Characterization of the Binding of Propionibacterium Granulosum to Glycosphingolipids Adsorbed on Surfaces

AN APPARENT RECOGNITION OF LACTOSE WHICH IS DEPENDENT ON THE CERAMIDE STRUCTURE*

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The binding properties of a strain of Propionibacterium granulosum derived from human skin was investigated with reference to glycosphingolipids separated on thin layer chromatograms or coated in microtiter wells using externally (^35S) methionine labeled bacteria. Binding was found to lactosylceramide (LacCer; Galβ1-4Glcβ1-Cer) but not to glycolipids lacking the lactose sequence (i.e. Glcβ1-Cer, Galβ1-Cer or Galα1-4Galβ1-Cer). In microtiter wells, binding occurred at 50 ng and became half-maximal at the theoretical value for a monomolecular layer of LacCer (i.e. 100-200 ng/well). The bacteria also bound to glycolipids with various substitutions (e.g. GalNACβ1-4, Galβ1-3GalNACβ1-4, Fucα1-2Galβ1-3GalNACβ1-4, Galβ1-3, GlcNACβ1-3, Galβ1-3GalNACβ1-3, Galβ1-4GlcNACβ1-3, and Galβ1-3(Fucα1-4/GlcNACβ1-3) at the Gal of LacCer, although only those species with GalNACβ1-4 or Galβ1-3GalNACβ1-4 were as active as LacCer itself. Glycolipids with other additions (e.g. Galα1-4 and NeuAcα2-3) were negative. For unsubstituted LacCer, the binding required either a trihydroxy base or 2-hydroxy fatty acid, specifying the epithelial type of ceramide; LacCer composed of a dihydroxy base and nonhydroxy fatty acid was negative. This is interpreted as indicating that the proper presentation of the binding epitope depends on the ceramide structure. The relevance of this to biological membranes has not yet been established. Neither free lactose (up to 20 mg/ml) nor lactose-bovine serum albumin (5 mg/ml) prevented the binding of LacCer to LacCer, two facts that support the solid-phase binding data demonstrating a low affinity binding and the crucial importance of a particular lactose epitope.

The carbohydrate residues of the animal cell surface glycolipids and glycoproteins appear to be involved in a variety of recognition phenomena (1-4), including the attachment of bacteria (2, 3) and viruses (4) and the interaction between cells during embryogenesis (1). Analysis of the capacity of ligands to interact with non-biological surfaces with bound carbohydrates is one way to characterize such carbohydrate-based receptors (5). Recently, a method was developed which allows the testing of glycolipids resolved in thin layer chromatograms to mediate attachment of bacteria (6, 7). Screening for bacterial receptors using this technique, revealed binding of diverse bacteria to lactosylceramide, LacCer (8, 9). The aim of the present study was to describe in detail the binding of Propionibacterium granulosum, typical of skin habitats, to a large number of glycosphingolipids separated on thin layer chromatograms and coated in microtiter wells. One reason for selecting this bacterial species was the possibility of making a detailed comparison with a related species, Propionibacterium freudenreichii, which apparently recognizes a separate binding epitope on lactose (10). Furthermore, P. granulosum seems to bind very similarly to other LacCer-recognizing bacteria characterized, including Neisseria gonorrhoeae (11) and other genera (8, 9).

MATERIALS AND METHODS

Preparation of Total Glycosphingolipid Fractions—Total neutral and acidic glycolipids from the sources given in Table I were prepared as previously described (12). Total lipid fractions, obtained by extraction with chloroform/methanol, were treated with mild alkali, dialyzed, and subjected to repeated ion exchange and silicic acid chromatography as acetylated and native compounds. For the preparation of total human brain gangliosides, glycolipids were separated into polar and nonpolar fractions by solvent partition (13) before further purification.

Isolation of Individual Glycolipids—Most of the individual neutral glycolipids were prepared as acetylated derivatives by repeated Iatrobeads (G5R-8060, Iatron Laboratories Inc., Tokyo, Japan) column chromatography using a continuous gradient of methanol in chloroform, 0-6% by volume. After deacetylation, the final purification was performed on the same column type using the chloroform/methanol/water gradient 65:25:4 to 50:40:10, by volume. Glycolipids nos. 6, 10, 14-20, and 27 were prepared according to the references given in Table I. Glycolipids nos. 21a and 31b and 32b were generated by enzymatic and acid hydrolysis of more complex glycolipids, as described elsewhere (11).

