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Amperometric monitoring of redox activity in intact, permeabilised and lyophilised cells of the yeast *Hansenula polymorpha*

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Abstract

An effect of permeabilisation and lyophilisation of the yeast cells *Hansenula polymorpha* on their electrochemical behaviour in the presence of mediators, substrates (formaldehyde, glucose, methanol, ethanol), and cofactors (NAD^+, $NADP^+$, NADH, NADPH, glutathione) has been studied. Two amperometric techniques differing in the cell immobilisation methods were applied. The cells of a wild strain (*356*) and mutant strains (*C-105* and *KCA 33*) of the yeast, grown in the presence of glucose or methanol, were used in the experiments. The intact cells revealed the highest reduction rates of mediators, 2,6-dichlorphenolindophenol (DCIP) and 2,4-benzoquinone (BQ), as measured by amperometry. The addition of formaldehyde significantly enhanced the response, if the cells were grown in the presence of glucose. The permeabilised cells showed the lowest current level in the presence of DCIP and BQ and no response to the addition of formaldehyde and NAD^+. However, the addition of NADH gave significant current surge. All these phenomena imply that the permeabilised cells lost cofactors and the activity of dehydrogenases producing NADH, but they remained the activity of NADH-ubiquinone oxidoreductase and of some components of the electron transport chain. The electrochemical behaviour of the lyophilised cells shows they are heterogeneous. The partial degradation of the outer membrane of the cells after their lyophilisation was electrochemically confirmed.

Keywords: Yeast *Hansenula polymorpha*; Intact, permeabilised and lyophilised cells; Amperometry; Mediators

1. Introduction

Electrochemical activity of living cells, *i.e.*, generation of current at cell modified electrodes in the presence of a mediator, can be considered as an indicator of their metabolic activity. Nonliving cells are electrochemically inactive [1–3]. The changes in the redox activity of the cells mean that the electron transport in the biological system and the physiological state of living cells has been modified [4]. The electrochemical studies of the redox state of living cells might be useful for monitoring of different pathophysiological states of the cells, such as an oxidative and nutrient stresses, effect of cytotoxic, mutagenic, and carcinogenic preparations [4–6]. Many organic electroactive substances, *e.g.*, 2,6-dichlorophenolindophenol (DCIP), 2,4-benzoquinone (BQ) and other quinone derivatives, are hydrophobic enough and capable to permeate through the membrane and, thus, act as electron transfer mediators between intracellular redox processes and electrodes. Moreover, combination of mediators, *e.g.*, application of

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both organic and inorganic compounds together ($K_3[Fe(CN)_6]$ and BQ or DCIP), gives a significant increase of the electrochemical response [7]. All these single mediators and double mediators systems are often used in "cell electrochemistry" nowadays [2,4,5,7]. They collect electrons from intracellular reducing agents produced by metabolic reactions. Components of the respiratory chain and FAD containing dehydrogenases are the most active in the electron-exchange reactions with mediators [3,7,8]. The function of different mediator systems as shuttles between an electrode and different cells, such as yeast, bacteria, and mammalian, is well described in the literature [2,7–9].

The respiratory chain of the mitochondrial inner membrane includes a proton-pumping enzyme, complex I, which catalyses the electron transfer from NADH to ubiquinone pool [10,11]. The mitochondria also contain several nonproton-pumping alternative NAD(P)H dehydrogenases. As known these reactions are the firsts in the chain of electron transport. Thus, the detection of intracellular redox reactions using a mediator is based on its reduction by the cytosolic and mitochondrial enzymes and electron-carriers catalysing electron transfer from NADH and NAD(P)H along the electron transport system.

The goal of the present work was to elucidate the effect of the membrane permeability on the function of the electron transfer system and some dehydrogenases, as well as to clarify possible differences in the redox properties of intact, lyophilised, and permeabilised cells. Towards this end the amperometric monitoring of redox activity of intact, lyophilised, and permeabilised cells of different strains of the methylotrophic yeast *Hansenula polymorpha*, which differ in metabolic characteristics [12–14], was performed using two different electrochemical techniques and several redox mediators.

