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Evidence for the Presence of Collagenous Domains in *Candida albicans* Cell Surface Proteins

PILAR SEPÚLVEDA,[1] AMELIA MURGUI,[2] JOSÉ L. LÓPEZ-RIBOT,[3] MANUEL CASANOVA,[1] JOAQUÍN TIMONEDA,[2] AND JOSÉ P. MARTÍNEZ[1]*

Departamento de Microbiología[1] and Departamento de Bioquímica y Biología Molecular,[2] Facultad de Farmacia, Universitat de València, Valencia, Spain, and Department of Microbiology and Immunology, Texas Tech University Health Sciences Center, Lubbock, Texas 79430[3]

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Rabbit polyclonal antibodies (PAbs) directed towards the amino-terminal cysteine-rich 7S domain (PAb anti-7S), the major internal collagenous domain (PAb anti-type IV), and the C-terminal noncollagenous region (PAb anti-NC1) of the type IV collagen molecule were probed by indirect immunofluorescence against *Candida albicans* blastoconidia and germinated blastoconidia. Most nongerminating cells and mother blastoconidia from which germ tubes originated showed strong fluorescence when PAb anti-7S was used, whereas with PAb anti-type IV, fluorescence was found almost exclusively on the surface of filamentous forms. A patched fluorescent pattern rather than a homogenous confluent fluorescence was observed in all cases. No fluorescent cells were observed with PAb anti-NC1. By Western immunoblotting, PAb anti-type IV cross-reacted primarily with a polypeptide of 37 kDa present in wall extracts obtained from intact cells of both growth forms by treatment with β-mercaptoethanol, whereas PAb anti-7S recognized a major 58-kDa antigen also present in both extracts, along with some other high-molecular-mass (>106-kDa) polydisperse species present only in the material released from blastoconidia with β-mercaptoethanol. No reactive bands were observed when PAb anti-NC1 was used as a probe in Western immunoblotting experiments. The sensitivities or resistances to collagenase digestion of the different polypeptides that cross-reacted with PAbs anti-type IV and anti-7S suggest the existence of cell wall components in *C. albicans* that contain epitopes that mimic the collagenous domains of the type IV collagen molecule.

The dimorphic, imperfect fungus *Candida albicans* (and related species) is emerging as a leading cause of disseminated fungal infection, primarily in immunocompromised individuals; it is considered one of the most common sources of nosocomial (hospital-acquired) infections. The adherence of *C. albicans* to host epithelial cells is believed to be a critical initial stage in the pathogenesis of candidiasis. Once the host's mucosal surfaces have been colonized, fungal invasion through the endothelium and/or subendothelial extracellular matrix (ECM) barriers, mediated by the interaction between complementary molecules on both parasite and host surfaces, is thought to play an important role in the dissemination of *C. albicans* cells, giving rise to metastatic sites of infection throughout the body (25). In this regard, multiple adherence mechanisms appear to be utilized by *C. albicans* cells. Thus, several candidal adhesins (10, 18, 19, 25, 28, 49, 53, 54), receptors for ECM components such as fibronectin (29, 30), laminin (5, 37), and entactin (38), and receptors for mammalian serum proteins such as complement components (11, 26) and fibrinogen (14) have been characterized. Virtually all of the candidal adhesins and receptors identified to date appear to be cell surface (cell wall) proteins, and most appear to be mannoproteins (for reviews on adhesins and receptor-ligand relationships as putative virulence factors in *C. albicans*, see references 10, 18, and 25).

Type IV collagen is the major collagenous component of the basement membrane, forming a network structure with which other basement membrane components (e.g., laminin and entactin) interact. In addition, type IV collagen can interact with cells indirectly through laminin; in this context, strong binding of type IV collagen to laminin is mediated by entactin, a glycoprotein of about 150 kDa which binds tightly to laminin and also has binding sites for type IV collagen and cells (2, 45).

In this report we present enzymatic and immunological evidence for the existence of several cell wall-bound protein and mannoprotein species, among them the *C. albicans* 58-kDa fibrinogen-binding mannoprotein (14) and the 37-kDa high-affinity laminin receptor-like protein (37) previously identified by our group, which appear to have collagenous domains in their polypeptide sequences that may define additional motifs through which adhesive interactions of *C. albicans* with the host's tissues could be established.

MATERIALS AND METHODS

Organism, culture conditions, and preparation of cell wall extracts and protoplast homogenates. *C. albicans* ATCC 26555 (serotype A) was used in this study. Exponential-phase cultures of blastoconidia (yeast phase) and blastoconidia bearing germ tubes (also defined here as germinated blastoconidia or mycelium) were obtained by basically following the procedure described previously (12, 36) and using the minimal medium supplemented with amino acids described by Lee et al. (35).

β-Mercaptoethanol (βME) and Zymolyase 20T [(1→3)-β-glucanase complex] extracts containing protein and glycoprotein cell wall components, as well as protoplast homogenates from both cell growth forms, were obtained as previously reported (13, 14). The total sugar and protein contents in the different samples were determined colorimetrically (20, 40) with glucose and bovine serum albumin (BSA), respectively, as the standards. The different samples (βME and Zymolyase extracts and homogenates) were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and then blotted onto nitrocellulose paper as described below.

Proteolytic and deglycosylating treatments. Samples of the βME extract from blastoconidia were subjected to digestion with endo-β-*N*-acetylglucosaminidase H (endo H) (0.01 U/50 μg of protein in the sample) by the procedure described

* Corresponding author. Mailing address: Departamento de Microbiología, Facultad de Farmacia, Room 3-70, Universitat de València, Avda. Vicente Andrés Estellés, s/n, 46100-Burjassot, Valencia, Spain. Phone: 34-6-3864770. Fax: 34-6-3864770. Electronic mail address: JOSE.PEDRO.MARTINEZ@uv.es.

