Antibodies to $\alpha_5\beta_1$ and $\alpha_v\beta_3$ integrins react with *Candida albicans* alcohol dehydrogenase

Stephen A. Klotz,1,3 Micheal L. Pendrak2 and Raymond C. Hein2

Author for correspondence: Stephen A. Klotz. Tel: +1 520 629 4762. Fax: +1 520 629 1801.
e-mail: sklotz@u.arizona.edu

It has been hypothesized that *Candida albicans* possesses integrin-like receptors on its cell surface. This is because *C. albicans* binds numerous fluid-phase extracellular matrix (ECM) proteins on its cell surface and adheres to the same ECM proteins when immobilized. In addition, numerous antibodies to human integrins (receptors for ECM proteins) bind to the fungal cell surface and in so doing inhibit the binding of the respective proteins. To demonstrate the presence of such a cell surface integrin, a cDNA library of *C. albicans* yeast cells was screened with polyclonal antiserum to the human fibronectin receptor ($\alpha_5\beta_1$ integrin). Clones isolated by this screening technique also reacted specifically to antiserum against the human vitronectin receptor ($\alpha_v\beta_3$ integrin). DNA sequence analysis of the cloned insert predicted a 350 aa protein (37 kDa). This predicted protein showed 75% homology at the nucleotide sequence level to alcohol dehydrogenase (ADH) of *Saccharomyces cerevisiae*. In *vitro* transcription/translation of the cloned inserts yielded a 37 kDa protein that was immunoprecipitated with antibodies to the $\alpha_5\beta_1$ and $\alpha_v\beta_3$ integrins and an antibody to a *C. albicans* fibronectin receptor. These antibodies and an mAb to the human vitronectin receptor demonstrated an antigen of ~37 kDa present in the cell-wall preparations of *C. albicans* and in spent growth medium. All four antibodies reacted with authentic ADH. The possible significance of these results in relation to *C. albicans* adherence is discussed.

**Keywords:** *Candida albicans*, adherence, integrins, extracellular matrix proteins, alcohol dehydrogenase

INTRODUCTION

Adherence of *Candida albicans* to host tissue is thought to be an important event in the pathogenesis of all forms of candidiasis and one of the proposed targets of adherence is the extracellular matrix (ECM) (Calderone & Braun, 1991; Klotz, 1992). Several groups of investigators have reported *C. albicans* receptors and/or adhesins for a number of plasma and ECM proteins. ECM protein receptors described on the surface of *C. albicans* include vitronectin (Jakab et al., 1993), laminin (Bouchara et al., 1990), entactin (Lopez-Ribot & Chaffin, 1994), collagen (Klotz, 1994), fibronectin (Klotz & Smith, 1991; Negre et al., 1994) and tenascin-C (Lopez-Ribot et al., 1999).

Receptors that bind these proteins are termed integrins, cell-surface heterodimeric membrane glycoproteins involved in cell–cell and cell–substratum attachment (Ruoslhti, 1991). Interestingly, a number of polyclonal and monoclonal antibodies (pAbs, mAbs) to human integrins bind in a specific manner to the surface of *C. albicans*. For example, an antibody to the human fibronectin receptor, $\alpha_v\beta_1$, binds to the surface of *C. albicans* as determined by FACS analysis (Santoni et al., 1994) and in so doing inhibits the binding of fibronectin to the surface of *C. albicans* yeast cells (Klotz & Smith, 1991) and the adherence of yeast cells to immobilized fibronectin (Santoni et al., 1994) and to human endothelial cells (Frey et al., 1990). This same antibody, when labelled with fluorescein, is distributed in patches over the surface of the fungus (unpublished data). Similar results have been obtained using antibodies to the human vitronectin receptor (Spreghini et al., 1999).

Because *Candida* binds ECM proteins and antibodies to human integrins bind to *C. albicans*, a reasonable
speculation is that integrin-like molecules are present on the surface of this fungus (Gustafson et al., 1991). We have screened a \textit{C. albicans} CDNA library using polyclonal antiserum to the human fibronectin receptor and isolated cDNA clones which encode an alcohol dehydrogenase (ADH) homologous to ADH of \textit{Saccharomyces cerevisiae} and \textit{Kluyveromyces lactis}. We describe the cloning and characterization of this gene in addition to the possible adherence function of this extracellular protein.

\textbf{METHODS}

\textbf{Strains and media.} \textit{Escherichia coli} strains XL-1 Blue, SOLR, TOPP-2 and SURE were all obtained from Stratagene, stored in buffered glycerol at \(-20^\circ\text{C}\) and grown in Luria broth (LB) or on LB solidified with 1.5 \% (w/v) agar (Difco) at \(37^\circ\text{C}\). \textit{C. albicans} was cultured in Sabouraud dextrose (SD) broth (Difco) and maintained on SD solidified with 1.5 \% (w/v) agar.

