Hemoglobin Induces Binding of Several Extracellular Matrix Proteins to *Candida albicans*

**IDENTIFICATION OF A COMMON RECEPTOR FOR FIBRONECTIN, FIBRINOGEN, AND LAMININ***

(Received for publication, July 10, 1997, and in revised form, November 17, 1997)

Sizhuang Yan‡, Rui G. Rodrigues‡, Diego Cahn-Hidalgo‡§, Thomas J. Walsh¶, and David D. Roberts¶

From the §Laboratory of Pathology and ¶Pediatric Branch, NCI, National Institutes of Health, Bethesda, Maryland 20892

Host infection by the pathogenic fungus *Candida albicans* is initiated by adhesion and mediated by binding to several host extracellular matrix proteins. Previously, we demonstrated that hemoglobin supplemented into a chemically defined medium significantly and specifically induced fibronectin binding to *C. albicans*. We now report that hemoglobin also induces binding of laminin, fibrinogen, and type IV collagen but not of thrombospondin-1 or type I collagen. The binding of each protein was inhibited by the respective unlabeled ligand in a concentration-dependent manner. Fibronectin inhibited the binding of radiolabeled fibronectin, laminin, and fibrinogen with similar IC50 values, suggesting that a single promiscuous receptor recognizes these three proteins. Competitive binding studies indicated that a second class of receptor binds specifically to laminin. Growth of *C. albicans* in the presence of hemoglobin also increased cell adhesion to immobilized fibronectin, laminin, fibrinogen, and type IV collagen but not to thrombospondin-1 or type I collagen. Exposure to hemoglobin induced increased or de novo expression of several surface proteins on *C. albicans*. One of these proteins with a molecular weight of 55,000 recognized fibronectin, based on ligand protection and affinity chromatography on immobilized fibronectin. Thus, hemoglobin induces both promiscuous and specific receptors for extracellular matrix proteins and, therefore, may regulate matrix adhesion during dissemination of *C. albicans* infections.

*Candida albicans* is an important opportunistic pathogen for humans, causing both superficial and disseminated infections (reviewed in Refs. 1 and 2) with significant morbidity and mortality among immunocompromised patients (3). Adhesion of the organism to mucosal epithelium is a prerequisite for colonization and, therefore, is regarded as an initial step in the process leading to infections (3, 4). Additional adhesion events to endothelium and extracellular matrix (ECM) components are required for dissemination of *C. albicans*. A number of ECM proteins bind to *C. albicans*, including fibronectin (5–8), laminin (9), vitronectin (10, 11), complement (12), fibrinogen (13), gelatin (7), and types I and IV collagen (14).

Interaction with individual ECM proteins is mediated by binding to respective receptors on the surface of *Candida* cells (5–7, 9–11). Some of these receptors are probably homologs of mammalian integrins (15). However, fibronectin binds to *C. albicans* both through the cell binding domain recognized by mammalian integrins (8) and through the collagen binding domain (6), and laminin and fibrinogen bind to distinct classes of receptors on *C. albicans* that are probably not integrins (9, 13, 16). Thus, both integrin and non-integrin receptors may mediate adhesion of *C. albicans*.

Previously, we reported that hemoglobin induces a marked enhancement of fibronectin binding activity in *C. albicans* (17). This induction is reversible, requires cell growth in the presence of hemoglobin, and is not due to a bridge effect of hemoglobin between a receptor on the organism and fibronectin. In addition, adhesion of *C. albicans* to corneal endothelial cells was significantly increased when grown in hemoglobin-containing defined medium compared with those grown in defined medium alone. Although the ability to acquire iron has long been considered one of the most important adaptive responses for microbial pathogenesis (18, 19), the enhancement of fibronectin binding by hemoglobin was not simply due to iron acquisition from the hemoglobin, because other ferroproteins, ferrous ions, or iron-containing porphyrins were inactive (17). Thus, hemoglobin itself may act as a potent regulator for the fibronectin receptor in *C. albicans*, although its precise mechanism remains unclear.

We have now examined whether this specific induction by hemoglobin influences binding of *C. albicans* to other ECM proteins. We demonstrate that binding of *C. albicans* to laminin, fibrinogen, and type IV collagen, are increased to various degrees, but binding to thrombospondin-1 and type I collagen are not. In addition to inducing specific receptors for some ECM ligands on *C. albicans*, growth in the presence of hemoglobin induces a promiscuous receptor for several ECM proteins including fibronectin (FN), fibrinogen, and laminin. Adhesion to these proteins is also coordinately increased, and expression of specific cell surface proteins that bind FN is increased. Among these, a 55-kDa protein was identified as a hemoglobin-induced fibronectin receptor. Based on these findings, hemoglobin seems to be a potent regulator of the adhesive phenotype of *C. albicans*.

