MUCIDIN-RESISTANT ANTIMYCIN A-SENSITIVE MITOCHONDRIAL MUTANT OF *SACCHAROMYCES CEREVISIAE*

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1. Introduction

Mucidin, an antibiotic from *basidiomycete Oudemansiella mucida,* inhibits the growth of yeasts and filamentous fungi [1,2]. It has been found that the primary action of the antibiotic is an inhibition of respiration at the bc_1 complex of the mitochondrial respiratory chain [2,3] the biogenesis of which is under the control both of mitochondrial and nuclear genes [4–8].

In an attempt to obtain more information on the nature of the mucidin binding site especially in the relation to the other inhibitors of this region [9,10], mucidin-resistant mutants have been isolated. The present paper discusses genetic and biochemical studies of antimycin-sensitive mutant of *S. cerevisiae* resistant to mucidin as a result of mutation in mitochondrial DNA.

2. Experimental

Mucidin-resistant mutant, designated *Saccharomyces cerevisiae* 3D (α adel lys2 ρ^+ $C^SE^SO^SM^R$), was isolated by spreading cell suspensions of sensitive strain D 225-5A (α adel lys2 ρ^+ $C^SE^SO^SM^S$) on to agar plates composed 2% agar, 2% glycerol, 1% yeast extract, 1% bacteriological peptone, 0.01% adenine, mineral salts [2] and 0.25 μg mucidin/ml. Mutant colony appearing spontaneously after 7 days incubation at 30°C was isolated and purified by subcloning three times before rechecking the phenotype and stocking the culture. The resistance of the mutant to various inhibitors of mitochondrial functions was determined according to Rank and Bech-Hansen [11]. The techniques of mating of yeast strains, tetrad analysis [12,13], quantitative random diploid analysis and technique for analysis of mitotic segregants [14–16] will be described in more detail elsewhere (Šubík, Kováčová and Takácsová, in preparation). Oxygen uptake of isolated yeast cells and mitochondria was measured polarographically at 30°C.

Abbreviations: C^R/C^S, E^R/E^S, O^R/O^S, M^R/M^S: resistant and sensitive alleles of the mutational sites determining sensitivity to chloramphenicol, erythromycin, oligomycin and mucidin respectively.

3. Results and discussion

Mucidin caused inhibition of glycerol (ethanol) supported growth of yeast at a concentration of 0.2 μg/ml but had no effect on yeast strains grown fermentatively on glucose media at 50 μg/ml. The spontaneous mutant 3D resistant to mucidin was isolated from mucidin-sensitive strain D 225-5A as described in experimental. The mutant was found to tolerate 2 μg mucidin/ml of solid glycerol medium and was subjected to further analysis.

Study of the cross-resistance of this mutant to antimycin A, chloramphenicol, erythromycin, CCCP (carbonylcyanide *m*-chlorophenylhydrazone) and bongkrekic acid on solid media revealed that this strain is specifically resistant to mucidin and shows no significant cross-resistance to tested drugs.

Genetic studies, apart from being useful in elucidating nucleo-cytoplasmic interrelationships with respect to mitochondrial biogenesis, can be distinguishing between nuclear and cytoplasmic inheritance help define

Table 1
Genetic characterization of the mutant 3D

Mitotic segregation of M^R/M^S among diploids from the cross	
ρ^+M^R with ρ^+M^S tester	Yes (32–69% M^R)
ρ^- from M^R with ρ^+ M^S tester	No (all M^S)
ρ^+M^R with ρ^- from M^S tester	No (all M^R)
Meiotic segregation in tetrads (M^R/M^S)	
From mucidin-resistant diploids	4:0 (3:0)
From mucidin-sensitive diploids	0:4
Recombination between pairs of markers	
C–M	Yes
E–M	Yes
O–M	Yes

Diploids from the crosses indicated in the table were tested for mitotic segregation by replica plating onto glycerol and glycerol plus antibiotic containing media (mucidin: 0.5 μg/ml, chloramphenicol: 4 mg/ml, erythromycin: 2 mg/ml, oligomycin: 10 μg/ml). Tester strains: *S. cerevisiae* 26–4 (a leu1 thr2–1 ρ^+ $C^SE^SO^SM^S$), IL8–8D (a ura ρ^+ C^R_{321} E^R_{514} O^SM^S), IL126–1C (a ura ρ^+ C^R_{321} E^R_{221} O^SM^S), 55R5–3C/1 (a ura ρ^+ $C^SE^SO^R_1M^S$). Indicated petites (presumed to be ρ^0) were isolated after ethidium bromide treatment (25 μM, growth for 24 h). The detailes of the genetic analysis will be discussed extensively elsewhere (Šubík, Kováčová and Takácsová, in preparation).

the site of the mutation. Genetic analysis of the mutant 3D (table 1) indicates that the resistance allele is mitochondrially inherited, i.e. it obeys all the criteria established for yeast cytoplasmic genes including association with the rho factor [14–16].

