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Electron transport chain in aerobically cultivated *Zymomonas mobilis*

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Abstract

Respiratory chain composition and energy coupling in cytoplasmic membrane of *Zymomonas mobilis* was shown to depend on culture aeration. Aerobically grown cells contained mainly the non-energy-generating NADH dehydrogenase with K_M for NADH 58 mM. In anaerobically cultivated bacteria, the energy-coupling NADH dehydrogenase complex with K_M for NADH 7 mM predominated. In aerobically cultivated *Z. mobilis*, CoQ content and absorption peaks of cytochromes at 554–556 nm and 525–528 nm were significantly increased. Energy-coupling site I, linked to the NADH:CoQ oxidoreductase complex, could be eliminated under sulfate-deficient cultivation conditions. For anaerobically grown cells this resulted in loss of oxidative phosphorylation activity, while in aerobically grown cells energy coupling was not affected. These findings indicate a shift of energy coupling from site I to the cytochrome region of the respiratory chain in *Z. mobilis* under transition from anaerobic to aerobic growth conditions.

Keywords: *Zymomonas mobilis*; Oxidative phosphorylation; Electron transport chain; Aerobic cultivation; NADH oxidase; Sulfate deficiency

1. Introduction

Oxidative phosphorylation activity was recently demonstrated in non-growing cells and cytoplasmic membrane vesicles of the obligately fermentative aerotolerant bacterium *Zymomonas mobilis* [1]. This finding once again raised the question of the structure and function of the electron transport chain (ETCh) of *Z. mobilis*. So far, not much attention has been paid to the function of the ETCh in *Z. mobilis*, probably due to poor growth and low growth yields of aerated *Z. mobilis* culture.

According to our published data, only energy coupling site I, related to NADH:CoQ oxidoreductase, is present in the ETCh of this bacterium [2]. At the same time, Kim et al. [3] reported that NADH dehydrogenase in the cytoplasmic membranes of *Z. mobilis* belongs to type II (energy non-generating), and that membrane energization is coupled to electron transport between CoQ and oxygen, but not related to NADH:CoQ oxidoreductase. We found that the two studies used different culture conditions: in one case [2] *Z. mobilis* was grown anaerobically, while in the other case [3], it was grown aerobically. Therefore, in the present study we compared the composition of the ETCh and energy-coupling sites in anaerobically and aerobically cultivated *Z. mobilis*.

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The results show a strong dependence of ETCh composition on aeration in this species.

2. Materials and methods

Zymomonas mobilis ATCC 29191 was maintained, cultivated, harvested and starved as described previously [1,2]. Aerobic cultivation was carried out in 750 ml flasks with 50 ml culture volume on a shaker at 120 rpm. Cultivation under sulfate-deficient conditions, as well as the experimental routine of ethanol-dependent ATP synthesis in aerated, starved cells, and ATP determinations with the luciferin-luciferase assay were exactly as described earlier [2]. For permeabilization of cells a modification of the method described by Osman et al. [4] was applied: cell suspension at 8–10 mg dry weight ml^{-1} was vortexed for 1 min in the presence of 2% chloroform. Room-temperature dithionite-reduced versus ferricyanide-oxidized cytochrome spectra were taken in permeabilized cells using a Shimadzu UV 260 spectrophotometer. CoQ content was determined in heptane extracts spectrophotometrically at 275 nm, as described by Collins [5]. Anilinonaphthalenesulfonate (ANS^-) fluorescence in intact cells was monitored following the protocol of Griniuviene et al. [6] using a home-built fluorimeter. Oxygen consumption rate with ethanol was measured with a Clark electrode, as previously [2]. 50 mM potassium phosphate buffer with 5 mM $MgSO_4$, pH 6.9, was used throughout the experiments. Except for spectrophotometric analysis of cytochromes, all the experiments were carried out at 30°C.

Membrane fractions were obtained by sonication of washed cell suspensions at a concentration of 5–6 mg dry weight ml^{-1}, followed by removal of unbroken cells, and pelleting the membranes by centrifugation, as described [1]. For some unknown reason, similar amounts of aerobically or anaerobically grown cells subjected to the same cell disruption routine yielded membranes with different protein concentrations (less in aerated cells). Therefore, for quantitative comparison of the cytochrome content, spectra were taken in permeabilized whole cells and normalized per amount of dry biomass. In the NADH oxidase assay, membranes without washing were resuspended at 1–2 mg protein ml^{-1} in 50 mM potassium phosphate buffer with 5 mM $MgSO_4$, pH 6.9. NADH oxidase activity was monitored spectrophotometrically as described by Matsushita et al. [7]. Kinetic constants of NADH oxidase(s) were found by computer-aided fitting to a Michaelis function (or a sum of two functions in the case of a biphasic

