DEGRADATION OF MITOCHONDRIAL RESPIRATORY SYSTEM OF *SACCHAROMYCES CEREVISIAE*, INDUCED BY RESPIRATORY INHIBITORS, UNCOUPLERS AND ANAEROBIOSIS

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1. Introduction

The relation of oxidative phosphorylation to maintenance of mitochondrial integrity *in vitro* has been discussed extensively by many authors. A considerable part of the experimental data testified that under conditions favouring oxidative phosphorylation, mitochondrial swelling and related processes are slowed down [1–8]. It was also suggested that the level of endogenous ATP is of prime importance for preservation of the phosphorylating capacity of mitochondria and inhibition of swelling [9–12]. However, some results made it doubtful whether oxidative phosphorylation was necessary for the stabilization of mitochondria. In particular, it was shown that although 2,4-dinitrophenol [13, 14] and oligomycin [15] completely depress phosphorylation under certain conditions, they nevertheless retard swelling of mitochondria. It was also found that mitochondria are well preserved in anaerobic conditions [16] or in the presence of respiratory inhibitors: amytal, malonate, antimycin A, and cyanide [5, 17, 18].

On the other hand, a number of model experiments, carried out in our laboratory with submitochondrial particles and a reconstituted respiratory chain, showed that in the absence of an oxidizable substrate or oxygen, these systems are split readily by proteinases and cobra venom phospholipase, and undergo rapid thermal degradation [19–23].

In this connection it seems fundamentally important to ascertain how various agents, which interrupt oxidative phosphorylation, affect mitochondria *in vivo*. A convenient tool for these investigations is a facultative anaerobic yeast which can grow both in the presence and in the absence of oxidative phosphorylation. Several years ago Bartley and Tustanoff showed that certain respiratory inhibitors and 2,4-dinitrophenol hinder the development of respiration in anaerobically grown yeast [24]. According to data published recently by Kováč et al. [31], yeast cells grown in the presence of compounds capable of suppressing ATP synthesis in mitochondria do not possess a complete respiratory chain. In the present investigation we studied the influence of cyanide, antimycin A, carbonyl cyanide *m*-chlorophenylhydrazone (CCCP), 2,4-dinitrophenol (DNP) and anaerobiosis on the mitochondrial respiratory system of aerobically grown *Saccharomyces cerevisiae*. Our experiments show that incubation of mitochondria in a medium containing respiratory inhibitors or uncouplers caused the same decrease in respiratory activity as deaeration of the cell suspension.

2. Materials and methods

In this work we used *Saccharomyces cerevisiae*, wild type diploid (strain no. 11 of the National Collection of Yeast Cultures). The organism was maintained aerobically on agar slopes consisting of 1.5% agar-agar, 0.25% glucose, and 0.2% yeast extract. The growth medium contained (g per l): glucose 2.5, yeast extract 2, KH_2PO_4 1, ammonium sulphate 1, sodium chloride 0.5 and magnesium sulphate 0.5. The yeast was grown aerobically at 35° in a 10 litre fermenter. At the end of the exponential growth phase (12–13 hr) the medium was saturated with argon. In other experiments, a respiratory inhibitor (cyanide or

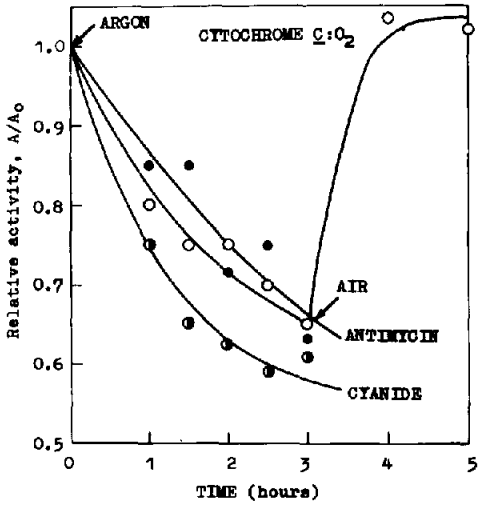

Fig. 1. Decrease in cytochrome oxidase activity of mitochondria during incubation of aerobically grown yeast cells under anaerobic conditions and in the presence of cyanide or antimycin A. A_0: specific activity of mitochondria isolated from yeast cells immediately after introducing argon or inhibitors. A_0 was 120–150 nmoles of cytochrome *c* oxidized per mg protein per min at 30° for mitochondria isolated by Kováč's method, and 300–400 nmoles of cytochrome *c* for mitochondria isolated by Tzagoloff's method. The results were reproduced in at least three experiments.

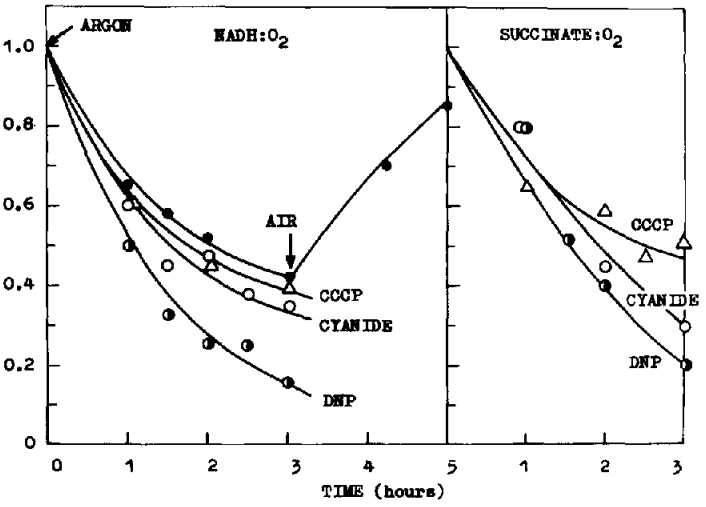

Fig. 2. Decrease in NADH oxidase and succinate oxidase activities of mitochondria upon incubation of aerobically grown yeast cells in anaerobic conditions and in the presence of cyanide, DNP, or CCCP. For details see fig. 1. A_0 was 250–320 nmoles NADH and 120–150 nmoles succinate oxidized per mg protein per min at 30°, when the mitochondria were isolated by Kováč's method or 700–800 nmoles NADH and 300–350 nmoles of succinate when the mitochondria were isolated by Tzagoloff's method.

