INFECTION AND IMMUNITY, Mar. 1996, p. 891–896 Vol. 64, No. 3
0019-9567/96/$04.00+0

Blood Group Glycolipids as Epithelial Cell Receptors for *Candida albicans*

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Received 30 October 1995/Returned for modification 28 November 1995/Accepted 19 December 1995

The role of glycosphingolipids as possible epithelial cell receptors for *Candida albicans* was examined by investigating the binding of biotinylated yeasts to lipids extracted from human buccal epithelial cells and separated on thin-layer chromatograms. Binding was visualized by the addition of ^{125}I-streptavidin followed by autoradiography. Five *C. albicans* strains thought from earlier work to have a requirement for fucose-containing receptors all bound to the same three components in the lipid extract. A parallel chromatogram overlaid with biotinylated *Ulex europaeus* lectin, which is a fucose-binding lectin with a specificity for the H blood group antigen, showed that two of these glycosphingolipids carried this antigenic determinant. Preparations of crude and purified adhesin (a protein with a size of 15.7 kDa which lacked cysteine residues) from one of the strains also bound to these same two components. The third glycosphingolipid, which bound whole cells but neither preparation of adhesin, was recognized by *Helix pomatia* lectin, indicating that it contained *N*-acetylgalactosamine, possibly in the form of the A blood group antigen. Overlay assays with a sixth strain of *C. albicans* (GDH 2023) revealed a completely different binding pattern of four receptors, each of which contained *N*-acetylglucosamine. These results confirm earlier predictions about the receptor specificity of the strains made on the basis of adhesion inhibition studies and indicate that blood group antigens can act as epithelial cell receptors for *C. albicans*.

The ability of *Candida albicans* to adhere to mucosal surfaces enables the organism to resist removal by the cleansing action of the fluids that bathe these surfaces. Adhesion thus facilitates colonization and can be regarded as the first step in the pathogenesis of *Candida* infections. Attachment of the yeast form of the fungus to epithelial cells appears to involve several kinds of adhesin-receptor interaction (3, 4, 9). Most experimental evidence indicates that the adhesins are mannoproteins which may be associated with fibrils or fimbriae on the yeast surface (8). Currently, four categories of mannoprotein adhesin have been proposed (9): lectin-like adhesins (6, 7, 29), fimbrial adhesins (33, 34), factor 6 (24), and integrin analogs (1, 15).

Adhesion of many strains of *C. albicans* to exfoliated buccal or vaginal epithelial cells involves predominantly lectin-like interactions between the protein portion of a mannoprotein adhesin and a glycoside receptor on the host cell surface. Early evidence for the participation of the protein moiety of the adhesin in the attachment process came from experiments in which crude adhesin preparations were subjected to heat or incubated with dithiothreitol or various proteolytic enzymes. Such procedures either partially or completely destroyed the ability of the adhesin to block yeast attachment to epithelial cells, whereas similar treatments with sodium metaperiodate or α-mannosidase had little or no effect (6). Preliminary attempts to characterize the epithelial receptors by means of adhesion inhibition tests with sugars and lectins indicated that most *C. albicans* strains bound to glycosides containing L-fucose, although one strain (GDH 2023) seemed to have a preference for *N*-acetylglucosamine-containing receptors (7).

Knowledge of the receptor specificity of a fucoside-binding strain of *C. albicans* was used to devise a scheme for purifying the adhesin (29). The starting material was crude mannoprotein isolated from culture supernatants, and the purification protocol involved stepwise treatment with *N*-glycanase, papain, and dilute alkali to cleave the protein and carbohydrate portions of the glycoprotein. Fucoside-binding protein fragments were then recovered by affinity adsorption with the trisaccharide determinant of the H (type 2) blood group antigen, which terminates in a residue of L-fucose. The purified adhesin was devoid of carbohydrate and inhibited yeast adhesion to buccal epithelial cells by 80% at a concentration of 10 μg/ml.