**EXPERIMENTAL PROCEDURES**

Strains and Growth Conditions—ATCC strain 44807 of *C. albicans* was purchased from the American Type Culture Collection and used throughout this study. The organism was initially grown in Sabouraud medium (6) to early stationary phase at 26 °C, and b sta 004 were removed. The paper is available online at http://www.jbc.org

---

* The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ Present address: Dept. of Medicine, University of Rochester Medical School, Rochester, NY 14624.

§ To whom correspondence and reprint requests should be addressed: Bldg. 10, Rm 2A33, 10 Center Dr. MSC 1500, NIH, Bethesda, MD 20892-1500. Tel.: 301-496-6264; Fax: 301-402-0043.

† The abbreviations used are: ECM, extracellular matrix; BSA, bovine serum albumin; FN, fibronectin; DPBS, Dulbecco’s phosphate-buffered saline; sulfo-SHPP: sulfosuccinimidyl-3-(4-hydroxyphenyl) propionate; YNB, yeast nitrogen base.
Hemoglobin-enhanced Binding of Matrix Proteins to C. albicans

5639

basis, a fresh culture was made as a stock for daily use. For each experiment, the organism was inoculated into the 4 × yeast nitrogen base (YNB) broth with or without hemoglobin at a final concentration of 0.1% and incubated at 26 °C for 48 h (17). YNB is a chemically defined medium, and under this growth condition no germination was found upon microscopic examination of cultures in the presence or absence of hemoglobin.

Extracellular Matrix Proteins—FN was purified from frozen human plasma as described previously (6). Thrombospondin-1 was isolated from human platelets (20). Mouse laminin and gelatin were purchased from Life Technologies, Inc., bovine fibrinogen was from Calbiochem, and collagen type I and IV were from Collaborative Research Incorporated (Bedford, MA). Iodination of individual ECM proteins was accomplished using Iodogen (Pierce), and unbound iodine was removed by gel filtration through a PD-10 column (6).

Cell Binding Assay—In a typical binding assay, 2 × 10⁶ C. albicans were exposed to each [³⁵S]matrix protein at a final concentration of 0.5 μg/ml in a total volume of 200 μl of Dulbecco’s phosphate-buffered saline (PBS) without CaCl₂ and MgCl₂, 0.1% BSA, pH 6.0, in 12 × 75-mm polystyrene tubes (PGC, Gaithersburg, MD) and incubated for 3 h at room temperature with shaking at 180 rpm. The cell suspensions were then transferred to microfuge tubes, and the blastoconidia were separated from unbound radiolabeled ligand by centrifugation through 100 μl of an oil mixture of dibutyl phthalate/diocyl phthalate (2:1). Radiolabeled ECM protein bound to the cell pellet was quantified in a gamma counter (Packard Instrument Company, Downers Grove, IL).

For inhibition assays, the binding of radiolabeled protein was determined in the presence of various concentrations of unlabeled homologous or heterologous ECM ligands.

Adhesion to Immobilized Matrix Proteins—Extracellular matrix proteins were coated onto glass chamber slides (Nalge Nunc International, Naperville, IL) by adding 300 μlwell of FN solution at 1 or 10 μg/ml into each well and incubating at 4 °C overnight. Adhesion was measured as described previously (17).

