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Development of amperometric L-tyrosine sensor based on Fe-doped hydroxyapatite nanoparticles

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ABSTRACT

A novel biosensor based on Fe-doped hydroxyapatite (Fe-HA) nanoparticles and tyrosinase has been developed for the detection of L-tyrosine. Nanostructured Fe-HA was synthesized by a simple microwave irradiation method, and its phase formation, morphology and magnetic property were examined by powder X-ray diffraction (XRD), transmission electron microscopy (TEM) and vibrating sample magnetometer (VSM). Electrochemical performance of the nano Fe-HA/tyrosinase modified glassy carbon electrode (GCE) for detection of L-tyrosine was investigated by cyclic voltammetry (CV) and amperometric methods. The fabricated biosensor exhibited a linear response to L-tyrosine over a wide concentration range of 1.0×10^{-7} to 1.0×10^{-5} M with a detection limit of 245 nM at pH 7.0. In addition, the fabricated sensor showed an excellent selectivity, good reproducibility, long-term stability and anti-interference towards the determination of L-tyrosine.

1. Introduction

Hydroxyapatite [$Ca_{10}(PO_4)_6(OH)_2$], a main inorganic component of bone, has been extensively applied in biomedical implants, bone regeneration, drug delivery, protein separation and immunosensors owing to its excellent biocompatibility, bioactivity and multi-adsorbing sites [1]. Due to the excellent biocompatibility and adsorbability, nanostructured hydroxyapatite (HA) has been used for the detection of hydrogen peroxide [2], cyanide [3], carbon monoxide [4], carbon dioxide [5], phenolic compounds [6] and glucose [7]. Iron (Fe) doping into HA resulted in a strong ferromagnetic material, which found applications in the fields of magnetic resonance imaging, cell separation, drug delivery, and as heat mediators for hyperthermia treatment of cancers and tumor masses [8,9]. Several techniques have been reported to date for the synthesis of iron incorporated nano HA, which includes wet chemical, gel, sol–gel, hydrothermal and microwave methods [10–14]. In this work, we have prepared nano Fe-HA by microwave irradiation method.

L-Tyrosine, one of the amino acids, is indispensable for humans to establish and maintain nutritional balance. Its content in the human body directly reflects the healthy state of people [15,16]. The addition of tyrosine to dietary and food products or pharmaceutical formulations is sometimes necessary due to its limited presence in food material. Tyrosine acts as a precursor for dopa, dopamine, thyroxin, noradrenalin and adrenalin as the hormone or non-tuberculosis mycobacterial (NTM) in mammalian central nervous systems [17]. For instance, tyrosinemia, a congenital metabolic defect, is caused by a deficiency of fumarylacetoacetate hydrolase in the tyrosine degradation pathway. The absence of L-tyrosine could cause hypochondrium, depression and other psychological diseases. However, sister chromatid exchange increases due to a high concentration of L-tyrosine [18,19]. Therefore, the nutritional importance emphasizes the need for reliable analytical methods for the determination of tyrosine in food. Many methods have been applied for the measurement of tyrosine, including fluorometric methods, chemiluminescence, spectrometric analysis, high-performance liquid chromatography and capillary electrophoresis [20–24]. However, most of these methods suffer from some disadvantages, such as high costs, long analysis times and requirement for sample pretreatment. These disadvantages make them unsuitable for routine analysis. Electrochemical methods provide a simple, cost-effective and quick way of analyzing biologically and environmentally important molecules. Unfortunately, the direct electrochemical response of the amino acid at solid electrodes is usually poor. Therefore, chemically modified electrodes are employed to improve the anodic oxidation of amino acids. The most serious drawback of these modified metal electrodes is that they are subject to rapid surface fouling. This problem could be overcome by applying an appropriate enzyme at the electrode surface. Tyrosinase is a copper-containing enzyme protein that catalyzes the oxidation of phenol derivatives, such as tyrosine or tyramine, in the presence of oxygen, to the respective catechol derivatives, e.g., L-dopa or dopamine, which are further oxidized by the enzyme to the respective quinone products [25].

In this work, we have prepared nano Fe-HA by a simple, rapid and efficient microwave irradiation method. These nanoparticles modified glassy carbon electrodes provide a well-defined microenvironment for tyrosinase immobilization and enhanced the direct electron transfer between tyrosinase and electrodes. Interestingly, the electron transfer ability of the nano Fe-HA sample was found to be higher than that of

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undoped HA. The constructed biosensor displayed fast electron transfer and a good electrochemical activity for the detection of L-tyrosine with high stability and low detection limit. Therefore, the present work offers a new avenue to broaden the applications of nanostructured Fe-HA in electrochemical biosensors.

2. Experimental procedure

2.1. Preparation of HA and Fe-HA nanoparticles

Calcium hydroxide ($Ca(OH)_2$) and diammonium hydrogen orthophosphate ($(NH_4)_2HPO_4$) were obtained from Merck. Ferric chloride ($FeCl_3$) was purchased from Sisco Research Laboratory, Mumbai and used without further purification. The reaction mixture was prepared by adding a stoichiometric amount of $(NH_4)_2HPO_4$ aqueous solution (0.24 M) to an aqueous suspension of $Ca(OH)_2$ (0.4 M) under constant stirring conditions at room temperature (Eq. 1)

$$10Ca(OH)_2 + 6(NH_4)_2HPO_4 \rightarrow Ca_{10}(PO_4)_6(OH)_2 + 6H_2O + 12NH_4OH \quad (1)$$

The mixture was then exposed to microwave radiation (600 W) for 35 minutes, which resulted in a white powder. This sample was washed with deionized water and dried at 80 °C in a hot air oven. To prepare Fe-doped HA nanoparticles, the above experimental procedure was repeated by mixing $FeCl_3$ (1 M%) with 0.4 M $Ca(OH)_2$. The final product Fe-doped HA was found to be a light-orange powder.

