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OXIDATIVE PHOSPHORYLATION IN YEAST

II. AN OXIDATIVE PHOSPHORYLATION-DEFICIENT MUTANT

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SUMMARY

1. Mitochondria from the mutant *Saccharomyces cerevisiae* DH 1 oxidized members of the tricarboxylic acid cycle, D(−)- and L(+)-lactates, ethanol, NADH, and *N,N,N',N'*-tetramethyl-*p*-phenylenediamine with P/O ratios found in manometric experiments to be as low as 0.1 to 0.4. The low phosphorylation efficiency was not improved by a large excess of ADP or glucose + hexokinase, by serum albumin, or by the supernatant from an homogenate of non-mutant yeast. The weak phosphorylation was suppressed by oligomycin. In short-term polarographic experiments, higher P/O ratios, determined by a radioactive phosphate incorporation technique, were consistently found.

2. The oxidation activity of the mutant mitochondria was the same in the presence and absence of hexokinase + glucose and was not raised by ADP.

3. 2,4-Dinitrophenol and carbonylcyanide *p*-trifluoromethoxyphenylhydrazone at uncoupling concentrations stimulated the oxidation of all substrates with the exception of L(+)-lactate. This indicates that the coupling mechanism was not abolished in the mutant mitochondria. In media of about 0.15 osmolarity, Mg^{2+} was required for dinitrophenol to stimulate the oxidation.

4. ATPase (ATP phosphohydrolase, EC 3.6.1.3) activity of mutant mitochondria displayed two pH optima like that from non-mutated yeast. However, the oligomycin-sensitive component with pH optimum at pH 9.5 was substantially reduced in the mutant mitochondria.

5. Cytochrome *a* content was found to be lower in the mutant mitochondria.

6. Possible changes in the oxidative phosphorylation system of the mutant mitochondria are discussed.

INTRODUCTION

Biochemical mutants with lesions in metabolic sequences have proved useful in solving many problems of the intermediary metabolism. The existence of yeast mutants with deficiencies in the respiratory chain or in mitochondrial structure[1–4]

Abbreviations: FCCP, carbonylcyanide *p*-trifluoromethoxyphenylhydrazone; PCMB, *p*-chloromercuribenzoate; TMPD, *N,N,N',N'*-tetramethyl-*p*-phenylenediamine.

as well as mutants with 'ineffectual respiration'[2,5–7] along with the elaboration of procedures for the isolation of intact yeast mitochondria[8–10] provide the opportunity of using the biochemical mutants in oxidative phosphorylation research.

A previous study[6] with intact cells of a mutant with ineffectual respiration[5] suggested a lesion in its oxidative phosphorylation system. Despite the normal respiration ability the mutant cells did not grow substantially on non-carbohydrate substrates and their molar growth yields on glucose were the same aerobically and anaerobically. Unlike wild-type yeast, the mutant oxidized ethanol completely with no assimilation of a part of the substrate carbon. By the procedure of Lynen and Königsberger[8], which was invented to measure phosphorylation ability of the yeast cell *in vivo*, no drop in intracellular inorganic phosphate was found in the mutant cells after ethanol addition and essentially equal patterns of the phosphate changes were obtained under aerobic and anaerobic conditions after glucose addition.

In this paper a study of mitochondria from this mutant is reported which corroborates changes in its oxidative phosphorylation ability. A short report summarizing genetic, physiological and biochemical properties of the mutant is being published simultaneously[7].

EXPERIMENTAL

The mutant used, designated *Saccharomyces cerevisiae* DH 1, is an adenine-requiring diploid strain, homozygous for the mutant gene op_1 (see refs. 5,7), prepared by a cross of two haploid mutant cells of opposite mating types. It was grown for 24 h at 30° in a semi-synthetic medium[10] with 0.5 % glucose as carbon source and supplemented with 0.004 % adenine. The other materials and methods as well as the wild-type strain, *S. cerevisiae* DT XII, employed in this study are the same as used previously[10].

