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EXPERIMENTAL ARTICLES

A Bioelectrochemical Study of a Suspension of *Escherichia coli* Cells Metabolizing Glucose and Lactose

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Abstract—The metabolism of glucose and lactose in *Escherichia coli* K-12 cells has been studied using a bioelectrochemical (BEC) approach. The magnitude and the duration of the response of a BEC anode were found to be functions of the composition of nutrient media and the concentration of bacterial cells. The amount of electricity that is generated enzymatically during the metabolism of a particular substrate depends on the activity of the relevant enzymes. This suggests that the BEC approach can be used for evaluating the activity of particular enzyme systems.

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Key words: metabolism, cells, mediator, bioelectrochemical approach.

In recent years, the catalytic activity of redox enzymes has been extensively studied with the aid of bioelectrochemical (BEC) systems. The mechanism of action of such systems is based on the biochemical oxidation of a substrate (e.g., glucose) and the transfer of derived electrons to electrode processes, which requires operation of a low-molecular-weight redox pair (mediator). The oxidized form of this mediator is reduced by cells, whereas its reduced form is oxidized on the electrode. The electric signal of the BEC system depends on the concentrations of the substrate and the mediator [1, 2]. The electrochemical response of live cells can be used to describe their growth curve [3]. BEC systems are at the basis of many biosensors [5]. The use of the BEC systems for studying microbial metabolism is of great interest.

In this work, we used the bacterium *Escherichia coli* K-12 because the enzyme systems of this bacterium involved in the metabolism of the low-molecular-weight substrates glucose and lactose have been well studied [6–8]. The metabolism of these substrates was investigated by the BEC approach, which is based on the ability of the *E. coli* K-12 cells to reduce the oxidized form of the redox indicator methylene blue (tetramethyldiaminophenothiazine) due to glucose oxidation.

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MATERIALS AND METHODS

The bacterium *Escherichia coli* K-12 was obtained from the collection at the Institute of Biochemistry and Physiology of Plants and Microorganisms. This bacterium was grown in one of the following media.

Medium 1 contained (g/l) yeast extract, 5; peptone, 5; and NaCl, 10.

Medium 2 contained peptone, 6; lactose, 6; Na_2HPO_4, 3; KH_2PO_4, 6; $CaCl_2$, 0.01; and NaCl, 1.8.

Medium 3 contained peptone, 6; glucose, 6; Na_2HPO_4, 3; KH_2PO_4, 6; $CaCl_2$, 0.01; and NaCl, 1.8.

Cultivation was performed aerobically at 30°C on a shaker (160 rpm) for 12 hours. Then, bacterial cells were harvested by centrifugation at 2800 *g* for 5 min, washed, and suspended in a phosphate buffer. The cell concentration was determined turbidimetrically [8].

Electrochemical measurements were carried out as described by Minell and Minell [9] using a sealed three-electrode glass cell with separate cathode and anode spaces. The anode was a carbon electrode. The cathode was a platinum electrode. The potential of the working electrode was measured relative to the Ag/AgCl reference electrode (E_0 = +0.201 V). The mediator was methylene blue. The experiments were conducted anaerobically in an argon atmosphere.

The electric response of the working electrode was recorded using a PDP4-002 two-axis recorder. The anode was polarized with a P5827M potentiostat.

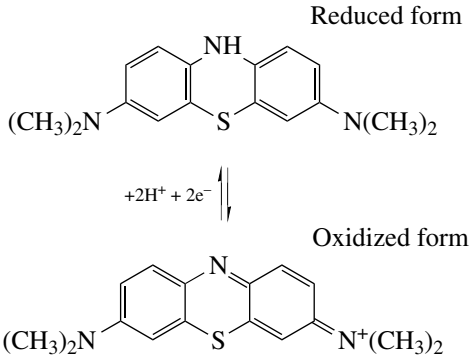

Fig. 1. Structural formula for methylene blue.

The concentrations of glucose and methylene blue were 27 μM and 3 mM, respectively. The cell concentration was varied from 2.5×10^{-4} to 12.2×10^{-4} g/ml.

