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A 160-Kilodalton Epithelial Cell Surface Glycoprotein Recognized by Plant Lectins That Inhibit the Adherence of *Actinomyces naeslundii*

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The adherence of *Actinomyces naeslundii* to human epithelial (KB) cells is mediated by the interaction of a fimbrial lectin on this oral bacterium with epithelial cell receptors exposed by sialidase. The D-galactose- and *N*-acetyl-D-galactosamine-reactive plant lectins from peanut and from *Bauhinia purpurea* inhibit this interaction. This report describes the partial purification and characterization of a 160-kilodalton (kDa) cell surface glycoprotein which is the principal receptor for these lectins. Radioiodinated lectins detected a band of 160 kDa on sialidase-treated Western blots of epithelial cell extracts but did not detect bands on nontreated filters. However, wheat germ agglutinin was reactive with the 160-kDa band on filters that were not treated with sialidase, suggesting that this lectin recognizes the sialic acid residues of this molecule. The 160-kDa component was partially purified from *n*-octylglucoside extracts of the epithelial cells by wheat germ agglutinin affinity chromatography. This molecule was metabolically labeled with D-[^{14}C]glucosamine and labeled at the cell surface by lactoperoxidase-catalyzed iodination or periodate oxidation followed by sodium borotritide reduction. Incubation of epithelial cells with sialidase before extraction resulted in the loss of the 160-kDa band and the appearance of a band at 200 kDa which was directly reactive with ^{125}I-labeled peanut agglutinin. These results indicate that the 160-kDa glycoprotein on the surface of the epithelial cell serves as a receptor for the agglutinins from the peanut and *B. purpurea* and presumably the fimbrial lectin of actinomyces.

A number of bacteria and viruses are known to have surface lectins which promote their adherence to and subsequent infection of host tissues. Lectin activities of bacteria are commonly associated with fimbriae or pili, and on some bacteria the presence of these structures has been correlated with virulence (2). Although a number of different fimbriae have been shown to possess lectin activities, little is known about the nature of their complementary receptors on mammalian cells. We have recently shown that the lectin associated with the type 2 fimbriae of *Actinomyces naeslundii* WVU45 (7) and *A. viscosus* T14V (5, 28) mediates the attachment of these oral bacteria to a human epithelial cell line (4). Bacterial adherence was inhibited by β-linked galactosides and was dependent upon the removal of sialic acid to expose the D-galactose and *N*-acetyl-D-galactosamine receptors. The removal of sialic acid from mammalian glycoconjugates, an initial event in the attachment process, may be mediated by a bacteria-derived enzyme, since sialidase is secreted by the actinomyces and, in addition, is found in association with the surface of these bacteria (8, 11).

Lectin-mediated adherence of the actinomyces to other bacteria (6) or mammalian cells (4) appears to depend on a cooperative effect of multivalent binding involving many low-affinity lectin sites (J. O. Cisar, *in* D. Mirelman, ed., *Microbial Lectins and Agglutinins*, in press). Whereas the low affinity prohibits the use of isolated fimbriae as probes for the identification of mammalian cell receptors, we have found that the peanut agglutinin (PNA) which is most specific for β-D-galactopyranosyl-(1→3)-*N*-acetyl-D-galactosamine (Galβ3GalNAc) and the lectin from *Bauhinia purpurea* (BPA) which reacts with this disaccharide and with other D-galactose and *N*-acetyl-D-galactosamine-containing receptors are potent inhibitors of the adherence of actinomyces to KB cells (4). In contrast, lectins with specificity for α-linked D-galactose or *N*-acetyl-D-galactosamine and D-mannose are not inhibitory. In this study, these two plant lectins have been used as probes to detect a major cell surface sialoprotein of 160 kilodaltons (kDa) on human epithelial cells which presumably serves as a receptor for the fimbrial lectin of the actinomyces.

