Wall Mannoproteins of the Yeast and Mycelial Cells of *Candida albicans*: Nature of the Glycosidic Bonds and Polydispersity of Their Mannan Moieties

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Zymolyase released between 20 and 25% of the total protein from purified walls of yeast (Y) and mycelial (M) cells of *Candida albicans*. The material released contained 92% carbohydrate (86% mannose and 6% glucose) and 7% protein. Over 85% of the carbohydrate was *N*-glycosidically linked to the protein and the rest (less than 15%) was linked *O*-glycosidically. Highly polydisperse, high molecular mass mannoproteins, resolved by electrophoresis as four defined bands in Y cells and two bands in M cells, had both types of sugar chains. A 34 kDa species found in both types of cells had a single 2.5 kDa *N*-glycosidically linked sugar chain and a 31.5 kDa protein moiety. Polydispersity in the high molecular mass mannoproteins was due to the *N*-linked sugar chains (mannan) with a molecular mass between 500 kDa and 20 kDa (average 100 kDa) in Y cells and between 400 kDa and 20 kDa (average 50 kDa) in M cells. Three mannoproteins of 34, 30 and 29 kDa secreted by protoplasts were associated with the high molecular mass mannoproteins, suggesting that this type of interaction might be related to the regeneration of the cell wall.

INTRODUCTION

Candida albicans is a dimorphic opportunistic pathogen that grows as a yeast (Y) or as a mycelial (M) fungus depending on the environmental conditions. The Y form is isolated when the organism is growing saprophytically in the intestinal ducts, but both forms are found in infected tissues. In this case formation of M cells is thought to be an important factor in adherence of the parasite to the host epithelium (Lee & King, 1983; Sobel *et al.*, 1984) and avoidance of ingestion by phagocytic cells (Smith, 1985).

To understand the mechanism of the dimorphic change and its relation to pathogenesis, it is important to understand the wall structure and also to know if the modifications occurring during the morphological change are the result of the preferential expression of cell wall mannoproteins as indicated by antigen analysis (Sundstrom & Kenny, 1984; Ponton & Jones, 1986; Brawner & Cutler, 1986).

β -Glucans, chitin and mannoproteins have been reported to be the most abundant components of the walls of *C. albicans* (Chattaway *et al.*, 1968; Sullivan *et al.*, 1983) and while glucans are insoluble, the mannoproteins are easily extracted on partial degradation by alkaline reagents (Kolarova *et al.*, 1973). We have described the solubilization of wall mannoproteins from several ascomycetous and non-ascomycetous yeast following digestion of the glucan skeleton with Zymolyase (Pastor *et al.*, 1984; Herrero *et al.*, 1987). Two main components are released: a polydisperse high molecular mass mannoprotein material (MPM, > 120 kDa) and a

Abbreviations: Y, yeast; M, mycelial; MPM, mannoprotein material; PMSF, phenylmethylsulphonyl fluoride; Endo H, endo- β -*N*-acetylglucosaminidase; ConA, concanavalin A.

mannoprotein of 31 to 34 kDa depending on the species studied. In *C. albicans* the MPM was resolved in four bands when obtained from the Y cells and in only two in the case of M cells. In addition, the walls of the two types of cells have a mannoprotein of 34 kDa which was originally characterized as 30 kDa (Elorza *et al.*, 1985). In the present work the type of carbohydrate linkages and the polydispersity of the sugar chains present in the mannoproteins solubilized by Zymolyase from both cellular forms of *C. albicans* were analysed and compared with the material secreted by protoplasts during the regeneration process.

METHODS

Strain. The organism used was *C. albicans* ATCC 26555. It was maintained by subculturing every 2–3 weeks on Sabouraud dextrose agar and propagated in the medium of Lee *et al.* (1975).