antimycin A) or uncoupler (CCCP or DNP) was added to the fermenter under aerobic conditions. At the times indicated on the graphs, portions of yeast suspension were taken*. After immediate centrifugation and washing, the cells were used for preparation of mitochondria by the method of Kovac et al. [25] or Tzagoloff [26]. The results were independent of the method of mitochondrial isolation.

NADH oxidase, cytochrome oxidase and succinate oxidase activities of the mitochondria were measured spectrophotometrically. For NADH oxidase the medium contained 50 mM potassium phosphate (pH 7.5), 1 mM EDTA and 0.75 mM NADH. For cytochrome oxidase, cytochrome *c* (1 mg per ml) reduced by an equivalent amount of ascorbic acid was added instead of NADH. 20 mM succinate was used for measuring succinate oxidase activity. All measurements were made at 30°.

* In the experiment with argon, to prevent respiratory adaptation of the yeast, the suspension was sampled in an intense current of argon. The suspension was cooled quickly to 0–2°. The medium contained cyanide in all procedures with cells until freezing of the yeast suspension or introduction of *Helix pomatia* juice.

3. Results

Under the experimental conditions used, the concentration of the yeast suspension, whole cell respiration, and specific respiratory activity of mitochondria reached limiting values by the 12–13th hr of growth. Deaeration of the medium at this moment resulted in a decrease in respiratory activity of the mitochondria, which recovered completely upon subsequent aeration (figs. 1, 2). Addition of cyanide (3 mM) or antimycin A (0.11 μg per ml) to the medium also resulted in gradual degradation of the respiratory system (fig. 1, 2). The cyanide was removed from the mitochondria during their isolation and therefore did not interfere with the enzymic measurements. In the case of antimycin A, only cytochrome oxidase activity was estimated; its

activity was independent of the presence of this inhibitor in the assay medium. Attention is drawn to the fact that degradation of the respiratory system proceeded practically at the same rate upon deaeration of the medium and in the presence of respiration inhibitors (fig. 1). The effect of cyanide, like that of anaerobiosis, was reversible. The respiratory activity of the mitochondria increased and reached its normal value when, after 3 hr incubation with cyanide, the cells were transferred to a cyanide-free medium (0.1 M KH_2PO_4).

Fig. 2. gives the results of experiments in which yeast cells were treated with DNP or CCCP, added in amounts sufficient for complete uncoupling of respiration and phosphorylation (10^{-4} and 10^{-6} M, respectively). It follows from fig. 2 that both agents, while not inhibiting respiration at all, caused profound changes in the mitochondrial respiratory system. The rate of decrease of NADH oxidase activity in the presence of DNP was slightly higher than that induced by anaerobiosis, CCCP, or cyanide. Similar results were obtained for succinate oxidase activity of the mitochondria.

In our experiments the yeast concentration varied within ±5%; thus all the changes observed in the mitochondria occurred under conditions where cell division was insignificant. The decrease in specific activities of mitochondrial enzymes cannot be attributed to dilution of the mitochondrial fraction with extraneous particles. In particular, spectrophotometric measurements showed that 3 hr incubation of the yeast cells with cyanide decreased the NADH oxidase activity of the mitochondria by more than 50%, whereas the content of cytochrome aa_3 in the mitochondrial fraction decreased only by 10–15%.

4. Discussion

In contrast to *in vitro* results [5, 13, 14, 16–18], this study offers evidence that anaerobiosis, cyanide, antimycin A, DNP, and CCCP cause degradation of mitochondria in yeast cells. The most interesting fact is that, except for DNP, all the agents and anaerobiosis are practically equally effective, although they act at different sites of oxidative phosphorylation. This suggests that the above effect is due to a disturbance in the functioning of mitochondria, especially since, by themselves, cyanide [18], antimycin A [18], and DNP [13] evidently do not impair the mitochondrial structure. The slightly higher effectiveness of DNP compared to the other agents may be a result of ATPase activation and a rapid decrease of the ATP content in the mitochondria [27]. Although CCCP and DNP at the concentrations used are not inhibitors of the respiratory chain, they nevertheless may interrupt its functioning indirectly. Indeed, uncouplers may impair the mitochondrial retention of cofactors [3, 9] and certain citric acid cycle enzymes [8, 30] and thus gradually stop the delivery of substrates to the respiratory system. The above results should be qualified as a confirmation of the assumption that oxidative phosphorylation or intramitochondrial ATP play an important part in stabilization of mitochondria.

At present we do not know the details of the changes induced in mitochondria by the above agents. It is not impossible that some of them display side effects associated with their interaction with mitochondrial proteins [28] or other metabolic processes apart from oxidative phosphorylation (e.g. see [29]). To ascertain these questions a comprehensive study must be made of the changes occuring in mitochondria for each respiratory inhibitor or uncoupler. Recently we were able to show that anaerobiosis causes destruction of mitochondrial membranes in *S. cerevisiae*, and that aeration restores the mitochondrial structure. The results of these studies will be described in the next paper.

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