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Mediated amperometry reveals different modes of yeast responses to sugars

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ABSTRACT

Menadione-mediated amperometry at carbon paste electrodes modified with various yeasts (*Saccharomyces cerevisiae*, *Candida pulcherrima*, *Pichia guilliermondii* and *Debaryomyces hansenii*) was employed to monitor redox activity inside the yeast cells induced by glucose, fructose, sucrose, maltose or galactose. Continuous measurements revealed distinct modes (transient or gradually increasing) of the current development during the first 2 to 3 min after subjection to glucose, fructose and sucrose at electrodes containing *S. cerevisiae* and non-*Saccharomyces* strains. Different modes (increasing or decreasing) of the current development after yeast subjection to galactose at electrodes with *S. cerevisiae* or *D. hansenii* and at electrodes with *C. pulcherrima* and *P. guilliermondii* suggested different mechanisms of galactose assimilation.

1. Introduction

Yeasts are able to assimilate various substances such as sugars, amino acids, nitrogen compounds. Basic metabolic pathways of sugar assimilation for various yeasts are considered similar with differences in sugar transport systems, equipment of phosphorylation, intracellular localization of proteins, or in regulation of the pathways [1–6]. These differences result in different rates of carbohydrate consumption.

Electrochemistry provides the possibility of monitoring redox processes inside the yeast without cell disruption. To achieve the electron transfer between the electrode and redox centers of the enzymes in the cell, an electroactive mediator is added to the solution. The mediator shuttles the electrons between the electrode and redox centers in the enzymes. Glucose is one of the main nutrients for all yeasts. Glucose metabolism has been investigated amperometrically by employing platinum electrodes, yeast *Saccharomyces cerevisiae* and the double mediator system consisting of lipophilic menadione/menadiol (2-methyl-1,4-naphtalenedione/2-methyl-1,4-naphtalenediol) and hydrophilic ferricyanide/ferrocyanide [7–12]. Menadione can cross the cell membrane and enter the cytoplasm where it is reduced to menadiol by the cytosolic and mitochondrial enzymes catalyzing the electron transfer from NAD(P)H to quinone substrates. Menadiol can diffuse outside the cell and reduce ferricyanide to ferrocyanide. Ferrocyanide is then oxidized to ferricyanide at the electrode, thus, an anodic current is recorded. The direct electrochemical oxidation of menadiol at Pt electrode is a competing process, however, occurring at a lower rate than oxidation via ferricyanide and usually is not taken into consideration. In the presence of glucose, an increase in amperometric response is observed due to the additional formation of NAD(P)H in glycolytic and pentose phosphate pathways [8,10–12]. The magnitudes of the mediated glucose currents were considered to reflect glucose assimilation rates.

Our previous research employing carbon paste electrodes modified with yeasts showed that intracellular redox activity could be monitored by using a single lipophilic mediator — menadione [13]. The continuous measurement of menadione-mediated glucose currents at carbon paste electrodes modified with starving yeasts revealed two distinct modes in the development of the currents during the first 2 to 3 min after subjection to glucose. Initial transient currents and delay in the current development were characteristic of electrodes with tested *S. cerevisiae*. The currents at electrodes with tested non-*S. cerevisiae* strains were gradually increasing with no delay in "switching on" glucose assimilation. The values of glucose-induced currents correlated with the capacity of wild type yeasts to secrete various substances.

This work was aimed to investigate how electrodes modified with various yeasts responded to other sugars (fructose, sucrose, maltose, galactose). Fructose, sucrose, maltose are the main sugars in grape must, beer wort or bread dough and are readily consumed by yeasts. Yeasts are also able to assimilate galactose [14], a sugar of medical importance in the context of galactosemia, a disorder that results from a defect in the galactose metabolic pathway (the Leloir pathway) [15,16].

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2. Materials and methods

2.1. Yeasts

Two samples of commercial baker's yeasts (Uniferm, Germany and Lallemand, Poland, GBY and PBY, respectively) were obtained from markets (shelf life not less than 2 weeks as specified by the producer). *Candida pulcherrima* (*Metschnikowia pulcherrima*) was obtained from the CBS-KNAN Fungal Biodiversity Center, an Institute of the Royal Netherlands Academy of Arts and Sciences (Utrecht, The Netherlands).

Wild type (wt.) wine yeast *S. cerevisiae* Rom K100 wt. HM/HM (kill-K2) and *S. cerevisiae* M437 wt. HM/HM (kill-K2) were from the collection of Institute of Botany (Nature Research Center, Vilnius, Lithuania). *S. cerevisiae* N1M Kn wt., *Pichia guilliermondii* P179 wt. (K+) and *Debaryomyces hansenii* DRV3 wt. were isolated from the spontaneous fermentations of apples, sea-buckthorns and cherries, respectively, collected at different localities of Lithuania. The yeasts were isolated from fermentations that terminated within 7 days and possessed only one dominating yeast strain. The isolated yeasts varied in biocide and biostatic properties and in abilities to secrete low molecular weight glycoproteins (killer toxins) [17,18] which were lethal to other yeasts.

The identification of yeasts was performed using the automatised API 20C AUX (bioMerieux, France) system for clinical yeast identification and applying classical methods such as assimilation of sugars and other substances. *S. cerevisiae* N1M Kn wt strain was additionally identified by polymerase chain reaction according to the protocols described in [19]. The yeasts were grown on the YEPD medium containing 1% yeast extract, 2% peptone, 2% glucose and 2.5% agar as continuous lawns for 3 days at 30 °C until biomass stopped growing. The nutrients were exhausted during this time and the cells began to starve. Some strains were also grown on medium containing 2% galactose instead of glucose. The cells were tested microscopically to be sure that non-budding cells comprised 90–95%. Yeasts were stored in the fridge and used for electrode preparation within 7 days.