In this study, we have used the purified adhesin, together with crude adhesin and whole-cell preparations of different *C. albicans* strains, to investigate binding to potential glycosphingolipid receptors separated on thin-layer chromatograms. Chromatogram overlay assays of this type have been widely employed in the characterization of bacterial adhesion mechanisms (18). The lipids tested were extracted from human buccal epithelial cells and sheep erythrocytes; yeast cells and adhesin preparations were labelled by means of a biotin-streptavidin technique.

MATERIALS AND METHODS

Organisms. Six strains of *C. albicans* were used in this study. Four of the strains were isolated in Glasgow and are now deposited with the National Collection of Yeast Cultures (NCYC), Food Research Institute, Norwich, United Kingdom. *C. albicans* GDH 2346 (NCYC 1467) and GDH 2023 (NCYC 1468) were isolated at Glasgow Dental Hospital from patients with denture stomatitis; strains GRI 681 (NCYC 1472) and GRI 682 (NCYC 1473) were obtained from routine cervical smears taken from asymptomatic women at Glasgow Royal Infirmary. *C. albicans* NCPF 3153 (MRL 3153) was obtained from the Mycological Reference Laboratory, Colindale, London, United Kingdom. *C. albicans* "outbreak strain" was isolated as the causative agent of an outbreak of systemic candidosis at The London Hospital and was supplied by J. P. Burnie. The organisms were maintained on slopes of Sabouraud dextrose agar (Difco)

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TABLE 1. Structures of glycosphingolipid standards tested by chromatogram overlay assay

Glycosphingolipid	Structure
CMH (ceramide monohexose)	Galβ1-1Cer
CDH (ceramide dihexose)	Galβ1-4Glcβ1-1Cer
CTH (ceramide trihexose)	Galα1-4Galβ1-4Glcβ1-1Cer
GL4 (globoside)	GalNAcβ1-3Galα1-4Galβ1-4Glcβ1-1Cer
FORS	GalNAcα1-3GalNAcβ1-3Galα1-4Galβ1-4Glcβ1-1Cer

and subcultured monthly. Every 2 months, cultures were replaced by new ones freshly grown from freeze-dried stocks.

Growth conditions. Organisms were grown at 37°C with shaking in yeast nitrogen base medium (Difco) containing 500 mM galactose as described previously (22). They grew exclusively in the budding yeast phase under these conditions. Cells were harvested after 24 h (stationary phase of growth) and washed twice in 0.15 M phosphate-buffered saline (PBS) (pH 7.2).

Isolation of EP. Extracellular polymeric material (EP) from strains GDH 2346 and GDH 2023 was prepared by freeze-drying dialyzed culture supernatants by using a minor modification of a method previously described (6). Batches of medium (500 ml in 2-liter Erlenmeyer flasks) were inoculated with overnight yeast cultures (50 ml) and incubated at 37°C for 5 days in an orbital shaker operating at 150 rpm. Yeast cells were removed by centrifugation, and the culture supernatants were subjected to ultrafiltration in an Amicon DC2 hollow-fiber concentration system, with a 3.5K filter. The supernatants were then dialyzed at 4°C for 3 days against five changes (5 liters each) of distilled water. The retentates (crude EP) were freeze-dried.

Purification of fucoside-binding adhesin. Purified adhesin was prepared as described previously (29) by stepwise treatment of EP from strain GDH 2346 with *N*-glycanase, papain, and dilute alkali followed by H-2 affinity adsorption with the trisaccharide determinant of the H (type 2) blood group antigen.

Analysis of EP and purified adhesin. The carbohydrate content was estimated by the method of Dubois et al. (11), with mannose as a standard. The amount of protein was determined by the method of Lowry et al. (19), with bovine serum albumin as a standard.

Adhesion assays. Yeast adhesion to exfoliated buccal epithelial cells was determined by light microscopy as described previously (10) except that a lower yeast cell concentration (10^7 organisms ml^{-1}) was used. The ability of crude EP or purified adhesin to inhibit adhesion was tested by the method of Tosh and Douglas (29). Epithelial cells were obtained from the buccal mucosa of a single, healthy, male, blood group A donor and were collected at the same time of day to minimize variability.