The separation of LacCer into different molecular species (Table II) was also accomplished by repeated Iatrobeads column chromatography of both acetylated and non-acetylated compounds. In this case, however, the gradients were made more shallow (0-1.5% and 85:15:1 to 60:35:8, respectively). For the preparation of LacCer species nos 4d and 4e, a ganglioside fraction isolated earlier from the rat small intestine (14) was degraded by Clostridium perfringens neuraminidase (Boehringer Mannheim GmbH, Mannheim, West Germany). Other lactosylceramides were prepared from the sources given in Table II.

The individual acidic glycolipids nos 5, 22, 34, and 35 were prepared by DEAE-Sepharose chromatography (15), followed by purification on Iatrobeads columns using a gradient of chloroform/methanol, 5 M

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1. N. Stromberg and K.-A. Karlsson, unpublished results.
2. The glycolipid nomenclature and symbols used in this paper follow the recommendations by the IUPAC-IUB Commission on Biochemical Nomenclature (CBN for lipids: (1977) Eur. J. Biochem. 79, 11-21 and (1982) J. Biol. Chem. 257, 3347-3351). It is assumed that Gal, Glc, GalNAC, GlcNAC, and NeuAc are of the D configuration, Fuc of the L configuration, and all sugars present in the pyranose form. The abbreviations used are: PBS, phosphate-buffered saline; BSA, bovine serum albumin.

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substance no. 30 by incubation of 25 μg of the glycolipid with 1 plus sheep agar plates (diameter 50 mm) streaked with bacteria. The comparison of copper acetate-stained (16) bands with serially diluted standard glycolipids. The glycolipid structures previously reported ZAB-HF mass spectrometer (VG-Analytical, Wythenshawe, United glycolipids were also analyzed by NMR spectroscopy (21-23) using a but also the ceramide composition (Table II) (17-20). Derivatized glycolipids were also analyzed by NMR spectroscopy (21-23) using a 270 MHz spectrometer (model WH-270, Bruker, Switzerland), and by gas chromatography following degradation (24-27).

Bacteria and Labeling—P. granulosum, strain ATCC 255.64 of human skin origin, and P-fimbriated Escherichia coli, strain S21, were kindly provided by Dr. A. A. Lindberg, Karolinska Institutet, Stockholm. For all binding assays, P. granulosum was grown for 3 days under standard conditions on Todd-Hewitt plus sheep agar, and E. coli overnight on CF agar. After being harvested, cells were labeled with 35S using the Bolton-Hunter reagent (28), as previously described (6). After this reagent (100 mCi in benzene, Du Pont-New England Nuclear) was dried in the reaction vessel and 5 × 10^7 bacteria in 0.5 ml 0.1 M borate buffer, pH 8.5, was added, the reaction mixture was aged for 15 min at 37°C. To examine the possible modification of bacterial surface proteins due to the external labeling and to simplify the labeling procedure, cells were also metabolically labeled by addition of [35S]methionine (600 μCi/mmol, 150 mCi/plate, Amersham, United Kingdom) diluted in 50 μl of broth media to Todd-Hewitt plus sheep agar plates (diameter 50 mm) streaked with bacteria. The labeled bacteria were then washed three times in PBS and counted for radioactivity in a gamma and scintillation counter, respectively. Both procedures incorporated about 5 × 10^6 cpm counted on 10^6 bacteria, corresponding to 0.05 cpm/cell. No difference in glycolipid binding patterns was observed between the two procedures.

Binding to Thin Layer Chromatograms—The binding of the bacteria to glycolipids separated on thin layer plates was carried out as described elsewhere (6, 7). Chromatograms on aluminum-backed Silicic Gel 60 high performance-thin layer chromatography plates (Merck, Darmstadt, West Germany) were treated with 0.5% polyvinylpyrrolidone (628, Rühm, GmbH, Darmstadt, West Germany), soaked in 1% BSA in PBS 7.5, 5% rotameric modification, and radiolabeled bacteria (2 ml of 10^8 cpm/ml, 10^6 cells/ml) for 2 h. The chromatogram was washed five times with PBS to remove unbound bacteria, then dried, and exposed to x-ray film (XAR-5, Eastman Kodak) for 70 h.

