Electrochimica Acta 126 (2014) 132–138

Contents lists available at ScienceDirect

Electrochimica Acta

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

Induced-fit binding of laccase to gold and carbon electrodes for the biological fuel cell applications

Maciej Karaskiewicz[a,c], Dominika Majdecka[a,c], Agnieszka Wieckowska[a], Jan F. Biernat[b], Jerzy Rogalski[d], Renata Bilewicz[a,*]

[a] Faculty of Chemistry, University of Warsaw, Pasteura 1, 02-093 Warsaw, Poland
[b] Department of Chemical Technology, Gdansk University of Technology, Narutowicza 11/12, 80-233 Gdańsk, Poland
[c] College of Inter-Faculty Individual Studies in Mathematics and Natural Sciences, Banacha 2, 02-097 Warsaw, Poland
[d] Department of Biochemistry, Maria Curie Sklodowska University, Akademicka 19, 20-031 Lublin, Poland

ARTICLE INFO

Article history:
Received 27 May 2013
Received in revised form 1 August 2013
Accepted 3 August 2013
Available online 17 August 2013

Keywords:
Laccase
Biocathode
Carbon nanotubes
Fuel cell
Biofuel cell

ABSTRACT

Analogues of laccase natural substrates (syringic, veratric, ferulic, vanillic, isovanillic, 3,5-dimethoxybenzoic aldehydes) were employed to bind and orient laccase molecules in a way which facilitates adsorption of the catalyst molecules and their electrical connection with the conductive support. Laccase was bound efficiently to these substrates both on gold and carbon electrodes forming, respectively, 2D and 3D films sensitive to oxygen. Gold electrodes were used for the surface plasmon resonance evaluation of the extent of laccase binding. Glassy carbon electrodes covered with single-wall carbon nanotubes (SWCNTs) covalently modified with laccase were shown to lead to higher catalytic reduction currents than the bare carbon nanotubes deposited in a similar film at the electrode. SWCNTs with ferulic group chosen for the practical application of the biocathode in the air-zinc biobattery allowed to improve its performance both in terms of power and stability in time. The open circuit potential of the cell was 1.71 ± 0.05 V and the maximum power density achieved was 5.1 mW/cm^2 at 0.6 V.

1. Introduction

Laccase – a multicopper enzyme capable of oxidizing phenolic compounds reduces oxygen to water in a 4ē process [1–3]. The active site of laccase contains four copper ions divided into three groups distributed within the protein. The copper ion of the T1 site is located on one side of the protein, at the bottom of the hydrophobic cavity. The other three copper ions constitute T2 and T3 sites and are located about a nanometer (1.2 nm) from the T1 site. The substrate molecule is oxidized on T1 site and the electron is transferred to T2/T3 cluster, where the reduction of oxygen takes place [1–10]. Appropriately designed chemical groups can facilitate the contact between the T1 centre surrounded by the hydrophobic environment formed by different aminoacids, and the electrode, and provide the path for direct electron transfer to the T1 site [6–8]. A most suitable group would be one that consists of the natural substrates in the catalytic reactions involving laccase, which show the highest affinity towards the enzyme.

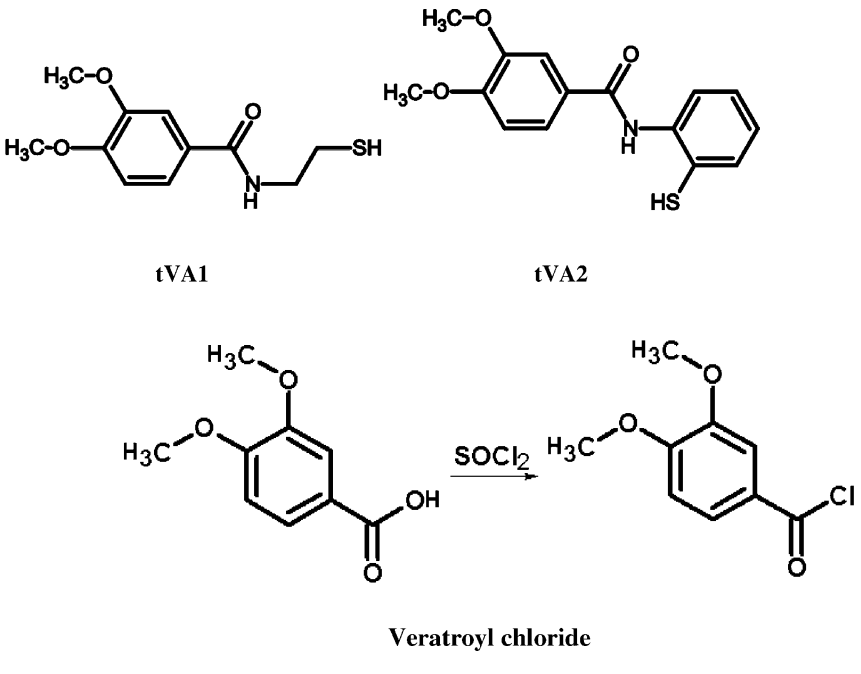
The aim of this study was to provide an induced-fit interaction between laccase and electrode surface in order to improve the communication between the active site of laccase and the electrode by using the natural substrates of laccase and their analogues. Surface plasmon resonance (SPR) is a convenient tool for the observation of the interactions between molecules in the real time [11–13]. The gold surfaces modified with thiolated compounds which mimic natural substrates for laccase were studied using SPR. While one type of the molecules is immobilized on the surface of the SPR sensor, the other one is in the solution flowing along the surface. The changes of the resonance angle depend on many factors including binding of biomolecules. In particular, they allow to determine the surface concentration of compound – in our case laccase, bound to the surface and relate it to the properties of the group attached to the electrode surface and responsible for addressing the enzyme.

