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Xanthine oxidase/laponite nanoparticles immobilized on glassy carbon electrode: Direct electron transfer and multielectrocatalysis

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ABSTRACT

In this work, colloidal laponite nanoparticles were further expanded into the design of the third-generation biosensor. Direct electrochemistry of the complex molybdoenzyme xanthine oxidase (XnOx) immobilized on glassy carbon electrode (GCE) by laponite nanoparticles was investigated for the first time. XnOx/laponite thin film modified electrode showed only one pair of well defined and reversible cyclic voltammetric peaks attributed to XnOx–FAD cofactor at about −0.370 V vs. SCE (pH 5). The formal potential of XnOx–FAD/$FADH_2$ couple varied linearly with the increase of pH in the range of 4.0–8.0 with a slope of −54.3 mV pH^{-1}, which indicated that two-proton transfer was accompanied with two-electron transfer in the electrochemical reaction. More interestingly, the immobilized XnOx retained its biological activity well and displayed an excellent electrocatalytic performance to both the oxidation of xanthine and the reduction of nitrate. The electrocatalytic response showed a linear dependence on the xanthine concentration ranging from 3.9×10^{-8} to 2.1×10^{-5} M with a detection limit of 1.0×10^{-8} M based on S/N = 3.

1. Introduction

Xanthine oxidase catalyzes the oxidation of hypoxanthine to xanthine and can further catalyze the oxidation of xanthine to uric acid. Thus, this enzyme plays an important role in the catabolism of purines in some species, including human (Hille, 2005; Harrison, 2002). Since hypoxanthine and xanthine are the key indicators for estimating fish freshness and human health, XnOx has been implicated as a key oxidative enzyme by electrochemists to construct electrochemical sensors for the determination of hypoxanthine and xanthine, providing a simple, fast and reliable proposed systems (Kilinc et al., 1998; Rahman et al., 2007; Villalonga et al., 2007; Kirgöz et al., 2004; Zhao et al., 1996; Shan et al., 2009a; Shan et al., 2009b). Generally, the electrochemical detection of hypoxanthine or xanthine was based on the electrochemical oxidation of the enzymatically generated H_2O_2 and uric acid, or the electrochemical reaction of the introduced redox mediators. Obviously, the analytical performance was not satisfactory enough, especially due to the poor selectivity and stability.

The most promising approach for the development of electrochemical biosensors is to establish a direct electrical communication between the biomolecules and the electrode surface (Freire et al., 2003), which can avoid the use of redox mediators, thus reducing side reactions and potential interferences, as well as being more compatible with in vivo conditions. Nevertheless, the direct electron transfer reaction of XnOx is still very difficult to be achieved due to the deeply hided electro-active centers, thus rare reports appeared in the literatures (Bernhardt et al., 2006; Wang and Yuan, 2004; Zhou et al., 2006; Wu and Hu, 2007). Furthermore, previous attempts at direct electrochemically driven catalysis of this enzyme have been ineffective, due to the protein denaturation and unfavorable interactions (Bernhardt et al., 2006; Wang and Yuan, 2004).

Direct electron transfer of protein depends strongly on the immobilization procedure, the nature of the support, and the properties and the stability of the biomolecule (Freire et al., 2003). Clay colloid provides a favorable microenvironment for electron transfer and catalytic reactions on electrode (Shumyantseva et al., 2005; Liu et al., 2005a,b). In the literature, there are several reports on direct electrochemistry based on clay–protein modified electrode (Sun, 2006; Zhou et al., 2002; Li and Hu, 2003; Shan et al., 2007).

Laponite (hydrous sodium lithium magnesium silicate) is an entirely synthetic crystalline layered silicate colloid with crystal structure and composition closely resembling the natural smectite clay hectorite (Cummins, 2007). Individual particles are disk-shaped crystals, 25 nm in diameter and 1 nm in thickness, with chemical composition of $(Mg_{5.5}Li_{0.5})Si_4O_{10}(OH)_2(Na^+_{0.73}{\cdot}nH_2O)$,

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significantly smaller than naturally occurring clays (Van Olphen, 1977). Within a single crystal, each sheet of octahedrally coordinated aluminum or magnesium oxide is sandwiched between two layers of tetrahedrally coordinated silica. An isomorphic substitution of magnesium by lithium atoms generates negative charge on its surface, which is counterbalanced by the positive charge of the sodium ions present in the interlayer. In an aqueous medium, sodium ions dissociate which, in turn, leads to a net negative charge on its surface. The edge of the laponite particle is composed of hydrous oxide, and depending on the pH of the solution, it appears that the sides of the disks can be charged positively (Van Olphen, 1977; Bonn et al., 1999). Its suspension in the aqueous medium leads to the formation of nonergodic soft solids that exhibit a rich variety of physical behavior (Joshi et al., 2008). Thus, laponite has been successfully used in the past as cast films onto electrodes for immobilization of redox proteins (Cosnier and Le Lous, 1996; Senillou et al., 1999; Mousty et al., 2001; Shan et al., 2002, 2003; Cosnier et al., 2006; Fan et al., 2007; Shan et al., 2003; Shi et al., 2008).

In this work, we continue to take advantage of the merit of laponite described above and attempt to design a novel third-generation biosensor based on immobilization of XnOx with colloidal laponite nanoparticles. Direct electron transfer and bioelectrocatalytic activity of immobilized protein were investigated by cyclic voltammetry and amperometry techniques. The XnOx/laponite film exhibits the feasibility in electrochemical catalysis towards xanthine and nitrate.

