeks after birth strongly suggest an organ-characteristic and developmentally regulated expression of the receptor carbohydrate.

Previous work on the binding specificity of the adhesin on S fimbriae encoded by the *sfaI* gene cluster had revealed that glycoproteins expressing the terminal disaccharide sequence NeuAcα(2-3)Gal represent the most potent inhibitors of hemagglutination by the bacterial cells or by the isolated fimbriae (12). No correlation between organ selectivity of infection and the tissue distribution or developmentally regulated expression of the proposed adhesin receptor can be postulated on the basis of available structural information.

The present contribution reports on the results of a reinvestigation performed on the basis of a solid-phase binding assay using isolated S fimbriae and structurally defined gangliosides or (neo)glycolipids as immobilized receptor analogs. It was the aim of this study to define on a molecular level the structural requirements of S-adhesin binding and to correlate these findings with information on receptor expression during fetal brain development. The investigations have mainly been performed with S fimbriae encoded by the *sfaI* gene cluster. Some of the results obtained were confirmed with S fimbriae encoded by the *sfaII* gene cluster present in *E. coli* from meningitis strains. There is evidence from DNA sequencing of the respective genes that both types of S fimbriae carry adhesins with identical primary structures (6a).

MATERIALS AND METHODS

Bacteria. Recombinant *E. coli* HB101(pANN801-13) and HB101(pAZZ50), which do not express type I fimbriae, were used for isolation of S fimbriae (7).

Isolation and purification of S fimbriae. S fimbriae were isolated essentially according to the procedure described by Salit and Gotschlich (28) as modified by Wevers et al. (34). The homogeneity of the preparation was confirmed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and examination by electron microscopy. Preparations used for the binding studies contained S fimbriae in the unaggregated state.

Gangliosides, glycoproteins and polysaccharides. Gangliosides GM3, GM1a, GD1a, GD1b, and GT1b were purchased from Sigma (Munich, Germany), while ganglioside GD3 was from Bio-Carb (Lund, Sweden). GM3-(NeuGc) was prepared in our laboratory according to the procedure described below. Neoglycolipids were synthesized by conjugation of

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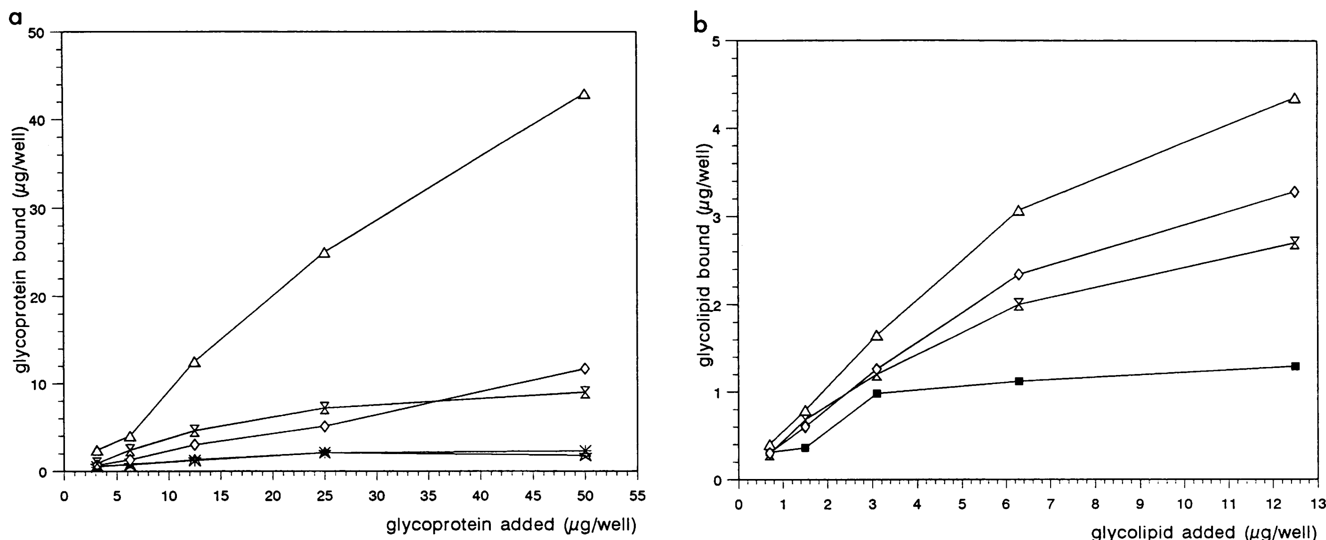

