SEQUENTIAL INCREASE IN ACTIVITY OF MITOCHONDRIAL ENZYMES DURING RESPIRATORY ADAPTATION OF ANAEROBICALLY-GROWN SYNCHRONOUS YEAST

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1. Introduction

A sequential step-wise increase in activities of a number of mitochondrial enzymes [1–4], as well as in concentrations of cytochromes [5] and of cardiolipin [5,6] were found to take place during the aerobic synchronous growth of yeast-cells. This points to a discontinuous and time-ordered formation, or at least complementation of mitochondria in the cell-cycle of this organism.

Similar step-wise changes in activities of mitochondrial enzymes were also observed in anaerobically-grown yeast during a subsequent synchronous growth under aerobic conditions [2]. In this case, not only the formation of new mitochondria but also a transformation of preformed anaerobic mitochondria (promitochondria) into aerobic mitochondria [7–11] took place. The question whether the latter process did also proceed in a step-wise manner has remained unresolved.

The present paper shows that the activities of cytochrome *c*:O_2 oxidase (EC1.9.3.1.), NADH: cytochrome *c* reductase (EC1.6.2.1), succinate:PMS reductase (EC1.3.99.1) and L-malate dehydrogenase (EC1.1.1.37) increase in a sequential step-wise manner when synchronous anaerobically-grown cells undergo respiratory adaptation under non-growing conditions. This strongly suggests that the transformation of promitochondria into aerobic mitochondria in the yeast cells is a synchronous time-ordered process.

2. Materials and methods

Cells of *Saccharomyces cerevisiae* DT XII were grown under strictly anaerobic conditions for 17 h at 30°C in a semisynthetic medium [12] with 5% glucose as carbon source, supplemented with 0.25% Tween-80, 0.005% ergosterol and 0.4% ethanol.

Synchronous cells were prepared from the asynchronous culture by isopycnic gradient centrifugation procedure [13] as described previously [1,2].

Synchronous non-budding cells representing a population in a middle of the G_1-phase of the cell-cycle were suspended in 50 mM potassium phosphate, pH 5.0, containing 0.25% glucose to a concentration 10^7 cells/ml and incubated under strong aeration at 30°C. Samples were taken in 15 min intervals directly into ice-cold mixture of cycloheximide and chloramphenicol (final concentrations 50 μg/ml and 4 mg/ml, respectively) to prevent protein synthesis. Washed cells were disintegrated with ballotini glass beads [6] and the homogenates analysed for enzyme activities. Spectrophotometric assays were performed to measure the activities of NADH:cytochrome *c* reductase [16], cytochrome oxidase [17], succinate:PMS reductase [18] and malate dehydrogenase using of Boehringer MDH UV Test.

3. Results and discussion

During four hours of respiratory adaptation of the anaerobically-grown synchronous cells in the buffered glucose solution the cells underwent practically no growth and no multiplication; total protein increased by 18–23% and the cell number by 5–10 (fig.1). At the same time, however, the activity of NADH:cytochrome *c* reductase rose as many as 11 times, that of cytochrome oxidase about 10.5 times, of malate dehydrogenase 4.5 times and of succinate:PMS reductase 5 times. As shown in fig.2 the increase of cytochrome oxidase and NADH: cytochrome *c* reductase activities were step-wise at a definite time interval. A minor antimycin A insensitive part of the latter increased at a different time interval than the antimycin A sensitive part (fig.3) which represented about 80% of the total NADH:cytochrome *c* reductase activity.

Malate dehydrogenase activity increased in two steps as it did during aerobic synchronous growth of anaerobically-grown yeast [2], the first minor component having probably corresponded the cytosolic enzyme.

As can be seen from fig.2, the rise in succinate dehydrogenase activity exhibited an oscillatory pattern which was not observed during the aerobic synchronous-growth of both aerobically- and anaerobically-grown *Saccharomyces cerevisiae* [1,2]. This may be an expression of a feed-back control of the synthesis of this dehydrogenase. Oscillation in enzyme activity in the cell-cycle was observed with other organisms and considered as a characteristic of unstable enzymes [14].

The relative time sequence of increase in activity of all the enzymes studied in these experiments with non-growing cells has been found the same as in

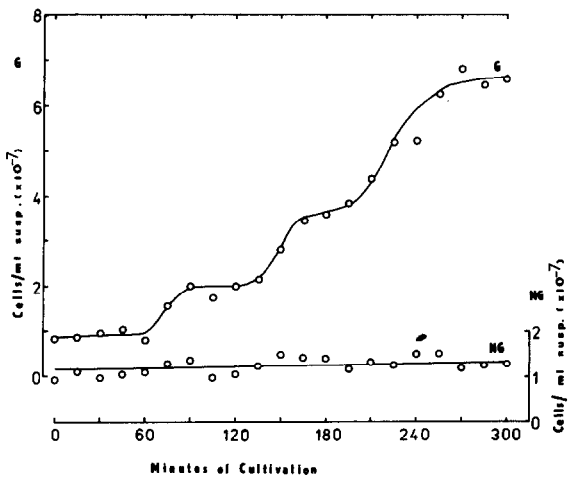

Fig.1. Time-course of the cell-number during the respiratory adaptation of the synchronous anaerobically-grown *Saccharomyces cerevisiae* under non-growing conditions (NG) and separately made growth curve of the same cell-population in the growth medium (G) for the control of cell-synchronicity.

