Direct Electrochemistry of Horseradish Peroxidase Immobilized on the Surface of Au Ring/AET/Au Electrode

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(Received April 18, 2005; CL-050527)

In this letter, single crystal gold nanorings were assembled on the surface of Au electrode with a self-assembly monolayer (SAM) of 2-aminoethanethiol hydrochloride (AET). The direct electrochemistry of horseradish peroxidase immobilized on the surface was explored using electrochemical methods.

Recently, nanomaterials have been wildly used in the research of biomolecules, among them, gold nanoparticles especially spherical gold nanoparticles have been widely used because of their good conductivity and biocompatibility. However, these nanomaterials are usually polycrystalline. Different from reported work, single crystal gold nanorings were assembled on the electrode surface in our research. These single crystal gold nanorings could be oriented on the electrode surface with (111) planes parallel to the substrate by a SAM of AET.

The research of direct electrochemistry for protein, particularly enzyme, like horseradish peroxidase (HRP) is very significant for understanding the structure–function relationship of protein, developing the third generation biosensor, and other functional devices.

In the present paper, single crystal gold nanorings have been successfully assembled on the electrode surface, and HRP was further immobilized on it. The assembly processes were characterized by atomic force microscopy (AFM, tapping mode). Further electrochemical studies indicated that single crystal gold nanorings might promote the direct electron transfer between electrode and biomolecules immobilized on the surface.

Gold nanorings were prepared according to the literature. Gold electrode was first polished with diamond paper and alumina (0.015 μm)/water slurry, and then it was rinsed with de-ionized water. The assembly processes are listed as following steps: (1) The cleaned gold electrode was immersed in 0.1 M of 2-aminoethanethiol hydrochloride (alcohol:water = 1:9) for 2 h, then the electrode was soaked in a solution of alcohol/water (alcohol:water = 1:9) for 4 h in order to remove excess AET. (2) Gold nanorings were dropped onto the surface of the electrode and dried in a refrigerator, and then the electrode was immersed in deionized water for several hours in order to remove excess gold nanorings. (3) Horseradish peroxidase (HRP) of 3 mg/mL was dropped on the surface of the gold electrode.

Figure 1 is the TEM image of the as-prepared gold nanorings, which had an average size of ca. 150 nm. These prepared gold nanorings were single crystals with (111) planes as basal planes. From the TEM, SAED images (Figure 1), and XRD pattern, it can be confirmed that these gold nanorings are highly oriented crystals with (111) planes parallel to the substrate.

Scheme 1 shows the surface assembly processes: Firstly, Au electrode surface was chemisorbed with an AET SAM; second-
redox peaks with regard to Fe(III) to Fe(II) conversion of the was immobilized on the surface, a pair of stable and well-defined on the surface of the electrode (Curves a, b, and c). When HRP was observed at the electrode surface before HRP was assembled compared with bare electrode showing better conductivity. No peak the surface of the gold electrode, the capacitance decreased com-

which AFM image clearly indicated that these gold nanorings were which was in accord with the TEM image. What is more, the AFM image clearly indicated that these gold nanorings were highly oriented on the substrate with (111) planes parallel to the substrate. Figure 2c is the AFM image after HRP immobilized on the electrode surface. Compared with Figures 2a and 2b, HRP had been successfully immobilized on the surface of the substrate by gold nanorings.

The electrochemical behavior of the HRP/Au ring/AET/ Au electrode was further studied in phosphate buffer solution (pH = 7.0). Figure 3 shows the cyclic voltammograms of the assembly process. When gold nanorings were assembled on the surface of the gold electrode, the capacitance decreased compared with bare electrode showing better conductivity. No peak was observed at the electrode surface before HRP was assembled on the surface of the electrode (Curves a, b, and c). When HRP was immobilized on the surface, a pair of stable and well-defined redox peaks with regard to Fe(III) to Fe(II) conversion of the immobilized HRP was observed. The anodic and cathodic peak potentials were −0.306 and −0.361 V (vs SCE), respectively (Figure 3d).

The effect of scan rate on the response of immobilized HRP in 0.1 M phosphate buffer solution (pH = 7.0) is shown in Figure 4. The anodic and cathodic peak currents (i_p and i_c) were proportional to the scan rate (inset in Figure 4), indicating a surface-controlled quasi-reversible process. When the scan rate varied from 30 to 450 mV/s, very little changes were observed in the peak–peak separation, indicating a faster heterogeneous electron-transfer rate. The electron-transfer rate constant k_e of HRP immobilized on the surface of the electrode could be determined to be 5.16 s⁻¹ using Laviron’s method. Therefore, the orientation of gold nanorings on the electrode surface might reduce the electron-tunneling distance between HRP and the electrode surface and promote the direct electron-transfer rate between HRP and the electrode.

In summary, we have successfully assembled gold nanorings and immobilized HRP on the electrode surface, and the further electrochemical studies indicated reversible electron transfer of HRP, which might help to develop biosensing devices.

We greatly appreciate the support of the National Natural Science Foundation of China (Grant Nos. 20325516 and 90206037) and 973 Project (2004CB520804) for this research work.

References