RESULTS

Oxidation and phosphorylation in mutant mitochondria

Mitochondria isolated from the mutant, like mitochondria from the wild strain[10], oxidized members of the tricarboxylic acid cycle, D- and L-lactates, ethanol, NADH and *N,N,N',N'*-tetramethyl-*p*-phenylenediamine (TMPD) reduced by ascorbate. However, the oxidation rates, determined by a manometric procedure, were the same in the presence and in the absence of glucose + hexokinase and in polarographic experiments they did not increase after the addition of ADP. Specific activities were generally lower than those of the wild strain.

In Warburg experiments, only very weak phosphorylation accompanied the oxidation of substrates (Table I), the P/O ratios being substantially lower than in the wild-type yeast[10]. The P/O ratio with citrate remained equally low during 5 to 60 min of incubation and did not depend on the concentration of mitochondria. It was not changed in the presence of 2 mM arsenite and it slightly increased in the presence of fluoride which did not affect the P/O ratio in the wild yeast under the same conditions. Serum albumin did not substantially affect the oxidation; it increased P/O ratios in some experiments but they still remained much lower than in the wild-type yeast. Raising the pH from 6.4 to 8.5 increased the oxidation but the phosphory-

TABLE I

OXIDATION AND PHOSPHORYLATION WITH VARIOUS SUBSTRATES IN MUTANT MITOCHONDRIA

The main compartment of the Warburg flasks contained in 2.0 ml: 0.48 M mannitol, 1.1 mM EDTA, 10 mM Tris–maleate, 10 mM (in Expt. 1) or 4 mM (in Expt. 2) potassium phosphate, 0.5 mM ATP, 7.5 mM $MgCl_2$, 0.15% serum albumin, 25 mM glucose, 0.5 mg hexokinase, substrate, and mitochondria (1.34 and 2.25 mg of mitochondrial protein in Expts. 1 and 2, respectively); final pH 6.4. The central well contained 0.2 ml 2 M KOH and a piece of fluted paper. The measurements commenced after 7 min of thermal equilibration at 30° by addition of glucose and hexokinase from the side arm. To obtain initial values of inorganic phosphate, mitochondria were added to 2 flasks containing the complete reaction mixture, with and without glucose + hexokinase acidified by 5% trichloroacetic acid.

Expt. No.	*Substrate*	*Time (min)*	$-\Delta O$ *(μgatoms)*	$-\Delta P_i$ *(μmoles)*	*P/O ratio*	*Oxidation rate (μgatom O/min per mg)*
1	None	20	0.6	(−0.5)*	0	0.02
	10 mM citrate	20	4.8	1.05	0.22	0.18
	10 mM succinate	20	3.6	0.32	0.09	0.13
	10 mM α-ketoglutarate	20	2.9	0.35	0.12	0.11
	10 mM D(−)-lactate	20	4.9	0.83	0.17	0.18
	10 mM L(+)-lactate	20	2.6	0.31	0.12	0.10
	10 mM ethanol	20	3.5	0.45	0.13	0.13
2	4 mM NADH	30	4.9	0.69	0.14	0.07
	150 μM TMPD + 7.5 mM ascorbate	20	23.2	0.92	0.04	0.52

* Increase of P_i.

lation was depressed. The weak phosphorylation was completely inhibited by oligomycin at concentrations which were effective in non-mutant yeast[10]. The phosphorylation did not increase with increasing amounts of ADP. An increase in the level of inorganic phosphate was actually observed due to hydrolysis of the ADP. These results are summarized in Table II.

The low P/O ratios found could not be due to the α-ketoglutarate-linked substrate-level phosphorylation as they were obtained also with lactates (in the absence of a sparker which would permit their oxidation *via* the Krebs cycle), NADH, TMPD and with citrate + arsenite. Furthermore, the P/O ratio with α-ketoglutarate was not higher than with other substrates despite the presumed substrate-level phosphorylation step. The phosphorylation with α-ketoglutarate was not increased in the presence of malonate or fluoride which would have inhibited its further oxidation *via* succinate and was also almost completely prevented by oligomycin (Table III).

