PROTECTION OF YEAST MITOCHONDRIAL STRUCTURE BY PHOSPHATE AND OTHER H^+-DONATING ANIONS

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1. Introduction

The energy-dependent uptake of cations by mammalian mitochondria can be balanced by an equivalent counterflux of other cations. In the case of the valinomycin-mediated uptake of K^+ the counterion is H^+ [1,2]. The pH difference accross the membrane, increased by this process, can be utilized to accumulate P_i via the P_i-carrier (which catalyses a H^+–anion symport) or other H^+-donating anions as acetate (which cross the membrane under the undissociated form) [3–5]. Some tricarboxylic acid cycle intermediates can also enter the matrix compartment by a series of exchange-diffusion carriers which require P_i [5,6]; therefore, the accumulation of these anions is also electroneutral and ΔpH-dependent [3,4]. The permeability properties of the yeast mitochondrial membrane are not basically different from those of mammalian mitochondria [7–10].

Brierley showed that an energy-linked pH increase in the matrix of isolated beef heart mitochondria modified the permeability of inner membrane to Cl^- and other non-permeant anions [11]. As shown in this paper a similar effect can lead to irreversible structural damage in yeast mitochondria. A protective effect of P_i, when it crosses the membrane via the P_i-carrier, is also demonstrated.

Abbreviations: BSA, bovine serum albumin; VAL, Valinomycin; P_i, inorganic phosphate (potassium salt); CCCP, carbonyl cyanide *m*-chlorophenylhydrazone

2. Materials and methods

Cells of the diploid yeast strain Yeast Foam were grown aerobically with 2% galactose as carbon source, and mitochondria were prepared as previously described [10]. Mitochondrial protein concentration was estimated by the biuret method. Oxygen uptake was measured with a Clark oxygen electrode (Gilson) at 27°C in 3 ml of the following basal medium: 10 mM Tris–maleate, 0.65 M mannitol, 0.3% BSA, pH 6.7. Swelling was measured at 27°C in a cuvette containing 3 ml basal medium. Electron microscopy was carried out as described elsewhere [12].

3. Results

Isolated yeast mitochondria oxidize added NADH at a high rate, in contrast to mitochondria from animal sources [13]. Respiration of mitochondria on NADH in the basal medium containing 10 mM KCl, in the absence or presence of P_i, are compared (fig.1). In the presence of 10 mM P_i, the respiration rate remained constant and was stimulated upon ADP addition (trace B). In the absence of P_i, a stimulation of the respiration rate during the course of the experiments was observed (trace A). The addition of valinomycin stimulated respiration rates, both in the presence or absence of P_i; however, the decrease of the respiration rate by P_i was always observed (fig.1, trace D and E). It was surprising to note a stimulation of the respiration rate by oligomycin when P_i was

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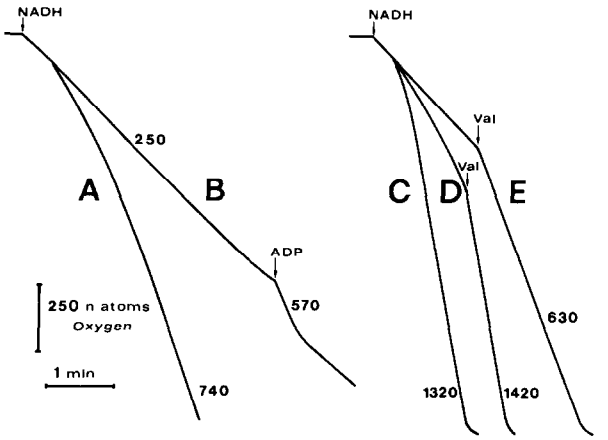

Fig.1. Respiration of yeast mitochondria in the presence of 10 mM KCl. Mitochondrial protein, 1 mg, was incubated in 3 ml basal medium, supplemented as follows: A and D, 10 mM KCl; B and E, 10 mM KCl + 10 mM P_i; C, 10 mM KCl + 30 μg oligomycin. As indicated on the curves, other additions are 5 μmol NADH, 300 nmol ADP, 0.1 μg valinomycin. The maximal respiration rate is indicated in natoms oxygen · min^{-1} · mg $protein^{-1}$.

