SHORT COMMUNICATION

The Porosity of the Cell Wall of *Candida albicans*

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Polyethylene glycols of different molecular sizes have been used to study the penetrability of the cell wall of *Candida albicans* harvested at the end of the growth phase and after prolonged incubation in the stationary phase. No differences were found which could account for the greater resistance of cells from stationary phase cultures of *C. albicans* to the antimycotic agents amphotericin B methyl ester and miconazole.

INTRODUCTION

The yeast form of *Candida albicans* develops a phenotypic resistance to the membrane-active polyene and imidazole antimycotics during the stationary phase of a batch culture (Gale, 1974; Hammond & Kliger, 1974; Kerridge *et al.*, 1976; Cope, 1980). Spheroplasts prepared from either sensitive or resistant cells are sensitive to both the polyene antibiotic amphotericin B methyl ester and the synthetic imidazole miconazole (Gale *et al.*, 1975; E. F. Gale, personal communication), suggesting involvement of the cell wall. The present work is an investigation of the possibility that there is a change in the porosity of the cell wall of *C. albicans* during the stationary phase of culture, so that antimycotic agents are unable to penetrate the cell wall of resistant cells to reach their site of action. This is feasible, since the cell wall in stationary phase *C. albicans* is thicker (Hammond & Kliger, 1974; Cassone *et al.*, 1979), contains more disulphide bonds and is more resistant to enzymes (Chattaway *et al.*, 1974; Kerridge *et al.*, 1976) than the cell wall in exponentially growing cells. The porosity of the cell wall of *Saccharomyces cerevisiae* has been studied using polyethylene glycols of different molecular sizes (Scherrer *et al.*, 1974); the threshold of permeability was estimated as corresponding to a molecular weight of 620 and an Einstein–Stokes hydrodynamic radius of 0.81 nm. Since the molecular weights of amphotericin B methyl ester and miconazole are 938 and 479, respectively, these molecules may be close to the limit of penetrability of the cell wall, although they may not behave in the same way as polyethylene glycols in their interaction with the cell wall.

METHODS

Abbreviations. AME, Amphotericin B methyl ester; DG, diethylene glycol; EG, ethylene glycol; M, molecular weight of monodisperse compounds; M_n, molecular weight of polydisperse compounds; MN, miconazole; PEG, polyethylene glycol; R, percentage of cell volume penetrated; r_{ES} , Einstein–Stokes hydrodynamic radius; SRC, standard release concentration; TG, triethylene glycol.

Organism and growth conditions. *Candida albicans* 6406 was grown in 1 or 5 l growth medium at 37 °C either in an orbital incubator (1 l cultures) or in rotating drums in a controlled temperature room (5 l

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cultures), using either a synthetic yeast medium containing 2% (w/v) glucose (SYG medium) or Yeast Nitrogen Base medium (Difco) containing 2% (w/v) glucose (YNBG medium) (see Cope, 1980). Each batch of cells consisted of material harvested from six 1 l cultures or one 5 l culture.

Antimycotic sensitivity testing. A K^+ -sensitive electrode was used as described by Cope (1980), and SRC values as defined by Gale (1974) for AME and by Cope (1980) for miconazole were determined. For AME, $SRC < 0.5 \mu\text{g ml}^{-1}$ was taken to indicate sensitivity and $SRC > 10 \mu\text{g ml}^{-1}$ to indicate resistance, while for miconazole, $SRC \leq 10 \mu\text{g ml}^{-1}$ was taken to indicate sensitivity and $SRC > 30 \mu\text{g ml}^{-1}$ to indicate resistance.

Porosity testing. Cells were harvested, washed twice and resuspended in distilled water. Approx. 1 g (wet wt) samples of cells were centrifuged in tared glass tubes (12×75 mm) at 6000 g for 20 min at 4 °C, and the supernatant fluids were discarded. The sides of the tubes were wiped dry before re-weighing and calculating exact values for wet weight. Solutions of ethylene glycol, its polymers and glycerol (BDH) were made up in water at 10% (w/v) and four to six determinations were carried out with each solution for each batch of cells. One ml solution was added to each sample of cells, the suspensions were mixed thoroughly and equilibrated for 2 h at 4 °C. The cells were re-sedimented using the same centrifugation procedure as before and the solute concentrations in the supernatant fluids were determined using a high accuracy Abbé refractometer (model 60/ED, Bellingham & Stanley Ltd, London N19) with a sodium light source. Dilutions of the original 10% (w/v) solutions were used as standards. Values for R were calculated as described by Macdonald & Gerhardt (1958), assuming that the percentage of the pellet volume penetrated by PEG 6000 represented the interstitial space within the pellet.