RESULTS AND DISCUSSION

The enzyme activity of *E. coli* K-12 cells metabolizing glucose and lactose was measured by the bioelectrochemical method as the amount of electricity generated due to the metabolism of these substrates. The electrode compartment with the BEC anode contained bacterial cells and one of the substrates. It is known that *E. coli* cells metabolize glucose via the Embden–Meyerhof–Parnas pathway. Glucose is oxidized through the formation of glucose-6-phosphate and then pyruvate. Under anaerobic conditions, the pyruvate molecule is cleaved to acetyl-CoA and formate by pyruvate formate-lyase [6]. The formate is converted to CO_2 and H_2 by formate hydrogenlyase. The electrons released in these reactions are transferred from the cells to the anode by the methylene blue mediator molecules (Fig. 1).

The bacterium *E. coli* is a facultative anaerobe. Under aerobic conditions, about half of glucose is oxidized to CO_2, whereas the other half is used for constructive metabolism. In this case, oxygen serves as the terminal electron acceptor [6]. Under anaerobic conditions, methylene blue can serve as the terminal electron acceptor.

Preliminary experiments showed a complete reversibility of the methylene blue redox pair. Figure 2 presents the potentiodynamic polarization curves for the BEC electrode in the pure electrolyte (curve *1*) and in the electrolyte containing methylene blue (curve *2*). The maxima of anode current in curve *2* correspond to the oxidation of methylene blue on anode ($E = +0.02$ V) and its reduction on cathode ($E = -0.3$ V). The exponential decline in the anode current of the carbon electrode at $E = +0.02$ V (Fig. 3) indicates the ability of the reduced form of methylene blue to completely convert to the oxidized form under the given experimental conditions.

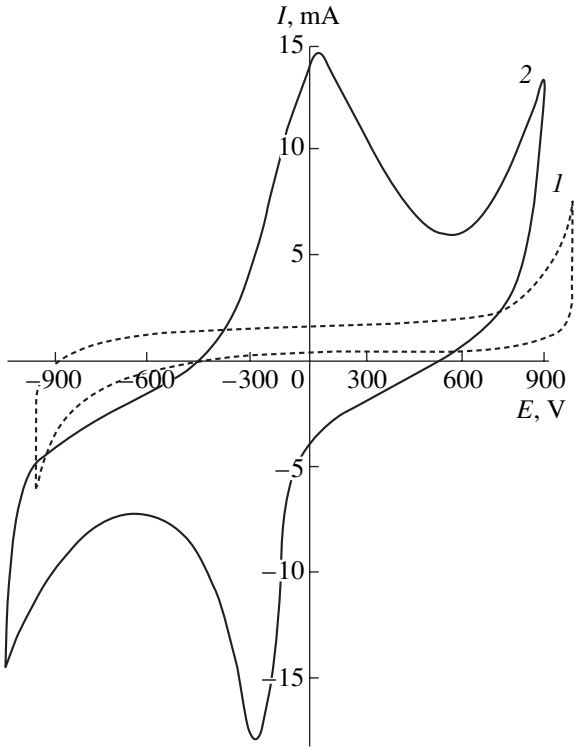

Fig. 2. Cyclic voltammetric curves for carbon electrode in the working electrolyte (curve *1*) and 3 mM methylene blue (curve *2*), pH 6.9.

