SOME PROPERTIES OF *CANDIDA LIPOLYTICA* YEAST MITOCHONDRIA

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1. Introduction

An approach to the study of energy metabolism in yeasts is the investigation of the endergonic mitochondrial functions of these organisms.

Most interesting is the study of the reversed electron transfer process. A number of scientists have shown the reduction of coenzyme Q [1] dependent on lactate oxidation energy and the reduction of NAD(P)* dependent on α-glycerophosphate oxidation energy [2,3].

This paper presents results of an investigation of the reduction of pyridine-nucleotides by yeast mitochondria from *C. lipolytica* during oxidation of various substrates.

It has been demonstrated that mitochondria of yeast grown on lactate or citrate are able to reduce endogenous pyridine-nucleotides using the oxidation energy of succinate. Reversed electron transfer from succinate to NAD(P) is absent in mitochondria of yeast grown on glucose but it is observed during α-glycerophosphate oxidation.

All these three types of mitochondria of yeast grown on glucose, lactate and citrate did not show a reversed electron transfer followed by reduction of pyridine-nucleotides on lactate oxidation.

* *Abbreviations:* DNP, 2,4-dinitrophenol; FCCP, carbonylcyanide-*p*-trifluoromethoxyphenylhydrazone; EDTA, ethylenediamintetraacetate; NAD(P), both nicotinamide adenine dinucleotide and nicotinamide adenine dinucleotide phosphate.

2. Methods

Candida lipolytica was grown in synthetic Reader's medium [5]. Glucose, lactate or citrate were used as growth substrates in concentrations 1%, 2% and 1% respectively.

Incubation period 22–24 hr (stationary phase).

Mitochondria were isolated by the method of Akimenko et al. [6].

The concentration of mitochondrial protein was determined by the biuret method [7].

The degree of reduction of pyridine-nucleotides was determined with a fluorometer, designed in the Laboratory of Mitochondrial Biophysics in the Institute of Biophysics, Academy of Sciences, USSR.

Incubation medium contained: 0.65 M mannite; 300 μM EDTA; 0.05% bovine serum albumin; 10 mM Tris–phosphate, pH 7.0. Sample volume – 2 ml, temperature 22–25°C.

3. Results

Studies of the ability of yeast mitochondria to reverse electron transfer showed up essential differences between yeast mitochondria grown on glucose, lactate or citrate (referred to subsequently a 'glucose', 'lactate' and 'citrate' mitochondria).

In fig. 1 are shown fluorometric measurements of pyridine-nucleotides reduction by 'glucose' mitochondria. From curve A it is seen that succinate causes a slow reduction of NAD(P), on ADP addition there occurs a cyclic and on addition of FCCP an irreversible oxidation of reduced pyridine-nucleotides. Such

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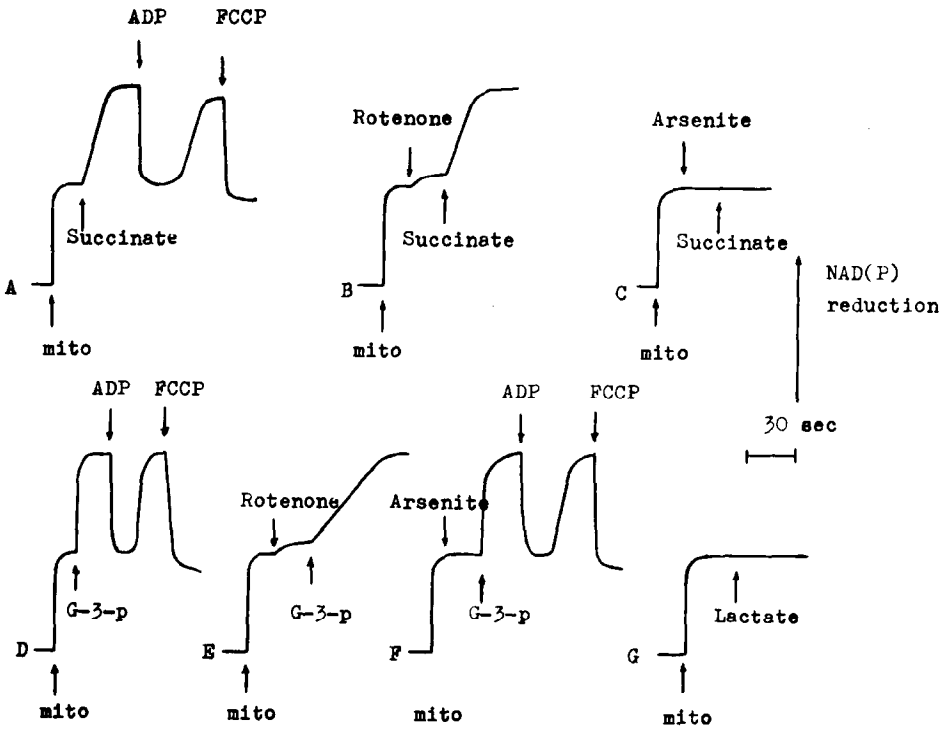

