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The Fibronectin Adhesin of *Candida albicans*

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***Candida albicans* possesses on its cell surface an adhesin which binds the whole viable fungus to subendothelial extracellular matrix and matrix proteins. The adhesin is composed of 75 to 80% carbohydrate and approximately 20 to 25% protein by weight. High-performance liquid chromatography of material eluted from a fibronectin-agarose affinity column demonstrates the presence of three peaks, all of which on sodium dodecyl sulfate-polyacrylamide gel electrophoresis show the presence of one protein of approximately 60 kDa. Molecular weight sizing column chromatography, however, demonstrates that the adhesin elutes with an apparent molecular mass of 42 kDa. The N terminus of the 60-kDa glycoprotein is blocked to Edman degradation. The fibronectin adhesin of *C. albicans* is a glycoprotein that may be present and functional as an aggregate or multimer of a 60-kDa protein.**

Candida albicans is the major cause of disseminated opportunistic fungal infections in North America. The ability of the fungus to adhere to endothelial cells, subendothelial extracellular matrix, and matrix glycoproteins such as laminin and type IV collagen may contribute to the pathogenicity of this microorganism. In vitro the whole microorganism adheres avidly to extracellular matrix and matrix proteins in the presence of calcium. The fungus possesses a detergent-extractable surface glycoprotein receptor or adhesin capable of adhering to immobilized plasma fibronectin (7). The adhesin is composed of two glycoproteins of approximately 60 and 105 kDa. The adhesin is produced by yeast cells and germ tubes alike and is eluted from fibronectin-agarose affinity columns with EDTA (7). This adhesin appears to function much like a lectin or mammalian integrin-like receptor by virtue of its requirement for calcium to function and its preferred ligands.

C. albicans is known to possess on its cell surface analogs, or perhaps homologs, of the integrin family of receptors (3). Integrins are phylogenetically conserved heterodimeric glycoproteins requiring divalent cations for function that bind to many extracellular matrix proteins (10). Interestingly, *C. albicans* adheres to many of the same extracellular matrix proteins as do the integrins on mammalian cells. Furthermore, monoclonal and polyclonal antibodies to several human integrins, such as CR3, the human complement receptor (6), and the human fibronectin and vitronectin (7) receptors, specifically bind to proteins in cell wall extracts of *C. albicans*. These glycoproteins on the cell surface of *C. albicans* have been dubbed integrin analogs (3); however, preliminary data indicate that they do not have extensive homology with known integrin receptors found on human cells (4).

This report provides biochemical information on the *C. albicans* fibronectin adhesin, which superficially appears to function like an integrin. Calcium must be present for the optimal adherence of whole, viable *C. albicans* to an immobilized surface (7). This surface phenomenon implies the presence of a structure on *Candida* cells that is capable of interacting with calcium. Because integrins are suspected to exist in *C. albicans*, we chose to isolate the receptors in a manner similar to that used for the integrins. Therefore, detergent extracts of *C. albicans* were prepared on the premise that a protein was in part critical to this interaction. The detergent extracts of *C. albicans* were then recirculated continuously over a fibronectin or gelatin affinity column in the presence of calcium ions for 3 h and then eluted with EDTA. This report characterizes the glycoprotein obtained in this manner.

C. albicans yeast cells were extracted with octylglucopyranoside (7). The extract at pH 7.4 was recirculated over a fibronectin- or gelatin-agarose affinity column and washed, and the adhesin was eluted with EDTA (7). In some cases, yeast cells were "extracted" with 12 mM dithiothreitol (DTT). The total polysaccharide content of the adhesin purified by high-performance liquid chromatography (HPLC) was determined by the phenol-sulfuric acid method with mannose as the standard (5). The carbohydrate moiety was also demonstrated by labeling of the periodate-oxidized sugar residues with biotin-LC-hydrazide and reaction with strepavidin-alkaline phosphatase after the adhesin had been transferred to nitrocellulose (Oxford GlycoSystems Inc., Rosedale, N.Y.). The total protein content of the adhesins was determined by the bicinchoninic acid method with bovine serum albumin as the standard (12). Reversed-phase HPLC (model 510; Waters, Milford, Mass.) was performed on yeast cell extract fractions eluted from the fibronectin-agarose column. An acetonitrile-water gradient was employed with either a C_4 or C_{18} silica column (Vydac, Hesperia, Calif.). Mobile phase A consisted of 10% acetonitrile. The samples were evaporated under N_2 and stored at 4°C. A Sephadex G-200 superfine (Sigma) column (80 by 12 cm) was equilibrated in 25 mM Tris-HCl, pH 7.4, with gravity flow for sizing column chromatography. Molecular weight standards included apoferritin, bovine serum albumin (which migrated as a dimer), and aprotinin. Proteins were analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) according to the method of Laemmli (8), transferred to nitrocellulose according to the method of Towbin et al. (13), and visualized with colloidal gold.

