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Hydrophilic carbon nanoparticle-laccase thin film electrode for mediatorless dioxygen reduction
SECM activity mapping and application in zinc-dioxygen battery

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ABSTRACT

Laccase from *Cerrena unicolor* was adsorbed on hydrophilic carbon nanoparticles (diameter = ca. 7.8 nm) modified with phenyl sulfonate groups and immobilized on an ITO electrode surface in a sol–gel processed silicate film. As shown by scanning electron and atomic force microscopies, the nanoparticles are evenly distributed on the electrode surface forming small aggregates of tens of nanometers in size. The mediator-free electrode exhibits significant and pH-dependent electrocatalytic activity towards dioxygen reduction. The maximum catalytic current density (95 $\mu A\,cm^{-2}$) is obtained at pH 4.8 corresponding to maximum activity of the enzyme. Under these conditions dioxygen electroreduction commences at 0.575 V vs. $Ag|AgCl_{sat}$, a value close to the formal potential of the T1 redox centre of the laccase. The scanning electrochemical microscopy images obtained in redox competition mode exploiting mediatorless electrocatalysis show that the laccase is evenly distributed in the composite film. The obtained electrode was applied as biocathode in a zinc-dioxygen battery operating in 0.1 M McIlvaine buffer (pH 4.8). It provides 1.48 V at open circuit and a maximum power density 17.4 $\mu W\,cm^{-2}$ at 0.7 V.

1. Introduction

The immobilization of laccases (*p*-diphenol:dioxygen oxidoreductases, EC 1.10.3.2) for efficient electron exchange between enzyme and electrode surface is important for the development of new types of fuel cells, battery systems, and in vivo power supplies. Some laccases catalyse the four electron electroreduction of dioxygen to water [1]. Since the dioxygen molecule is ubiquitous in nature it is utilized as oxidant in biofuel cells (see [2–6]).

Laccases are produced by numerous fungi and their isolation and purification methods are quite well developed [7]. The active site of most of the fungal laccases (i.e., blue oxidase) contain four copper (II) cations. A trinuclear Cu center serves as the catalytic site for O_2 reduction, whereas a remote single Cu ion (T1 site) has been proposed to serve as electron transfer channel [1]. The communication of this site with the electrode is possible either by mediator or direct electron transfer (DET). The devices employing DET between laccase and the electrode have been favoured recently, because of the simplicity and the lack of thermodynamic losses due to mismatch between enzyme and mediator redox potentials.

Mediatorless dioxygen electroreduction catalysed by laccase adsorbed on the electrode surface was observed on smooth electrodes like bare and thiol modified gold [8–14] or carbon-based materials [13,15–21].

With the advent of the nanotechnology era it has been found that higher efficiency of bioelectrocatalytic reaction can be obtained with nanostructured electrodes. This is probably due to favourable orientation of immobilized nanoparticles and enzyme decreasing the electron transfer distance from active site to conductive surface [22–24]. Indeed, efficient mediatorless dioxygen electroreduction catalysed by laccase was observed with electrodes composed of carbon black [25], mesoporous carbon [26], mesoporous carbon aerogel [27] or colloidal carbon [12] modified with laccase. Multiwalled or single walled carbon nanotubes films were also found to be promoters of this process [21,28–32]. Some of these

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nanostructured electrodes were already tested in biofuel cell models [26–30].

In the present study, a film composed of hydrophilic carbon nanoparticles (CNPs) with phenyl sulfonic acid functionalities is tested as substrate for mediatorless bioelectrocatalytic dioxygen reduction. These nanoparticles offer advantages of nanocarbon as conductivity, high surface area and adsorption sites together with charged functional groups. The presence of sulfonate groups increases their solubility, facilitating electrode preparation. For electrode modification CNPs have simply been embedded in polyaniline [33] or sol–gel processed silicate film [34]. Electrostatic immobilization by layer-by-layer assembly represents another option [35–39]. These electrodes exhibits high surface area and can be used for immobilization or accumulation of different redox probes. However, the films composed of hydrophilic CNPs were not yet used for enzyme immobilization.

Laccase and CNPs are immobilized on the electrode surface in a one step procedure-sol drop deposition [40]. Sol–gel processed silicate was chosen as a matrix, because it provides a suitable environment for protein immobilization [41,42]. It was earlier used for laccase immobilization on a smooth electrode surface [40,43–48], but mediatorless bioelectrocatalytic dioxygen reduction was not observed. The catalytic activity of the enzyme encapsulated in the composite film is studied by cyclic voltammetry and scanning electrochemical microscopy (SECM) [49–51]. SECM has already been widely used to monitor distribution and activity of enzymes immobilized on flat surfaces, e.g. on electrodes and within thin films [51–59] and was recently applied to laccase encapsulated in a silicate film [58].

