OBLIGATORY REQUIREMENT OF INTRAMITOCHONDRIAL ATP FOR NORMAL FUNCTIONNING OF THE EUCARYOTIC CELL

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Mass formation of respiration-deficient mutants takes place in the aerobic culture of S.cerevisiae when the synthesis of ATP in mitochondria is prevented by inhibiting respiration and, simultaneously, the influx of ATP from cytosol into mitochondria is blocked by bongkrekic acid. In addition, the multiplication of the respiration-deficient mutants in complex glucose medium is arrested by bongkrekic acid. Thus, continual presence of ATP inside mitochondria is necessary for normal replication of mitochondrial DNA and also for a function related to cellular multiplication.

Mitochondria are generally considered to be cellular organelles specialized for aerobic production of ATP. If this were their only essential function, facultatively anaerobic organisms, such as yeast, could dispense with mitochondria. Although a number of mutations are known affecting mitochondrial structure and function in yeast, mutants entirely lacking mitochondria have not been found. Also, mitochondrial structures are preserved in non-respiring anaerobically-grown cells and exhibit energy-transfer reactions and translocation of ions and adenine nucleotides (1,2).

An additional essential function of mitochondria has been implicated by the observation that the superposition of two mitochondrial mutations within a single cell, ρ^- and op_1, results in the arrest of multiplication ability (3). The former mutation entails the loss of some respiratory enzymes (4) and a lesion in

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the energy-transfer reactions in mitochondria (5) and the latter a modification of mitochondrial adenine nucleotide translocation (6). This implies a possible involvement of respiration and adenine nucleotide translocation in the control of cell multiplication. To further analyze this possibility, the effect of inhibitors of respiration and of adenine nucleotide translocation upon wild-type yeast has been studied.

EXPERIMENTAL

Wild-type yeast Saccharomyces cerevisiae DT XII was grown at 30° in a semi-synthetic medium (2), pH 5.0, with 2% glucose as carbon source. Growth was followed by counting cells in a haemocyte chamber. Respiration-deficient mutants were detected by differential plating on solid glucose and glycerol media and by the tetrazolium overlay method (7). Fermentation and respiration were determined by the conventional manometric technique. Cytochrome spectra were measured in the Hitachi-Perkin Elmer 356 spectrophotometer. Isolation of mitochondria and determination of their properties were done by previously employed procedures (2,5,6).

Bongkrekic acid was a gift from Dr. W. Berends and was used as a solution in methanol.

RESULTS

Bongkrekic acid as inhibitor of mitochondrial adenine nucleotide translocation in intact cells

Atractyloside, an inhibitor of adenine nucleotide translocation in isolated mitochondria, had no effect on intact yeast cells, probably owing to its inability to cross the cell membrane. Another translocation inhibitor, bongkrekic acid (8-10), acted on the cells of wild-type yeast in the following manner:

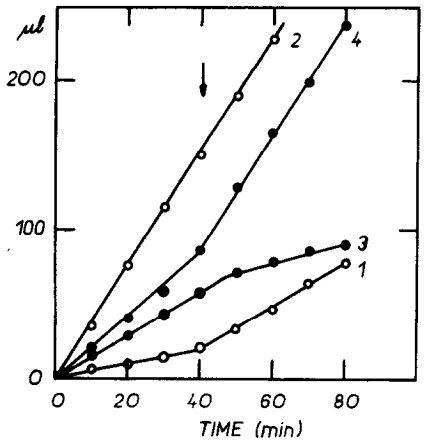

Fig. 1.

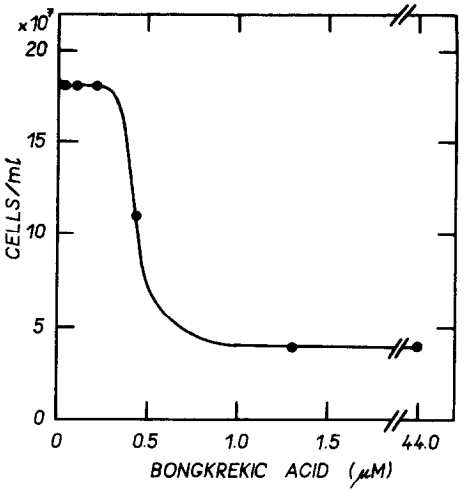

Fig. 2.

