ALTERNATING ELECTRIC FIELDS STIMULATE ATP SYNTHESIS IN *ESCHERICHIA COLI*

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Abstract: External alternating electric fields of low intensity stimulated membrane bound ATP synthesis in starving *Escherichia coli* cells with electric field amplitudes of 2.5–50 V/cm and a frequency optimum at 100 Hz. The model of electrocon-formational coupling was used to analyze the frequency and amplitude responses of ATP synthesis. Two relaxation frequencies of the system were obtained at 44 Hz and 220 Hz, and an estimate of roughly 12 was obtained as the effective charge displacement for the catalytic cycle of ATP synthesis.

Key Words: Electrical Stimulation, Electroconformational Coupling, ATP, *E. Coli*

INTRODUCTION

Pulsed as well as oscillating electric fields were shown to influence the activity of membrane proteins [1-3]. The theoretical basis for this is the electroconformational coupling model [4]. An important use of the model was emphasized by Robertson *et al.* in their study of membrane protein dynamics [5]. Their hypothesis postulates that the *steady-state rate of enzyme turnover should be a function of the frequency of an applied periodic membrane potential of small amplitude*. The frequency dependence is described by a sum of Lorentzians, and the measured frequency response functions can be used to determine the relaxation frequencies and the displacement charge of the system. In this study, motivated by these findings, the steady-state dynamics of ATP synthesis in *E. coli* were examined.

MATERIALS AND METHODS

E. coli (wt) cells were grown in a liquid LB medium. After harvesting, cytoplasmic ATP was depleted, and the bacteria resuspended in 1.5 mL of 250 mM sucrose, 15 mM NaCN and 100 mM Tris-HCl (pH 8) [1], and incubated for 2 h at room temperature. They were then transferred to the stimulation cell at 3°C and incubated for 3 min prior to the onset of electrical stimulation. The same medium was used during stimulation in order to block the electron transport chain. The alternating electric field in the stimulation cell was established by means of two parallel platinum electrodes (0.75 cm^2 active area, 2

mm apart), connected to a frequency generator (Metex MS-9150). The stimulated sample was thermostated and kept at 3°C during the entire duration of the experiment, with a temperature variation of under 0.2°C. The amplitude of the alternating voltage on the electrodes was from 0 V to ±10 V with zero DC component and a frequency from 50 Hz to 100 kHz. The duration of exposure was 30 min. Intracellular ATP was then extracted with a boiling buffer (3 min in 100 mM Tris-HCl, pH 8, at 100°C), and its relative concentration determined with the bioluminescence reaction (Sigma FL-AA).

RESULTS AND DISCUSSION

An externally applied alternating electric field with an amplitude of intensities between 2.5–50 V/cm stimulated ATP synthesis in *E. coli* cells, with a maximum at 100 Hz (Fig. 1a). The amplitude dependence of ATP synthesis was approximated by a quadratic function (Fig. 1b). Electric field induced ATP synthesis was repressed in the presence of an ATP synthase inhibitor (1.5 mM N,N'-dicyclohexylcarbodiimide) and an ionophore (0.2 mM 2,4-dinitrophenol) (data not shown).

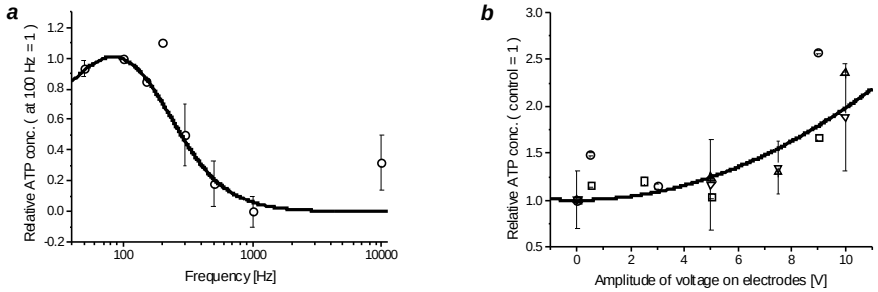

Fig. 1. *De novo* ATP synthesis in *E. coli* cells stimulated by alternating electric fields. The procedure was as described in the text. Vertical bars represent the standard error of the mean. a) Frequency dependence of ATP synthesis, the voltage on electrodes was 9 V, nine independent repetitions were performed (summaric graph). b) Amplitude dependence of ATP synthesis, the frequency of the applied electric field was 100 Hz, four independent repetitions were performed (all data shown).

From the amplitude response it was possible to estimate the magnitude of the displacement charge [6] to 12 elementary charges for the catalytic cycle of ATP synthesis. The results of the frequency response (Fig. 1a) were fitted to a sum of Lorentzians [5] and the values of two relaxation frequencies of the system were estimated at 44 Hz and 220 Hz. Three kinetically relevant states are therefore sufficient to describe the steady-state activity of ATP synthesis, but further experiments are needed to determine the individual rate constants of the

reaction, and verify the relaxation frequency at 44 Hz, which was out of the experimental range of frequencies.

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