Biotinylation and Extraction of Surface Proteins of C. albicans—Biotinylation of Candida surface proteins was achieved using sulfo-2-mercaptoethanesulfonic acid (DMDES, 2-mercaptoethanol) hexanoyl (Pierce). Briefly, 20 ml of Candida cultures grown in 4 × YNB with or without hemoglobin at 26 °C for 48 h were harvested by centrifugation, and the cells were washed twice with DPBS, pH 7.4, without Ca²⁺ and Mg²⁺ (Life Technologies, Inc.) to remove trapped or precipitated hemoglobin. The pellets were resuspended in 1 ml of 50 mM sodium bicarbonate buffer, pH 8.5, in microfuge tubes, and 74 μl of 1 mg/ml sulfo-2-mercaptoethanesulfonic acid-6-(biotinamido) hexanoyl were added. The cell suspension was incubated at room temperature with shaking for 1 h, and excess biotinylation reagent was removed by washing the cells with DPBS. After biotinylation, cell surface proteins were extracted. Candida cells were suspended either in lysis buffer (Sigma) at a final concentration of 2 mg/ml, 8 mM dithiothreitol, or a combination of both and incubated at 37 °C for 1 h with vigorous shaking. Cell pellets were removed by centrifugation, and the cell surface proteins in the supernatant fluids were either separated through 10% SDS-polyacrylamide gel electrophoresis containing 8 M urea or stored at −70 °C for future use. After electrophoresis, proteins were transferred onto a nitrocellulose membrane, blocked with 3% BSA in 50 mM Tris, pH 7.5, 150 mM NaCl, and incubated with streptavidin-horseradish peroxidase (Amersham Life Science, Inc.). Biotinylated cell surface proteins were visualized using an ECL chemiluminescent detection kit (Amersham). To identify surface proteins of hemoglobin-induced C. albicans that bound FN, the above procedure was modified. Briefly, 20 ml of Candida cultures grown in 4 × YNB with hemoglobin at 26 °C for 48 h were harvested by centrifugation, and the cells were washed twice with Dulbecco’s PBS. The cultures were divided into three parts. The cells were incubated with 1 ml of FN (100 μg/ml) in DPBS at room temperature for 2 h with shaking, and the remaining fraction was incubated in DPBS without FN. The cells were centrifuged to remove excess unbound FN, and the pellets were suspended in 1 ml of sulfo-SHPP solution (0.5 mg/ml sulfo-2-mercaptoethanesulfonic acid-3-(4-hydroxyphenyl) propionate (Pierce) in 50 mM sodium bicarbonate buffer, pH 7.8, and incubated at room temperature for 2 h with shaking. The pellets were then washed with Dulbecco’s PBS 4 times, 15 min each, to remove bound FN. Finally, each protein portion of cell surface proteins were subjected to biotinylation, and each cell surface proteins were extracted, separated, and visualized as described above.

Purification of a FN-Binding Protein Using Affinity Chromatography—For preparation of the affinity gel, FN was conjugated to Reacti-Gel (Pierce) at a concentration of 1 mg/ml at 4 °C overnight, and excess FN was removed by washing. Unbound reactive groups were blocked by incubating with 100 mg Tris buffer, pH 7.5, at room temperature for 1 h. Extracted Candida biotinylated surface proteins were incubated with a suspension of the affinity gel at room temperature for 4 h and transferred to a glass column. The column was washed with 10 column volumes of DPBS, pH 7.4, containing 300 mM NaCl. Bound proteins were eluted stepwise with DPBS buffer, pH 7.4, containing 650 mM NaCl followed by 0.2 M imidazole acetate, pH 4.0. Eluted fractions were dialyzed, freeze-dried, and analyzed by SDS-polyacrylamide gel electrophoresis. FN-binding cell surface proteins were visualized by ECL detection.

RESULTS

Induction of ECM Protein Binding by Hemoglobin Is Not Limited to Fibronectin—After growth in YNB supplemented with 0.1% hemoglobin for 48 h, binding of Candida cells to several radiolabeled ECM proteins was examined. Among the ECM proteins tested, binding of fibronectin, laminin, fibrinogen, and type IV collagen increased more than 10-fold relative to their binding to cells grown in the absence of hemoglobin (Fig. 1). In contrast, hemoglobin failed to enhance the binding of type I collagen or thrombospondin-1 to C. albicans. Binding of FN, laminin, fibrinogen, and type IV collagen were consistently and markedly enhanced in all experiments. Some enhancement of gelatin binding was observed but varied from 2 to 16-fold in three separate experiments (results not shown).

Hemoglobin Enhances Adhesion of Candida albicans to Several Immobilized Ligands—Although increased binding of soluble matrix proteins could have biological significance, the ability of immobilized ECM proteins to promote C. albicans adhesion to a surface is the parameter that is required for dissemination of the pathogen. We previously reported that hemoglobin induced an increased adhesion of C. albicans to immobilized FN coated on glass Chamber slides (17). Using the same technique, adhesion of C. albicans grown in hemoglobin-containing medium was examined on slides coated with various ECM proteins (Fig. 2). Compared with the cells grown in the absence of hemoglobin, hemoglobin-induced C. albicans demonstrated a significantly greater adhesion (p < 0.05 by a two-tailed t test) to immobilized FN, laminin, fibrinogen, or type IV collagen at a concentration of 10 μg/ml, respectively. Although adhesion to type I collagen increased slightly for Candida cells prepared from cultures grown in the presence of hemoglobin, the difference was not statistically significant (p > 0.1). Growth in medium containing hemoglobin did not alter
adhesion of *C. albicans* to immobilized thrombospondin-1 or gelatin (*p* > 0.2).