Fig. 1. Effect of bongkrekic acid on respiration and fermentation of aerobically grown wild-type yeast. Main compartments of Warburg vessels contained in 2.5 ml: 80 mM citrate phosphate buffer pH 4.3, 50 mM glucose, inhibitor and 0.88 mg yeast (dry weight). 1 - oxygen consumption in the presence of 4.2 µM bongkrekic acid; at arrow, 50 µM 2,4-dinitrophenol was tipped from the side-arm; 2 - as 1, but CO_2 production; 3 - oxygen consumption; no inhibitor; at arrow, 4.2 µM bongkrekic acid was tipped from the side arm; 4 - as 3, but CO_2 production.

Fig. 2. Growth yield on glucose as a function of concentration of bongkrekic acid. Cells were grown aerobically for 60 h in the medium containing 0.5% glucose as carbon source and bongkrekic acid at concentrations indicated on the abscissa. Growth yield of cells grown anaerobically in the absence of inhibitor was 4×10^7 cells/ml.

(a) It prevented growth on non-fermentable substrates and lowered the aerobic growth yield on glucose to the anaerobic level. (b) It inhibited respiration on glucose by 65%, the extent of the inhibition corresponding to that found previously with oligomycin (11). The inhibition of respiration was released by uncoupler (Fig.1). (c) It enhanced the rate of aerobic fermentation to the anaerobic level, thus completely inhibiting the Pasteur effect (Fig.1). It did not affect the anaerobic fermentation.

All these effects are consistent with the assumption that, in intact yeast cells, bongkrekic acid specifically inhibits adenine nucleotide translocation across the mitochondrial membrane, leaving the other processes unaffected. This is also supported by the fact that the inhibitor was effective in intact cells at such low concentrations as in isolated mitochondria (cf. ref. 8,9)(Fig.2).

Cells grown aerobically on glucose in the presence of 22 μM bongkrekic acid were able to respire with Q_{O_2} on glucose of 17.7 as compared with the value of 97.2 of control cells and possesed all cytochromes, although the amount of cytochrome a was considerably diminished with respect to cytochrome b and especially to cytochrome c.

Induction of respiration-deficient mutants by combined arrest of respiration and adenine nucleotide translocation

The formation of respiration-deficient mutants, the spontaneous rate of which was lower than 1 per cent in the strain employed, was not enhanced when the cells were grown aerobically on glucose in the presence of bongkrekic acid. However, when bongkrekic acid was supplemented with respiratory inhibitors, antimycin A or cyanide, mass production of the respiration-deficient mutants ensued (Fig.3). The respiratory inhibitors themselves, in the absence of bongkrekic acid, did not induce the mutants (11).

The respiration-deficient mutants were also formed at a high rate when bongkrekic acid was added to anaerobically growing cells of the wild-type strain or to aerobically growing cells of a cytochrome a-deficient mutant.

Active growth was prerequisite for mutant formation. No mutants arose in the presence of bongkrekic acid and antimycin A