2.2. Electrode preparation and modification

Tyrosinase (TY, T38 1.14.18.1, 3610 units/mg, from mushroom) was purchased from Sigma-Aldrich (St. Louis, MO). A glassy carbon electrode (GCE) was polished with alumina slurry (1.0 and 0.05 μm) and ultrasonically cleaned with 1:1 distilled water and ethanol. The modified electrodes were prepared by a simple drop casting method. The HA nanoparticles were dispersed into doubly-distilled water (5 mg/mL) by sonicating for about 20 min until a homogenous solution was obtained. Typically, 10 μL of HA nanoparticles suspension was mixed with 10 μL of tyrosinase (1 mg/mL, 0.1 M PBS; pH 7.0) solution. Then, 10 μL of the mixed dispersion was cast onto the GC electrode, dried at room temperature (Scheme 1). The electrode was rinsed in 0.1 M phosphate buffer saline (pH 7.0) to eliminate the unadsorbed tyrosinase and stored in pH 7.0 PBS at 4 °C when it was not in use.

2.3. Characterization

Powder X-ray diffraction (XRD) patterns were recorded using a Bruker AXS D8 advanced diffractometer in the 20 range of 20° to 80° (CuKα radiation; λ = 1.5406 Å). Surface morphology and particle size of Fe-HA were characterized by transmission electron microscopy (JEOL 2100 F) with an accelerating voltage of 200 keV. Magnetic measurements were carried out using a Lakeshore VSM 7410 vibrating sample magnetometer (VSM). X-ray photoelectron spectra were recorded at room temperature by using ESCA 3400 apparatus with an Mg Kα (1253.6 eV) X-ray source. All electrochemical measurements were carried out on CHI 608D electrochemical workstation (CH Instruments, Austin, TX) at room temperature. A three-electrode cell was used with a modified glassy carbon electrode (GCE, 3 mm dia) as the working electrode, Ag/AgCl (3 M KCl) as the reference electrode and a platinum wire electrode as the counterelectrode. Cyclic voltammograms (CVs) were recorded between a potential window of −0.2 V and 0.6 V at a scan rate of 50 mV/s in 1 M KCl containing 1.0 mM $[Fe(CN)_6]^{3-/4-}$ redox couple. The electrochemical impedance spectroscopy (EIS) measurements were made by applying AC potential of amplitude 5 mV over the DC potential of 250 mV in the frequency range 100 kHz to 1 Hz. The amperometric measurement was performed in stirred 0.1 M PBS solution of pH 7.0 by applying desired potential to the working electrode and by allowing the steady state current to be reached.

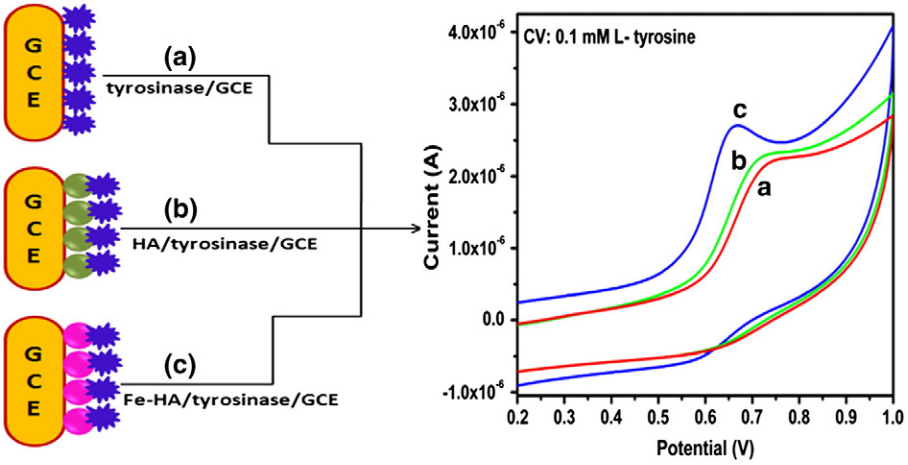
3. Results and Discussion

3.1. Structural analysis

Powder X-ray diffraction (XRD) analysis of nano Fe-HA revealed that the system crystallizes in the hexagonal structure with space group $P6_3/m$ (ICDD card no. 09–0432). The XRD patterns are shown in Fig. 1. Average crystallites size of the products was calculated by using Scherrer's equation (Eq. 2).

$$D = K\lambda/\beta\cos\theta \quad (2)$$

where K is the shape factor (0.9), β is the full-width at half-maximum (fwhm) of diffraction peaks ($h\ k\ l$) measured in radians, λ is the wavelength of the X-rays (λ = 1.5406 Å) and θ is Bragg's diffraction angle. The average crystallite size of undoped HA and Fe-HA were found to be 22.87 and 18.85 nm, respectively. The reduced crystallite size and a small upward shift in peak positions suggest that the dopant Fe^{3+} (ionic radius 0.645 Å) substitutes at the Ca^{2+} (ionic radius 0.99 Å) site in the HA system.



Scheme 1. L-Tyrosine detection scheme using Fe-HA/tyrosinase modified GCE.