Hemoglobin Induces a Shared Receptor for Fibronectin, Laminin, and Fibrinogen—Binding of fibronectin to *C. albicans* induced by hemoglobin was previously shown to be saturable and described by a single class of binding sites (17). Similarly, induced binding of fibrinogen and laminin were also saturable (Fig. 3), but cross-competition experiments suggested that these three proteins partially share a common binding site. Fibrinogen was equally active as an inhibitor of iodinated laminin, fibrinogen, or FN binding, and the binding of all three labeled proteins was completely inhibited by excess fibrinogen (Fig. 3A). This result demonstrates that fibrinogen binds to a site recognized by all three proteins. In contrast, laminin was a better inhibitor of iodinated laminin binding than of fibronectin binding, suggesting that more than one class of receptor(s) interacts with these two proteins (Fig. 3B).

Additional heterologous and homologous displacement assays were analyzed using the LIGAND program (21, 22) to assess the extent of cross-competition among these three ligands (Table I). The apparent association constants for fibrinogen binding were identical when measured by displacement of either labeled fibronectin or fibrinogen, indicating that both bind to a common receptor. Although laminin displayed some cross-competition with both fibrinogen and fibrinogen, the apparent association constant for laminin measured by heterologous displacement of labeled fibrinogen differed significantly from the association constant determined by homologous displacement of iodinated laminin by unlabeled laminin. Conversely, the binding constant for fibrinogen measured by displacement of labeled laminin differed from that measured by displacement of either fibrinogen or fibronectin. Furthermore, in most cases the heterologous displacement curves for laminin could only be fit by assuming that part of laminin binding was to a class of sites not recognized by fibrinogen or fibronectin.

Approximately 25% of iodinated laminin binding was to a site not displaceable by fibronectin, and laminin displaced less than 50% of labeled fibronectin (Fig. 3B). Thus, the population of sites recognized by laminin overlaps with those binding fibronectin/fibrinogen, but distinct laminin-specific binding sites are also present.

Cross-competition was limited to those ECM proteins that showed enhanced binding and adhesion following growth in hemoglobin, as type I collagen did not inhibit labeled fibrinogen binding (Fig. 4). Cross-competition by thrombospondin-1, the other protein without enhanced binding, could not be examined because thrombospondin-1 binds to fibrinogen (23). Thrombospondin-1 and type I collagen also could not be tested for cross-competition with labeled fibronectin due to direct binding between these proteins (24). As reported previously for fibronectin (17), BSA did not inhibit fibrinogen binding to *C. albicans* (Fig. 4).

Expression of Novel Surface Proteins Induced by Hemoglobin—Since hemoglobin dramatically induced binding of several ECM proteins involving both distinct receptors and a class of promiscuous receptor, we expected that hemoglobin may induce increased or *de novo* expression of proteins on the surface of *Candida* cells. To detect these changes in cell surface protein expression, *Candida* cells grown in medium with or without hemoglobin supplement were labeled with biotin. Labeled surface components were extracted with either dithiothreitol alone or together with lyticase. Equal amounts of protein for each extract were loaded onto SDS gels and detected using peroxidase-streptavidin (Fig. 5). Biotinylated cell surface proteins ranging from 14 to >100 kDa were observed in hemoglobin-induced and noninduced *C. albicans*. The proteins detected by streptavidin binding should represent exclusively cell wall or cell membrane proteins, since the sulfonated biotin derivative used is not membrane-permeable. This was verified by comparing electrophoretic profiles of surface-labeled cells to those of...
Radioactive fibrinogen (●) from unbound radioactive ligand by centrifugation through 100,000 × g. The cell suspensions were centrifuged, and the blastoconidia were separated by microfuge tubes, and the blastoconidia were separated from unbound radioactive ligand by centrifugation through 100,000 × g. The cell suspensions were centrifuged, and the blastoconidia were separated from unbound radioactive ligand by centrifugation through 100,000 × g. The cell suspensions were centrifuged. The cell suspensions were centrifuged. Unlabeled fibrinogen (●), fibronectin (■), and laminin (■) bound to the cell pellet was quantified in a gamma counter, and binding is presented as the percent of that measured in the absence of inhibitors, mean ± S.D., n = 3.

