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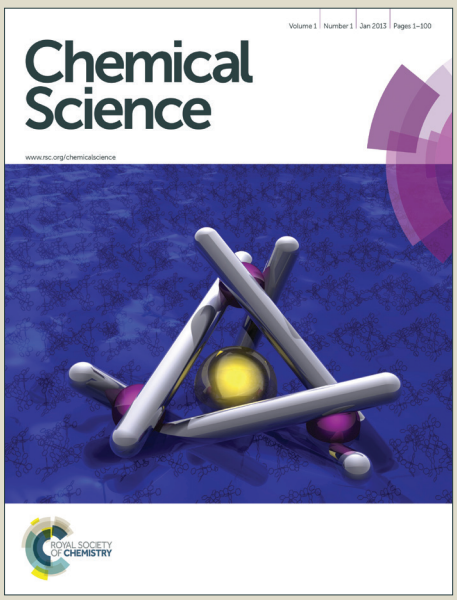

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Edge Article

Freestanding redox buckypaper electrodes from multi-wall carbon nanotubes for bioelectrocatalytic oxygen reduction via mediated electron transfer

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An efficient and easy way of designing free standing redox buckypaper electrodes via the elegant combination of multi-walled carbon nanotube (MWCNT) and a bis-pyrene derivative is reported. This bis-pyrene 2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (bis-Pyr-ABTS) that acts as a cross-linker between nanotubes, assures the formation of mechanically reinforced buckypaper obtained by a classical filtration technique of MWCNT suspension in presence of bis-Pyr-ABTS. In addition, the ABTS derivative assumes the mediated electron transfer to laccase. The electroactive buckypapers were characterized in terms of morphology, conductivity, and electrochemical properties. Two setups were evaluated: the first consisted in the immobilization and wiring of laccase enzymes via inclusion complex formation between the hydrophobic cavity of laccase and pyrene groups of bis-Pyr-ABTS that are not π-stacked to the nanotubes. The second approach was to evaluate the mediated electron transfer using laccase in solution. For this setup, the developed mediator electrodes demonstrate high performances with maximum currents up to 2 mA ± 70 µA and an excellent operational stability during two weeks under daily one hour discharges with refreshed laccase solutions.

Introduction

Glucose biofuel cells are promising candidates to replace lithium batteries in implanted devices one day [1, 2]. In fact, the possibility to convert the chemical energy of glucose and oxygen into electric energy is particularly interesting for in vivo applications since both, glucose and oxygen are present in body fluids at relatively low but constant concentration. Such physiological liquids generally contain about 5 mmolL^{-1} glucose and 50 µmolL^{-1} oxygen. Due to the low oxygen content, the total power output is often limited by the biofuel cell cathode and therefore, the efficiency of the oxygen reduction reaction (ORR) and the resulting electron transfer is of particular importance. Certain multicopper enzymes possess highly favorable catalytic properties for ORR compared to inorganic catalysts in terms of over potentials and activity in physiological environment. Furthermore, multicopper oxidases like laccase or bilirubin oxidase (BOD) are capable of transferring electrons used for ORR with various types of electrode materials via direct (DET) [3] or mediated electron transfer (MET). Concerning MET, there are a wide variety of artificial mediators for efficient electron transfers. ABTS [2.2-azino-bis-(3-ethylbenzothiazoline-6-sulphonic acid)] is one of the mostly used mediators for multicopper enzymes [4].

Carbon nanotubes (CNTs) have great advantages as electrode material in biofuel cell design due to their high conductivity and shape which allow enhanced DET or MET with the biocatalysts [5]. Furthermore, CNTs can be shaped to pellets, [6] fibers, [7] or films (Buckypapers, BPs) [8] that makes them easy to process as mesoporous pure CNT bioelectrodes in biofuel cell designs. Beside pellets, which are exclusively obtained by compression, and few fiber spinning procedures [9], there are many different ways to form CNT BP-electrodes [10-13]. The widely used method is vacuum filtration of CNT dispersions where important factors are the CNT quality and their homogeneous dispersion to form stable, free standing BP films.

Here, we present an original approach using ABTS modified with two pyrene units (Bis-Pyr-ABTS) as cross-linker to form free standing redox-BP for efficient wiring of multicopper enzymes represented by laccase from *Trametes Versicolor*.