RESULTS

Table 1 records the average percentage of cell volume penetrated (R) for each solute for cells which were AME- and miconazole-sensitive, AME-sensitive and miconazole-resistant, and AME- and miconazole-resistant. Although variation between similar batches of cells was considerable, the standard deviations for replicate samples taken from each individual batch were below 2.0 percentage units in each case. Although resistant cells showed higher values for the penetration of solutes than those which were sensitive to AME and miconazole, *P* values calculated using Student's *t* test show that the differences are not significant.

Table 1. Average values for the percentage of the cell volume of *C. albicans* penetrated by solutes of different molecular sizes

Solute	M or M̄	r_{ES} (nm)*	Average percentage ($\pm$ S.D.) of cell volume penetrated (R) for different organisms			<i>P</i> value (%) for R values between batches†	
			AME _s MN _s †	AME _s MN _r	AME _r MN _r	1 to 3 and 4 to 6	1 to 3 and 7 to 9
			Batches 1 to 3§	Batches 4 to 6§	Batches 7 to 9§		
EG	62	0.33	35.7 $\pm$ 5.6	51.5 $\pm$ 13.6	32.0 $\pm$ 9.6	9.8	> 20
Glycerol	92	0.31	24.0 $\pm$ 4.2	40.6 $\pm$ 8.1	44.3 $\pm$ 3.2	2.7	3.9
DG	106	0.41	23.0 $\pm$ 0.7	32.9 $\pm$ 6.7	30.2 $\pm$ 7.1	4.3	13.7
TG	150	0.46	21.4 $\pm$ 5.0	26.5 $\pm$ 6.2	26.5 $\pm$ 8.4	> 20	> 20
PEG 200	200	0.56	18.5 $\pm$ 2.9	23.3 $\pm$ 4.2	21.8 $\pm$ 7.9	14.2	> 20
PEG 400	400	0.68	10.1 $\pm$ 2.1	17.8 $\pm$ 4.9	16.8 $\pm$ 7.3	4.7	16.2
PEG 600	600	0.80	7.2 $\pm$ 3.0	13.0 $\pm$ 7.0	16.9 $\pm$ 8.1	18.8	9.1
PEG 1000	908	1.00	4.3 $\pm$ 4.6	6.6 $\pm$ 4.4	14.7 $\pm$ 8.6	> 20	10.0
PEG 1500	1450	1.20	4.0 $\pm$ 3.6	3.5 $\pm$ 3.1	10.6 $\pm$ 8.5	> 20	13.0
PEG 4000	3650	1.90	4.3 $\pm$ 3.8	3.5 $\pm$ 9.4	11.1 $\pm$ 7.8	> 20	19.3
PEG 6000	6750	3.00	0	0	0		

Abbreviations are defined in Methods.

* Values taken from Scherrer & Gerhardt (1971).

† s, Sensitive; r, resistant (as defined in Methods).

‡ Values > 5% not significant.

§ Batches 1 to 3: cells grown for 15 to 16 h in SYG medium; batches 4 to 6: cells grown for 65 to 96 h in SYG medium; batches 7 to 9: cells grown for 75 to 113 h in YNMG medium.

DISCUSSION

Previous authors who have undertaken porosity measurements using PEGs plotted graphs of R against either molecular weight or r_{ES} , and drew three straight lines through the resulting points, forming two intersections which were taken to represent the limits of penetrability of the cell wall and plasma membrane (Scherrer & Gerhardt, 1971; Scherrer *et al.*, 1974). However, there is no evidence that such distinct discontinuities should exist, and Trevithick *et al.* (1966) had previously suggested that a continuous hyperbola might be a better fit to such data. These authors also pointed out the problem of having to choose which points should be assigned to each section of the graph. The scatter in the R values obtained by all authors, due in part to the polydispersity of the glycol preparations used, often results in a number of possible ways of drawing the graphs, which may lead to rather different estimates of limits of penetrability. Since the equations used in calculating R values are, in any case, only approximate (Macdonald & Gerhardt, 1958), the degree of error in such estimates is too high to give very meaningful results. Since the present work was undertaken to compare cells of *C. albicans* sensitive and resistant to antimycotic agents, data were analysed by comparing statistically the penetration of each individual solute into sensitive and resistant cells, rather than by attempting to calculate absolute values for limits of penetrability. Since no significant differences were found, any further investigation of the penetrability properties of the cell wall of *C. albicans* is not likely to lead to a greater understanding of the action of antimycotic agents.

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