ASSEMBLY OF THE MITOCHONDRIAL MEMBRANE SYSTEM XVI. MODIFIED FORM OF THE ATPase PROTEOLIPID IN OLIGOMYCIN-RESISTANT MUTANTS OF *SACCHAROMYCES CEREVISIAE*

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Received 20 April 1976

1. Introduction

Cytoplasmic mutations in *Saccharomyces cerevisiae* causing a resistance to oligomycin have been shown to be associated with two genetically separate and unlinked loci on the mitochondrial DNA – OLI 1 and OLI 2 [1,2]. It has been postulated that the mutations occur in gene products of the oligomycin-sensitive adenosine triphosphatase [3]. This notion is substantiated by the finding that the ATPase of resistant strains is less sensitive to the inhibitor. To date, however, the lesion at the level of the protein components of the ATPase has not been identified. In this brief communication we report evidence showing a modification in OLI 1 resistant mutants of one of the mitochondrially synthesized subunits of the ATPase.

2. Materials and methods

The yeast strains and their origin are listed in table 1. With the exception of D273-10B and its derivative mutants all the strains were isolated in other laboratories and have already been described in the literature, Spontaneous oligomycin-resistant mutants of D273-10B/A were obtained by plating an exponential culture on glycerol medium containing 5 μg per ml rutamycin. Resistant colonies were picked after 5 days incubation at 30°C. The mutants were purified and further selected first on paromomycin and then on erythromycin media. This yielded strains that were triply resistant to oligomycin, paromomycin and erythromycin.

In order to establish whether the oligomycin-resistance was due to the OLI 1 or OLI 2 resistance alleles, the mutants were mated to standard strains containing known oligomycin resistance loci and the mitotic segregants issued by the diploids were scored for growth on rutamycin (oligomycin). The results presented in table 2 indicate that D273-10B/A21 contains the OLI 1 resistance locus since no sensitive recombinants were found when it was crossed to a strain (D22A16) known to contain the OLI 1 marker. In a cross to D22A15, a strain with the OLI 2 resistance allele D273-10B/A21 was found to produce 9% recombinants that were sensitive to rutamycin. D273-10B/A16 has a mutation that is closely linked to OLI 2 (1.5% recombinants) and may be an OLI 4 type of mutant. The OLI 4 resistance allele has been found to be closely linked to OLI 2 [5].

2.1. *Gel electrophoresis*

The mitochondrially synthesized proteins in the various yeast strains studied were labeled with [^{35}S]-methionine in the presence of cycloheximide as described previously [6]. The radioactively labeled products were analyzed in two ways. Mitochondria were depolymerized in sodium dodecyl sulfate (SDS) and the dissociated proteins separated on slab gels [6]. The radioactive products were vizualized by exposure of the dried slabs to no-screen X-ray film. Alternatively, the SDS treated mitochondria were separated on disc gels which were then sliced and counted as described previously [7].

Table 1
Strains of Saccharomyces cerevisiae

Name	Chromosomal genotype	Mitochondrial genotype	Oligomycin-resistant locus	Reference
D273-10B/A	α,met	$\rho^+,\omega^+,E^SO^SP^SC^S$	none	
D273-10B/A21	α,met	$\rho^+,\omega^+,E^R_{624}O^R_{625}P^R_{626}C^S$	OLI 1	This study
D273-10B/A16	α,met	$\rho^+,\omega^+,E^R_{621}O^R_{622}P^R_{623}C^S$	OLI 4 (?)	This study
D22	a,ade2	$\rho^+,\omega^+,E^SO^SP^SC^S$	none	[1]
D22A16	a,ade2	$\rho^+,\omega^+,E^SO^R_{145}P^SC^S$	OLI 1	[1]
D22A15	a,ade2	$\rho^+,\omega^+,E^SO^R_{144}P^SC^S$	OLI 2	[1]
KL14-4B	α,aux	$\rho^+,\omega^+,E^SO^R_1P^R_{454}C^R_{321}$	OLI 1	[4]

Table 2
Mitotic segregation of oligomycin-resistance markers in mutants of D273-10B

Cross	Number of colonies scored			
	Rutamycin-resistant	Rutamycin-sensitive	Total	Percent sensitive recombinants
D273-10B/A21 × D22	61	83	144	57.6
D273-10B/A21 × D22A16	191	0	191	0
D273-10B/A21 × D22A15	159	16	175	9.1
D273-10B/A16 × D22	63	144	207	69.5
D273-10B/A16 × D22/A16	253	19	272	7.5
D273-10B/A16 × D22A15	257	4	261	1.5

The strains were mixed on complete glucose medium and incubated at 30°C for 24 h. The diploids were selected by replication on minimal glucose medium and allowed to grow for 2 days. The prototrophically selected diploids were spread for single colonies on minimal glucose medium. The plates were replicated on glycerol medium without and with 5 μg per ml rutamycin. Growth was scored after 2 days.