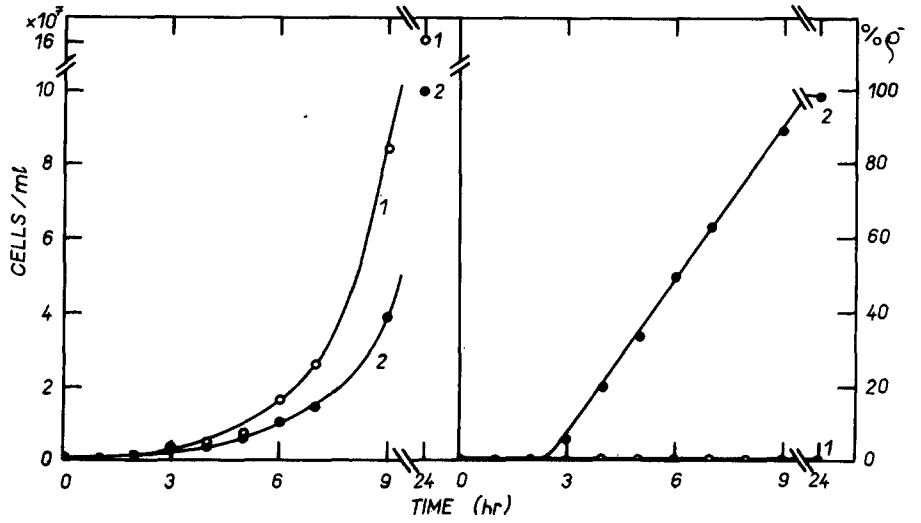

Fig. 3. Growth curves and formation of respiration-deficient mutants (♀⁻) in the presence of bongkrekic acid and antimycin A. Cells were grown aerobically in the medium containing 2% glucose. 1 - with 22 μM bongkrekic acid; 2 - with 22 μM bongkrekic acid and 2 μg/ml antimycin A. Similar curves were obtained when antimycin A was replaced by 1 mM KCN.

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if the growth was inhibited by cycloheximide or if the cells were suspended in buffer and incubated with the two inhibitors for as long as 50 hours.

Inability of respiration-deficient mutants to grow in the presence of bongkrekic acid

As glycolysis is the only source of ATP in respiration-deficient mutants, one would expect that the growth yield of the mutants would not be influenced by bongkrekic acid. It was found, however, that respiration-deficient mutants could not grow on glucose in the presence of bongkrekic acid (4.4 to 44 μM) for more than three generations. The cells not able to further multiply were not dead: they did not stain with methylene blue, fermented glucose at normal rate and resumed growth when washed and transferred into medium not containing bongkrekic acid.

Mitochondria were isolated from these cells with the same yield as from control cells grown in the absence of inhibitor; they possesed ATPase activity and cytochrome c as did the control mitochondria.

The growth in media containing bongkrekic acid could not be restored by adding a number of low-molecular compounds, including amino acids and nucleic acid bases, or by enriching the media by yeast extract and casein hydrolyzate.

DISCUSSION

The following are the implications of the results:

(1) The inhibition of the exchange of adenine nucleotides across the mitochondrial membrane in aerobically-growing cells does not prevent the formation of new complete mitochondria. The mitochondrial biogenesis consists in a complex interplay of intra- and extramitochondrial protein synthetizing systems, the two systems drawing energy from ATP produced within mitochondria and in cytosol, respectively. The connection between the two energy pools is apparently not necessary. Cytosolic ATP can supply all energy required for the genesis of mitochondria (12), but, in such a case, the translocation system must be at least partly functional to make the cytosolic ATP available inside mitochondria.

A diminished formation of cytochrome a in cells growing aerobically in the presence of bongkrekic acid may be explained by a high catabolic repression that is associated with inhibition of the Pasteur effect (11,12).

(2) The continual presence of ATP inside mitochondria is necessary for normal replication of mitochondrial DNA in growing cells. The ATP can be furnished either by respiration or

by glycolysis *via* the mitochondrial translocation system. When ATP is supplied by neither way, the mitochondrial DNA may either cease to replicate or be replicated in an aberrant fashion and the cells are converted into respiration-deficient mutants.

(3) Even when total energy expenditure can be covered by glycolytically-produced ATP, the eucaryotic cell cannot multiply if ATP is not available inside mitochondria. It seems that the need of intramitochondrial ATP cannot be simply explained by its requirement for synthesis of some low-molecular compounds in mitochondria. It may be required for the synthesis of a cell constitutent whose dilution within three cell generations gave rise to cells that were viable but unable to multiply. On the basis of these results, it is concievable that the cellular multiplication may depend upon a component formed, and perhaps also localized, inside mitochondria.

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