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OXIDATIVE PHOSPHORYLATION IN YEAST

I. ISOLATION AND PROPERTIES OF PHOSPHORYLATING MITOCHONDRIA FROM STATIONARY PHASE CELLS

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SUMMARY

1. A procedure for the preparation of protoplasts from yeast *Saccharomyces cerevisiae* harvested in their stationary phase of growth on glucose was modified and mitochondria were isolated from the protoplasts.

2. Oxidative activity, phosphorylation efficiency and sensitivity to 2,4-dinitrophenol and oligomycin of these mitochondria were similar as previously reported for mitochondria from lactate-grown *Saccharomyces carlsbergensis*.

3. The mitochondria exhibited a Mg^{2+}-dependent ATPase (ATP phosphohydrolase, EC 3.6.1.3) activity with two pH optima, one at pH 6.2 and another at 9.5. Properties of the ATPase at the two pH optima with respect to activation by dinitrophenol, requirement for cations, stimulation by detergents, as well as dependence on ATP and Mg^{2+} concentrations, were studied. The activity at pH 9.5 was strongly inhibited by oligomycin and azide and only moderately by fluoride. Inverse sensitivity towards the three inhibitors was observed at pH 6.2.

4. The mitochondria catalyzed ATP–inorganic phosphate exchange reaction which was inhibited by dinitrophenol, azide and oligomycin.

INTRODUCTION

Yeast appears to be a particularly suitable organism for studies of oxidative phosphorylation since the structure and enzymic composition of its mitochondria can be modified to a large extent by conditions of cultivation and by genic or extrachromosomal mutations[1–6]. A successful preparation of intact mitochondria is prerequisite for such studies. In this paper, a modification of the procedure[7] for the isolation of mitochondria from *Saccharomyces cerevisiae* harvested in their stationary phase of growth is described providing a method which may be generally applicable to yeast. Previous studies on oxidative phosphorylation in yeast[8–17] are extended and new data about the partial reactions of oxidative phosphorylation in yeast are reported.

Abbreviation: PCMB, *p*-chloromercuribenzoate.

EXPERIMENTAL

Chemicals

Oligomycin and hexokinase (Type III, 300 Kunitz units/mg) were donated to us by Upjohn Chemical Company and Sigma Chemical Company, respectively. ADP, D(−)- and L(+)-lactates originated from Calbiochem, ATP (sodium salt) from Reanal and Boehringer, atebrine and chlorpromazine from Spofa, Prague, bovine serum albumin from Mann Res. Lab., and *p*-chloromercuribenzoate (PCMB) from Light. All other chemicals were purchased from Lachema, Brno; most of them were recrystallized in the laboratory. Glass-redistilled water was employed. Snail gut juice was prepared from the digestive tract of *Helix pomatia*. After centrifugation at 10000 × *g* it was dialyzed or filtered through a Sephadex G-25 column and lyophilized. It has been found that the active preparation may be regenerated from the solution used in digestion of yeast walls and employed at least twice providing useful economy of this material.

Microorganism and culture procedure

The laboratory strain *S. cerevisiae* DT XII was grown in a semi-synthetic medium containing per l: 1 g KH_2PO_4, 0.7 g $MgSO_4 \cdot 7H_2O$, 0.5 g NaCl, 0.4 g $CaCl_2$, 1.2 g $(NH_4)_2SO_4$, 5 mg $FeCl_3$, 5 g peptone, 3.75 g yeast autolysate and 2.5 g glucose. The cells were cultivated aerobically on a shaker at 30° for 24 h in erlenmeyer flasks filled to one-tenth of their volume with the medium. The medium in the flasks was inoculated with 1/1000 of its volume of 24-h-old inoculum. The harvested cells were washed twice with distilled water.

Preparation of protoplasts

The cells (1 g dry wt.) were suspended in 20 ml of 0.5 M sodium thioglycollate buffered with 0.1 M Tris to pH 9.3 and incubated at 30° for 30 min. The suspension was centrifuged, the cells washed with a solution containing 0.7 M sorbitol, 0.3 M mannitol, 10 mM citrate–phosphate buffer (pH 5.8) and 1 mM EDTA and finally suspended in the same medium to a final vol. of 10 ml. About 200 mg of lyophilized snail gut preparation dissolved in a minimal volume of 1.0 M sorbitol were added and the suspension incubated at 30° with occasional stirring. The formation of protoplasts was followed by differential counting of samples diluted with water and 0.8 M mannitol[7]. After the formation of protoplasts was completed (20–60 min) the suspension was centrifuged at 3000 × *g* for 10 min and the protoplasts washed 3 times with 0.8 M mannitol in 2 mM EDTA (pH 7.6) being centrifuged each time at 1600 × *g* for 10 min.