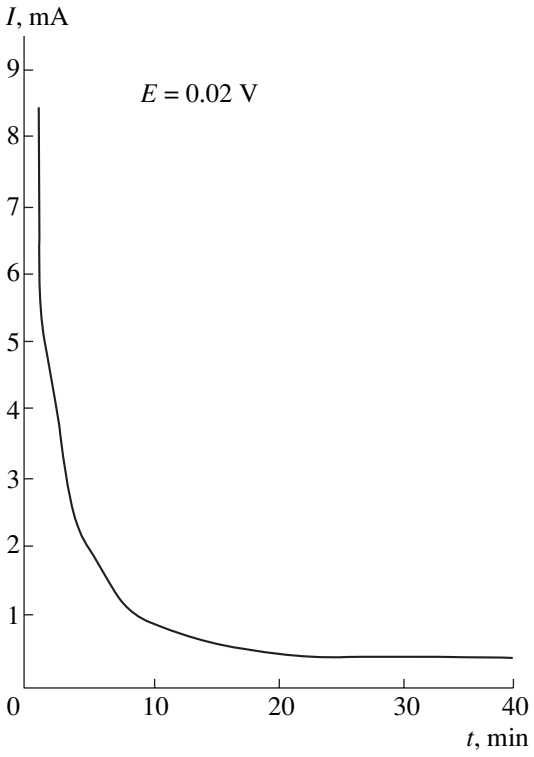

Fig. 3. The potentiostatic curve of the anode oxidation of 3 mM methylene blue on carbon electrode.

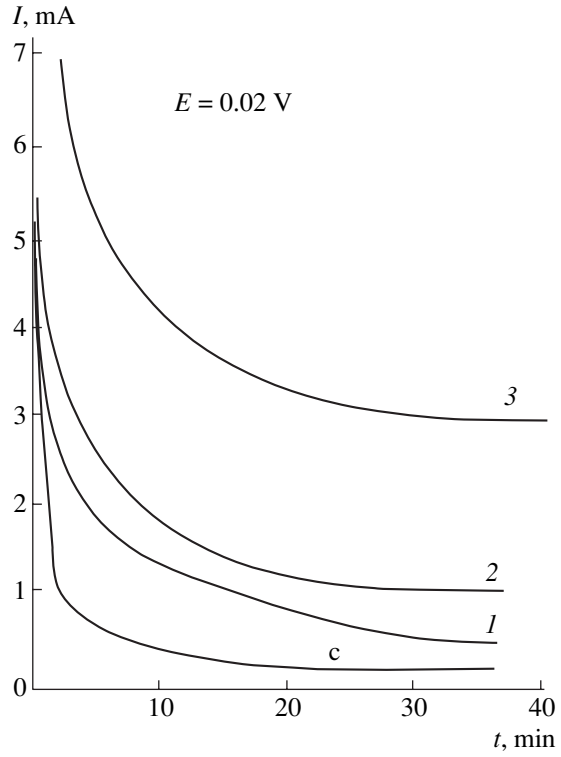

Fig. 4. The potentiostatic curves of the anode oxidation of 3 mM methylene blue on carbon electrode in the working electrolyte containing 27 μM glucose without bacterial cells (control, curve c) and in the presence of 3.7×10^{-4} g/mL of *E. coli* cells grown in LB broth (curve *1*), LB broth with lactose (curve *2*), and LB broth with glucose (curve *3*).

