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Fabrication of cytochrome *c*-poly(5-amino-2-napthalenesulfonic acid) electrode by one step procedure and direct electrochemistry of cytochrome *c*

A. Balamurugan, Shen-Ming Chen*

Electroanalysis and Bioelectrochemistry lab, Department of Chemical Engineering and Biotechnology, National Taipei University of Technology, No. 1, Section 3, Chung-Hsiao East Road, Taipei, Taiwan 106, ROC

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ABSTRACT

Herein, we reported for the first time one step procedure for the preparation of cytochrome *c* (cyt c)-poly (5-amino-2-napthalenesulfonic acid) (PANS) modified glassy carbon electrode by cyclic voltammetrically (CV). Hereafter, we called the above modified electrode as cyt c-PANS electrode. The presence of cyt c on modified electrode was investigated with electrochemical quartz crystal microbalance (EQCM), CV, and superoxide radicals reaction studies. The reaction between cyt c in the modified electrode and superoxide radicals in solution, was exemplified by cyclic voltammetric measurements. Surface morphology of the modified electrode was investigated by using atomic force microscopy (AFM). The modified electrode showed a pair of well defined redox peak in PBS solution, pH 6.7. The modified electrode utilized for electrocatalytic reduction as well as amperometric determination of hydrogen peroxide (H_2O_2). The detection limit and linear range for H_2O_2 were 5 and 50 μM to 7 mM, respectively.

1. Introduction

Cytochrome *c* (cyt c) is one of the well studied redox protein. It contains one Fe(III) redox center located in a haem unit which is approximately spherical in shape with 34 Å diameter and 12,384 Da molecular weight. However, on metal surfaces it usually shows a short-lived, transient response (Eddowes and Hill, 1977; Yeh and Kuwana, 1977), because cyt c molecule lacks direct electrical contact with the electrode surface due to the non-appropriate orientation of the heme site in respect to the electrode surface lead to poisoning and deactivation of a bare electrode (Szucs et al., 1992; Fedurco, 2000; Tarlov and Bowden, 1991). In order to improve the direct electron transfer (ET) between an electrode and redox protein, an important step for electrochemical process, requires interfaces that exhibit reasonably fast ET kinetics. The desired interfacial properties are accessible only through electrode modification (Armstrong et al., 1988; Harmer and Hill, 1985). There are different strategy employed to modify the electrode in order to improve interfacial properties between cyt c and electrode namely by means of formation of self assembled monolayer, DNA modification, polymer modification, deposition of metal nano particles, etc.

There are number of modified interfaces available to immobilize cyt c such as $-CO_2H$ terminated self assembled monolayers (SAMs) through electrostatic interactions between carboxylate and the exterior of the protein (Collinson et al., 1992; Yamamoto et al., 2001; El Kasmi et al., 1998), layer by layer surface (Lvov et al., 1998), or multilayer film surface (Beissenhirtz et al., 2004), on the surface of DNA modified electrode (Lisdat et al., 2001, 1999a; Chen and Chen, 2003; Liu et al., 2003), by covalent linkage (McNeil et al., 1995; Koh et al., 2008), protein modification (Heller, 1990; Dronov et al., 2008), and on the surface of inorganic porous material (Xu et al., 2003; Yu and Ju, 2002). Although these new materials have been proven to be excellent as the immobilization matrices due to their high stability and good absorbability, some inherent defects are inevitable for the application of these multilayer and inorganic porous materials in electrochemical sensing such as low conductivity in inorganic material and in thicker films or multilayers, generally only protein molecules near the electrode surface are electroactive.

In order to improve good communication between cyt c and electrode surface, cyt c has been immobilized on the conducting polymer modified electrode (Bartlett and Faington, 1989; Caselli et al., 1991) that resulted good electron transfer. Since polymer modified electrodes are stable and amount on the electrode surface can be varied in a controlled manner. Recently, Jiang et al. (2006) immobilized cyt c on surface of poly(aniline-co-*o*-aminobenzensulfonic acid) (PANABS) through electrostatic interaction between cyt c and sulfonic group of PANABS. The above all reported the literature need two steps to achieve cyt c modified electrode. The two steps are (i) first preparation of modified or interface surface and (ii)

* Corresponding author. Tel.: +886 2270 17147; fax: +886 2270 25238.
E-mail address: smchen78@ms15.hinet.net (S.-M. Chen).

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immobilization or entrapment or covalent linking of cyt c. The enzyme or heme protein entrapment or immobilization on pre-prepared film is a more complicated and time consuming technique, which limits the heme quantity to a few monolayers and makes it possible to reduce the activity (Gooding et al., 1999). Therefore, to retain the heme protein activity and to limit the time consumption during preparation of cyt c electrode, we have prepared cyt c-PANS electrode by one step procedure. To the best of our knowledge, there is no such literature available for one step preparation of cyt c electrode.

In this present work, we report for the first time, that ANS polymerized electrochemically and during polymerization cyt c was entrapped. In this way, we achieved cyt c-PANS electrode by one step procedure. The presence of cyt c on modified electrode was investigated with electrochemical quartz crystal microbalance (EQCM), CV, and superoxide radicals reaction studies. The modified electrode exhibited electrocatalytic as well as good amperometric response towards H_2O_2 with the detection of limit of 5 μM.

2. Experimental

2.1. Reagents and solutions

ANS, bovine heart cytochrome *c* (cyt c), peroxidase and catalyse were purchased from Sigma–Aldrich. Stock solutions H_2O_2 were prepared each time freshly from 34% solution (purchased from Wako). All reagents were of analytical grade and used without any further purification. Solutions were prepared with doubly distilled water. High purity nitrogen was used for deaeration. The buffer and sample solutions were purged with highly purified nitrogen for at least 10 min prior to the experiments. Nitrogen atmosphere was maintained over the solutions during experiments.