Isolation of mitochondria

The protoplasts from 0.5 to 1 g (dry wt.) of original cells were suspended in a minimal volume of 0.8 M mannitol in 2 mM EDTA and mixed with 10 ml of 0.44 M mannitol in 1 mM EDTA (pH 7.6) containing 0.1 % bovine serum albumin. Instead of mannitol, 0.44 M sucrose was employed occasionally. The suspension was homogenized for 8 sec in a stainless steel blendor at medium speed and then centrifuged at 1500 × *g* for 10 min. Mitochondria were isolated from the supernatant by centrifugation at 8000 × *g* for 10 min and suspended in 4 ml of the homogenization medium

from which serum albumin had been omitted. The suspension was centrifuged at 1200 × *g* for 5 min to remove contamination of unbroken protoplasts and cell-wall fragments and the mitochondria sedimented from the supernatant at 18000 × *g* for 12 min. The surface of the sediment was gently washed with a small amount of 0.8 M mannitol and the mitochondria suspended in 0.8 M mannitol.

Assay of oxidative phosphorylation

O_2 consumption was measured manometrically in a Warburg apparatus or polarographically with a vibrating gold electrode[18]. In both assays, incubation proceeded at 30°. In manometric experiments, reaction was terminated by adding chilled trichloroacetic acid to flasks to a final concentration of 5 % and inorganic phosphate was determined in the supernatant[19].

Determination of ATPase and ATP–phosphate exchange activities

Mitochondria (0.2 ml in 0.44 M sucrose) were added to 0.8 ml of reaction mixture and incubated in open tubes kept in a water thermostat at 30°. The reaction was terminated by adding trichloroacetic acid to a final concentration of 5 % and the precipitated protein removed by centrifugation. In the ATPase assay, the supernatant was analyzed for inorganic phosphate[19]. In the ATP–phosphate exchange assay, inorganic phosphate (containing $3 \cdot 10^5$ to $2 \cdot 10^6$ counts/min of ^{32}P per tube) was separated from an aliquot of the supernatant by a modified procedure of LINDBERG AND ERNSTER[20]. The modification consists in extraction of molybdate complex twice with isobutanol and once with isobutanol–benzene (1:1, v/v) and then filtering the water phase through a paper filter. Radioactivity of the water phase was counted by a thin-window Geiger-Müller tube. Since a relatively high concentration of P_i was employed, the correction of the ATP–phosphate exchange activity for concomitant ATP hydrolysis was not applied[21].

Determination of protein content

Protein was determined by the method of LOWRY *et al.*[22] or by the biuret procedure[23].

RESULTS

Preparation of protoplasts and mitochondria

The following modifications have been introduced into the original procedure of DUELL, INOUE AND UTTER[7] for the preparation of protoplasts: (1) Glucose concentration in culture media was decreased to 0.25 or 0.5 %. (2) Before digestion with snail-gut juice, yeast was preincubated with much higher concentrations of thiol compounds than used in the original procedure; 0.5 M thioglycollate or mercaptoethanol were found optimal. (3) The preincubation mixture was buffered with an alkaline buffer. These modifications have substantially increased the digestibility of yeast cells with snail-gut enzymes as well as the stability of protoplasts in the course of the digestion. By this procedure, intact protoplasts were readily obtained from a large number of haploid and diploid wild-type strains and mutants of *S. cerevisiae* grown either aerobically or anaerobically.

The protoplasts could be easily lysed by suspending in water or solutions of

0.25 to 0.4 osmolarity with mild agitation as shown by DUELL, INOUE AND UTTER[7]. However, it has been observed that such lysates contained large aggregates of subcellular particles. The particles did not agglutinate substantially if the lysis was accomplished with simultaneous vigorous mixing of the homogenation medium in the blendor.