FIG. 1. (a) Immobilization of sialoglycoproteins to polystyrene plates. Tritiated sialoglycoproteins were solubilized in 0.2 M sodium carbonate buffer, pH 9.6 (1 mg/ml), and 50-μl aliquots were dried down at 37°C after twofold serial dilution in the same buffer. After washing off nonbound sialoglycoproteins, immobilized sialic acid was quantitated by hydrolysis in 200 μl of 0.2 M H_2SO_4 for 1 h at 70°C followed by radiometric analysis of the solubilized products △, bovine glycophorin; ⧖, human glycophorin; ◇, human meconium glycoproteins; ☆, *Collocalia* mucin; ×, bovine submaxillary mucin. (b) Ganglioside immobilization to polystyrene plates. Tritiated gangliosides were solubilized in methanol (0.25 mg/ml), and 50-μl aliquots were dried down at 37°C after twofold serial dilution in methanol. Nonbound gangliosides were washed off with 3 × 200 μl of water, and the remaining glycolipids were eluted from the plastic surface by addition of 2 × 200 μl of *n*-butanol. The combined aliquots from the washing or elution steps were filled up to 1 ml with *n*-butanol or water to ensure equal solvent compositions and were prepared for radiometric analysis. ◇, GD1a; △, GM3-NeuGc; ⧖, GD1b; ■, GT1b.

sialyloligosaccharides 3′-sialyllactose, 6′-sialyllactose, and sialyllactotetraose b to dipalmitoylphosphatidylethanolamine according to the method of Stoll et al. (29). Derivatives of *N*-acetylneuraminic acid on GM3 (C-7 analog, methyl ester) were prepared according to methods published in reference 25. Commercial gangliosides, neoglycolipids, and GM3 derivatives were analyzed for their identity and purity by high-performance thin-layer chromatography, gas-liquid chromatography of sialic acid, and fast atom bombardment-mass spectrometry in the negative ion mode (4). Colominic acid and bovine submaxillary mucin were commercial products of Sigma, while ovine submaxillary mucin and sialyllactotetraose c-HSA were obtained from Bio-Carb. Sialoglycoproteins from erythrocyte membranes (human or bovine) or mucins from the nest-cementing substance of Chinese swiftlets (members of the genus *Collocalia*) and from human meconium were prepared according to the method of Klenk and Uhlenbruck (10).

Chemical modification of gangliosides and sialoglycoproteins. Sialic acid on human or bovine glycophorins which had been immobilized to polystyrene plates was chemically modified by oxidative cleavage of the side chain with $NaIO_4$ at pH 5.5 for 2 h at 4°C and reduction with sodium borohydride resulting in formation of the C-7 analog (26). Similarly, the sialoglycoproteins were desialylated after immobilization by incubation with 10 mU of *Vibrio cholerae* sialidase for 2 h at 37°C.

Formation of the methyl ester of NeuAc on GM3 was performed by incubation of ganglioside (0.1 mg) in dry methanol containing Dowex 50WX8 in the hydrogen form at ambient temperature (26). After stirring for 48 h, the product was recovered by filtration and washing with 3 ml of methanol. The derivative was analyzed by high-performance thin-layer chromatography on silica 60 plates (Merck, Darmstadt, Germany) with chloroform-methanol-2.5 M ammonium acetate as the mobile phase. Gangliosides used for binding assays were solubilized in methanol either on a weight basis or according to their sialic acid content as quantified by the thiobarbituric acid assay (1).

Solid-phase binding and binding inhibition assay of isolated S fimbriae. Ninety-six-well polystyrene microtiter plates were coated with glycoconjugates by drying at 37°C of 50-μl solutions in 0.1 M carbonate buffer, pH 9.6 (glycoproteins), or in methanol (gangliosides). The actual amounts of sialoglycoproteins immobilized to the wells were quantitated by analysis of hexose (19) and sialic acid (1). Moreover, binding of sialoglycoproteins and gangliosides to the plastic surface was measured by radiometric analysis after mild periodate oxidation of sialic acid and reductive labeling with NaB^3H_4.

Binding of sialoglycoproteins varied considerably with the compound immobilized (Fig. 1a). On addition of 50 μg per well, the amounts of adsorbed glycoprotein ranged from 2 to 43 μg per well, corresponding to 0.1 to 2 μg of sialic acid per well. Accordingly, because of the high variability of sialic acid immobilization, comparative analyses of S-fimbrial binding to sialoglycoproteins (Fig. 2a) are difficult to interpret.

In the case of gangliosides, 0.75 to 1.25 μg was bound to the 0.5-cm^2 plastic surface, corresponding to 0.2 to 0.5 μg of sialic acid per well, if 50-μl solutions of the various gangliosides in methanol (50 μg/ml) were dried down (Fig. 1b). The binding profile varied with the type of ganglioside, particularly at higher concentrations exceeding 0.1 mg/ml. In the context of this study, it is important to indicate that GM3-NeuGc and GD1b exhibited 77 and 63%, respectively, of the binding activity measured for GD1a at 250 μg/ml (Fig. 1b).

