Antigenic cell wall mannoproteins in *Candida albicans* isolates and in other *Candida* species

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Polyclonal antibodies (pAbs) and monoclonal antibodies (mAbs), raised against mannoprotein components from *Candida albicans* ATCC 26555 (serotype A) blastoconidia and mycelial cell walls, were used to investigate antigenic similarities among wall mannoproteins from other *C. albicans* serotype A and B strains, and from *C. tropicalis* and *C. guilliermondii*. Radioactively labelled walls isolated from cells grown at either 28 °C or 37 °C were digested with a β -glucanase complex (Zymolyase 20T) to release cell-wall-bound mannoproteins. Numerous molecular species with different electrophoretic mobilities were released from the various isolates. Differences appeared to be related to both the organism and the growth temperature. Among the major protein components solubilized were mannoproteins larger than 100 kDa (high molecular mass mannoproteins), heterogeneous in size in most cases. Antigenic homology was detected among the cell wall high molecular mass mannoproteins of the two *C. albicans* serotype A isolates, whereas significant qualitative and quantitative differences were detected between serotype A and serotype B cell-wall-bound antigenic profiles. Moreover, *C. tropicalis* and *C. guilliermondii* wall antigenic determinants were not recognized by the preparations of pAbs and mAbs raised against *C. albicans* walls. A mannoprotein with a molecular mass of 33–34 kDa was present in the enzymic wall digests of all the organisms studied. When probed with pAbs raised against the protein moiety of the 33 kDa cell wall mannoprotein of *Saccharomyces cerevisiae*, antigenic cross-reactivity was observed in all cases except *C. tropicalis*. There appear to be significant antigenic differences between the mannoproteins of different isolates of *C. albicans*, and between those of *C. albicans* and other *Candida* species.

Introduction

Candida albicans is one of the most pervasive species of pathogenic fungi, being able to invade virtually all kinds of animal tissues and to opportunistically infect a wide variety of immunocompromised hosts (Bodey, 1984; Meunier, 1989; Odds, 1988). Among the diverse clinical manifestations of candidiasis, diagnosis of the disseminated form, which is a major cause of death in compromised patients (Meunier, 1989), is difficult as its symptoms are often non-specific (Meunier, 1989; Odds, 1988).

C. albicans is a dimorphic fungus which grows either by budding or by production of germ tubes. Although both morphologies (blastoconidia and mycelium) are found simultaneously in infected tissue, formation of

mycelial filaments is thought to play an important role in pathogenesis (Odds, 1988). In this context, as the cell wall constituents act as the most immediate elicitors of the host immune response during infection, detection of antigens which are expressed specifically or preferentially in the mycelial cell walls might be useful in the diagnosis of invasive candidiasis.

Analysis of antigen expression with polyclonal antibodies (pAbs) and monoclonal antibodies (mAbs) has revealed the complex antigenic composition of the surface of *C. albicans* cells and suggests that mannoproteins are the main antigenic cell wall components (Brawner & Cutler, 1984, 1986a, b; Casanova *et al.*, 1989; Chaffin *et al.*, 1988; Hopwood *et al.*, 1986; Poulain *et al.*, 1985; Sundstrom *et al.*, 1988). In addition to common components, antigenic determinants specific to the yeast cell wall (Chaffin *et al.*, 1988; Elorza *et al.*, 1985) and to the mycelium wall (Casanova *et al.*, 1989; Ponton & Jones, 1986; Sundstrom & Kenny, 1985) have been described. Moreover, studies with pAbs have

Abbreviations: ConA, concanavalin A; mAb, monoclonal antibody; pAbs, polyclonal antibodies; BpAbs, pAbs directed towards purified blastoconidia; MpAbs, pAbs directed towards purified mycelial walls.

shown that immunodeterminants may not be expressed in all strains or under all growing conditions in a single strain (Poulain *et al.*, 1985). Given that most of the methods currently available to diagnose the invasive forms of candidiasis are based on recognition of *Candida* surface antigens which are predominantly polysaccharide in nature (the carbohydrate component of mannoproteins, sometimes termed mannan), the variability of antigen expression may partially explain the poor specificity and sensitivity observed in such tests.

