NOTES

Binding of Actinomyces naeslundii to Glycosphingolipids

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The fimbrial lectin of Actinomyces naeslundii WVU45 mediated the binding of this bacterium to glycosphingolipid chromatographed on thin-layer silica gel plates. Radioiodinated bacteria attached to GM1, GDIb, and globoside. After chromatograms were treated with sialidase, the bacteria also bound to GD1a and GT1b. The actinomyces lectin apparently recognized the Galβ3GalNAc termini of these gangliosides and the GaNAcβ3Gal terminus of globoside, suggesting that glycolipids containing these sequences may serve as receptors for A. naeslundii on mammalian cells.

The D-galactose (Gal)-, N-acetyl-D-galactosamine (GalNAc)-reactive lectin associated with the type 2 fimbriae of Actinomyces naeslundii WVU45 and A. viscosus TI4V mediates the interaction of these gram-positive oral bacteria with other bacteria (5, 11) and with sialidase-treated human erythrocytes (7), epithelial cells (3), and polymorphonuclear leukocytes (12). The most potent inhibitor of actinomyces lectin-mediated interactions is β-D-galactopyranosyl-(1→3)-N-acetyl-D-galactosamine (Galβ3GalNAc) (4, 11). Peanut agglutinin, a plant lectin that specifically recognizes this disaccharide, inhibits the attachment of A. naeslundii to monolayers of human oral epithelial cells (3) and recognizes a 160-kilodalton cell surface glycoprotein that apparently serves as a receptor for this bacterium on epithelial cells (2). Since certain glycosphingolipids contain the carbohydrate sequence Galβ3GalNAc, the actinomyces fimbrial lectin may also interact with mammalian cell surface glycolipids. Therefore, the binding of viable actinomyces to a number of defined glycolipids has been examined by a procedure previously utilized to detect receptors for viruses (8), bacteria (1), and antibodies (9, 10).

The gangliosides Gm2 (a gift from William Young, Jr., University of Virginia, Charlottesville), Gm2 (Bachem, Inc., Torrance, Calif.), asialo Gm1 (Supelco, Inc., Bellefonte, Pa.), and Gm1 (Supelco) were chromatographed on high-performance thin-layer chromatography silica gel plates (article no. 5547; Camag Scientific, Inc., Wrightsville Beach, N.C.) with chloroform-methanol-water (50:40:10, vol/vol) for 45 min and detected with orcinol reagent (Fig. 1a). Attachment of radioiodinated bacteria to the gangliosides was assessed on a duplicate chromatogram. The chromatogram was dipped in polyisobutylmethacrylate (Polysciences Inc., Warlington, Pa.) for 1 min and blocked with 0.5% bovine serum albumin in phosphate-buffered saline (0.02 M PO₄³⁻, 0.15 M NaCl [pH 7.2]) for 2 h. A. naeslundii WVU45 was cultured as previously described (7); washed in Hanks balanced salt solution (Flow Laboratories, Inc., McLean, Va.) containing 0.2 mg of CaCl₂ per ml, 0.2 mg of MgSO₄ per ml, and 0.2% bovine serum albumin (HBSS⁺); adjusted to 10⁸ cells per ml; and incubated for 1 h at 22°C with 25 μg of R64 rabbit immunoglobulin G per ml that is reactive with nonfimbrial bacterial surface antigens (6). The bacteria were washed with HBSS⁺, incubated with 10⁶ cpm of ¹²⁵I-protein A (Amersham Corp., Arlington Heights, Ill.) for 1 h at 22°C, washed thoroughly, and resuspended in HBSS⁺. The radioabeled bacteria retained lectin activity as determined by hemagglutination and epithelial cell adherence assays (3, 7). Radioiodinated actinomyces (5 × 10⁶ cells, 4 × 10⁻⁵ cpm) were layered on chromatograms, and the plates were incubated for 90 min at 25°C and washed three times with HBSS⁺. The chromatograms were air-dried, and attached bacteria were detected by autoradiography with XAR-5 film (Eastman Kodak, Rochester, N.Y.) and Cronex Quanta III intensifying screens (Du Pont Co., Wilmington, Del.) at −70°C.

