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Short communication

Laccase electrodes based on the combination of single-walled carbon nanotubes and redox layered double hydroxides: Towards the development of biocathode for biofuel cells

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ABSTRACT

Single-walled carbon nanotubes (SWCNT) were combined with layered double hydroxides (LDH) intercalated with 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonate) diammonium salt [ZnCr-ABTS] to entrap and electrically connect laccase enzyme. The resulting laccase electrodes exhibited an electro-enzymatic activity for O_2 reduction. To improve this electrocatalytic activity, varying SWCNT quantities and loading methods were tested to optimize the configuration of the laccase electrodes. Furthermore, the resulting bioelectrode was successfully used as a biocathode for the elaboration of a membrane-less glucose/air biofuel cell. In 0.1 M phosphate buffer (PBS) of pH 6.0, containing glucose (5 mM) under ambient conditions, the assembled biofuel cell yielded a maximum power density of 18 $\mu W\,cm^{-2}$ at a cell voltage of 0.3 V whereas this power decreased to 8.3 $\mu W\,cm^{-2}$ for a biofuel cell based on the identical biocathode setup without SWCNT.

1. Introduction

Laccase electrodes have aroused a considerable attention as biocathode for the development of biofuel cells [1–7]. Laccase catalyses the four-electron reduction of oxygen directly to water without intermediate formation of hydrogen peroxide by oxidizing a variety of mediators like 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonate) diammonium salt (ABTS). Among the various procedures for immobilizing and electrical connecting of laccase, a faster, simpler and cheaper approach consists in the use of anionic redox clays like layered double hydroxides (LDH), intercalated by ABTS anions ($Zn_2Cr(OH)_6ABTS_{1/2}$) as host matrix [8]. However, one of the limitations of common enzyme-clay modified electrodes resides in the non-conductive nature of these inorganic lamellar particles. For instance, the electron transfer within redox LDH (denoted as [ZnCr-ABTS]) occurs only by an electron hopping mechanism involving a small amount of accessible intercalated anions (i.e. ABTS), located at the edge sites of the crystallites and at the proximity of a conductive surface. The charge neutralization is determined by a diffusion process of electrolyte ions through channels in between the LDH platelets [9,10].

With the aim to enhance charge transport within the redox LDH coating and electrical communication with immobilized laccase, an original approach consists in the intercalation of single-walled carbon nanotubes (SWCNT) within the laccase-[ZnCr-ABTS] LDH biofilm. In this context, we report here an easy and fast procedure for the construction of SWCNT-laccase-[ZnCr-ABTS] coatings based on the electrostatic interactions between SWCNT and [ZnCr-ABTS] particles. For this purpose, carbon nanotubes were chemically oxidized for generating hydroxyls and carboxylic groups on the nanotube sidewall. This functionalization allows the dispersion of SWCNTs in aqueous media and leads to negatively charged SWCNTs interacting with the positively charged LDH particles. Thus, glassy carbon electrodes covered by laccase, bovine serum albumin and [ZnCr-ABTS] with and without SWCNT were prepared and characterized in the presence of oxygen. These modified electrodes were then successfully used as a biocathode for elaborating a membrane-less glucose/air biofuel cell and its performance was investigated.

2. Experimental

2.1. Materials and chemicals

Laccase (EC 1.10.3.2 from *T. versicolor*, 26 U mg^{-1}) and glutaraldehyde (25%) were purchased from Fluka. Bovin serum

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albumin (BSA), glucose oxidase (GOX) (EC 1.1.3.4, from *Aspergillus niger*, 110 units mg^{-1}), ferrocene (Fc) and graphite powder were obtained from Sigma. Layered double hydroxides (LDH) $Zn_2Cr(OH)_6ABTS_{1/2}$, denoted as [ZnCr-ABTS] was synthesized by a co-precipitation method [9]. Single-walled carbon nanotubes (SWCNTs) produced by arc discharge and supplied by Nanoledge SA, were oxidized by refluxing in 69% nitric acid (10.8 M) at 110 °C for 3 h. After the reaction mixture was cooled down to room temperature and the solid was settled out, the acidic supernatant was decanted off and the residue was diluted with distilled water. This suspension was filtered over cellulose membrane (pore size 0.45 μm) and rinsed until the filtrate reaches a neutral pH value. The resulting solid was re-dispersed in water with ultra sound and insoluble particles were allowed to sediment. The obtained black solution was again filtered over a cellulose membrane filter and the obtained oxidized SWCNTs were dried at 70 °C. Such chemically modified nanotubes can be dispersed in distilled water up to concentrations around 0.5 mg ml^{-1}.

