ENERGETIC ASPECTS OF THE MITOCHONDRIAL BIOGENESIS

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Received 22 February 1972

1. Introduction

Little is known about the origin of energy required for the maintainance of the structure of mitochondria *in vivo* and for their biogenesis. Either the energy generated by the mitochondria themselves may be necessary to maintain their integrity and to drive the synthesis of new mitochondrial components or the energy may be derived from extramitochondrial sources.

Synthesis of the respiratory chain components during respiratory adaptation of anaerobically-grown yeast was found not to be inhibited by cyanide [1], antimycin A [2] or by oligomycin [3] indicating that the energy generated by mitochondria is not required for respiratory adaptation. The respiratory adaptation, however, appears to reflect mainly a transformation of preformed "promitochondria" into aerobic mitochondria [4] and may not represent a true biogenesis of new mitochondria. The present paper indicates that even in aerobically growing cells the formation of mitochondria is not obligatorily dependent upon energy generated by the organelles.

2. Experimental

Diploid strains of wild-type yeast *Saccharomyces cerevisiae* DT XII and of the oxidative phosphorylation-deficient mutant DH 1 [5] were cultured aerobically at 30° in a semi-synthetic medium [6] with 2% glucose or galactose as carbon source. Respiration of the cells was measured by the standard Warburg technique and the difference spectra (reduced by endogenous substrates or by DL-lactate + dithionite, minus oxidized by 60 mM H_2O_2) were recorded in the Hitachi-Perkin Elmer 356 spectrophotometer. With cells grown in the presence of antimycin A, absolute reduced spectra with filter paper as a reference were also measured in order to reveal cytochrome *b* which remained reduced in cells oxidized by H_2O_2. Respiration-deficient mutants were detected on solid glucose medium by tetrazolium overlay method [7] or by selective plating on glycerol medium.

Antimycin A was purchased from Sigma, erythromycin lactobionate from Abbot, and oligomycin was a gift from the Upjohn Company.

3. Results

As reported previously, the wild-type strain grown aerobically on glucose in the presence of antimycin A or oligomycin exhibited normal amount of cytochrome *c*, a decreased amount of cytochrome *b* and virtually no cytochrome $a.a_3$ [8]. Although this may have implied that generation of energy by the mitochondrial respiratory chain is prerequisite for the synthesis of normal mitochondria, another interpretation has been adopted: the diminished synthesis has been assumed to be due to enhanced catabolic repression that ensued as a consequence of the elimination of the Pasteur effect by antimycin A or oligomycin [8].

This interpretation is borne out by the results presented in fig. 1. The wild-type strain used was

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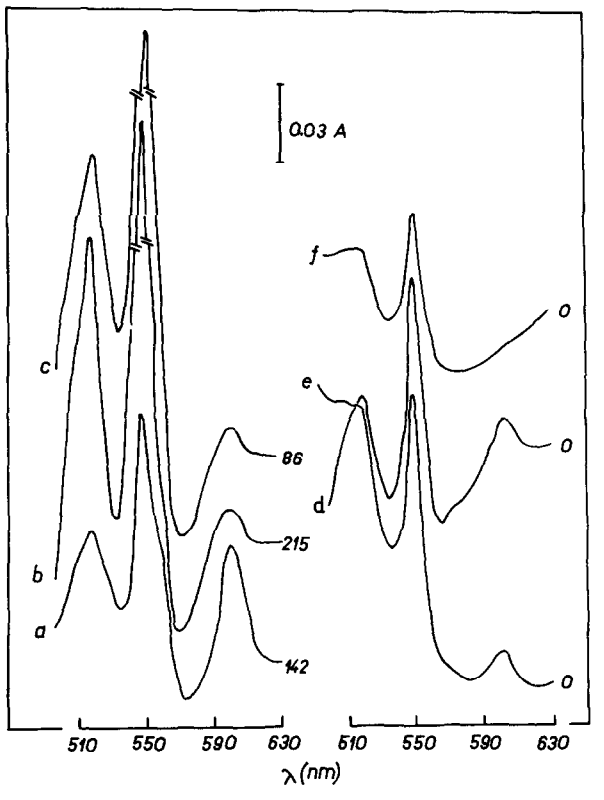