Binding in Microtiter Wells—The assay was conducted as described elsewhere (7). Glycolipids, dissolved in methanol (50 μl), were dried in round-bottom 96-well polyvinylchloride plates (Cooks M24, Nutaxon, Holland) followed by blocking with 2% BSA in PBS for 2 h. After being washed three times with PBS, the wells were incubated with radiolabeled bacteria (2.5 × 10^3 cpm and 5 × 10^3 bacteria/well and 50 μl) for 4 h. The wells were then washed five times with PBS, dried, cut out, and measured for radioactivity in a gamma counter.

3H Labeling of LacCer—LacCer (no. 4a) was labeled by the galactose oxidase and NaBH₄ method (29, 30). LacCer (1 mg) and taurodoxycholate (1 mg) were dissolved in 0.5 ml of PBS and sonicated for 4 min, followed by addition of 15 units of galactose oxidase (type V, Dactylium dendroides, Sigma) in 100 μl of PBS, and incubation of the reaction mixture at 37°C overnight. The incubation with 15 units of galactose oxidase was repeated twice. The sample was then incubated with 100 μl of NaBH₄ (50 mCi/ml in 0.1 M NaOH) for 30 min at room temperature. A knife edge of solid unlabeled NaBH₄ was added and the reduction was completed by incubation overnight. The sample was partitioned (13) and the phase further purified by DEAE-Sepharose and silicic acid column chromatography. The 3H-LacCer end product was pure as revealed by autoradiography of thin layer chromatograms and the specific activity 5 × 10⁶ cpm/mg as determined by scintillation counting.

Adsortion of 3H-Labeled LacCer to Microtiter Wells—To study the efficiency and stability of LacCer under microtiter well assay conditions, serial dilutions of 3H-labeled LacCer according to Fig. 2 were each coated in 6 microtiter wells, as described above. After incubation in 2% BSA in PBS for 2 h, the wells were thoroughly washed five times with PBS. The 6 wells of each dilution were then dried, cut out, and all transferred to a single scintillation vial, where they were extracted with 10 ml of scintillation liquid (Instagel, Packard, Zurich, Switzerland) and counted. Over the whole range of dilutions of LacCer that were possible to measure, e.g. down to 50 ng, a 90% recovery of radioactivity was found in the test samples as compared with the controls. Thus, lactosylceramide is highly efficiently adsorbed to microtiter wells and stable under aqueous assay conditions.

Chemicals—Lactose was obtained from Sigma, (lactose-3-O-CF₃C₆H₅OCH₂CH₂OH)2-BSA (2-(2-carboxyethylthio)ethylglycopolis coupled in amide linkage to Lys; 20–30 mol/mol of BSA) and lactose/OCH₂CH₂S(CH₂)₇CHOH were purchased from Sockerbolaget, År, Sweden.

RESULTS

Binding of P. granulosum to Glycosphingolipids—The binding of radiolabeled P. granulosum to glycosphingolipids separated on thin layer chromatograms was examined using a broad spectrum of reference glycolipids with different carbohydrate and lipidic portions (Fig. 1 and Tables I-II). As inferred from these data, the minimal binding requirement on the oligosaccharide was terminally or internally located lactose (Galβ1–4Glc). We also found that this recognition was dependent on the ceramide structure (Table II).

When various mixtures of glycolipids were screened (Fig. 1), P. granulosum was found to bind selectively to molecular species in the 2- to 6-sugar interval (e.g. nos 4a, 11, 13, 14, 16, 21a, 31a, 32a). Many glycolipids (i.e. nos. 8, 9, and 12), including monohexosylceramides (nos. 1 and 2), were negative even though present in amounts of 2 μg or more. As documented in Table I, the recognized glycolipids all contained Galβ1–4Glc (lactose), and the most potent binder, being active down to about 50 ng, was LacCer with a heterogeneous ceramide composition (no. 4a). Glycolipids lacking the lactose sequence were negative (nos. 1–3). Further arguments for lactose being the minimal binding requirement were the inactivity of Glcβ1–1-Cer (no. 2) and various structures with a Galβ1-terminus (nos. 1, 10, 16, 25, 30, and 34). We therefore interpret the weaker binding to the 3-, 4-, and 5-sugar glyco-
### Table I