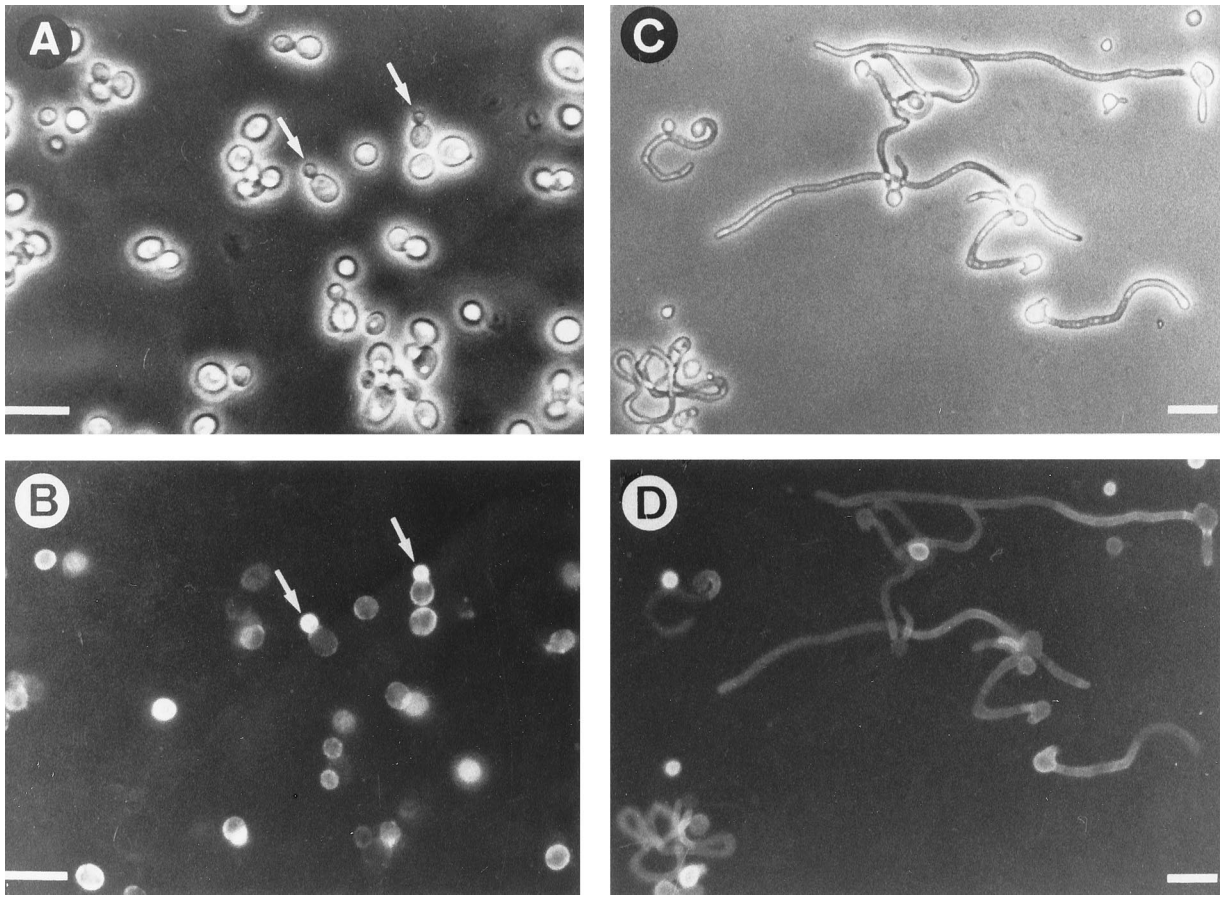

FIG. 1. Phase-contrast (A and C) and fluorescence (B and D) microscopy of cells incubated with PAb anti-7S (1:10 dilution). *C. albicans* ATCC 26555 was incubated under conditions that induce budding growth (A and B) or germ tube formation (C and D). Bars, 10 μm.

elsewhere (15) or to β-elimination by the method of Elorza et al. (21), to release N- or O-linked carbohydrate moieties, respectively. Likewise, blastoconidium βME extracts were treated with 6 M guanidine-HCl (to solubilize any aggregated materials which appeared after prolonged storage at 4°C), desalted by gel filtration chromatography (Sephadex G-25), and finally digested (22 to 24 h at 37°C) with collagenase (17.5 μg of enzyme per 500 μg of sample expressed as total sugar content) in 50 mM HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid)-Na buffer (pH 7.5) containing 10 mM $CaCl_2$, 0.02% sodium azide, and the following protease inhibitors: 5 mM *N*-ethylmaleimide, 4 mM benzamidine-HCl, 25 mM δ-aminohexanoic acid, and 1 mM phenylmethylsulfonyl fluoride.

IIF. Specimens of blastoconidia and germinated blastoconidia were prepared for indirect immunofluorescence (IIF) by the procedure described elsewhere (12, 23). The following rabbit polyclonal antibodies (PAbs) against different domains of bovine glomerular basement membrane type IV collagen were used as probes: PAb anti-7S, which is directed against the N-terminal cross-linking domain (7S domain); PAb anti-type IV collagen, which was raised against reduced and alkylated type IV collagen and reacts mainly with the large central triple-helical domain between the 7S and the C-terminal (NC1) domains; and PAb anti-NC1, which recognizes the C-terminal noncollagenous NC1 domain of the type IV collagen molecule (2, 33). Cells were reacted for 1 h at room temperature with the antisera (assayed separately) diluted 1:10 in 10 mM phosphate (pH 7.4)–0.15 M NaCl containing 1% BSA.

SDS-PAGE and Western immunoblotting techniques. SDS-PAGE was performed basically as described by Laemmli (32) with slab gradient gels (5 to 15%; ratio of acrylamide to bisacrylamide, 30:0.2) and 3.5 to 4% polyacrylamide stacking gels. Samples were prepared for electrophoresis by a procedure reported previously (12). Electrophoretic transfer (Western blot) of proteins from polyacrylamide gels to nitrocellulose paper was carried out as described by Burnette (9) with minor modifications (12).

Immunochemical detection of proteins on blots was performed by the protocol described in the Bio-Rad Immun-Blot (GAR-HRP) assay kit, which is based on the procedures of Burnette (9) and Towbin et al. (50). In Western immunoblot experiments, antibodies were used at final concentrations of 1:250 (for PAb anti-7S and PAb anti-NC1) and 1:1,000 (for PAb anti-type IV) in 0.01 M Tris hydrochloride buffer (pH 7.4) containing 0.9% NaCl, 0.05% Tween 20, and 3% (wt/vol) BSA as the blocking agent (TBSTB buffer). Peroxidase-labelled goat anti-rabbit immunoglobulin G at a 1:2,000 dilution in TBSTB was used as an indicator antibody. Colored reactive bands were developed with hydrogen peroxide and with 4-chloro-1-naphthol as the chromogenic agent.