MATERIALS AND METHODS

Materials. Affinity-purified PNA, wheat germ agglutinin (WGA), and BPA were purchased from E-Y Laboratories, Inc., San Mateo, Calif. Concanavalin A (ConA) was obtained from Calbiochem-Behring, La Jolla, Calif. Sialidase (neuraminidase from *Clostridium perfringens*) Type V and Type X and saccharides were obtained from Sigma Chemical Co., St. Louis, Mo. Lactoperoxidase and *n*-octylglucoside were from Boehringer Mannheim Biochemicals, Indianapolis, Ind. D-[U-^{14}C]glucosamine hydrochloride (278 mCi/mmol), sodium boro[^{3}H]hydride (6.2 Ci/mmol), and sodium [^{125}I]iodide (15 mCi/μg of iodine) were obtained from Amersham Corp., Arlington Heights, Ill. The source of [*methyl*-^{3}H]thymidine (6 Ci/mmol) was Schwarz/Mann, Orangeburg, N.Y. [^{3}H]formaldehyde was from New England Nuclear Corp., Boston, Mass.

Bacteria. *A. naeslundii* WVU45 (ATCC 12104) was cultured in complex media (8), and the presence of fimbriae was verified by electron microscopy as previously described (4, 5). For adherence assays, the bacteria were harvested after 48 h of growth in media containing 0.01 mCi of [*methyl*-^{3}H]thymidine per ml.

Cells. The human KB epithelial cell line (ATCC CCL17) was purchased from the American Type Culture Collection,

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Rockville, Md. The cells were routinely cultured in tissue culture flasks (75 cm^2; Costar, Cambridge, Mass.) or roller bottles (490 cm^2; Corning Glass Works, Corning, N.Y.) containing Dulbecco modified essential medium (Flow Laboratories, Inc., McLean, Va.) supplemented with 5% fetal bovine serum (M. A. Bioproducts, Walkersville, Md.), 100 U of penicillin per ml, and 100 μg of streptomycin per ml. Cell cultures were maintained in a 5% CO_2 atmosphere at 37°C.

Attachment assay. The adherence of radiolabeled *A. naeslundii* to confluent monolayers of KB cells in 24-well cluster dishes (Costar) was performed as described previously (4). The effect of lectins on bacterial attachment to cell monolayers which had been preincubated for 60 min at 37°C with 3×10^{-3} U of sialidase (type X) per ml of attachment buffer (Hanks balanced salt solution containing 0.2 mg of $CaCl_2$ per ml, 0.2 mg of $MgSO_4$ per ml, and 0.2% bovine serum albumin) or attachment buffer alone was examined. Lectins (50 μg/ml) were incubated with the cell monolayers for 60 min at 25°C, 2×10^8 radiolabeled bacteria in attachment buffer containing sialidase at a final concentration of 3×10^{-3} U/ml were added to each well, and the number of bacteria attached per mammalian cell was determined after a 30-min incubation as described previously (4).

Lectin-binding assay. Lectins were radiolabeled by reductive methylation with 1 mM [^{3}H]formaldehyde in the presence of sodium cyanoborohydride (19) and 0.1 M inhibitory saccharides. ^{3}H-labeled lectins were diluted 10-fold with unlabeled lectins to a final concentration of 50 μg of lectin per ml, and approximately 50,000 cpm per well was added to confluent monolayers of KB cells cultured in 96-well tissue culture plates (Costar) which had been preincubated with sialidase as described above. After a 90-min incubation at 25°C, the wells were washed three times, the contents were solubilized in 1% sodium dodecyl sulfate (SDS) and 4 M urea and were counted in a Beckman LS9000 scintillation counter after suspension in Ultrafluor (National Diagnostics Inc., Somerville, N.J.). Specific binding was determined by performing the assay in the presence of inhibitory saccharides as indicated in the text.

Cell labeling and extraction. KB cells were metabolically labeled by addition of 10 ml of Dulbecco modified essential medium containing 50 μCi of D-[^{14}C]glucosamine hydrochloride to 10^7 cells for 24 h at 37°C. KB cells were surface labeled by the method of Gahmberg and Anderson (15) by incubating a flask of cells with 1 mM sodium metaperiodate in phosphate-buffered saline (PBS) (0.15 M NaCl, 0.02 M PO_4, pH 7.2), containing 0.2 mg of $CaCl_2$ per ml and 0.2 mg of $MgSO_4$ per ml, for 10 min at 4°C, followed by incubation with 1 mCi of sodium borotritide for 30 min at 25°C. Labeled cells were scraped from tissue culture flasks into PBS, washed three times, and extracted in 0.2 M *n*-octylglucoside containing 1 mM phenylmethylsulfonyl fluoride and 1 mM *N*-ethylmaleimide for 20 min at 25°C. Extracts were centrifuged at 12,000 × *g* for 10 min in a Beckman Microfuge-2, and the supernatant was collected. Washed and suspended KB cells (8×10^7 cells) were radioiodinated with 2 mCi of sodium [^{125}I]iodide and 0.2 mg of lactoperoxidase per ml by the method of Lebien et al. (26) as modified by Pytela et al. (31), and cells were extracted as described above.