Cell wall preparation. *C. albicans* blastospores were grown to early stationary phase at 28 °C in 100 ml Lee medium on a gyratory shaker at 200 r.p.m. The cells were collected after 22 h, washed twice with cold distilled water and resuspended in water. The suspension was then aerated for 3 h and stored at 4 °C for 16–22 h. To obtain yeast cells the starved cells were inoculated in Lee medium (1 mg ml⁻¹) and incubated at 28 °C in a shaker, whereas to induce mycelial cells, the suspension kept at 4 °C was diluted in prewarmed medium at 37 °C to give a final concentration of 1 mg ml⁻¹ and incubated with shaking at 37 °C. In both cases, cells were collected from the culture medium by centrifugation at 3000 g for 10 min, washed twice with distilled water at 0 °C and resuspended in a small volume of 0.001 M-phenylmethylsulphonyl fluoride (PMSF). Glass beads (0.45–0.50 mm) were added in a proportion of 100 mg per mg dry cells and the cells were disrupted in a tube maintained at an angle of 45° by heavily shaking at the top speed of a vortex mixer (Super-Mixer, Lab Line Instruments) for 2 min. Walls were sedimented (1200 g, 10 min) and washed eight times in 0.001 M-PMSF. Cell rupture was assessed by examination of the preparation under a phase-contrast microscope.

Preparation of walls and spent medium from regenerating protoplasts. Protoplasts were obtained and regenerated as previously described (Elorza *et al.*, 1983). Protoplasts were separated from the spent regeneration medium by centrifugation (1200 g, 10 min), resuspended in 0.001 M-PMSF, broken by shaking with glass beads in a vortex mixer and the walls were sedimented (1200 g, 10 min) and washed eight times with PMSF.

Wall labelling with ¹⁴C-labelled protein hydrolysate and [¹⁴C]mannose. Cells were suspended (0.5 mg dry wt ml⁻¹) in 50 ml Lee medium containing 10 µCi (370 kBq) ¹⁴C-labelled protein hydrolysate [specific activity 56 mCi (2072 MBq) per mg-atom carbon] and incubated at 28 °C or 37 °C. Protoplasts, adjusted to an OD₆₀₀ of 0.22, were suspended in 50 ml of Lee medium supplemented with 0.6 M-KCl and 10 µCi ¹⁴C-labelled protein hydrolysate and incubated at the temperature described in each experiment. In some experiments, tunicamycin (20 µg ml⁻¹) was added to the incubation medium to inhibit N-glycosylation. For labelling with [¹⁴C]mannose, cells were resuspended in Lee medium supplemented with 1% (w/v) casein hydrolysate, and 1.2% (w/v) galactose and 11.1 Bq ml⁻¹ [¹⁴C]mannose (11.1 GBq mol⁻³) as carbon source. Addition of galactose was necessary to reduce the interconversion of mannose to glucose (Farkaš *et al.*, 1974).

Solubilization of wall mannoproteins and endo-β-N-acetylglucosaminidase (Endo H) treatment. Labelled walls from 30 mg cells were initially treated with 0.05 M-dithiothreitol and incubated at 30 °C for 30 min. Walls were separated by centrifuging at 1200 g for 10 min and then washed in 1 mM-PMSF, resuspended in the same solution containing Zymolyase (1 mg ml⁻¹) and incubated at 30 °C for 2.5 h. The suspension was then centrifuged (1200 g, 10 min) and the radioactivity in the supernatant measured. Mannoproteins in the supernatants were concentrated by freeze drying, resuspended in 100 µl double distilled water and prepared for polyacrylamide gel electrophoresis (PAGE). Treatment with Endo H, an enzyme that cleaves the high mannose structures present in glycoproteins, was done as described by Pastor *et al.* (1984). The wall mannoproteins were resuspended in 10 µl 0.05 M-citrate buffer pH 4.5 and 0.4% SDS and boiled for 2 min, and then 4 mU of Endo H in 10 µl 0.05 M-citrate buffer pH 5.5 without SDS added to the suspension. This was made up to 1 mM and incubated for 18 h at 37 °C. [One unit (U) of Endo H is that amount of enzyme which releases 1 µmol dansyl-Asp(GlcNAc)₄(Man)₆ min⁻¹ at 37 °C under the assay conditions described.]