In vivo resistance of mitochondrial electron transport to mucidin might result either from impermeability of cell membrane or from a change in the mitochondrial membrane itself. A form of detoxification mechanism is a third but unlikely possibility. To clarify this problem the effect of mucidin on the respiration of intact cells and isolated mitochondria was studied. It was found that not only the whole cells but also the isolated mutant mitochondria are resistant to mucidin. In spite of this fact the mutant 3D showed no significant change in sensitivity to antimycin A as measured in vivo or in vitro (fig.1, table 2).

At present the strain 3D is the first mitochondrial mutant of *S. cerevisiae* with apparently functional oxidative phosphorylation resistant to the specific inhibitor of mitochondrial respiratory chain. Resistance to mucidin in mutant 3D can be demonstrated both with growing (or resting) cells and isolated mitochon-

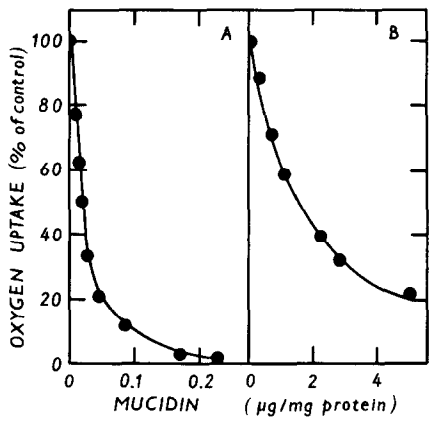

Fig.1. Effect of mucidin on respiration of isolated yeast mitochondria. The polarographic vessel contained in 2 ml: 0.6 M mannitol, 20 mM KCl, 1.5 mM EDTA, 10 mM Tris–maleate, 10 mM potassium phosphate, 10 mM citrate, 0.48% ethanol, 0.25 mM ADP, mitochondria (1.16–3.18 mg protein) and inhibitor as indicated. Final pH 6.4. Mitochondria were prepared by the protoplast method according to Kováč et al. [25]. (A), wild-type D 225-5A; (B), mutant 3D.

Table 2
Inhibition by mucidin and antimycin A of respiration of whole cells and mitochondria isolated from wild-type and mucidin-resistant mutant

Strain	Inhibitor I_{50} values			
	Mucidin		Antimycin A	
	cells (μg/ml)	mitochondria (μg/mg protein)	cells (μg/ml)	mitochondria (μg/mg protein)
Wild-type D 225–5A	0.016	0.015	0.0045	0.035
Mutant 3D	2.4	1.5	0.0050	0.042

The polarographic vessel contained in 2 ml: 50 mM potassium glutarate, 10 mM potassium phosphate, 100 mM KCl, 0.25% glucose, 0.48% ethanol, inhibitor and yeast cells (1.5–4.5 mg dry weight) grown in semi-synthetic medium [2] with 0.5% glucose to the stationary phase. Final pH 4.3. Oxygen uptake of isolated mitochondria was determined as indicated in fig.1. The cells and mitochondria were preincubated with antimycin A for 7 min before the activity was measured. I_{50} values are defined as the amount of inhibitor required to inhibit the respiratory activity to 50% of the maximal control activity.

dria. The lack of cross-resistance to antimycin A (in vivo and in vitro) and genetic evidences would suggest that mucidin reacts on mitochondrial membrane at a binding or inhibition site different from that of antimycin A.

In yeast resistance on the mitochondrial level to antimycin seems to be inherited nuclearly [17,18]. On the other hand, mucidin resistance in mutant 3D is the result of mutation in mitochondrial DNA. Thus, in mutant 3D resistance to mucidin - an inhibitor of mitochondrial electron transport between cytochromes b and c - may be associated with specific modification of the membrane bound polypeptide (proteolipid) subunit(s) of the bc_1 complex of the respiratory chain [19–21] which are synthetized in the mitochondrion [22–24] and coded for by mitochondrial DNA. In addition, identification of new mitochondrial genetic marker facilitates mapping studies of the mitochondrial genome and will assist in the identification of mitochondrial gene product(s) which are components of the respiratory chain.

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