Biotinylation of yeasts, EP, and purified adhesin. Yeast cells were biotinylated by a modification of the method of Casanova et al. (5), with a biotinylation kit from Amersham. PBS-washed cells were collected by centrifugation, resuspended in 0.05 M sodium borate buffer (pH 8.6), and adjusted to a concentration of 2×10^8 organisms ml^{-1}. Biotinylation reagent (40 μl) was added to 1 ml of cell suspension, and the mixture was incubated for 1 h at room temperature on a tumbling mixer. The yeast cells were washed twice in 0.05 M Tris-HCl (pH 7.8) containing 0.15 M NaCl and 1% bovine serum albumin (Tris-BSA) and adjusted to a concentration of 10^8 cells ml^{-1} in the same buffer. EP and purified adhesin were biotinylated by using the Amersham biotinylation kit according to the manufacturer's protocol. EP (10 mg) or adhesin (200 μg) was dissolved in 1 ml of 0.05 M sodium borate buffer (pH 8.6). Biotinylation reagent (40 μl) was added, and the mixture was incubated for 1 h at room temperature on a tumbling mixer. Unreacted biotinylation reagent was removed by desalting on G-25 Sephadex, and the EP and adhesin were recovered by freeze-drying.

Lectins. Biotinylated lectins from *Ulex europaeus* (I) and *Helix pomatia* were obtained from Sigma. Biotinylated *Griffonia simplicifolia* lectin II was from Vector Laboratories, Peterborough, United Kingdom.

Glycosphingolipids. Neutral glycolipid thin-layer chromatography standards were purchased from Calbiochem. These glycolipids included galactosylceramide (CMH), lactosylceramide (CDH), globotriaosylceramide (CTH), globotetraosylceramide (GL4), and globopentaosylceramide (Forssman glycolipid [FORS]) (Table 1). A lipid extract of human buccal epithelial cells was prepared by adaptation of the methods of Magnani et al. (20) and Esselman et al. (12) as follows. Buccal epithelial cells were obtained by gently rubbing the inside cheek area of a healthy, male, blood group A donor with sterile cotton swabs. The cells were washed three times in PBS and stored frozen until required. Cells collected over 10 days were thawed, washed in PBS, resuspended in methanol (7 volumes), and homogenized with a pestle and mortar. Chloroform (14 volumes) was added, and the mixture was homogenized again and filtered through coarse-grade, solvent-washed filter paper in a Buchner funnel. The residue was reextracted with 10 volumes of chloroform-methanol (2:1), and the combined filtrates were evaporated to dryness. Sheep erythrocyte lipids were a gift from Chris Brotherston (Division of Infection and Immunity, Institute of Biomedical and Life Sciences, University of Glasgow) and were prepared by a similar procedure.

Chromatogram overlay assay. A modification of the method of Jimenez-Lucho et al. (17) was used. Glycolipids were chromatographed on aluminium-backed silica gel 60 thin-layer plates (E. Merck AG, Darmstadt, Germany) developed with chloroform-methanol-water (65:25:4). Total lipids were visualized with orcinol reagent. Plates to be used in overlay assays were coated three times for 1 min in polysiobutylmethacrylate (0.1% in hexane) and air dried between coatings. The plates were again air dried, blocked for 1 h in Tris-BSA, and overlaid for 2 h at room temperature with biotin-labelled yeast cells (10^7 cells ml^{-1} in Tris-BSA; 10 ml), biotinylated EP (1 mg ml^{-1} in Tris-BSA; 10 ml), biotinylated purified adhesin (20 μg ml^{-1}; 10 ml), or biotinylated lectins (5 μg ml^{-1}; 10 ml). The plates were gently washed in distilled water to remove unbound material, blocked for 1 h in Tris-BSA, and then overlaid with 5 μCi ^{125}I-streptavidin (Amersham) in 10 ml of Tris-BSA. The plates were finally washed, dried, and exposed for 2 to 5 days to blue sensitive X-ray film (Genetic Research Instrumentation Ltd., Dunmow, Essex, United Kingdom).

Amino acid analysis of purified adhesin. Amino acid analysis was done by Gordon Curry and Maggie Cusack (Department of Geology and Applied Geology, University of Glasgow), using an Applied Biosystems 420-H amino acid analyzer with an automatic hydrolysis head. Affinity-adsorbed, freeze-dried, purified adhesin (1 μg dissolved in 50 μl of 0.1% trifluoroacetic acid) was used. A blank sample was also analyzed (i.e., the product of the purification protocol without EP in the initial digest).