Preparation of N-glycosidically linked sugar chains (mannan). Walls (100 mg dry wt) from Y and M cells were extracted with 2 M-NaOH at 100 °C for 90 min. The alkaline supernatant was treated with Fehling solution (Haworth *et al.*, 1941) to precipitate mannan. The precipitate was collected, washed with hot water, solubilized with 0.5 M-HCl and precipitated with 3 vols ethanol.

Concanavalin A (ConA)-Sepharose binding. ConA-Sepharose (10 ml) as supplied by the manufacturer was washed four times with 0.05 M-Tris/HCl buffer (pH 7.5) containing 0.25 M-CaCl₂ and 1 mM-MgCl₂. A column (1 × 12 cm) was partially filled with the resuspended particles and washed with 10 vols of the same buffer. Samples obtained from the digestion of walls (50 mg dry wt) with Zymolyase were added to the ConA-Sepharose column and washed with 50 ml buffer. Mannoproteins retained by the lectin were eluted with 0.5 M-methyl α-D-mannoside, dialysed and freeze dried.

Gel electrophoresis of mannoproteins. Samples of protein (80 µg) were run in PAGE as described previously (Herrero *et al.*, 1982). In experiments with labelled proteins, constant radioactivity was loaded for each sample. In all the electrophoresis experiments, the following molecular mass standards were run in parallel: lysozyme (14 kDa), β -lactoglobulin (18.4 kDa), ovalbumin (45 kDa), bovine serum albumin (BSA, 66 kDa), phosphorylase b (97 kDa), β -galactosidase (116 kDa) and myosin (205 kDa).

Fluorography of gels. Fluorography of the radioactive gels was done as described Murgui *et al.* (1985).

ConA-peroxidase staining of mannoproteins. Proteins separated in acrylamide gels were blotted onto nitrocellulose membranes by the method of Towbin *et al.* (1979) and mannoproteins were detected by indirect ConA-mediated peroxidase staining, using the protocol described by Millette & Scott (1984).

β -Elimination and paper chromatography. β -Elimination of the carbohydrate moiety of [14 C]mannose-labelled mannoproteins was done in 0.1 M-NaOH at 22 °C for 24 h. The released sugars were separated by descending paper chromatography in ethyl acetate/butanol/acetic acid/water (3:4:2.5:4, by vol.) and the radioactivity was measured as described by Sentandreu & Northcote (1968). The radioactivity remaining at the origin was considered to be due to *N*-glycosidic bonds that were resistant to β -elimination.

β -Elimination of the mannoproteins labelled with 14 C-labelled protein hydrolysate was done at 4 °C in the presence of 0.4 M-sodium borohydride for 24 h. The excess of sodium borohydride was destroyed by the addition of 0.5 M-acetic acid and the solution desalted by passing through a Sephadex G-25 column (5 × 1 cm).

Analytical gel filtration of mannoproteins. Samples resuspended in 0.01 M-sodium phosphate buffer pH 7.0 plus 0.05 M-NaCl were passed through a Bio-Gel A-1.5 m column (1.6 × 90 cm) or Sephacryl S-300 column (1.6 × 100 cm) previously equilibrated with the above buffer. Elution was carried out at a flow rate of 15 ml h⁻¹ and 1.8 ml volume fractions were collected. Dextran, invertase, BSA and lysozyme were used as molecular mass markers and chromatographed separately on the same column under identical conditions.

Analytical methods. Total sugars were determined by the phenol/sulphuric acid method of Dubois *et al.* (1956) using D-glucose as standard. Hydrolysis of the carbohydrate moiety (mannan) of mannoproteins was done in 2 M-HCl at 100 °C for 2 h following neutralization with Dowex AG 1 × 8 resin. Glucose was measured using the glucose oxidase method of Lloyd & Whelan (1969). Invertase activity was assayed essentially as described by Gascon & Lampen (1968). Protein was estimated by the method of Lowry *et al.*

Chemicals. Methyl α -D-mannoside, ConA-Sepharose 4B, tunicamycin and protein standards were obtained from Sigma. Zymolyase 20T and Endo H were purchased from Miles Laboratories. 14 C-Labelled protein hydrolysate and [14 C]mannose were obtained from, respectively, Amersham and the Commissariat à l'Energie Atomique, centre d'Études Nucleaires, Gif-sur-Yvette (France).