In the procedure generally used in this work, the homogenation proceeded in solutions of 0.44 osmolarity. It has been shown afterwards in collaboration with Dr. Y. YOUTSUYANAGI (Laboratoire de Génétique physiologique du CNRS, Gif-sur-Yvette) that resulting mitochondria were swollen in isolation media of about 0.4 osmolarity while little swelling occurred in 0.8 M sorbitol.

Oxidation and phosphorylation activity of mitochondria

With respect to oxidation activity, phosphorylation efficiency, osmotic stability, and inhibitions with dinitrophenol and oligomycin, as measured by the polarographic procedure, the mitochondria from *S. cerevisiae* harvested in their stationary phase of growth on glucose resembled the mitochondria of *S. carlsbergensis* harvested in exponential phase of growth on lactate as described by OHNISHI, KAWAGUCHI AND HAGIHARA[11].

Linear respiration and phosphorylation rates were maintained in manometric experiments for at least 60 min with citrate as substrate and the respiratory control was preserved under these conditions. The oxidation rates and P/O ratios found in a typical manometric experiment are listed in Table I. The P/O ratios are lower than ADP/O ratios found polarographically which generally corresponded to values found by OHNISHI, KAWAGUCHI AND HAGIHARA[11]. On the other hand, the oxidation rates were higher in manometric than in polarographic experiments. The differences might be due to the different compositions of the reaction mixtures, especially the presence of Mg^{2+} in manometric experiments.

TABLE I

OXIDATION RATES AND PHOSPHORYLATION EFFICIENCIES IN A MANOMETRIC EXPERIMENT

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 potassium phosphate, 0.5 mM ATP, 7.5 mM $MgCl_2$, 0.15% serum albumin, 25 mM glucose, 1 mg hexokinase, substrates as indicated below, and 1.4 mg of mitochondrial protein; 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, by the addition of the glucose and hexokinase from the side arms and after 20 min of additional incubation were terminated.

Substrate	*Oxidation rate* (*μgatoms O/min per mg protein*)	*P/O ratio*
No	0.03	1.2
10 mM citrate	0.49	1.5
10 mM α-ketoglutarate	0.34	1.5
10 mM D(−)-lactate	0.49	1.0
10 mM L(+)-lactate	0.18	1.0
10 mM ethanol	0.46	1.4
10 mM succinate	0.28	1.4
10 mM pyruvate + 2 mM malate	0.42	1.5

Although, polarographically, respiratory control with various substrates was always observed with freshly prepared mitochondria, the addition of 0.25 % serum albumin usually increased the respiratory control ratios. This was mostly due to the increased State-3 oxidation (as defined in ref. 24) as exemplified in Table II. The ADP/O ratios were not affected by the serum albumin. Respiratory activity in both States 3 and 4 of mitochondria stored at 0° for 24 h was substantially decreased, and completely reestablished in the presence of serum albumin.

Some differences were observed in the effect of dinitrophenol and oligomycin under conditions of manometric and polarographic experiments. 5 μM dinitrophenol showed an observable stimulation of State-4 oxidation in polarographic experiments and the maximal effect was observed at 100 μM; in manometric experiments (Table III) 100 μM dinitrophenol displayed only a slight uncoupling effect in the oxidation

TABLE II

THE EFFECT OF SERUM ALBUMIN ON OXIDATION IN A POLAROGRAPHIC EXPERIMENT

The incubation mixture contained in 2.0 ml: 19 mM KCl, 0.95 mM EDTA, 9.5 mM Tris–maleate, 9.5 mM potassium phosphate, 570 mM mannitol, 1.8 mg of mitochondrial protein; final pH 6.4. The values are expressed in ngatoms/min.

Substrate	*Addition*			
	None		*0.25 % serum albumin*	
	State 4	*State 3*	*State 4*	*State 3*
Endogenous	54	54	91	112
5 mM citrate	182	300	182	364
5 mM α-ketoglutarate	118	182	118	374
5 mM succinate	146	164	155	265

TABLE III

THE EFFECT OF DINITROPHENOL AND OLIGOMYCIN ON OXIDATIVE PHOSPHORYLATION

Conditions as in Table I, with 10 mM citrate as substrate, except for modifications indicated in the table. The flasks contained 1.0 mg of mitochondrial protein in Expt. 1 and 0.9 mg in Expt. 2. With oligomycin, methanol was added to the final concn. of 0.5 %.

