Self-assembly of Horseradish Peroxidase on Biocompatible Gold Nanoparticles–Vaterite Core–Shell Composite and its Direct Electrochemistry

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Biocompatible AuNPs–vaterite core–shell composite, which combines the advantages of mineral compound and nanoparticles, was firstly used in the construction of biosensor. Direct electron transfer process of HRP on it was investigated.

Direct electrochemistry of metalloenzymes is of great interest in fundamental studies of electron transfer (ET) in proteins and development of highly selective and sensitive biosensors. Horseradish peroxidase (HRP), as the most commonly used enzyme, does not easily undergo direct electrochemistry in solution with "naked" solid electrodes. Various materials,[1] such as films, single wall carbon nanotubes (SWNTs) and nanoparticles were employed and the direct electron transfer for the HRP heme Fe(III)/Fe(II) redox couple was greatly enhanced.

Vaterite is one of the polymorphs of calcium carbonate. It has been considered as a safe material for administration into biological system,[2] and has been widely used in industry, technology, medicine, microcapsule fabrication, and many other bio-related fields.[3] The functional carboxyl groups on its surface can interact with proteins and improve their electron transfer.[4] When gold nanoparticles (AuNPs) are assembled on it, the AuNPs–vaterite composite will couple the advantages of both the nanoparticles and minerals. Like core–shell composite of gold nanoparticles–polyurethane microsphere,[5] the hybrid material will provide a native environment and increase the enzyme loading. However, to our knowledge, so far there is no report on the assembly of AuNPs on vaterite and the direct electrochemistry of HRP using the AuNPs–vaterite composite modified electrodes. Thus, in this study, we firstly report the assembly of AuNPs on vaterite and the direct electron transfer properties of the HRP using the composite modified on a glassy carbon electrode. The electrocatalytic activity of the as-assembled HRP-composite for H_2O_2 reduction and the potential application of the composite for developing novel biosensors were also evaluated.

AuNPs[6] and vaterite particles[3] were prepared according to the literatures. The step-by-step process of forming the AuNPs–vaterite core–shell composite and assembling HRP on it was illustrated in Scheme 1. 10 mg of vaterite were mixed with 1 mL of AuNPs and sonicated for 20 min. After centrifuguting at 4000 rpm, a purple deposit was obtained. Then the AuNPs–vaterite composite was suspended in 1 mL of HRP solution (1 mg/mL) and shaked for one hour for the enzyme assembly. The HRP-composite was separated by centrifuging and washed to remove the loosely assembled HRP moleculars, and was evenly re-suspended in 0.5 mL distilled water. Then 10 mL of the suspension was dropped on a glassy carbon electrode. After drying, a layer of silica sol–gel was coated to the electrode surface for
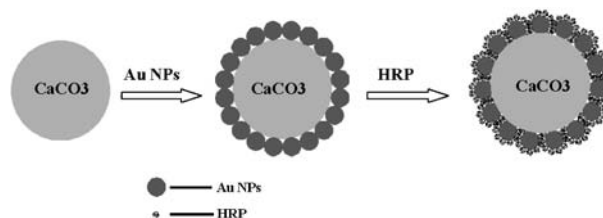
Scheme 1. Schematic representation of the fabrication of AuNPs–vaterite composite and the assembly of HRP on it.

the HRP-composite immobilization.

Figure 1 showed the scanning electron images of vaterite (a), AuNPs–vaterite (b), HRP–AuNPs–vaterite (c) and HRP–vaterite (d) composits. Specific morphology with spheres was observed in Figure 1 a, b, and c, which corresponded to the shape of vaterite. The spheres were composed of a great number of carbonate nanoparticles.[3] These nanoparticles offered a large area for the adsorption of AuNPs and HRP. Although the AuNPs on the surface of vaterite could not be observed due to the resolution of the SEM measurement, EDAX (energy dispersive analysis of X-ray) measurement showed the appearance of the Au signal for AuNPs–vaterite and HRP–AuNPs–vaterite. It is well known that carboxyl group can interact with calcium ions,[7] so the citrate groups around AuNPs can interact with calcium ions of vaterite. On the other hand, with the isoelectric point (IEP) at pH 8.5,[8] vaterite is positively charged in neutral aqueous solution, while the AuNPs prepared by citrate reduction were negatively charged. Therefore, the negatively charged AuNPs could assemble on positively charged vaterite surface via electrostatic interaction and other supramolecular interactions.[9] EDAX measurement also confirmed the presence of the enzyme in the HRP–vaterite and HRP–AuNPs–vaterite composite through a sulfur
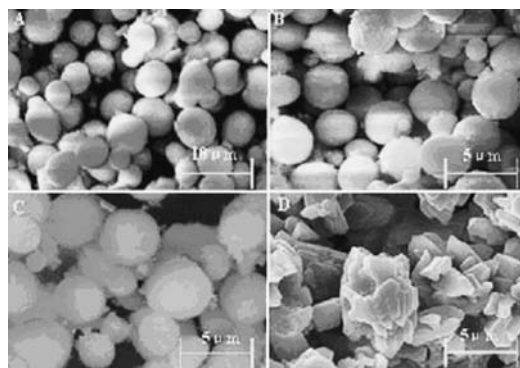

