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Direct Electrochemical Reaction of Horseradish Peroxidase Immobilized on the Surface of Active Carbon Powders

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Abstract: It is reported for the first time that horseradish peroxidase (HRP) immobilized on the active carbon can undergo a direct quasi-reversible electrochemical reaction. In addition, the immobilized HRP showed the stable bioelectrocatalytic activity for the reduction of H_2O_2.

Keywords: Direct electrochemical reaction, horseradish peroxidase, active carbon.

The direct electrochemical reactions of biomacromolecules have been received more and more attention because it can supply the valuable information on the mechanisms of the biological electron transfer reaction and find potential applications in biotechnology[1]. However, it is difficult to undergo a direct electrochemical reaction for biomacromolecules because the active center of a biomacromolecule usually buried deeply in the center of the molecule. In this paper, it is reported for the first time that horseradish peroxidase (HRP) immobilized on the active carbon can undergo a direct quasi-reversible electrochemical reaction.

HRP was immobilized on Vulcan XC-72 active carbon with the physical adsorption. 2 mg of HRP-adsorbed active carbon are mixed with 80 μL 5% Nafion solution. 1μL of this mixture was cast on the surface of the glassy carbon (GC) electrode (4 mm in diameter) surface and then dried at ambient temperature before use. The electrode obtained with or without HRP is called the HRP-C/GC or C/GC electrode, respectively.

The electrochemical experiments were carried out using an EG&G PAR 273A Potentiostat/Galvanostat with a conventional three-electrode cell. The Pt wire and the saturated calomel electrode were used as the counter electrode and the reference electrode, respectively. All experiments were performed at 25 ± 2 °C.

Figure 1 shows the cyclic voltammograms (CVs) of a C/GC electrode (**Curve a**) and a HRP-C/GC electrode (**Curve b**) in the 0.2 mol/L phosphate buffer solution (PBS, pH 6.8) at a scan rate of 100 mV/s. It can be found that no any observable voltammetric response is observed at the C/GC electrode, but a pair of well-defined and nearly symmetric redox peaks at –0.345 and –0.380 V was observed at the HRP-C/GC electrode, indicating that the redox peaks produced from the electrochemical reaction of

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HRP immobilized on the active carbon. It demonstrated that the HRP immobilized on the active carbon undergoes a direct quasi-reversible electrochemical reaction. Its formal potential is -0.363 V. The value is similar to that previously reported for HRP entrapping in the tributylmethyl phosphonium chloride polymer which bound to an anionic exchange resin by Ferri *et al.*[2] and incorporating into Eastman AQ film by Hu *et al.*[3]. The anodic and cathodic peak currents are linearly proportional to scan rate at least up to 700 mV/s, demonstrating that the reaction is a surface-controlled process, as expected for immobilized systems.

Figure 2 is the CVs of a HRP-C/GC electrode in 0.2 mol/L PBS (pH 6.8) in the absence (**Curve a**) and presence (**Curve b**) of 5 mmol/L H_2O_2 at a scan rate of 100 mV/s. The electrocatalytic cathodic current of H_2O_2 (**Curve b**) can be clearly observed. In addition, after the HRP-C/GC electrode was stored at 4 °C for several months without any observable loss of the enzyme activity. The results indicated that HRP keeps its bioelectrocatalytic activity with the good stability after immobilized on the active carbon.

Figure 1 CVs of (a) a C/GC and (b) a HRP-C/GC electrode in 0.2 mol/L PBS.

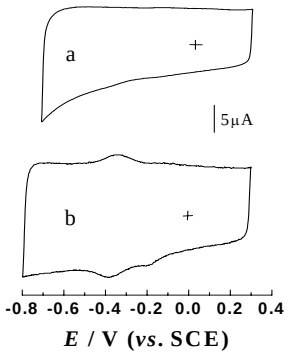
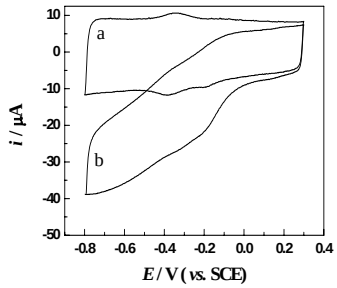


Figure 2 CVs of a HRP-C/GC electrode in 0.2 mol/L PBS in the absence (a) and presence (b) of 5 mmol/L H_2O_2.



The results mentioned above indicated that HRP immobilized on the active carbon could undergo the direct quasi-reversible electrochemical reaction. The immobilization method is simple. Moreover, this method may be suitable for other biomacromolecules.

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