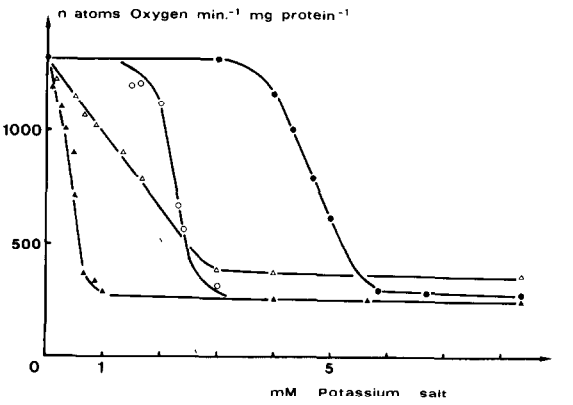

Fig.2. Inhibition of respiration rate stimulation in presence of KCl by H^+-donating anions. To 3 ml basal medium, containing 10 mM KCl and different concentrations of indicated K^+ salts, 30 μg oligomycin, 1 mg protein and 5 μmol NADH, were added sequentially. Maximal respiration rate is plotted against potassium salt concentrations. (▲———▲) Phosphate, (△———△) Arsenate, (○———○) Propionate, (●———●) Acetate.

omitted (trace C). Similar results were observed with ethanol as substrate (not shown here).

The stimulation of the respiration under the conditions of fig.1A was not specific for Cl^-. Nitrate, pyruvate, glutamate or fumarate, for example, gave the same results (table 1). In all these cases, P_i prevented the stimulation of respiration rate. The valinomycin-induced permeability of the membrane to K^+ increased the respiration rate when one of the potassium salts was present in the medium.

As shown in fig.2, a protective effect on the respiration rate was obtained not only by P_i but also by arsenate, propionate and acetate. In this experiment, the incubation medium contained oligomycin to prevent arsenate-stimulated respiration [14]. These weak acids share a common denominator, the ability to cross the inner membrane in undissociated form either under carrier-mediated (phosphate, arsenate) or carrier-free (acetate, propionate) transport [5]. The acids' efficiency limiting respiration rate can be compared to their pK value:

$$pK_2 \text{ phosphate} > pK_2 \text{ arsenate} > pK \text{ propionate} > pK \text{ acetate}.$$

A protective effect was also observed with weak bases as ammoniac (not shown here).

In order to correlate stimulation of respiration

Table 1
Stimulation of mitochondrial respiration by potassium salts

	Control	Phosphate	Chloride	Nitrate	Pyruvate	Glutamate	Fumarate
– Valinomycin	310	250	740	760	800	810	800
+ Valinomycin	310	630	1420	1320	1380	1200	1440

To 3 ml basal medium, containing 10 mM potassium salt, were added 1 mg mitochondrial protein, ± 0.1 μg valinomycin and 5 μmol NADH. The maximal respiration rate is indicated in natoms oxygen · consumed · min^{-1}. mg $protein^{-1}$

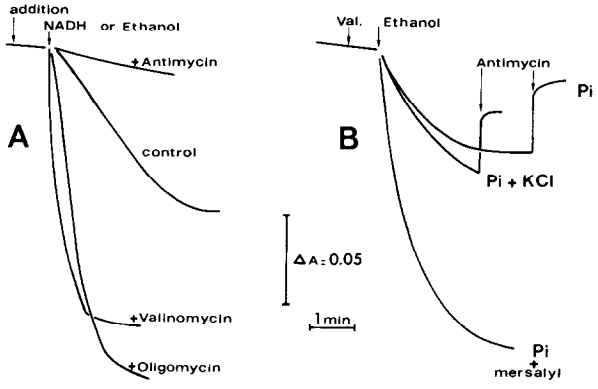

Fig.3. Low amplitude swellings of yeast mitochondria. Swelling was monitored at 546 nm using an Eppendorf photometer. (A) 1 mg mitochondrial protein incubated in 3 ml basal medium containing 10 mM KCl. As indicated, the various additions were: 1 μg antimycin, 0.1 μg valinomycin or 30 μg oligomycin. (B) 1 mg mitochondrial protein was incubated in basal medium supplemented, as indicated, with 10 mM P_i or 10 mM P_i + 10 mM KCl. In order to test the action of mersalyl, 1 mg mitochondrial protein was preincubated with 25 nmol mersalyl and assayed in the presence of 10 mM P_i. Other additions were: 0.1 μg valinomycin, 5 μmol NADH, 0.003% (v/v) ethanol and 1 μg antimycin.

with salt uptake, the swelling of yeast mitochondria was measured under conditions of fig.1. NADH induced a low amplitude swelling of yeast mitochondria suspended in medium containing 10 mM KCl; this swelling was stimulated by valinomycin or oligomycin addition and inhibited when respiration was blocked by antimycin (fig.3A); antimycin or CCCP did not reverse swelling (not shown). In contrast, when 10 mM P_i was added to the medium (in the presence or absence of KCl) a swelling, induced by valinomycin and reversed by CCCP, antimycin or anaerobiosis, was observed as for rat liver mitochondria [15] (fig.3B). When mitochondria was preincubated with mersalyl, valinomycin addition induced a greater swelling in medium containing 10 mM P_i than observed with KCl in the absence of P_i; however