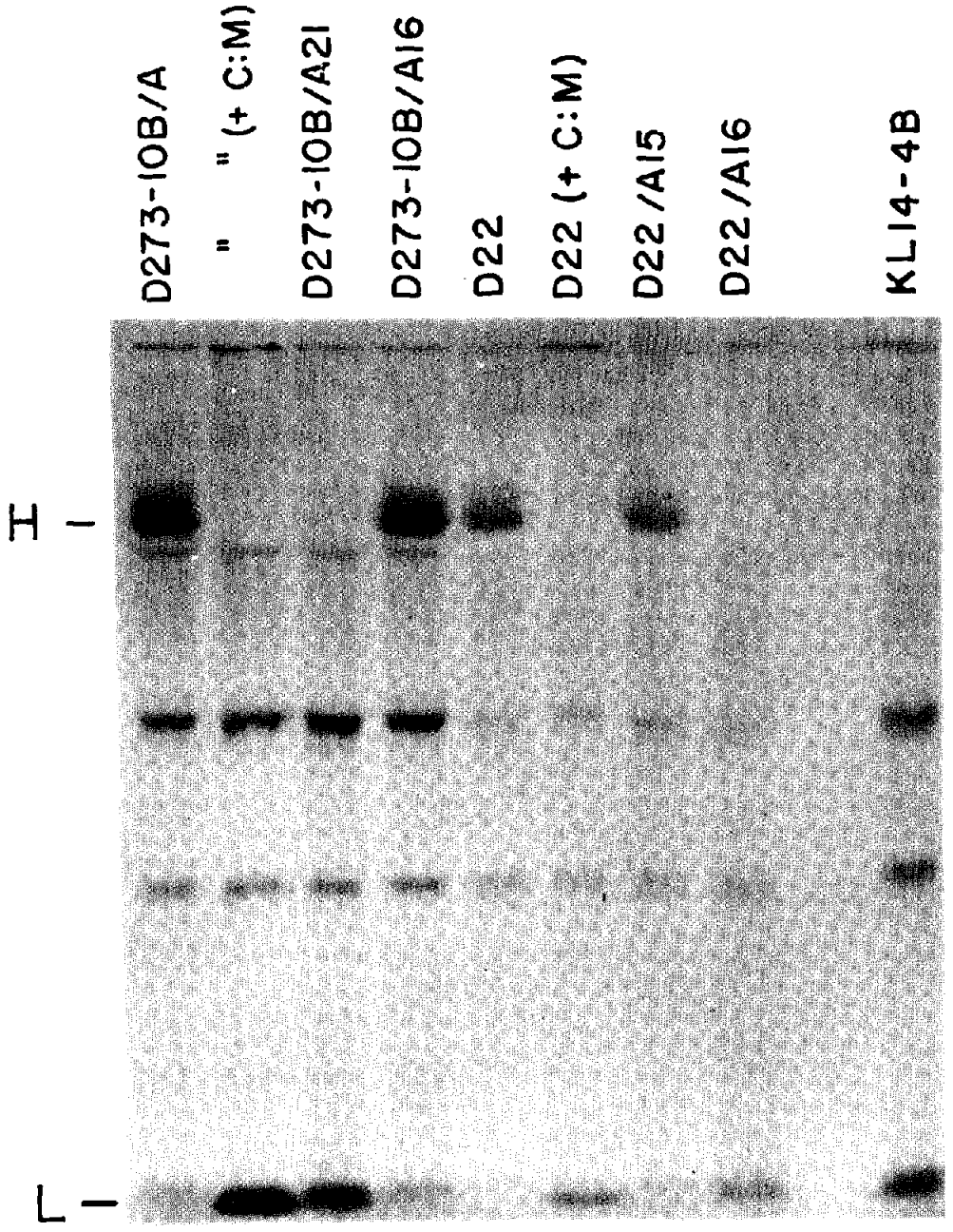

Fig.1. Slab gel electrophoresis of mitochondrial products in wild-type and oligomycin-resistant strains of *Saccharomyces cerevisiae*. The strains were labeled with [^{35}S]methionine in the presence of cycloheximide. Mitochondria were isolated, dissociated in 1% sodium dodecyl sulfate and samples representing 20 μg of protein were separated on a 15% polyacrylamide slab as described previously [6]. The high- and low mol. wt. forms of the ATPase proteolipid are indicated by the letters H and L, respectively. The genotypes of the strains are described in table 1.