All other chemical reagents were of analytical grade. Double-distilled water was used for aqueous solution. Phosphate buffer solution was prepared by K_2HPO_4 and KH_2PO_4. Stock solutions of glucose were allowed to mutarotate at room temperature for 24 h before use and were kept refrigerated.

2.2. Instruments

The electrochemical characterization and the biofuel cell tests were performed with an Autolab potentiostat 100 (Eco Chemie, Utrecht, The Netherlands). All electrochemical experiments were carried out in a conventional three-electrode cell except for the biofuel cell experiments. A Pt wire, placed in a separate compartment containing the supporting electrolyte and a saturated calomel electrode (SCE) were used as counter electrode and reference electrode respectively, while the working electrodes were glassy carbon electrode (diameter 3 mm).

2.3. Enzyme immobilization and biocathode construction

The [ZnCr-ABTS] colloidal suspension (4 mg ml^{-1}) was prepared by dispersing LDH in deionized and decarbonated water and stirred overnight. A defined amount of an aqueous mixture of [ZnCr-ABTS] (20 μg), laccase (28 μg), BSA (12 μg) and 5–10 μg of SWCNT was directly adsorbed on a glassy carbon electrode. This method is qualified as mixed method and noted laccase-composite electrode. The second procedure consists in modifying glassy carbon electrode first by varying the amount (5–20 μg) of the SWCNT coating by spreading and drying in air an aqueous solution of SWCNTs (0.5 mg ml^{-1}) onto the electrode surface. Then, an aqueous mixture containing [ZnCr-ABTS] (20 μg), laccase (28 μg) and BSA (12 μg) was spread and dried on the SWCNT-modified electrode. This 'separated-method' called electrode configuration, is denoted as laccase-LDH/SWCNT electrode. The resulting electrodes were placed in saturated-glutaraldehyde vapor for 1 h in order to induce chemical cross-linking of the entrapped proteins. Before use, the biocathodes were rinsed for 20 min in stirred 0.1 M phosphate buffer (pH 6) to remove not firmly immobilized enzymes.

2.4. Preparation of glucose/air biofuel cell

A bioanode was prepared by mechanical compression of a mixture of graphite particles (275 mg), glucose oxidase (10 mg), ferrocene (15 mg) and glycerol (45 μL) at 10,000 kg cm^{-2} to form a compact graphite disc (diameter 1.33 cm) following a described procedure [11]. This bioanode was associated to laccase-LDH or laccase-LDH/SWCNT electrodes as biocathodes to elaborate a compartmentless glucose/air biofuel cell.

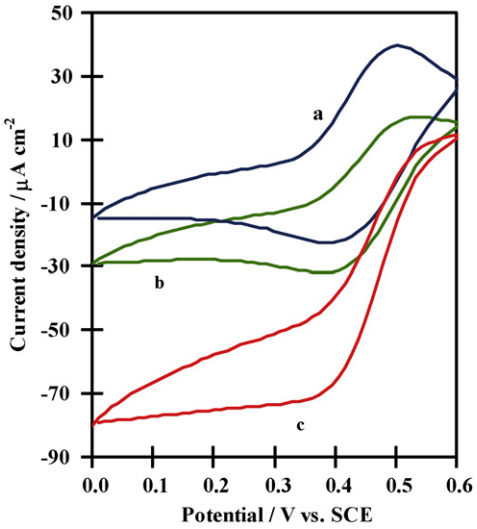

Fig. 1. Cyclic voltammograms recorded at a laccase-LDH/SWCNT electrode in 0.1 M PBS (pH 6) under argon (a), ambient air (b) and oxygen (c), $\nu = 10$ mV s^{-1}.

3. Results and discussions

3.1. Electrochemical characterization of laccase electrodes

In order to confer a higher conductivity to redox LDH, the innovative combination of SWCNTs and [ZnCr-ABTS] LDH was attempted by mixing all components (called 'mixed method') or by depositing first SWCNT (called 'separated-method'). These two kinds of configurations were composed of laccase (28 μg), BSA (12 μg) and [ZnCr-ABTS] (20 μg) while the SWCNT amount varied from 5 to 20 μg. Cyclic voltammetry was used to characterize the bioelectrocatalytic activity of laccase electrodes toward the reduction of oxygen. For instance, Fig. 1 shows the voltammograms recorded at a laccase-LDH/SWCNT electrode in 0.1 M PBS (pH 6.0) saturated with argon, ambient air, or oxygen. Under argon atmosphere, the cyclic voltammogram displayed a reversible peak system at 0.46 V *vs* SCE (ΔE_p = 110 mV at 10 mV s^{-1}) corresponding to the one-electron oxidation of ABTS. This potential value is similar to those previously reported for the immobilization of ABTS in polypyrrole films (0.46 V) or onto multiwalled carbon nanotubes (0.47 V) and even in pure [ZnCr-ABTS] matrix (0.47 V) [2,8,12,13]. This seems to indicate that the presence of SWCNT does not affect the potential value of the ABTS oxidation.

