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pH-Sensitive syringaldazine modified carbon ceramic electrode for bioelectrocatalytic dioxygen reduction

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Abstract

Voltammetrically stable water insoluble 3,5-dimethoxy-4-hydroxybenzaldehydrazine (syringaldazine) modified electrode was obtained by sol–gel processing of methyltrimethoxysilane based sol with dissolved syringaldazine together with dispersed graphite particles. This silicate carbon ceramic electrode exhibits stable voltammetry corresponding to the surface confined oxidation–reduction process. The pH dependence of the immobilized syringaldazine redox potential obeys the Nernst equation in the range of 3–10. After further modification with extracellular lyophilized laccase from *Cerrena unicolor* encapsulated within a thin hydrophilic silicate film the electrode exhibits catalytic activity towards dioxygen reduction. Both catalytic current and potential are pH dependent.

Keywords: Laccase immobilization; Syringaldazine; Dioxygen reduction; Sol–gel; pH Sensing; Biofuel cell

1. Introduction

The preparation of laccase [1,2] modified electrodes is an important task, because of their potential application for oxidation of phenolic substrates [3,4] and electrocatalytic reduction of dioxygen [5–9] for biofuel cells [9–12].

The design of laccase based electrocatalytic system depends on the way electrons are exchanged between the enzyme and the electrode substrate. Numerous molecules serving as electron relay between the electrode and the active multicopper site of the enzyme were already examined [1,13]. One of them 3,5-dimethoxy-4-hydroxybenzaldehydazine (syringaldazine) is commonly used for laccase activity determination [14,15] and identification [17]. When dissolved in solution it is able to shuttle electrons between the oxidized laccase and the electrode [8,18].

Immobilization of the mediator on the electrode is essential for the preparation of an efficient and stable biocatalytic system. A close contact of the enzyme and mediator is a prerequisite for efficient electron transfer and electrocatalysis. For bioelectrocatalytic reduction of dioxygen the reduction of fully oxidized four Cu atoms in laccase is the slowest reaction in the catalytic cycle [19,20]. In its first step the oxidized mediator is produced by electron transfer to Cu(II) at the T1 center [9]. Therefore a close contact between the mediator and the T1 copper center of laccase has to be provided. Next electrons are shuttled to T3/T2 trinuclei centers to reduce the remaining part of the enzyme. This trinuclear center is the binding site of molecular oxygen and the site of its catalytic reduction to H_2O [9]. The catalytic reaction requires re-oxidation of fully reduced Cu(I) atoms in the enzyme to regenerate the enzyme.

In this present paper, we apply a simple procedure for the non covalent immobilization of syringaldazine and laccase. As previously proposed [21] the mediator is immobilized into a hydrophobic porous silicate–carbon heterogeneous structure [22,23] of carbon ceramic electrode (CCE) [24]. Such a material allows for stable confinement of organic molecules by their addition to the sol [24]. If the mediator is almost insoluble in water and both

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substrate and product of the electrochemical reaction are neutral, the voltammetrically stable behaviour is expected.

In the next step, laccase is immobilized in the hydrophilic silicate thin film deposited on the syringaldazine modified CCE (Syr-CCE) surface. It is already known that encapsulation of protein in a sol–gel processed silicate matrix does not much affect its properties [25]. Here it prevents laccase leaching to the surrounding solution [6,7,10,20] ensuring contact with the mediator present within a heterogeneous matrix. Additionally, the sol–gel processing of silicate films on the top of a silicate based heterogeneous material of CCE allows for strong bonding between both parts of the electrode.

In order to evaluate Syr-CCE electrochemical properties cyclic voltammetry, differential pulse voltammetry and chronoamperometry were performed at different pHs, because of expected participation of protons in the electrode reaction [8]. After immobilization of laccase within the silicate layer experiments were conducted in a dioxygen saturated solution at different pHs. This is because of the pH dependent activity of fungal laccase [26] and the interest in biocathode performance at neutral pH [27].

2. Experimental

2.1. Chemicals and materials

Tetramethoxysilane (TMOS) (99+%), methyltrimethoxysilane (MTMOS) (98%) and syringaldazine (99%) were purchased from Aldrich. Graphite powder (MO-300, average particle size 20 μm) was purchased from carbon GmbH, ethanol (96%), H_3PO_4 (85%) and NaOH were from Chempur. HCl (35%) was from POCH. H_3PO_4 and NaOH were used to prepare 0.1 mol dm^{-3} phosphate buffers of pH 3–10. All solutions were prepared with water purified by a Milipore-ELIX system (>15 MΩ cm). Oxygen (99.9%) was purchased from Multax.