Sources of materials. Gel electrophoresis and blotting reagents, peroxidase- and fluorescein isothiocyanate-conjugated goat anti-rabbit immunoglobulin G, and prestained molecular mass markers were from Bio-Rad. Endo H and Zymolyase 20T were from Miles Laboratories. CLSPA collagenase was obtained from Worthington Biochemical Corp. All other chemicals used were purchased from Sigma Chemical Co.

RESULTS

IIF staining of blastoconidia and germinated blastoconidia. Two distinct reactivity patterns were observed when PAbs anti-7S and anti-type IV were tested by IIF against cells of both *C. albicans* growth forms. Most nongerminating yeast cells (ca. 80%) (Fig. 1B) and mother blastoconidia from which germ tubes originated (Fig. 1D) showed strong fluorescence when PAb anti-7S was used as a probe, whereas hyphal elements were not labelled or exhibited a faint fluorescence (Fig. 1D). The strongest fluorescent reaction was generally observed on the surface of daughter bud cells (Fig. 1A and B, arrows). However, with PAb anti-type IV, fluorescence was found almost exclusively on the surface of filamentous forms (Figs. 2B to D). Thus, hyphal elements that occasionally appear in the cultures of nongerminating blastoconidia and that were hardly visible by phase-contrast microscopy (Fig. 2A) were clearly distinguished by fluorescence microscopy following reaction

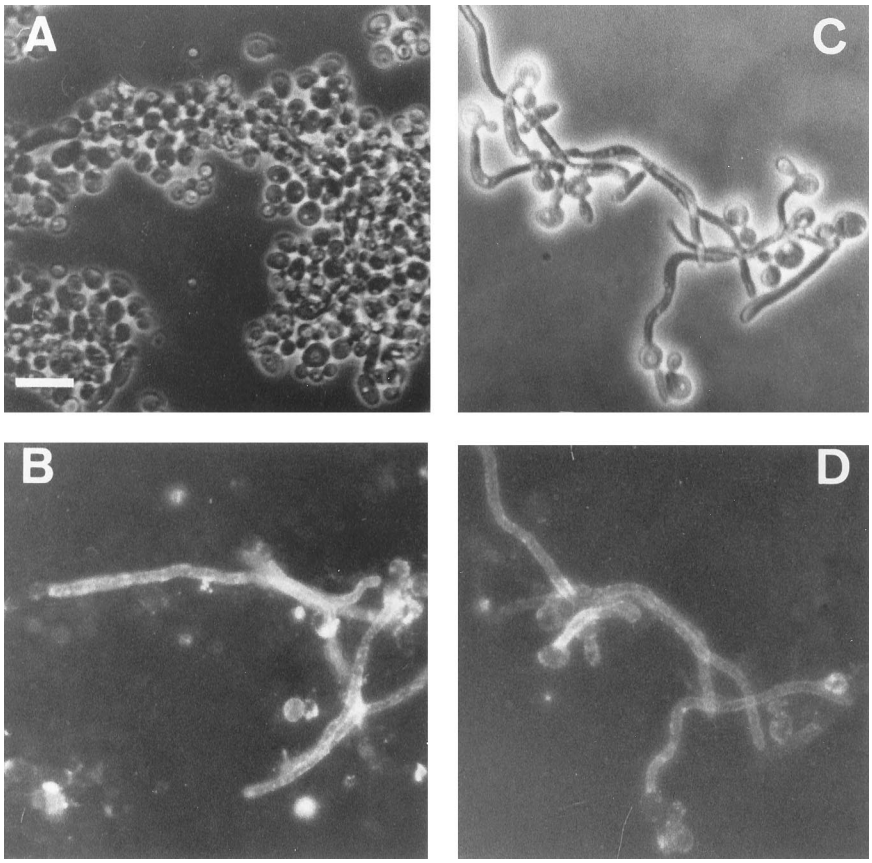

FIG. 2. Phase-contrast (A and C) and fluorescence (B and D) microscopy of *C. albicans* cells incubated under conditions that induce budding growth (A and B) or germ tube formation (C and D) and reacted with PAb anti-type IV collagen (1:10 dilution). Fluorescence microscopy allowed visualization of several filamentous forms, which eventually appeared in cultures of yeast cells (panel B; see text). Bar, 10 μm (the magnification is the same for all panels).

with PAb anti-type IV collagen molecules (Fig. 2B). In all cases, the reactivity appeared to depend on the previous interaction of the specific antibodies (PAb anti-7S and anti-type IV) with cells, since fluorescence was not observed when the cells were reacted with the second fluorescein isothiocyanate-labelled marker antibody alone. Fluorescence was also not observed on either yeast or mycelial form cells when PAb anti-NC1 was used as a probe. A patched fluorescent pattern rather than a homogeneous confluent fluorescence was generally observed, which suggests that candidal cell surface constituents exhibiting reactivity towards PAbs anti-7S and anti-type IV are not uniformly distributed within the cell wall structure. Similar observations have been reported for the 58-kDa fibrinogen-binding mannoprotein and the high-affinity laminin receptor-like molecule present in the wall of *C. albicans* cells (14, 37, 43).

Identification of surface (cell wall) components that cross-reacted with antibodies against collagen. Wall components that could promote the reactivity observed by IIF towards PAbs anti-7S and anti-type IV (Fig. 1 and 2) were extracted by βME treatment followed by Zymolyase digestion of intact cells. βME and Zymolyase extracts are known to contain a complex array of genuine cell wall protein and glyco(manno) protein components (from 21 to 25 individual species) within a very wide molecular mass range (from 650 to 13 kDa) (13). Both cell wall extracts and the protoplast homogenates were subjected to SDS-PAGE under reducing conditions on slab gradient gels (5 to 15% polyacrylamide) and electrophoretically transferred to nitrocellulose paper for Western immunoblotting analysis.