In the presence of O_2, the comparison with the cyclic voltammogram recorded in the argon-saturated solution (curve a in Fig. 1) clearly indicates an increase in the cathodic peak currents and a decrease in the corresponding anodic currents (curves b and c in Fig. 1). This catalytic process is due to the enzymatic reduction of oxygen, reflecting thus the laccase wiring by ABTS. In the O_2-saturated solution (about 1250 μM O_2), the electrocatalytic current (77.6 μA) is much larger than that (30 μA) recorded in air-saturated solution (about 250 μM O_2). As expected, this catalytic current is proportional to the O_2 concentration. It should be noted that this maximum current density obtained for a deposited amount of 28 μg of laccase in O_2-saturated solution is twice higher than the catalytic current (34.7 μA cm^{-2}) theoretically generated by a laccase-[ZnCr-ABTS] biosensor, the reported performance of the latter being: 6.2 μA cm^{-2} for 5 μg of laccase [8]. The electrocatalytic property of the different configurations of bioelectrodes was therefore evaluated under O_2-saturated atmosphere by chronoam-

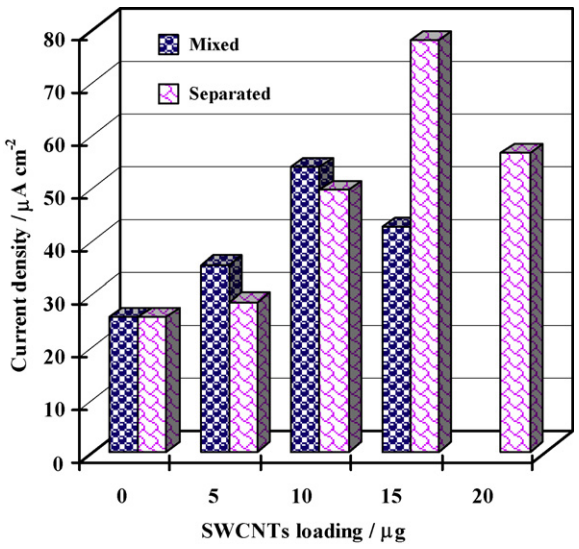

Fig. 2. Influence of SWCNT loading amount and loading methods on the electrocatalytic current of laccase electrodes for O_2 reduction under O_2-saturated conditions in 0.1 M PBS (pH 6).

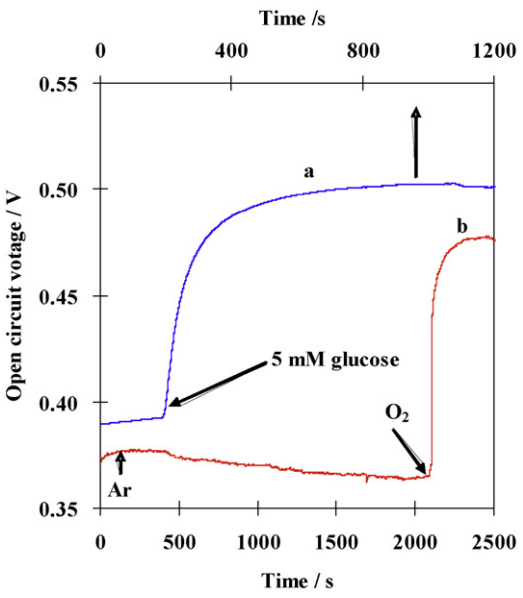

Fig. 3. Influence of the fuel (glucose) (a) and the oxidizer (O_2) (b) on the open circuit voltage of the assembled compartmentless glucose/air biofuel cell in 0.1 M PBS (pH 6) at 37 °C.