The presented paper combines few recently published approaches to construct efficient biocathode for mediatorless dioxygen reduction. They include: (i) the application of high potential laccase from *Cerrena unicolor* [60], (ii) immobilization of this enzyme in sol–gel processed matrix [46] and (iii) the use of hydrophilic carbon nanoparticles as electroactive component [36]. The favourable conditions for mediatorless dioxygen allow to study this electrode material with redox competition SECM with pulsed O_2 generation as introduced by Eckhard et al. [61]. Finally, this electrode is applied as cathode in a zinc-dioxygen cell. Such a hybrid device was recently proposed by Heller [3] and constructed by Smolander et al. [62]. The combination of the low potential of the Zn/Zn^{2+} redox couple and the high potential of the bioelectrocatalytic dioxygen reduction provides a much larger voltage than that obtained from a dioxygen glucose biofuel cell [3].

2. Experimental

2.1. Chemicals and materials

Tetramethoxysilane (TMOS) (99%) and Nafion (5% solution in 2-propanol) were purchased from Aldrich. $K_3Fe(CN)_6$, $K_4Fe(CN)_6$, Na_2HPO_4, NaH_2PO_4 and HCl all of analytical grade were from POCH. Citric acid and KCl were from Chempur. 2-Propanol was obtained from Merck. Carbon nanoparticles with phenylsulfonic acid surface functionalities (ca. 7.8 nm mean diameter, with a typical bulk density of 320 g L^{-1}, Emperor 2000) were obtained from Cabot Corporation. Tin-doped ITO coated glass (resistivity 15 Ω per square) was obtained from Image Optics Components Ltd., Basildon, Essex, UK. Zinc wire (diameter 0.25 mm) was obtained from Goodfellow, Cambridge Ltd., UK. Water was filtered and demineralized with an ELIX system (Millipore). All reagents were used as received. Lyophilised laccase was obtained from the *Cerrena unicolor* fungus and purified as described earlier [60,63,64].

2.2. Electrode modification

The ITO electrodes were prepared as earlier described [34].

CNP solution was prepared by adding 3 mg of CNPs to 1 mL of water followed by 1 h sonication. For preparation of the film 500 μL of TMOS was mixed with 27.5 μL of 0.04 M aqueous HCl and 125 μL of CNPs solution. Next 250 μL of the sol was diluted with 225 μL of CNP solution and 25 μL of 0.1 M phosphate buffer (pH 5.8). Then a 25 μL aliquot of the diluted sol was mixed with 125 μL of laccase (145 μg of laccase dissolved in 2 mL of CNPs solution), 75 μL CNP solution and 25 μL of phosphate buffer. Finally, 25 μL of the sample obtained in last step was diluted in the same way. Every step of solution or sol preparation was followed by sonication.

The ITO electrode was modified by drop coating with 20 μL of the solution obtained in the final step using microsyringe. For sol–gel processing and drying these samples were left for at least 20 h at room temperature (22 ± 2 °C) and a relative humidity of 40–50%. The amount of enzyme and CNPs in the film is estimated to 2.7×10^{-10} mol and 8.5 μg respectively. This electrode is later called $TMOS_{gel}$/CNP/Lc. For control experiments the electrodes modified with $TMOS_{gel}$ film ($TMOS_{gel}$) and the same film with embedded laccase ($TMOS_{gel}$/Lc) [46] or carbon nanoparticles ($TMOS_{gel}$/CNP) [34] was prepared.

A zinc-dioxygen battery was assembled from $TMOS_{gel}$/CNP/Lc (cathode) and a zinc wire (anode) immersed in buffer solution

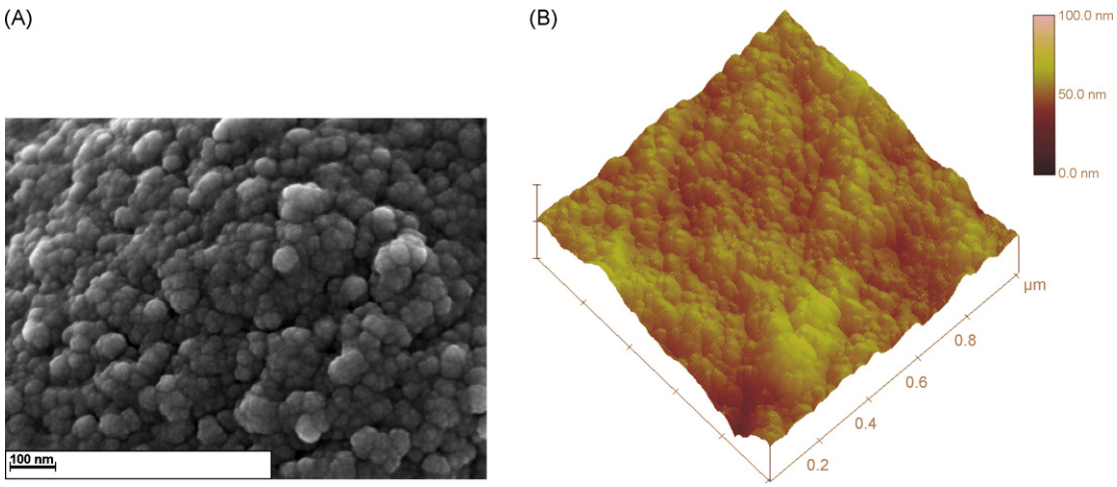

Fig. 1. SEM (A) and AFM (B) images of the $TMOS_{gel}$/CNP/Lc electrode. Scale bar 100 nm.