Figure 1. SEM images of vaterite (A), AuNPs–vaterite (B), HRP–AuNPs–vaterite (C) and HRP–vaterite (D).

signal from residues of HRP. Without AuNPs, the HRP–vaterite composite was not stable and the calcite crystals would be formed by the recrystallization of the vaterite in aqueous solution. In Figure 1d, thermodynamically stable rhombohedral shaped calcite crystals were observed.

In a 0.1 M and pH 7.0 of PBS solution, a pair of current peaks (current peaks) located at −0.03 V and −0.118 V were observed on the HRP–AuNPs–vaterite composite-modified electrode, which were characteristic of the HRP heme Fe(III)/Fe(II) redox couple. This potential is lower than the potential of Fe (III/II) redox couple in HRP solution. The low redox potential might imply special interactions between the molecular 3D structure of HRP and the morphology of the composites. The interactions strongly affected the heme microenvironment. On the other hand, both vaterite and AuNPs have functional groups containing oxygen such as CO_3^{2-}, which should facilitate the adsorption of heme peptide of HRP by the interactions including hydrogen bonding and electrostatic interaction, thus the heme edge will be oriented towards its electron donor or acceptor so as to facilitate the electron-transfer process.[10] Hill and his coworkers have implied that multivalent cations such as Mg^{2+} and $Cr(NH_3)_6^{3+}$ could promote the heterogeneous electron transfer of protein,[11] so the presence of Ca^{2+} might accelerate the electron transfer. Direct electrochemistry of HRP was also observed when HRP–vaterite modified electrode was used, but it is not stable. The vaterite spheres in HRP–vaterite composite would quickly transfer into calcite crystals as shown in Figure 1d. Therefore, we speculate that the AuNPs may form a shell to stabilize the vaterite[12] surfaces and prevent its phase transformation. To test the importance of vaterite, calcite was used for a comparison. Calcite is the most stable polymorph of $CaCO_3$, which has the low dispersibility in water. When calcite was mixed with AuNPs solution, it should be noted that the color of the obtained composite was nearly white. Therefore, few AuNPs were adsorbed on the calcite. Direct electrochemistry of HRP was not observed when HRP–AuNPs–calcite composite was used for the modification. As a result, direct and stable electron transfer process of HRP could be performed by the synergetic effect of vaterite and AuNPs.

Figure 2a showed the CVs of the HRP-composite-modified electrode in pH 7.0, 0.1 M of phosphate buffer at scan rates from 0.01 to 0.75 V/s. At this range of scan rates, the CVs were almost symmetrical with equal reduction and oxidation peak heights. This indicated that all the electroactive Fe(III) was reduced to ferrous Fe(II) on the forward scan and the Fe(II) produced was reoxidized to Fe(III) on the reverse scan. The peak current had a linear relationship with the scan rates from 0.01 to 0.75 V/s (Figure 2b), as expected for the thin-layer electrochemical behavior,[13] so HRP was self-assembled on the surface of the AuNPs–vaterite composite to form a layer.

When the biosensor was used to determine the concentration of hydrogen peroxide, wide linearity and high sensitivity were obtained. The response was linear in the concentration range of 5×10^{-6}–5×10^{-5} M (RSD% = 0.9968) and 5×10^{-5} M–1×10^{-2} M (RSD% = 0.9996). The detection limit was 1×10^{-6} M based on a signal-to-noise ratio of 3.

In conclusion, the present study has demonstrated the feasi-
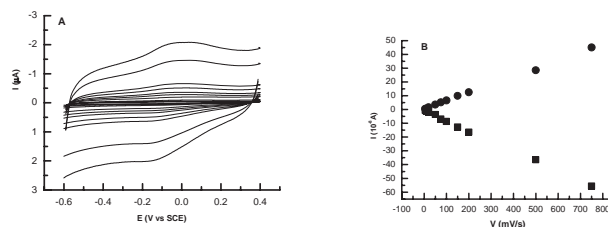

Figure 2. Cyclic voltammograms of HRP–AuNPs–vaterite modified GCE in 0.1 M and pH 7.0 of PBS at 10, 20, 50, 75, 100, 150, 200, 500, 750 mV/s (A), and the plot of peak current vs scan rate (B).

bility of the direct electrochemistry of HRP self-assembled on the core–shell composite of AuNPs–vaterite. The composite combined the advantages of mineral compound and nanoparticles. Though we cannot fully understand the mechanisms of the direct electrochemistry in this system, this knowledge may have good practical applications in the field of biosensors, enzyme immobilization, and other regions as well.

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