3. Results and discussion

We have previously reported that in *Saccharomyces cerevisiae,* a major product of mitochondrial protein is a subunit of the oligomycin-sensitive ATPase that has the properties of a proteolipid [7,8]. This protein was purified by extraction of mitochondria with chloroform–methanol and shown to have a high proportion of non-polar amino acids [8]. An interesting property of the proteolipid was its mol. wt. which in SDS gels was estimated to be either 45 000 or 8000 depending on the treatment of the protein [8,9]. When mitochondria or the purified ATPase complex was dissolved directly in SDS, the protein migrated with an apparent mol. wt. of 45 000. If, however, mitochondria or the ATPase were pretreated with strong acid or base or with organic solvents including a mixture of chloroform–methanol, the protein acquired a faster migration and its mol. wt. was decreased to 8000 [9].

The fact that the proteolipid is one of the major products synthesized by yeast mitochondria and because its mobility in SDS gels differs from all the other mitochondrial products, it is possible to detect and study this component in gels of whole mitochondria. Fig.1 shows the mitochondrial products observed in different strains of *Saccharomyces cerevisiae.* In each case the cells were labeled in vivo with [^{35}S]methionine in the presence of cycloheximide to block cytoribosomal protein synthesis. The gel on the extreme left is that of wild-type D273-10B/A. The mitochondria were dissolved directly in 1% SDS prior to electrophoresis. The second gel is also of wild-type except that the mitochondria were first treated with 20 volumes of chloroform–methanol (2:1, v/v). After evaporation of the organic solvent, the particles were dissolved in SDS and subjected to electrophoresis. A comparison of the first two gels shows that the chloroform–methanol treatment causes a quantitative conversion of the proteolipid from the high (H) to the low-molecular weight form (L) as reported previously [9]. The third gel shows the products of D273-10B/A21, the strain with the OLI 1 resistance allele. The mitochondria were dissolved in SDS without prior treatment with organic solvents. The gel shows that in this strain the proteolipid is present in the L form. The fourth gel shows the products in D273-10B/A16

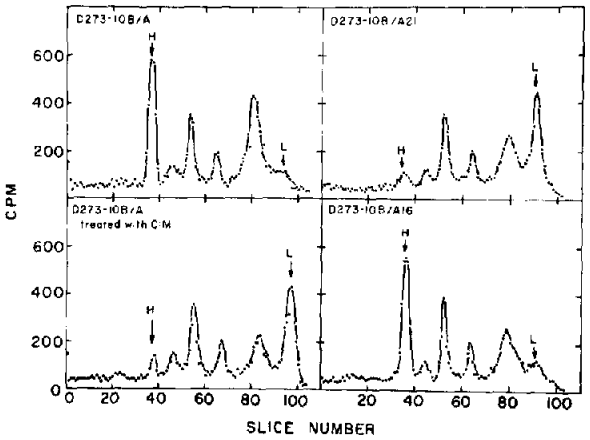

Fig.2. Disc gel electrophoresis of mitochondrial products of wild-type and oligomycin-resistant mutants D273-10B/A. Mitochondrial samples (20 μg) from the experiment of fig.1 were separated on 10% polyacrylamide disc gels prepared according to the procedure of Weiss et al. [10]. The discs were frozen, sliced into 1 mm sections, digested with 6% H_2O_2 and counted [7]. The high- and low mol. wt. forms of the ATPase proteolipid are indicated by the letters H and L, respectively.

which has mutation linked to OLI 2. It is seen that the proteolipid in this strain is in the H form. The different mitochondria were also analyzed on disc gels and the proteins localized by slicing and counting the gel. These results with the disc gels shown in fig.2 are in agreement with those obtained by the slab gel technique.

In order to establish whether the presence of the proteolipid in the low mol. wt. form is a general property of strains carrying the OLI 1 resistance marker, other independently isolated strains were also examined. The results with the D22 and KL14 strains (cf. fig.1) confirm the generality of the phenomenon. In both strains, mutants having the OLI 1 resistance allele showed the presence of the proteolipid in the L form.

Although the results of this study point to a clear correlation between mutations in the OLI 1 locus and modification of the proteolipid component of the ATPase, the nature of the modification is still not known. This will require an understanding of the chemical basis for the difference between the high- and low mol. wt. forms of the proteolipid.

Acknowledgment

This work was supported by Grant 5 ROl HL13003 from the National Institutes of Health, US. Public Health Service.

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