Laccase was obtained from *Cerrena unicolor* fungus according to an already reported procedure [28,29]. This protein was purified by ion exchange chromatography on DEAE Sepharose (fast flow) and concentrated on a Pellicon 2 ultrafiltration semiprep cell (10 kDa cut off). Then laccase was distributed into vial and lyophilized in Labconco FreeZone 12. Each vials contained 1.4 mg of protein and 3×10^6 nkat of laccase. The enzyme activity was determined following the oxidation of 0.025 mM syringaldazine in 0.1 mol dm^{-3} citrate–phosphate buffer at pH 5.0 [14]. The oxidation of this compound was monitored by the increase in the A_{525} ($\varepsilon_{525} = 6.5 \times 10^4$ M^{-1} cm^{-1}) at 25 °C. The protein content was determined with bovine albumin as standard [15].

2.2. Procedures

The hydrophobic sol–gel matrix for CCE was prepared as described elsewhere [24]. The hydrolyzed sol was obtained by mixing 1 ml of MTMOS with 1.5 ml of methanol. For Syr-CCE preparation 78 mg of syringaldazine was added to the sample. After addition of 50 μl of 11 mol dm^{-3} HCl it was sonicated for 2 min. Next, 1.25 g of graphite powder was added and the obtained mash was sonicated for another minute. Next, it was immediately placed into a 2 mm deep cavity of a 1.55 mm inner diameter glass tubing filled tightly with a copper wire. The electrode was left for drying at room temperature for at least 48 h and then polished with emery paper. The geometric surface area of the exposed disc is 0.019 cm^2. However, the electrode surface is heterogeneous consisting of graphite and hydrophobic silicate parts as judged by optical microscopy (Fig. 1) (see also Refs. [22,23]).

For hydrophilic gel (TMOS$_{gel}$) film TMOS, H_2O and 0.04 mol dm^{-3} aqueous HCl were mixed in a volume ratio 18:4.5:1. This mixture was sonicated for 20 min and then mixed with water, in a volume ratio 1:1. This stock sol was sonicated for 3 min, diluted with water in a 1:100 volume ratio and sonicated for another 3 min. Finally, 145 μg of laccase was added to 250 μl diluted stock sol [7,26,30]. Ten microliters of 1:100 diluted sol was placed on the Syr-CCE surface. For sol–gel processing and drying these electrodes were left for at least 20 h at room temperature, at an air humidity equal to 40–50%.

2.3. Instrumentation and procedures

For visualization of the electrode surface a Nikon Eclipse LV150 microscope was used. Electrochemical experiments were done with an Autolab PGSTAT30 (EcoChemie) electrochemical system in a conventional three-electrode cell with dedicated software. The modified CCE was used as working electrodes. Ag/AgCl/KCl (saturated) electrode and Au wire served as reference and counter electrode respectively. These experiments were performed in 0.1 mol dm^{-3} phosphate buffers. Their pH was checked using CG 837 pH-meter (SCHOTT). Most

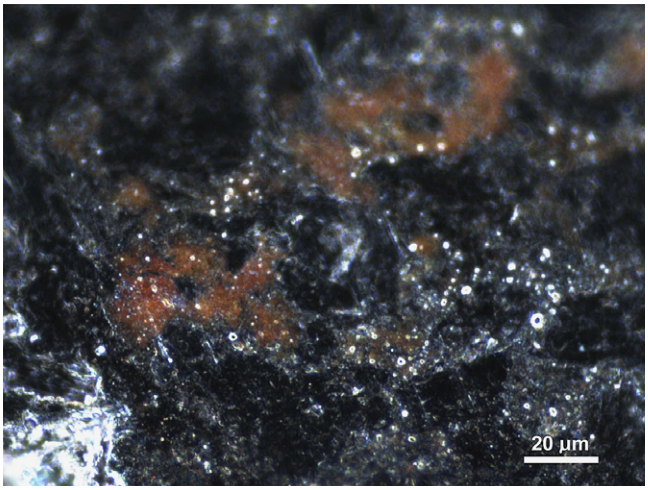

Fig. 1. Optical micrograph of syringaldazine modified CCE surface. Red islands correspond to syringaldazine deposit.