The active surface area remaining after immobilization of the glycoconjugates was blocked by incubation of the wells

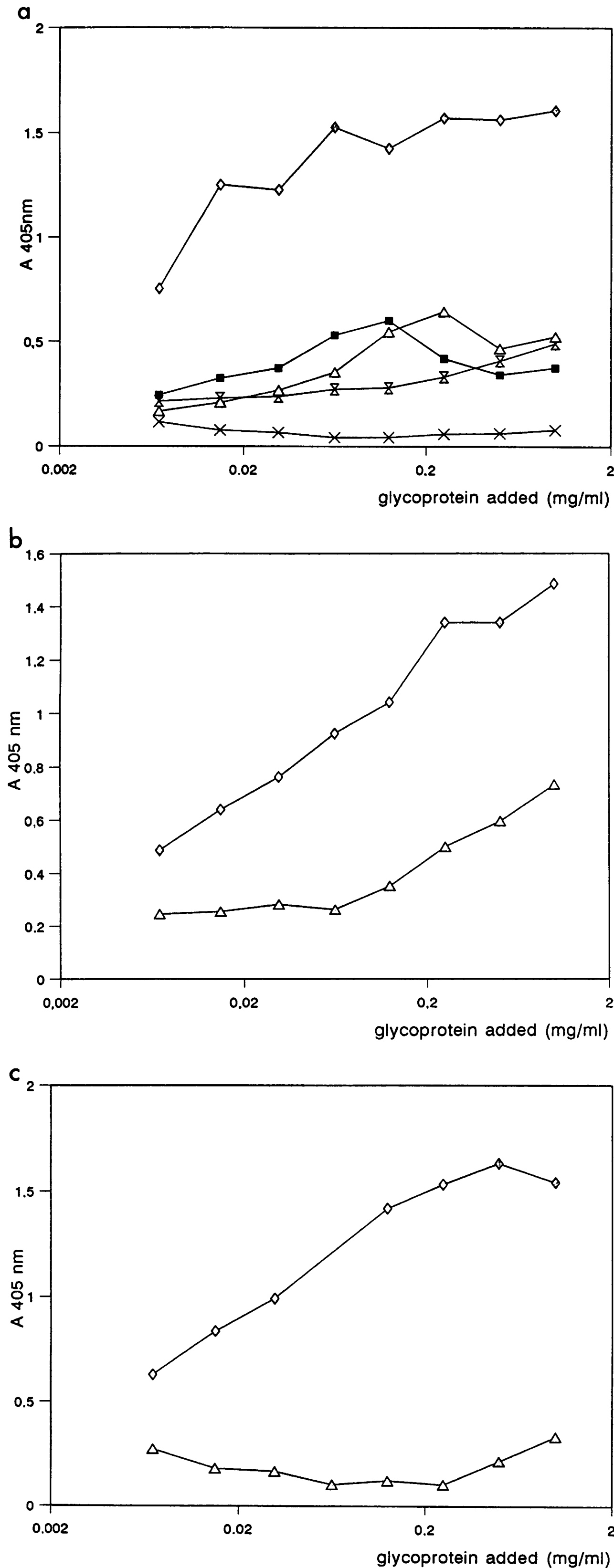

FIG. 2. Binding of S fimbriae to sialoglycoproteins. Sialoglycoproteins in 0.1 M carbonate buffer, pH 9.6 (1 mg/ml), were dried down (37°C) as 50-μl aliquots after twofold serial dilution. S fimbriae of the *sfaII* gene cluster were used at 50 μg of protein per ml of PBS, and 1,000-fold-diluted antibody A21356 A1 was used for the detection. In panels b and c, background binding of S fimbriae to the BSA-coated plastic surface was subtracted. (a) Comparative binding analyses were performed with human glycophorin (△), bovine glycophorin (◇), and mucins from bovine submaxillary glands (×), from human meconium (■), or from saliva of Chinese swiftlets (genus *Collocalia*) (⧖). Background binding of S fimbriae to the BSA-coated plastic surface was not subtracted and corresponded to 0.151 (A_{405}). (b) Bovine glycophorin was immobilized to the wells at various concentrations and treated with *V. cholerae* sialidase at 10 mU/ml for 2 h at 37°C. ◇, untreated control; △, desialylated samples. (c) Bovine glycophorin was immobilized to the wells at various concentrations and treated with 0.5 mM $NaIO_4$ in 0.1 M acetate buffer, pH 5.5, for 2 h at 4°C and then reduced with 50 mM $NaBH_4$ in PBS for 30 min at ambient temperature. The controls were treated equally except for omission of the periodate. ◇, untreated control; △, periodate-treated samples.