Results and Discussion

Bis-Pyr-ABTS was synthesized with the aim to immobilize the redox mediator for ORR on sp² hybridisized carbon allotropes via π-stacking interactions. The sulfonamides were formed using the coupling reagent benzotriazol-1-yloxy-tris(dimethylamino) phosphoniumhexa fluorophosphate (BOP) in presence of ABTS and pyrenemethylamide. Bis-Pyr-ABTS serves furthermore for the cross-linking of CNTs forming stable free standing redox active BPs with enhanced electron transfer rates with laccase. The BP electrodes were formed via filtration of CNTs in presence of Bis-Pyr-ABTS. As-received MWCNTs (30 mg) were dispersed in 250 ml dimethylformamide (DMF 99%, Sigma Aldrich), under sonication 3 times for 5 min to achieve homogeneous black suspension. Then, 20 mg of Bis-Pyr-ABTS was added to the MWCNTs dispersion under magnetic stirring overnight. This mixture was then filtered under vacuum over a PTFE membrane filter with 0.45 µm pore size. Once filtration of the MWCNTs suspension was complete, the resulting MWCNTs film was washed with deionized water to remove the excess of the cross-linker. The Bis-Pyr-ABTS-BP, supported on a PTFE membrane, was then taken out of the glass funnel and dried in a vacuum oven at 50°C for 15 min preventing the deformation of the film. Once dried, the supported film was gently handled and could be carefully peeled off from the surface of the membrane filter with a scalpel. Bis-Pyr-ABTS-BPs with defined thickness can be controlled by adjusting the volume of CNT suspension. For instance, using a 35 mm filtration system, BP of about 100

µm thickness can be obtained after filtration of 250 mL of the MWCNT/ABTS dispersion. It has to be noted that such MWCNT samples do not form BPs under identical conditions without the presence of Bis-Pyr-ABTS. The coupling reaction and the BP formation are sketched in **Figure 1A**.

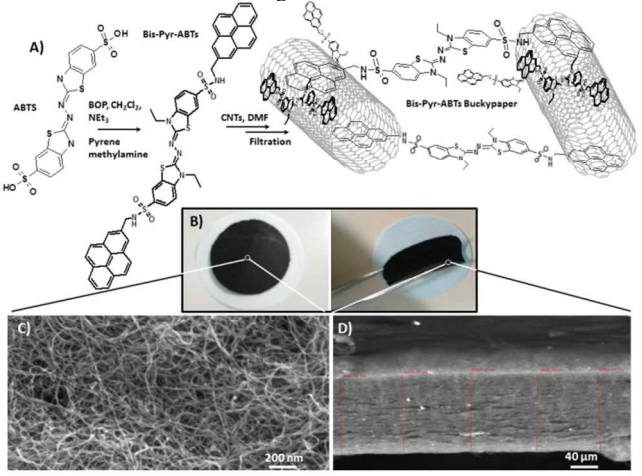

Figure 1. A) Reaction scheme for the synthesis of Bis-Pyr-ABTSand its capacity to crosslink CNTs giving a free-standing BP for mediated electron transfer. B) Photographs of an as formed BP on a membrane filter and bent with a tweezers. C) SEM images of the Bis-Pyr-ABTS BP surface. D) SEM image of Bis-Pyr-ABTS BP cross section.

Figure 1 B presents photographs of the as obtained free standing ABTS-BP with a satisfying mechanical stability. **Figure 1C** and **Figure 1D** show representative SEM images of as-prepared Bis-Pyr-ABTS-BPs. The MWCNTs are randomly oriented throughout the sample, predominantly parallel to the filter membrane surface (**Figure 1D**). Even under high magnification (**Figure 1C**), no excess of Bis-Pyr-ABTS can be observed.

Such obtained Bis-Pyr-ABTS-BPs were finally cut by a razor blade into rectangular strips of 1 cm width. For all electrochemical investigations, these BP strips were connected with an alligator clip and placed in a conventional three-electrode electrochemical cell giving an effective geometric area 1 cm^2 of the electrode in the electrolyte.

Likewise, it was observed that the fabricated BPs shows thicknesses about 100 ± 2 µm for a BP weight of 23.7 g m^{-2} and a specific volume equal to 4.2 ± 0.1 cm^3 g^{-1}. This is similar to the results found for BPs produced for ORR using bilirubin oxidase (BOD) [8].