Fig. 1. Difference spectra of wild-type yeast grown in the presence of inhibitors. The yeast was grown aerobically on 2% galactose for 38 hr without inhibitor (a), with 2 mg/ml erythromycin (b), with 10 μg/ml oligomycin (c), with 10 μg/ml oligomycin + 0.5 μg/ml antimycin A (d), with 0.5 μg/ml antimycin A (e), with 0.5 μg/ml antimycin A + 2 mg/mg erythromycin (f). The cuvettes contained about 50 mg (dry weight) cells/ml. Numbers on curves indicate respiratory activity (μl O_2/mg dry weight/hr) determined manometrically with 50 mM glucose as substrate.

found to exhibit a very low affinity for galactose (the K_m for galactose being about 0.1 M after adaptation to this sugar) so that the catabolic repression can be virtually abolished when the strain grows on galactose instead of glucose as carbon source. As shown in fig. 1, in the galactose medium neither antimycin A, nor oligomycin and even not their combination did prevent the synthesis of all the cytochromes. It has been established in a control experiment that the two compounds were equally effective in inhibiting the respiration of galactose grown cells as they were that of glucose-grown cells [8].

Interestingly, even erythromycin at a concentration of 2 mg/ml did not significantly inhibit the formation of the respiratory pigments when the cells were grown on galactose. This substance strongly inhibited the synthesis of both cytochromes $a.a_3$ and b during growth on glucose and prevented growth on solid media with glycerol or lactate as carbon source. This indicated that, in glucose media, the primary inhibition of the respiratory enzyme synthesis by erythromycin may not be complete (cf. [9, 10]), but is potentiated by the ensuing catabolic repression. As shown in fig. 1, only the combined action of both erythromycin and antimycin A was able to eliminate the cytochrome $a.a_3$ synthesis. As can be seen in the figure, synthesis of cytochrome c was considerably higher in the cells grown on galactose in the presence of oligomycin or erythromycin than in the cells grown in the absence of inhibitor.

The formation of cytoplasmic respiration-deficient mutants which, in the wild-type strain used, occurs spontaneously at a frequency lower than 1%, was not enhanced by antimycin A and oligomycin or by the combination of the two inhibitors during growth on both glucose and galactose media. The cells grown in the presence of the inhibitors were able to resume normal growth on non-fermentable substrates once the inhibitors were eliminated.

The same effect of the inhibitors on the synthesis of mitochondrial components was also observed in the oxidative phosphorylation-deficient mutant DH 1. The deficiency in this mutant is due to a modification of the mitochondrial adenine nucleotide translocation system, the rate of translocation being approx. 2.5 times lower in the mutant than in wild-type yeast [17]. It appeared desirable to prevent the adenine nucleotide translocation across the mitochondrial membrane entirely and then to observe the pattern of the synthesis of respiratory pigments. Unfortunately, the yeast cells were found to be impermeable for the inhibitor of adenine nucleotide translocation, atractyloside.

4. Discussion

The following is the implication of the present results:

1) Provided that the interference of catabolic re-

pression is eliminated, mitochondria with a complete respiratory chain and apparently normal function can be formed in the cells even if the generation of energy by these organelles is prevented by inhibiting respiration and energy-transfer reactions. Thus, the energy generated by mitochondria is not obligatory for their own biogenesis or for the genesis of new mitochondria and the energy required can be supplied by cytosolic reactions.

2) A normal respiratory chain is found in the cells in which the formation of the mitochondrial high-energy state is prevented by a combined presence of antimycin A and oligomycin. Processes which derive energy directly from the high-energy state, such as mitochondrial ion transport and reversed electron transport in the respiratory chain seem not to be obligatory for the biogenesis of mitochondria.

3) A diminished transport of cytosolic ATP into mitochondria by a defective adenine nucleotide translocation system does not interfere with the synthesis of a complete respiratory chain.

4) Neither functioning of the respiratory chain nor the maintenance of the mitochondrial high-energy state seem to be prerequisite for the maintenance and normal replication of the mitochondrial DNA. This is indicated by the observation that neither antimycin A and oligomycin, nor their combined presence induced cytoplasmic respiration-deficient mutants in the strain employed. This result is relevant to the observation that energy-transfer reactions are preserved in yeast promitochondria [12]. It indicates that the presence of the energy-transfer system in these incomplete mitochondria may have another function than assuring the replication of mitochondrial DNA.

Acknowledgements

The experimental part of this work was accomplished while the author was employed at the former Department of Biochemistry, Komensky University, Bratislava. The devoted technical assistance of Mrs. E. Böhmerová is gratefully acknowledged. I am grateful to Dr. J. Pogády, chief of the Psychiatric hospital in Pezinok, for support and encouragement.

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