RESULTS

Effect of Zymolyase on Y and M cell walls

When walls purified from Y and M cells grown in a medium with 14 C-labelled protein hydrolysate were digested with Zymolyase (mainly a β -glucanase complex), 20–25% of the radioactivity of the walls (2% of that of the whole cells) was solubilized. Analysis of the material retained in a ConA-Sepharose column showed that the MPM released from both types of walls contained mainly carbohydrate (92%) and protein (7%). Mannose was the main sugar present (86%) but there were also significant amounts of glucose (6%).

Linkages present in the wall mannoproteins

To obtain information on the nature of the carbohydrate linkages present in the mannoprotein molecules, MPM obtained from cells labelled with [14 C]mannose was treated with 0.1 M-NaOH (β -elimination) and the mobility of the radioactive sugars released was analysed by paper chromatography. About 88% of the radioactivity in the case of Y cells and 86% in the case of mycelial cells remained at the origin of the chromatogram whereas the rest (Table 1) ran as authentic mannose, mannobiose and mannotriose (results not shown in detail).

These results indicated that most of the wall carbohydrate was linked *N*-glycosidically to protein whereas the residual carbohydrate was *O*-glycosidically linked. Additional information on the existence of both types of linkages was obtained when the β -elimination was done with the MPM obtained from cells grown in the presence of tunicamycin. The percentage of radioactivity remaining at the origin of the chromatogram was reduced to 7% (Table 1), confirming that the bulk of the sugar chains present in the wall mannoproteins were joined *N*-glycosidically.

In order to determine the type of carbohydrate-protein linkages present in individual mannoproteins, the material released by Zymolyase from Y and M cell walls labelled with 14 C-

Table 1. *Distribution of radioactivity in the form of N- and O-glycosidically linked sugar chains*

Exponentially growing cells grown in the presence of [^{14}C]mannose at 28 °C (Y cells) and at 37 °C (M cells) and in either the absence or the presence of tunicamycin (20 $\mu\text{g ml}^{-1}$) were broken and the cell walls obtained. Purified walls were digested with Zymolyase and the MPM collected. Protoplasts obtained from Y and M cells were incubated as the whole cells supplemented with 0.6 M-KCl. After 5 h the protoplasts were separated from the spent medium by centrifugation. Both the MPM and the spent medium of partially regenerated protoplasts were β -eliminated and the released sugars analysed by paper chromatography. The values shown are the means of three experiments; the SD values were never higher than $\pm 10\%$.

	Tunicamycin	Radioactivity			
		N-linked sugars		O-linked sugars	
		$10^{-2} \times$ Amount incorporated (c.p.m.)	Percentage of total	$10^{-2} \times$ Amount incorporated (c.p.m.)	Percentage of total
MPM (Y cells)	—	220.6	89.2	27.9	11.2
	+	26.6	9.4	255.5	90.6
MPM (M cells)	—	307.7	86.6	48.0	13.3
	+	26.5	6.7	366.9	93.2
Protoplasts spent medium (Y)	—	127.5	89.2	15.4	10.7
	+	0.8	1.2	68.8	98.8
Protoplasts spent medium (M)	—	232.6	91.0	22.8	8.9
	+	2.3	2.6	84.1	97.3

labelled protein hydrolysate was analysed by PAGE before and after treatment with Endo H. Four bands (of 160 kDa, 205 kDa and higher molecular mass) and another of 34 kDa were observed before Endo H treatment (Fig. 1*a*). Following Endo H addition, the high molecular mass mannoproteins were still present whereas the 34 kDa band disappeared and a new one with a molecular mass of 31.5 was detected (Fig. 1*b*). Modification of the experimental conditions (concentration of Endo H and time of incubation) did not result in any further changes to the band pattern shown in Fig. 1*b*, indicating that the 34 kDa mannoprotein consists of a 31.5 kDa protein with a single 2.5 kDa carbohydrate chain. The results were similar for the material solubilized from M cell walls (data not shown). The results obtained for the high molecular mass mannoproteins were similar to those previously observed with equivalent material from the walls of *Zygosaccharomyces rouxii*, *Hansenula wingei*, *Saccharomycopsis lipolytica* and *Schizosaccharomyces pombe* (Herrero *et al.*, 1987).