SDS-PAGE and lectin overlays. KB cell extracts or partially purified cell constituents were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (25) on a 4 to 15% SDS gradient gel with a 3% stacking gel. Samples were boiled in the presence of 2-mercaptoethanol, and aliquots equivalent to 10^5 KB cells were added to each lane for analysis of whole-cell extracts. Some gels were stained with Coomassie blue or silver (10) for detection of protein bands. Molecular mass protein standards from Bio-Rad Laboratories (Richmond, Calif.) included myosin (200 kDa), β-galactosidase (116 kDa), phosphorylase *b* (92 kDa), bovine serum albumin (66 kDa), and ovalbumin (45 kDa). For lectin overlays, material was electrophoretically transferred (33) from the polyacrylamide gels to sheets of nitrocellulose (Schleicher & Schuell, Inc., Keene, N.H.). As indicated, some nitrocellulose transfers were treated with 0.7 U of Type V sialidase per ml or 0.03 U of Type X sialidase per ml in 0.05 M sodium acetate buffer containing 0.01 M $CaCl_2$, pH 5.5, for 2 h at 37°C. Transfers were blocked with 0.5% bovine serum albumin in PBS containing 0.02% sodium azide for 2 h at 37°C and washed in PBS containing 0.02% Tween 20. Lectins (0.2 mg) were radioiodinated in the presence of 0.1 M inhibitory saccharide with Iodo-Beads (Pierce Chemical Co., Rockford, Ill.) and 1 mCi of sodium [^{125}I]iodide as recommended by the manufacturer. ^{125}I-labeled lectins (25,000 cpm/ml) were incubated with the transfers in PBS-Tween 20 for 90 min at 25°C, washed, dried, and exposed for autoradiography with XAR-5 film (Eastman Kodak Co., Rochester, N.Y.) and Cronex Quanta III intensifying screens (Du Pont Co., Wilmington, Del.) at −70°C. To assess the specificity of the lectin blots, overlays were also performed in the presence of 0.2 M inhibitory saccharide.

WGA affinity chromatography. WGA was coupled to cyanogen bromide-activated Sephacryl S-1000 (Pharmacia Fine Chemicals, Piscataway, N.J.) to yield 1 mg of lectin per ml of Sephacryl S-1000. The gel was washed with PBS containing 0.5% bovine serum albumin and 0.02% sodium azide before use. Lysates of unlabeled or radiolabeled KB cells extracted with *n*-octylglucoside were incubated with WGA-Sephacryl overnight at 4°C. Extracts of 10^7 cells were mixed with 0.1 ml of a 50% solution of the affinity matrix. Unbound material was removed by washing three times with PBS containing 0.05 M *n*-octylglucoside and once with PBS only. Bound material was eluted with 0.2 M *N*-acetyl-D-glucosamine in PBS. Samples of the column fractions were analyzed by SDS-PAGE. In certain cases, material in the fractions was concentrated by precipitation with 0.2% phosphotungstic acid in 0.2 M HCl by the method of Kerjaschki et al. (22) and then resuspended in SDS-PAGE sample buffer.

RESULTS

Effect of plant lectins on the adherence of *A. naeslundii* to KB cells. Several plant lectins of known specificities were examined for their ability to compete with *A. naeslundii* WVU45 for receptors on epithelial cells (Table 1). WGA was a potent inhibitor of bacterial adherence when added to KB cells before sialidase treatment but was a weak inhibitor when preincubated with sialidase-treated cells. BPA and PNA, which do not bind to *A. naeslundii*, inhibited bacterial attachment by 54 and 38%, respectively, when incubated with KB cells that were treated with sialidase. They did not, however, inhibit attachment of the actinomyces to KB cells which were not preincubated with sialidase. The mannose-reactive lectin, ConA, had no effect on bacterial attachment.