2. Experimental

2.1. Materials

Xanthine oxidase (XnOx) (EC1.1.3.22, from microbial source, 8.1 U mg^{-1}) was obtained from Sigma. Laponite is a synthetic hectorite (monovalent cation exchange capacity: CEC = 0.74 mmol g^{-1}) purchased from Laportes Industries.

All other reagents were of analytical grade and used as received without further purification. All aqueous solutions were prepared in deionized distilled water. Phosphate buffer solution (PBS) was 0.1 M Na_2HPO_4 and NaH_2PO_4 and its pH was adjusted with H_3PO_4 or NaOH solutions.

2.2. Measurements and apparatus

Atomic force microscopy (AFM) images were obtained with a multimode digital instrument (Vecco-DI). Static contact angle measurements were performed at 20 °C with a sessile drop method using an OCA 40 system (German). A droplet of deionized water (3 μl) or diiodomethane, CH_2I_2 (1.5 μl) was gently placed onto the surface. The angle between the edge of the droplet and the surface was measured. Measurements were made in different regions on the surface. A CHI 660 electrochemical workstation (CHI Co., USA) was used for cyclic and amperometric voltammetry. All electrochemical studies were performed with a conventional three-electrode system. A saturated calomel electrode (SCE) and a Pt foil electrode were used as reference electrode and counter electrode, respectively. The working electrode was a glassy carbon electrode (GCE) (diameter 3 mm), polished carefully with 0.05 μm alumina particles on silk followed by rinsing with the distilled water and dried in air before use. Unless otherwise indicated, electrolytic solutions were purged with highly purified nitrogen for at least 20 min prior to the series of experiments. The electrochemical experiments in aqueous solutions were performed under a nitrogen atmosphere.

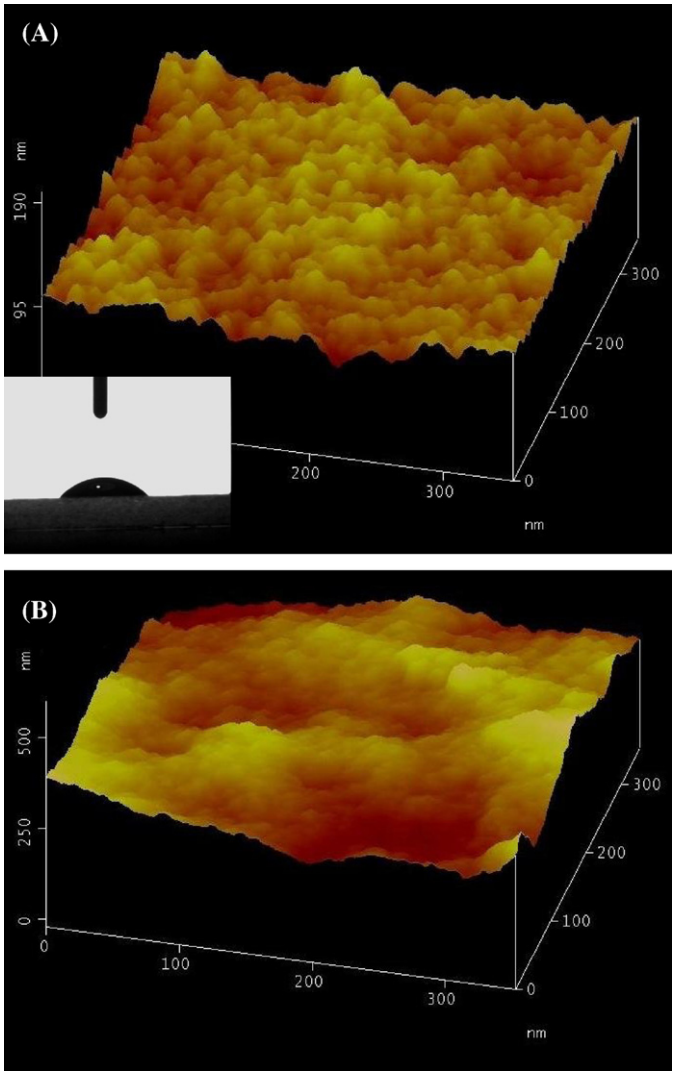

Fig. 1. Tapping mode AFM topography images of: (A) laponite film and (B) XnOx/laponite (w/w, 3/4) film on glassy carbon. Inset (A) shows the image of the initial droplet on laponite film.

2.3. Preparation of enzyme electrodes

The laponite colloidal suspension (2 mg ml^{-1}) was prepared by dispersing laponite in the deionized water with stirring overnight. XnOx was also dissolved in deionized water with a concentration of 2 mg ml^{-1}. A defined amount of aqueous XnOx/laponite mixture (for instance, containing 12 μg XnOx, 16 μg laponite) was spread on the surface of GCE. The mixture was dried in air at room temperature, leading to an adherent laponite film in which enzymes were entrapped. Before use, the enzyme electrode was rinsed under stirring for 20 min with buffer solution to remove the enzyme not firmly immobilized.

3. Results and discussion

3.1. Atomic force microscopy characterization

Topographical characterization was conducted using AFM in tapping mode. Fig. 1 shows surface topography images of laponite thin film and XnOx/laponite thin film, respectively. Fig. 1 A reveals the characteristic rag-like surface with domains of ridges and valleys formed by the deposition and the imperfect stacking of colloidal laponite nanoparticles on glassy carbon. The thickness of this