Among the polypeptides released by βME, a 58-kDa component, which was present in extracts from both growth forms and corresponds to the previously identified candidal receptor for fibrinogen (14), was the main species revealed by incubation of the nitrocellulose sheet with PAb anti-7S (Fig. 3, lanes 1 and 2, arrow), along with other components with apparent molecular masses higher than 106 kDa that were present in the βME extracts from blastoconidia and exhibited reactivity towards the antibody (Fig. 3, lane 1, stars; see Fig. 6A, lane 1). Reactions of PAb anti-7S with several bands, mostly in the high-molecular-mass range (>106 kDa), that were present in the Zymolyase digests from germinated blastoconidia (Fig. 3, lane 4) were also seen. Essentially no reactive bands were observed in the Zymolyase digests from blastoconidia (Fig. 3, lane 3) and in homogenates from yeast (Fig. 3, lane 5) and hyphal (Fig. 3, lane 6) protoplasts. Immunodetection of blotted polypeptides with PAb anti-type IV revealed reactivity with a 37-kDa species that was consistently detected in all βME extracts from both blastoconidia (Fig. 4, lane 1, arrow; Fig. 5, lane 2, arrow) and germinated blastoconidia (Fig. 4, lane 2, arrow) assayed, yet reactivity of PAb anti-type IV towards a moiety with a higher apparent molecular mass (ca. 50 kDa) was occasionally noticed in some βME extract batches (Fig. 5, lane 2). The identity and biological function of this larger component remain to be determined, but the 37-kDa species was previously identified by our group as a high-affinity laminin

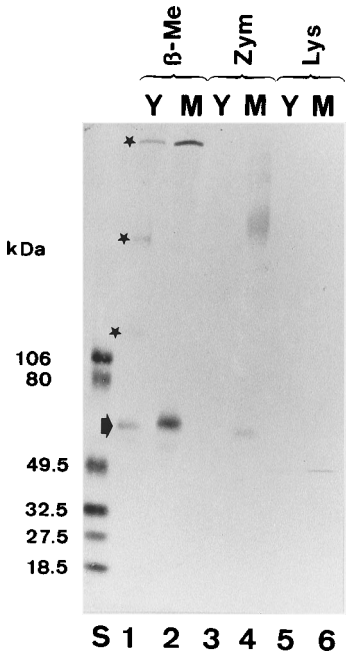

FIG. 3. Western blot of a 5 to 15% slab gradient gel loaded with βME extracts (lanes 1 and 2), Zymolyase (Zym) extracts (lanes 3 and 4), and protoplast homogenates (Lys) (lanes 5 and 6) from blastoconidia (Y) and germinated blastoconidia (M). Samples applied to each well contained 200 μg of material (expressed as total sugar content). The nitrocellulose sheet was stained with PAb anti-7S. Lane S shows a mixture of prestained molecular mass standards run in parallel, with molecular masses indicated at the left. Arrow, 58-kDa component; stars, high-molecular-mass components (see text).

receptor-like polypeptide in the wall of *C. albicans* cells (37). In any case, the identities of the 37- and 58-kDa species that exhibited reactivities towards PAb anti-type IV and PAb anti-7S, respectively, were additionally confirmed by establishing their abilities to specifically interact with laminin (in the case of the 37-kDa component) or fibrinogen (in the case of the 58-kDa species) in βME samples subjected in parallel to SDS-PAGE and Western blotting by the procedure previously described (14, 37) (not shown). No reactive bands were observed when the nitrocellulose sheets were incubated with the PAb anti-NC1 or the second peroxidase-labelled indicator antibody alone, indicating that the reactions visualized in Fig. 3 and 4 were dependent on the previous interaction of the blotted polypeptides with the specific antiserum preparations.

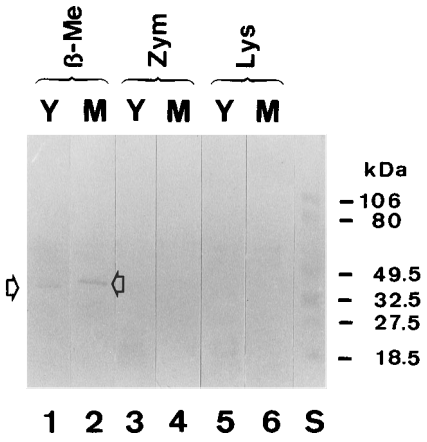

FIG. 4. Western immunoblot analysis of βME extracts (lanes 1 and 2), Zymolyase (Zym) extracts (lanes 3 and 4), and protoplast homogenates (Lys) (lanes 5 and 6) from blastoconidia (Y) and germinated blastoconidia (M) reacted with PAb anti-type IV collagen. Samples applied to each well contained 200 μg of material (expressed as total sugar content). Arrows point to a 37-kDa species that exhibited reactivity after detection with the antibody. The molecular masses of prestained proteins run in parallel in lane S are indicated at the right.

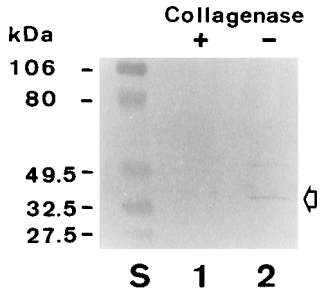

FIG. 5. Effect of collagenase treatment on the reactivity of the material released by βME treatment of yeast cells towards PAb anti-type IV collagen. βME-solubilized material was digested with collagenase (+) and compared with untreated material (−). Samples containing 200 μg of material (expressed as total sugar content) were separated by SDS-PAGE (5 to 15% slab gradient gels), transferred to nitrocellulose, and reacted with the antiserum. The arrow points to a 37-kDa species that exhibited reactivity towards the antiserum only in the untreated sample. The molecular masses of prestained markers run in parallel (lane S) are shown on the left.

Since the cell wall-bound components that cross-reacted with PAbs anti-7S and anti-type IV appeared to be present in both growth forms of the fungus, qualitative differences in the reactivities of yeast and hyphal elements towards both antisera against collagen as detected by IIF (see above) are possibly due to the distinct locations or distributions of the immunoreactive components within the wall structure, which is thicker in yeast cells than in mycelial cells (14).