with 250 μl of 5% bovine serum albumin (BSA) in phosphate (20 mM)-NaCl (0.15 M), pH 7.2 (phosphate-buffered saline [PBS]), for 1 h at 37°C prior to addition of S fimbriae (0.25 mg of protein per ml of PBS). Following incubation of the covered plates for 1 h at 37°C, the 50-μl suspension was removed by aspiration and the wells were washed three times with 0.5% BSA-PBS. Bound fimbriae were detected after washing with monoclonal antibody A21356 A1 (18) diluted 1/500 in 0.5% BSA-PBS and incubation for 16 h at 4°C. The plate was developed by a double sandwich assay using goat anti-mouse immunoglobulin (Z 259; Dako, Hamburg, Germany) and alkaline phosphatase–anti-alkaline phosphatase complex (Boehringer, Mannheim, Germany) followed by 4-nitrophenyl-phosphate in diethanolamine buffer, pH 9.8, containing 0.5 mM $MgCl_2$. Hydrolysis of the substrate was quantified spectrophotometrically at 405 nm in a Dynatech MR 5000 enzyme-linked immunosorbent assay reader. Specificity of binding was confirmed by appropriate control experiments using chemically desialylated NeuGc-GM3 (1 N acetic acid, 2 h, 100°C) and by binding inhibition assays. For this purpose, the fimbrial suspension was coincubated with serial twofold dilutions of oligosaccharide inhibitors (up to 10 mg/ml).

Hemagglutination inhibition assays. Hemagglutination caused by isolated S fimbriae and inhibition assays were performed with 2% suspensions of human blood group O and bovine erythrocytes in the cold as described by Korhonen (11).

Isolation of gangliosides. GM3-(NeuGc) was isolated from membranes of equine erythrocytes as described by Nohara-Uchida and Ohashi (20) with minor modifications. Briefly, membranes (300 mg) were extracted with chloroform-methanol mixtures in the order 2/1, 1/1, and 1/2 by ultrasonication for 15 min. The combined supernatants obtained after centrifugation were evaporated in vacuo, applied to anion-exchange chromatography on DEAE-Sephadex A25 (acetate form, 30 ml), and run in chloroform-methanol-water, 3/7/0.2, with chromatography being followed by evaporation of the solvents, desalting by dialysis against water, and freeze-drying. GM3-(NeuGc) was separated from other monogangliosides by preparative thin-layer chromatography on silica 60 (Merck) with chloroform–methanol–2.5 M ammonium hydroxide (60/35/8, vol/vol/vol) as the mobile phase. The bands were visualized by staining with iodine vapor, scraped from the plates, and extracted with chloroform-methanol-water, 10/10/1.

The same procedure was followed for the isolation of

mono-, di-, and trisialogangliosides from human fetal brain starting from 75.2 g of wet tissue.

Analytical methods. Ganglioside fractions from human fetal brain (26th week of gestation) or equine erythrocytes (0.1 mg) were methanolyzed in 1 ml of 0.05 N HCl-CH_3OH at 70°C for 2 h. The methanolysate was extracted with *n*-hexane (3 × 1 ml) to remove fatty acid methyl esters and dried down in a stream of nitrogen. After extensive drying in vacuo over KOH, the samples were taken up in 100 μl of *N*-methyl-*N*-trimethylsilyltrifluoroacetamide mixed with 10 μl of trifluoroacetic acid. Following heating at 70°C for 30 min, 2-μl aliquots were analyzed by gas chromatography-mass spectrometry using a fused silica capillary column wall-coated with SE54 (17 cm) for separation of the per-*O*-trimethylsilylmethylester-2-*O*-methylglycosides of sialic acids in a temperature gradient from 150 to 320°C (15°C per min). Eluting compounds were registered by single-ion monitoring at *m/z* 298,386,420 and 508 with a Hewlett-Packard MSD 5970 (dwell time, 20 ms) (9).

The identity of GM3-NeuGc isolated from equine erythrocytes was established by fast atom bombardment-mass spectrometry analysis on a ZAB HF mass spectrometer (VG-Analytical, Manchester, United Kingdom) after methylation with methyl sulfoxide carbanion-methyliodide.

RESULTS

Adhesion of S fimbriae to sialoglycoproteins and sialomucins. Isolated S fimbriae from recombinant *E. coli* HB101 (pANN801-13) (data not shown) and HB101(pAZZ50) were tested in a solid-phase enzyme immunoassay for their capacity to bind to various sialoglycoproteins before and after biochemical modification of their carbohydrates (Fig. 2). Both types of S fimbriae showed identical binding patterns on the different glycoproteins. Specificity of fimbrial adhesion was verified by enzymatic removal of sialic acid (Fig. 2b) and by mild selective periodate oxidation of the C-7–C-8–C-9 side chain of sialic acids resulting in the formation of the corresponding C-7 analogs (Fig. 2c).

The binding capacities of the various sialoglycoproteins can be related neither to their sialic acid contents nor to particular structural features of the proposed receptors because of the heterogeneity of their carbohydrate portions and substance-specific variations in their plate binding observed over a broad range of glycoprotein concentrations (refer to Materials and Methods). As a general feature of S-fimbrial binding to sialoglycoproteins, their adhesion to α-6-linked sialic acid as found on bovine submaxillary mucin (Fig. 2a) or ovine submaxillary mucin (data not shown) or on sialyllactotetraose c-HSA (data not shown) does not exceed the level of background binding.