Levels of ethanol in spontaneous fermentations were determined by gas chromatography at the National Veterinary laboratory according to LS EN ISO/IEC requirements.

2.2. Chemicals

All chemicals were of analytical grade and were used without purification. Glucose and fructose were purchased from Fluka. Sucrose and maltose were from Reakhim (Russia). Galactose was from Applichem (Germany). Phosphate buffer was prepared from 0.1 mol/L KH_2PO_4 (Fluka) and contained 0.1 mol/L KCl (Fluka). The pH value was adjusted with KOH. Menadione (Sigma) solution was prepared in ethanol.

2.3. Electrode preparation

Plain carbon paste was prepared by mixing 100 mg of graphite powder (Merck) with 50 μL of paraffin oil (Fluka). The paste was packed into an electrode body consisting of a plastic tube (diameter 2.9 mm) and a copper wire serving as an electrode contact. The layers of the yeast cells on the surfaces of plain carbon paste electrodes were formed by dipping the electrode into the suspensions prepared from 40 mg of yeast in 0.5 mL of phosphate buffer at pH 6.5. This concentration of yeasts was considered as optimal since further increase of yeast amount resulted in lower currents due to slower diffusion of the mediator through a thick layer of cells. The electrodes were allowed to dry at the room temperature for 25–30 min and were then covered with a dialysis membrane (Aldrich-Sigma). Yeast suspensions were only used for 1 day. To repeat the experiments, new yeast suspensions for electrode modification were prepared.

To test the effect of pretreatment of yeast cells with galactose, yeast suspensions contained 1.5 mmol/L galactose additionally. These suspensions were allowed to stay for several days before electrode modification. All experiments were repeated at least 3 times.

2.4. Electrochemical measurements

Electrochemical experiments were carried out with a BAS-Epsilon Bioanalytical system (West Lafayette, USA) and a three-electrode cell arranged with a magnetic stirrer. The platinum wire and Ag/AgCl, 3 M NaCl served as counter- and reference electrodes, respectively. The modified carbon paste electrode served as a working electrode. The electrochemical measurements were performed immediately after the electrode preparation. Amperometry was carried out in a stirred solution at an operating potential 0.1 V (vs. Ag/AgCl, 3 M NaCl) in phosphate buffer at pH 6.5. The electrode was poised at an operating potential until the steady state of the background current was obtained. Thereafter, menadione was added (final concentration 67 μmol/L). After the new steady state of the current was established (25 to 40 min.), glucose-, fructose-, maltose-, sucrose- or galactose-containing solutions were added. For repetitive measurements with the same electrode, the electrode was taken out from the solution, rinsed with water and replaced in the phosphate buffer. The electrode was again poised at an operating potential until the steady state of the background was obtained. Menadione and sugar solutions were added successively again.

All measurements were carried out at the room temperature.

3. Results and discussion

Due to quasi-reversible menadiol/menadione redox process at the carbon paste electrode [13], ferricyanide was not necessary as the second mediator for the signal amplification. Besides, electrodes containing tested *D. hansenii* and *P. guilliermondii* also responded to glucose and fructose in the presence of ferricyanide only. The origin of these currents is obscure. Therefore, by using menadione only, additional ferricyanide-mediated currents were eliminated. Menadione is known to produce reactive oxygen species inside the yeast cells that can cause oxidative yeast damage [20,21]. To avoid detrimental effects on yeast cells, its concentration should not exceed 100 μmol/L [10,11]. Yeast-modified electrode responses to menadione started immediately after the injection and after some time approached their steady states. Upon injection of the definite amount of glucose, fructose, sucrose or maltose, the increases of currents were observed due to increased menadiol concentrations caused by the additional formation of NAD(P)H during glycolysis and pentose phosphate pathway. Gradually increasing currents were recorded at electrodes containing commercial baker's yeasts PBY and GBY and non-*S. cerevisiae* strains (*C. pulcherrima*, *P. guilliermondii*

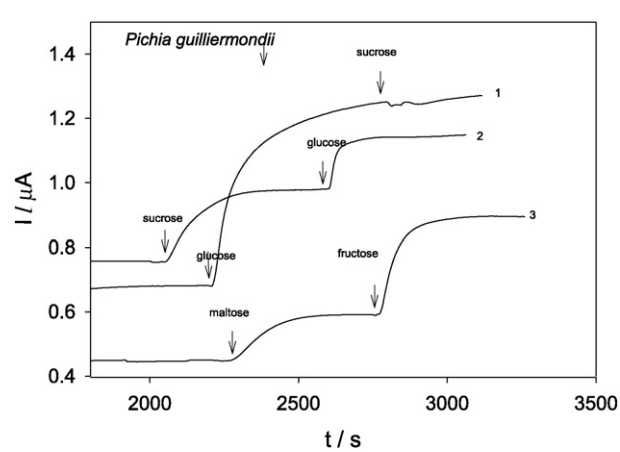

Fig. 1. Menadione-mediated current responses of electrodes containing yeast *P. guilliermondii* to additions of various sugars (as indicated) in phosphate buffer at pH 6.5, menadione concentration 67 μmol/L, glucose and fructose concentrations 10 mmol/L, sucrose and maltose concentrations 5 mmol/L, operating potential 0.1 V.