In order to ascertain whether both the N and the O types of linkages were present in these mannoproteins, we determined their mobility from cells grown in the presence of tunicamycin. The total incorporation of radioactivity extractable with Zymolyase was reduced to 50% relative to control cells. A similar inhibition has also been found in *Saccharomyces cerevisiae* (Pastor *et al.*, 1984) and other species (Herrero *et al.*, 1987). The 31.5 kDa band was detected in the material extracted with Zymolyase but the high molecular mass mannoproteins were hard to visualize (Fig. 1*c*), which is in accordance with Table 1. To obtain further information on these proteins, the medium and the 'walls' of partially regenerated protoplasts incubated in ^{14}C -labelled protein hydrolysate and in the presence and absence of tunicamycin were analysed. In the absence of the antibiotic the partially regenerated walls (5 h) showed a band of 34 kDa, a polydisperse material of lower molecular mass (16–28 kDa), a faint band of 65 kDa and significant amounts of polydisperse high molecular mass material (> 150 kDa). In the presence of the antibiotic the radioactivity fell to 63% and only a small part of this radioactivity (15%) was solubilized by Zymolyase (Fig. 1*e*; Murgui *et al.*, 1986). When the control protoplast medium was analysed large amounts of a high molecular mass polydisperse material together with the 34 kDa and other species were evident (Fig. 5). When this material was β -eliminated and blotted onto nitrocellulose paper, no change in the mobility of the mannoproteins, including

Fig. 1. Fluorography of mannoproteins obtained from Y cell walls and partially regenerated protoplast walls labelled with ^{14}C -labelled protein hydrolysate. (a) MPM from untreated Y cells; (c) MPM from tunicamycin-treated Y cells; (b) MPM treated with Endo H. (d, e) Zymolyase-solubilized mannoproteins from walls of partially regenerated protoplasts (5 h) grown in the absence (d) and presence (e) of tunicamycin. Molecular mass markers are indicated on the left.

Fig. 2. ConA-peroxidase staining of the material secreted by protoplasts in the absence (a, c) and presence (b, d) of tunicamycin. (c) and (d) correspond, respectively, to the material in (a) and (b) after β -elimination. Molecular mass markers are indicated on the left.

the 34 kDa species, was detected (though a significant degradation was evident cf. Frevert & Ballou, 1985) confirming that the *O*-mannosidic chains represent a small part of these molecules (Fig. 2). In the case of tunicamycin-treated protoplasts, the high molecular mass material was not present but several bands with molecular masses of 60, 68, 72 and 97 kDa were detected together with the 31.5 kDa band (Fig. 5). These bands may correspond to the original high molecular mass molecules that had lost their *N*-glycosidically linked sugar chains. They reacted with ConA-peroxidase when transferred to nitrocellulose sheets, confirming that they were still mannoproteins in nature (Fig. 2). The 31.5 kDa band did not react with ConA, confirming that the original 34 kDa mannoprotein band had only a single *N*-glycosidically linked sugar chain (Fig. 2). The type of sugar linkage present in the other bands was shown to be *O*-glycosidic when the protoplasts were labelled with [^{14}C]mannose in the presence of tunicamycin and the spent medium β -eliminated, bands were not detected with ConA-peroxidase staining (Fig. 2) and no radioactivity remained at the origin of the chromatogram (Table 1).