Two proteins are eluted from fibronectin or gelatin affinity columns with EDTA (7). These proteins can also be eluted from fibronectin affinity columns with α-methylmannopyranoside, a 23-mer hydrophobic fibronectin peptide containing the RGD sequence (PepTite-2000; Telios Corp., San Diego, Calif.) and the N-terminal hydrophobic sequence from the α-1 chain of type I collagen, QLSYGYDEK (7). However, only PepTite-2000 and not α-methylmannoside or QLSYGYDEK

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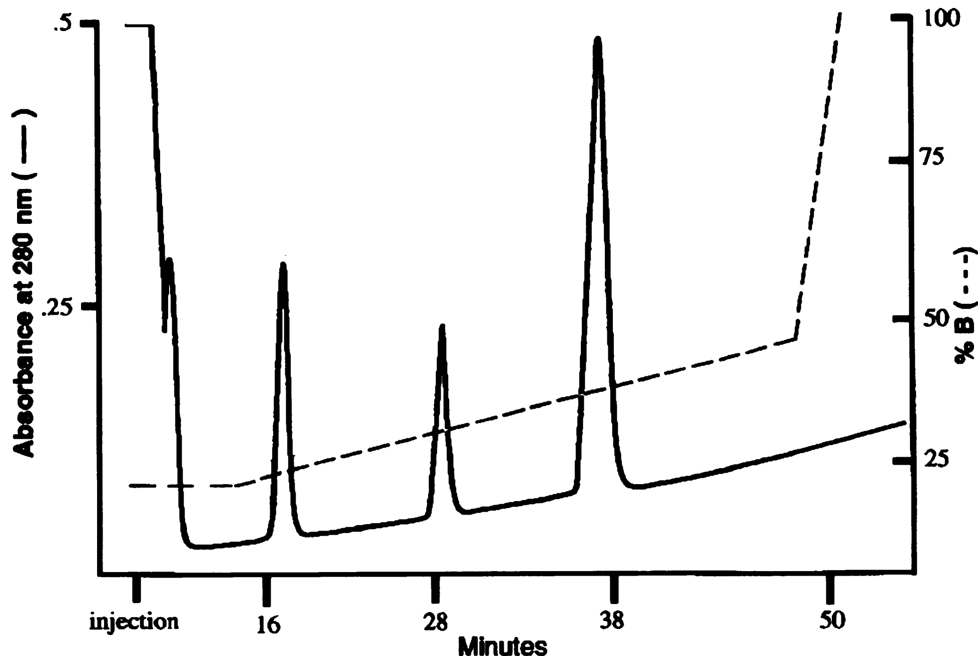

FIG. 1. Results of reversed-phase HPLC with a C_4 column. The characteristic three elution peaks are shown. B, mobile phase B.

reduces the adhesion of whole viable *C. albicans* yeast cells to immobilized fibronectin (7). These proteins in the unreduced state as determined by SDS-PAGE migrate at approximate molecular masses of 60 and 105 kDa. When adhesin samples are reduced with DTT, two proteins, one of 62 kDa and one of 72 kDa, are detectable (7). The unreduced 105-kDa protein was often difficult to detect by SDS-PAGE after several days of storage of the extract at 4°C. This suggested to us that it might be a dimer or an aggregate of the 60-kDa protein which was consistently present in large quantities even following storage. This possibility was pursued further in studies described below.

Numerous attempts were made with increasing amounts of isolated adhesin to obtain the N-terminal amino acid sequence of the protein. The N-terminal portion of the protein was blocked to all attempts at Edman degradation, even after treatment of the adhesin with a large molar excess of cyanogen bromide.

Previous work had demonstrated that concanavalin A bound to proteins of 60 and 105 kDa extracted from cell walls of *C. albicans*, suggesting that the adhesins were glycoproteins. This was also demonstrated by detection of biotinylated sugars with strepavidin-alkaline phosphatase. The quantitative phenol-sulfuric acid method of carbohydrate analysis performed on samples separated by HPLC demonstrated that the glycoprotein was approximately 75 to 80% carbohydrate by dry weight. The results of the bicinchoninic acid method of protein determination were consistent with the carbohydrate results, demonstrating that the glycoprotein was approximately 20 to 25% protein by weight. Carbohydrate contents of >70% (by weight) of *Candida* cell wall products usually indicate that the glycoprotein is a structural cell wall protein, whereas *Candida* cell wall enzymes and secreted enzymes are typically about 50% or less carbohydrate by weight (9).