The present study was undertaken to extend previous observations (Casanova *et al.*, 1989; Elorza *et al.*, 1985) on the proteins and mannoproteins from various *Candida* spp. cell walls, solubilized by digestion of the structural network of the wall with Zymolyase (a 1,3- β -glucanase complex). Moreover, it was hoped that identification of common antigens among isolates would improve serological tests capable of identifying, in a fast and reliable manner, patients suffering invasive candidiasis.

Methods

Organisms used and maintenance of stock cultures. *C. albicans* DMFM1 (serotype A), DMFM2 (serotype B), *C. guilliermondii*, and *C. tropicalis*, all isolated from clinical specimens, were kindly provided by Dr J. M. Nogueira from the Departamento de Microbiología, Facultad de Medicina (DMFM), University of Valencia, Spain. The identity of isolates was established by biochemical (API 20C AUX system) and serological (Cand-Check, Iatron Laboratories, Tokyo, Japan) procedures. *C. albicans* ATCC 26555 (serotype A) was employed as a reference strain. Stock cultures of each organism were maintained by subculturing every 2–3 weeks on 1.5% (w/v) Bacto-Agar slopes of Sabouraud-dextrose medium.

Culture and labelling conditions. Organisms were propagated as blastoconidia (yeast phase) at 28 °C in a minimal medium supplemented with amino acids as described by Lee *et al.* (1975), harvested and stored at 4 °C for 72–96 h (starvation period) as reported previously (Casanova *et al.*, 1989). Starved blastoconidia were inoculated (200 μ g dry wt cells ml⁻¹) in fresh Lee medium at 28 °C to obtain cultures of blastoconidia or at 37 °C for the formation of blastoconidia bearing germ tubes (described as mycelia throughout) (Casanova *et al.*, 1989). For radioactive labelling of peptides, Lee medium was supplemented with 11.1 kBq U-¹⁴C-labelled protein hydrolysate ml⁻¹ (specific activity 2.07 GBq milliatom⁻¹).

Cell wall purification and protein solubilization. Cell walls from the respective species grown at either 28 °C or 37 °C were isolated and purified as described previously (Casanova *et al.*, 1989). During the purification process walls were boiled for 5 min with 2% (w/v) SDS in glass-distilled water, although for some experiments this step was omitted (see below). Purified walls were freeze-dried, and stored at –20 °C until used.

Proteins were solubilized from purified walls by treatment with Zymolyase 20T, a β -glucanase complex, as described by Casanova *et al.* (1989). After treatment, the insoluble wall residue was removed by centrifugation (1200 g, 15 min) and discarded, and the solubilized material concentrated by freeze-drying. The total sugar and protein content in the lyophilized soluble fraction was determined by the method of Dubois *et al.* (1956) and by the Lowry method respectively.

Radioactivity was measured using a Beckman LS-7500 scintillation counter in samples adsorbed to Whatman GF/C glass-fibre filters, which were placed in a toluene-based scintillation fluid designed for dried samples.