Glycolipids tested for ability to bind *P. granulosum*

Assessment of binding was performed with the chromatogram binding assay as described in “Materials and Methods.”

| Series designation | Structure* | Symbol | Binding* | Source (Ref.) |
|--------------------|------------|--------|----------|---------------|
| **Simple** basic structures | | | | |
| 1 | Galβ1-Cer(h) | **+** | Various sources (51) |
| 2 | Glcβ1-Cer(h) | **+** | Various sources (51) |
| 3 | Galβ1-4Galβ1-Cer(h) | | Human meconium (52) |
| 4a | Galβ1-4Galβ1-Cer(h), LacCer | | Dog small intestine (53) |
| 5 | NeuAcα2-3Galβ1-4Glcβ1-Cer(h) | | Rat small intestine (15) |
| 6 | Fucα1-2Galβ1-4Glcβ1-Cer(h) | | Rat small intestine (54) |
| **Globo series** | | | | |
| 7 | Galα1-4Galβ1-4Glcβ1-Cer | Gb0a | Human erythrocytes (51) |
| 8 | GalNAcα1-3Galβ1-3Galβ1-4Glcβ1-Cer | Gb0a | Human erythrocytes (51) |
| 9 | Galβ1-4GalNAcα1-3Galβ1-4Glcβ1-Cer | | Dog small intestine (53) |
| 10 | Galβ1-3Galβ1NAcα1-3Galβ1-4Glcβ1-Cer | | Vero cells (55) |
| **Isoglobo series** | | | | |
| 11 | Galα1-3Galβ1-4Glcβ1-Cer | Gb0b | (+) Dog small intestine (53) |
| 12 | GalNAcα1-3Galβ1-3Galβ1-4Glcβ1-Cer | Gb0b | Rat intestinal carcinoma (56) |
| **Lacto series** | | | | |
| 13 | GlcNAcβ1-3Galβ1-4Glcβ1-Cer | (+) | Malignant Melanoma* |
| 14 | Galβ1-3GlcNAcβ1-3Galβ1-4Glcβ1-Cer | Lc4a | Human meconium (28) |
| 15 | Fucα1-2Galβ1-4GlcNAcβ1-3Galβ1-4Glcβ1-Cer | | Human meconium (52) |
| 16 | Galβ1-3(Fucα1-4GlcNAcβ1-3Galβ1-4Glcβ1-Cer | Le5* | Human small intestine (57) |
| 17 | Fucα1-2Galβ1-3(Fucα1-4GlcNAcβ1-3Galβ1-4Glcβ1-Cer | Le6* | Human small intestine (58) |
| 18 | Galα1-3(Fucα1-2Galβ1-3GlcNAcβ1-3Galβ1-4Glcβ1-Cer | | Human meconium (62) |
| 19 | GalNAcα1-3(Fucα1-2Galβ1-3GlcNAcβ1-3Galβ1-4Glcβ1-Cer | | Human meconium (52) |
| 20 | GalNAcα1-3(Fucα1-2Galβ1-3(Fucα1-4GlcNAcβ1-3Galβ1-4Glcβ1-Cer | | Human meconium (52) |
| **Lactoneo series** | | | | |
| 21a | Galβ1-4GlcNAcβ1-3Galβ1-4Glcβ1-Cer | Le4b | (+) Dog small intestine (58) |
| 22 | NeuAcα2-3Galβ1-4GlcNAcβ1-3Galβ1-4Glcβ1-Cer | | Human erythrocytes (51) |
| 23 | Fucα1-2Galβ1-4GlcNAcβ1-3Galβ1-4Glcβ1-Cer | H-5-I | Dog small intestine (58)* |
| 24 | Galβ1-3Galβ1-4GlcNAcβ1-3Galβ1-4Glcβ1-Cer | | Rabbit erythrocytes (59) |
| 25 | Galβ1-4(Fucα1-3GlcNAcβ1-3Galβ1-4Glcβ1-Cer | | Dog small intestine (58)* |
| 26 | Fucα1-2Galβ1-4(Fucα1-3GlcNAcβ1-3Galβ1-4Glcβ1-Cer | | Dog small intestine (58)* |
| 27 | Galα1-3(Fucα1-2Galβ1-4GlcNAcβ1-3Galβ1-4Glcβ1-Cer | | Human small intestine (51) |
| 28 | GalNAcα1-3(Fucα1-2Galβ1-4GlcNAcβ1-3Galβ1-4Glcβ1-Cer | | Human small intestine (58)* |
| 29 | GalNAcα1-3(Fucα1-2Galβ1-4(Fucα1-3GlcNAcβ1-3Galβ1-4Glcβ1-Cer | | Dog small intestine (58)* |
| 30 | Galβ1-4GlcNAcβ1-6(Galβ1-4GlcNAcβ1-3Galβ1-4Glcβ1-Cer | | Human erythrocytes (51) |
| **Ganglio series** | | | | |
| 31a | GalNAcβ1-4Galβ1-4Glcβ1-Cer | Gg03 | Mouse small intestine (29)* |
| 32a | Galβ1-3GalNAcβ1-4Galβ1-4Glcβ1-Cer | Gg04 | Mouse small intestine (29)* |
| 33 | Fucα1-2Galβ1-3GalNAcβ1-4Galβ1-4Glcβ1-Cer | (+) | Mouse large intestine (29)* |
| 34 | Galβ1-3GalNAcβ1-4(NeuAcα2-3Galβ1-4Glcβ1-Cer | | Human brain (60) |
| 35 | NeuAcα2-3Galβ1-4GalNAcβ1-4(NeuAcα2-3Galβ1-4Glcβ1-Cer | | Human brain (60) |