\textbf{cDNA libraries.} A yeast cell library of \textit{C. albicans} strain B311A (titre \(1.6 \times 10^{11}\) p.f.u. \(\mu\text{m}^{-1}\)) was a gift of G. Livi, Beecham SmithKline, Philadelphia, PA, USA. A germ tube library (titre \(4 \times 10^{10}\) p.f.u. \(\mu\text{m}^{-1}\)) was a gift of W. Fonzi, Georgetown University, Washington, DC, USA. Both libraries were prepared \(\lambda\) ZAP II (Stratagene) and propagated in \textit{E. coli} strain XL-1 Blue.

\textbf{cDNA library screening.} Screening of both cDNA libraries was carried out by plating on NZYM medium with duplicate nitrocellulose filters and represented approximately 10 000 recombinants (Sambrook et al., 1989). Briefly, phage lysates diluted to \(10^{-4}\) p.f.u. \(\mu\text{m}^{-1}\) were used to infect \textit{E. coli} XL-1 Blue and were plated on NZY plates and allowed to grow at \(37^\circ\text{C}\) for 3–4 h before being overlaid with IPTG-soaked (5 mM) filters and incubated for an additional 4 h at \(37^\circ\text{C}\). Non-specific binding sites were blocked with Superblock (SB) in TBS (Pierce) for 30 min at ambient temperature. Rabbit polyclonal antiserum to the human fibronectin or vitronectin receptors (1:500 dilution in SB) was allowed to react with the filters at \(4^\circ\text{C}\) for 18 h. Filters were then washed with TBS and developed using an anti-rabbit alkaline-phosphatase substrates. The antibody to ADH was prepared in the following manner. Approximately 100 \(\mu\text{g}\) \textit{S. cerevisiae} ADH was run on an SDS gel, transferred to nitrocellulose and blocked with 3 \% bovine serum albumin. Antiserum to the \textit{C. albicans} fibronectin adhesin was diluted 1:500 with PBS and incubated with the antigen for 5 h at 26 \(^\circ\text{C}\). The nitrocellulose strip was washed and stained with alkaline-phosphatase labelled anti-rabbit antibody. A strip corresponding to the detected antigen was cut out and the antibody was eluted with 0.2 M glycine, 1 mM EGTA (pH 2.8), transferred to a microfuge tube and brought to neutrality with Tris base (Harlow & Lane, 1988).

ADH type I from \textit{S. cerevisiae} was purchased from Boehringer Mannheim. Cell-wall extracts of \textit{C. albicans} were prepared by octylglucopyranoside extraction as reported previously (Klotz et al., 1993).

\textbf{Sequence analysis.} Dideoxy sequence analysis was performed according to the method of Sanger et al. (1977) using T7 DNA polymerase (Sequenase; USB).

\textbf{RESULTS}

The yeast cell and germ tube CDNA libraries were screened with polyclonal antiserum to the human fibronectin receptor \(\alpha_\text{FN}\). Both libraries expressed clones reactive to the antibody. Twelve clones were isolated from the yeast cell library that were specifically recognized by the antibody. The clones identified by the pAb to the human fibronectin receptor \(\alpha_\text{FN}\beta_1\) were even more strongly reactive to pAb to the human vitronectin receptor \(\alpha_\text{VN}\beta_3\). We then probed these isolates for identity with the human fibronectin receptor. Southern hybridization performed with oligonucleotides of the \(\beta_1\)
and $\alpha_5$ chains of the human fibronectin receptor under conditions of low stringency, however, did not detect homology between the isolated clones and the human integrins. Furthermore, the same 12 clones did not react with Mo-1 or OK-M1 mAbs which recognize the human iC3b receptor $\alpha$ integrin (where $\alpha_2$). Mo-1 and OK-M1 bind in a specific manner to the surface of C. albicans and so do not share homology with the human fibronectin receptor gene, nor do all anti-integrin antibodies recognize the protein, since Mo-1 and OK-M1 do not bind.

Plasmids from the 12 clones which bound the antibodies to human fibronectin and vitronectin receptors were excised from $\lambda$ ZAP II bacteriophage vectors for further characterization. The nucleotide sequence of the first 200–300 bp of each was determined and the clones were placed into four groups based on the similarity of these sequences. Expression of the proteins from these clones was accomplished using an in vitro coupled transcription/translation system. Clones from only two of the groups yielded protein products in the in vitro translation system. The expressed protein products were 37 kDa. This protein was immunoprecipitated using polyclonal sera to human fibronectin and vitronectin receptors and the C. albicans fibronectin adhesin (data not shown). Thus, these three antibodies recognize not only the protein expressed by Escherichia coli, but also the protein expressed in vitro.