PAGE and Western blot techniques. Mannoproteins solubilized after enzymic digestion of the walls were separated by SDS-PAGE as described by Laemmli (1970) with minor modifications as reported previously (Casanova *et al.*, 1989). Electrophoretic transfer (Western blot) to nitrocellulose paper (Bio-Rad) was done as described by Burnette (1981) except that the transfer buffer used contained 0.025 M-Tris/0.192 M-glycine (pH 8.3) and 20% (v/v) methanol. Electrophoretic transfer was done at 6–8 V cm⁻¹ for 15–17 h at 4 °C in a Trans-Blot cell (Bio-Rad) using an LKB 2197 power supply. Immunochemical detection of proteins on blots was performed using the Bio-Rad Immun-Blot (GAR-HRP) assay kit (Burnette, 1981; Towbin *et al.*, 1979). Polyclonal rabbit antisera enriched for mannoprotein antibodies directed toward purified blastoconidia (BpAbs) or mycelial walls (MpAbs) of *C. albicans* ATCC 26555, and a mouse monoclonal antibody (mAb 4C12) raised against a mannoprotein band from mycelium having an average apparent molecular mass of 260 kDa, were prepared as reported previously (Casanova *et al.*, 1989) and used as probes. Antibodies were used at a final concentration of 1:500 for pAbs or 1:3000 for mAbs. Diluted (1:2000) peroxidase-labelled goat anti-rabbit or goat anti-mouse IgG (Bio-Rad) were used as indicator antibodies with 4-chloro-1-naphthol as the chromogenic reagent. Indirect concanavalin A (Con A)-mediated peroxidase staining of mannoproteins in nitrocellulose blots was done according to Hawkes (1982) with the modifications of Millette & Scott (1984). For fluorography, the gels were treated with Amplify (Amersham), dried, and exposed for appropriate times on Kodak X-Omat films at –70 °C.

Indirect immunofluorescence. The immunofluorescence assay was done as described by Casanova *et al.* (1989).

Chemicals. Radioactively labelled protein hydrolysate was obtained from Amersham, gel electrophoresis and blotting reagents were from Bio-Rad and Zymolyase 20T was from Miles Laboratories. SDS molecular mass markers were from Sigma and Pharmacia. All other chemicals used were from Sigma.

Results

Effect of incubation temperature on growth of the various Candida strains and species

Incubation of the *C. albicans* strains and other *Candida* species used in the present study was done according to the procedure currently used in our laboratory to induce germ-tube formation in *C. albicans* ATCC 26555 (Casanova *et al.*, 1989; Elorza *et al.*, 1983). Formation of mycelia was observed following resumption of growth of starved blastospores at 37 °C only in serotype A isolates of *C. albicans*; as assessed by phase-contrast light microscopy, after 6 h at 37 °C, more than 75% of the yeast cells from *C. albicans* strains ATCC 26555 (Fig. 1a) and DMFM1 (Fig. 1c) possessed well-defined mycelial tubes whose surface was specifically labelled by mAb 4C12, as revealed by indirect immunofluorescence (Fig. 1b and 1d respectively). In contrast, starved blastospores of *C. albicans* strain DMFM2 (serotype B) (Fig. 1e), *C.*

Fig. 1. Phase-contrast microscopy (a, c, e, f, g) and immunofluorescence (adsorbed mAb 4C12; 1:400 dilution) (b, d) of intact cells of *C. albicans* ATCC 26555 (a, b), *C. albicans* DMFM1 (c, d), *C. albicans* DMFM2 (e), *C. guilliermondii* (f) and *C. tropicalis* (g) grown under conditions that induce myceliation (see Methods). Bars, 10 µm.

guilliermondii (Fig. 1f) and *C. tropicalis* (Fig. 1g) did not form germ tubes when incubated under the same conditions. In all cases, starved yeast cells resumed growth at 28 °C giving rise to homogeneous populations of blastospores.

Effect of Zymolyase treatment on the solubilization of radioactively labelled molecules from isolated walls

Purified walls obtained from cells of the five organisms

studied, grown either at 28 °C or 37 °C in the presence of a mixture of radioactive amino acids, contained 2–4% of the total cell radioactivity. These values are in agreement with those reported in similar experiments done with other yeast species (Herrero *et al.*, 1987).