**TABLE I**

| Unlabeled ligand | $K_r$ for unlabeled ligand assessed using the $^{125}$I-labeled ligand (nM) | Fibrinogen | Fibronectin | Laminin |
|------------------|---------------------------------------------------------------|-------------|-------------|---------|
| Fibronectin       | $2.17 \pm 3.3 \times 10^7$ | $3.4 \pm 1.2 \times 10^6$ | $(5.9 \pm 2.6 \times 10^7)^a$ |
| Fibrinogen        | $2.3 \pm 0.2 \times 10^7$ | $2.0 \pm 0.6 \times 10^6$ | $(2.4 \pm 1.7 \times 10^7)^a$ |
| Laminin           | $(2.0 \pm 10^7)^a$ | $(4.3 \pm 0.7 \times 10^6)^a$ | $2.1 \pm 0.4 \times 10^7$ |

$a$ Fitted parameters required the assumption that a significant fraction (>25%) of the labeled ligand bound to sites not displaceable by unlabeled ligand.

The quantity or exposure of several surface proteins from the cells grown in hemoglobin medium increased (Fig. 5, lanes b and d) as compared with those from noninduced cells (Fig. 5, lanes a and c). Increased labeling of proteins with apparent molecular weights of 30,000, 65,000–70,000, and 100,000 were consistently seen in extracts prepared by extraction using dithiothreitol (Fig. 5, lane d). Subsequent lyticase treatment released increased levels of proteins with apparent molecular weights of 55,000 and 100,000–150,000 in hemoglobin-induced cells (Fig. 5, lane b).

Identification of a FN-binding Protein from the Cell Surface—Several approaches were used to identify which of these induced surface proteins bind to the ECM proteins. Ligand blots of lyticase extracts with $^{125}$I-fibronectin or fibrinogen identified a 70-kDa protein, but this protein was identified as...
hemoglobin-enhanced Binding of Matrix Proteins to C. albicans

Inhibition of a fibronectin binding activity by biotinylation. Fibronectin binding activity was determined from cells with (modified, striped bar) or without (control, solid bar) biotinylation. FN binding activity fromthese respective cells was assayed by incubating 2 × 10⁵ Candida cells with 125I-FN in DPBS in a total volume of 200 μl for 3 h at room temperature. The bound radioactivity was quantified as described under “Experimental Procedures” and are presented as mean ± S.D., n = 3.

an impurity in the lyticase.² In preliminary experiments, we observed that modification of exposed amino groups on C. albicans inhibited binding of 125I-fibronectin (Fig. 6). This implied that amino groups on a surface protein are required for fibronectin binding to its receptor. We therefore used ligand protection from chemical modification to identify a fibronectin-binding protein. C. albicans cells were incubated with 0, 10, or 100 μg/ml FN for 2 h to allow FN to bind to its receptors on the cell surface. Excess FN was removed, and exposed amino groups on surface proteins were modified using sulfo-SHPP in the presence of the bound FN. After removal of unreacted sulfo-SHPP and dissociation of the bound FN by thorough washing, the cells were biotinylated. By this approach, biotinylation was limited to those amino groups that were protected by the bound FN. Surface proteins were released by dithiothreitol/lyticase digestion, and biotin-labeled proteins were identified by blotting with streptavidin-peroxidase after separation by SDS gel electrophoresis (Fig. 7). Protection by 10 or 100 μg/ml FN resulted in prominent labeling of a 55-kDa protein (Fig. 7, lanes b and c) that was absent in the cells without FN protection (Fig. 7, lane a). At the higher FN concentration, additional bands were revealed at molecular masses of 45 and 66 kDa (Fig. 7, lane c). In contrast to specific protection of the 55-kDa protein, biotin labeling of the 30- and 70-kDa hemoglobin-induced proteins identified in lane d of Fig. 5 were not altered by preincubation with FN (Fig. 7, lanes a–c).