*A Cer indicates ceramide with nonhydroxy fatty acid and dihydroxy long-chain base; Cer(h), a ceramide based on hydroxy fatty acid and/or trihydroxy long-chain base, that both types of ceramide were present in the fraction tested.

* A plus sign, +, indicates optimal binding (detection level of about 50 ng of glycolipid). A plus sign within parentheses, (+), indicates suboptimal binding (detection level of 500 ng or more). A minus sign, -, indicates no binding (even at a level of 2 pg of glycolipid). LacCer (no. 4a), the glycolipid selected to serve as a reference, showed intense autoradiographic staining at 2 pg of glycolipid (Fig. 1), an amount therefore used in the initial testing of other glycolipids. Glycolipids showing no autoradiographic staining at this amount (nos. 8 and 9 in Fig. 1), on at least twice occasions, were considered negative. Glycolipids that did result in autoradiographic staining (Fig. 1) were classified as follows. Optimal binders repeatedly showed intense autoradiographic staining at 2 pg and weak residual staining down to 50 ng when serially diluted on the plate. Suboptimal binders usually, but not always (no. 11 in Fig. 1), showed a much weaker staining at 2 pg and could not be diluted below 500 ng without complete loss of staining. Nonetheless, glycolipids classified as suboptimal binders showed a clear and distinct staining at 2 μg compared with the negative glycolipid species.

* A plus sign, +, indicates positive binding. A minus sign, -, indicates no binding.

* A main source and a reference is indicated for each compound and for most glycolipids at least two different sources were used.

* K. A. Karlsson and B. E. Samuelsson, unpublished results.

* N. Stromberg and K. A. Karlsson, unpublished results.

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lipids carrying various sugar extensions on their common LacCer core structure (nos. 11, 13, 14, 16, 21a, 31a, and 32a) to mean that the bacteria also accommodate lactose in the internal position. However, the inactivity of the majority of the complex glycolipids analyzed in Fig. 1 indicates that many other sugar extensions on LacCer block access to the internal lactose. In addition, the lactosaminyl structure (Galβ1-4GlcNAcβ1-3, no. 11), lacto (Galβ1-3GlcNAcβ1-3, no. 14), and neolacto series (Galβ1-4GlcNAcβ1-3, no. 21a), indicating limited steric hindrance.

On the other hand, the GalNAcβ1-4 substituent, specifying the ganglio series of glycolipids, allowed optimal binding activity in three ways. One group of substituents at this position abolished the binding completely. This was the case for Galα1-4 (no. 7) and, consequently, all tested globo-series glycolipids were found to be negative (nos. 7–10). Similarly, NeuAcα2-3 and Fuca1-2 blocked binding (nos. 5 and 6, respectively).

