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Analytical Methods

A microbial biosensor based on *Lactobacillus delbruecki* sp. bacterial cells for simultaneous determination of lactic and pyruvic acid

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ABSTRACT

The aim of this study was mainly to develop a microbial biosensor for the simultaneous determination of lactic acid and pyruvic acid. In developing biosensor, lyophilised *Lactobacillus delbruecki* sp. bacterial cells were immobilised with polypyrrole on a platin electrode surface using electropolymerization method. Lactate concentration was determined based on the differences in amperometric responses at cathodic peak (+0.2 V) of potassium ferricyanide, whereas pyruvate concentration was determined using the differences at anodic peak (+0.1 V). The response of biosensor showed linearity between 0.1 and 1.0 mM for both of two substrates. Optimisation studies were carried out for amount of microorganism, pyrrole concentration, pH and temperature. In the characterisation studies, substrate specificity, interference effect of some substances on the biosensor response, and storage stability were established.

1. Introduction

In the development of biosensors biologically active materials such as enzymes, cells, plant and animal tissue have been mostly used. Because of their high specific activities and analyte sensitivities, enzymes are mostly used in the biosensor construction. But most of enzymes used in the microbial biosensor construction are unstable and cost affair for routine analysis of the specific substances (Akyılmaz & Dinçkaya, 2005). On the other hand, whole cell microbial biosensors have received recent attention; because enzyme purification is unnecessary, whole cell microbial sensors are simple and inexpensive systems to construct the biosensors and enzymes are usually more stable in their natural environment in the cell (Reshetilov et al., 1997). In addition their short responsive time makes them suitable for online and in time field monitoring (D'Souza, 2001; Lei, Chen, & Mulchandani, 2006).

Lactic acid is one of the most studied analytes since its concentration is an important parameter for many applications in food (Zaydan, Dion, & Boujtita, 2004) and beverages industry (De Luca et al., 2005), as well as clinical diagnostics (Burmeister, Palmer, & Gerhardt, 2005) and sports medicine (Volpe, Moscone, Compagnone, & Palleschi, 1995). Pyruvate is a key intermediate in the glycolytic and pyruvate dehydrogenase pathways, which are involved in biological energy production. It may also have cardiac and skeletal muscle inotropic activity as well as bariatric activity, and also antioxidant activity (Stanko, Tietze, & Arch, 1992). Pyruvate may help some obese individuals lose weight. There is also the suggestion in current research that it might help some overweight individuals lower their blood pressure, and it may favourably modify lipid profiles (Kalman, Colker, Wilets, Roufs, & Antonio, 1999; Stanko, Reynolds, Hoyson, Janosky, & Wolf, 1994). Many methods have been reported for lactate and pyruvate determination, such as chromatographic and spectrometric analysis (Barıskaner et al., 2003; Fernandes, Relva, Da Silva, & Freitas, 2003; Posner, Li, Bethell, Tsuji, & Benkovic, 1996; Wulkan, Verwers, Neele, & Mantel, 2001). However, these methods are relatively expensive, time consuming, complex to perform and require laborious sample pre-treatment. Thus, there is an increasing demand for inexpensive, rapid and reliable methods for lactate and pyruvate determination.

Lactate dehydrogenase (LDH) is an enzyme present in a wide variety of organisms, including plants and animals. It catalyses the interconversion of pyruvate and lactate with concomitant interconversion of NADH and NAD^+. Lactate biosensors can be based on two enzymes, either lactate oxidase or lactate dehydrogenase (Laval, Bourdillon, & Moiroux, 1984; Wang, Xu, & Chen, 2006; Yang, Atanasov, & Wilkins, 1999). On the other hand, there are only a few microbial biosensors for lactic acid based on baker's yeast *Saccharomyces cerevisiae* (Garjonyte & Malinauskas, 2003; Garjonyte, Melvydas, & Malinauskas, 2006, 2008) genetically modified *Hansenula polymorpha* yeast cells (Shkil et al., 2009), *Lactobacillus bulgaricus* and *Streptococcus thermophilus* mixed culture (Chen & Jin, 2011). There is not any study based on microbial biosensor for pyruvate determination.

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In this study, a novel microbial biosensor method has been developed based on *Lactobacillus delbruecki* bacteria for simultaneous determination of lactate and pyruvate. For this purpose, *L. delbruecki* was used as a biomaterial because it has either lactate or pyruvate metabolism and also lactate dehydrogenase enzyme, providing lactate–pyruvate conversion.

2. Experimental

2.1. Chemicals

Potassium ferricyanide, polypyrrole, glutaraldehyde (2.5%), sodium lactate, sodium pyruvate and all other chemicals were purchased from Sigma Chemical Co. (USA). All solutions used in the experiments were prepared just before their use.

2.2. Apparatus

In the experiments PalmSens potentiostat (Palm Instruments, Houten, Netherlands), a three-electrodes system from CH Instruments (USA) that contains a CHI 102 model platinum working electrode, a CHI 111 model Ag/AgCl reference electrode and a CHI 115 model platinum wire counter electrode, Gilson P100 and P1000 automatic pipettes (France), Yellow-Line magnetic stirrer (Germany) and Nuve model thermostat (Turkey) were used.