It could be inferred from these results that the phosphorylation in the mutant mitochondria was essentially uncoupled from oxidation, in the same manner as in wild-yeast mitochondria by the action of uncoupling agents. This was not the case, however, as 2,4-dinitrophenol raised the oxidation rate of substrates when added to mutant mitochondria. In polarographic experiments, dinitrophenol in concentrations as low as 25 μM was active in increasing the oxidation rate, the maximal effect being achieved at about 100 μM which is the same as in wild-strain mitochondria[10] (Fig. 1). From the results of a typical experiment, presented in Table IV, it is evident that 100 μM dinitrophenol raised the oxidation rates with all substrates tested except for L-lactate. This corresponds to the observation that the State-4 to State-3 transition was ob-

served after the addition of ADP to wild-type mitochondria with all substrates, with the exception of L-lactate. The oxidation rates were also increased in the presence of 0.5 μM carbonylcyanide *p*-trifluoromethoxyphenylhydrazone (FCCP). The oxidation was inhibited by azide (Fig. 1), but at much higher concentrations than in wild-

TABLE II

OXIDATION AND PHOSPHORYLATION WITH CITRATE AS SUBSTRATE IN MUTANT MITOCHONDRIA

Incubation mixture similar to that in Table I with 4 mM potassium phosphate except for modifications listed below. Substrate was 10 mM citrate.

Expt. No.	*Mitochondrial protein (mg)*	*Change in flask content*	*Time (min)*	$-\Delta O$ *(μgatoms)*	$-\Delta P_i$ *(μmoles)*	*P/O ratio*
1	5.04	None	5	3.7	0.44	0.12
		None	10	8.1	2.04	0.25
		None	20	17.2	2.42	0.14
		Hexokinase omitted	20	16.2	(−1.82)*	0
		40 mM NaF added	20	14.8	4.95	0.33
2	1.60	None	40	8.3	1.42	0.17
		Mg^{2+} omitted	40	6.0	0.62	0.10
		0.28 M instead of 0.48 M mannitol	40	10.3	1.50	0.16
		0.08 M mannitol and 0.75 M sorbitol instead of 0.48 M mannitol	40	8.9	2.30	0.26
3	2.25	None	20	12.1	2.16	0.18
		2 mM arsenite added	20	10.3	1.13	0.11
		Ten times more hexokinase added	20	14.3	1.27	0.09
		Instead of glucose + hexokinase, 5 mM ADP added	20	12.1	(−3.02)*	0
		Final pH 8.4 instead of 6.4	20	17.5	(−0.31)*	0
		Serum albumin omitted	20	13.5	0.37	0.03
		Serum albumin omitted, 40 mM NaF added	20	13.9	2.16	0.16
		20 μg oligomycin added	20	13.7	0.12	0.01
		5 μg oligomycin added	20	12.0	(−0.73)*	0

* Increase of P_i.

TABLE III

OXIDATION AND PHOSPHORYLATION WITH α-KETOGLUTARATE IN MUTANT MITOCHONDRIA

Conditions as in Table II except for modifications listed below. Substrate was 10 mM α-ketoglutarate and each flask contained 2.25 mg of mitochondrial protein. Incubation time was 20 min.

Change in flask content	$-\Delta O$ *(μgatoms)*	$-\Delta P_i$ *(μmoles)*	*P/O ratio*
None	6.2	2.22	0.32
20 mM NaF added	2.8	0.30	0.11
6 mM malonate added	4.4	0.38	0.09
NaF + malonate added	1.7	(−0.29)*	0
20 μg oligomycin added	5.8	0.21	0.04
5 μg oligomycin added	6.0	0.48	0.08

* Increase of P_i.

yeast mitochondria in which 20 μM azide considerably inhibited State-3 and slightly inhibited State-4 oxidations.