Another group of substituents reduced, but did not block, the binding. This was the case for glycolipids of the isoglobo (Galα1-3, no. 11), lacto (Galβ1-3GlcNAcβ1-3, no. 14), and neolacto series (Galβ1-4GlcNAcβ1-3, no. 21a), indicating limited steric hindrance.

As documented in Table I, the sugar substitutions at the Gal of LacCer specifying the different series of glycolipids modulate the binding activity in three ways.

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TABLE II

Assessment of binding was performed with the chromatogram binding assay as described under "Materials and Methods."

| Designation Glycolipid | Lipophilic part | Binding | Origin (species; Ref.) |
|------------------------|----------------|---------|-----------------------|
| 4a LacCer              | Varying ceramide components | +       | Mucosal epithelium (dog; 53) |
| 4b LacCer               | d18:1–16:0/24:0 | –       | Human erythrocytes     |
| 4c LacCer               | d18:1–h24:0   | +       | Mucosal epithelium (dog; 53) |
| 4d LacCer               | t18:0–24:0    | +       | Mucosal epithelium (rat; 14) |
| 4e LacCer               | t18:0–h16:0   | +       | Mucosal epithelium (rat; 14) |
| 4f LacCer               | OCH2(CH2)nS(CH2)mCH3 | –       | Synthetic compound     |
| 31a Gg03                | t18:0–h16:0/h24:0 | +       | Mucosal epithelium (mouse; 27) |
| 31b Gg03                | d18:1/h20:0–18:0 | +       | Human brain            |
| 32a Gg04                | t18:0–h16:0/h24:0 | +       | Mucosal epithelium (mouse; 27) |
| 32b Gg04                | d18:1/h20:0–18:0 | +       | Human brain            |
| 21a Le4b                | d18:1/t18:0–h16:0/h24:0 | (+)   | Mucosal epithelium (dog; 53) |
| 21b Le4b                | d18:1–16:0/24:0 | (+)   | Human erythrocytes     |

*The structure of the carbohydrate portion of each glycolipid is given in Table I.

†According to an earlier recommendation (61), d stands for dihydroxy long-chain base, t for trihydroxy long-chain base, and h for 2-hydroxy fatty acid. Whereas the figures before a colon mean paraffin chain length, those after denote the number of double bonds. Varying components mean a mixture of di- and trihydroxy bases and hydroxy and nonhydroxy fatty acids.

+ indicates optimal binding (detection level of about 50 ng), (+) suboptimal binding (detection level of 500 ng or more), – no binding even at a level of 2 μg. See also Table I and text.

To illustrate the characteristic variation in the structure of the ceramide between different tissues (38), the source of each glycolipid is indicated. The structures were isolated and characterized as described in Table I and "Materials and Methods."

* N. Strömberg, G. C. Hansson, J. Thurin, and H. Leffler, unpublished results.

FIG. 2. Binding of P. granulosum ATCC 255.84 and E. coli SS1 to varying amounts of selected glycolipids adsorbed on microtiter wells. Abbreviations to the right of each curve refer to the structures listed in the tables: 4a, LacCer with more hydroxylated ceramide; 4b, LacCer with less hydroxylated ceramide; 4c, Gb4a; 4d, LacCer with less hydroxylated ceramide; 8, Gb4a; 23, H-5-II. The binding assay was performed with [125I]-labeled bacteria, as described under "Materials and Methods." Data are the average value for triplicate determinations.
when measured as a function of the amount added to each well. The results obtained are consistent with the binding studies summarized above, both in view of carbohydrate specificity (compare nos. 4a and 23) and ceramide dependence (compare nos. 4a and 4b). In this analysis, binding was also detectable at a level of about 50 ng and became half-maximal close to 200 ng, the theoretical value of a monomolecular layer of glycolipids. When ²H-labeled glycolipid was used, 90% of LacCer was found to remain attached to the wells throughout the assay procedure (see "Material and Methods"). To put the binding data in perspective with a receptor glycolipid for bacteria reported earlier (33-35), we next examined the Galα1-4Gal-specific binding of uropathogenic E. coli to Galβ1-4Glc (8) in this assay; binding occurred then at 10-100-fold lower amounts of glycolipid (Fig. 2). Consequently, the two bacteria also differed with regard to the blocking ability of their corresponding free receptor disaccharides. Binding of P. granulosum to the thin layer chromatogram was not affected by preincubation of the bacteria with lactose up to 20 mg/ml or lactose-BSA (~25 mol/mol, 5 mg/ml). On the other hand, free Galα1-4Gal clearly prevented the binding of E. coli to glycolipids separated on thin layer chromatograms (33).