Binding of the lectins to untreated or sialidase-treated KB cells was consistent with their ability to inhibit bacterial attachment. WGA bound to untreated KB cells, and its binding was decreased approximately 50% by sialidase treatment, whereas binding of BPA and PNA was significantly

TABLE 1. Inhibition by plant lectins of *A. naeslundii* adherence to KB cells or KB cells pretreated with sialidase

Lectin	Lectin specificity	Inhibition of bacterial adherence (%)	
		KB cells[a]	Sialidase-pretreated KB cells[b]
WGA	Sialic acid, *N*-acetyl-D-glucosamine	46	7
BPA	*N*-acetyl-D-galactosamine, D-galactose	11	54
PNA	Galβ3GalNAc	3	38
ConA	D-Mannose, D-glucose	0	2

[a] KB cells were incubated with buffer for 60 min and then with lectins (50 μg/ml) for an additional 60 min. Radiolabeled bacteria were then allowed to attach for 30 min in the presence of sialidase as described in Materials and Methods. Attachment of *A. naeslundii* with no lectins present (control) was 15 bacteria per KB cell.

[b] KB cells were pretreated for 60 min with 3×10^{-3} U of sialidase. Lectins were added for 60 min, and radiolabeled bacteria were then allowed to attach for 30 min in the presence of sialidase as described in Materials and Methods. Attachment of *A. naeslundii* with no lectins present (control) was 22 bacteria per KB cell.

enhanced by sialidase treatment (Fig. 1). Sialidase treatment did not affect the binding of ConA to the KB cells.

Detection of lectin-reactive glycoproteins in KB cell extracts. To identify glycoproteins of the KB cell that were reactive with WGA, PNA, BPA, and ConA, *n*-octylglucoside extracts of KB cells were separated by SDS-PAGE and transferred to nitrocellulose filters, and strips were probed with

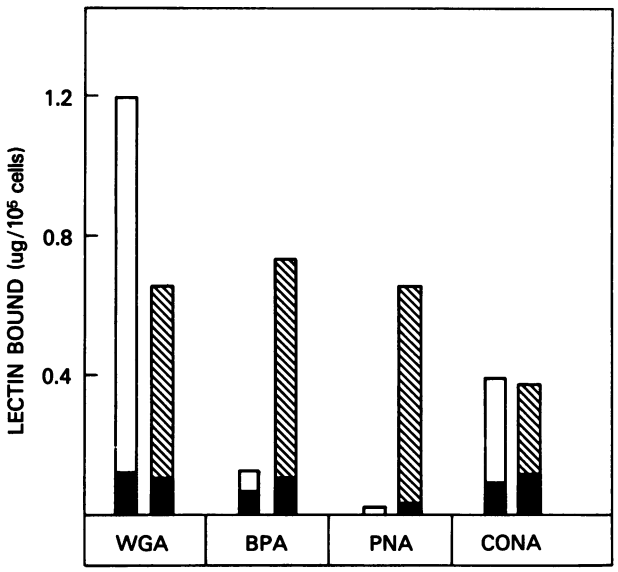

FIG. 1. Specific binding of ^{3}H-labeled plant lectins to KB cell monolayers in the presence and absence of sialidase. Confluent KB cell monolayers in 96-well plates were pretreated for 60 min at 37°C with Hanks balanced salt solution (□) or with Hanks balanced salt solution containing 10^{-3} U of sialidase per ml (▨). Tritiated lectins (50 μg/ml; approximately 50,000 cpm per well) were added for 90 min. Binding was determined in the absence or presence (■) of 0.1 M lactose for PNA and BPA, 0.1 M *N*-acetyl-D-glucosamine for WGA, or 0.1 M D-mannose for ConA. Micrograms of lectin bound per 10^5 cells was determined from the specific activity of each ^{3}H-labeled lectin, and data represent the average of bound radioactivity in three wells.