Affinity chromatography with immobilized FN was used to purify FN-binding proteins from biotin-labeled cell surface extracts (Fig. 8). After eluting unbound proteins with DPBS (Fig. 8, lane c), sequential elution with DPBS containing 300 mM and 650 mM NaCl eliminated most of the contaminating proteins (data not shown). Final elution with 0.2 M sodium acetate, pH 4.0, yielded a major prominent band with an apparent molecular mass of 55 kDa (indicated by the line on Fig. 8, lane d) that had identical mobility to the 55-kDa protein identified by the ligand protection method (Fig. 7, lane b, and Fig. 8, lane a) and was absent in the unbound fractions from the FN column (Fig. 8, lane c). This protein was further purified by preparative SDS gel electrophoresis using a Mini Prep Cell (Bio-Rad). The resultant protein was homogeneous by silver staining (Fig. 8, lane c) and was derived from cell surface proteins based on streptavidin labeling (Fig. 8, lane f). Microsequencing of the purified protein revealed that the amino terminus was blocked.²

DISCUSSION

Both solution phase cell binding assays and adhesion to immobilized extracellular matrix proteins demonstrate that growth of C. albicans in a defined medium containing hemoglobin coordinately up-regulates interactions with laminin, fibronectin, fibrinogen, and type IV collagen. This up-regulation is specific in that interactions with at least two other extracellular matrix proteins, type I collagen and thrombospondin-1 are unaffected. Both binding to the proteins in solution and adhesion to the immobilized proteins are increased by growth in the presence of hemoglobin. A larger increase was observed in binding to the soluble proteins than in adhesion to immobilized proteins. This was noted previously using fibronectin (17) and probably reflects a greater sensibility of soluble ligand binding assays to changes in receptor number compared with the multivalent avidity measured in adhesion assays. Growth in the

² S. Yan, H. Krutzsch, and D. D. Roberts, unpublished results.
presence of hemoglobin induced increased expression of several proteins on the surface of \textit{C. albicans}. FN binding to \textit{C. albicans} protected amino groups on a 55-kDa protein from chemical modification, identifying this surface protein as a candidate for the hemoglobin-induced ECM receptor. A 55-kDa protein was also identified as a potential FN receptor by purification using affinity chromatography on immobilized FN.

To address whether a single promiscuous receptor or a family of receptors with varying specificities are responsible for the enhancement of ECM protein binding by hemoglobin, we performed quantitative analysis of heterologous displacement experiments. By comparing the \( K_i \) values for a single protein to inhibit binding of the three labeled proteins, fibronectin, fibrinogen, and laminin, and fibrinogen, we demonstrated that fibronectin and fibrinogen bind to a common class of sites that overlap only partially with those sites recognizing laminin. Therefore, growth in the presence of hemoglobin induces expression on \textit{C. albicans} of a class of promiscuous receptors that bind fibronectin, fibrinogen, laminin, and type IV collagen and specific receptors for some ECM proteins, such as laminin. Binding of both fibronectin and fibrinogen to the promiscuous site produces a linear Scatchard plot, indicating that a homogeneous class of receptors accounts for binding of each of these ligands. Because some of these ECM proteins bind to each other, we could not examine heterologous displacement between all pairs of proteins. Using fibrinogen as a tracer for binding to the promiscuous receptor, however, we can show that native type I collagen does not bind to this receptor. Although \textit{C. albicans} apparently has receptors that mediate adhesion to type I collagen and thrombospondin-1, these receptors are probably distinct from the promiscuous receptor because their binding and ability to promote adhesion are not affected by growth with hemoglobin.