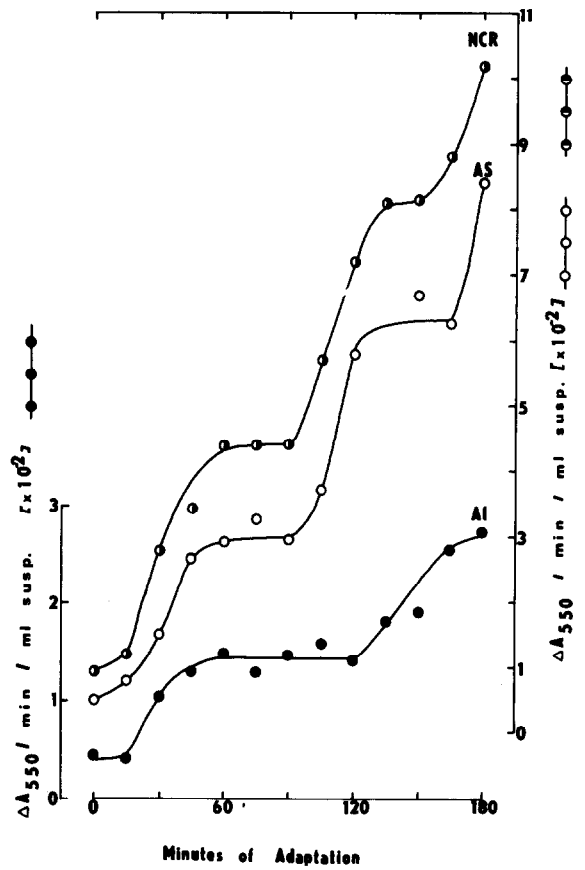

Fig.2. Time-course of the increase in NADH:cytochrome *c* reductase (NCR), cytochrome oxidase (CO), malate dehydrogenase (MDH) and succinate dehydrogenase (SDH) activities during the respiratory adaptation of the synchronous anaerobically-grown yeast-cells under non-growing conditions.

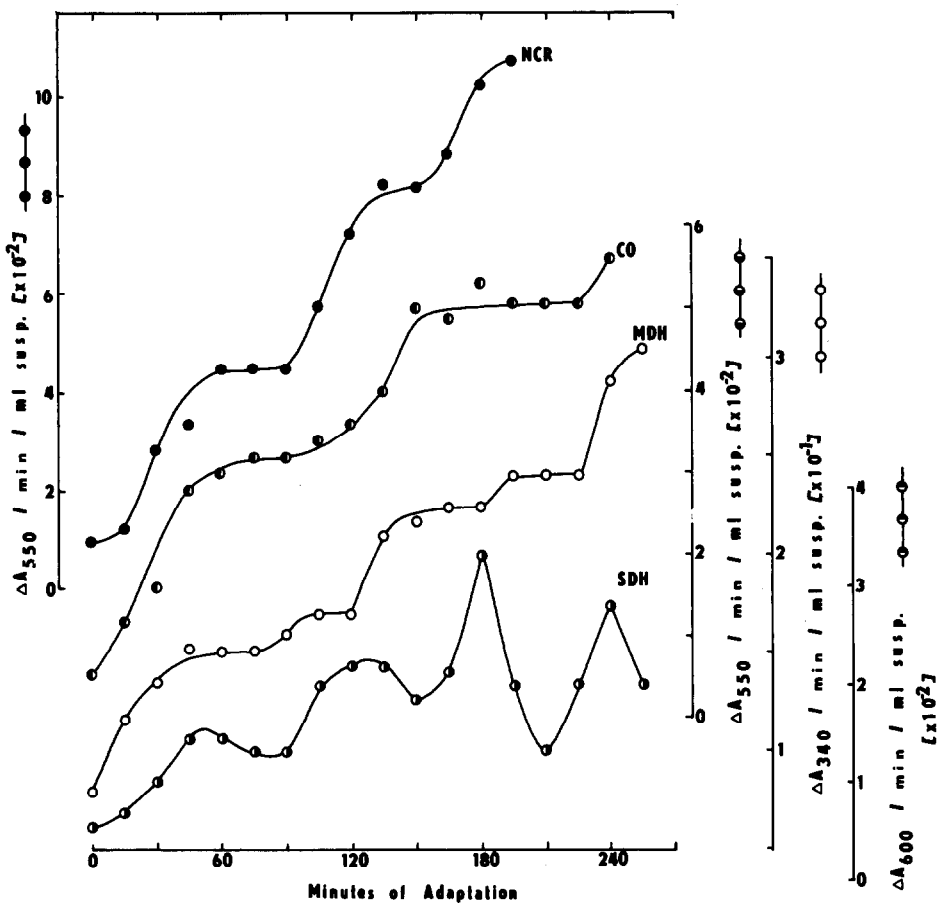

Fig.3. Time-course of the increase in total NADH:cytochrome *c* reductase (NCR), antimycin sensitive (AS) and antimycin insensitive (AI) parts of this enzyme activity during the respiratory adaptation of the synchronous anaerobically-grown yeast-cells under non-growing conditions.

experiments with aerobic synchronously growing cells [1,2].

It might be inferred that the synthesis of the respiratory-chain components in a cell is a sequential time-ordered process independently of whether the components are then being inserted into preformed mitochondria during respiratory adaptation of anaerobically-grown cells or into newly arising mitochondria during cell-growth and multiplication. This would be in accord with the hypothesis of sequentional transcription of genes in the cell-cycle of yeast [15]. It is quite clear, however, that such an interpretation would be too simple to account for a highly complex and coordinate assembly of mitochondria in which transcription of both nuclear and mitochondrial genes, as well as translational and a 'pool-size' controls have been implicated by many studies. Synchronous and synchronized yeast-cells offer excellent opportunities for further exploration of this problem which is tightly connected with the 'cell clock' running even at the stopped cell-growth and division.

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