Expt. No.	*Change in flask content*	$-\Delta O$ (*μgatoms*)	$-\Delta P_i$ (*μmoles*)	*P/O ratio*
1	None	7.7	11.5	1.5
	25 μM dinitrophenol added	7.8	10.6	1.4
	100 μM dinitrophenol added	8.9	9.1	1.0
	500 μM dinitrophenol added	7.1	(−0.9) *	0
2	None	9.4	15.1	1.6
	Hexokinase and glucose omitted	6.2	0.2	0.03
	0.5 % methanol added	9.2	13.4	1.5
	1 μg oligomycin added	3.3	0.3	0.1
	5 μg oligomycin added	3.6	(−0.4) *	0
	5 μg oligomycin added; hexokinase and glucose omitted	3.3	0.2	0.06
	30 μg oligomycin added	7.0	0.6	0.09

* Increase of P_i.

of citrate and the concentration as high as 500 μM was required for total uncoupling which is much higher than in mammalian mitochondria and similar to mitochondria of *Endomyces magnusii*[25]. However, as shown in Fig. 1, 100 μM dinitrophenol did raise the rate of State-4 oxidation to the rate of State-3 oxidation in Warburg experiment.

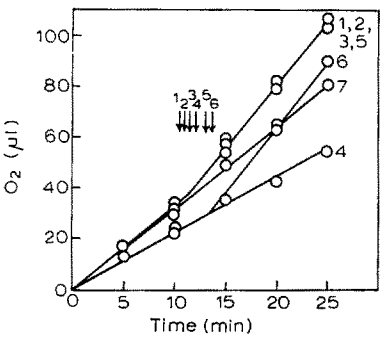

Fig. 1. The effect of glucose + hexokinase, dinitrophenol and oligomycin on the oxidation of citrate in Warburg apparatus. Conditions were similar to those in Table I, except that glucose + hexokinase were added only if indicated below. The flasks contained 0.96 mg of mitochondrial protein. 1, at the time indicated by Arrow 1, hexokinase (0.5 mg) and glucose (final concn., 25 mM) were added from the side arm; 2 and 3, at times indicated by Arrows 2 and 3, dinitrophenol was added from the side arm (final concns., 0.5 and 0.1 mM, respectively); 4, the flask contained 1 μg of oligomycin and 0.5 % methanol. At the time indicated by Arrow 4, hexokinase (0.5 mg) and glucose (25 mM) were added from the side arm; 5, at the time indicated by Arrow 5, oligomycin (1 μg), methanol (final concn. 0.5 %), hexokinase (0.5 mg) and glucose (25 mM) were added from the side arm; 6, the flask contained 1 μg oligomycin and 0.5 % methanol. At the time indicated by Arrow 6, dinitrophenol (final concn., 0.5 mM), hexokinase (0.5 mg) and glucose (25 mM) were added from the side arm; 7, no additions were made.

This would indicate a competition of dinitrophenol and glucose + hexokinase system for a common high-energy intermediate. At least 5 μg of oligomycin per mg of mitochondrial protein were required to attain an observable effect in polarographic experiments, while, in the Warburg apparatus, 1 μg oligomycin per mg protein inhibited oxidative phosphorylation completely (Table III). As shown in Fig. 1, 1 μg oligomycin per mg protein inhibited oxidation if mitochondria had been preincubated with it during the thermal equilibration period but did not inhibit oxidation when added from the side arm. This suggests that the inhibition of oxidative phosphorylation by oligomycin requires preincubation of yeast mitochondria with the inhibitor. Similar indications were obtained in polarographic experiments.

It is noteworthy that small amounts of oligomycin depressed the oxidation of citrate in the presence or absence of glucose + hexokinase below the State-4 rate (Table III and Fig. 1). The point of inhibition remained the energy-transfer step[26,27] as the inhibition was completely relieved by 0.5 mM dinitrophenol (Fig. 1).

ATPase activity of mitochondria

Isolated mitochondria, incubated with ATP in a buffered medium, showed only very weak ATPase activity. The activity increased enormously after the addition of Mg^{2+}. The rate of hydrolysis of 4 mM ATP was linearly dependent on concentration of mitochondria and on time of incubation until about one-quarter of the added ATP was hydrolyzed.