Adhesion of S fimbriae to gangliosides and neoglycolipids. To establish the detailed structural requirements of adhesin binding, S fimbriae were tested on monospecific glycoconjugates (gangliosides and neoglycolipids) immobilized to polystyrene microtiter plates (Fig. 3). In accordance with previous reports (12, 23) on the binding specificity of S fimbriae, the position of linkage of sialic acid to subterminal sugars was found to be of major importance. As already suggested by the binding studies performed for sialoglycoproteins and sialomucins, α-3-linked sialic acid (structures 8, 9, 13, 15, and 16 in Table 1) represents the preferred receptor compared with α-6-linked sialic acid (structures 10 and 12 in Table 1), which shows only low binding capacities (Fig. 4). Substitution of GM3 in position 4 of the subterminal galactose by the structural element Galβ(1-3)GalNAc as found in GM1a reduced the fimbrial binding (Fig. 4) only slightly. In contrast to earlier findings by Parkkinen et al. (21, 23), the structural element NeuAcα(2-8)NeuAc as found on b-series gangliosides (structures 11, 14, and 16 in Table 1) shows strong binding capacities, which regularly exceed those of NeuAcα(2-3)Gal-expressing gangliosides (Fig. 3). In particular, GD3, GD1b, and GT1b (Fig. 3) were highly active in the solid-phase enzyme immunoassay, suggesting that the conditions of the assay (solid phase versus solution) or the mode of receptor presentation (lipid bound versus free) may be critical for the binding event.

Finally, the most potent adhesin receptor was *N*-glycolylneuraminic acid (NeuGc) on GM3 derived from equine erythrocytes (Fig. 3) which expresses the terminal disaccharide NeuGcα(2-3)Gal (structure 13 in Table 1). Binding activity of GM3-NeuGc exceeded that of GM3-NeuAc by a factor of at least 16, if the ganglioside concentrations at half-maximal binding were compared.

Binding to native gangliosides is readily inhibited by 3′-sialyllactose, while background adhesion to desialylated gangliosides, cholesterol, or lecithin is not. This hydrophobic interaction of S fimbriae does not interfere with the event of specific binding to gangliosides.

To summarize the data obtained with defined gangliosides and (neo)glycolipids, the binding specificity of S fimbriae can be described by the following receptor structures ordered according to decreasing activities: NeuGcα(2-3) > NeuAcα(2-8)NeuAc > NeuAcα(2-3)Gal > NeuAcα(2-6)Gal(NAc).

Thus, S fimbriae exhibit with regard to their specificities striking similarities with other sialic acid-specific *E. coli* adhesins like those on K99 fimbriae (15) (see Discussion).

Adhesion of S fimbriae to structural variants of sialic acids. In using NeuAcα(2-3)Gal on GM3 (structure 9) as a reference for comparative binding studies of structural variants of sialic acid, further details of the binding characteristics of S fimbriae became evident (Fig. 4 and 5). As shown above, increased binding was observed when C-5 of sialic acid was N-glycolylated instead of N-acetylated. The reverse effects on the binding capacity of sialic acid were measured, when the hydroxyl groups at C-8 and C-9 were not available as with the C-7 sialic acid analog of NeuAc-GM3 (Fig. 4) or with sialic acids on human or bovine glycophorins (Fig. 2) obtained after oxidative cleavage of the side chain. A less pronounced reduction of the binding capacity by GM3-NeuAc was observed, when the carboxyl group was converted to the methyl ester (Fig. 4 and 5).

In summary, enhancement of binding seems to be strongly dependent on the availability of hydroxyl groups in C-8, C-9, and N-acyl groups and to some extent also on electrostatic interactions of the adhesin with the carboxyl group of sialic acid.

Inhibition of S fimbriae binding in hemagglutination and in solid-phase enzyme immunoassay. Since earlier findings on the binding specificity of S fimbriae were in several respects contradictory to our present results (23), purified S fimbriae from recombinant *E. coli* HB101(pANN801-13) were characterized also by hemagglutination inhibition studies and compared with the corresponding data from binding inhibition in solid-phase enzyme immunoassay. In both systems, the findings of Parkkinen et al. and Korhonen et al. (12, 21, 23) were confirmed. While 3′-sialyllactose caused 50% inhibition at 0.97 mM and 6′-sialyllactose caused 50% inhibition at 2.58 mM, colominic acid did not affect the binding of S fimbriae over the entire range of concentrations tested (up to 10 mM total sialic acid measured by the thiobarbituric acid assay). It should be noted in this context that the actual