Effect of enzymatic and chemical treatments on the reactivity of βME extracts with antibodies against collagen. The effects of several deglycosylating (endo H digestion and β-elimination) and proteolytic (collagenase digestion) treatments on the species solubilized with βME from blastoconidia were assayed in order to assess the role played by the carbohydrate and polypeptide moieties of the different molecules in their abilities to cross-react with PAbs anti-7S and anti-type IV.

Collagenase treatment of βME extracts was performed as described in Materials and Methods, and the treated and control (i.e., treated with buffer only or with boiled collagenase) samples were tested by Western immunoblotting with PAb anti-type IV. Digestion with the protease caused the abolition of reactivities of the 37-kDa band and the larger (50-kDa) species with the antiserum (compare lanes 1 and 2 in Fig. 5), thus indicating that the immunodeterminants recognized by PAb anti-type IV in both molecules are sensitive to treatment with collagenase. Sensitivity to collagenase suggests the existence of a collagenous domain in these candidal cell wall components (47).

Treatment of βME extracts with endo H, which cleaves high-molecular-mass, N-linked mannose polysaccharides of *C. albicans* cell wall mannoproteins (21), resulted in the conversion of the major 58-kDa antigen recognized by PAb anti-7S (Fig. 6A, lane 1, closed arrow) to a 47-kDa species which was still able to cross-react with the antiserum (Fig. 6A, lane 2, open arrow). Digestion with the glycosidase also resulted, apparently, in the transformation of the poorly resolved high-molecular-mass (i.e., >106-kDa) material (possibly due to the large carbohydrate content in these molecules and the poly-

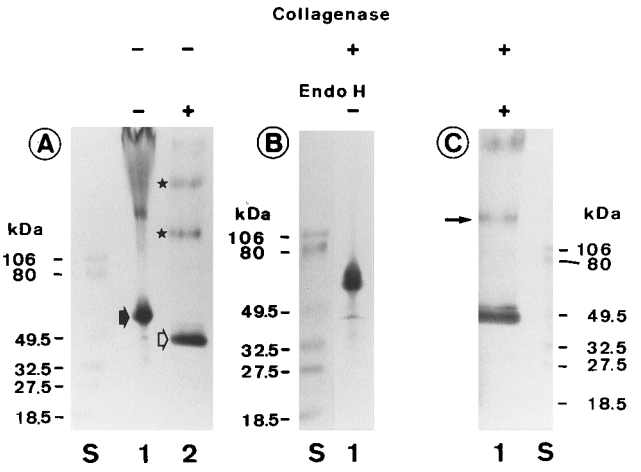

FIG. 6. Effect of proteolytic (collagenase) and/or deglycosylation (endo H) treatments on the reactivity of the material released by βME treatment of yeast cells towards PAb anti-7S. βME-solubilized material was independently or sequentially digested with collagenase and endo H and compared with untreated material (panel A, lane 1) Samples (200 μg per well [expressed as total sugar content]) were separated by SDS-PAGE, blotted onto nitrocellulose sheets, and reacted with the antiserum. Molecular masses of prestained markers run in parallel (lanes S) are shown. Closed arrow in panel A, 58-kDa antigen; open arrow in panel A, 47-kDa species; stars, high-molecular-mass material; arrow in panel C, collagenase-resistant species in high-molecular-mass material (see text).

dispersity of their mannan moieties [21]) to two discrete bands that still retained reactivity towards PAb anti-7S (Fig. 6A, lane 2, stars). Subsequent deglycosylation by β-elimination (not shown), which removes O-linked oligosaccharides (21), or collagenase digestion of endo H-treated material did not modify the pattern of reactivity against this antiserum (Fig. 6C, lane 1) compared with that obtained with the βME extract treated with endo H only (Fig. 6A, lane 2). However, a single collagenase digestion of βME extracts caused the abolition of reactivity of the high-molecular-mass species towards PAb anti-7S, although the broad 58-kDa band retained essentially unaffected its reactivity with the antiserum in this case (compare Fig. 6A, lane 1, with Fig. 6B, lane 1). These observations suggest that an epitope(s) recognized by PAb anti-7S in the 58-kDa molecule mimics the amino-terminal region of the type IV collagen molecule (7S domain), which is collagenous and totally resistant to collagenase (8, 34). On the other hand, the lack of reactivity of high-molecular-mass species (>160 kDa; Fig. 6A, lane 1) towards PAb anti-7S subsequent to digestion with collagenase alone (Fig. 6B, lane 1) suggests the existence of collagenous, collagenase-sensitive domains in these candidal cell wall species, yet the removal of N-glycosidically linked mannose residues by treatment with endo H also indicated the existence of at least one collagenase-resistant species in the high-molecular-mass material released by βME from the wall of blastoconidia (Fig. 6C, lane 1, arrow). In any case, the possibility that endo H digestion may cause conformational or structural changes of the molecules involved, resulting in the absence of sensitivity towards collagenase, cannot be ruled out.

Since the 37-kDa band recognized by PAb anti-type IV (Fig. 4) showed no reactivity towards concanavalin A, thus indicating the absence of sugar (mannose) residues linked to the polypeptide moiety of this molecule (13, 37), the effects of both deglycosylation treatments (digestion with endo H and β-elimination) on the reactivity of βME extracts towards PAb anti-type IV were not assayed.

DISCUSSION

Different studies have shown that the proteinaceous constituents of the wall, most of them glycoproteins containing N- and O-linked mannose polymers (mannoproteins), appear to play an essential role in the modulation of several cell surface properties that can represent survival and/or virulence factors in *C. albicans* (10, 16, 18, 25, 46, 48).

The results obtained in this study suggest that several genuine cell wall-bound proteins and mannoproteins of *C. albicans*, including the already characterized 58-kDa fibrinogen-binding mannoprotein and the 37-kDa laminin receptor-like protein (14, 37), possess epitopes that mimic collagenous domains or sequences of the type IV collagen molecule. Changes in the reactivity patterns towards PAbs anti-7S and anti-type IV observed following deglycosylation (endo H digestion or β-elimination) and/or proteolytic (collagenase digestion) treatments indicate that the molecular motifs conferring the ability of these molecules to cross-react with both anticollagen antisera are determined by the amino acid sequence rather than by the sugar residues eventually linked to the polypeptide moieties. The cross-reactivity of antibodies to type IV collagen with the cell wall components described in this work is not a surprising observation, since the ability of antisera raised against animal proteins (e.g., the high-affinity human laminin receptor and the chicken integrin β1 subunit) to specifically recognize candidal cell surface and/or cytosolic proteins has been already reported (37, 42). In this regard, it has to be stressed that the βME wall extracts examined by Western immunoblotting are known to contain a complex array of protein species (about 17 polypeptide chains) (13, 14, 37). The fact that only one species was recognized by PAb anti-type IV whereas one to four bands (depending on the growth form considered) were revealed following immunostaining with PAb anti-7S, along with the absence of reactivity against PAb anti-NC1, which recognized the C-terminal noncollagenous region of the type IV collagen molecule (2), supports the contention of the specificity of the immunological evidence presented here.