The electric resistance of the buckypaper was measured using a four-point probe and revealed a high conductivity of in average (5 samples) 51± 5 $S.cm^{-1}$.

According to literature, ABTS is an efficient electron donor in the laccase-catalyzed reduction of oxygen to water [14] where both, the oxidized and reduced species of ABTS are chemically stable and do not inhibit the enzymatic reaction. Two setups were evaluated by cyclic voltammetry. Firstly, the redox activity of Bis-Pyr-ABTS used as cross-linker was measured for the as obtained BPs. In order to saturate the BP surface (BP_{sat}) by Bis-Pyr-ABTS, the BP strips were incubated in DMF containing Bis-Pyr-ABTS (5mg/ml) in order to cover both sides of the strip. This serves, on the one hand, to increase the amount of ABTS groups of the BP structure, thus enhancing the mediated electron transfer with laccase. On the other hand, by saturating the BP with the bis-pyrene groups, a statistic amount of one of the two pyrene groups is not adsorbed to the CNTs and should be available for the oriented immobilization of laccase [5]. The electrochemical behavior of the as prepared BP and BP_{sat} was evaluated by cyclic voltammetry at a scan rate of 1 mVs^{-1} in phosphate buffer solution (pH 5) at ambient temperature. The resulting cyclic voltammograms reveal a reversible peak system characteristic of the one-electron oxidation of ABTS at $\Delta E_{½}$ = 0.46 and 0.47 V vs. SCE for as prepared BP and BP_{sat}, respectively (**Figure 2A**). These values are similar to those previously reported for ABTS entrapped in polypyrrole (0.46 V) and for ABTS immobilized on MWCNT (0.47 V) [16-18]. This concordance indicates that the pyrenyl groups, attached to MWCNTs, do not affect the redox potential of ABTS.

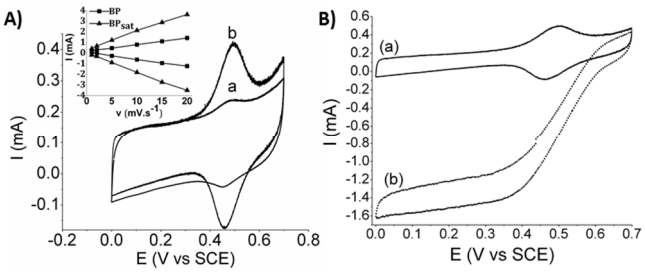

Figure 2. A) Cyclic voltammograms of a) as formed Bis-Pyr-ABTS BPs and b) BPs_{sat}in PBS (0.1 M, pH 5, scan rate 1 mV s^{-1}.) Plot of cathodic and anodic peak currents as a function of the scan rate for BP (squares) and BP_{sat} (triangle) under argon. B) Electrode responses recorded in 0.1 $molL^{-1}$ phosphate buffer (pH 5) solution containing 0.1 mg/mL laccase with BPs under (a) argon and (b) oxygen. Reference: SCE. Scan rate: 0.001 $V.s^{-1}$.

A significant difference between the as prepared BPs and the Bis-Pyr-ABTS saturated BPs can be observed for the intensity of the peak current and the related charge transfer (**Figure 2A**).

The integration of the charge under the redox waves allows to estimate the amount of immobilized ABTS, i.e 0.23 µmol cm^{-2} for BP (9 wt % ABTS/MWCNTs) and 1.03 µmol cm^{-2} for BP_{sat} (40 wt % ABTS/MWCNTs). The amount of immobilized ABTS could therefore be increased by factor 4.5after further incubation in a Bis-Pyr-ABTS solution giving BP_{sat}.

The current peak amplitudes of I_{pa} and I_{pc} increase linearly with the scan rate confirming a surface-controlled redox process without diffusion of ABTS into solution (inset, **Figure 2A**).

Figure 2B shows the cyclic voltammograms of as prepared BPs at 1 mVs^{-1} in 0.1 $molL^{-1}$ phosphate buffer solution (pH 5) containing 0.1 $mg.mL^{-1}$ of laccase under argon and under oxygen atmosphere. In presence of oxygen, the disappearance of the anodic peak and the marked increase in the cathodic redox wave clearly reflects an electrocatalytic process [19, 20]. The latter corresponds to the enzymatic oxidation of the immobilized mediator by the freely diffusing laccase, leading thus to the electro-enzymatic reduction of oxygen.

