Biosensors and Bioelectronics 50 (2013) 186–193

Contents lists available at SciVerse ScienceDirect

Biosensors and Bioelectronics

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

Fabrication of High performance bioanode based on fruitful association of dendrimer and carbon nanotube used for design O_2/glucose membrane-less biofuel cell with improved bilirubine oxidase biocathode

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ARTICLE INFO

Article history:
Received 8 March 2013
Received in revised form
23 May 2013
Accepted 29 May 2013
Available online 24 June 2013

Keywords:
Dendrimer
Bilirubine oxidase
Glucose dehydrogenase
Safranin O
MWCNTs
Biofuel cell
Membraneless

ABSTRACT

In this study, the preparation of an integrated modified electrode based on the covalent attachment of glucose dehydrogenase (GDH) enzyme and safranin O to amine-derivative multiwalled carbon nanotubes (MWCNTs-NH_2) modified glassy carbon (GC) electrode using G2.5-carboxylated PAMAM dendrimer (Den) as linking agent is reported. The obtained results indicated that the proposed system has effective bioelectrocatalytic activity toward glucose oxidation at 100 mV with onset potential of −130 mV (vs. Ag/AgCl). The performance of the prepared hybrid system of GC/MWCNTs-NH_2/Den/GDH/Safranin as anode in a membraneless enzyme-based glucose/O_2 biofuel cell is further evaluated. The biocathode in this system was composed of bilirubin oxidase (BOX) enzyme immobilized onto a bilirubin modified carbon nanotube GC electrode. Immobilized BOX onto CNTs/bilirubin not only show direct electron transfer but also it has excellent electrocatalytic activity toward oxygen reduction at a positive potential of 610 mV. The open circuit voltage of the cell was 590 mV. The maximum current density was 0.5 mA cm^{-2}, while maximum power density of 108 μW cm^{-2} was achieved at voltage of 330 mV. The immobilized enzymes in anode and cathode are very stable and output power of the BFC is approximately constant after 12 h continues operation.

1. Introduction

Major research efforts have been recently directed at utilizing the biocatalytic electron transfer functions of enzymes to assemble biofuel cells (BFCs). BFCs are a type of energy conversion devices that use biocatalysts (either complete living cells or enzymes) to convert the chemical energy of a fuel into electrical energy. The typical fuels employed in BFCs have been glucose, methanol, ethanol, and lactate. BFCs are usually classified on the basis of biocatalyst employed which contains three types of microbes, organelles, and enzymes. Each type of these BFCs has their own advantages and disadvantages, but considerable interest has focused on enzymatic biofuel cells (EBFCs). However, EBFCs have a short lifetime, low current and power density, which are related to enzyme instability, electron transfer kinetics on the electrode surface, enzyme loading and inefficient electron conduction.

Different methods have been developed to electrically communicate of enzymes with electrodes including employment of diffusional electron transfer mediators (Brito and Turner, 2010; Willner et al., 2009b; Yu and Scott, 2010), the functionalization of enzymes with electron relays (Schuhmann et al., 1991; Willner et al., 1994) and the immobilization of enzymes in redox polymers (Beyl et al., 2011; Heller et al., 2006, 2004, 2003, 2002, 2001). Also, the alignment of redox enzymes on electrodes by the reconstitution of apo-enzymes on relay-cofactor monolayer modified electrodes led to effectively wired enzyme electrodes that were unaffected by environmental interfering reagents (Willner et al., 2004, 2005). The effective electrical wiring of the enzymes with electrodes prevents the interfering biocatalytic oxidation of the fuel substrate by oxygen, thus enabling the assembly of membraneless, noncompartmentalized biofuel cells (Willner et al., 2011, 2007; Gorton et al., 2008). Such biofuel cell elements can be used as implants that generate electrical power from living organisms (Mano. et al., 2003; Katz. et al., 2012; Scherson et al., 2012).

In recent years, a variety of nanoengineered materials such as oligoaniline crosslinked nanocomposites (Willner et al., 2009a, 2011), graphene oxide/metal hydroxide/chitosan nanocomposite

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http://dx.doi.org/10.1016/j.bios.2013.05.047

(Lee et al., 2013), carbon nanotubes (CNTs)(Holzinger et al., 2012) and CNTs forest (Miyake et al., 2011) have been developed for effective electrically communicates of enzymes with electrodes and BFCs design.

Dendrimers (Den) are monodisperse synthetic polymers with a regular and highly branched three-dimensional structure. The most important characteristics of dendrimers compared with other polymers are their greater uniformity and highly functionalized surface, which allows for the use of different techniques for enzyme immobilization, such as multilayer adsorption (Villalonga et al., 2012), hydrophobic or hydrophobic/ionic interaction between enzyme and electrode surface (Forti et al., 2011), self-assembly methodology (Aquino Neto et al., 2013) and covalent immobilization (Aminur Rahman et al., 2008). So, the combination between two "hot" topics of nanotechnologies: Den and CNTs, can give rise to many exciting material properties, finding applications in many fields. As a result of this association, enzyme loading increased remarkably because of the multiplicity of functional groups on the Den surface. On the other hand, CNTs can be effectively used for wiring the enzyme molecules to the electrode surfaces and fabrication of bioanodes and biocathodes with high performance and stability.