perometry at applied potential of 0.35 V, corresponding to the plateau of the electrocatalytic wave. The results are summarized in Fig. 2. The catalytic current increased with increasing amount of SWCNTs reaching a maximum value of 54.2 μA cm^{-2} for 10 μg SWCNTs and 77.6 μA cm^{-2} for 15 μg SWCNTs for each, the laccase-composite and laccase-LDH/SWCNT configuration, respectively. The optimal configuration was consequently ascribed to the separated-method with 15 μg loading of SWCNT. Since the oxidized SWCNTs are negatively charged, this material should not display electrostatic interactions with laccase molecules that are also negatively charged (isoelectric point ≅ 4). Taking into account that the different bioelectrode configurations were elaborated with the same amount of laccase. The increase in catalytic current with increasing amount of SWCNTs from 0 to 10–15 μg is more likely ascribed to an improvement of the enzyme wiring process than to an enhancement in the immobilized amount of enzyme. It should be noted that this maximum current density was markedly stronger than that (28 μA cm^{-2}) measured for a laccase-[ZnCr-ABTS] electrode without SWCNTs highlighting the beneficial effect of nanotubes on the enzyme wiring and electron transport processes [8–10]. In fact, the ABTS percentage implied in the electro-enzymatic process was increased thanks to the conductive SWCNTs situated in the vicinity of the LDH platelets. As expected, the electroactive SWCNT network formed in an underlying coating seems to be more efficient than the carbon nanotubes dispersed within the non-conductive enzyme-LDH film. Moreover it should be noted that no deactivation of the bioelectrocatalytic system was observed during repetitive voltammetric experiments. This seems to indicate that the electrostatic interactions between SWCNT and LDH increases the stability of the bioelectrodes.

3.2. Use of the laccase electrodes as biocathode for biofuel cells

A membrane-less glucose/air biofuel cell was built by association of a composite GOX-Fc graphite disc as bioanode and the optimized laccase-LDH/SWCNT electrode as biocathode. Fig. 3 shows the influence of the fuel (glucose) and the oxidizer (O_2) on the open circuit voltage (OCV) of the resulting biofuel cell. In air-saturated 0.1 M PBS (pH 6), the biofuel cell displays an OCV of 0.39 V. Upon addition of glucose (5 mM), a stable OCV value of 0.510 V was reached after 10 min. According to the Nernst equation, this OCV increase reflects the negative shift of the half-wave potential of ferrocene due to the enzymatic oxidation of glucose, GOX consuming the oxidized form of ferrocene at the bioanode. Similarly, in argon-saturated PBS containing glucose (5 mM), the OCV value was stabilized to 0.37 V after 33 min. Upon addition of O_2, the OCV sharply increased to 0.48 V. In presence of oxygen and laccase, the half-wave potential of ABTS shifts positively due to the enzymatic oxidation of ABTS. Fig. 4 displays the relationship between the power density and the cell voltage of the assembled glucose/O_2 biofuel cell in air-saturated 0.1 M PBS (pH 6.0) containing 5 mM glucose. The maximum power output of the biofuel cell was 18 μW cm^{-2} at 0.3 V while the OCV was equal to 510 mV. To further illustrate the efficient role of SWCNTs in the biocathode functioning, the parameters of a biofuel cell composed of an identical bioanode and a laccase-LDH electrode without SWCNTs as biocathode, were evaluated (Fig. 4). Although a similar OCV was obtained, the maximum power output value of 8.3 μW cm^{-2} is

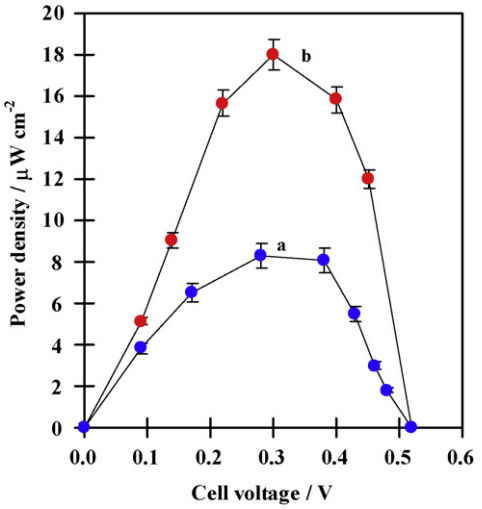

Fig. 4. Dependence of power output density on the operating cell voltage for the assembled compartmentless glucose/air biofuel cell using as biocathode: (a) laccase-LDH electrode without SWCNT and (b) laccase-LDH/SWCNT electrode; in air-saturated 0.1 M PBS (pH 6) containing 5 mM glucose.

markedly lower. This demonstrates the beneficial role of SWCNT within the LDH coating for the electron transport.

4. Conclusion

In this preliminary study, we have demonstrated the possibility of closely mixing oxidized SWCNT and redox LDH nanoparticles and to create stable electrode materials capable to immobilize enzyme molecules. It appears that specific electrostatic interactions between negatively charged nanotubes and redox LDH enhance the biofilm stability and the electron hopping mechanism, improving thus the electrochemical properties of laccase-LDH electrodes for oxygen reduction. It is expected that this easy construction of composite materials will be helpful for the development of electrically wired enzyme electrodes and could open new research paths in biofuel cells and biosensor fields.

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