As with wild-yeast mitochondria, the oxidation of α-ketoglutarate and of suc-

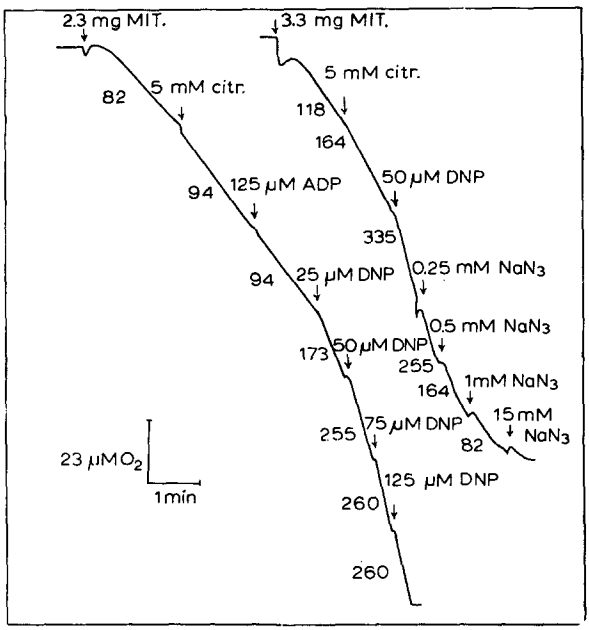

Fig. 1. The effects of ADP, 2,4-dinitrophenol (DNP) and azide on the oxidation of citrate by mutant mitochondria. The polarographic vessel contained in 2.0 ml: 0.6 M mannitol, 20 mM KCl, 1.5 mM EDTA, 10 mM Tris–maleate and 10 mM potassium phosphate; final pH 6.4. Numbers at curves indicate oxidation rates in ngatoms O/min. Additions are expressed in final concns. in the incubation mixture.

TABLE IV

INCREASE OF OXIDATION RATES IN MUTANT MITOCHONDRIA BY DINITROPHENOL

The polarographic vessel contained in 2.0 ml: 0.6 M mannitol, 1.5 mM EDTA, 20 mM KCl, 10 mM Tris-maleate, 10 mM potassium phosphate, 2.4 mg of mitochondrial protein and substrates as indicated below; final pH 6.4. Before dinitrophenol, 125 μM ADP was added which did not change the oxidation rates. The oxidation rates are expressed in μgatoms O/min per g protein.

Substrate	*Oxidation rate*	
	Without dinitrophenol	*In the presence of 0.1 mM dinitrophenol*
None	21	21
5 mM citrate	64	137
5 mM α-ketoglutarate	39	92
5 mM succinate	59	94
5 mM pyruvate + 1 mM maleate	63	78
0.75 mM NADH	73	149
5 mM ethanol	65	76
5 mM L(+)-lactate	32	32
5 mM D(−)-lactate	63	76
75 μM TMPD + 3.2 mM ascorbate	236	302

cinate was inhibited by higher concentrations of dinitrophenol which still stimulated the oxidation of citrate. As shown in Table V, the weak phosphorylation coupled both to the citrate and α-ketoglutarate oxidations was totally prevented by dinitrophenol.

It may be important that P/O ratios with mutant mitochondria found in short-term polarographic experiments (lasting 2 to 3 min) from ^{32}P-labelled inorganic phosphate incorporation were higher than those usually found in Warburg flasks by chemical analysis. On the other hand, P/O ratios with wild-strain mitochondria were lower with the former than with the latter method so that P/O ratios obtained in polarographic experiments with mutant mitochondria were about 60 % of those found with wild-strain mitochondria (Table VI).

Mg^{2+} did not substantially affect the oxidation by mutant mitochondria in incubation media with 0.2 to 1.5 M mannitol or sorbitol. The oxidation seemed to be less affected by high tonicities than in wild-strain mitochondria. At mannitol concen-

TABLE V

THE EFFECT OF DINITROPHENOL ON OXIDATION AND PHOSPHORYLATION IN MUTANT MITOCHONDRIA

Conditions similar as in Table II. Each Warburg flask contained 2.25 mg of mitochondrial protein. Incubation time 20 min.