SDS-PAGE and Western blot analysis of purified adhesin. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was carried out in a minigel apparatus based on the Bio-Rad Mini-Protean II electrophoresis system. A modification of the procedure of Schagger and Von Jagow (26) was employed as described in Technical Bulletin no. MWM-100 (Sigma). Samples (biotinylated purified adhesin) and molecular weight markers (MW-SDS-17S; Sigma) were electrophoresed for 3 to 4 h at a constant current of 100 V. The markers were stained with Brilliant Blue G (Sigma). Biotinylated adhesin was transferred to nitrocellulose (Hybond C; Amersham) for Western blot (immunoblot) analysis according to the method of Towbin and Gordon (30) in a Trans-Blot electrophoretic transfer apparatus (Bio-Rad). The transfer was performed at 0.13 A (50 V) for 1 h, at 0.08 A (30 V) for 18 h, and then at 0.13 A (50 V) for 1 h. The nitrocellulose was immersed in a blocking solution consisting of 3% (wt/vol) skimmed milk and 0.2% Tween 20 in PBS at 4°C overnight. This solution was replaced with 10 ml of fresh blocking solution to which 5 μCi of ^{125}I-streptavidin (Amersham) had been added. After incubation with gentle shaking for 2 h, the nitrocellulose was washed extensively in distilled water to remove unbound ^{125}I-streptavidin, allowed to air dry, and exposed for 2 to 5 days to blue sensitive X-ray film (Genetic Research Instrumentation Ltd.) with intensifying screens.

RESULTS

Purification and analysis of fucoside-binding adhesin. The fucoside-binding adhesin of *C. albicans* GDH 2346 was isolated from culture supernatants by a procedure described previously (29) involving stepwise treatment with *N*-glycanase, papain, and dilute alkali, followed by affinity adsorption with the trisaccharide determinant of the H (type 2) blood group antigen. The purified material was completely free of carbohydrate and inhibited yeast adhesion to buccal epithelial cells by an extent (75%, at an adhesin concentration of 6.4 μg ml^{-1}) similar to that observed for earlier preparations (29). Purified adhesin was biotinylated and then subjected to SDS-PAGE and Western blotting. Subsequent probing with ^{125}I-streptavidin and autoradiography revealed a strong band corresponding to a protein with a size of 15.7 kDa (Fig. 1) and the complete absence of material with a larger molecular mass. However, the intact mannoprotein from which the fucoside-binding fragment was derived by chemical and enzymic degradation is likely to be considerably larger than 15.7 kDa.

The amino acid composition of the purified adhesin is detailed in Table 2. The most abundant amino acids were Thr, Pro, Ala, Ser, Val, Gly, Asx, and Glx. No Cys was detected, and there was very little Met, Phe, or Arg. The percentage of nonpolar amino acids was 52%. This compositional analysis, together with the observed molecular mass of 15.7 kDa, suggests that the purified adhesin consists of approximately 152 amino acid residues.

Binding of biotinylated *C. albicans* to glycolipids on chromatograms. The binding of biotinylated whole cells of five

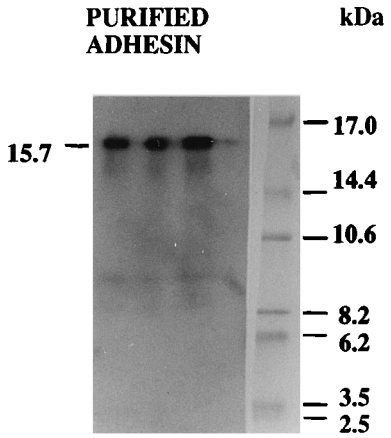

FIG. 1. Western blot analysis of purified fucoside-binding adhesin. Purified adhesin (200 μg) was biotinylated as described in Materials and Methods and resuspended in 100 μl of solubilizing buffer. Adhesin samples (25 μl; lanes 1 to 3) were subjected to SDS-PAGE, transferred to nitrocellulose, probed with ^{125}I-streptavidin, and examined by autoradiography. Molecular mass markers (lane 4), with sizes indicated on the right, were stained with Brilliant Blue G. The position of the 15.7-kDa protein is indicated on the left.

