Superoxide Radical Biosensor Based on a 3D Enzyme/Carbon Nanotube Conductive Networks

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We report on a novel 3-dimensional (3D) network of crosslinked Cytochrome C/Carbon Nanotube (CytC/CNT) on a thiol-modified gold surface which can establish direct electrical communication between the redox center of Cytochrome C and the electrode. Cyclic voltammograms (CVs) results showed a pair of well defined redox peaks for Cytochrome C, located at about −0.03 and +0.06 V, cathodic and anodic respectively. Additionally, the formal potential \( E_0 \) of adsorbed Cyt c was found to be 15 mV, a value close to that of native Cyt c. Based on 3D Cytochrome c and carbon nanotube network, a sensitive superoxide radical biosensor has been proposed. The biosensor showed high sensitivity and lower detection limit of 0.3 \( \mu \)M of superoxide.

Keywords: 3D Crosslinked Networks, Cytochrome C, Superoxide, Biosensor.

1. INTRODUCTION

The superoxide anion radical (\( O_2^\cdot - \)) is the primary species of the so-called reactive oxygen species (ROS) is generated in significant quantities as result of univalent reduction of oxygen.\(^1\) In appropriate production of superoxide anion (\( O_2^\cdot - \)) has been known to be directly or indirectly involved in various pathologies such as cardiovascular dysfunction, ischemia and neurodegenerative diseases.\(^2\) Therefore, the quantitative detection of superoxide radicals and the characterization of the influence of antioxidants on their concentration are of great interest.\(^3\) However, due the lack of a sensitive and specific method the detection of superoxide is a challenging analytical problem.\(^4\) At the same time, the measurement of superoxide generated in biological systems is a difficult task because of its high reactivity and short half-life.\(^5\) Till now, few detecting methods for the determination of \( O_2^\cdot - \) have been proposed, such as electron spin resonance (ESR), spectrophotometry, and chemiluminescence method.\(^6\) However, nearly all of these assays have some drawbacks for various reasons. As an alternate sensitive detection method for \( O_2^\cdot - \), electrochemical biosensors have received extension attention because of easy construction, easy use and high sensitivity.\(^7\)-\(^9\) The electrochemical biosensor for superoxide radical is based on either superoxide dismutase (SOD) or cytochrome c (Cyt c) modified electrodes.\(^10\) It is already reported that SOD based biosensors for superoxide detection faces reproducibility problem due to poor immobilization technique.\(^11\) On the other hand, Cyt c based biosensors have shown more stability. Direct electron transfer (DET) of Cyt c has been investigated in order to construct sensitive electrochemical biosensor at various matrixes.\(^12\) However, DET of Cyt c on those matrixes has shown poor performances. Various kind of materials including nanostructured martials play an important role in electron shuttling for electrochemical biosensor, biofuel and capacitors.\(^13\)-\(^20\) Recently, we have reported an excellent method to generate three-dimensional electrically wired Hemoglobin (Hb) electrodes that showed high electron-transfer turnover rates.\(^21\) In that system, 4-aminophenol-modified Hb in the presence of 4-aminophenol-functionalized carbon nanotube (CNT) had created a three-dimensional bis-aniline-crosslinked CNT/Hb network. The resultant bis-aniline crosslinked network was redox active and able to medicate the electron from redox site of Hb to the base electrode. In this work, we have extended work to a new application of the 3D enzyme/CNT network by focusing on a construction of three-dimensional (3-D) conductive Cyt c network with CNT onto the thiol-modified gold (Au) electrode. In order to obtain the 3-D network, CNTs modified with electropolymerizable aniline and 4-aminophenol-modified Cyt c were coelectrolytically polymerized on the thiol-modified Au electrode surface. Proteins have many NH2-containing...