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

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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,[1–10] among them, gold nanoparticles especially spherical gold nanoparticles have been widely used because of their good conductivity and biocompatibility.[1,4–10] 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.[11,12]

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.[13] Gold electrode was first polished with diamond paper and alumina (0.015 µm)/water slurry, and then it was rinsed with deionized 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.[13] From the TEM, SAED images (Figure 1), and XRD pattern,[13] 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; secondly, gold nanorings were assembled on the electrode surface by amino groups; finally, HRP was adsorbed on Au ring/AET/Au electrode surface. Figure 2 is the AFM image of the assembly processes. The AFM characterization was preceded on the silicon wafer with a gold layer of 100 nm. The surface of the silicon wafer is very flat (AFM not shown here). When electrode surface was loaded with a monolayer of AET, no obvious changes was observed. Figures 2a and 2b show the AFM images of Au ring/AET/Au electrode surface. As is shown in Figure 2, the as-prepared gold nanorings had an average size of ca. 150 nm,

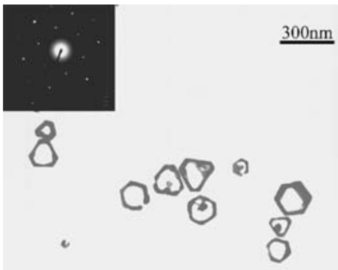
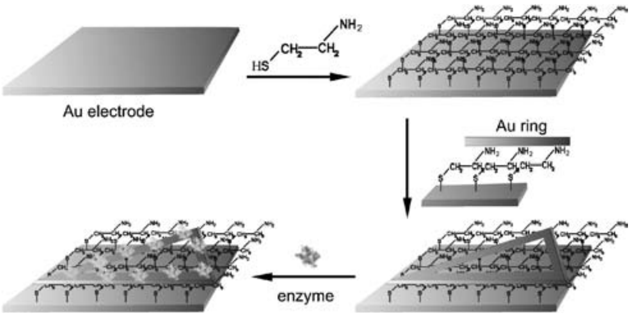


Figure 1. TEM image of prepared gold nanorings (inset was the SAED pattern of a single gold nanoring).



Scheme 1. The surface assembly processes of the HRP/Au ring/AET/Au electrode.

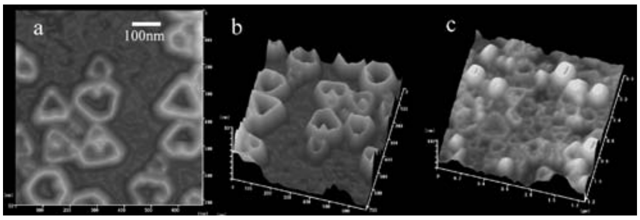


Figure 2. AFM image of the single crystal surface before (a, b) and after (c) HRP was immobilized on electrode surface.

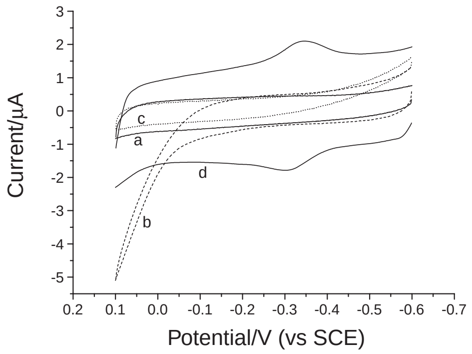


Figure 3. Cyclic voltammograms of (a) bare Au electrode, (b) AET/Au electrode, (c) Au ring/AET/Au, and (d) HRP/Au ring/AET/Au in phosphate buffer solution (pH = 7.0) at the scan rate of 100 mV/s.

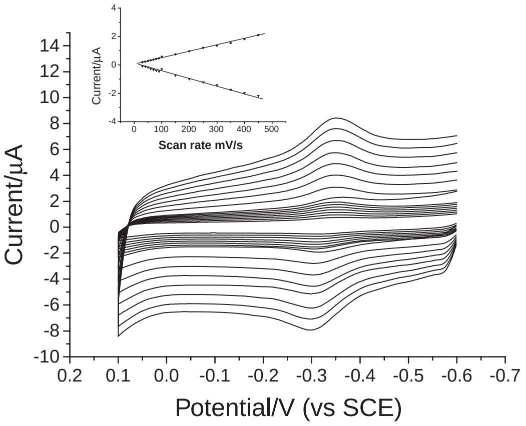


Figure 4. Cyclic voltammograms of HRP/Au ring/AET/Au electrode in PBS and the calibration plot of the peak current at different scan rates (from inner to outer): 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250, 300, 350, 400, and 450 mV/s.

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_{pa} and i_{pc}) 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_s of HRP immobilized on the surface of the electrode could be determined to be 5.16 s^{-1} using Laviron's method.[14] Therefore, the orientation of gold nanorings on the electrode surface might reduce the electron-tunneling distance[2] 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.

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