The dependence of the ATPase activity on pH is shown in Fig. 2. In the absence of dinitrophenol, the Mg^{2+}dependent ATPase activity had two distinct pH optima, at pH 6.2 and 9.5, respectively. In the presence of Mg^{2+}, dinitrophenol increased the activity at pH's higher than 6. The activation effect of dinitrophenol was displayed by concentrations as low as 0.25 mM and did not further increase with increasing concentrations. In the absence of Mg^{2+}, the activation effect of dinitrophenol was negligible.

Optimal concentrations of Mg^{2+} were different at the two pH optima, the optimal ATP/Mg^{2+} ratios being 1:1 and 2:1 at pH 6.2 and 9.5, respectively (Fig. 3).

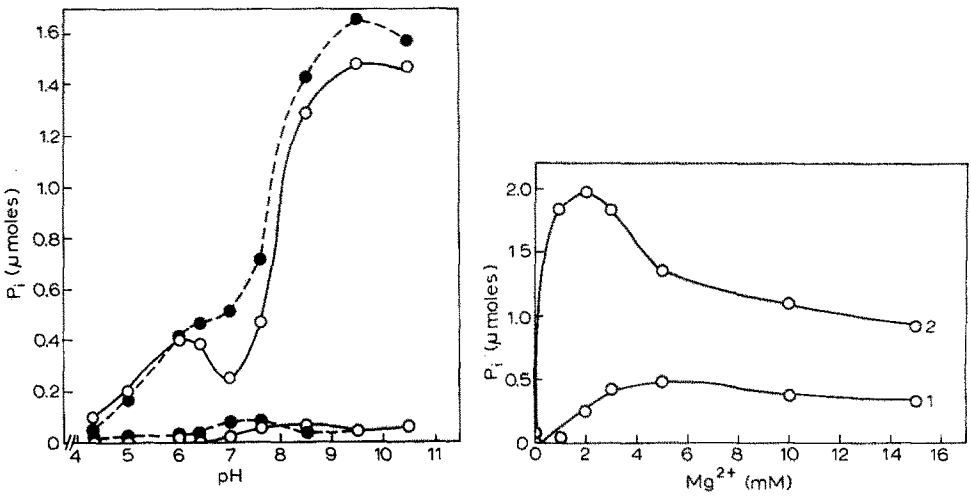

Fig. 2. Dependence of ATPase activity on pH. The incubation mixture contained in 1 ml: 4 mM ATP, 40 mM KCl, 0.4 mM EDTA, 5 mM $MgCl_2$, 30 mM buffer (Tris–maleate for pH 4.3 to 7.0 and Tris–chloride for pH 7.6 to 10.5), 88 mM sucrose and 0.11 mg of mitochondrial protein. Reaction time: 10 min. Dotted line indicates the activity in the presence of 0.5 mM dinitrophenol. The two lowest curves represent the activity in the absence of Mg^{2+}.

Fig. 3. Dependence of ATPase activity on concentration of Mg^{2+}. The reaction mixture contained in 1 ml: 4 mM ATP, 80 mM KCl, 0.5 mM EDTA, 20 mM Tris–maleate (Curve 1) or Tris–chloride (Curve 2), 88 mM sucrose, 0.05 mg of mitochondrial protein and $MgCl_2$ in concentrations indicated on the abscissa. Incubation time: 10 min. Curve 1, final pH 6.2; Curve 2, final pH 9.5.

TABLE IV

EFFECT OF BIVALENT CATIONS ON ATPASE ACTIVITY

Conditions similar to those in Fig. 3. The cations were used as chloride salts in final concns. of 5 mM and 2 mM at pH 6.2 and 9.5, respectively. The results are means from 2 experiments. The activities are expressed in per cent relative to the activity in the presence of Mg^{2+} which is taken as 100%.

Cation	*Relative activity* (%)	
	pH 6.2	*pH 9.5*
Mg^{2+}	100	100
Ca^{2+}	0	27
Mg^{2+} + Ca^{2+}	33	68
Mn^{2+}	33	100
Mg^{2+} + Mn^{2+}	33	100

As shown in Table IV, Mg^{2+} could not be replaced by Ca^{2+} and only partly by Mn^{2+} at pH 6.2. On the other hand, at pH 9.5, Mn^{2+} was as effective as Mg^{2+}. At pH 9.5, Ca^{2+} partly activated the activity in the absence of Mg^{2+} and inhibited the activity in the presence of Mg^{2+}.