strains of *C. albicans* to glycolipids extracted from human buccal epithelial cells and sheep erythrocytes, and to a number of neutral glycolipid standards, was examined by the chromatogram overlay assay. The solvent system used for thin-layer chromatography gave a satisfactory separation of the glycolipid standards as revealed by staining with orcinol reagent (Fig. 2). In the overlay assay, bound yeast cells were visualized by the addition of ^{125}I-streptavidin followed by autoradiography. All five strains (GDH 2346, GRI 681, GRI 682, MRL 3153, and Outbreak), which were thought from earlier work (7) to have a requirement for fucose-containing receptors, showed similar binding patterns (Fig. 3). All bound to the same three components in the buccal cell extract (lane 2). A parallel chromatogram overlaid with biotinylated *U. europaeus* I lectin, which is a fucose-binding lectin with a specificity for the H blood group antigen, showed that two of these glycolipids carried this antigenic determinant. The third glycolipid was not recognized by

TABLE 2. Amino acid composition of *C. albicans* fucoside-binding adhesin

Amino acid residue[a]	Mol%	Estimated no. of residues/adhesin molecule
Asx	8.5	13
Glx	6.5	10
Ser	8.0	12
Gly	7.8	12
His	2.3	4
Arg	1.9	3
Thr	17.0	26
Ala	11.1	17
Pro	13.3	20
Tyr	2.6	4
Val	7.9	12
Met	0.5	1
Cys	0.0	0
Ile	2.9	4
Leu	4.8	7
Phe	1.5	2
Lys	3.5	5

[a] Trp was destroyed during analysis.

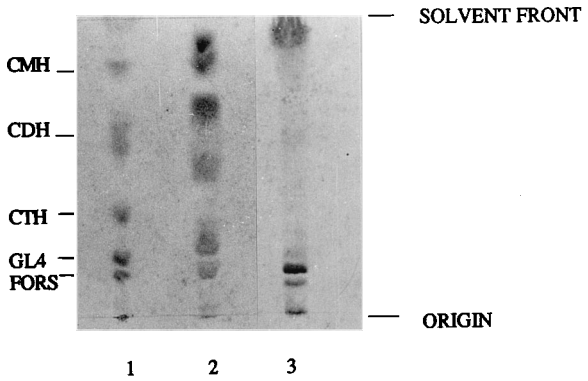

FIG. 2. Thin-layer chromatography of lipid extracts and neutral glycolipid standards. Lipids were separated with chloroform-methanol-water (65:25:4) and visualized with orcinol. Lanes: 1, 2 μg each of CMH, CDH, CTH, GL4, and FORS; 2, lipid extract of buccal epithelial cells (80 μg); 3, lipid extract of sheep erythrocytes (500 μg).

U. europaeus I lectin but bound to a lectin from *Helix pomatia* specific for *N*-acetylgalactosamine (Fig. 3). None of the five *C. albicans* strains recognized any of the glycosphingolipid standards used, including lactosylceramide (lane 1), but all bound to two fucose-containing glycolipids in the sheep erythrocyte extract (lane 3), one of which had a mobility identical to that of one of the buccal cell lipids.

Binding of biotinylated EP and purified adhesin to glycolipids on chromatograms. The binding of EP and purified adhesin from *C. albicans* GDH 2346 to glycolipids was also examined by the chromatogram overlay assay (Fig. 4). Their binding patterns were remarkably similar. Neither preparation bound to any of the glycosphingolipid standards (lane 1). However, comparison with a parallel chromatogram overlaid with biotinylated *U. europaeus* I lectin showed that both EP and purified adhesin bound to the fucose-containing lipids from buccal cells (lane 2) and sheep erythrocytes (lane 3) that had been recognized by intact yeast cells of strain GDH 2346 (and four other strains). Interestingly, neither EP nor purified adhesin bound to the third, *N*-acetylgalactosamine-containing lipid in buccal cell extracts, which was also recognized by whole cells of all five *C. albicans* strains. It is possible that the component on the yeast cell surface that binds this lipid is not released during growth and is therefore not recovered in EP preparations. Alternatively, its ability to bind the *N*-acetylgalactosamine-containing lipid may be lost during the isolation of EP.

