Available online at www.sciencedirect.com
SCIENCE DIRECT
Sensors and Actuators B 107 (2005) 768–772
SENSORS AND ACTUATORS B CHEMICAL
www.elsevier.com/locate/snb

Development of a lactate biosensor based on conducting copolymer bound lactate oxidase

S. Suman [a], Rahul Singhal [b], Amit L. Sharma [b], B.D. Malthotra [b], C.S. Pundir [a,*]

[a] *Biochemistry Research Laboratory, Department of Bio-Sciences, M.D. University, Rohtak, Haryana 124001, India*
[b] *Biomolecular Electronics and Conducting Polymer Research Group, National Physical Laboratory, New Delhi 110012, India*

Received 17 July 2004; received in revised form 13 October 2004; accepted 9 December 2004
Available online 19 February 2005

Abstract

An amperometric enzyme electrode was developed for determination of lactate in serum. To prepare this electrode, commercial lactate oxidase from Pediococcus species has been immobilized through glutaraldehyde coupling onto polyaniline-co-fluoroaniline film deposited on an Indium tin oxide (ITO) coated glass plate. This plate acted as working electrode when combined with Pt electrode as counter electrode to the electrometer for the development of a biosensor. The method is based on generation of electrons from H_2O_2, which is formed from lactic acid by immobilized lactate oxidase. The concentration of lactic acid is directly proportional to the current measured. The enzyme electrode showed optimum response when operated at 42 °C in 0.05 M, sodium phosphate buffer pH 6.5 for 50 s. The biosensor showed a good performance with a linear response range from 0.1 to 5.5 mM/l. The minimum detection limit of the method is 0.1 mM/l and sensitivity of the sensor is 1.18 μA/mM/l lactate. This electrode was employed for determination of lactate in serum. The serum values in healthy and diseased persons were in the range 0.51–2.9 and 5.0–15.0 mM, respectively. The analytical recovery of added lactic acid was 71%. Within batch and between batch CV were <4 and <5%, respectively. Among the various serum substances tested only 8-hydroxyquinoline, urea, ammonium molybdate and uric acid caused 64, 38, 34 and 31% inhibition, respectively. The electrode was used 150 times over 26 days without any considerable loss of activity, when stored at 4 °C.

Keywords: Lactate; Lactate oxidase; Immobilization; Polyaniline-co-fluoroaniline; Lactate biosensor; Serum

1. Introduction

Lactate is a metabolite formed from pyruvate in muscles and liver due to inadequate supply of oxygen. Normal range of lactate in blood is 0.5–2.5 mM. The pattern of change or the trend towards an increase of blood lactate is a sensitive indicator of survival [1]. Lactate level gives an indication of the oxygenation state of tissues, warning of ischemic condition. Lactate sensor is mainly applied in critical cases, during surgical operation and intensive therapy. Lactate sensors may find application in sports, medicine and spatial medicine also [2,3]. Different biosensors for lactate monitoring are based on immobilized lactate monooxygenase (LMO) [4], lactate oxidase [5]. Bienzyme system such as LOD/LDH [6], cytochrome *b*2/LDH [7], GPT/LDH [8] have also been described. Recently, the use of conducting polymers in biosensor development has attracted significance attention. Trojanovicz et al. [9] have electrochemically immobilized LOD onto conducting polypyrrole films and Gros et al. [10] employed same film containing Fe(CN)63, as pseudo-reference electrode for amperometric biosensor. Chaubey et al. [11] immobilized LDH onto conducting PPY–PVS films via cross-linking by glutaraldehyde. These LDH/PPY–PVS electrodes showed linearity from 0.5 to 6 mM lactate

Abbreviations: CV, coefficient of variation; PPY, polypyrrole; PPY–PVS, polypyrrole–polyvinyl sulphonate; LOD, lactate oxidase; GPT, glutamate pyruvate transaminase; PGIMS, postgraduate institute of medical sciences; Poly(An-co-FAn), poly-aniline-co-fluoroaniline; DMGA, dimethylglutaric acid; DMA, *N*,*N*-dimethylaniline; DBS, dodecylbenzene-sulphonic acid

* Corresponding author. Tel.: +91 1262 272012; fax: +91 1262 294817.
E-mail address: pundircs@rediffmail.com (C.S. Pundir).

0925-4005/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.snb.2004.12.016

concentration. Although dehydrogenases are highly selective in many cases, as oxygen is not involved, the oxidation of coenzymes (NADH or NADPH) at higher voltage would reverse the action of LDH. Therefore LOD was preferred over LDH due to its simple reaction, which involves aerobic oxidation of lactic acid in to pyruvate and H_2O_2.