Although some qualitative differences in the electrophoretic patterns of cell wall proteins (particularly in the high-molecular-mass range) containing epitopes that cross-react with anticollagen antisera were observed between yeast form cells and germinated blastoconidia, the major antigenic bands recognized by PAb anti-7S and PAb anti-type IV (i.e., the 58- and 37-kDa antigens) appeared to be common to both *C. albicans* growth phases. Similarly, the 58-kDa fibrinogen-binding mannoprotein was found to be present and fully functional (i.e., it showed the ability to interact specifically with fibrinogen in all cases) in the wall of blastoconidia and germ tubes (14). However, the biologically functional 37-kDa laminin receptor was detected exclusively in the cell wall of nongerminating yeast cells; yet, βME extracts from germinated blastoconidia also contained a 37-kDa species that cross-reacted with antibodies directed against the human high-affinity laminin receptor but, in contrast to its counterpart present in the wall of yeast cells, was unable to bind laminin (37). In this regard, the existence of collagenous domains in these species could represent a supplementary mechanism by which such molecules, particularly those present in the hyphal cell wall, interact with laminin indirectly through entactin (22). In this context, the presence of collagenous domains in candidal wall protein and mannoprotein constituents may account for the reported ability of *C. albicans* cells to bind entactin (38). In addition, it has been recently reported that the gelatin/collagen-binding domain of fibronectin contains a high-affinity binding site for *C. albicans* (44). Accordingly, the presence of collagenous motifs in sev-

eral cell wall-bound candidal proteins (this work) may account for the suggested presence of more than one fibronectin receptor on the *C. albicans* surface (29, 30) In this regard, the adherence of *C. albicans* to the ECM and basement membrane appeared to be mediated by a polyvalent receptor-ligand system that promotes binding to laminin, fibronectin, and type IV collagen.

The collagen-like domains present in some cell wall-bound protein and glycoprotein moieties may play a role in protein-protein interactions, which are one of the three categories of adhesive interactions proposed for *C. albicans* and which appear to be mediated by integrin analogs (CR2- and CR3-like molecules) located at the surface of *C. albicans* cells (10, 18, 25). Because of a number of features (e.g., similar molecular weight and affinity for ligands) shared by candidal adhesins displaying integrin-like properties, it has been suggested that some of these adhesins represent the same molecule, which is able to recognize the protein portion of host glycoproteins with the arginine-glycine-aspartic acid (RGD) sequence, such as iC3b, fibronectin, type I and IV collagen, laminin, fibrinogen, fibrin, and C3d (18). This idea seems to be less tenable for several reasons. First, it is unlikely that adhesive domains for distinct large-molecular-size ligands could be simultaneously accommodated on a single candidal protein (25); in this regard, it has to be stressed that molecules exhibiting receptor-like activities are found among the medium- and low-molecular-mass species of *C. albicans* cell wall extracts on immunoblots of denatured proteins. Second, there is evidence indicating that the ligands are not interchangeable (3, 4, 29, 51). Third, the existence of different receptor-like molecules for the same ligand (laminin) has been reported (5, 37). Last, two of the best-characterized candidal adhesins, the C3d receptor (11) and the 58-kDa fibrinogen-binding mannoprotein (14), appear to be functionally and biochemically distinct molecular entities (39). However, the presence of collagenous motifs in several candidal cell wall moieties, including the previously characterized receptors for fibrinogen and laminin (14, 37) and possibly also the C3d receptor (the broad band recognized by PAb anti-7S in the medium-molecular-mass range may well represent a cluster including the C3d and fibrinogen receptors, since both have a similar apparent molecular mass [around 60 kDa]), may account for the functional cross-reactivity among receptors observed in various studies. Particularly in the case of fibronectin, the existence of a candidal promiscuous receptor that mediates binding to this ECM component has been suggested (44); this receptor may be analogous to the macrophage scavenger receptor, which contains a collagenous domain (31), or to the staphylococcal fibronectin receptor, which recognizes multiple domains of fibronectin with different affinities (6). The observations reported in this communication may account for the existence of the suggested promiscuous fibronectin receptor. In any case, because of the variety of ligands that can bind to *C. albicans* cells under similar experimental conditions, stringent controls are essential in studies on binding to determine the precise molecular nature of candidal adhesins, the relatedness between these adhesins (if they actually are different entities), and the interactions between the candidal receptors and host ligands. The isolation of the gene(s) encoding integrin-like proteins in both growth forms of the fungus will be essential to the resolution of these possibilities. A research line in this context is currently being developed in our laboratory.

Finally, taking into account the existence of candidal cell surface-related protein and glycoprotein moieties displaying lectin-like adhesive (7, 17, 49) and complement-binding (1, 11, 24) properties and of proteins exhibiting collagenous motifs (this work), it is tempting to speculate that the wall of *C. albicans* cells possesses species analogous to mammalian collectins, a term coined to describe the members of a group of proteins that have a collagen-like domain and/or are associated with a complement receptor and that are able to bind carbohydrates (lectin activity) (41), which seem to be involved in various effector and immunomodulatory functions (27, 52), functions that at least in part (immunomodulation) have been assigned to candidal cell wall mannoproteins (16).