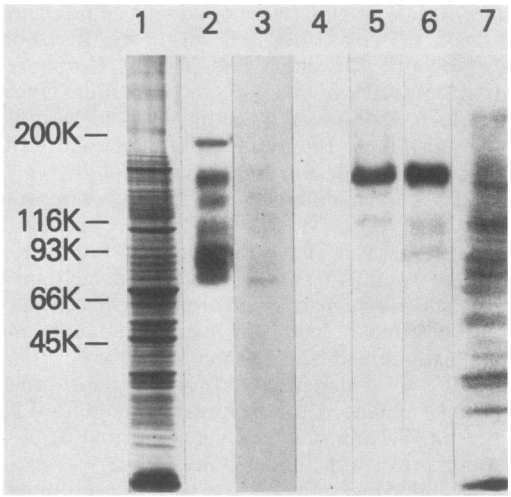

FIG. 2. Detection of lectin receptors on Western blots of KB cell extracts. *n*-Octylglucoside extracts of KB cells (10^5 cells per lane) were separated on 4 to 15% SDS gradient gels. Gels were stained with Coomassie blue (lane 1) or electroblotted onto nitrocellulose and incubated with ^{125}I-labeled lectins (lanes 2 to 7). Nitrocellulose strips in lanes 3, 5, 6, and 7 were treated with sialidase before incubation with lectins. Transfers were incubated with ^{125}I-labeled WGA (lanes 2 and 3), ^{125}I-labeled PNA (lanes 4 and 5), ^{125}I-labeled BPA (lane 6), or ^{125}I-labeled ConA (lane 7). Molecular mass markers (in kilodaltons) are listed to the left of the figure.

radioiodinated lectins (Fig. 2). Numerous protein bands were present in the extract, as indicated by Coomassie blue staining of the gel (Fig. 2, lane 1). ^{125}I-labeled WGA detected several bands, including one at 160 kDa on non-sialidase-treated filters (Fig. 2, lane 2), but reactivity was significantly decreased when ^{125}I-labeled WGA was incubated with filters which had been pretreated with sialidase (Fig. 2, lane 3). In contrast, only one prominent band of 160 kDa was detected by ^{125}I-labeled PNA and ^{125}I-labeled BPA after the nitrocellulose strips were pretreated with sialidase (Fig. 2, lanes 5 and 6). Minor bands of 110 and 85 kDa were also present. Since these bands were barely detectable in fresh extracts of KB cells, they may be the products of proteolytic digestion. In the absence of sialidase, no bands were identified by incubation of the nitrocellulose strips with either ^{125}I-labeled PNA (Fig. 2, lane 4) or ^{125}I-labeled BPA (data not shown). ConA detects numerous components in the total KB cell extract (Fig. 2, lane 7).

Partial purification of the 160-kDa glycoprotein by WGA affinity chromatography. Extracts of unlabeled KB cells or cells radiolabeled with D-[^{14}C]glucosamine were chromatographed on a WGA-Sephacryl affinity column. Specific fractions were analyzed by SDS-PAGE and transferred to nitrocellulose, and the filters were either developed with ^{125}I-labeled PNA after pretreatment of the filter with sialidase (Fig. 3a) or subjected directly to autoradiography for the detection of glycoproteins incorporating D-[^{14}C]glucosamine (Fig. 3b). The components detected by PNA in the cell extract are shown in Fig. 3a, lane 1. Most of the PNA-reactive components in the extract were bound to the column, as noted by the loss of PNA reactivity in the flow-through fraction (Fig. 3a, lane 2). The bound material was not eluted by 0.1 M D-mannose (Fig. 3a, lane 3), but the 160-kDa band was detected by ^{125}I-labeled PNA in the fraction specifically eluted with 0.1 M *N*-acetyl-D-glucosamine (Fig. 3a, lane 4).