Studies in other organisms provide precedent for both specific and shared promiscuous receptors for ECM proteins. Promiscuous integrins have been identified in mammalian cells. The best defined of these are the platelet integrin \( \alpha IIb/\beta 3 \), which binds to fibrinogen, thrombospondin, fibronectin, and von Willebrand factor (25), and the leukocyte \( \beta 2 \) integrin \( \alpha \mathrm{CR}3 \) (CD11b/CD18, reviewed in Ref. 26). Mammalian cells also express several families of scavenger receptors that bind multiple ligands (27). Among these, the low density lipoprotein receptor-related protein and CD36 have been demonstrated to bind to several ECM proteins (28–30). Microbial interactions with multiple ECM proteins are also frequently observed. Staphylococcus aureus interacts with fibronectin, laminin, collagen, thrombospondin-1, and elastin (31) reviewed in (32, 33). Some of these interactions are mediated by distinct receptors, but a \textit{S. aureus} protein that binds several ECM proteins has also been reported (34). Blood stages of the protozoan pathogen responsible for malaria, \textit{Plasmodium falciparum}, recognize the host proteins thrombospondin-1, CD36, VCAM1, E-selectin, and ICAM1 via a family of related cell surface receptors (35). The promiscuous receptor on \textit{C. albicans} resembles a mammalian scavenger receptor (27) in that the proteins bound are apparently unrelated in sequence, but only a subset of proteins are recognized by the receptor. Previous studies of extracellular matrix interactions with \textit{C. albicans} have identified candidate receptors for fibronectin (5, 8), laminin (16), fibrinogen (36), and entactin (37) and an analog of mammalian integrin \( \beta 2 \) subunits that may mediate adhesion to epithelial cells (reviewed in Ref. 15). Limited evidence has been obtained for partial competition between laminin, fibronectin, and entactin for binding to cell wall extracts of \textit{C. albicans} (37). However, no competition was observed between fibrinogen and the complement receptor (36). Heparin inhibited binding of \textit{C. albicans} to several ECM proteins, including fibronectin, laminin, and types I and IV collagen. This inhibition does not reflect binding of the glycosaminoglycans to a \textit{C. albicans} binding site shared with these proteins but probably resulted from sequestration of ligands after binding of heparin to the proteins (38).

Up-regulated expression of potential receptors for selected ECM proteins was observed at the functional level by increased binding activity and adhesion to the immobilized proteins as well as at the protein level as increased expression of several surface proteins on \textit{C. albicans}, including a 55-kDa protein. Binding of FN to the cells differentially protected the protein from chemical modification (Fig. 7), suggesting that it may directly mediate hemoglobin-induced interactions with fibronectin. The same 55-kDa protein was also identified as a major protein bound to a FN affinity column and eluted with acetic acid. Proteins of 68–72 kDa were identified previously as putative receptors for laminin, fibrinogen, and C3d (9) and are reviewed in (3). Proteins with molecular masses of 60 and 105 kDa were identified as potential FN receptors in uninduced \textit{C. albicans} by affinity chromatography on immobilized FN (5, 14). Because lyticase was used to release the surface proteins, protease contamination in the lyticase could result in some degradation of the receptor. Thus, the native molecular mass of the receptor identified here may be larger than 55 kDa.

The finding that hemoglobin is a potent regulator of the binding of \textit{C. albicans} to several ECM proteins may contribute to understanding the pathogenesis of systemic candidiasis. Hemoglobin is an abundant circulating protein in the body, and it is often present in sites of tissue injury. \textit{C. albicans} may readily encounter hemoglobin at sites of tissue injury, which may in turn change its binding to ECM proteins in the tissue. Furthermore, virulent strains of \textit{C. albicans} express a hemolytic activity that could release hemoglobin from erythrocytes exposed to \textit{C. albicans} (39). Recently, hyphal cells of \textit{C. albicans} were reported to bind to human hemoglobin, suggesting that this organism expresses hemoglobin receptors (40). Induction of ECM binding to \textit{C. albicans} by hemoglobin would facilitate colonization of this organism after it enters the vascular compartment. Therefore, defining of the mechanism by which hemoglobin regulates adherence in this pathogen could identify new targets to prevent or treat \textit{C. albicans} infections.