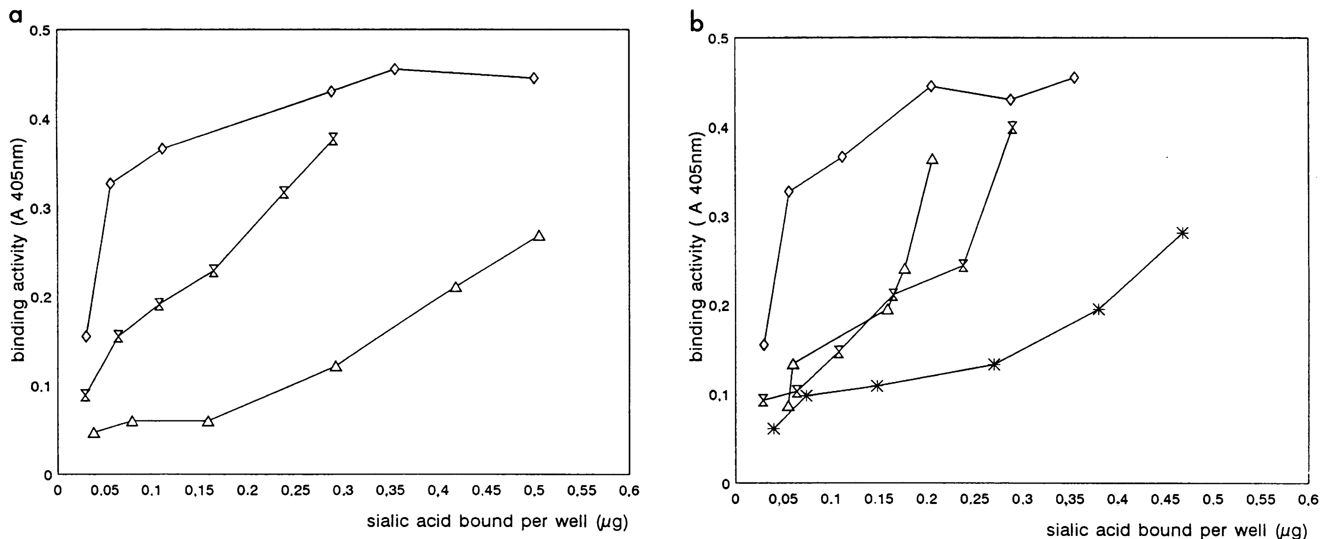

FIG. 3. Binding of S fimbriae to gangliosides. Gangliosides were immobilized as described in the legend to Fig. 1, and S fimbriae of the *sfaI* gene cluster were tested for adhesion at 100 μg of protein per ml of PBS. Detection of bound fimbriae was performed with a 500-fold dilution of monoclonal antibody A21356 A1. Mean values of duplicate assays are given. Background binding of S fimbriae to the BSA-coated plastic surface was subtracted. (a) ◇, GM3-NeuGc; ⧖, GD1b; △, GD1a. (b) ◇, GM3-NeuGc; △, GT1b; ⧖, GD3; ×, GM3-NeuAc.

concentration of terminal sialic acid in a solution of colominic acid is lower by a factor corresponding to the degree of polymerization. Agglutination titers obtained with bovine erythrocytes regularly exceeded those in the human system, again indicating that NeuGc on gangliosides, but in particular on glycophorin, should be regarded as the preferred receptor.

Analysis of sialic acids. The high binding activity of *N*-glycolylneuraminic acid (NeuGc) on gangliosides and on sialoglycoproteins raised the question of whether this variant of sialic acid, which is not found in normal tissue of adult humans, could show a tissue distribution during fetal (and neonatal) life that correlates with the selective organ colonization of S-fimbriated *E. coli*. Accordingly, gangliosides from brain tissue of a 24-week-old human fetus were extracted, and extraction was followed by isolation of monogangliosides, which were finally analyzed for the presence of NeuGc as described recently by Kawai et al. (9). The methyl ester 2-*O*-methylglycosides of sialic acids were separated by gas-liquid chromatography and identified by single-ion monitoring of specific fragment ions registered at *m/z* 420,298 (NeuAc) or *m/z* 508,386 (NeuGc). On the basis of an estimated detection limit of 50 pg, it can be stated that NeuGc was not present in the fetal brain gangliosides analyzed.

Under the same conditions, gangliosides from a human colon carcinoma were demonstrated to contain a 2.3% proportion of NeuGc versus NeuAc, confirming the validity of the method.

DISCUSSION

E. coli strains with type K1 capsulae and S fimbriae are a major cause of age-specific sepsis and meningitis in neonatal infants (6, 13). Disease is associated with a mortality of up to 31% and with 29% neurological sequelae (16, 27). The hematogenous infection of cerebrospinal fluid and brain tissue has been demonstrated in a rat model to be initiated by bacterial adhesion to the vascular endothelium and to the epithelial lining of the choroid plexus and brain ventricles (22). Since this adhesion event is mediated by S fimbriae (22), a structural definition of their host receptor is of major clinical relevance.