2.3. Culture medium of L. delbruecki cells

L. delbruecki is a gram-positive rod that may appear long and filamentous. This bacterium is regarded as aciduric or acidophilic, since it requires a low pH (around 4.6–5.4) to grow effectively (Axelsson, 1998).

L. delbruecki was grown in potato dextrose broth (PDB) at 30 °C for 18 h. The bacteria strain was cultured in 250 ml of erlenmeyer flasks containing of 50 ml fermentation medium (PDB) consisting of (g/l): 4 potato extract and 20 g dextrose, at pH 5.5. Lactic acid was added to the fermentation medium at the concentration of 10 μg mL^{-1} after membrane filter sterilisation. The erlenmeyer flasks were inoculated with 1% (v/v) of 18 h old *L. delbruecki* cells and flasks were kept on a rotary incubator shaker at 30 °C with agitation (1500 rpm) for 12 h. The culture medium contains agar extract (3.0 g L^{-1}), $(NH_4)_2SO_4$ (10.0 g L^{-1}), $MgSO_4{\cdot}7H_2O$ (0.5 g L^{-1}), $CaCl_2$ (0.1 g L^{-1}), peptone (0.5 g L^{-1}), KCl (1.0 g L^{-1}) and lactic acid (20 mg L^{-1}). After 12 h of fermentation period that is the log phase of cell growth, culture medium was centrifuged at 5000*g* for 15 min. The cells were resuspended with physiological saline water for two times and recentrifuged with the same condition. At the end of the process the cells of *L. delbruecki* were lyophilised and used for the preparation of amperometric microbial biosensor for simultaneous determination of lactic acid and pyruvic acid.

2.4. Preparation of the biosensor

Before the immobilisation of microorganism on the surface of the electrode, Pt electrode was polished with Gamma alumina 1.0 and 0.1 μm, respectively on microfiber cloth. Then Pt electrode was rinsed with double distilled water and it was sonicated to remove adsorbed particles first in ethanol solution (96%) and then in double distilled water for 10 min. After that, for the electrochemical cleaning the surface was cleaned by fifteen successive cyclic voltammetric sweeps between −1.0 and +1.0 V potentials in the 0.1 M HCl.

0.4 M pyrrole solution was prepared in 0.1 M KCl solution for the electropolymerization experiment. 2.5 mg microorganism, 300 μl (50 mM, pH: 5.5) citrate buffer and 30 μl 2.5% glutaraldehyde solution were added and mixed in an eppendorf tube. This mixture was added to the pyrrole solution. The triplet electrode system was submerged into this solution found in reaction cell and it was provided to occur a layer on the surface of the electrode with amperometric screening for 45 s at 0.7 V (Guerrieri, De Benedetto, Palmisano, & Zambonin, 1998).

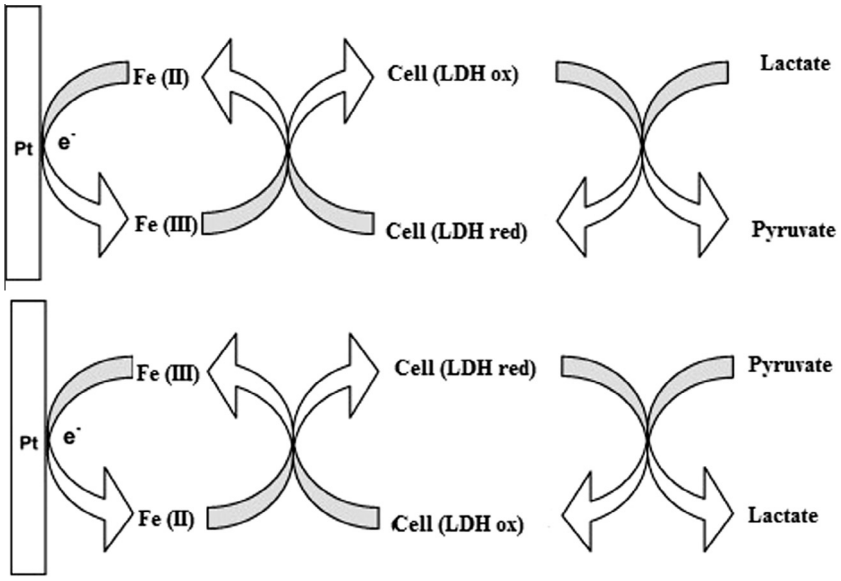
2.5. Principle of measurements

Principle of measurements was based on monitoring of amperometric responses which occurs during the reaction on the cathodic peak (+0.2 V) for determination of lactate and on the anodic peak (+0.1 V) for determination of pyruvate in the presence of potassium ferricyanide (1.0 mM) as a mediator (Scheme 1).

3. Results and discussion

3.1. Cyclic voltammograms and amperometric measurements

Cyclic voltammogram (CV) is an important method that could show electrochemical interaction and also differences. So, the bio-



Scheme 1. Suggested mechanism of the microbial biosensor.