REFERENCES

1. Odds, F. C. (1994) \textit{Am. Acad. Dermatol.} 31, S2–S5
2. Odds, F. C. (1994) \textit{ASM News} 60, 313–318
3. Calderone, R., and Braun, P. (1991) \textit{Microbiol. Rev.} 55, 1–20
4. Segal, E. (1987) \textit{Microbiol. Rev.} 4, 344–347
5. Klotz, S. A., Hein, R. C., Smith, R. L., and Rouse, J. B. (1994) \textit{Infect. Immun.} 62, 4679–4681
6. Negre, E., Vogel, T., Levanon, A., Guy, R., Walsh, T. J., and Roberts, D. D. (1994) \textit{J. Biol. Chem.} 269, 22039–22045
7. Klotz, S. A., and Smith, R. L. (1995) \textit{Microbiology} (Washington D C) 141, 2681–2684
8. Klotz, S. A., and Smith, R. (1991) \textit{J. Infect. Dis.} 163, 604–610
9. Bosshara, J., Tronchin, G., Annaux, V., Robert, R., and Senet, J. (1990) \textit{Infect. Immun.} 58, 48–54
10. Jakab, E., Paulsson, M., Ascencio, F., and Ljunch, A. (1992) \textit{APMIS} 101, 187–193
11. Limper, A. H., and Stading, J. E. (1994) \textit{Immunol. Lett.} 42, 139–144
12. Calderone, R., Diamond, R., Senet, J. M., Warmington, J., Filler, S., and Edwards, J. E. (1994) \textit{J. Med. Vet. Mycol.} 32, Suppl. 1, 151–168
13. Casanova, M., Lopez-Ribot, J. L., Montenegro, C., Llombart-Bosch, A., Sentandreu, R., and Martinez, J. P. (1992) \textit{Infect. Immun.} 60, 4221–4229
14. Klotz, S. A., Rutten, M. J., Smith, R. L., Babcock, S. R., and Cunningham, M. D. (1993) \textit{Microb. Pathog.} 14, 133–147
15. Hostetter, M. K. (1996) \textit{Pediatr. Res.} 33, 569–573
16. Lopez-Ribot, J., Casanova, M., Montenegro, C., Sepulveda, P., and Martinez, J. (1994) \textit{Infect. Immun.} 62, 742–746
17. Yan, S., Negre, E., Cashel, J. A., Guo, N., Lyman, C. A., Walsh, T. J., and Roberts, D. D. (1996) \textit{Infect. Immun.} 64, 2930–2935
18. Eyster, N., Brammer, S., and Crounse, R. (1967) \textit{J. Invest. Dermatol.} 49, 437–442
19. Weinberg, E. (1978) \textit{Microbiol. Rev.} 42, 45–66
20. Roberts, D. D., Cashel, J., and Guo, N. (1994) \textit{J. Tissue Cult. Methods} 16, 217–222
Hemoglobin-enhanced Binding of Matrix Proteins to C. albicans

21. Munson, P. J., and Rodbard, D. (1980) Anal. Biochem. 107, 220–239
22. Thakur, A. K., Munson, P. J., Hunston, D. L., and Rodbard, D. (1980) Anal. Biochem. 103, 240–254
23. Bacon-Baguley, T., Ogilvie, M. L., Gartner, T. K., and Walz, D. A. (1990) J. Biol. Chem. 265, 2317–2323
24. Dardik, R., and Lahav, J. (1989) Eur. J. Biochem. 185, 581–588
25. Yamada, K. (1991) J. Biol. Chem. 266, 12809–12812
26. Thornton, B. P., Vetter, V., Pitman, M., Goldman, R. C., and Ross, G. D. (1996) J. Immunol. 156, 1235–1246
27. Krieger, M., and Herz, J. (1994) Ann. Rev. Biochem. 63, 601–637
28. Mikhailenko, I., Kounnas, M. Z., and Strickland, D. K. (1995) J. Biol. Chem. 270, 9543–9549
29. Asch, A. S., Barlow, J., Silverstein, R. L., and Nachman, R. L. (1987) J. Clin. Invest. 79, 1054–1061
30. Tandon, N. N., Kralisz, U., and Jamieson, G. A. (1989) J. Biol. Chem. 264, 7576–7583
31. Park, P., Roberts, D., Grosso, L., Park, W., Rosenbloom, J., Abrams, W., and Mecham, R. (1991) J. Biol. Chem. 266, 23399–23406
32. Roberts, D. (1990) Am. J. Respir. Cell Mol. Biol. 5, 181–186
33. Patti, J. M., and Hook, M. (1994) Curr. Opin. Cell Biol. 6, 752–758
34. McGavin, M., Krajewska-Pietrasik, D., Ryden, C., and Hook, M. (1993) Infect. Immun. 61, 2479–2485
35. Borst, P., Bitter, W., McCulloch, R., Van Leeuwen, F., and Rudenko, G. (1995) Cell 82, 1–4
36. Lopez-Ribot, J., Martinez, J., and Chaffin, W. (1995) Infect. Immun. 63, 2126–2132
37. Lopez-Ribot, J. L., and Chaffin, W. L. (1994) Infect. Immun. 62, 1454–1457
38. Klotz, S. A., and Smith, R. L. (1992) PNAS Microbiol. Lett. 78, 205–209
39. Manns, J., Mosser, D., and Buckley, H. (1994) Infect. Immun. 62, 5154–5156
40. Watanabe, T., Tanaka, H., Nakao, N. Mikami, T., and Matsumoto, T. (1997) Biochem. Biophys. Res. Commun. 232, 350–353