In a series of previous studies (12, 21, 23), it was shown by several lines of evidence that S fimbriae have the highest affinity for (α-2-3)-linked sialic acid, while (α-2-6)-linked sialyl galactosides and disialyllactose or colominic acid with terminal (α-2-8)-linked sialic acid were less effective or not inhibitory at all (12, 23). The erythrocyte receptors of S-fimbriated *E. coli* were identified with the ubiquitous tri- and tetrasaccharides NeuAcα(2-3)Galβ(1-3)GalNAc and NeuAcα(2-3)Galβ(1-3)[NeuAcα(2-6)]GalNAc on human glycophorin (23). Although the trisaccharide sequence also occurs as a structural component of gangliosides, which are abundant in the brain, no binding of S-fimbriated *E. coli* to thin-layer chromatograms of these gangliosides could be observed (23), in contradiction to the present results.

The present reinvestigation was based on a different assay system replacing hemagglutination inhibition by a solid-phase binding assay. Under the conditions of this test system, isolated S fimbriae exhibited a binding characteristic which partially deviates from that previously established.

(i) The highest affinity of S fimbriae was measured for NeuGcα(2-3)Gal, exceeding that of NeuAcα(2-3)Gal by a factor of at least 16.

(ii) S fimbriae show a preferential binding to b-series gangliosides having in common the structural element NeuAcα(2-8)NeuAc. The latter results, which conflict with previous findings (21, 23), could be explained by suggesting that under the conditions of our assay system the receptor structures were presented to the adhesin in a more favorable manner because of higher densities (clustering effect).

Some of the results were confirmatory by demonstrating the strong influence of the sialic acid linkage position (α-3 versus α-6). With regard to their binding specificity for NeuGcα(2-3)Gal and NeuAcα(2-8)NeuAc, S fimbriae resemble other sialic acid-specific fimbriae, in particular K99 (15) and colonization factor antigen (CFA) I (33), suggesting that

TABLE 1. Terminal carbohydrate sequences on glycoproteins or gangliosides used in this study

Structure no.	Relevant carbohydrate structure	Source[a]
1	NeuAcα2 | 6 GalNAc	B, C
2	NeuGcα2 | 6 GalNAc	C
3	NeuAcα2 | 6 Galβ(1-3)GalNAc	A, C
4	NeuAcα(2-3)Galβ(1-3)GalNAc	A, D
5	NeuAcα2 | 6 NeuAcα(2-3)Galβ(1-3)GalNAc	A
6	NeuGcα(2-3)Galβ(1-4)GlcNAc-	E
7	NeuAcα2 | 6 Galβ(1-4)GlcNAc-	J
8	NeuAcα(2-3)Galβ(1-4)GlcNAc-	F
9	NeuAcα(2-3)Galβ(1-4)Glc	G, L
10	NeuAcα2 | 6 Galβ(1-4)Glc	K
11	NeuAcα(2-8)NeuAcα(2-3)Galβ(1-4)Glc	H
12	NeuAcα2 | 6 Galβ(1-3)GlcNAcβ(1-3)Galβ(1-4)Glc	I
13	NeuGcα(2-3)Galβ(1-4)Glc	M
14	Galβ(1-3)GalNacβ(1-4)\\ Galβ(1-4)Glc NeuAcα(2-8)NeuAcα(2-3)/	N
15	NeuAcα(2-3)Galβ(1-3)GalNacβ(1-4)\\ Galβ(1-4)Glc NeuAcα(2-3)/	O
16	NeuAcα(2-3)Galβ(1-3)GalNAcβ(1-4)\\ Galβ(1-4)Glc NeuAcα(2-8)NeuAcα(2-3)/	P

[a] A, human glycophorin; B, ovine submaxillary mucin; C, bovine submaxillary mucin; D, *Collocalia* mucin; E, bovine glycophorin; F, sialylparagloboside; G, GM3; H, GD3; I, sialyllactotetraose b; J, sialyllactotetraose c; K, 6′-sialyllactose; L, 3′-sialyllactose; M, GM3-NeuGc from equine erythrocytes; N, GD1b; O, GD1a; P, GT1b.

the respective adhesins belong to a family of closely related proteins.

The high affinity of S fimbriae for N-glycolylated sialic acid may indicate that S-fimbriated *E. coli* should have evolved in primates (3), since this variant of the acidic sugar is a regular constituent of glycoconjugates in the chimpanzee (32) but not in humans (3). Recent studies, however, have revealed evidence for the expression of the specific monooxygenase converting NeuAc into NeuGc in human tissue of fetal or cancerous origin (26). On the other hand, this