Binding of biotinylated whole cells and EP from strain GDH 2023 to glycolipids on chromatograms. Earlier work indicates that *C. albicans* GDH 2023 has an unusual type of adhesion mechanism and, unlike the other strains tested in this study, interacts principally with *N*-acetylglucosamine-containing receptors on epithelial cells (7). When biotinylated whole-cell and EP preparations from this strain were used in the chromatogram overlay assay, binding patterns completely different from those observed previously were obtained (Fig. 5). In each case there was binding to four lipid components in the buccal cell extract (lane 2) and to four components in the sheep erythrocyte extract (lane 3); two of the lipids appeared to be common to both extracts. A parallel chromatogram overlaid with biotinylated *G. simplicifolia* lectin II, which has a specificity for *N*-acetylglucosamine, showed that all of the lipids contained this amino sugar. As with the other strains tested, there was no binding by whole cells of *C. albicans* GDH 2023, or by EP, to any of the neutral glycolipid standards, including lactosylceramide.

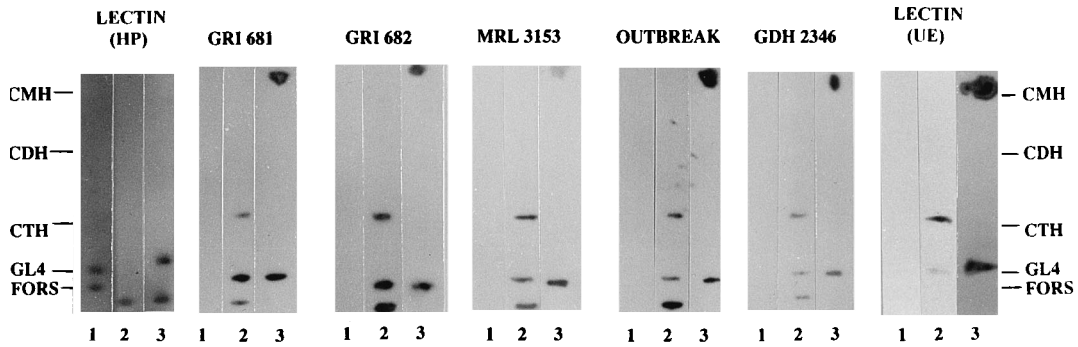

FIG. 3. Binding of different *C. albicans* strains to glycolipids separated on thin-layer chromatograms. Glycolipids were obtained and chromatographed as described in Materials and Methods. Chromatograms were overlaid with biotinylated *C. albicans* GRI 681, GRI 682, MRL 3153, Outbreak, or GDH 2346 or with the biotinylated lectins from *U. europaeus* (lectin I; UE) or *H. pomatia* (HP). Plates were then treated with ^{125}I-streptavidin, and binding was detected by autoradiography. Lanes: 1, 2 μg each of CMH, CDH, CTH, GL4, and FORS; 2, lipid extract of buccal epithelial cells (80 μg); 3, lipid extract of sheep erythrocytes (500 μg).

DISCUSSION

L-Fucose-containing glycoconjugates were first proposed as epithelial receptors for *C. albicans* on the basis of adhesion inhibition studies using sugars and lectins as blocking agents (7, 27). More recently, Brassart et al. (2) employed complex carbohydrates derived from human milk to demonstrate that the minimum structure required to inhibit yeast cell attachment to buccal cells was the Fucα1→Galβ determinant of the H blood group antigen. We have previously described a scheme for purifying the fucoside-binding adhesin of *C. albicans* in which the protein and carbohydrate portions of the molecule are separated enzymically and chemically and fucoside-binding protein fragments are recovered by affinity adsorption (29). In the present investigation, adhesin purified in this way was biotinylated and then subjected to SDS-PAGE and Western blot analysis. This analysis revealed a 15.7-kDa component, which presumably includes the receptor-binding domain of a mannoprotein whose intact molecular mass is substantially greater. Amino acid analysis of the purified adhesin indicated that it lacked cysteine and contained very little methionine.