Substrate	*Dinitrophenol (mM)*	$-\Delta O$ *(μgatoms)*	$-\Delta P_i$ *(μmoles)*	*P/O ratio*
10 mM citrate	—	12.1	2.16	0.18
	0.2	25.0	(−0.98) *	0
10 mM α-ketoglutarate	—	6.2	2.22	0.32
	0.2	6.2	(−0.37) *	0
	0.5	4.8	(−1.00) *	0

* Increase of P_i.

TABLE VI

P/O RATIOS WITH WILD-TYPE STRAIN AND MUTANT MITOCHONDRIA DETERMINED IN SHORT-TERM POLAROGRAPHIC EXPERIMENTS

The polarographic vessel contained in 2.0 ml: 0.6 M mannitol, 20 mM KCl, 10 mM Tris–maleate, 10 mM potassium phosphate (containing ^{32}P with about $5 \cdot 10^5$ counts/min), 1.5 mM $MgCl_2$, 0.5 mM ADP, 5 mM substrate, 25 mM glucose, 0.5 mg of hexokinase (Sigma, Type III), 0.25 % bovine serum albumin and about 2.5 mg of mitochondrial protein; final pH 6.4. The reaction was started by the addition of mitochondria and terminated, after O_2 concentration decreased to about 50 %, by adding 0.1 ml 100 % (w/v) trichloroacetic acid. Inorganic phosphate was extracted as the phosphomolybdate complex and the radioactivity of the non-extractable residue determined as described previously[10]. Controls without substrate, without glucose + hexokinase and with oligomycin, as well as samples deproteinized at the beginning of the experiments, were also measured. The results are means of 2 or 3 experiments.

Origin of mitochondria	*Substrate*	*P/O ratio*
Wild-type strain	Citrate	1.27
	α-Ketoglutarate	1.46
Mutant	Citrate	0.76
	α-Ketoglutarate	0.90

trations lower than 0.1 M (and the final osmolarity about 0.18) the oxidation rates showed little or no increase in the presence of dinitrophenol. In the presence of Mg^{2+}, dinitrophenol raised the rates even at these low tonicities (Fig. 2). Such a requirement of Mg^{2+} for dinitrophenol action, which could not be accounted for by an increase in the tonicity by Mg^{2+}, has not been observed with wild-yeast mitochondria.

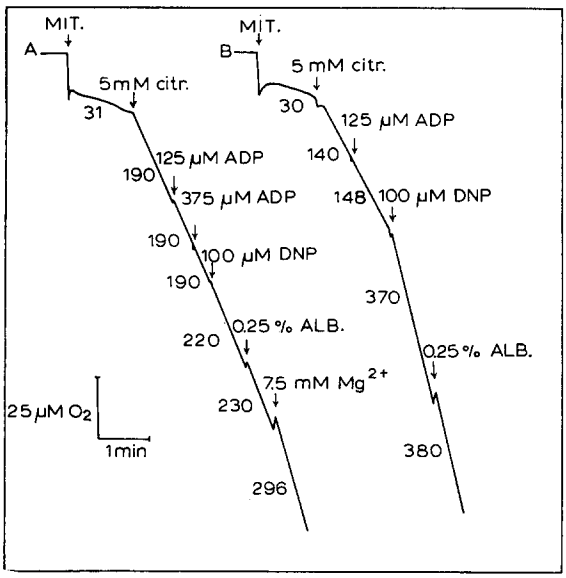

Fig. 2. The effects of ADP, 2,4-dinitrophenol (DNP), Mg^{2+} and serum albumin on the oxidation of citrate by mutant mitochondria at a low tonicity. The polarographic vessel contained in 2.0 ml: 80 mM mannitol, 20 mM KCl, 10 mM Tris–maleate, 10 mM potassium phosphate and 2.25 mg of mitochondrial protein; final pH 6.4. In B, it contained, in addition to the above, 7.5 mM $MgCl_2$. Numbers at curves indicate oxidation rates in ngatoms O/min. Additions are expressed in final concns. in the incubation mixture.