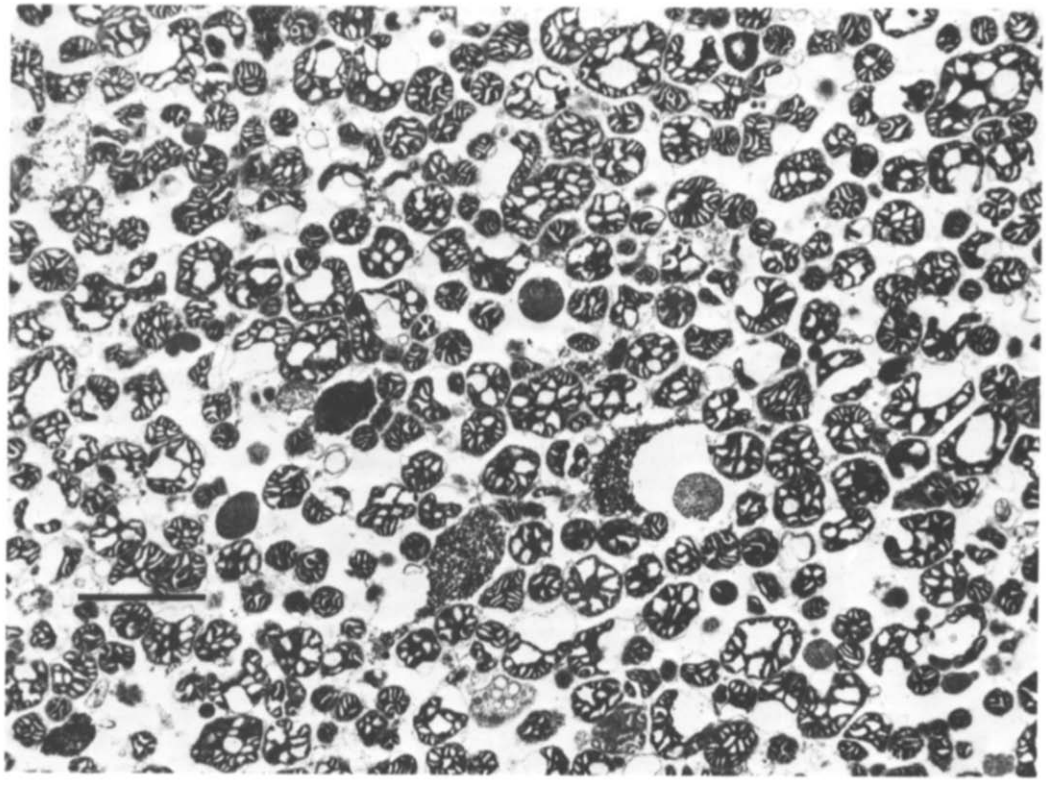

Fig.4A

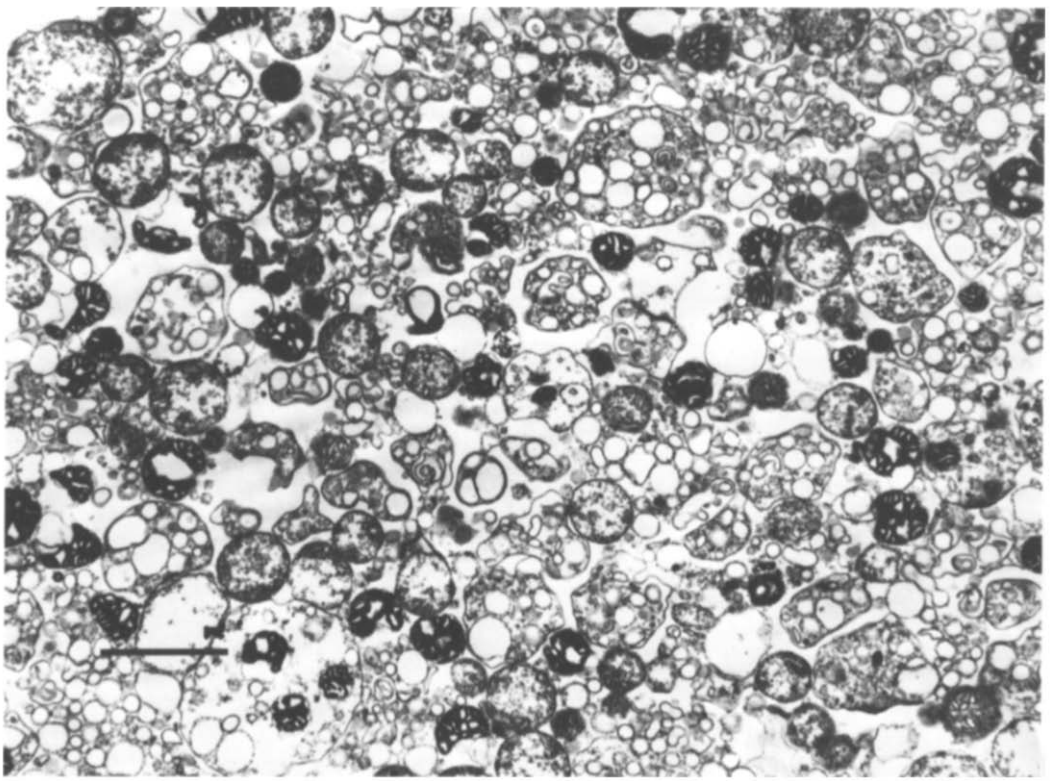

Fig.4B

Fig.4. Electron micrographs of yeast mitochondria. The organelles were incubated at 27°C in 3 ml basal medium for 2 min after addition of 5 μmol NADH. Mitochondria were then immediately centrifuged and the pellets prefixed with 2.5% glutaraldehyde and prepared for electron microscopy. (A) Incubation in the presence of 10 mM KCl + 10 mM P_i. (B) Incubation in the presence of 10 mM KCl. Bar 2 μm.

this stimulation was maximal, only with ethanol as substrate, since the external NADH dehydrogenase was partially inhibited by the amount of mersalyl needed to block P_i transport.

The morphological changes which accompany stimulation of respiration under the conditions of fig.1A are presented in fig.4. Mitochondria were incubated 2 min in medium containing 10 mM KCl and NADH, in the presence or absence of 10 mM P_i. The material was immediately treated for morphological observations. In the presence of P_i, the densely-stained matrix was interspersed with clear vesicular areas (twisted configuration) (fig.4A). In the absence of P_i, very little of this configuration was observed; however, the material appeared significantly swollen and disintegrated (fig.4B).

4. Discussion

In this communication it is shown that, in the presence of potassium phosphate, valinomycin increases the respiration rate in isolated yeast mitochondria (see also [7]) and induces swelling which can be reversed by uncoupler, respiratory inhibitors or anaerobiosis as for mammalian mitochondria [15]. These results are in agreement with an energy-linked K^+/H^+ exchange followed by a transmembrane ΔpH driven H^+–P_i uptake into the matrix.

However, KCl or other salts were able to support stimulation of respiration at low external concentration, in the absence of valinomycin; this effect was enhanced by the ionophore addition. The respiration was accompanied by swelling basically different from

