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OXIDATIVE PHOSPHORYLATION IN YEAST

III. ATPase ACTIVITY OF THE MITOCHONDRIAL FRACTION FROM A CYTOPLASMIC RESPIRATORY-DEFICIENT MUTANT

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SUMMARY

1. Mitochondria were isolated from a cytoplasmic respiratory-deficient variant of yeast *Saccharomyces carlsbergensis* NCYC 74.

2. Mg^{2+}-dependent ATPase (ATP phosphohydrolase, EC 3.6.1.3) activity of the mitochondrial fraction displayed two pH optima, at pH 6.2 and 9.5. The activity at pH 6.2 corresponded closely to that found in non-mutant yeast. The specific activity of the reaction at pH 9.5 was lower than in wild-yeast mitochondria and, unlike wild-type yeast ATPase, was resistant to oligomycin inhibition.

3. The possibility is discussed that the ATPase activity of the mutant mitochondria may represent a preserved part of the oxidative phosphorylation system either structurally modified or lacking a component which would make it oligomycin sensitive.

INTRODUCTION

A cytoplasmic mutation which occurs in yeast spontaneously or can be induced by acridines and other treatments (for review see refs. 1 and 2) involves a change in mitochondrial DNA[3] and results in cells with hereditarily impaired respiratory ability. Several components of the respiratory chain, such as cytochrome *a* and *b*, ubiquinone, NADH–cytochrome-*c* reductase and succinate–cytochrome-*c* reductase have been reported to be substantially reduced or entirely lacking in such mutants[1–8] and electron microscopic evidence indicates an abnormal structure of their mitochondria[9].

Although the formation of ATP by oxidative phosphorylation is not possible in these mutants with a functionally incomplete respiratory chain, it has been found previously[10,11] that compounds interfering with oxidative phosphorylation, 2,4-dinitrophenol and azide, inhibited synthetic processes in such a mutant. This implied that partial reactions of oxidative phosphorylation might be preserved in the mutant mitochondria and be eventually of importance in the coupling of catabolic and anabolic reactions. In this paper the occurrence of a dinitrophenol- and azide-sensitive ATPase activity in mutant mitochondria is described.

Abbreviation: PCMB, *p*-chloromercuribenzoate.

EXPERIMENTAL

The respiratory-deficient mutant was prepared from a diploid yeast, *Saccharomyces cerevisiae* NCYC 74 (kindly provided by Prof. A. A. EDDY, Department of Biochemistry, Manchester, England), by acriflavin treatment[12]. As the acriflavin treatment results in mass production of cytoplasmic yeast mutants with impaired respiratory ability[1,2,4,12] the mutant was assumed to be a cytoplasmic one although genetic analysis could not be performed because of its inability to sporulate. The mutant was cultivated in semi-synthetic medium with 0.5 % glucose as carbon source[13] at 30° for 24 h. Mitochondria were isolated from it by a procedure described in previous papers[13,14] which also give other details of experimental methods and materials employed in this study.

RESULTS

Although difficulties with preparations of mitochondria from respiratory-deficient yeast have been reported[15,16] the procedure employed provided a good fraction of mitochondria from the mutant as proved by visual inspection in a phase-contrast microscope. The only difference from wild-yeast mitochondria mentioned in the course of the preparation procedure was a relatively higher tendency of the mutant mitochondria to agglutinate. Only cytochrome *c* could be demonstrated in the mutant mitochondria when inspected for difference spectra in a spectrophotometer.

The mutant mitochondria exhibited a very low ATPase activity in the absence of Mg^{2+} but the addition of Mg^{2+} elicited a high ATPase activity. As in wild-type yeast mitochondria[13] the activity had two pH optima, one at pH 6.2 and another at about 9.5 (Fig. 1). Dinitrophenol inhibited the activity below pH 7.5 and stimulated above this pH. This dual effect of dinitrophenol was observed in several experiments similar to that presented in Fig. 1. The Michaelis constant for ATP at pH 9.5 was

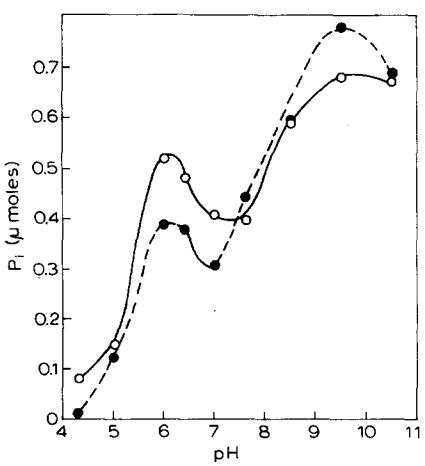

Fig. 1. pH curve of ATPase activity. The incubation mixture contained in 1.0 ml: 4 mM ATP, 40 mM KCl, 0.4 mM EDTA, 5 mM $MgCl_2$, 30 mM buffer (Tris–maleate at pH 4.3 to 7.0 and Tris–chloride at pH 7.6 to 10.5), 88 mM sucrose and 0.13 mg of mitochondrial protein. Reaction time, 10 min. Dotted line indicates the activity in the presence of 0.5 mM dinitrophenol.