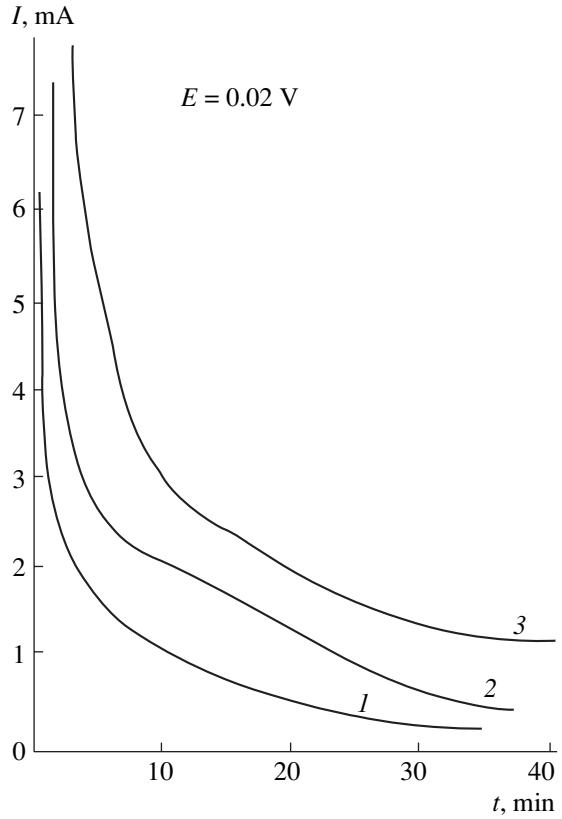

Fig. 5. The potentiostatic curves of the anode oxidation of 3 mM methylene blue on carbon electrode in the working electrolyte containing 27 μM glucose and *E. coli* cells at concentrations of 3.7×10^{-4} (curve *1*), 9.5×10^{-4} (curve *2*), and 12.2×10^{-4} (curve *3*) g/ml.

Potentiometric measurements confirmed the ability of *E. coli* cells to reduce methylene blue. After the removal of oxygen with argon sparging, oxidized methylene blue remained the only electron acceptor in the medium. The addition of *E. coli* cells to the methylene blue–containing electrolyte of the working electrode resulted in a shift of the potential of the carbon electrode in this electrolyte from –(0.09–0.1) to –(0.39–0.4) V. The *E. coli* cells deprived of oxygen were forced to trigger to anaerobic respiration. Methylene blue was reduced, as was evident from discoloring of the methylene blue solution. The availability of cellular reducers to mediator molecules is determined by the permeability of the cell walls and membranes. The number and the disposition of cellular reducing entities (enzymes, pyridine nucleotides, quinone intermediates, and cytochromes) may vary. The mediator molecules really interact with specific intracellular electron donors. However, for the sake of convenience, the electrons derived from these donors can be considered to form a reducing pool in the cytoplasm. The mechanism through which methylene blue is reduced can be conceived of as follows. The permeability of lipid membranes allows penetration of the mediator molecules into the cell. Then, the mediator molecules accept electrons from the intracellular reducing pool and transfer them to the electrode.

In order to understand the effect of enzymes involved in glucose and lactose metabolism on the electric response of the BEC anode, further experiments were carried out with bacterial cells grown in media containing (i) peptone and yeast extract, (ii) peptone and lactose, and (iii) peptone and glucose. Figure 4 shows the temporal dynamics of the bioelectrochemical oxidation of glucose by three types of bacterial cells grown on the aforementioned substrates. As is evident from this figure, the amount of electricity, which is expressed by the area below the curve, depends on the activity of enzymes. In turn, the enzyme activity depends on the composition of the nutrient medium. Consequently, the electric response of the BEC anode is a function of substrate metabolism. The oxidation of glucose by the *E. coli* cells grown in the medium containing peptone and glucose influenced the electric response of the BEC anode to the greatest degree.

Figure 5 shows potentiostatic curves for the anode oxidation of glucose by the bacterial cells grown on peptone and yeast extract. The data presented in this

figure suggest that the magnitude and the duration of the amperometric response of the working electrode under the given experimental conditions are directly proportional to the concentration of the *E. coli* cells. Specifically, the higher the cell concentration, the greater the amount of electricity generated by the electrode. Similar results were obtained for the *E. coli* cells grown on lactose and glucose.

Thus, the amount of electricity generated by the BEC anode depends on the activity of the enzymes involved in the metabolism of particular substrates. Since the activity of these enzymes is determined by the composition of the nutrient medium, the electric response of the BEC anode is a function of substrate metabolism. The oxidation of glucose by the *E. coli* cells grown in the medium containing peptone and glucose influences the electric response of the BEC anode to the greatest degree. The study of the amperometric response of the working electrode as a function of the concentration of bacterial cells in the amperometric cell showed that the amount of electricity generated by the electrode is proportional to the cell concentration. A similar dependence was observed for the *E. coli* cells grown on lactose and glucose.

To conclude, this study showed that the amount of electricity generated during the metabolism of particular substrates depends on the activity of the enzymes involved in this metabolism. Studies with the use of the BEC anode make it possible to evaluate the activity of enzyme systems and, eventually, to gain information on the metabolic processes occurring in microbial cells.

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