Applied Surface Science 383 (2016) 283–293

Contents lists available at ScienceDirect

Applied Surface Science

journal homepage: www.elsevier.com/locate/apsusc

Self-assembled monolayers of 1-alkenes on oxidized platinum surfaces as platforms for immobilized enzymes for biosensing

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ARTICLE INFO

Article history:
Received 10 February 2016
Received in revised form 28 April 2016
Accepted 2 May 2016
Available online 4 May 2016

Keywords:
Self-assembled monolayers
Platinum
Enzyme immobilization
Lactate biosensor

ABSTRACT

Alkene-based self-assembled monolayers grafted on oxidized Pt surfaces were used as a scaffold to covalently immobilize oxidase enzymes, with the aim to develop an amperometric biosensor platform. NH_2-terminated organic layers were functionalized with either aldehyde (CHO) or N-hydroxysuccinimide (NHS) ester-derived groups, to provide anchoring points for enzyme immobilization. The functionalized Pt surfaces were characterized by X-ray photoelectron spectroscopy (XPS), static water contact angle (CA), infrared reflection absorption spectroscopy (IRRAS) and atomic force microscopy (AFM). Glucose oxidase (GOX) was covalently attached to the functionalized Pt electrodes, either with or without additional glutaraldehyde crosslinking. The responses of the acquired sensors to glucose concentrations ranging from 0.5 to 100 mM were monitored by chronoamperometry. Furthermore, lactate oxidase (LOX) and human hydroxyacid oxidase (HAOX) were successfully immobilized onto the PtOx surface platform. The performance of the resulting lactate sensors was investigated for lactate concentrations ranging from 0.05 to 20 mM. The successful attachment of active enzymes (GOX, LOX and HAOX) on Pt electrodes demonstrates that covalently functionalized PtOx surfaces provide a universal platform for the development of oxidase enzyme-based sensors.

1. Introduction

Self-assembled monolayers (SAMs) provide an excellent platform for the development of biosensors. The basic structure of the biosensor comprises a biological recognition element (typically enzymes, nucleic acids or antibodies) that interacts with the analyte, and a transducer which converts that interaction into a quantifiable electronic signal [1,2]. For better precision and reproducibility biosensors often require that the sensing element is immobilized on a solid substrate. SAMs enable the covalent and non-covalent attachment of biomolecules onto surfaces and provide a good control over the accessibility and orientation of the immobilized biomolecules [3,4]. SAMs can be formed on metal substrates to make them compatible with biosensor applications involving current or potential measurements [5]. However, the examples of SAMs on truly conductor materials are practically restricted to thiols on noble metals (Au, Ag, Pt or Pd) [6], and the thermal and hydrolytic stabilities of those SAMs are rather limited.

To overcome such limitations we have recently reported the functionalization of oxidized Pt (PtOx) substrates via the covalent attachment of L-alkenes [7]. In this process, Pt is first oxidized, and upon surface modification yields Pt—O—C linked monolayers. Pt and PtOx are appropriate materials to explore since their extensive use in catalysis, electrochemistry and biosensing [8]. Furthermore, they are compatible with silicon processing in micro and

Abbreviations: SAMs, self-assembled monolayers; RG, reactive group; GOX, glucose oxidase; LOX, lactate oxidase; HAOX, hydroxyacid oxidase; BSA, bovine serum albumin; NHS, *N*-hydroxysuccinimide; TFAAD, trifluoroacetyl-protected 10-amino-1-decene; TFA, trifluoroacetyl; TFEE, 2,2,2-trifluoroethyl undec-10-enoate; TFE, trifluoethyl ester; TAEA, tris-(2-aminoethyl)amine; DIPEA, *N,N*-diisopropylethylamine; DMF, *N,N*-dimethylformamide; DNA, deoxyribonucleic acid; FMN, flavin mononucleotide; SDS-PAGE, sodium dodecyl sulfate- polyacrylamide gel electrophoresis; BS3, bis(sulfosuccinimidyl) suberate sodium salt; DSC, *N,N'*-disuccinimidyl carbonate; HMF, hydroxymethylferrocene; DI, deionized; PBS, phosphate buffered saline; SSC, saline-sodium citrate buffer; GC, gas chromatography; HPLC, high-performance liquid chromatography; FPLC, fast protein liquid chromatography; IRRAS, infrared reflection absorption spectroscopy; XPS, X-ray photoelectron spectroscopy; UHV, ultrahigh vacuum; CA, contact angle; AFM, atomic force microscopy; QCM, quartz crystal microbalance.

