Available online at www.sciencedirect.com

ScienceDirect

Electrochimica Acta 53 (2008) 2408–2415

ELECTROCHIMICA Acta

www.elsevier.com/locate/electacta

Enhancement of bio-electrocatalytic oxygen reduction at the composite film of cobalt porphyrin immobilized within the carbon nanotube-supported peroxidase enzyme

Barbara Kowalewska [a], Magdalena Skunik [a], Katarzyna Karnicka [a], Krzysztof Miecznikowski [a], Malgorzata Chojak [a], Grazyna Ginalska [b], Anna Belcarz [b], Pawel J. Kulesza [a,*]

[a] *Department of Chemistry, University of Warsaw, Pasteura 1, PL-02-093 Warsaw, Poland*
[b] *Chair and Department of Biochemistry, Medical University, PL-20-031 Lublin, Poland*

Received 1 August 2007; received in revised form 3 October 2007; accepted 4 October 2007
Available online 7 October 2007

Abstract

Combination of multi-walled carbon nanotubes, cobalt porphyrin, and peroxidase (horseradish, cabbage) enzyme in the film (deposited onto glassy carbon electrode substrate) produces a bio-electrocatalytic system capable of effective reduction of oxygen in such neutral media as 0.1 mol dm^{-3} KCl and 0.1 mol dm^{-3} KCl + 0.01 citrate buffer (pH 6).

Carbon nanotubes have been modified with ultra-thin layers of 4-(pyrrole-1-yl) benzoic acid, or phosphododecamolybdate, to form stable colloidal suspensions of carbon nanostructures. The resulting inks have been utilized during sequential deposition of components. Co-existence of cobalt porphyrin, peroxidase enzyme together with dispersed carbon nanotubes leads to synergistic effect that is evident from some positive shift of the oxygen reduction voltammetric potentials (more than 50 mV in citrate buffer) and significant (ca. twice) increase of voltammetric currents (relative to those of the enzyme-free system). The multi-component bio-electrocatalytic film has also exhibited relatively higher activity towards reduction of hydrogen peroxide. It is reasonable to expect that the reduction of oxygen is initiated at cobalt porphyrin redox centers, and the undesirable hydrogen peroxide intermediate is further reduced at the horseradish or cabbage peroxidase enzymatic sites. An important function of carbon nanotubes is to improve transport of electrons within the bio-electrocatalytic multi-component film.
© 2007 Elsevier Ltd. All rights reserved.

Keywords: Oxygen reduction; Hydrogen peroxide reduction; Neutral electrolyte; Cobalt porphyrin; Multi-walled carbon nanotubes; Horseradish and cabbage peroxidases

1. Introduction

Electrocatalytic systems that would be useful in biological media, or the systems utilizing biocatalysts (enzymes), have to operate in neutral solutions. Recently, there has been growing interest in the fabrication of stable highly effective bio-electrocatalytic systems for oxygen reduction, particularly with respect to potential applications in biofuel cells [1–5]. Unless highly specific and expensive enzymes belonging to a group of proteins with the copper active centers, such as bilirubin oxidase or laccase, are considered, the reduction of oxygen in neutral media is a two-step process suffering from the formation of hydrogen peroxide as undesirable intermediate product. Although the above enzymes are capable of the effective four electron reduction of oxygen to water in neutral media, and significant progress have recently been made in their practical utilization [1–3], there is a need to look for alternate bio-electrocatalytic systems.

In the present work, we refer to the recent concept of bifunctional electrocatalysis [6] in which significant enhancement of the oxygen electroreduction in acid medium has been achieved by immobilization of the electrocatalyst ($RuSe_x$) within the reactive matrix (WO_3) capable of inducing reduction of the hydrogen peroxide intermediate. To fabricate the analogous bio-electrocatalytic systems operating effectively in neutral media, we have utilized macromolecular metalloporphyrin redox centers (at which the reduction of oxygen, mostly to hydrogen peroxide, is initiated) and such an enzyme as horseradish

* Corresponding author. Tel.: +48 22 8220211x289; fax: +49 22 8225996.
E-mail address: pkulesza@chem.uw.edu.pl (P.J. Kulesza).

0013-4686/$ – see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.electacta.2007.10.005

peroxidase (HRP), or cabbage peroxidase (CP), that is capable of catalyzing electroreduction of hydrogen peroxide to water as a final product.

Peroxidases are widely used for the construction of biosensors [7], and as enzyme labels in immunoassays [8]. HRP, is probably the most studied member of heme-containing plant peroxidases that is capable of accepting electrons and catalyzing oxidation of variety of reductants by hydrogen peroxide [9]. Electrochemical behavior of HRP [10–13] can be studied directly following adsorption on electrode surface, upon immobilization together with promoters or within redox polymer matrix. Cabbage peroxidase (CP) [14,15] which is also utilized in the present study, can be considered as the robust analogue of HRP.

To facilitate electron transfers between the electrode surface and the redox protein centers, we explore here the concept of codeposition of multi-walled carbon nanotubes (CNTs) within the bio-electrocatalytic film [16–19]. Good electronic conductivity of CNTs, together with their mechanical stability, have made them attractive for potential applications in electrochemistry. Representative examples include cases of electrocatalytic reductions of hydrogen peroxide and oxygen [20–22]. In the latter case, metalloporphyrins can be used as suitable catalysts for reduction of oxygen [20], but almost all reported monomeric complexes of porphyrins can catalyze only the reduction of O_2 to H_2O_2 [23–25]. An importat issue is to activate metalloporphyrin catalytic centers to make the oxygen electroreduction more efficient in terms of current densities and occur at more positive potentials.