The surface nature of the adhesin was also demonstrated by treatment of whole viable yeast cells with 12 mM DTT. This yielded an "extract" from the yeast cell surface which when passed over a fibronectin affinity column and eluted with EDTA yielded a protein of about 62 kDa. This molecular mass is identical to that of the detergent-isolated glycoprotein adhesins when they are reduced with DTT.

Reversed-phase HPLC with a C_{18} or C_4 column and an acetonitrile-water gradient gave three characteristic sharp protein peaks when samples of fungal proteins that had been eluted from fibronectin columns with EDTA were analyzed. The elution times were reproducible and similar with either a C_4 or C_{18} column (Fig. 1). When the three separate peaks were then subjected to SDS-PAGE, all three peaks contained a single major protein band at a molecular mass of approximately 60 kDa (Fig. 2), again suggesting that the fibronectin

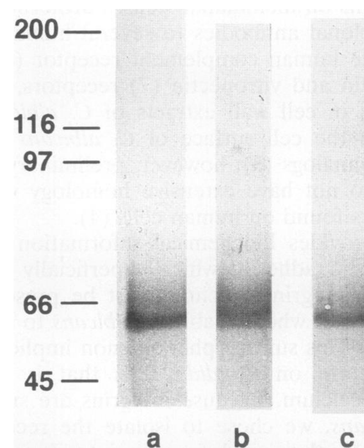

FIG. 2. The results of SDS-PAGE (7.5%) of samples of peaks from HPLC with a C_4 column, similar to those peaks shown in Fig. 1. Sizes of standard molecular weight markers (in thousands) are shown at left. Lane a, sample with elution time of ~22 min; lane b, sample with elution time of ~32 min; lane c, sample with elution time of ~38 min.

adhesin exists as an aggregate or multimer of a basic 60-kDa unit.

In further attempts to estimate the size of the adhesin, EDTA eluates from the fibronectin-agarose affinity column containing the adhesin(s) were applied to a G-200 Sephadex column. Repeated experiments demonstrated that the adhesin (60 and 105 kDa by SDS-PAGE) eluted with an apparent molecular mass of 42 kDa, considerably smaller than the molecular mass estimated by SDS-PAGE (data not shown).

The *C. albicans* fibronectin adhesin is composed of two proteins of ca. 60 and 105 kDa as established by SDS-PAGE. The adhesin is a cell surface structure of yeast cell and germ tube forms of the fungus. Isolation and detection of the adhesin have been accomplished by a functional assay by elution of the glycoprotein from a fibronectin-agarose affinity column by EDTA. By use of this method it was determined that the adhesins were present in the cell wall and cell membrane of detergent-extracted yeast cells (7). However, the adhesin was not detected in cell cytosol by this method, suggesting that precursor molecules are present only at very low concentrations or are not functional (7). The surface nature of this protein is corroborated by the fact that DTT-treated yeast cells release a ca. 62-kDa protein that can also be eluted from fibronectin-agarose affinity columns with EDTA. The DTT-extracted protein is similar in size to the detergent-extracted proteins, which are about 62 and 72 kDa after reduction with DTT.

This report also establishes that the adhesin is heavily glycosylated, being approximately 75 to 80% carbohydrate and 20 to 25% protein by weight. This composition is similar to that of the adhesin described by Critchley and Douglas (1). Furthermore, the high carbohydrate level may indicate that the adhesin is a structural glycoprotein on the *Candida* surface (9). The glycoprotein is blocked at the N terminus.

The HPLC data demonstrated three separate proteins on either a C_4 or a C_{18} column. When each peak was then subjected to SDS-PAGE, these three separate peaks all yielded a single band which migrates at an approximate molecular mass of 60 kDa. This suggests that the adhesin is present as a multimer, since a protein aggregate, if present, would be likely to yield less-reproducible elution times and peaks on HPLC and would probably occur on HPLC only as a monomer. The use of a molecular weight sizing column indicates that the molecular mass is closer to 42 kDa. This is reminiscent of data by Saxena and Calderone demonstrating that the C3d receptor on *C. albicans*, which appears to be a protein of about 60 kDa by SDS-PAGE, is only about 14 kDa when the molecular mass is determined by gel permeation chromatography (11).

This work has not resolved whether the *C. albicans* fibronectin adhesin is a lectin or an integrin-like glycoprotein. It does appear that the glycoprotein is present as an aggregate or multimer in vitro and perhaps in vivo as well. It is interesting that an integrin found on human macrophages has features that suggest lectin-like activity as well as features more like those of conventional integrins (2).

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