Superoxide radical was generated according to the previously reported literature (McNeil et al., 1989).

2.2. Apparatus

Electrochemical experiments were performed with CH Instruments (Model CHI-400) using CHI-750 potentiostat. Glassy carbon electrode (geometric area = 0.07 cm^2) obtained from BAS served as a working electrode. Pt wire act as counter electrode and Ag/AgCl with the saturated KCl solution used as reference electrode. All the potentials given in this paper were referred with respect to Ag/AgCl (saturated KCl solution) reference electrode. The EQCM experiment was performed using CH instruments EQCM oscillator. An Au coated working electrode (area 0.196 cm^2, 8 MHz, AT-cut quartz crystal) was used.

Ultraviolet visible (UV–vis) spectra were recorded on a model U-3300 UV–vis spectrophotometer (Hitachi). Films of cyt c-PANS and PANS electrode were prepared by cyclic voltammetrically, respectively. Also, cyt c solution in pH 6.7 PBS was used as control.

2.3. Fabrication of cyt c-PANS electrode

Prior to modification, glassy carbon electrode (GCE) was polished with 0.05 μm alumina on Buehler felt pads and then ultrasonically cleaned for about a minute in water. Finally, the electrode was washed thoroughly with double distilled water and used. After being cleaned, the electrode was immersed into 0.05 M PBS solution, pH 6.7 containing 0.5 mM ANS and 2 mg/ml cyt c and the potential of working electrode was cycled between −0.5 and 0.8 V at the 100 mV s^{-1} for 260 s to fabricate cyt c-PANS electrode.

3. Results and discussion

3.1. Fabrication of cyt c-PANS electrode by one step procedure

Fig. 1 shows the CVs obtained from solution containing ANS monomer and cyt c in 0.05 M PBS solution, pH 6.7. During potential scanning of the electrode from −0.5 to 0.8 V at the scan rate of 100 mV s^{-1}, the anodic peak noticed at more positive potential in the first sweep corresponds to oxidation of ANS. In the subsequent sweeps, the redox peak appeared at around 0 V (versus Ag/AgCl) and the continuous increase of peak currents was observed. These observations can be ascribed as that cyt c was entrapped during oxidation followed by polymerization of ANS on the electrode surface. It might be due to electrostatic interaction between negatively charged sulfonic acid group of ANS and the positively charged cyt c (Armstrong et al., 1988). It is well known that cyt c has nine positive charges at pH 7.0 (Armstrong et al., 1988), indicating that amino groups in cyt c were protonated in pH 6.7 PBS solution and then interacted with sulfonic group of ANS to form organic complexes. Jiang et al. (2006) were observed similar type of interaction between cyt c and PANABS modified electrode. For comparison, we have given CV of PANS electrode in the absence of cyt c, is shown in Fig. 1B.

In order to estimate, the amount of cyt c entrapped in PANS during polymerization, we have carried out EQCM experiment and recorded the frequency changes on Au electrode surface in the presence and absence of cyt c. There was a decrease in frequency in the range of 132 Hz, which corresponds to a mass change (Δm) of ≈184 ng, in the presence of cyt c and only 60 Hz, which corresponds to mass change (Δm) of ≈84 ng, in the absence of cyt c (figures are given in supplementary file). This significant frequency and mass variation between these two electrodes confirmed and that excess frequency as well as mass change is due to the presence of cyt c while it was entrapped during

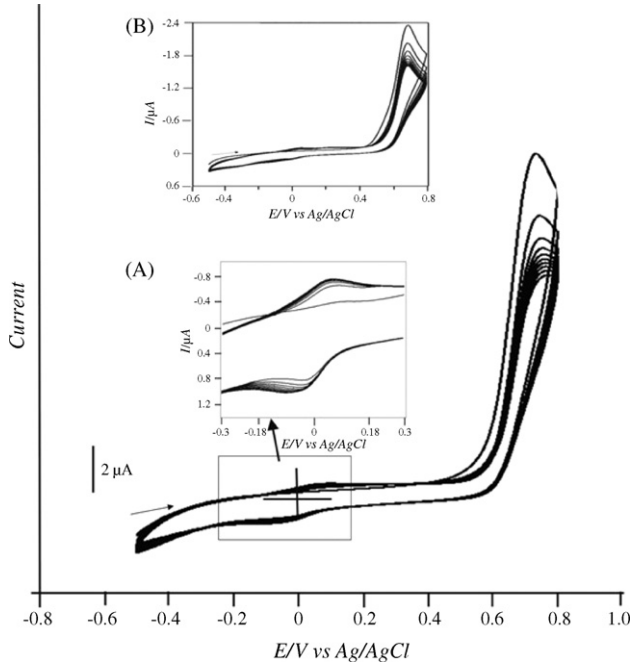

Fig. 1. CVs of cyt c-PANS electrode from solutions containing 0.5 mM ANS and 2 mg/ml cyt c in 0.05 M PBS solution, pH 6.7, 0.05 M PBS for modification. Scan rate: 0.1 V s^{-1}. (A) Magnified CV of cyt c-PANS electrode in the region of −0.3 to 0.3 V. (B) CVs of PANS electrode from solutions containing 0.5 mM ANS in 0.05 M PBS solution, pH 6.7, for modification. Scan rate: 0.1 V s^{-1}.