Analytical gel filtration of the mannoproteins released from cell walls by Zymolyase and secreted by protoplasts

Having established that the high molecular mass mannoproteins had both *O*- and *N*-glycosidic linkages, their molecular behaviour on gel filtration was studied. When the MPM obtained from Y cells labelled with ^{14}C -labelled protein hydrolysate was passed through a Sephacryl S-300 column, two main peaks of radioactive material were detected (Fig. 3); the first was eluted in the exclusion volume region (V_e/V_0 from 1.00 to 1.30) and was less polydisperse than the glycosylated *S. cerevisiae* invertase [the polymeric forms of this enzyme are heterogeneous in size (V_e/V_0 from 1.00 to 1.49) from below 180 to higher than 500 kDa (Chu *et*

Fig. 3. Analytical gel filtration on a Sephacryl S-300 column of MPM from Y (●) and M (■) cells labelled with ^{14}C -labelled protein hydrolysate. Identical amounts of radioactivity (96 000 c.p.m.) were loaded for both samples and the eluted radioactivity in each fraction determined. The elution positions of several dextrans of known mean size are indicated as well as that of BSA (66 kDa) and lysozyme (14 kDa). The elution range of glycosylated invertase (measured enzymically) is also shown (I).

Fig. 4. PAGE analysis of the mannoproteins found in fractions collected from the Sephacryl S-300 column (see Fig. 3) corresponding to the MPM from Y (a–d) and M (e–h) cells. (a, e) Unfractionated MPM. Pooled fractions: (b, f) V_e/V_0 from 1.00 to 1.30; (c, g) V_e/V_0 from 2.00 to 2.37; (d, h) from 2.37 to 2.75. The pooled fractions were dialysed in distilled water, concentrated by lyophilization and run in a polyacrylamide gel, followed by fluorography.

al., 1983; Esmon *et al.*, 1987; Tammi *et al.*, 1987)]. A molecular mass for this material from above 500 kDa to 190 kDa was calculated by extrapolation from the elution volume of several dextran markers. When the fractions were pooled and the material obtained analysed by SDS-PAGE and fluorography of the gels, four bands with a relative mobility higher than 150 kDa were detected (Fig. 4). The second peak eluted from the Sephacryl column in the included region was larger (Fig. 3). The trailing edge of this peak contained material that was resolved by electrophoresis into two bands with molecular masses of 34 and 33 kDa. The leading part of the peak contained other radioactive material that could not be resolved under the electrophoretic conditions used.

Fig. 5. PAGE analysis of the material secreted by protoplasts after gel filtration. (a–d) Protoplasts grown in the absence of tunicamycin. (e–f) Protoplasts grown in the presence of tunicamycin. (a, e) Unfractionated spent medium. Pooled fractions: (b) V_e/V_0 from 1.00 to 1.25; (c) V_e/V_0 from 1.25 to 1.45; (d, g) V_e/V_0 from 1.9 to 2.2; (f) V_e/V_0 from 1.5 to 1.9; (h) V_e/V_0 from 2.2 to 2.75. The pooled fractions were processed as described in Fig. 4. Molecular mass markers are indicated on the left.

The elution pattern of the material solubilized by Zymolyase from M cell walls was similar to that from the Y cell walls. The material detected in the first peak extended from the void volume to a V_e/V_0 of 1.37 with molecular masses between 500 and 150 kDa (Fig. 3). Fluorography of the pooled fractions after gel electrophoresis demonstrated the presence of the high molecular mass material. On the other hand, the first part (V_e/V_0 from 2.00 to 2.37) of the second peak was enriched in three bands of 34, 33, and 31 kDa and in a faint broad band of about 24 kDa. The material eluted last from this peak (V_e/V_0 from 2.37 to 2.75) was shown to contain a band of 31 kDa and a material which ran with the indicator dye. Detection of a band of 33 kDa in the case of Y cells, and bands of 33, and 31 kDa in M cells in addition to the 34 kDa protein previously detected, points to an enrichment of these by gel filtration.