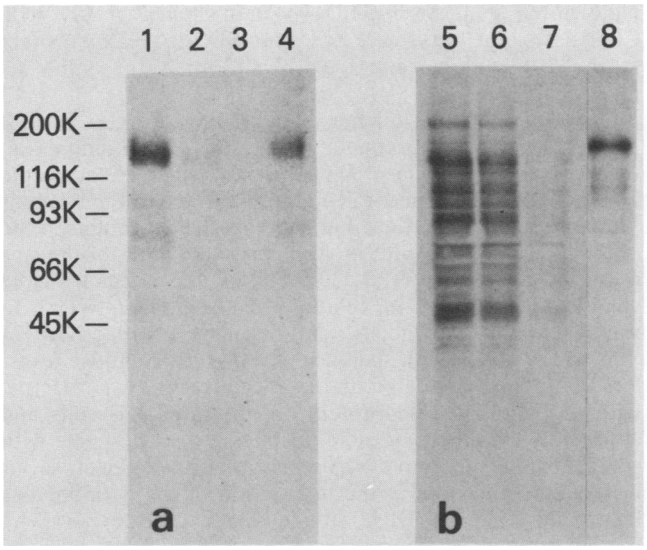

FIG. 3. Partial purification of the 160-kDa lectin receptor by WGA affinity chromatography and incorporation of D-[^{14}C]glucosamine into this component. Lysates of unlabeled (a) or D-[^{14}C]glucosamine-labeled (b) KB cells (10^7 cells) were fractionated on WGA-Sephacryl. Fractions were collected, separated on a 4 to 15% SDS gradient gel, and transferred to nitrocellulose. (a) Nitrocellulose filter treated with sialidase (lanes 1 to 4) and probed with ^{125}I-labeled PNA. Lanes: 1, pre-column extract of KB cells; 2, flow through; 3, 0.2 M mannose eluate; 4, 0.2 M *N*-acetyl-D-glucosamine eluate. (b) Autoradiogram of D-[^{14}C]glucosamine-labeled KB cells fractionated on WGA-Sephacryl. Lanes: 5, pre-column extract; 6, unbound material; 7, 0.05 M *n*-octylglucoside wash; 8, 0.2 M *N*-acetyl-D-glucosamine eluate. Molecular mass markers (in kilodaltons) are listed to the left of the figure.

Although numerous components of the KB cell extract were intrinsically labeled with D-[^{14}C]glucosamine (Fig. 3b, lane 5), most of them did not bind to the WGA-Sephacryl affinity column (lanes 6 and 7). A prominent band incorporating D-[^{14}C]glucosamine was present in the fraction eluted with *N*-acetyl-D-glucosamine (Fig. 3b, lane 8), and this band comigrated with the 160-kDa glycoprotein detected by ^{125}I-labeled PNA on the nitrocellulose strips containing the unlabeled KB cell extract (Fig. 3a).

Identification of the 160-kDa lectin receptor as a cell surface sialoprotein. Intact, viable cells were radiolabeled by lactoperoxidase-catalyzed iodination and extracted with *n*-octylglucoside, and the extract was chromatographed on the WGA-Sephacryl affinity matrix. Fractions were separated by SDS-PAGE and transferred to nitrocellulose, and labeled material was detected by autoradiography (Fig. 4a). Many bands were detected in both the cell extract before WGA affinity chromatography (Fig. 4a, lane 1) and the flow-through fraction (lane 2). A radioiodinated protein of 160 kDa was specifically eluted from the column with 0.1 M *N*-acetyl-D-glucosamine (Fig. 4a, lane 3). This fraction also contained a minor band at 85 kDa.

Evidence that the 160-kDa protein is a cell surface sialoprotein was obtained by radiolabeling the KB cell with periodate-[^{3}H]borohydride, which preferentially labels the sialic acid residues of cell surface glycoconjugates (15). Numerous bands were labeled, as shown in the autoradiogram of the whole-cell extract (Fig. 4b, lane 4). However, when this material was chromatographed on a WGA-Sephacryl affinity column, a major band visualized by autoradiography in the eluted fraction (Fig. 4b, lane 5) comigrated with the 160-kDa protein (arrow).

The susceptibility of the 160-kDa cell surface glycoprotein to sialidase was examined by incubation of the intact KB cells with the enzyme. Extracts were then prepared, separated by SDS-PAGE, and transferred to nitrocellulose (Fig. 5). For comparative purposes, an extract of untreated KB cells is included which demonstrates the reactivity of ^{125}I-labeled PNA with the 160-kDa band on a sialidase-treated filter (Fig. 5, lane 1). However, incubation of the cells with sialidase before extraction resulted in a shift of the PNA-reactive band to approximately 200 kDa on both sialidase-treated (Fig. 5, lane 2) and untreated (lane 3) filters.