In the present work, to facilitate charge distribution and to stabilize the composite film, we utilize multi-walled carbon nanotubes [26,27] that have been modified with ultra-thin layers of organic 4-(pyrrole-1-yl) benzoic acid (PyBA) or inorganic phosphododecamolybdate (PMo_{12}). We expect here attractive interactions between anionic adsorbates and positively charged domains of the enzymatic and metalloporphyrin sites. The other important issues are stability and mediating capabilities of adsorbates. In this respect, stable but rather insulating PyBA layers [28,29] are compared with less stable (in neutral media) but better conducting molybdates (PMo_{12}). Finally co-porphyrin centers are expected to induce reduction of oxygen at the initial stage, and the enzyme should act as highly reactive catalyst capable of further reducing hydrogen peroxide intermediate.

2. Experimental

Electrochemical measurements were done with CH Instruments (Austin, USA) Model 660B workstation. A standard three-electrode cell was used for all experiments. A platinum flag was used as the counter electrode. A saturated (KCl) Ag/AgCl electrode was used as a reference electrode. Glassy carbon disk (geometric area, 0.071 cm^2) was utilized as a working electrode. Before modification, it was activated by polishing with successively finer grade aqueous alumina slurries (grain size, 5–0.5 (m) on a Buehler polishing cloth.

Transmission electron microscopy (TEM) images were obtained using a JEOL 2010F microscope (and the voltage of 200 kV was applied). The samples were prepared by placing a drop of the colloidal suspension of bare or modified CNTs (diluted 100 times) onto a carbon coated copper mesh grid (and followed by drying in air).

Infrared spectra were measured with Shimadzu 8400 Fourier transform infrared (FTIR) spectrometer. The infrared reflectance absorption spectra (IRRAS) were recorded using a specular reflectance accessory model 500 produced by spectra tech. The beam incidence angle was equal to 80° with respect to the surface normal. Typically 1000 scans were averaged for single reflectance spectrum.

Solutions were prepared from triply-distilled subsequently-deionized (Millipore Milli-Q) water. They were de-aerated (using pre-purified argon) or saturated with oxygen for at least 10 min prior to the electrochemical experiment. Argon was used to de-aerate solutions and to keep air-free atmosphere over the solution during the measurements. Experiments were conducted at room temperature (20 ± 0.5 °C).

All chemicals were of analytical grade purity, and they were used as received. Co(III) protoporphyrin IX was from Frontier scientific, multi-walled carbon nanotubes and 4-(pyrrole-1-yl) benzoic acid (PyBA) were from Aldrich. Horseradish peroxidase (HRP, EC 1.11.1.7) and phosphododecamolybdic acid, $H_3PMo_{12}O_{40}$ (PMo_{12}) were from Fluka. Cabbage peroxidase (CP) was extracted from spring cabbage (*Brassica oleracea*) collected from autumn (2005) harvest from fields near Lublin, Poland. Supernatant from head homogenate (specific activity, 7.47 U mg^{-1} of protein) was subjected to 40–75% ammonium sulfate precipitation followed by exhausting dialysis and centrifugation (twice) to remove undesired precipitates. Resulting solution was lyophilized and stored at −20 °C. The lyophilizate specific activity was 98.7 U mg^{-1} of protein (with purification coefficient of 13.21). Peroxidase activity was measured against guaiacol as a substrate according to the procedure described elsewhere [14,15]. One unit of enzymatic activity was defined as amount of enzyme that oxidizes 1 mol min^{-1} of guaiacol under assay conditions.

To produce PyBA protected (stabilized) carbon nanotubes, the carbon suspension was formed by dispersing ca. 50 mg of CNTs (length, 1–10 m; outer diameter, 10–30 nm; inner diameter, 3–10 nm) in 5 cm^3 aqueous PyBA solution (50 mg/dm^3). The suspension was sonicated for 12 h. Subsequently, it was centrifuged, and the supernatant solution was removed and replaced with fresh PyBA solution. The centrifuging procedures were typically repeated three to four times. Later, the PyBA solution was decanted. The procedures of washing out and centrifuging were done with water and repeated at least twice. Thus, stable colloidal solution of PyBA-stabilized nanostructured carbon was obtained. To form colloidal suspensions of PMo_{12}-modified CNTs [26,27], 50 mg of CNTs was dispersed in 5 cm^3 of 5 mmol dm^{-3} aqueous solution of $H_3PMo_{12}O_{40}$. The suspensions were sonicated, centrifuged and subjected to decanting as described earlier above for the PyBA-modified CNTs.

In order to form bio-electrocatalytic layer (thickness, ca. 20–30 μm), first the glassy carbon electrode was covered with the film of PyBA- or PMo_{12}-modified CNTs by dropping onto the electrode surface (without spinning) an ink (5 μl) of the

Download English Version:

https://daneshyari.com/en/article/193874

Download Persian Version:

https://daneshyari.com/article/193874

Daneshyari.com