Further information on the wall mannoproteins was obtained by analysing the material secreted by protoplasts obtained from Y cells incubated in the presence and absence of tunicamycin (data not shown). In both cases the radioactivity eluted in two main peaks, indicating that each consists of two main components of different molecular mass. In the absence of tunicamycin the first component eluted with V_e/V_0 from 1.0 to 1.45 and a shoulder up to 1.70 whereas the second component was included in the gel (V_e/V_0 from 1.87 to 2.30). A large part of the material in the trailing half of the first peak had a mobility in SDS-PAGE similar to the mannoproteins released by Zymolyase from the cell walls, but, in addition, a band of 30 kDa was present. Furthermore, in the leading part of this peak there was a polydisperse material with an apparent molecular mass > 150 kDa and three relatively low molecular mass bands (34, 30, and 29 kDa) (Fig. 5). Specific interactions may therefore exist between the high molecular mass mannoproteins and the relatively low molecular mass species.

When the gel filtration was done with the medium obtained from protoplasts grown in the presence of tunicamycin, both the elution profile from the Sephacryl column and the electrophoretic patterns were different. In the first peak, material was included in the gel (V_e/V_0 from 1.50 to 1.90) whereas the second peak had the same V_e/V_0 as that obtained from the

supernatant of protoplasts grown in the absence of the drug. In the trailing edge of the first peak, the species with a molecular mass >150 kDa was not present but three new bands with molecular masses of 60, 68 and 72 kDa were the most abundant. The 34 kDa and 30 kDa bands of the control preparation also disappeared and new bands of 31.5, 28 and 27 kDa were detected, suggesting that these corresponded to deglycosylated forms of the 34, 30 and 29 kDa proteins (Fig. 5). These bands eluted from the Sephacryl column in the same fractions as the 60, 68 and 72 kDa molecules, in place of the elution volumes corresponding to their apparent molecular masses (V_e/V_0 2.05 and higher), implying that the absence of *N*-linked carbohydrate in the 60, 68 and 72 kDa species was not essential for their interaction.

Analytical gel filtration of the N-glycosidic sugar chains present in wall mannoproteins

N-linked sugar chains of the mannoproteins (mannan) of both cellular forms of *C. albicans* were obtained by alkaline extraction of isolated walls following ethanol precipitation. Initially, we analysed the mannans by gel chromatography in a column (1×100 cm) of Bio-Gel A-1.5 m. The elution patterns indicated that each mannan was formed by a collection of highly polydisperse molecules. Some of the Y mannan material eluted in the void volume whereas most was retarded by the gel. The molecular mass range of this mannan extended from over 500 kDa down to about 20 kDa. In the case of the M mannan, practically all the molecules were included in the gel and also extended down to 20 kDa. Further information on the molecular mass distributions of mannans was obtained by molecular filtration in Sephacryl S-300 columns (1.6×100 cm). Two peaks were obtained in both cases, one of them in the void volume and another one included in the gel (data not shown). The elution profiles indicated that the mannan obtained from M cells had more material with lower molecular mass than that from Y cells. The average molecular mass of the mannan was 100 kDa in the Y cells and 50 kDa in the M cells.

DISCUSSION

Several groups have tried to obtain information on the molecular mechanism of dimorphism in *C. albicans* (Ahrens *et al.*, 1983; Niimi *et al.*, 1980; Sullivan *et al.*, 1983). In an attempt to detect structural differences in the wall mannoproteins, Ponton & Jones (1986) extracted with dithiothreitol a mycelium specific component with an estimated molecular mass of 235–250 kDa whereas Sundstrom *et al.* (1987) extracted M walls with Zymolyase and obtained a 200 kDa material that seemed to be absent from the walls of Y cells. Shibata *et al.* (1986) suggested that Y–M transformation results in the formation of a phosphomannan protein complex containing a mannan moiety with incomplete structure.

The novel features of the present study are the determination of some specific characteristics of the wall mannoproteins which are released by Zymolyase from the Y and M cell walls. The material solubilized (MPM) contained 7% protein and 92% carbohydrate. These values closely resemble those found in an equivalent material (12% and 88%, respectively) extracted from *S. cerevisiae* walls by Frevert & Ballou (1985). The carbohydrate component is mainly composed of mannose residues, except for a small but significant amount of glucose. The presence of glucose residues in the MPM is of interest, as they might have formed part of the linkage between the wall glucan and the mannoproteins, as has been suggested for *S. cerevisiae* (Tkacz, 1984). Although, as far as we know, this is the first reported detection of glucose in *C. albicans* mannoproteins, this hexose has been described as forming, with mannose residues, part of a cell surface epitope recognized by a monoclonal antibody (Brawner & Cutler, 1986).