Nucleotide sequence analysis of one of the clones revealed a 37 kDa protein with $>70\%$ homology with ADH from S. cerevisiae (ADH1) and K. lactis (ADH1, ADH3), and $61\%$ identity with the Schizosaccharomyces pombe ADH gene (the sequence is assigned NCBI accession no. U15924; the online version of this paper at http://mic.sgmjournals.org contains a supplementary figure showing the sequence alignment). The sequence possessed no pre-sequences identified for organelle targeting or membrane insertion, e.g. the mitochondrial presequence of the K. lactis ADH3 gene. The C. albicans gene encodes a 350 aa polypeptide with a calculated mass of 36866 Da. The sequence displays the consensus pattern for zinc-containing ADHs with the histidine in the second position of the signature predicted to be the zinc-coordinating ligand. The sequence contains 13 potential myristic acid sites and two sites for N-glycosylation. A portion of this sequence has been reported by Shen et al. (1991) from nt 310 to 940. Interestingly, the latter authors obtained this partial sequence after probing a C. albicans cDNA library with mAbs to a major allergen of C. albicans. Their antisera would presumably be recognizing a cell-surface-associated or extracellular protein of the fungus.

We then turned our attention to demonstrating whether a protein of the size of the encoded protein could be demonstrated in the cell wall of C. albicans. ADH of S. cerevisiae and cell-wall extracts of C. albicans were electrophoresed and analysed by Western blotting with pAbs to the human fibronectin receptor, the human vitronectin receptor and the C. albicans fibronectin adhesin, and with a mAb to the human vitronectin receptor (Fig. 1). These same antibodies had immunoprecipitated the in vitro transcription/translation product of the gene and/or identified the clones in the original screening. Each antibody reacted with bona fide S. cerevisiae ADH (arrow). These antibodies also reacted to multiple proteins from detergent extracts of C. albicans. However, each antibody demonstrated the presence of a cell-wall antigen of about 37 kDa, similar to ADH. The secondary antibodies did not react with the enzyme, nor with the C. albicans cell-wall proteins (data not shown).

Next we asked the question whether the antigen is secreted extracellularly. To answer this we used a monospecific antibody to ADH that was derived from the anti-fibronectin adhesin antibody. Supernatant from the growth medium of C. albicans was immunoblotted and reacted with the monospecific antibody to ADH.

![Fig. 1. Immunoblot demonstrating the presence of a protein consistent with ADH in the cell-wall preparations of C. albicans using anti-integrin antibodies. Lanes: 1, S. cerevisiae ADH1; 2, detergent-extracted cell wall of C. albicans (12% SDS-PAGE reduced with DTT). The arrow points to 37 kDa.](http://mic.sgmjournals.org)
DISCUSSION

Plasma fibronectin was the first ECM protein reported to bind C. albicans (Skerl et al., 1984) and an expanding number of publications have followed describing ECM protein interactions with the fungus (Jakab et al., 1993; Klotz, 1994; Klotz & Smith, 1991; Lopez-Ribot et al., 1999, 1994; Lopez-Ribot & Chaffin, 1994; Negre et al., 1994; Santoni et al., 1994; Spreghini et al., 1999). A pAb to the human integrin, \(\alpha_5\beta_1\), inhibits the binding of fluid-phase fibronectin to the surface of C. albicans (Klotz & Smith, 1991), inhibits the adherence of the yeast cell to human endothelial cells (Frey et al., 1990) and to immobilized fibronectin (Santoni et al., 1994), and does so by binding to the surface of the fungus. This same antibody was used in this work to identify the cDNA clones which expressed ADH. In addition to the integrin antibodies used in this work and that of Santoni et al. (1994) and Spreghini et al. (1999), cDNA libraries of C. albicans have been probed successfully with an mAb to \(\alpha_5\beta_1\) (Santoni et al., 1993) and with an antibody to the transmembrane portion of the \(\beta_1\) protein of the \(\alpha_5\beta_1\) integrin (Marcantonio & Hynes, 1988). Recently, an integrin-like gene, INT1, has been described in C. albicans by screening C. albicans genomic DNA with a cDNA probe from the transmembrane domain of the human integrin, \(\alpha M\) (Gale et al., 1996). The homologue of this protein is confined to this small domain. The N terminus does not possess homology with an integrin.