Zymolyase treatment of walls isolated from *C. albicans* strains ATCC 26555, DMFM1 and DMFM2 and *C. guilliermondii* released similar amounts of radioactivity (values obtained differed by less than 10–12%) regardless of the temperature of incubation (Table 1). However, the

Fig. 2. Proteins extracted by Zymolyase from purified walls (boiled for 5 min with 2% w/v, SDS in glass-distilled water; see Methods) of cells of *C. albicans* ATCC 26555 (lanes 1, 2), *C. albicans* DMFM1 (lanes 3, 4), *C. albicans* DMFM2 (lanes 5, 6), *C. guilliermondii* (lanes 7, 8) and *C. tropicalis* (lanes 9, 10), grown at 28 °C or 37 °C as shown. Solubilized components were analysed by SDS-PAGE (5–15%, w/v, acrylamide slab gradient gels) and fluorography (30000 c.p.m. per well). The electrophoretic mobility of standard proteins of known molecular mass is indicated on the left of the figure. The arrowhead (►) indicates the absence from these electrophoretic patterns of the 33–34 kDa band, as a result of boiling with SDS prior to zymolyase treatment (see text).

solubilizing efficiency of Zymolyase was significantly lower on walls from *C. tropicalis* cells grown at 37 °C (Table 1). These results suggest that there are quantitative and/or qualitative changes in the amounts of structural polymers not susceptible to Zymolyase degradation, such as chitin and/or 1,6- β -glucan, or differences in the way the wall constituents are spatially organized, in the walls of *C. tropicalis* cells grown at 37 °C.

Identification of proteins solubilized by Zymolyase digestion of the walls

The material released by Zymolyase from isolated radioactively labelled walls was analysed by SDS-PAGE and fluorography (Fig. 2). All the organisms studied, grown both at 28 °C and 37 °C, produced conspicuous

proteinaceous components with molecular masses >100 kDa. This material exhibited a marked polydispersity that was specially noticeable in the case of *C. guilliermondii* (Fig. 2, lanes 7 and 8) and *C. tropicalis* (Fig. 2, lanes 9 and 10). The components solubilized from the *C. albicans* strains examined (Fig. 2, lanes 1–6) gave rise to more well-defined bands of higher molecular mass than those from *C. guilliermondii*.

Although intrinsic differences were observed among the isolates, significant variations in the electrophoretic pattern with respect to the high molecular mass material appeared to be related to growth conditions. Thus, material released by Zymolyase from the walls of all strains except *C. tropicalis* (Fig. 2, lane 10) grown at 37 °C had a greater electrophoretic mobility (Fig. 2, lanes 2, 4, 6 and 8) than that obtained from cells grown at 28 °C

Table 1. Solubilization by Zymolyase of wall proteins from isolated cell walls of *Candida* spp.

Radioactively labelled walls obtained from exponentially growing cells incubated in the presence of U-¹⁴C-labelled protein hydrolysate were digested with Zymolyase as described in Methods (samples contained in all cases about 10000 c.p.m.). Values are expressed with respect to total radioactivity in purified walls before treatment and are means of two experiments; SD values differed by less than 10%.

Organism	Percentage of cell wall radioactivity solubilized by Zymolyase	
	Cells grown at:	
	28 °C	37 °C
<i>C. albicans</i> ATCC 26555	67	69
<i>C. albicans</i> DMFM1	46.7	51.6
<i>C. albicans</i> DMFM2	62.3	57
<i>C. guilliermondii</i>	71	63
<i>C. tropicalis</i>	61.5	30

(Fig. 2, lanes 1, 3, 5 and 7). This was particularly evident in the case of the two *C. albicans* serotype A strains (ATCC 26555 and DMFM1) (Fig. 2, lanes 2 and 4 respectively). The existence of form-specific high molecular mass wall mannoproteins in *C. albicans* has been reported previously (Casanova *et al.*, 1989; Elorza *et al.*, 1985). Interestingly, the electrophoretic patterns of the material solubilized by Zymolyase from the walls of *C. albicans* ATCC 26555 (Fig. 2, lane 2) and DMFM1 (Fig. 2, lane 4) cells grown at 37 °C were basically similar, though differences in the concentration of individual components were evident. As stated previously, these strains were the only ones able to form true mycelial filaments (Fig. 1a, c).