Overall, the amino acid composition of the purified adhesin is quite similar to that of some other microbial adhesins (16) and lectins (14) which also typically have few, if any, residues of sulfur-containing amino acids. However, it is not identical with that of the fimbrial adhesin recently isolated from a different strain of *C. albicans* by Yu et al. (33). The purified fimbrial subunit had a molecular mass of 66 kDa, with a protein portion of around 8.6 kDa. The most abundant amino acids were Asx, Glx, Gly, and Leu, but there was relatively little Pro. By contrast, the fucoside-binding adhesin has a high proline content (Table 2). A comparison of sequence data is required to determine whether the two adhesins are in any way related. The fucoside-binding adhesin is isolated from mannoprotein in culture supernatants which seems to originate mainly from the fibrillar layer on the yeast cell surface (23). This adhesin is therefore likely to be fibrillar (or fimbrial) in nature.

In studies on the role of glycosides as epithelial receptors for microorganisms, most investigators have preferred to use glycosphingolipids rather than glycoproteins for binding analysis (18). Glycosphingolipids carry only one oligosaccharide chain and are easily extracted from tissues. Glycoproteins, on the other hand, may express two or more different glycoside moieties, which makes identification of receptors more difficult. Here, we used chromatogram overlay assays with glycolipids extracted from human buccal epithelial cells and sheep erythrocytes, together with neutral glycolipid standards. Our results indicated that purified adhesin, crude adhesin (EP), and intact yeast cells of *C. albicans* GDH 2346 all bound to glycolipids carrying the H blood group antigen. Four other strains of *C. albicans*, thought from previous work to have a similar requirement for fucose-containing receptors, also recognized these glycolipids. By contrast, a sixth strain (GDH 2023) revealed a completely different binding pattern and bound only to glycolipids containing *N*-acetylglucosamine. All of these results confirm earlier predictions about the receptor specificity of the strains made on the basis of adhesion inhibition studies (7).

Recent reports indicate that glycosphingolipids other than those carrying blood group determinants may also function as epithelial cell receptors for *C. albicans*. Using a chromatogram overlay assay with ^{125}I-labelled organisms, Jimenez-Lucho et al. (17) obtained evidence for lactosylceramide as a possible receptor for *C. albicans*, *Saccharomyces cerevisiae*, *Cryptococcus neoformans*, and other fungal species. We have been unable to confirm this finding with the strains of *C. albicans* used here. Similarly, Yu et al. (34) failed to detect any affinity of *C. albicans* fimbriae for lactosylceramide. On the other hand, the latter group reported that isolated fimbriae bound to asialo-GM_1 and asialo-GM_2 gangliosides separated on thin-layer chromatograms (34). The fimbriae also bound in a concentration-dependent manner to synthetic βGalNAc(1-4)βGal-protein conjugates which had been immobilized on microtiter

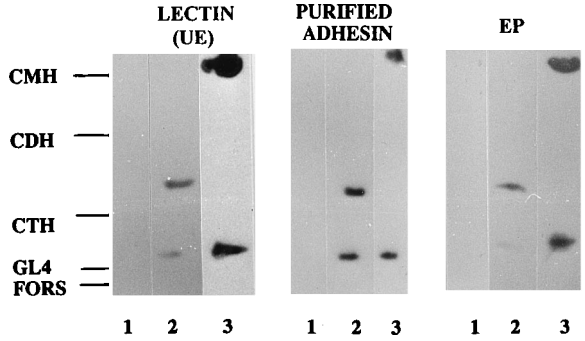

FIG. 4. Binding of EP and purified adhesin from *C. albicans* GDH 2346 to glycolipids separated on thin-layer chromatograms. Glycolipids were obtained and chromatographed as described in Materials and Methods. Chromatograms were overlaid with biotinylated EP or purified adhesin or with biotinylated *U. europaeus* lectin I (UE). Plates were then treated with ^{125}I-streptavidin, and binding was detected by autoradiography. Lanes: 1, 2 μg each of CMH, CDH, CTH, GL4, and FORS; 2, lipid extract of buccal epithelial cells (80 μg); 3, lipid extract of sheep erythrocytes (500 μg).

