The Release of Acid Phosphatase and Polysaccharide- and Protein-containing Components from the Surface of the Dimorphic Forms of *Candida albicans* by Treatment with Dithiothreitol

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INTRODUCTION

It is well established that in the preparation of protoplasts from yeasts by enzymic treatment, a sulphhydryl reagent accelerates the process by reducing disulphide bonds in proteins on the cell surface (Davies & Elvin, 1964; Kidby & Davies, 1970). Acid phosphatase is released from *Saccharomyces mellis* by exposure to 2-mercaptoethanol (Weimberg, 1971), and invertase from *S. carlsbergensis* and *S. cerevisiae* by the action of dithiothreitol (Sommer & Lewis, 1971). In the course of work on the nature of components released from the surface of both blastospore and mycelial forms of *Candida albicans* during protoplast formation by enzyme digestion it was found that a number of products containing saccharide and peptide material, and including acid phosphatase, were released by exposure of the cells to dithiothreitol.

METHODS

The organism used was *Candida albicans*, ca 1. It was grown on medium C, and blastospores or mycelium were harvested as described by Chattaway, Holmes & Barlow (1968). Cells (10 mg dry wt for 4 h cells and 30 mg dry wt for 18 h cells) were suspended in mannitol (800 mM)-tris-HCl (50 mM) buffer, pH 7.5; when required, dithiothreitol (12 mM, BDH) was added. The suspension was incubated under nitrogen for 24 h at 37 °C and then centrifuged at 800 g. The supernatant was freeze-dried and the residue (1 g/10 ml) redissolved in tris-HCl buffer (50 mM), pH 7.5 and then fractionated on a Sephadex G-75 column with elution by the same buffer. Fractions were monitored for protein content by measurement of E_{280} , for carbohydrate by the phenol-sulphuric acid method of Dubois *et al.* (1956), and for acid phosphatase according to Linhardt & Walter (1963) (specific activity as $\mu\text{mol } p\text{-nitrophenol/h/mg protein}$). Isoelectric focusing was carried out at 4 °C with an LKB 440 ml I.E.F. column using standard sucrose density gradient with 1% ampholine (pH range 3 to 10) at 300 V. Eluates were monitored as above. Protein was determined from extinction measurements at 260 and 280 nm.

RESULTS AND DISCUSSION

Blastospores and mycelium grown for 4 and 18 h were treated with dithiothreitol and the qualitative pattern of material released was the same in all cases. Figure 1 shows its fractionation on Sephadex G-75. There was a carbohydrate-rich fraction (Fig. 1: 1 and 3)

Fig. 1. Fractionation on Sephadex G-75 of material released by the action of dithiothreitol from (—) blastospores and (---) mycelia of *Candida albicans* harvested after 4 h (left) and 18 h (right). For methods see text; figures for 4 h cells corrected to 30 mg dry wt cells.

which had low peptide content but which contained all the acid phosphatase activity. Acid hydrolysis of this material and paper chromatography of the hydrolysate showed the presence of glucose and mannose. It was released in greater amount per unit dry weight of cells from blastospores than from mycelium at 18 h, and more material was released from 4 h cells than from 18 h cells. The specific activities of the acid phosphatase after 4 and 18 h were 289 and 735 units for blastospores and 16 and 41 units for mycelia. The greater activity (approx. 18 $\times$) for blastospores probably reflected differences in the composition of the peptide material released from the two forms.

A further fraction (Fig. 1: 2 and 4) was released in almost identical amounts from both forms at 18 h but in considerably greater amounts from 4 h cells than from 18 h cells. It was glycopeptide in nature, both glucose and mannose being present, and quantitatively represented the major peptide material released.

Tris-mannitol buffer alone released only very small amounts of any of the above fractions. Isoelectric focusing of the original supernatant showed separation into at least four components, each containing both peptide and saccharide material, the acid phosphatase from both forms having an isoelectric point at pH 6.8.

Total surface acid phosphatase of untreated cells was assayed. Cells grown for 4 h and then incubated in tris-mannitol buffer alone for 24 h released 4 % of this activity from mycelia and 18 % from blastospores. In the presence of dithiothreitol, a further 42 % was released from both forms. Cells grown for 18 h and suspended in tris-mannitol buffer alone showed a release of 4 and 12 % from mycelia and blastospores respectively, with a further release of 23 and 17 % in the presence of dithiothreitol. These results indicate that in 4 h cells there is a difference in the cell surface between the two forms and release of

acid phosphatase occurs more readily from blastospores, since the action of dithiothreitol in reducing disulphide bonds is required before any significant amount is released from the mycelial surface. A smaller proportion of the enzyme was released from stationary phase cells (18 h growth), as also found by Sommer & Lewis (1971) for invertase release from *Saccharomyces* spp.

The results indicate that in *Candida albicans*, as in certain *Saccharomyces* spp., the reduction of disulphide bonds alone will release acid phosphatase and other glycopeptide components, without any action of hydrolytic enzymes or sphaeroplast formation. The amounts released vary between blastospores and mycelia and with the age of the cells, thus indicating changes in the nature of the cell surface.

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