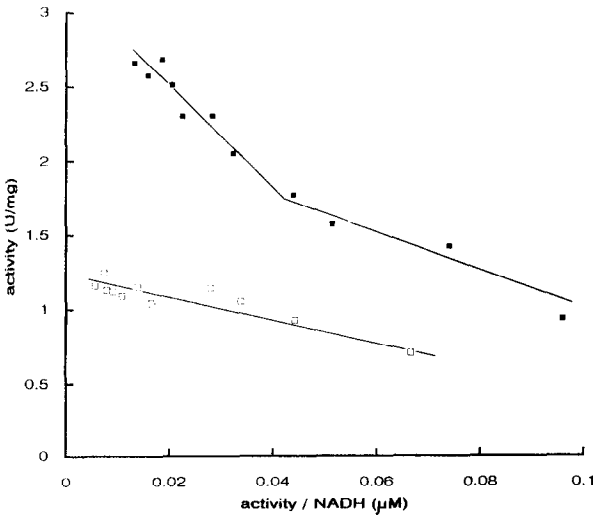

Fig. 1. Kinetic analysis of NADH oxidase in membranes of anaerobically (□) and aerobically (■) cultivated cells. An Eadie-Hofstee plot is shown of activities measured over the range of 10–2000 μM NADH with 0.03 mg of membrane protein/ml in the spectrometer cuvette. Parameters for anaerobic cells: K_M=7 μM, V_{max}=1.2 U/mg. Aerobic cells, high affinity component: K_M = 7 μM, V_{max} = 1.1 U/mg. Aerobic cells, low affinity component: K_M = 60 μM, V_{max} = 2.1 U/mg.

Eadie-Hofstee plot) to the experimental curve of NADH oxidation rates versus respective concentrations. The least squares criterion was used for fit optimization.

ANS^- ammonium salt was purchased from Serva, Heidelberg, Germany, deamino-NADH was from Sigma, Deisenhofen, Germany. Other chemicals were as described [2].

3. Results and discussion

3.1. Kinetic parameters of NADH oxidase

The concentration dependence of NADH oxidase activity was examined in membrane preparations of aerobically and anaerobically cultivated cells. The results are presented in Eadie-Hofstee coordinates in Fig. 1. The plot for aerobically grown cells was biphasic, as was demonstrated for *E. coli* [7]. However, for anaerobically grown cells the Eadie-Hofstee plot was monophasic. The apparent K_M of NADH oxidase for NADH in anaerobically grown cells was around 7 mM, typical for the energy-coupling NADH dehydrogenase complex I [7–9]. The same K_M value was found for one of the components in aerobically grown cells. The apparent K_M of the second component prevailing in aerobically grown cells was 58 mM. This enzyme probably corresponds to the NADH oxidase described by Kim et al. [3], who reported a single apparent K_M value of 66 mM and attributed *Z. mobilis* NADH dehydrogenase to the energy non-generating type (type II). We speculate that Kim et al. might have lost some of the complex I activity during preparation of membranes (by double passage of cells through a French press [3]). In our preparations obtained by sonication the total NADH oxidase activity was significantly higher than reported by these authors.

3.2. Effect of aeration on cytochrome and CoQ content

The α- and β-regions of cytochrome spectra are shown in Fig. 2A,B. The peak at 554–556 nm in the α-region and the peak around 525–528 nm in the β-region of the cytochrome spectrum were significantly higher in aerated cells. Both b and c type

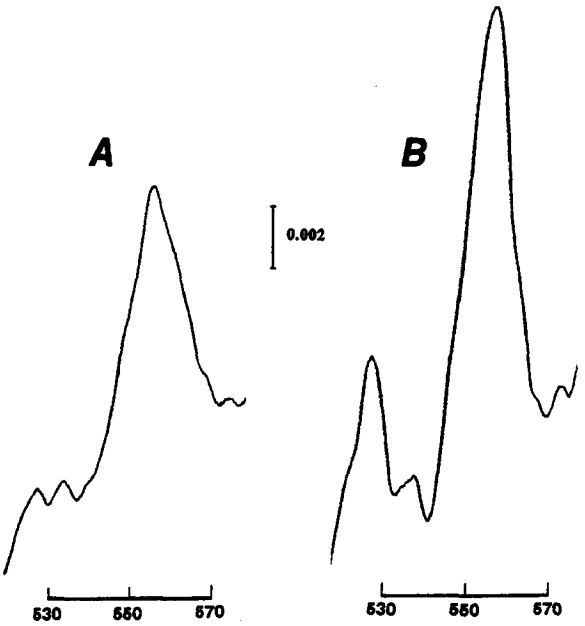

Fig. 2. Dithionite-reduced minus ferricyanide-oxidized room temperature cytochrome spectra of permeabilized cells. A: Anaerobically cultivated cells. B: Aerobically cultivated cells. Concentration of cells in the cuvette was 9 mg dry weight ml^{-1}.

cytochromes have been previously described for *Z. mobilis* [10,11], and could, in principle, contribute to the increase of absorption at the given interval of wavelengths. Identification of the cytochrome species induced by aeration in *Z. mobilis* is a further question of interest which needs investigation. Although earlier reported for *Z. mobilis* [10,11], cytochrome d in our preparations was present in trace amounts (not shown). CoQ content also was induced by aerobic growth conditions. As judged from absorption at 275 nm in heptane extracts, under aerobic conditions it reached 0.23–0.25 nmol mg^{-1} dry weight. In extracts from anaerobically grown cells the absorption peak at 275 nm was absent.