Fig. 1. Reduction of pyridine-nucleotides in mitochondria of yeast grown on glucose. *Additions:* 2.1–2.4 mg protein/ml of mitochondria (mito); 5 mM of succinate; α-glycerophosphate (G-3-P) and lactate; 178 μM ADP; 1 μM ADP; 1 μM FCCP; 10 μM rotenone; 1 mM arsenite.

data can indicate the existence of reversed electron transfer to NAD(P), but the reduction of pyridine-nucleotides is not inhibited by rotenone (curve B) and is prevented by arsenite, which inhibits the oxidation of NAD-linked substrates (curve C).

On oxidation of α-glycerophosphate by 'glucose' mitochondria reduction of NAD(P) takes place (fig. 1, curve D), the reduction velocity being higher than on succinate oxidation. In the presence of rotenone, α-glycerophosphate reduces pyridine-nucleotides more slowly (curve E) and arsenite does not inhibit the reduction (curve F). These results support the presence of reversed electron transfer to NAD(P) in 'glucose' mitochondria, oxidizing α-glycerophosphate.

Lactate oxidation by 'glucose' mitochondria is not coupled with the reduction of pyridine-nucleotides (fig. 1, curve G).

Measurements of reduction of pyridine-nucleotides in yeast mitochondria, grown on lactate, are shown in fig. 2. From curve A one can see that succinate caus-

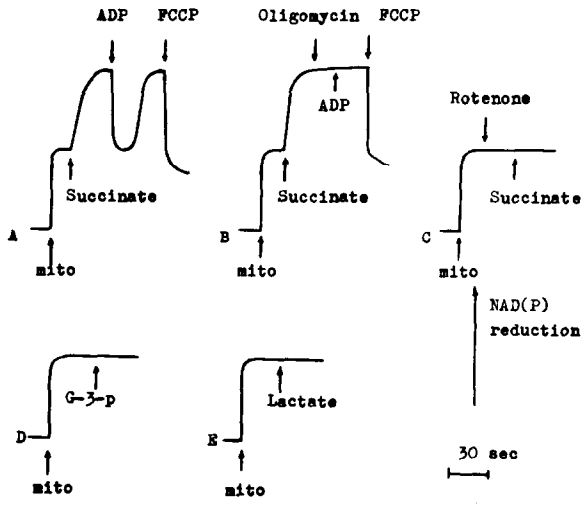

Fig. 2. Reduction of pyridine-nucleotides in mitochondria of yeasts grown on lactate. *Additions:* 2.6 mg protein/ml of mitochondria (mito); 5 mM of succinate; α-glycerophosphate (G-3-P) and lactate; 178 μM ADP; 1 μM FCCP; 5 μg/ml of oligomycin; 10 μM rotenone.