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http://dx.doi.org/10.1016/j.apsusc.2016.05.006
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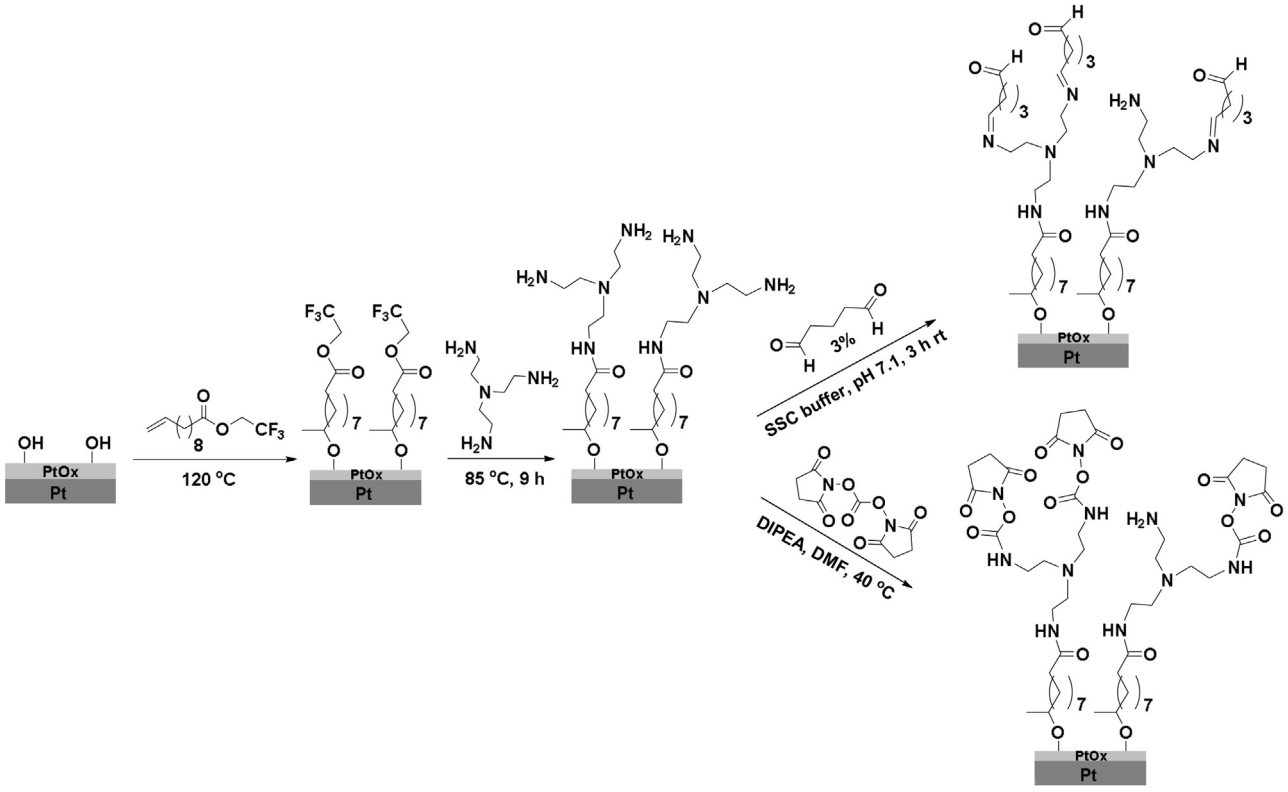

Scheme 1. Route for the functionalization of PtOx with aldehyde (CHO) or *N*-hydroxysuccinimide (NHS) ester groups.