β -Elimination of walls revealed that about 12% of the total mannosyl residues were in the form of mannose and small oligosaccharides connected to the protein through *O*-glycosidic linkages. The other mannosyl residues are linked to the protein by *N*-glycosidic linkages resistant to mild alkaline treatment. The type of carbohydrate linkages present in individual mannoproteins was determined with the use of Endo H and tunicamycin. The four high molecular mass bands detected in the control walls disappeared from the 'walls' of protoplasts grown in the presence of tunicamycin. New bands of 60, 68, 72 and 98 kDa were detected in high amounts in the supernatant of these protoplasts, suggesting that they correspond to the original

glycosylated high molecular mass mannoproteins. These mannoproteins, which lack the *N*-glycosidic sugar chains but still retain *O*-glycosidically linked carbohydrate, were prevented from translocating into the growing wall structure and as a result accumulated in the surrounding medium of the regenerated protoplasts.

When MPM was passed through a Sephacryl column highly polydisperse material was eluted in and near the exclusion volume of the gel. The range of molecular masses of this material was from 190 to over 500 kDa in the case of Y cells and 150 to 500 kDa in the M cells. The polydispersity of this material seems to be due to its carbohydrate moiety and specifically to *N*-linked sugar chains (mannan). The mannan molecules have a molecular mass from 20 to 500 kDa with an average of 100 kDa (125 to 3000 mannose residues; average 600 residues) and from 20 to 400 kDa, average 50 kDa (125 to 2500 mannose residues; average 300 residues) in the case of Y and M cells, respectively. The high polydispersity of the sugar chains present in the high molecular mass mannoproteins was confirmed when they were detected with ConA-peroxidase after blotting onto nitrocellulose sheets (results not shown). The question is raised as to why, with a fluorography technique, the mannoproteins appeared as defined bands, whereas by blotting and ConA-peroxidase staining, they appear as highly polydisperse. One explanation is that the impression of the photographic film by the β -particles of the radioactive material, is inversely proportional to the distance from the film; this phenomenon would result in the apparent focusing in defined bands in places where the concentration of the ^{14}C -labelled material is higher. The physiological significance of the very high polydispersity of the wall mannoproteins is totally unknown. The observations that (i) changes in a single sugar residue affect the overall conformation of a macromolecule, and (ii) the carbohydrate moiety of glycoconjugates changes during development, differentiation and transformation (Stanley, 1987), point to the important functional role of mannoproteins in controlling the cell wall structure (Murgui *et al.*, 1986; Elorza *et al.*, 1987).

The observation that two main mannoproteins of 31 and 34 kDa were secreted by protoplasts associated with the high molecular mass species was also of interest. This association was disrupted by SDS and the mannoproteins released independently of each other when solubilized from walls by Zymolyase. We know nothing about the meaning of this association but it could be related to the process of wall assembly in a similar way to microtubules and actin filaments that can assemble spontaneously from different subunits to give long polymers. After elimination of *N*-glycosidic chains this association is maintained, which suggests that the protein alone is essential for this association, as the *O*-linked chains play no role in the assembly of glycoproteins (Matzuc *et al.*, 1987).

Cytochemical studies have shown that the wall mannoproteins are distributed throughout the wall although they accumulate on both surfaces (Valentin *et al.*, 1987). The location of these mannoproteins in the external surface is of interest because they may regulate the interaction with mammalian cells, e.g. adhesion to epithelial cells and the immunological response of the host. In relation to this it is important to note that some antigenic determinants (mannoproteins) are only intermittently expressed in the cell surface of *C. albicans* (Brawner & Cutler, 1986). We do not know if this phenomenon is due to newly secreted molecules or if they are modified *in situ*. In any case the result is a change in the cell wall immunological structure which in turn provides a means of perturbing the host defences.

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