2. Experimental

2.1. Chemicals

K_2HPO_4, glucose, yeast extract, methanol, and ethanol were from "Reachim" (Moscow, Russia). Digitonin, $CaCl_2$, KCl, $MgSO_4$, $(NH_4)_2SO_4$, and KH_2PO_4 were from "Merck" (Darmstadt, Germany). Tris, paraformaldehyde, succinate, 2,6-dichlorophenolindophenol (DCIP), 2,4-benzoquinone (BQ), 1,2-naphthoquinone, $K_3[Fe(CN)_6]$, reduced and oxidised glutathione, NAD^+, $NADP^+$, NADH, and NADPH were from "Sigma" (St. Louis, MO, USA). Buffers were prepared using double-distilled water.

2.2. Materials and cells

The solution of formaldehyde (1 M) was prepared by hydrolysis of the corresponding amount of paraformaldehyde (300 mg; 10 ml water) in sealed test-tubes placed in water bath for 3 h at 100 °C.

The wild type and mutant strains of the thermotolerant methylotrophic yeast *H. polymorpha* constructed in Institute of Cell Biology (Lviv, Ukraine) were used, namely intact cells of the wild type strain *356*, mutant strain *KCA 33* (*gcr1 catX*), and permeabilised cells of a mutant strain *C-105* [13,14]. The strain *H. polymorpha KCA 33* has impairment in glucose catabolite repression of alcohol oxidase (AOX) synthesis, it is catalase-defective, and it has the ability to perform constitutive synthesis of AOX in a glucose-containing growth medium, in contrast to the wild type strain *356* unable to synthesise this enzyme in the presence of glucose.

2.3. Cultivation of the yeast H. polymorpha and preparation of lyophilised and permeabilised cells

Cells of *H. polymorpha* (any strain) were cultivated in flasks on shaker (200 rpm) at 30 °C to the middle of the exponential growth phase (~24 h) in the medium containing (g/L): glucose – 10; $(NH_4)_2SO_4$ – 3.5; KH_2PO_4 – 1.0; $MgSO_4$ – 0.5; $CaCl_2$ – 0.1; yeast extract – 3.0. The pH of the medium was 5.5.

Permeabilised cells of the *H. polymorpha C-105* yeast were prepared as described herewith [15]. Briefly, yeast cells at a concentration of 4–5 mg/ml were treated with 0.1% digitonin in 50 mM K-phosphate buffer, pH 7.0 for 15 min at 30 °C on shaking periodically. The cells were washed twice with the initial buffer, separated by centrifugation at 1000 rpm at 4 °C, lyophilised, and kept at −15 °C. Before each experiment lyophilised yeast cells were re-suspended in 0.1 M K-phosphate buffer, pH 7.0 at the concentration of 0.8 mg/ml and allowed to swell during 30 min at 37 °C. Just after the swelling the suspension of cells was used in the electrochemical experiments.

2.4. Amperometric studies

2.4.1. Measurements by using a glassy-carbon electrode pressed to the filtered cells

The electrochemical measurements were performed by using glassy-carbon electrode according to [3,4]. The suspension of cells of *H. polymorpha* (0.5 ml) was filtered through Schleicher & Schull filter (Germany, diameter 7 mm, with pore diameter 0.45 μm). The filter was placed on the bottom of the electrochemical cell and the working glassy-carbon electrode (Bioanalytical Systems, West Lafayette, IN, USA) recorded to investigate whether proofs of direct heterogeneous was lightly pressed to the filter together with the salt bridges of the $Ag/AgCl/KCl_{sat}$ reference electrode, and auxiliary platinum electrode. Then 0.5 ml of the K-phosphate buffer containing 0.2 mM mediator with or without cell's substrate (formaldehyde) was introduced to the yeast cell sample being on the filter. The potential of the electrode was measured by open circuit for 5 min using BAS CV-50W Electrochemical Analyser with BAS CV-50W software v. 2.1. Then the potential of 250 mV was applied and the current was recorded for