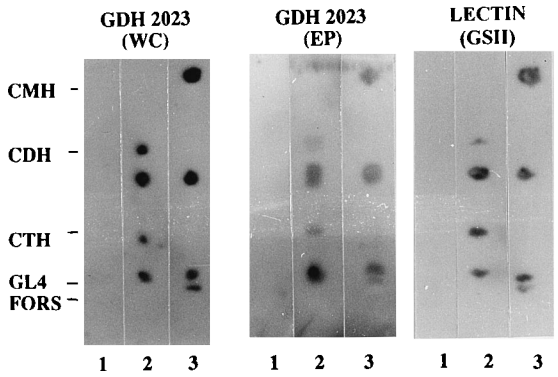

FIG. 5. Binding of whole cells and EP of *C. albicans* GDH 2023 to glycolipids separated on thin-layer chromatograms. Glycolipids were obtained and chromatographed as described in Materials and Methods. Chromatograms were overlaid with biotinylated whole cells (WC) or EP of *C. albicans* GDH 2023 or with biotinylated *G. simplicifolia* lectin II (GS II). Plates were then treated with ^{125}I-streptavidin, and binding was detected by autoradiography. Lanes: 1, 2 μg each of CMH, CDH, CTH, GL4, and FORS; 2, lipid extract of buccal epithelial cells (80 μg); 3, lipid extract of sheep erythrocytes (500 μg).

plates, suggesting that this disaccharide represents the minimal structural requirement for the fimbrial receptor. Neither of these studies examined yeast cell binding to lipids containing L-fucose.

An *N*-acetylgalactosamine-containing lipid extracted from buccal epithelial cells acted as a receptor for five strains of *C. albicans* in the present investigation. The yeast component responsible for binding was clearly different from the fucoside-binding adhesin, which showed no affinity for this glycolipid. The glycolipid has not yet been fully characterized. Its reactivity with *H. pomatia* lectin (specific for GalNAc end groups) suggests a possible identity with asialoganglioside-GM_2, which contains a trisaccharide that terminates in *N*-acetylgalactosamine. However, its low mobility on thin-layer chromatograms (lower than that of either GL4 or FORS with tetrasaccharide and pentasaccharide moieties, respectively; Fig. 3) seems to discount this possibility. A more likely candidate would be a lipid carrying the blood group A determinant, which is a hexasaccharide terminating in *N*-acetylgalactosamine. This antigenic determinant is not recognized by *U. europaeus* I lectin, which is highly specific for the H blood group antigen (14). Both H and A antigens are present on buccal cell glycolipids of blood group A individuals, one of whom donated the buccal cells used for lipid extraction in these experiments. Blood group A antigen tends to be present on a greater number of superficial cells than does blood group H antigen, and this phenomenon is believed to be related to cell differentiation (31).

The existence of several adhesin-receptor systems undoubtedly contributes to the versatility of *C. albicans* as an opportunistic pathogen. Previously, preliminary evidence has been produced in support of the Lewis^a blood group antigen as a possible receptor (21, 28, 29). In general, blood group antigens seem quite commonly to function as epithelial receptors for microorganisms (25); *Helicobacter pylori*, for example, attaches to the Lewis^b antigen and other fucosylated blood group determinants (13). Surface carbohydrates on buccal epithelial cells may be altered during critical illness. Weinmeister and Dal Nogare (32) recently compared the surface carbohydrate content of buccal cells in normal subjects and critically ill patients. Buccal cells of the patients had unchanged levels of fucose but decreased amounts of sialic acid and galactose compared with those of normal subjects. The investigators proposed that such changes in surface carbohydrate composition on buccal cells might explain the high prevalence of colonization with gram-negative bacteria among these patients. It could also partly account for the well-known predisposition of the critically ill to colonization by *C. albicans*.

ACKNOWLEDGMENTS

We thank C. Brotherston (University of Glasgow) for a generous gift of sheep erythrocyte lipids.

B.J.C. was the recipient of a research studentship from the Medical Research Council.

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Editor: D. H. Howard