A. naeslundii bound to asialo Gm1 and Gm1, which have terminal Galβ3GalNAc (Fig. 1b, lanes 7 and 8), but did not bind to asialo Gm2 or Gm2 (Fig. 1b, lanes 5 and 6), the immediate biosynthetic precursor to Gm1 which lacks only the β-linked terminal D-galactose (Table 1). A mixture of brain gangliosides containing Gm1, Gd1a, Gd1b, and GT1b (Sigma Chemical Co., St. Louis, Mo.) was also separated by thin-layer chromatography and detected by orcinol staining

FIG. 1. Binding of A. naeslundii WVU45 to thin-layer chromatograms of gangliosides. Duplicate chromatograms were (a) stained with orcinol or (b) incubated with radioiodinated A. naeslundii WVU45, and bands were detected by autoradiography, as described in the text. Approximately 3 μg of asialo Gm2 (AsGm2; lanes 1 and 5), Gm2 (Gm2; lanes 2 and 6), asialo Gm1 (AsGm1; lanes 3 and 7), or Gm1 (Gm1; lanes 4 and 8) was applied.

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TABLE 1. Oligosaccharide structure of glycosphingolipids

<table>
<thead>
<tr>
<th>Glycolipid</th>
<th>Oligosaccharide structure</th>
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<tbody>
<tr>
<td>( \text{G}_M^1 )</td>
<td>( \text{GalNAcB1-4GalB1-4GlcB1}\rightarrow\text{NeuAc} )</td>
</tr>
<tr>
<td>( \text{G}_M^1 )</td>
<td>( \text{GalB1-3GalNAcB1-4GalB1-4GlcB1}\rightarrow\text{NeuAc} )</td>
</tr>
<tr>
<td>( \text{G}_D^1\alpha )</td>
<td>( \text{GalB1-3GalNAcB1-4GalB1-4GlcB1}\rightarrow\text{NeuAc})</td>
</tr>
<tr>
<td>( \text{G}_D^1\beta )</td>
<td>( \text{GalB1-3GalNAcB1-4GalB1-4GlcB1}\rightarrow\text{NeuAc})</td>
</tr>
<tr>
<td>( \text{G}_T^1\beta )</td>
<td>( \text{GalB1-3GalNAcB1-4GalB1-4GlcB1}\rightarrow\text{NeuAc})</td>
</tr>
</tbody>
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Neutral glycolipid

\( \text{CTH} \) | \( \text{Gaia1-4GalB1-4GlcB1} \) |

\( \text{Globoside} \) | \( \text{GalNAcB1-3GalB1-4GlcB1} \) |

\( \text{Forssman glycolipid} \) | \( \text{GalNAcB1-3GalNAcB1-3GalB1-4GlcB1} \)

* Abbreviated according to Svennerholm (13).

(Fig. 2, lane 1). \( A. \text{naceslundii} \) bound directly to \( \text{G}_M^1 \) and \( \text{G}_D^1\beta \), and also to a band that was not routinely detected by orcinol staining but comigrated with asialo \( \text{G}_M^1 \) (Fig. 2, lane 2). Recognition of this latter band by the bacterium overlay suggests that this technique is more sensitive for detection of glycolipids than is chemical staining, as previously indicated (1, 8). An identical chromatogram was treated with 3 \( \times 10^{-2} \) U of sialidase (type X from \( \text{Clostridium perfringens} \); Sigma) per ml in 0.05 M sodium acetate [pH 5.5] containing 0.01 M \( \text{CaCl}_2 \) for 2 h at 37°C (Fig. 2, lane 3). After this treatment, \( A. \text{naceslundii} \) bound to \( \text{G}_D^1\alpha \) and \( \text{G}_T^1\beta \) in addition to \( \text{G}_M^1 \), \( \text{G}_D^1\beta \), and the band that comigrated with asialo \( \text{G}_M^1 \). The actinomycoses therefore bound directly to the gangliosides asialo \( \text{G}_M^1 \), \( \text{G}_M^1 \), and \( \text{G}_D^1\beta \), which have a terminal Galβ3GalNAc (Table 1), and to \( \text{G}_D^1\alpha \) and \( \text{G}_T^1\beta \) after exposure of this carbohydrate sequence by sialidase. This enzyme, which is produced by the actinomycoses (7), has also been found to enhance bacterial attachment to mammalian cells (3, 12). The sialic acids linked to the internal \( \alpha \)-galactose did not appear to interfere with bacterial binding, as indicated by the direct bacterial binding to \( \text{G}_M^1 \) and \( \text{G}_D^1\beta \). Also, removal of internal sialic acids apparently did not expose alternative bacterial lectin receptors, since no reactivity was seen with asialo \( \text{G}_M^2 \) (Fig. 1b).