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REFERENCES

1. **Alaei, S., C. Larcher, C. Ebenbichler, W. M. Prodinger, J. Janatova, and M. P. Dierich.** 1993. Isolation and biochemical characterization of iC3b receptor of *Candida albicans*. Infect. Immun. **61:**1395–1399.
2. **Bejarano, P. A., J. P. M. Langeveld, B. G. Hudson, and M. E. Noelken.** 1989. Degradation of basement membranes by *Pseudomonas aeruginosa* elastase. Infect. Immun. **57:**3783–3787.
3. **Bouali, A., R. Robert, G. Tronchin, and J. M. Senet.** 1986. Binding of human fibrinogen to *Candida albicans* in vitro: a preliminary study. J. Med. Vet. Mycol. **24:**345–348.
4. **Bouali, A., R. Robert, G. Tronchin, and J. M. Senet.** 1987. Characterization of binding of human fibrinogen to the surface of germ-tubes and mycelium of *Candida albicans*. J. Gen. Microbiol. **133:**545–551.
5. **Bouchara, J. P., G. Tronchin, V. Annaix, R. Robert, and J. M. Senet.** 1990. Laminin receptors on *Candida albicans* germ tubes. Infect. Immun. **58:**48–54.
6. **Bozzini, M., L. Visai, P. Pignatti, T. E. Petersen, and P. Spezial.** 1992. Multiple binding sites in fibronectin and the staphylococcal fibronectin receptor. Eur. J. Biochem. **207:**327–333.
7. **Brassart, D., A. Woltz, M. Golliard, and J. R. Neeser.** 1991. In vitro inhibition of adhesion of *Candida albicans* clinical isolates to human buccal epithelial cells by Fucα1→2 Galβ-bearing complex carbohydrates. Infect. Immun. **59:**1605–1613.
8. **Brinker, J. M., M. T. Pegg, P. S. Howard, and N. A. Kefalides.** 1985. Immunochemical characterization of type IV procollagen from anterior lens capsule. Collagen Relat. Res. **5:**233–244.
9. **Burnette, W. N.** 1981. "Western blotting": electrophoretic transfer of proteins from sodium dodecyl sulfate-polyacrylamide gels to unmodified nitrocellulose and radiographic detection with antibody and radioiodinated protein A. Anal. Biochem. **112:**195–203.
10. **Calderone, R. A., and P. C. Braun.** 1991. Adherence and receptor relationships in *Candida albicans*. Microbiol. Rev. **55:**1–20.
11. **Calderone, R. A., L. Linehan, E. Wadsworth, and A. L. Sandberg.** 1988. Identification of C3d receptors on *Candida albicans*. Infect. Immun. **56:**252–258.
12. **Casanova, M., M. L. Gil, L. Cardeñoso, J. P. Martínez, and R. Sentandreu.** 1989. Identification of wall-specific antigens synthesized during germ tube formation by *Candida albicans*. Infect. Immun. **57:**262–271.
13. **Casanova, M., J. L. López-Ribot, J. P. Martínez, and R. Sentandreu.** 1992. Characterization of cell wall proteins from yeast and mycelial cells of *Candida albicans* by labelling with biotin: comparison with other techniques. Infect. Immun. **60:**4898–4906.
14. **Casanova, M., J. L. López-Ribot, C. Monteagudo, A. Llombart-Bosch, R. Sentandreu, and J. P. Martínez.** 1992. Identification of a 58-kilodalton cell surface fibrinogen-binding mannoprotein from *Candida albicans*. Infect. Immun. **60:**4221–4229.
15. **Casanova, M., J. P. Martínez, M. L. Gil, R. Sentandreu, and J. Ruiz-Herrera.** 1987. Different molecular forms of invertase in the *slime* variant of *Neurospora crassa*: comparison with the wild-type strain. J. Gen. Microbiol. **133:**2447–2456.
16. **Cassone, A.** 1989. Cell wall of *Candida albicans*: its functions and its impact on the host. Curr. Top. Med. Mycol. **3:**249–314.
17. **Critchley, I. A., and L. J. Douglas.** 1987. Isolation and partial characterization of an adhesin from *Candida albicans*. J. Gen. Microbiol. **133:**629–636.
18. **Cutler, J. E.** 1991. Putative virulence factors of *Candida albicans*. Annu. Rev. Microbiol. **45:**187–218.
19. **Douglas, L. J.** 1987. Adhesion of *Candida* species to epithelial surfaces. Crit. Rev. Microbiol. **15:**27–43.