In the medium-to-low molecular mass range a great assortment of polypeptide bands (from 80 to 14 kDa) appeared in the fluorographs (Fig. 2; Fig. 3a). Again, marked intrinsic and growth-dependent differences in polypeptide composition were evident. However, a band of molecular mass 33–34 kDa was consistently detected in all cases (Fig. 3a, lanes 1–10, →). This molecule seemed to be bound to the wall structure through low-strength bonds, since boiling the isolated walls with 2% (w/v) SDS prior to Zymolyase treatment caused its disappearance from the electrophoretic patterns (Fig. 2, ►). The reactivity towards Con A (Fig. 3b) indicated the mannoprotein nature of this molecule. The presence of this or a similar mannoprotein has been already reported for *C. albicans* ATCC 26555 (Elorza *et al.*, 1985), *Saccharomyces cerevisiae* (Pastor *et al.*, 1984; Sanz *et al.*, 1987), and *Hansenula wingei* and *Kluyveromyces lactis* (Herrero & Boyd, 1986; Herrero *et al.*, 1987).

In the present work, we have extended these observations to other *C. albicans* strains and *Candida* species and

found that the pAbs (Sanz *et al.*, 1987) against the 31.5 kDa protein moiety (Fig. 3c, lane 11, marked ►) of the 33 kDa mannoprotein from *S. cerevisiae* (Fig. 3c, lane 11, marked ►) gave a significant reaction with the 33–34 kDa molecule of *C. albicans* strains DMFM1 (Fig. 3c, lanes 3 and 4) and DMFM2 (Fig. 3c, lanes 5 and 6), and *C. guilliermondii* (Fig. 3c, lanes 7 and 8), though no reactivity was observed with the analogous 33–34 kDa molecule from *C. tropicalis* walls (Fig. 3c, lanes 9 and 10).

Western blot (immunoblot) analyses of wall proteins solubilized by Zymolyase

Wall proteins and mannoproteins solubilized by Zymolyase were separated by SDS-PAGE, blotted to nitrocellulose paper, and immunodetected using as probes pAbs and a mAb raised against wall components of mycelial (MpAbs and mAb 4C12) and yeast (BpAbs) cells of *C. albicans* ATCC 26555 (Casanova *et al.*, 1989).

BpAbs reacted with similar antigens in the two serotype A strains (ATCC 26555 and DMFM1) of *C. albicans* grown at 28 °C (Fig. 4a, lanes 1 and 2), the major common antigens detected having molecular masses of 650, 500, 200 and 67 kDa. However, the 650, 500 and 67 kDa antigens did not appear in the immunoblot pattern of strain DMFM2 (Fig. 4a, lane 3), although large amounts of polydisperse material were detectable by autoradiography in the very high molecular mass range (Fig. 2, lane 5). Instead, there were reactive bands in the range 200–500 kDa (Fig. 4a, lane 3); a 260 kDa antigen was particularly noticeable, and was also detected in the enzymic wall digests from strain DMFM1 (Fig. 4a, lane 2) but not in those from strain ATCC 26555 (Fig. 4a, lane 1). A very weak and diffuse reactivity of BpAbs was observed against wall proteins from *C. tropicalis* (Fig. 4a, lane 5), whereas no reactivity was detected in the case of *C. guilliermondii* (Fig. 4a, lane 4).

Immunodetection with MpAbs (Fig. 4b) gave similar results. Thus, the pattern of antigens exhibiting reactivity towards this antiserum was very similar for the two *C. albicans* serotype A strains (Fig. 4b, lanes 1 and 2), whereas a weak and qualitatively different reactivity was observed with the serotype B strain (Fig. 4b, lane 3), all growing at 37 °C. The general pattern of reactive bands against the MpAbs preparation shown in Fig. 4(b), lanes 1 and 2, is basically similar to that previously reported for *C. albicans* ATCC 26555 (Casanova *et al.*, 1989). MpAbs did not cross-react with the material solubilized by Zymolyase from the walls of *C. guilliermondii* (Fig. 4b, lane 4) and *C. tropicalis* (Fig. 4b, lane 5) growing at 37 °C.