DISCUSSION

The lectin associated with the fimbriae of *A. naeslundii*, PNA, and BPA may all interact with the same receptor on KB epithelial cells, since the two plant lectins inhibit the attachment of actinomyces to the mammalian cells. This receptor appears to be a 160-kDa glycoprotein which has been detected on Western blots of KB cell extracts incubated with the radioiodinated plant lectins. Recognition of this glycoprotein only occurs on nitrocellulose transfers that have been treated with sialidase to expose receptors that are reactive with these lectins. Similarly, the binding of PNA and BPA to intact KB cells is significantly enhanced after incubation of the epithelial cells with sialidase (4), a finding consistent with other studies which have demonstrated that the binding of these lectins to other cells (12, 18, 21, 32) is dependent upon the removal of sialic acid. Likewise, the adherence of the actinomyces to KB cells, which is mediated by the fimbrial lectin (4), is enhanced by sialidase treatment

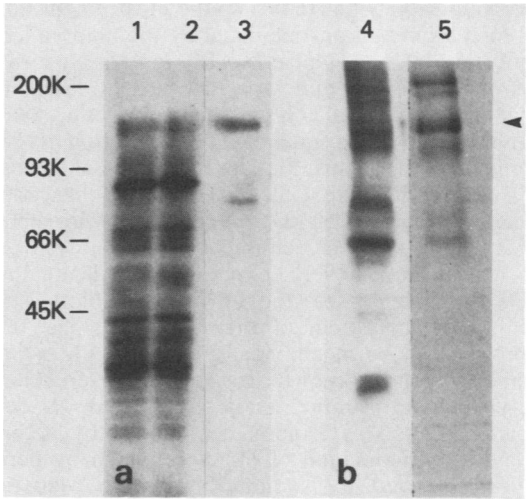

FIG. 4. Surface labeling of the 160-kDa glycoprotein. KB cells were radiolabeled by lactoperoxidase-catalyzed iodination (a) or by periodate-[^{3}H]borohydride (b). Cell lysates were chromatographed on WGA-Sephacryl, and fractions were analyzed by SDS-PAGE autoradiography. (a) Autoradiogram of [^{125}I]-surface-labeled proteins. Lanes: 1, KB cell extract; 2, flow through; 3, 0.2 M *N*-acetyl-D-glucosamine eluate. (b) Autoradiogram of ^{3}H-labeled KB cell extract (lane 4) and material subsequently eluted from WGA-Sephacryl with 0.2 M *N*-acetyl-D-glucosamine (lane 5). The arrowhead denotes the position of the 160-kDa band detected by ^{125}I-labeled PNA on sialidase-treated companion strips containing unlabeled KB cell extract. Molecular mass markers (in kilodaltons) are listed to the left of the figure.

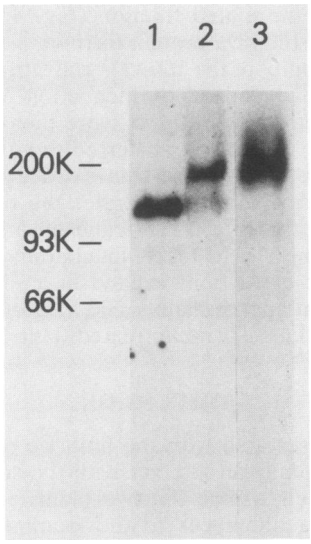

FIG. 5. Effect of sialidase on the 160-kDa cell surface glycoprotein. Nitrocellulose transfers containing lysates of KB cells (lane 1) or KB cells incubated with sialidase before extraction (lanes 2 and 3) were incubated with ^{125}I-labeled PNA. Filters in lanes 1 and 2 were treated with sialidase, and the filter shown in lane 3 was not treated. Molecular mass markers (in kilodaltons) are listed to the left of the figure.

of the epithelial cells, as is the agglutination of erythrocytes by these bacteria (8).