In the presence of optimal concentrations of Mg^{2+}, the ATPase activity obeyed Michaelis–Menten kinetics with respect to ATP concentration. Michaelis constants for ATP were found to be 1.3 mM and 2.2 mM at pH 6.2 and 9.5, respectively. The constants remained unchanged in the presence of dinitrophenol.

Under the conditions employed, the ATPase activity was not affected by increasing tonicity of incubation media. In the presence of sucrose concentrations of 0.2 to 0.7 M, the activity at pH 6.2 and 9.5 remained unchanged. Likewise, ageing of mitochondria for 1 h at 30° or for 24 h at 0° had no effect on the ATPase activity. Rapid freezing and thawing of mitochondrial suspension or incubation with 0.1 to 1 mM oleate or 0.1 % deoxycholate increased the ATPase activity at pH 9.5 leaving the activity at pH 6.2 unchanged. The increase in the activity at pH 9.5 by 0.1 mM oleate varied with different batches of mitochondria from 30 to 180 %.

TABLE V

INHIBITORS OF ATPASE ACTIVITY

The reaction mixture contained in 1 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) $MgCl_2$, 0.014 to 0.14 mg of mitochondrial protein and inhibitors in final concns. indicated below. The values are means of 2 to 5 experiments.

Inhibitor	*Inhibition* (%)	
	pH 6.2	*pH 9.5*
Atebrin, 1 mM	68	91
Chlorpromazine, 1 mM	74	80
PCMB, 1 mM	71	88
0.1 mM	75	80
Ouabain, 0.1 mM	0	0
NaN_3, 20 mM	28	91
4 mM	5	85
0.4 mM	0	53
NaF, 80 mM	98	52
10 mM	82	10
1 mM	15	0

Inhibition of the ATPase activity by various compounds is shown in Table V. With respect to some inhibitors, the activities at pH 6.2 and 9.5 did not differ substantially. However, the activity at pH 6.2 was much more sensitive to the inhibition by fluoride than the activity at pH 9.5 which in turn was much more sensitive to azide. While the activity at pH 6.2 was only slightly inhibited by oligomycin, there was a strong inhibition at pH 9.5 (Table VI). The extent of the inhibition depended on the ratio oligomycin/mitochondria, 50 % inhibition at pH 9.5 being achieved at about 1 μg oligomycin per mg of mitochondrial protein.

TABLE VI

THE EFFECT OF OLIGOMYCIN ON ATPase ACTIVITY

Conditions similar to those in Table V except that the incubation mixture contained oligomycin and 1% methanol (added with the inhibitor). The methanol alone did not significantly affect activities.

pH	*Mitochondrial protein (mg)*	*Oligomycin (μg)*	*Inhibition (%)*			
			30	*5*	*1*	*0.1*
6.2	0.1		15	6	0	—*
6.2	0.09		17	10	0	0
9.5	0.014		96	95	90	90
9.5	0.03		95	95	88	71
9.5	0.14		86	70	—*	26

* Not tested.

When the ATPase activity was measured in a total non-fractionated homogenate of yeast protoplasts a similar pattern of inhibition by fluoride and oligomycin was found: fluoride inhibited strongly at pH 6.2 and slightly at pH 9.5 while oligomycin acted inversely. This suggests that most of the ATPase activity of the yeast homogenate might correspond to the activity which in differential centrifugation sedimented with mitochondria.

ATP–inorganic phosphate exchange activity of mitochondria

The incubation of ^{32}P-labelled inorganic phosphate with ATP and mitochondria in the absence of oxidizable substrate resulted in the incorporation of ^{32}P into ATP.

TABLE VII

INHIBITION OF ATP–P_i EXCHANGE ACTIVITY

The reaction mixture contained in 1 ml: 4 mM ATP, 80 mM KCl, 0.5 mM EDTA, 20 mM Tris–chloride, 88 mM sucrose, 2.5 mM $MgCl_2$, 20 mM radioactive potassium phosphate and 0.1 to 0.15 mg of mitochondrial protein; final pH 7.5. Methanol (1%) added with oligomycin did not affect the activity. The values are means of 2 experiments.