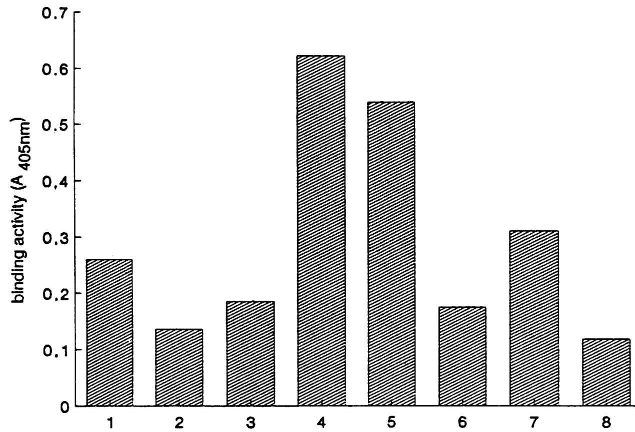

FIG. 4. Comparative binding studies with isolated S fimbriae on gangliosides, neoglycolipids, and structural variants of GM3. The assay was performed as described in the legend to Fig. 3. Mean values of duplicate assays are given. For structural variants of sialic acid, see Fig. 5. Background binding of S fimbriae to the BSA-coated plastic surface was subtracted. Bars: 1, GM3-NeuAc; 2, GM3-NeuAc (C-7 analog); 3, GM3-NeuAc (methyl ester); 4, GM3-NeuGc; 5, GD3; 6, GM1a; 7, 3'-sialyllactose (neoglycolipid); 8, 6'-sialyllactose (neoglycolipid).

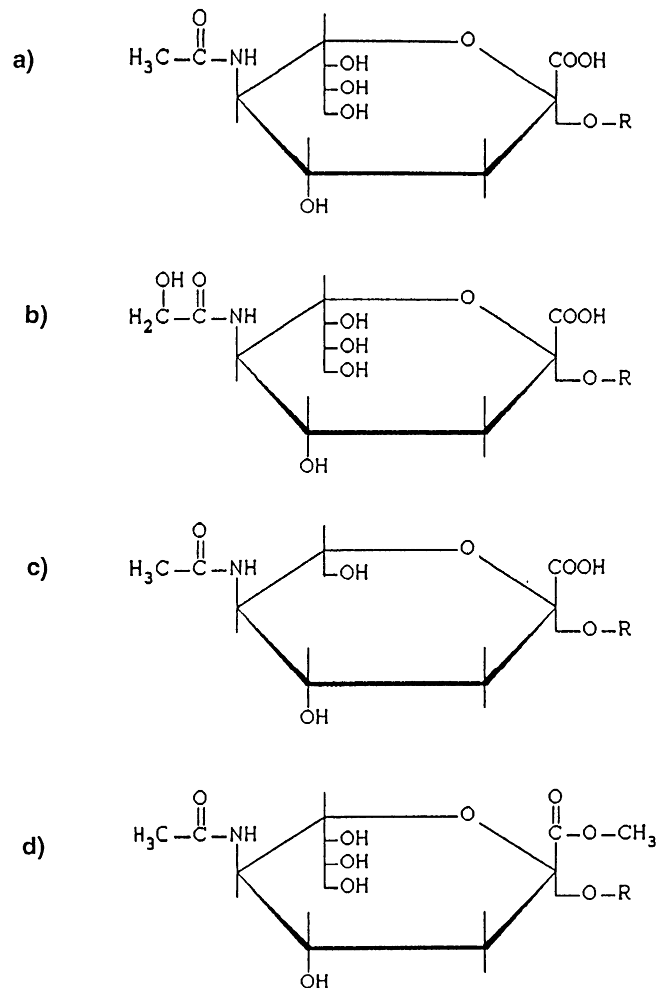

FIG. 5. Structural variants and chemical modifications of sialic acids on GM3 used in this study. (a) *N*-Acetylneuraminic acid (Neu5Ac); (b) *N*-glycolylneuraminic acid (Neu5Gc); (c) C-7 analog of *N*-acetylneuraminic acid (C-7–Neu5Ac); (d) methyl ester of *N*-acetylneuraminic acid.

atavistic NeuGc-versus-NeuAc expression in human meconium gangliosides never exceeds 1% (8) and, accordingly, should not play a mediatory role in *E. coli* adhesion to gastrointestinal epithelia in neonates. Moreover, no chemical evidence could be obtained for the presence of NeuGc in gangliosides from fetal brain (this study), suggesting that the oncofetal expression of the sugar should not be involved in the observed organ selectivity of S-fimbriated *E. coli.*

Of possible medical relevance could be that S fimbriae bind with a high affinity to the structural element NeuAcα2-8NeuAc.

This structure on b-series gangliosides, on the other hand, reaches its lowest level of expression in brain tissue at birth, followed by a slow, but pronounced, increase during childhood (24, 30, 31). The time course of b-series ganglioside expression during human ontogenesis shows, accordingly, an inverse relationship to the susceptibility for infection by S-fimbriated *E. coli*. On the other hand, expression of α-2-8-linked sialic acid polymers on glycopeptides in human and rat fetal brain (5) is developmentally regulated and comprises approximately 10% of the total protein-bound sialic acid.

Further investigations will have to establish whether the structural elements showing a preferential binding of S fimbriae under in vitro conditions have any biological relevance as receptors in human neonates. In particular, biochemical analyses of NeuGcα(2-3)Gal and NeuAcα(2-8)NeuAc expression in the neonatal intestine and brain tissue form an essential prerequisite for defining more precisely the molecular basis of infection by S-fimbriated *E. coli.*

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