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Fabrication of 3-dimensional Cross-linked redox enzyme/Nanomaterials

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A grand challenge for the scientific community in the rapidly progressing field of biosensors and bioelectronics is the coupling of biomolecules with electronics to achieve high current outputs at low concentration of substrates, thus leading to highly sensitive biosensors and ultimately enabling their applicability for real applications in complex matrices, e.g. biological residue, environmental samples, etc. Communication between the biological and non-living worlds is effected by the passage of electrons and designing appropriate interfaces to facilitate this has been extremely difficult to achieve to date. One aspect of it aims to integrate biomaterials in electronic devices by using complex biomolecules to fabricate transducers and read-out systems for the development of novel biosensors. In order to construct an electrochemical sensitive biosensor, the biological recognition materials must be in intimate contact with the transducer. Redox enzyme/proteins are the most widely used biomolecules to study and configure an electrochemical biosensor. To solve the biomolecule-electrode communication problem, researchers have used various methods to establish direct electron transfer between enzymes and electrodes. Nanoscale surfaces such as nanotubes or nanoparticles can overcome some limitations because they contain regions of high curvature that energetically limit biomolecule adsorption, and reduced steric hindrance of the substrate to binding site on the enzyme. We have recently developed a three dimensional (3D) of carbon nanotube/haem cross-linked network that enhanced electrical contact of enzyme to the solid substrate (Kafi et al., 2013). In this system, 4-aminothiophenol-modified Hb in the presence of 4-aminothiophenol-functionalized carbon nanotube (CNT) has 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 made a new advance in the study of electrical communication with Horseradish peroxidase (HRP) by creating 3D networks of HRP/CNT onto the gold nanotube (AuNT) arrays that yields a high surface areas. Increasing attention has been paid to apply highly ordered gold nanotube arrays as a matrix as it provides more surface and electroactive sites in comparison with other conventional electrodes (Guangming et al., 2011; Min et al., 2014).

In this work, for the first time, we have constructed a three-dimensional (3-D) conductive HRP network with CNT onto the Au nanotube arrays electrode. In order to obtain the 3-D network, CNTs modified with electropolymerizable aniline and 4-aminothiophenol-modified HRP were coelectroplolymerized on the thiol-

modified Au electrode surface. Our results demonstrate for the first time that that the created 3-D HRP/CNT composite effectively builds a good electrical communication between the redox centers of the HRP and an electrode. Based on this, a H_2O_2 biosensor has been developed bearing in mind that the sensitive and accurate H_2O_2 detection is of great interest to researchers because of its importance in the pharmaceutical, clinical and industrial settings.

The morphology and composition of the AuNT/SAM/HRP/CNT electrode were examined by SEM observation and EDS analysis. The AuNT/SAM/HRP/CNT composite film exhibited a net structure in fig 1 C and D. On the AuNT/SAM/HRP/CNT composite film many netlike structure were found homogeneously distributed on the surface, which indicated that HRP molecules were connected with carbon nanotube and built up a 3D networks.). And the electrochemical properties of the modified electrodes is investigated with Cyclic voltammogram (CV) and amperometry. Experimental results reveals the establishment of the direct electron transfer between the redox active unit of HRP and the AuNT arrays. Electrochemical measurements prove that the immobilized HRP exhibits high biological activity and stability and a quasi-reversible redox peak of the redox centre of HRP was observed at about −0.398 V and −0.285 V vs. Ag/AgCl. As a biosensor, the examination of electrocatalytic activity of the GCE/AuNT/SAM/HRP/CNT electrode to H_2O2 has been performed by the typical cyclic voltammograms. Figure 1 presents the CVs of the GCE/AuNT/SAM/HRP/CNT electrode in the absence and presence of H_2O_2. It is seen that when 2.5×10^{-5} M H_2O_2 was added into PBS (pH 7.0), the reduction current increased and the oxidation current decreased significantly at the GCE/AuNT/SAM/HRP/CNT electrode, indicating that an obviously catalytic reduction of H_2O2 was observed. This specific CV feature of the GCE/AuNT/SAM/HRP/CNT electrode is evidenced that the current response of the electrode to H_2O_2 was mainly due to the catalytic reduction effect of HRP and the immobilized HRP remained its activity in 3D HRP/CNT networks.
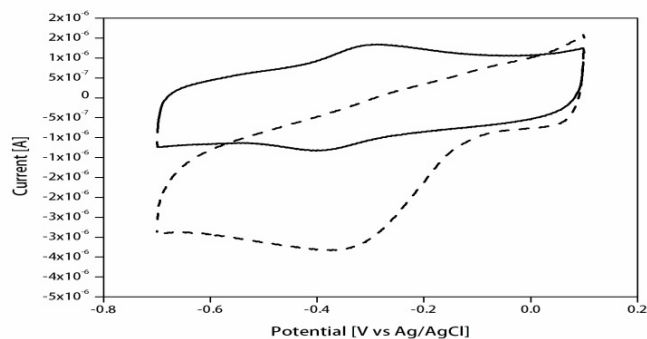

Fig. 1. Cyclic voltammograms of the GCE/AuNT/SAM/HRP/CNT electrode in the absence of H2O2 (Solid line) and in the presence (Dashed line) of 2.5×10−5 M in PBS with pH 7.0 at the scan rate of 0.05 V/s.

The developed biosensor exhibits excellent electrocatalytic activity for the reduction of H2O2. The proposed biosensor modified displays a broader linear range and a lower detection limit for H2O2 determination. The linear range is from 5.0×10−7 to 1.5×10−4 M with a detection limit of 2.0×10−7 M at 3σ.

Reference

[1] Kafi AKM, Crossley MJ. Synthesis of a Conductive Network of Crosslinked Carbon Nanotube/Hemoglobin on a thiol-modified Au Surface and its Application to Biosensing , *Biosens. Bioelectron*, 42; 2013: 273-279.