3.3. Energy coupling in anaerobically and aerobically grown cells

Membrane energization and aerobic ATP synthesis was compared in aerobically and anaerobically grown *Z. mobilis* under sulfate-deficient conditions. As demonstrated previously [2], sulfate-deficient conditions in *Z. mobilis* lead to a loss of coupling site I, similar to what has been shown for *E. coli* [12] and *Paracoccus denitrificans* [13]. With this approach it was possible to examine directly energy coupling downstream of coupling site I in whole-cell experiments using ethanol as a substrate for oxidative energy generation [1]. Cytochrome and CoQ contents in sulfate-deficient cultures were similar, and depended on aeration in the same way as in bacteria grown on complete medium (not shown).

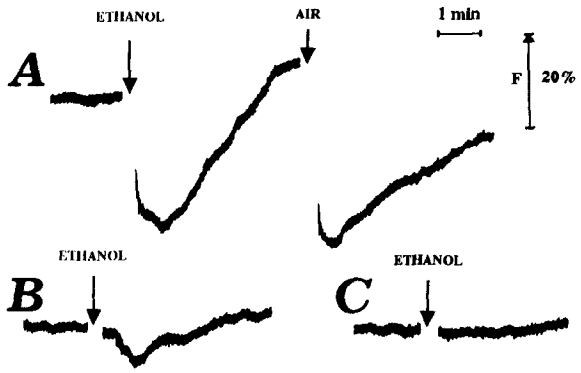

Fig. 3. Quenching of ANS^- fluorescence upon ethanol addition to the suspension of cells grown under sulfate-deficient conditions. A: Aerobically cultivated cells. B: Anaerobically cultivated cells. C: Aerobically cultivated cells in the presence of 0.5 mM KCN. Concentration of cells in the cuvette was 3 mg dry weight ml^{-1}; Concentration of ANS^- was 50 mM. Ethanol was added to 1 mg ml^{-1} final concentration.

Generation of a transmembrane electric potential was monitored by quenching of ANS^- fluorescence (Fig. 3). Addition of ethanol caused a profound, transient fluorescence quenching in aerobically grown sulfate-deficient cells (Fig. 3A), which could be restored by aeration of the cuvette contents. The fluorescent response in anaerobically grown sulfate-deficient cells was much smaller (Fig. 3B). However, the oxygen consumption rate with ethanol was higher in anaerobically grown cells (by 10–30%; data not shown), indicating that in anaerobic sulfate-deficient culture the coupling between respiration and mem-

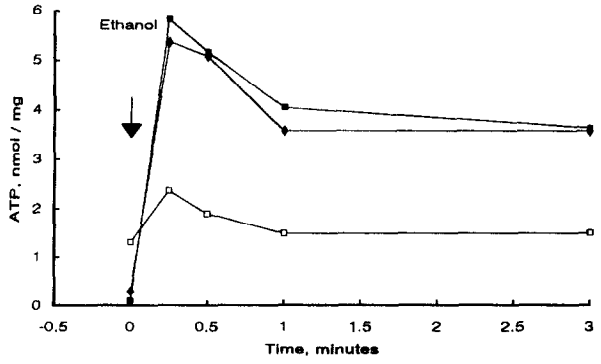

Fig. 4. Aerobic ATP synthesis in response to ethanol addition. Aerobically cultivated cells under sulfate-deficient conditions (■); aerobically cultivated cells in complete medium (◆); anaerobically cultivated cells under sulfate-deficient conditions (□). Ethanol was added to 1 mg ml^{-1} final concentration to a suspension of cells on a shaker at concentration of 7 mg dry weight ml^{-1}.

brane energization was strongly reduced. Fluorescence quenching was absent in aerobically (Fig. 3C) or anaerobically (data not shown) cultivated cells treated with 0.5 mM KCN. The increase of intracellular ATP level observed after ethanol addition was in good agreement with the membrane energization data: it was significantly higher in aerobically cultivated sulfate-deficient *Z. mobilis* (Fig. 4). In anaerobically cultivated cells only a residual ATP synthesis was seen, as demonstrated previously [2]. At the same time, ATP level in aerobically grown sulfate-deficient cells did not differ significantly from that in aerobic control cultivated on complete medium (Fig. 4).

The presented data allow us to suggest that in aerobically cultivated *Z. mobilis* oxidative energy coupling is linked mainly to the cytochrome region of ETCh, but not to site I. Kim et al. [3] have come to the same conclusion. On the other hand, in anaerobically grown *Z. mobilis* mainly site I is active, as was shown previously [2] and confirmed by the present data. Such a shift of energy coupling in *Z. mobilis* ETCh in response to aeration might result from the variation of relative amounts of type I and type II NADH dehydrogenases, and also from the changes of CoQ and cytochrome contents. This regulatory phenomenon probably points to some special, yet unknown physiological function of oxidative phosphorylation in *Z. mobilis*. So far, it has been shown that *Z. mobilis* does not employ oxidative phosphorylation for energy supply into biomass synthesis during aerobic growth [11,14], as do all the known bacteria carrying a respiratory chain.

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