The most active inhibitor of both PNA and BPA is Galβ3GalNAc (20, 29, 34), and earlier studies have demonstrated that PNA reacts specificially with glycoproteins having O-linked oligosaccharides containing Galβ3GalNAc (12, 17, 27). Galβ3GalNAc is also the most potent inhibitor of cell-to-cell interactions mediated by the fimbrial lectin of actinomyces (28), and Heeb et al. (16), in an assay measuring aggregation of glycopeptide-coated beads by actinomyces, demonstrated that O-linked asialooligopeptides are ten times more inhibitory than an equivalent concentration of N-linked asialooligopeptides. Thus, the presence of O-linked oligosaccharide chains with Galβ3GalNAc termini would account for the reactivity of the 160-kDa glycoprotein with PNA and BPA, as well as with the actinomyces fimbrial lectin.

The results from several experiments indicate that the 160-kDa component detected on Western blots is a cell surface sialoglycoprotein of the KB cells. The 160-kDa glycoprotein can be metabolically labeled with D-[^{14}C]glucosamine, which is known to serve as a precursor for *N*-acetyl-D-galactosamine, sialic acid, and *N*-acetyl-D-glucosamine (23). This component is labeled by lactoperoxidase-catalyzed iodination of intact cells and by periodate oxidation followed by sodium borotritide reduction, a method which preferentially labels sialic acid-containing surface molecules (15). The 160-kDa glycoprotein on intact cells is also susceptible to the action of sialidase, which results in the loss of the 160-kDa band and the appearance of a diffuse band at 200 kDa which can be directly detected by PNA. The detection of a higher-molecular-weight band on sialidase-treated cells is consistent with a previous study which reported that a membrane sialoprotein from HeLa cells migrates more slowly in SDS-PAGE after sialidase treatment (24). This is thought to be due to the partial desialylation of the glycoprotein and the anomalous behavior of sialic acid-rich proteins in SDS-PAGE. In addition, we have noted that the 160-kDa protein stains weakly with Coomassie blue but is detected upon SDS-PAGE by a silver stain (10) which reacts with sialoproteins such as glycophorin (13).

Adherence assays indicate that binding of WGA to KB cells prevents the subsequent attachment of actinomyces in the presence of sialidase. WGA has a similar effect on the binding of radiolabeled PNA and BPA to epithelial cells (unpublished data). These findings may be attributed to the interaction of WGA with multivalent sialic acid residues as proposed previously (1, 3, 30). This is consistent with the significant decrease in binding of ^{3}H-labeled WGA to sialidase-treated KB cells and the lack of reactivity of ^{125}I-labeled WGA with sialidase-treated nitrocellulose transfers containing KB cell extracts. Because WGA is reactive with the 160-kDa glycoprotein on untreated KB cells and apparently with the sialic acid residues, it may compete with sialidase to prevent unmasking of subterminal galactose, or it may sterically inhibit the interaction of the actinomyces lectin and PNA and BPA with these receptors exposed by partial removal of sialic acid from the KB cell surface glycoprotein.

The 160-kDa sialoprotein of the KB cell is similar in some respects to membrane proteins that have been described previously. These include the following: a 165-kDa transmembrane sialoprotein of the HeLa cell (24), the 160-kDa low-density lipoprotein receptor which contains 12 to 18 O-linked oligosaccharides (9), a 140-kDa membrane glycoprotein which serves as a cell surface receptor for fibronectin (31), and the major integral membrane sialoprotein of the erythrocyte (13, 14). Its lectin-reactive properties are also very similar to the major sialoprotein of the glomerulus, podocalyxin (22).

Biochemical and immunological characterization of the 160-kDa glycoprotein may clarify its endogenous function, as well as define the oligosaccharide structure of the 160-kDa glycoprotein. To date, evidence suggests that the specificity of the interaction of the actinomyces with KB cells is determined not only by the structure of the terminal and penultimate saccharides but also by the linkages. Evidence also indicates that the bacterial lectin receptor on cells of different origin may vary and that they may include saccharides which terminate with *N*-acetyl-D-galactosamine (6), as well as galactose. Defining the cell surface molecules that function as receptors for the actinomyces should further our understanding of the initial recognition processes involved in the colonization of specific host tissues by these and other microorganisms.

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