Two types of BPs (geometric area: $1cm^2$) were separately tested in terms of electrocatalytic oxygen reduction: as prepared BPs and BPs_{sat}. Furthermore, beside the evaluation of the electron transfer efficiency between the BPs and laccase (0.1 mg mL^{-1}) in solution, the performances of the BPs were also examined with immobilized laccase. For this, the BPs were incubated in phosphate buffer solution (0.1 mol L^{-1}) containing 5 $mg.mL^{-1}$of laccase and kept at 4°C overnight to assure most efficient immobilization of laccase via hydrophobic or supramolecular interactions with some available pyrene moieties [15]. The cyclic voltammograms were recorded by scanning the potential between 0 and 0.7 V vs. SCE while starting the scan from the open circuit potential. Under saturation with oxygen, a stable open-circuit potential value of 0.52 V was measured for all BPs whether laccase was immobilized or present in solution, this indicates

clearly that the electron transfer is dominantly realized by the ABTS mediators.

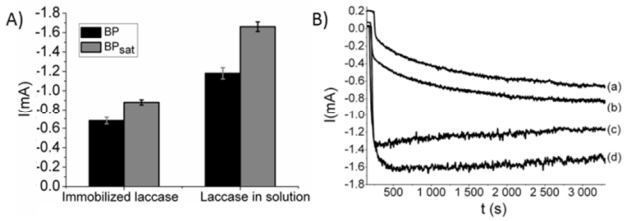

Figure 3. A) Diagram of the maximum currents obtained with as prepared BP and BPs_{sat} with immobilized laccase and laccase in solution. B) Chronoamperometric response for immobilized laccase on a) BP and b) BP_{sat}. For laccase in solution (0.1 $mg.mL^{-1}$): c) BP and d) BP_{sat}. Experimental conditions: 0.1 $mol.L^{-1}$ phosphate buffer (pH 5.0) saturated with O_2; applied potential 0.3 V vs. SCE.

Figure 3A shows the performance of BP and BP_{sat} in phosphate buffer containing 0.1 mg mL^{-1} of laccase and in enzyme free phosphate buffer after incubation with laccase. To compare the performance and stability of all configurations, chrono-amperometric measurements were carried out to determine the catalytic current under full load discharge for about 1 hour (3300 s) at 0.3 V vs SCE in oxygen saturated solution. All values were determined after 3000 s. It appears that the catalytic current using the laccase in solution setup was 1.20 ± 0.07 mA for BP and 1.55 ± 0.07 mA for BP_{sat}. Taking into account that both sides of the buckypaper provide efficient electron transfer, the corresponding current densities are therefore 0.60 mA cm^{-2} for BP, and 0.76 mA cm^{-2} for BP_{sat}. It should be noted that the catalytic current for the most efficient configuration (BP_{sat}) decreased by only 6% after 1 h illustrating the remarkable operational stability of this electrode. For the electrodes incubated with laccase, the efficient non-covalent binding and wiring of laccase leads to relatively high maximum currents that reach 44 to 50 % of the preceding catalytic current values with laccase in solution. As expected, the highest electroenzymatic activity is recorded with BP_{sat} (0.83 ±0.03 mA; current density 0.42 $mAcm^{-2}$) compared to BP (0.67 ±0.02 mA , current density 0.34 $mAcm^{-2}$).

Due to the fact that these mediator BPs show clear advantages when the multicopper enzyme is in solution, an alternative design of future glucose fuel cells can be envisioned. By eliminating the need of immobilized enzymes, these biocatalysts, in solution, can be exchanged after the end of their lifetime.

Conclusions

In summary, the possibility to form redox active BPs with a high density of mediators for enhanced electron transfer of ORR represents a promising alternative approach for the design of biofuel cells. The fact that high catalytic currents could be obtained in presence of laccase in solution circumvents the need of targeted immobilization and wiring techniques. Enzyme solutions can be exchanged when the enzymes lose their activity. This principle can be envisioned for implantable biofuel cells where one of the constant issues is the lifetime of the biocatalysts. With an appropriate design using enzyme solution containing dialysis bags with pierceable septa, the exchange of the enzymes can be done by injections. More appropriate biocatalysts with high activities at physiological pH values and with certain inertness to molecular inhibitors are under evaluation.

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Notes and references

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