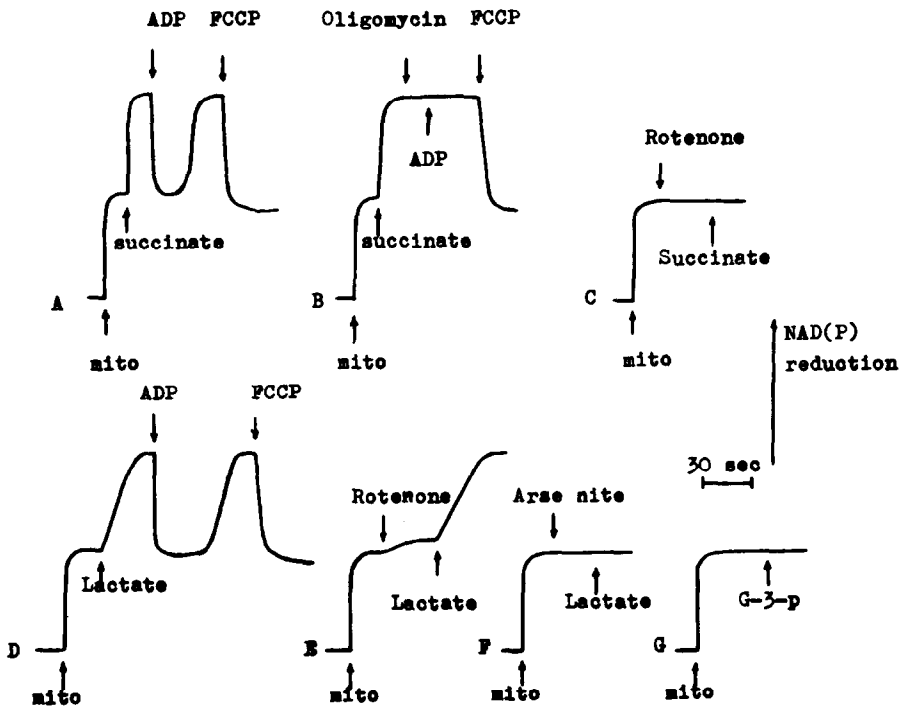

Fig. 3. Reduction of pyridine-nucleotides in mitochondria of yeasts grown on citrate. *Additions:* 2.6 mg protein/ml of mitochondria (mito); 5 mM of succinate; α-glycerophosphate (G-3-P) and lactate; 178 μM ADP; 1 μM FCCP; 5 μg/ml of oligomycin; 10 μM of rotenone; 1 mM of arsenite.

es NAD(P) reduction which can be reversibly removed by ADP addition and irreversibly by FCCP addition. The ADP effect is inhibited by oligomycin (curve B). The addition of succinate after rotenone does not lead to pyridine-nucleotides reduction (curve C).

On oxidation of α-glycerophosphate or lactate by 'lactate' mitochondria, NAD(P) reduction does not take place (curves D and E).

Fig. 3 represents pyridine-nucleotide reduction curves in yeast mitochondria, grown on citrate. As with 'lactate' mitochondria succinate reduces NAD(P) which is being reversibly oxidized by ADP and irreversibly by FCCP (curve A). Oligomycin prevents the effect of ADP (curve B) and rotenone inhibits pyridine-nucleotide reduction by succinate (curve C).

On lactate oxidation by 'citrate' mitochondria a slow NAD(P) reduction takes place (curve D), but in the presence of rotenone the lactate also reduces NAD(P) (curve E) and arsenite inhibits the reduction (curve F).

α-Glycerophosphate does not reduce pyridine-nucleotides in 'citrate' mitochondria (curve G).

4. Discussion

In a number of papers the authors did not report reversed electron transfer from succinate to NAD(P) in yeast mitochondria [4,8]. But our results show that in yeast mitochondria, grown on lactate or citrate, a reversed electron transfer from succinate to NAD(P) exists (figs. 2 and fig. 3). Indeed, as is seen from curve A, succinate reduces pyridine-nucleotides which are reversibly oxidized on ADP addition and irreversibly at addition of FCCP. Oligomycin prevents the ADP effect, but not that of FCCP (curves B). Rotenone, which inhibits electron transfer at the level of NADH-dehydrogenase, prevents NAD(P) reduction (curves C), which confirms the fact that pyridine-nucleotide reduction is taking place by reversed electron transfer from succinate.

It must be noticed that in 'citrate' mitochondria lactate is also able to reduce pyridine-nucleotides (fig. 3, curve D), but rotenone does not inhibit the reduction (curve E). Probably in this case NAD(P) is being reduced by pyruvate formed by lactate oxidation. If this is so, the reduction of pyridine-nucleotides by lactate should be removed by arsenite, which inhibits the oxidation of NAD-linked substrates*, as was in fact observed (curve F).

By analogy, in 'glucose' mitochondria the succinate reduced pyridine-nucleotides not on account of reversed electron transfer but because of the formation of NAD-linked substrates (fig. 1, curves A, B, C). Reduction of NAD(P) by 'glucose' mitochondria, oxidizing α-glycerophosphate, was going on, probably, by reversed electron transfer as the velocity of pyridine-nucleotides reduction was rather slower in the presence of rotenone (curves D and E) and arsenite did not inhibit their reduction (curve F).

The absence of reversed electron transfer from succinate to NAD(P) in yeast mitochondria, grown on glucose, may probably be explained by the great activity of glycolytic enzymes in cells which are able to supply the required pool of reduced pyridine-nucleotides. Under these conditions the loss of tight coupling between the succinate dehydrogenase system and the main respiratory chain probably occurs.

* Concentration of arsenite – 1 mM which does not inhibit succinate or lactate oxidation by mitochondria.

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