20. **Dubois, M., K. A. Gilles, J. K. Hamilton, P. A. Rebers, and F. Smith.** 1956. Colorimetric method for determination of sugars and related substances. Anal. Chem. **28:**350–356.
21. **Elorza, M. V., A. Marcilla, and R. Sentandreu.** 1988. Wall mannoproteins of the yeast and mycelial cells of *Candida albicans*: nature of the glycosidic bonds and polydispersity of their mannan moities. J. Gen. Microbiol. **134:** 2393–2403.
22. **Fox, J. F., U. Mayer, R. Nischt, M. Aumailley, R. Reinhardt, H. Wiedemann, K. Maun, R. Timpl, T. Krieg, J. Engel, and M. L. Chu.** 1991. Recombinant nidogen consists of three globular domains and mediates binding of laminin to collagen type IV. EMBO. J. **10:**3137–3146.
23. **Gil, M. L., M. Casanova, J. P. Martínez, and R. Sentandreu.** 1991. Antigenic cell wall mannoproteins in *Candida albicans* isolates and in other *Candida* species. J. Gen. Microbiol. **137:**1053–1061.
24. **Gilmore, B. J., E. M. Retsinas, J. S. Lorenz, and M. K. Hostetter.** 1988. An iC3b receptor on *Candida albicans*: structure, function, and correlates for pathogenicity. J. Infect. Dis. **157:**38–46.
25. **Hostetter, M. K.** 1994. Adhesins and ligands involved in the interaction of *Candida* spp. with epithelial and endothelial surfaces. Clin. Microbiol. Rev. **7:**29–42.
26. **Hostetter, M. K., J. S. Lorenz, L. Preus, and K. E. Kendrick.** 1990. The iC3b receptor on *Candida albicans*: subcellular localization and modulation of receptor expression by glucose. J. Infect. Dis. **161:**761–768.
27. **Jensenius, J. C., S. B. Laursen, Y. Zheng, and U. Holmoskov.** 1994. Conglutinin and CL-43, two collagenous C-type lectins (collectins) in bovine serum. Biochem. Soc. Trans. **22:**95–100.
28. **Klotz, S. A., and R. L. Penn.** 1987. Multiple mechanisms may contribute to the adherence of *Candida* yeast to living cells. Curr. Microbiol. **16:**119–122.
29. **Klotz, S. A., and R. L. Smith.** 1991. A fibronectin receptor on *Candida albicans* mediates adherence of the fungus to extracellular matrix. J. Infect. Dis. **163:**604–610.
30. **Klotz, S. A., R. L. Smith, and B. W. Stewart.** 1992. Effect of an arginine-glycine-aspartic acid-containing peptide on hematogenous candidal infections in rabbits. Antimicrob. Agents Chemother. **36:**132–136.
31. **Krieger, M.** 1992. Molecular flypaper and the atherosclerosis: structure of the macrophage scavenger receptor. Trends Biochem. Sci. **17:**141–146.
32. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227:**680–685.
33. **Langeveld, J. P. M., J. Wieslander, J. Timoneda, P. Mckinney, R. J. Bulkowski, B. J. Wisdom, and B. G. Hudson.** 1988. Structural heterogeneity of the noncollagenous domain of basement membrane collagen. J. Biol. Chem. **263:**10481–10488.
34. **Langeveld, J. P. M., A. W. T. Yem, J. Timoneda, J. Saus, B. J. Wisdom, and B. G. Hudson.** 1987. Molecular structure of collagen IV of glomerular basement membrane, p. 25–39. *In* R. G. Price and B. G. Hudson (ed.), Renal basement membrane in health and disease. Academic Press, London.
35. **Lee, K. L., H. R. Buckley, and C. C. Campbell.** 1975. An amino acid liquid synthetic medium for development of mycelial and yeast forms of *Candida albicans*. Sabouraudia **13:**148–153.
36. **López-Ribot, J. L., M. Casanova, J. P. Martínez, and R. Sentandreu.** 1991. Characterization of cell wall proteins of yeast and hydrophobic mycelial cells of *Candida albicans*. Infect. Immun. **59:**2324–2332.
37. **López-Ribot, J. L., M. Casanova, C. Monteagudo, P. Sepúlveda, and J. P. Martínez.** 1994. Evidence for the presence of a high-affinity laminin receptor-like molecule on the surface of *Candida albicans* yeast cells. Infect. Immun. **62:**742–746.
38. **López Ribot, J. L., and W. L. Chaffin.** 1994. Binding of the extracellular matrix component entactin to *Candida albicans*. Infect. Immun. **62:**4564–4571.
39. **López-Ribot, J. L., J. P. Martínez, M. Casanova, and W. L. Chaffin.** 1994. The C3d-receptor and the 58-kDa fibrinogen-binding mannoprotein of *C. albicans*: a comparative study, abstr. F40, p. 595. *In* Abstracts of the 94th General Meeting of the American Society for Microbiology 1994. American Society for Microbiology, Washington, D.C.
40. **Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall.** 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193:**265–275.
41. **Malhotra R. J., J. Haurum, S. Thiel, and R. B. Sim.** 1992. Interaction of C1q receptor with lung. Eur. J. Immunol. **22:**1437–1445.
42. **Marcantonio, E. E., and R. O. Hynes.** 1988. Antibodies to the conserved cytoplasmic domain of the integrin β1 subunit react with proteins in vertebrates, invertebrates, and fungi. J. Cell Biol. **106:**1765–1772.
43. **Martínez, J. P., J. L. López-Ribot, and W. L. Chaffin.** 1994. Heterogeneous surface distribution of the fibrinogen-binding protein on *Candida albicans*. Infect. Immun. **62:**709–712.
44. **Nègre, E., T. Vogel, A. Levanon, R. Guy, T. J. Walsh, and D. D. Roberts.** 1994. The collagen binding domain of fibronectin contains a high affinity binding site for *Candida albicans*. J. Biol. Chem. **269:**22039–22045.
45. **Olsen, B. R., and Y. Ninomiya.** 1993. Basement membrane collagens (type IV), p. 35–37. *In* T. Kreis and R. Vale (ed.), Guidebook to the extracellular matrix and adhesion proteins. Oxford University Press, Oxford.
46. **Poulain, D., V. Hopwood, and A. Vernes.** 1985. Antigenic variability of *Candida albicans*. Crit. Rev. Microbiol. **12:**223–270.
47. **Reid, K. B. M.** 1976. Isolation by partial pepsin digestion of the three collagen-like regions present in subcomponent C1q of the first component of human complement. Biochem. J. **155:**5–17.
48. **Sentandreu, R., J. P. Martínez, M. V. Elorza, and S. Mormeneo.** 1991. Relationships between dimorphism, cell wall structure and surface activities in *Candida albicans*, p. 72–88. *In* R. Prasad (ed.), *Candida albicans*: cellular and molecular biology. Springer-Verlag, New York.
49. **Tosh, F. D., and L. J. Douglas.** 1992. Characterization of a fucoside-binding adhesin of *Candida albicans*. Infect. Immun. **60:**4734–4739.
50. **Towbin, H., T. Staehelin, and J. Gordon.** 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. Proc. Natl. Acad. Sci. USA **76:**4350–4354.
51. **Tronchin, G., R. Robert, A. Bouali, and J. M. Senet.** 1987. Immunocytochemical localization of in vitro binding of human fibrinogen to *Candida albicans* germ tube and mycelium. Ann. Inst. Pasteur Microbiol. **138:**177–187.
52. **Turner, M. W.** 1994. Mannose binding proteins. Biochem. Soc. Trans. **22:** 88–94.
53. **Yu, L., K. K. Lee, K. Ens, P. C. Doig, M. R. Carpenter, W. Staddon, R. S. Hodges, W. Paranchych, and R. T. Irvin.** 1994. Partial characterization of a *Candida albicans* fimbrial adhesin. Infect. Immun. **62:**2834–2842.
54. **Yu, L., K. K. Lee, H. B. Sheth, P. Lane-Bell, G. Srivastava, O. Hindsgaul, W. Paranchych, R. S. Hodges, and R. T. Irving.** 1994. Fimbria-mediated adherence of *Candida albicans* to glycosphingolipid receptors on human buccal epithelial cells. Infect. Immun. **62:**2843–2848.