found to be 3.0 mM, which did not differ significantly from the value of 2.2 mM found with wild-type mitochondria[13]. The effect of various inhibitors on the ATPase activity at the two pH optima is demonstrated in Tables I and II. The inhibitions at pH 6.2 were similar to that found in non-mutated yeast[13]. The mean specific activities (in μmoles P_i liberated per mg protein per h) at this pH were also similar in mitochondria from a wild strain and the mutant, being 33 and 36, respectively. The specific activity at pH 9.5 was lower in the mutant (72 as compared with 183 of the wild strain[13]) and the inhibitions at this pH were different, the activity in the mutant mitochondria being less sensitive to all inhibitors tested except for fluoride. The inhibition pattern strikingly resembled that found in an oxidative phosphorylation-deficient mutant[14] with one important exception: the ATPase activity at pH 9.5 of mitochondria from the respiratory-deficient mutant was almost completely resistant to inhibition by oligomycin (Table II).

TABLE I

INHIBITORS OF ATPASE ACTIVITY IN MUTANT MITOCHONDRIA

The reaction mixture contained in 1.0 ml: 4 mM ATP, 80 mM KCl, 0.5 mM EDTA, 20 mM Tris–maleate or Tris–chloride, 88 mM sucrose, 5 mM (at pH 6.2) or 2 mM (at pH 9.5) $MgCl_2$, and 0.02 to 0.135 mg of mitochondrial protein and inhibitors in final concns. indicated below. The values are means from 2 to 4 experiments.

Inhibitor	*Inhibition* (%)	
	pH 6.2	*pH 9.5*
Atebrine, 1 mM	80	79
Chlorpromazine, 1 mM	77	61
PCMB, 1 mM	81	62
0.1 mM	86	49
Ouabain, 0.1 mM	0	0
NaN_3, 20 mM	30	75
4 mM	6	71
0.4 mM	0	55
NaF, 80 mM	94	87
10 mM	85	25

TABLE II

THE EFFECT OF OLIGOMYCIN ON ATPASE ACTIVITY IN MUTANT MITOCHONDRIA

Conditions similar to those in Table I except that incubation mixture contained oligomycin and 1% methanol (added with the inhibitor).

pH	*Mitochondrial protein (mg)*	*Oligomycin* (μg)	*Inhibition* (%)			
			30	*5*	*1*	*0**
6.2	0.13		12	6	0	7
6.2	0.135		25	26	11	10
9.5	0.02		23	25	9	7
9.5	0.065		7	6	0	2
9.5	0.135		26	11	8	0
9.5	0.140		17	13	10	0

* Methanol alone.

DISCUSSION

It is generally supposed that mitochondrial DNA may determine the synthesis of structural components of mitochondria especially that of structural protein. Respiratory enzymes may be coded by nuclear genes and synthesised outside mitochondria[6,17–23]. The enzyme components of the mitochondrial ATPase system might also be synthesised outside mitochondria; however, experiments on protein synthesis by isolated mitochondria[23] suggest that the mitochondrial ATPase may be formed directly in mitochondria and thus coded by mitochondrial DNA. The fact that the mutant ATPase is almost insensitive to oligomycin, unlike the ATPase of non-mutant mitochondria, may reflect structural changes in the protein. Alternatively, a factor conferring oligomycin sensitivity to the mitochondrial ATPase[24] may be absent from the mutant mitochondria. If so, the ATPase activity demonstrated in the mutant mitochondria may well be a preserved part of the oxidative phosphorylation system despite its oligomycin insensitivity. It may be either an unnecessary remnant or a functionally important component equilibrating cellular ATP with non-phosphorylated high-energy compounds or states. The mitochondrial localisation of the ATPase studied has been recently proved by a gradient-centrifugation technique[25].

Dinitrophenol only slightly activated this ATPase reaction and azide inhibited it at rather high concentrations as compared with its efficiency *in vitro*[10,11]. Therefore, the possibility remains that an interaction with some hitherto unexplored reactions may be responsible for the inhibitory effect of the two compounds on synthetic processes in the respiratory-deficient mutants and in yeast generally.

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