The lectin associated with the type 2 fimbriae mediates the binding of \( A. \text{naceslundii} \) to the gangliosides. The actinomycoses did not bind to these glycolipids in the presence of 0.1 M lactose (Fig. 2, lane 4), and a radiolabeled mutant strain of \( A. \text{naceslundii} \) WVU45, WVU45M, that specifically lacks the type 2 fimbriae (6) did not bind to any of the gangliosides (Fig. 2, lane 5).

![FIG. 2. Binding of actinomyces to mixed brain gangliosides separated by thin-layer chromatography. Approximately 3 μg of gangliosides was applied to each lane, and chromatograms were stained with orcinol prior to sialidase (+S) treatment and autoradiography. The autoradiogram was exposed to X-ray film for 2 weeks.](http://iai.asm.org/)

The globo-series glycosphingolipids globotriaosylceramide (CTH; Supelco), globotetraosylceramide (globoside; Supelco), and 1'\(^2\)-N-acetylgalactosaminyl-β-globotetraosylceramide (Forssman glycolipid; a gift from William Young, Jr., University of Virginia, Charlottesville) were also chromatographed, stained with orcinol (Fig. 3a), and overlaid with radioiodinated \( A. \text{naceslundii} \) WVU45 (Fig. 3b). The oligosaccharide structures of these glycolipids (Table 1) contain the P blood group determinant Galα4Gal, which is a receptor for the uropathogenic \( \text{Escherichia coli} \) (1). This sequence is not recognized by the actinomycoses lectin, since

![FIG. 3. Binding of \( A. \text{naceslundii} \) WVU45 to thin-layer chromatograms of neutral glycosphingolipids. Duplicate chromatograms were stained with orcinol or incubated with radioiodinated \( A. \text{naceslundii} \) WVU45, and bands were detected by autoradiography, as described in the text. Approximately 3 μg of globo-series (Glob; lanes 1 and 4), CTH (lanes 2 and 5), or Forssman glycolipid (Fors; lanes 3 and 6) was applied.](http://iai.asm.org/)
A. naeslundii bound to globoside (Fig. 3b, lane 4) but not to CTH (Fig. 3b, lane 5). The terminal GalNACβ3Gal of globoside was specifically recognized by the actinomyces lectin, whereas the terminal GalNACβ3GalNAc of the Forssman glycolipid (Fig. 3b, lane 6) and the terminal GalNACβ4-Gal of asialo GM2 (Fig. 1b) were not. Bacterial attachment to globoside was inhibited by 0.1 M lactose, and the A. naeslundii mutant specifically lacking the type 2 fimbriae did not bind to this glycolipid (data not shown).

The direct binding of actinomyces to glycolipids on thin-layer chromatograms has significantly extended our knowledge of the reactivity of the actinomyces lectin. Although the physiological relevance of glycolipids as cell surface receptors for the actinomyces remains to be determined, these studies demonstrate that actinomyces can bind to specific glycosphingolipids and suggest that these molecules should be considered potential receptors on eucaryotic cell surfaces.

We are indebted to William Young, Jr., University of Virginia, Charlottesville, for his gift of glycolipids and to John Magnani, National Institute of Arthritis, Diabetes, and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Md., for his advice on the bacterial binding procedure.

LITERATURE CITED