Inhibitor	*Inhibition (%)*
Chlorpromazine, 1 mM	100
PCMB, 0.1 mM	100
NaN_3, 10 mM	95
2 mM	83
NaF, 40 mM	32
Potassium oleate, 1 mM	100
Sodium deoxycholate, 0.1%	100
Dinitrophenol, 0.4 mM	88
0.1 mM	65
Oligomycin, 6 μg/ml	100
1 μg/ml	92
0.1 μg/ml	64

This exchange activity had a pH optimum at pH 7.5 and Michaelis constant for ATP 0.75 mM. No exchange reaction occurred in the absence of ATP. In the absence of Mg^{2+}, the activity was reduced to 60%. 1 mM $MgCl_2$ was sufficient to achieve the maximal activity.

As shown in Table VII, chlorpromazine, PCMB, azide and oligomycin strongly inhibited the exchange activity. Treatment of mitochondria with the detergents deoxycholate and oleate completely eliminated the exchange activity.

DISCUSSION

The present results on oxidation and phosphorylation activity of yeast mitochondria corroborate and extend the previous findings of OHNISHI, KAWAGUCHI AND HAGIHARA[11] and DUELL, INOUE AND UTTER[7]. At variance with the report of SCHUURMANS STEKHOVEN[12], they support the contention that Saccharomyces mitochondria lack the phosphorylation site corresponding to Coupling site I of animal mitochondria[11,13,15,17].

The data on the ATPase activity of isolated mitochondria confirm and extend other observations on yeast ATPases[28–31,11]. The ATPase activity found in our mitochondrial preparations had two pH optima, one at pH 6.2 and another at pH 9.5. There are several indications in favour of the view that these two different pH optima may correspond to two different ATPases: (1) The enzyme with the pH optimum at pH 6.2 (referred to as the pH 6.2 enzyme) was only active in the presence of Mg^{2+} and to some extent with Mn^{2+}, while the pH 9.5 enzyme was equally active with Mg^{2+} and Mn^{2+}, and to some extent even with Ca^{2+}; (2) with 4 mM ATP, the optimal concentration of Mg^{2+} was 4 mM at pH 6.2 and 2 mM at pH 9.5; (3) only the pH 9.5 enzyme seemed to be activated by dinitrophenol; (4) the pH 6.2 enzyme was strongly inhibited by fluoride, which inhibited the activity only slightly at pH 9.5; the pH 9.5 enzyme was in turn inhibited by low concentrations of azide and oligomycin which were less effective at pH 6.2; (5) the activity at pH 9.5 was raised by freezing and thawing or by the addition of oleate and deoxycholate, which did not affect the reaction at pH 6.2. On the other hand, the activities at both pH's were inhibited by PCMB, atebrine and chlorpromazine while ouabain inhibited activity at neither pH. The Michaelis constants for ATP were found to be similar at pH 6.2 and 9.5. The evidence for the two distinct ATPases cannot, of course, be considered to be conclusive and further studies are required. It should be noted that the pH curve of the ATPase activity in yeast mitochondria is strikingly similar to that found in aged or fragmented rat-liver mitochondria[32].

The high specific activity of the yeast ATPase, highly exceeding the rate of oxidative phosphorylation, and its apparent non-latency would suggest that this enzyme, along with its presumed participation in oxidative phosphorylation, might have other functions as well. For instance, a role of the partial reactions of oxidative phosphorylation (which, under certain conditions, may be apparent as ATP hydrolysis) in synthetic processes in the cell has been envisaged[33–35] which would be of quantitatively higher importance in the rapidly growing microbial cell than in an animal cell. The high resting metabolism of microbial cells might be due to this ATPase; owing to its relatively high Michaelis constant for ATP it might decompose

surplus ATP under resting conditions with little interference in conditions of active cellular syntheses.

The ATP–P_i exchange activity conforms more closely to that found in animal mitochondria with regard to both its specific activity and sensitivity towards inhibitors[21,26,27,34–38]. Because of the presumed absence of the Coupling site I, the occurrence of the ATP–P_i exchange reaction in yeast mitochondria would argue against the assumption that the partial reactions along the Coupling site I can mainly account for the ATP–P_i exchange activity found in isolated mitochondria[38].

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