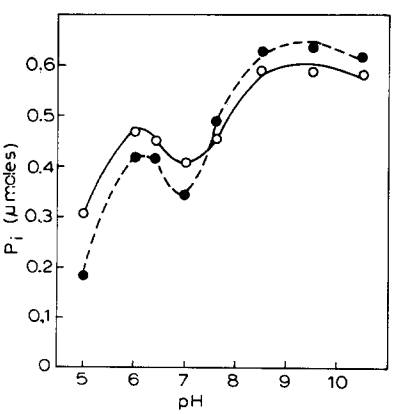

Fig. 3. pH curve of ATPase activity. The incubation mixture contained in 1.0 ml: 4 mM ATP, 40 mM KCl, 0.4 mM EDTA, 5 mM $MgCl_2$, 30 mM buffer (Tris–maleate at pH 5 to 7 and Tris–chloride at pH 7.6 to 10.5), 88 mM sucrose and 0.10 mg of mitochondrial protein. Reaction time: 10 min. Dotted line indicates the activity in the presence of 0.5 mM dinitrophenol.

ATPase activity of mutant mitochondria

As wild-strain mitochondria[10], the mutant mitochondria displayed only very weak ATPase activity in the absence of Mg^{2+}. In the presence of Mg^{2+}, the highly increased ATPase activity had two pH optima, at pH 6.2 and a rather broad optimum at about 9.5 (Fig. 3). These optima might correspond to two different enzymes or enzyme systems, similar to what has been observed in wild-strain mitochondria[10]. However, the activity at pH 9.5 was lower than in the wild-yeast mitochondria. The mean specific activities (in μmoles P_i liberated per mg protein per h) were 33 and 39 at pH 6.2 and 183 and 68 at pH 9.5, with wild and mutant mitochondria, respectively. Dinitrophenol inhibited the activity at pH values lower than 7.5 and stimulated above this pH.

The effect of various inhibitors on the ATPase activity is summarized in Tables VII and VIII. The inhibitions at pH 6.2 were so strikingly similar to those found in wild-strain mitochondria[10] that it may be safely concluded that the activity at pH 6.2

TABLE VII

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) $MgCl_2$, 0.02 to 0.18 mg of mitochondrial protein and inhibitors in the final concns. indicated below. The values are means from 2 or 3 experiments.

Inhibitor	*Inhibition* (%)	
	pH 6.2	*pH 9.5*
Atebrine, 1 mM	72	79
Chlorpromazine, 1 mM	76	40
PCMB, 1 mM	81	78
0.1 mM	80	60
Ouabain, 0.1 mM	0	0
NaN_3, 20 mM	25	74
4 mM	8	60
0.4 mM	0	45
NaF, 80 mM	96	86
10 mM	73	30

TABLE VIII

THE EFFECT OF OLIGOMYCIN ON ATPASE ACTIVITY IN MUTANT MITOCHONDRIA

Conditions similar to those in Table VII except that the incubation mixtures contained oligomycin and 1% methanol (added with the inhibitor). The methanol alone only slightly inhibited the activity (0 to 7%).

pH	*Mitochondrial protein (mg)*	*Oligomycin* (μg)	*Inhibition* (%)			
			30	*5*	*1*	*0.1*
6.2	0.11		7	5	5	—*
9.5	0.022		76	76	71	44
9.5	0.11		67	59	45	11
9.5	0.18		71	66	54	20

* Not tested.

was not affected by the mutation. On the other hand, the ATPase activity of the mutant mitochondria at pH 9.5 was less sensitive to atebrine, chlorpromazine, *p*-chloromercuribenzoate (PCMB), azide, and oligomycin and more inhibited by fluoride than the activity in wild-strain mitochondria[10]. This need not mean, however, that the activities were qualitatively different in the two strains. If the absolute amount of the pH 9.5 enzyme is lower in the mutant than in the wild strain the contribution of the pH 6.2 enzyme to the total activity at pH 9.5 becomes relatively more important in the mutant. As the pH 6.2 enzyme is almost insensitive to oligomycin, only slightly inhibited by azide and strongly inhibited by fluoride, the differences in inhibitions at pH 9.5 in the two strains can be explained.