Finally, mAb 4C12, raised against a *C. albicans* ATCC 26555 mycelium-specific wall mannoprotein band with an apparent molecular mass of 260 kDa (Casanova *et al.*, 1989), reacted with its homologous antigen and with a

Fig. 3. Proteins extracted by Zymolyase from isolated walls (in these experiments boiling for 5 min with 2%, w/v, SDS in glass-distilled water was omitted during the process of wall purification; see Methods) of cells of *C. albicans* ATCC 26555 (lanes 1, 2), *C. albicans* DMFM1 (lanes 3, 4), *C. albicans* DMFM2 (lanes 5, 6), *C. guilliermondii* (lanes 7, 8) and *C. tropicalis* (lanes 9, 10), grown at 28 °C or 37 °C as shown. Solubilized components were analysed by SDS-PAGE (5–15%, w/v, acrylamide slab gradient gels) followed by fluorography (30000 c.p.m. per well; *a*), or Western blotting followed by staining with Con A-peroxidase (*b*), or immunodetection with pAbs against the peptide moiety of the 33 kDa wall mannoprotein of *S. cerevisiae* (*c*). In (*a*), (*b*) and (*c*) arrows (→) on the left indicated a mannoprotein ranging in size from 33–34 kDa that was present in the enzymic wall digests of the organisms studied. A mixture of the 33 kDa species from *S. cerevisiae* (►) and its deglycosylated 31.5 kDa form (▷) was run in parallel in lane 11. Only the relevant regions of the fluorograph and the blots are shown.

180 kDa band (Fig. 5, lane 2), and with two antigens with apparent molecular masses of 220 and 280 kDa (Fig. 5, lane 4) released by Zymolyase from the walls of strain DMFM1 growing at 37 °C (myceliation conditions). No reactivity of mAb 4C12 with the remaining antigenic preparations was observed (Fig. 5, lanes 1 and 3, 5–10).

Discussion

The antigenic variability of the *C. albicans* cell wall appears to be dependent on multiple environmental factors such as length of incubation or growth conditions and organism-related factors such as growth state,

Fig. 4. Western blots of 5–15% (w/v) slab gradient gels loaded with Zymolyase-released material (about 400 µg per well, expressed as total sugar content in the sample subjected to electrophoresis) from purified wall preparations (boiled in 2% w/v, SDS for 5 min) of cells of *C. albicans* ATCC 26555 (lanes 1), *C. albicans* DMFM1 (lanes 2), *C. albicans* DMFM2 (lanes 3), *C. guilliermondii* (lanes 4) and *C. tropicalis* (lanes 5), grown at 37 °C (a) or 28 °C (b). Blots were stained with BpAbs (a) or MpAbs (b). Only the relevant region of the blots are shown. The electrophoretic mobility of standard proteins of known molecular mass (in kDa) is indicated on the left of the figure.

morphology of the cells or serotype (Brawner & Cutler, 1984, 1986a, b; Brawner *et al.*, 1990; Chaffin *et al.*, 1988; Hopwood *et al.*, 1986; Poulain *et al.*, 1985; Sundstrom *et al.*, 1987, 1988). Unfortunately, in most cases the individual surface constituents containing the antigenic determinants have not been identified.

In the present work we have used the methodology currently employed in our laboratory (Casanova *et al.*, 1989; Elorza *et al.*, 1985; Herrero *et al.*, 1987; Pastor *et al.*, 1984) to characterize intrinsic wall proteins and mannoproteins in cells of *C. albicans* strains and other *Candida* species grown in a defined synthetic medium under controlled incubation conditions. Significant differences in the overall pattern of wall-bound proteins were observed, not only between the separate species (*C. albicans*, *C. guilliermondii* and *C. tropicalis*), but also

between serotypes A and B of *C. albicans*. In addition to indigenous differences between organisms that were evident when wall polypeptides from cells grown at 28 °C (blastospores in all cases) were compared, temperature-induced and morphology-related differences were also detected by fluorography (Fig. 2). Firstly, there was an increase in the electrophoretic mobility of the high molecular mass material solubilized by Zymolyase from the walls of cells grown at 37 °C (myceliation conditions), which in turn may reflect changes in the glycosylation levels of these molecules and/or the way they are linked to other wall constituents. Secondly, different protein species were detected. As already reported (Casanova *et al.*, 1990; Elorza *et al.*, 1989) some of the high molecular mass mannoproteins appear to play an essential role in the construction of the wall specific for mycelial