Because of our prior work with the fibronectin receptor of C. albicans, we attempted the detection of a fibronectin-binding integrin in C. albicans. Multiple clones from a C. albicans yeast cell cDNA library were isolated using polyclonal serum to the human fibronectin receptor. The clones also reacted strongly to polyclonal serum to the human vitronectin receptor. These clones were probed by Southern hybridization with the human \(\alpha_5\) and \(\beta_1\) chains of the human fibronectin receptor (\(\alpha_5\beta_1\)) and no homology was detected, suggesting that although similarity exists at the level of protein structure there is no homology at the DNA sequence level. The protein encoded by the clones is an ADH with a homology of \(~ 75\%\) with ADH1 of S. cerevisiae and ADH3 of K. lactis. pAbs to the human fibronectin receptor, vitro-

Fig. 2. Photomicrograph of C. albicans yeast cells stained with antibody prepared to protein bound by fibronectin. (a) Yeast cells stained with fluorescein-tagged goat anti-rabbit antibody; (b) the same cells reacted with a pAb to the anti-fibronectin receptor and a fluorescein-tagged second antibody; (c) different field of cells prepared in the same manner as (b).

Fig. 3. Immunoblot of C. albicans growth medium. (a) Authentic ADH (37 kDa; arrow); (b) supernatant of minimal broth medium in which C. albicans had grown for 24 h. These were immunoblotted with a pAb to the C. albicans fibronectin adhesin.

Fig. 3 demonstrates the presence of a 37 kDa protein in the supernatant, a size and antibody reaction compatible with the protein being ADH.
nectin receptor and the C. albicans fibronectin adhesin, and an mAb to the human vitronectin receptor reacted strongly to pure ADH and the protein encoded by the isolated clones. Furthermore, a monospecific antibody demonstrated that C. albicans ADH appears in the growth medium.

The gene reported here does not contain a secretory leader sequence; therefore the manner in which it is secreted to the cell surface is unknown. However, C. albicans 3-phosphoglycerate kinase appears on the surface of C. albicans yet lacks a secretory leader sequence (Alloush et al., 1997). GAPDH of Schistosoma mansoni (Goudot-Crozel et al., 1989) and ADH of Entamoeba histolytica, which is a putative ECM adhesin (Yang et al., 1994), are found on the cell surface of these micro-organisms but also lack a leader sequence. In the absence of a signal sequence it is unknown how these particular proteins get to the surface of micro-organisms. However, multiple means exist for the secretion of peptides lacking a leader sequence, such as the use of 'traffic wardens’ (Salmond & Reeves, 1993), charged amino acids in the sequence (Salmond & Reeves, 1993) or even the presence of certain amino acids in the C terminus of the protein may determine extracellular secretion (Stanley et al., 1991).

The possibility that a cell-surface-associated cytosolic enzyme could serve in the capacity of an ECM protein receptor and/or adhesin is well accepted in other micro-organisms. For example, E. histolytica ADH binds fibronectin, collagen type II and laminin (Yang et al., 1994). Along similar lines, the fibronectin receptor of Streptococcus pyogenes has been shown to be a glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Pancholi & Fischetti, 1992). Furthermore, these cytosolic enzymes are found on the cell surface. GAPDH is a major protective antigen found on the surface of Schistosoma mansoni (Goudot-Crozel et al., 1989). GAPDH also functions as a surface lectin responsible for flocculation of the yeast Kluyveromyces marxianus (Fernandes et al., 1992). Pertinent to C. albicans it has been shown that the immunodominant C. albicans glycolytic enzyme enolase is found in culture supernatants and on the surface of the fungus (Sundstrom & Aliaga, 1994), and the cytosolic enzyme 3-phosphoglycerate kinase is located on the surface of the fungus (Alloush et al., 1997). GAPDH of C. albicans is cell-wall-associated and even binds fibronectin and laminin (Gozalbo et al., 1998). These examples of cytosolic enzymes performing in different capacities are perhaps examples of gene sharing, a concept best demonstrated in the vertebrate cornea and lens where the enzyme ADH serves as a major structural protein with little or no enzymic activity (Cooper et al., 1993).

Our preliminary data in this report suggest that C. albicans ADH is found on the cell surface and in the culture supernatant. This protein was identified by screening a C. albicans cDNA library with antibodies to human integrins which are known to bind to the fungal cell surface. The cross-reactivity of human integrin antibodies with this fungal protein is quite likely to be the explanation for the phenomenon of integrin antibodies binding to the C. albicans cell surface. This will need to be confirmed by examining antibody reactivity in cells deleted for both copies of the ADH gene. Although ADH added exogenously did not inhibit adherence of C. albicans to fibronectin, this fact does not rule out ADH as an adhesin. There are other C. albicans adhesins whose function is not inhibited upon the addition of excess ligand (e.g. Gozalbo et al., 1998 or Gaur & Klotz, 1997). Therefore, whether or not ADH serves as a receptor for fibronectin or vitronectin remains unknown.

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REFERENCES


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