The practical utility of the gold electrode covered with a monolayer of the thiol compound binding molecules of laccase is limited due to a small population of catalyst present on the surface [14,15].

The 3-dimensional network of modified carbon nanotubes on the electrode is able to accommodate and accumulate huge amount of catalyst [16–19]. In our recent papers [20–22, and citations therein] we demonstrated that arylated carbon nanotubes (phenylated, naphthylated or terphenylated) provide more efficient pathways for the transfer of electrons between the active centre of laccase and the conducting support. The groups bound to the nanotubes allow stable and durable adsorption of the enzyme and increase the amount of enzyme molecules in a suitable orientation for the exchange of electrons without the need of adding low

* Corresponding author. Tel.: +48 228220211; fax: +48 228225996.
E-mail address: bilewicz@chem.uw.edu.pl (R. Bilewicz).

0013-4686/$ – see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.electacta.2013.08.024



Scheme 1. Veratric acid thiolated derivatives.

molecular mass mediators. Natural substrates of laccase attached to these nanotubes should optimize the fit and improve the contact of laccase active site with the conductive support.

Formerly, we presented the synthesis, purification and characterization of arylated SWCNTs [22]. In the present paper, we describe the synthesis of SWCNTs equipped with laccase natural substrates that correspond to the lignin monomers. These lignin-related aromatic compounds can be produced in fungi during ligninolysis [23] as well as in de novo synthesis from glucose [24]. We prove that these materials assure accommodation of a larger amount of catalyst at the electrode. They retain the laccase binding feature of the arylated carbon nanotubes but are advantageous because they fit better to the cavity surrounding the T1 site of the enzyme as in the case of the natural laccase substrates employed in the enzymatic reactions in the solution.

2. Experimental

2.1. Materials and chemicals

All reagents were of analytical grade; aqueous solutions were prepared using Milli-Q water. SWCNTs and MWCNTs were from CheapTubes, Brattleboro, USA (purity > 90%); or from Nanocyl, Sambreville, Belgium.

Laccase *Cerrena unicolor* C-139 was obtained from the culture collection of the Regensburg University and deposited in the fungal collection of the Department of Biochemistry (Maria Curie-Sklodowska University, Poland) under the strain number 139. Laccase from the fermentor scale cultivation was obtained according to already reported procedure after ion exchange chromatography on DEAE-Sepharose (fast flow) and lyophilized on Labconco (Kansas City, USA, FreeZone 12 Lyophiliser) in clear stoppering chambers [25,26].

Laccase activity was measured spectrophotometrically with syringaldazine as the substrate. The protein content was determined according to Bradford with bovine albumin as the standard [27]. The concentration of isolated and frozen (−18 °C) enzyme was C_{lacc} = 178 μg/cm^3. After lyophilizing, the laccase activity in each vial containing 1 mg of protein after dilution in 1 ml of water was 256 U/ml.

2.2. Compounds used for the modification of gold and carbon electrodes

Chloride of 3,4-dimethoxybenzoic acid (veratroyl chloride) was obtained in the following way: 364 mg (2 mmol) of the acid was treated with thionyl chloride (2 ml) and 0.01 ml dry pyridine. The chloride dissolves immediately. The solution was kept at 50 °C for 4 h. Next, the volatiles were removed under reduced pressure and the solid residue was dissolved in 2 ml of dry toluene. The solvent and traces of thionyl chloride were removed in vacuum. Yield ~410 mg (100%), m.p. 68–72 °C (Scheme 1).

The thiolated derivative, tVA1 was synthesized by adding 200 mg (~1 mmole) of the above veratroyl chloride to solution of cysteamine hydrochloride (170 mg, 1.5 mmole) and pyridine (1 ml) in 5 ml of ice cooled water. The mixture was vigorously stirred maintaining the temperature close to 0 °C for 4 h. Then, the mixture was acidified and the product was extracted with ethyl acetate. The solid product obtained upon removal of the solvent was triturated with saturated sodium bicarbonate solution, filtered off and washed with water. Yield ~70%. Thiolated derivative tVA2 was obtained by reacting veratroyl chloride (200 mg) with 1.1 mmole of o-aminothiophenol in water and in the presence of pyridine as above. The product was isolated as in the previous experiment. Yield ~70%.

2-Aminoethyl side-functionalized SWCNTs (SWCNT-$(CH_2)_2$-NH_2) [22] were used for covalent immobilization of lignin monomers in form of N-acyl derivatives, Schiff bases and their reduction products.

The mixtures of SWCNT-$(CH_2)_2$-NH_2 and syringic, veratryl, vanillic, isovanillic, ferulic aldehydes or 3,5-dimethoxybenzaldehyde were sonicated for 4 h and during 8 hrs rotated in a rotator (Neolab, Heidelberg, Germany) at 10 rpm. Next, the samples were centrifuged at 10,000 × *g* force for 8 min, washed with MilliQ water and centrifuged under the same as above conditions. The reduction of the Schiff bases was done by adding $NaBH_4$ under 1 h rotation at 10 rpm. After centrifugation at