DISCUSSION

The availability of a broad spectrum of glycosphingolipids (Fig. 1 and Table I), together with novel glycolipid overlay assays (6, 7), offers a convenient way to characterize carbohydrate-based attachment sites for microbial ligands. This has been demonstrated for the recognition of Galα1-4Gal by both uropathogenic E. coli (31-33) and the Shiga toxin (36). LacCer by diverse bacteria (8, 9, 11) including P. granulosum, GaINαcβ1-4Gal by pulmonary pathogens (34), and gangliosides by Sendai virus (9, 35).

In the present study, the interaction between P. granulosum and glycolipids has been characterized (Figs 1-2 and Tables 1-11). The specific importance of terminally or internally located lactose (Galβ1-4Glc) with regard to this interaction and glycolipids has been characterized (Figs 1-2 and Tables 4a,b). In this analysis, binding was also detectable at a level of about 50 ng and became half-maximal close to 200 ng, the theoretical value of a monomolecular layer of glycolipids. When ²H-labeled glycolipid was used, 90% of LacCer was found to remain attached to the wells throughout the assay procedure (see "Material and Methods").

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strated whether the assay surface conditions are fully relevant to the situation in the plasma membrane. In LacCer, the active saccharide is directly linked to the ceramide and, consequently, the ceramide may be expected to affect receptor function, either directly (by influences on conformation) or indirectly (by interacting with the plastic assay surface). Nevertheless, the sophisticated dependence on the structure of the ceramide (Table II), correlating with a distinct ceramide variation in potential target tissues, is provocative and should be subjected to further studies using model membranes and conformation methods. Slight variations in assay conditions may explain incidental negative binding for LacCer despite unaltered activities of the more complex ceramide-independent glycolipids, as discussed earlier (7). Complete lack of activity of some batches of bacteria may be due to lack of expression of the adhesin. Our collaborative efforts with molecular geneticists on N. gonorrhoeae, which also recognizes LacCer, may shed light on this problem (11).

Most of the bacteria hitherto classified as LacCer binders (8, 9, 41) display glycolipid-binding patterns very similar to those reported here for P. granulosum. On the other hand, our recent studies on N. gonorrhoeae (11) and P. freudenreichii (10) raise the possibility of a variety of LacCer-binding adhesins that differ in their detailed binding specificities. This may hypothetically correspond to only slight changes in the amino acid sequence of the adhesin-binding sites, analogous to receptor variants of influenza virus (42). The finding that several bacteria have selected a binding to LacCer may indicate an important factor in the colonization of mucosal surfaces. In the case of N. gonorrhoeae relevant glycolipids exist in target cells for infection (11). Several of the bacteria in question belong to the normal bacterial flora of the large intestine. It is therefore of interest that recent studies have identified LacCer of the more hydroxylated type in isolated epithelial cells of the human colon (43) but not in those of the human small intestine (44). Furthermore, both in the rat (45) and humans (46) the epithelial type of LacCer also exists in relatively large amounts in feces as a result of bacterial degradation of more complex glycolipids of extruded cells. However, at the membrane, in contrast to mannose, another common receptor for bacteria (2, 3), LacCer is considered cryptic on normal cells and not directly accessible from the outside (30, 47, 48). This and the fact that lactose is absent in glycoproteins (49) may suggest that LacCer is used to establish a firm attachment after initial binding to other sites followed by a more intimate collision with the membrane after lateral diffusion of masking surface components. Clearly, lactose is a common nutrient for bacteria, but ability to transport or ferment lactose (50) does not correlate with binding ability to LacCer in our assay. Therefore, it is unlikely that the binding detected is based on a surface-located transport site for lactose.

The biological relevance of the present findings from in vitro assays has to be further tested. A possible continuation is to identify the adhesin by genetic cloning studies, as in the current work with N. gonorrhoeae (11). Synthesis of soluble receptor analogues appears to be necessary before adequate experiments can be performed on the inhibition of bacterial binding to target cells.

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