The possibility that the pH 9.5 enzyme may be changed in the mutant in quantity and not in quality is supported by the observation that the Michaelis constant for ATP at pH 9.5 in the mutant mitochondria was found to be 2.5 mM, while the corresponding constant in the wild-type yeast is 2.2 mM (see ref. 10).

Other differences in mitochondria from the mutant and from wild-type yeast

It has been found previously that the cytochrome spectrum of the mutant cells grown in a complex medium with 2 % glucose as carbon source was similar to that in wild-type yeast[6,7]. This has now been confirmed. However, the cells grown under the standard conditions employed in this work, *i.e.* in semi-synthetic medium with 0.5 % glucose, contained less relative amounts of cytochrome *a* than cytochromes *b* and *c*. The same has been found in mitochondria isolated from the mutant. Difference spectra of mitochondria isolated from wild-type and mutant yeast are shown in Fig. 4. The decreased content of cytochrome *a* may account for the observation (unpublished) that the mutant colonies grown on solid media stained less intensely with triphenyltetrazolium chloride than colonies of wild-type yeast. The possibility is not excluded that the level of cytochrome *b* and *c* is higher in the mutant than in the wild strain; this will be explored in the future.

The question should be considered if the decrease in oxidative phosphorylation efficiency, especially that *in vivo*, could not be accounted for by an uncoupling compound produced in the mutant cells. This might be already partly excluded from observations obtained *in vitro* since serum albumin, which might bind presumed uncoupling agents[12], did not restore the phosphorylation (Table II). As shown in Table IX, supernatant obtained from an homogenate of the mutant cells after sedimentation of mitochondria did not substantially inhibit the oxidative phosphorylation with

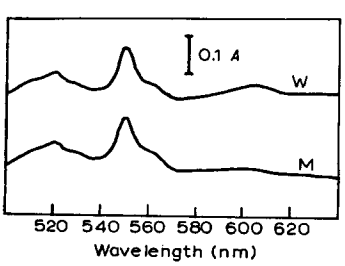

Fig. 4. Difference spectra of mitochondria from wild-type (W) and mutant (M) yeast. The spectra were measured by the procedure of WILLIAMS[11] in the spectrophotometer SF-10. Cuvettes of 10 mm light path contained 35 and 27 mg of mitochondrial protein per ml from wild-type and mutant yeast, respectively.

mitochondria from wild-type yeast. Similarly, the supernatant from wild-type yeast did not improve phosphorylation efficiency of the mutant mitochondria. In both cases, the supernatant from mutant cells had the same effect as the supernatant from wild-type yeast.

TABLE IX

THE EFFECT OF SUPERNATANTS ON OXIDATIVE PHOSPHORYLATION BY WILD-TYPE YEAST AND MUTANT MITOCHONDRIA

Conditions similar to those in Table I with 10 mM citrate as substrate. With wild-strain mitochondria, the Warburg flasks contained 10 mM phosphate and 1.3 mg of mitochondrial protein; with mutant mitochondria, the flasks contained 4 mM phosphate and 2.4 mg of mitochondrial protein. Supernatants were obtained from homogenates after sedimentation of mitochondria at 20000 × *g*; they contained 17 and 21 mg protein per ml from wild and mutant yeast, respectively; 0.1 ml of each was added to the flask.

Expt. No.	*Origin of mitochondria*	*Origin of supernatant*	*Time (min)*	$-\Delta O$ *(μgatoms)*	$-\Delta P_i$ *(μmoles)*	*P/O ratio*
1	Wild	—	20	8.1	10.5	1.30
		Wild	20	7.5	8.2	1.10
		Mutant	20	7.6	7.9	1.04
2	Mutant	—	30	8.9	0.8	0.09
		Wild	30	8.6	(−1.2)*	0
		Mutant	30	8.6	(−2.0)*	0

* Increase of P_i.