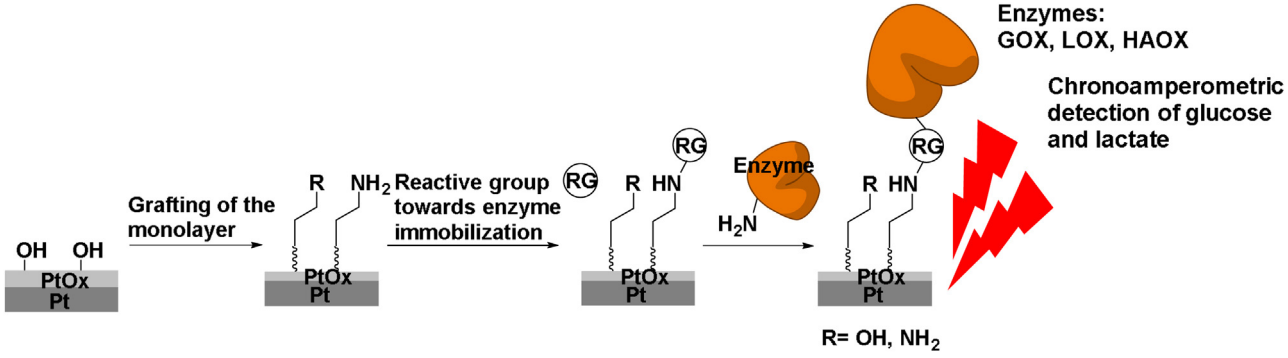

Fig. 1. Overview of the immobilization of enzymes on PtOx for the amperometric sensing of glucose and lactate. Note: RG = reactive group.

nanofabrication techniques, which is not the case for Au [9]. Herein we exploit the potential of this surface modification method for the immobilization of oxidase enzymes such as glucose oxidase (GOX), lactate oxidase (LOX) and human (S)-2-hydroxy-acid oxidase (HAOX) on functionalized Pt electrodes (Fig. 1). These enzymes were selected because their substrates, glucose and lactate, are important analytical targets in biomedical and industrial applications [10–12]. No assay is performed more frequently than that for glucose, as the global prevalence of diabetes approaches 10% among adults aged 18 years and above [13]. On the other hand lactate levels are used as biomarker for physical exercise and for several pathological conditions: cardiogenic shock, renal failure and tissue hypoxia [12].

The activity of immobilized enzymes was assessed by chronoamperometry according to the reactions depicted in Eqs. (1)–(3). First, glucose or lactate is oxidized enzymatically: glucose oxidase (GOX, Eq. (1)) catalyzes the oxidation of glucose to gluconolactone, while lactate oxidase (LOX, Eq. (2)) and (S)-2-hydroxy-acid oxidase (HAOX Eq. (2)) catalyze the conversion of lactate to pyruvate. The O_2 required in the oxidation process is reduced, resulting in the formation of hydrogen peroxide (H_2O_2). Finally, electrochemical oxidation (Eq. (3)) of the enzymatically produced H_2O_2 at these electrodes is used to generate a quantifiable electronic signal. Unlike for gold electrodes, which need an electron mediator to facilitate electron transfer from the enzyme to the electrode, H_2O_2 can be detected directly using Pt electrodes.

$$\beta\text{-D-glucose} + O_2 \rightarrow \text{D-glucono-1,5-lactone} + H_2O_2 \quad (1)$$

$$\text{L-lactate} + O_2 \rightarrow \text{pyruvate} + H_2O_2 \quad (2)$$

$$H_2O_2 \rightarrow O_2 + 2H^+ + 2e^- \quad (3)$$

In the current paper different enzyme coupling methods were tested. Electrode surfaces were characterized by static contact angle (CA), infrared reflection absorption spectroscopy (IRRAS), X-ray photoelectron spectroscopy (XPS) and atomic force microscopy (AFM). Our results show the successful immobilization of active oxidase enzymes (GOX, LOX and HAOX) on Pt electrodes. Moreover,