Fig. 5. Western blot of 5–15% (w/v) slab gradient gels loaded with Zymolyase-released material (about 400 µg per well expressed as total sugar content in the sample subjected to electrophoresis) from purified wall preparations (boiled in 2%, w/v, SDS for 5 min) of cells of *C. albicans* ATCC 26555 (lanes 1, 2), *C. albicans* DMFM1 (lanes 3, 4), *C. albicans* DMFM2 (lanes 5, 6), *C. guilliermondii* (lanes 7, 8) and *C. tropicalis* (lanes 9, 10), grown at 28 °C or 37 °C as shown. The blot was stained with mAb 4C12 (see Methods). The electrophoretic mobility of standard proteins of known molecular mass is indicated on the left of the figure.

morphology. In support of this contention, the two *C. albicans* serotype A strains examined in the present study had a closely similar cell-wall-bound protein and mannoprotein composition at 37 °C (Fig. 2).

Attempts to characterize surface antigens in *C. albicans* have shown heterogeneity in expression of immunodeterminants not only among cells of different strains but also among cells in a population (Chaffin *et al.*, 1988; Poulain *et al.*, 1985). This variability, in addition to the cross-reactivity that exists between antibodies prepared against surface immunodeterminants of *C. albicans* and antigens present in other *Candida* species and fungi (Brawner & Cutler, 1984; Chaffin *et al.*, 1988; Elorza *et al.*, 1985; Herrero *et al.*, 1987; Kumar *et al.*, 1985; Miyakawa *et al.*, 1986; Poulain *et al.*, 1985; Sundstrom *et al.*, 1988; this work), would partially explain the difficulties experienced in defining surface antigens.

The preparations of BpAbs and MpAbs used in this

work appear to contain antibodies against both protein and carbohydrate immunodeterminants present in the yeast and mycelial walls of a common serotype A laboratory strain of *C. albicans* (ATCC 26555) (Casanova *et al.*, 1989; unpublished observations). Hence, the great differences observed in cell wall antigenic composition among serotypes of *C. albicans* and *Candida* species using such pAbs indicate the existence of strain-specific epitopes that require further characterization.

Studies with mAbs suggest that surface antigenic variability in *C. albicans* is, at least in part, the result of heterogeneity in surface carbohydrates (Brawner & Cutler, 1984, 1986a, b; Chaffin *et al.*, 1988; Hopwood *et al.*, 1986; Miyakawa *et al.*, 1986; Sundstrom *et al.*, 1988). Results reported here are in partial agreement with these observations. The mAb 4C12, which was used as a probe in the present study, recognizes an epitope present in an *O*-glycosylated protein which is secreted by mycelial protoplasts (Elorza *et al.*, 1989) and solubilized from the wall of mycelial cells forming part of two complexes of different molecular masses (Casanova *et al.*, 1989; Elorza *et al.*, 1989). Differences in the electrophoretic mobility of these two complexes is due to the contaminating proteolytic activity present in the Zymolyase preparation (Elorza *et al.*, 1989). Nevertheless, in other cases differences in the molecular mass of species recognized by mAb 4C12 (Fig. 5) may also reflect differences in the glycosylation levels of the polypeptide carrying the epitope recognized by the mAb, and/or the existence of distinct types of linkages binding the polypeptide moieties to the wall with different sensitivities to digestion with β -glucanases. In this respect, phenotypic colonial switching in *C. albicans* is a phenomenon that is thought to modify the antigenic characteristics of the cell surface (Slutsky *et al.*, 1985), and it might be associated with changes in the degree of glycosylation in high molecular mass mannoproteins (Martínez *et al.*, 1990).

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