Bilirubin oxidase from blue multi copper oxidizes (BMCO) is highly active and efficient for oxygen reduction. BOX adsorbed on CNTs has been used as cathode in many constructed BFCs (Willner et al., 2007, 2011). T1 center located in a hydrophilic substrate-binding pocket and enzyme molecules exhibits affinity towards appropriately hydrophilic moieties, these groups may orient BOX on the electrode in a way that favors direct electron transfer (Blanford et al., 2011; Tominaga et al., 2008)

Dehydrogenase enzymes are dioxygen insensitive enzymes that catalyze the oxidation of corresponding fuels with the accompanying reduction of cofactor NAD^+ to nicotinamide adenine dinucleotide (NADH). For continuous reaction, the enzymatically produced NADH needs to be oxidized. Since the oxidation of NADH requires a large overvoltage on a conventional electrode, typically diaphorase and/or a mediator have been coupled with the NAD^+/dehydrogenase systems (Ohsaka et al., 2012; Salimi et al., 2012; Mao et al., 2011). Different redox mediators can be used for this purpose (Luz et al., 2008; Salimi et al., 2005, 2010; Manesh et al., 2008). Different redox-active organic dyes are known to act as active electrocatalysts for the oxidation of NADH and the regeneration of the NAD^+ cofactors (Willner et al., 2007; Saleh et al., 2011a, 2011b). Safranin O is one of the phenazine dyes that show excellent electrocatalytic activity for NADH oxidation (Ohsaka et al., 2012). It can generate flavin-type moiety which can effectively perform as a biological catalyst for the purpose of NADH/NAD^+ regeneration.

In the present study, we tried to combine the advantages of Den and CNTs for construction of an integrated modified electrode as bioanode. Safranine usually binds to CNTs through π–π interactions and electropolymerization (Saleh et al., 2011a, 2011b). For improving the electrocatalytic activity of safranine molecules, we used dendrimer with large number of functional groups for covalent attachment of GDH and safranin O on the electrode surface. GDH as oxygen insensetive enzyme was effectively used in fabrication of bioanode. The resulting integrated modified electrode showed excellent electrocatalytic activity toward glucose oxidation. Furthermore, the GC/MWCNTs-NH_2/Den/GDH/Safranin electrode was successfully applied as the bioanode of a glucose/O_2 biofuel cell. The biocathode consisted of the BOX immobilized onto GC electrode modified with MWCNTs and bilirubin. MWCNTs/bilirubin nanocomposite provided a more hydrophilic microenvironment suitable for direct electron transfer of immobilized BOX. The proposed bioanode and biocathode have been assembled to construct an efficient BFC. The performance of BFC was evaluated by connecting external resistances between bioanode and biocathode.

2. Experimental

2.1. Chemicals and reagents

NAD^+ (disodium hydrate), NADH, glucose dehydrogenase from Pseudomonas sp (GDH, EC number 1.1.1.47) and bilirubin oxidase from Myrothecium verrucaria (BOX) (EC number 1.3.3.5), G2.5 PAMAM dendrimer-COO^-Na^+ (Den) 10% in methanol, D-glucose (anhydrous 96%) N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC), uric acid (UA), dopamine (DA), acetaminophen (AP), NaH_2PO_4 and Na_2HPO_4 were prepared from Sigma. Multiwalled carbon nanotubes (MWCNTs) with purity of 95%, surface specific area of 480 $m^2\ g^{-1}$, diameter of 20–30 nm and 1 μM length were obtained from Nanolab (Brighton, MA).

2.2. Apparatus and electrochemical measurements

Cyclic voltammetry (CV) was performed on an AUTOLAB modular electrochemical system (ECO Chemie, Ultrecht, The Netherlands) equipped with a PGSTAT 101 module and driven by GPES (ECO Chemie) in conjunction with a conventional three electrode system and a personal computer for data storage and processing. A GC electrode ($A=0.0314\ cm^2$) employed as the working electrode and a platinum wire as the counter electrode. The effective surface area of GC/MWCNTs-NH_2/Den and GC/MWCNTs-bilirubin modified electrodes were 0.08 and 0.0314 cm^2 from cyclic voltammogram of 1 mM $K_3[Fe(CN)_6]$ in 0.1 mol L^{-1} PBS (pH 7). All potentials were referred to an Ag/AgCl/KCl (3 M) electrode. In order to construct the BFC, the BOX/Bilirubin/CNTs/GC electrode as cathode and the GC/MWCNTs-NH_2/Den/GDH/Safranin electrode as anode were placed into a 4 mL saturated oxygen of phosphate buffer solution (PBS) (pH=6) containing 50 mM glucose and 10 mM of NAD^+. The effect of pH and temperature on cathodic and anodic current response was investigated (Supplementary information). The biofuel discharge (polarization) curves were recorded under O_2 at variable external resistances (1 kΩ–2 MΩ) by using an electrometer.

2.3. Fabrication of bioanode

At first, 3 μL of MWCNT-NH_2 dispersed in DMF solution(1 mg/mL) was cast on the GC surface and air dried to form a MWCNT-NH_2 film. A 5 μL of Den was allowed to dry under N_2 stream and then diluted to 100 μL with PBS (pH=7). 5 μL of the prepared Den solution mixed with 5 μL of 30 mM EDC, 6 μL of which cast onto the MWCNT-NH_2 modified electrode surface. After 12 h, this modified electrode rinsed with distilled water to remove unbound Den and excess EDC. In order to covalent attachment of safranin O molecules, 10 μL PBS (pH 7) containing 10 mM safranin O was further cast on the modified electrode for 6 h. The as-prepared modified electrode was washed with distilled water and denoted as GC/MWCNT-NH_2/Den/Safranin. Subsequently, this modified electrode incubated with GDH solution in PBS (pH=7) for 24 h to fabricate GC/MWCNTs-NH_2/Den/GDH/Safranin bioanode. The bioanode fabrication procedure is schematically shown in Fig. S1 (Supporting information, SI).

2.4. Preparation of biocathode

For formation of MWCNTs film on the electrode surface, 3 μL of MWCNTs dispersed in DMF solution (1 mg/mL) was cast onto GC electrode. 4 μL aliquot of bilirubin solution (3 mM, dissolved in