that obtained in the presence of P_i, since antimycin or CCCP did not induce shrinkage. This process lead to damage of the mitochondrial structure. In contrast with mammalian mitochondria, the inner membrane of isolated yeast mitochondria seemed relatively permeable to K^+; such a permeability was induced during respiration under conditions described, in the absence of P_i. It is known that the entrance of K^+ into the matrix collapses the membrane potential and stimulates respiration. In the absence of an H^+-donating anion, the internal pH must have been largely increased by this process and probably lead to salt uptake and structural damages.

Brierley proposed that the alkalization, either in the matrix or inner membrane of heart mitochondria, caused the membrane to become permeable to Cl^- or other non-permeant anions [11]. In this model, the entrance of anions into the matrix occured down their chemical gradient. However, in our experimental system, the external potassium salt concentration seemed too low to induce passive swelling. Azzone et al. proposed an alternative model for the energy-linked movement of strong electrolytes in rat liver mitochondria [16]: the ΔpH caused by an energy-linked H^+/K^+ exchange drives the uptake of H^+, which in turn drives the uptake of anions; the flux of H^+ and anions are assumed to be electrically coupled. This model can account for our experimental data if we admit that the membrane became permeable to H^+ only when a critical transmembrane ΔpH was reached. This H^+-permeability change seems to be irreversible in yeast mitochondria. This hypothesis can explain why H^+-donating anions prevented respiration rate stimulation and structural damages in limiting the ΔpH increase. Mersalyl inhibits H^+–P_i transport via the P_i carrier in yeast mitochondria [9,10]; therefore P_i entrance, in the presence of energy supply, into the matrix of mersalyl-treated mitochondria must be not basically different from the one of Cl^- [10] (probably non-carrier-mediated transport). The effect of oligomycin, stimulation of both respiration and swelling in the presence of KCl and absence of H^+-donating anions, can be explained by this model. Oligomycin, fixed on the ATPase complex, decreases the natural permeability of the membrane to H^+ [17–20] and can then accelerate H^+-permeability change of the membrane by increasing transmembrane ΔpH.

This work shows an essential role of P_i-carrier in permitting the maintenance of the mitochondrial membrane structure.

Acknowledgements

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