Haccoun et al. [12] modified conducting polymer poly(5-hydroxy-1,4-naphthoquinone-co-5-hydroxy-3-thioacetic acid-1,4-naphthoquinone) and used it for amperometric determination of lactate. However, these polymers were unsuitable for device fabrication either due to low conductivity, amorphous nature or large number of defects presents in the polymer [13].

The commercial exploitation of most of these conducting polymers for biosensor preparation, electronic displays, molecular electronics and opto-electronic devices is linked with their ease of processability. The processability of conducting polymers was enhanced either by making substitution into the aromatic nucleus or copolymerizing in such a way that there is variation in the torsion angle between adjacent phenyl rings of the polymer. Copolymerization is the easy and powerful method of making systematic changes in the polymer properties. Sharma et al. [14] synthesized a co-polymer poly(aniline-co-fluoroaniline)[poly(An-co-FAn)], which showed good electrical conductivity, thermal stability, high solubility in organic solvents, partial crystallinity and ability to produce free standing films. To the best of our knowledge no report is available for the immobilization of lactate oxidase onto this conducting copolymer.

The present paper describes the immobilization and characterization of LOD onto polyaniline-co-fluoroaniline films deposited onto indium tin oxide (ITO) coated glass plate, and its use for the detection of lactate in serum.

2. Experimental

2.1. Reagents and materials

Lactate oxidase (LOD, EC 1.1.3.2, 39 units/mg, from Pediococcus species), L(+) lactic acid, glutaraldehyde (Grade 1, 25% solution) from Sigma Chem. Co., USA. ITO (indium tin oxide) glass plates (Balzers), aniline and 2-fluoroaniline from Fluka (95% GC), the interferants used such as uric acid, urea, glycine, succinic acid, sodium dithiozite, L-ascorbic acid, ammonium molybdate and 8-hydroxyquinoline from SISCO Research Laboratory Pvt. Ltd., Mumbai were used. All other chemicals used were of analytical reagent grade. Amperometric measurements were conducted with a Keithley Electrometer (Model 617).

2.2. Preparation of lactic acid solution and reaction cocktail

Lactic acid was used as a substrate for lactate oxidase. Solutions of different concentration of lactic acid ranging from 0.1 to 8 mM were prepared and stored at 4 °C until use.

The reaction cocktail was prepared, as described by Lockridge et al. [15] and consisted of 200 mM Dimethylglutaric acid (DMGA) (2.0 ml), horseradish peroxidase (50 U/1.0 ml), 10 mM lactic acid (1.0 ml) and deionized water (3.0 ml). The reaction cocktail was stored in amber colored bottle at 4 °C until use. The reaction cocktail older than a week was discarded.

2.3. Assay of free lactate oxidase

The assay of native/free LOD was carried out, as described by Lockridge et al. [15]. The reaction mixture contained 0.80 ml reaction cocktail, 0.20 ml dimethylaniline (0.2% DMA). The contents were mixed well and equilibrated at 37 °C for 2 min followed by addition of 0.02 ml of dissolved enzyme. The contents were mixed again and preincubated at 37 °C for 2 min 2.0 ml of dodecylbenzene sulphonic acid (0.25% DBS) was added, reaction mixture was mixed and absorbance was recorded at 565 nm (A_{565}) against control in Spectronic-20 (Milton and Roy, USA) and the content of H_2O_2 generated in the reaction was calculated from standard curve between A_{565} versus H_2O_2 concentration.

2.4. Preparation of poly(An-co-FAn) film

A poly(aniline-co-fluoroaniline) {poly(An-co-FAn)} film was prepared electrochemically in potentiostatic mode at a potential of 1.0 V by 0.1 M aniline and fluoroaniline (1:1) in 1 M HCl using a three electrode system electrochemical interface: (Schlumberger SI 1286) [14]. Indium tin-oxide (ITO)-coated glass plate was used as working electrode, and a platinum plate and Ag/AgCl electrode were used as the counter and reference electrode, respectively. The area used for deposition of the films was about 1 cm^2. The time of electrodeposition was kept 5 min, as the thickness of the films depends on the total charge passed for copolymerization. The electrical conductivity of these films measured by the four-point-probe method was found to be about 10^{-1} S/cm.

2.5. Immobilization of lactate oxidase

Activation of poly(An-co-FAn) film. Poly(An-co-FAn) film was activated by adding 0.1 ml of 2.5% glutaraldehyde in 0.05 M sodium phosphate (pH 7.0). The film was kept for 2 h at room temperature for activation.

Immobilization of enzyme. 0.1 ml lactate oxidase (39 Units/mg) dissolved in 1 ml of 0.1 M sodium phosphate buffer, pH 7.0, was spread evenly onto glutaraldehyde activated poly(An-co-FAn) film and kept for 24 h at 4 °C for coupling.