DISCUSSION

The low P/O ratios found with isolated mutant mitochondria could be due simply to damage to the mitochondria caused by the isolation procedure used. Even though the procedure is mild enough to furnish well preserved mitochondria with an efficient phosphorylation ability from several wild-type strains, the modification might occur during preparation if the mutation rendered particularly fragile mitochondria. Although the mutant mitochondria did not respond to the addition of ADP, respiratory control was maintained in them as high as in wild-type mitochondria, as dinitrophenol and FCCP activated the respiration in the absence of phosphate acceptors to the same extent in mutant and wild-type mitochondria. The presumed preparative modification would therefore be rather unique leaving the first step of the oxidative phosphorylation machinery, the coupling reaction, entirely preserved and affecting subsequent energy-transfer reactions. It is the common experience with wild-type yeast mitochondria, as well as with mitochondria from other sources, that preparative damage first affects the coupling process, resulting in loosely coupled mitochondria without respiratory control. We favour, therefore, the interpretation that the decreased phosphorylation efficiency is displayed by the mutant mitochondria *in situ* as well. This fully accounts for the properties of intact cells of the mutant as studied previously and summarized in INTRODUCTION.

At least three explanations can be proposed to account for the decreased phosphorylation efficiency of the mutant mitochondria. First, the energy-transfer part of the oxidative phosphorylation system could be structurally modified allowing the formation of the primary high-energy compounds or states but largely preventing

their transformation in ATP. At first sight, this interpretation would seem to be supported by the decrease observed in the oligomycin-sensitive ATPase activity of the mutant mitochondria. However, as pointed out in RESULTS, this decrease in activity might be simply due to a decrease in quantity of the catalytic protein. Further, the specific activity of the mutant ATPase, even though lower than in wild-type yeast mitochondria, is still at least twice as high as the specific activity of net oxidative phosphorylation in non-mutant yeast.

This would favour another interpretation, namely that the change in ATPase activity is a consequence of more profound changes in organization of proteins or structures in the mutant mitochondria. This interpretation is supported by the fact that the mutation has a pleiotropic effect, the amount of cytochromes being affected as well. The differences between wild-strain and mutant mitochondria in the sensitivity of oxidation to changes in tonicity and to Mg^{2+} would be in line with this interpretation. It may also partly explain the observation that the P/O ratio with α-ketoglutarate in the mutant mitochondria was as low as with other substrates and was suppressed by dinitrophenol and oligomycin despite the substrate-level phosphorylation which should accompany the α-ketoglutarate oxidation and should not be inhibited by the two compounds[13–16]. The same inefficiency of the substrate-level phosphorylation linked to α-ketoglutarate oxidation should also occur *in vivo*; if not, the mutant may grow relatively well on non-carbohydrate substrates supported by the ATP produced at this phosphorylation step, which had not been observed. Because of a structural modification of mitochondria, GTP formed by the substrate-level phosphorylation[17] may not be sufficiently rapidly transformed to extramitochondrial ATP and may be used in side reactions[18]. In addition, a communication between the substrate-level and respiratory chain phosphorylation compartments[19,25] might be influenced by the mutation.

This leads to the third possibility. The mutation lesion may concern the translocation of adenine nucleotides through the mitochondrial membrane[20–22]. Such a lesion would explain why both substrate- and respiratory chain-linked phosphorylations are affected. Furthermore, the decreased ATPase activity would be explained by a changed accessibility of ATP to the enzyme. The higher P/O ratios found in short-term polarographic experiments by the ^{32}P-labelled inorganic phosphate incorporation technique would also be explained if one were to assume that the internal ATP formed in short-term experiments is used to phosphorylate some components inside mitochondria. The fact that a one-gene mutation has affected simultaneously the phosphorylation at two coupling sites (which correspond to the Coupling sites II and III of mammalian mitochondria, Site I supposedly being absent from Saccharomyces[8,22–24]) would also be accounted for by the defect in the nucleotide translocation.

The three interpretations are not mutually exclusive and may have a common, hitherto unknown, denominator.

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