Biosensors and Bioelectronics 24 (2009) 1171–1176

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Development of a high analytical performance-xanthine biosensor based on layered double hydroxides modified-electrode and investigation of the inhibitory effect by allopurinol

Dan Shan[a,*], Yanna Wang[a], Mingjuan Zhu[a], Huaiguo Xue[a,*], Serge Cosnier[b], Chengyin Wang[a]

[a] College of Chemistry & Chemical Engineering, Yangzhou University, Si Wangting Road 180, Jiangsu 225002, China
[b] Département de Chimie Moléculaire UMR-5250, ICMG FR-2607, CNRS Université Joseph Fourier, BP-53, 38041 Grenoble, France

ARTICLE INFO

Article history:
Received 25 March 2008
Received in revised form 6 June 2008
Accepted 1 July 2008
Available online 23 July 2008

Keywords:
Xanthine oxidase
Layered double hydroxides
Xanthine
Allopurinol
Inhibition
Biosensor

ABSTRACT

The determination of xanthine has considerable importance in clinical and food quality control. Therefore, in this present work, we developed a novel xanthine biosensor based on immobilization of xanthine oxidase (XnOx) by attractive materials layered double hydroxides (LDHs). Amperometric detection of xanthine was evaluated by holding the modified electrode at 0.55 V (versus saturated calomel electrode (SCE)). Due to the special properties of LDHs, such as chemical inertia, mechanical and thermal stability, anionic exchange ability, high porosity and swelling properties, XnOx/LDHs-modified electrode exhibited a developed analytical performance. The biosensor provided a linear response to xanthine over a concentration range of 1×10^{-6} M to 2×10^{-4} M with a sensitivity of 220 mA M^{-1} cm^{-2} and a detection limit of 1×10^{-7} M based on S/N = 3. In addition, the immobilized XnOx layers have been characterized using atomic force microscopy under both air atmosphere and liquid environment, which exhibited the interesting swelling phenomenon of LDHs. The investigation of inhibition of XnOx by allopurinol was carried out using this XnOx/LDHs-modified electrode. The experimental results indicated that inhibitory effect could be achieved by allopurinol with a quasi-reversible competitive type.

1. Introduction

Xanthine (3,7-dihydro-1H-purine-2,6-dione) is a degradation product of purine derivatives; it can penetrate cell membranes and accumulate in extracellular fluids (Metzler, 1997). The concentration level of xanthine in blood, plasma and urine may provide sensitive indicators of certain pathologic states, especially for xanthinuria (Amigo et al., 2005). Xanthinuria is a rare genetic disorder where the lack of xanthine oxidase (XnOx) leads to high concentration of xanthine in body fluids and can cause heath problems such as renal failure. Besides, the level of xanthine is generally used in the food industry as an index for evaluating the freshness of meat or fish. Hence the quantity of xanthine is of clinical and industrial significance.

A variety of analytical methods for determining xanthine and hypoxanthine have been reported to date. The most common analytical method for detecting and quantifying xanthine is high performance liquid chromatography (HPLC) (Czauderna and Kowalczyk, 1997; Cooper et al., 2006) or high performance capillary electrophoresis (HPCE) (Chen et al., 2002; Caussé et al., 2007). The main disadvantages of these methods are the complexity and cost of the equipments needed that required skilled operators as well as the time of sample treatment and labor intensive measurement protocols. Moreover, these centralized analytical techniques are not adapted to real time monitoring and in situ continuous monitoring. In contrast, biosensors appear as a suitable alternative ensuring attractive advantages such as its ease of use in turbid samples, portability, low cost and sensitivity. In particular, XnOx-based electrochemical biosensors have been frequently used for the determination of xanthine, providing a simple, fast and reliable proposed system (Zhao et al., 1996; Kirgöz et al., 2004; Liu et al., 2004; Casero et al., 2006; Villalonga et al., 2007; Rahman et al., 2007). XnOx (EC.1.1.3.22) is a complex metalloprotein involved in purine catabolism, catalyzing the oxidation of hypoxanthine to xanthine and further catalyzing the oxidation of xanthine to uric acid (UA) and hydrogen peroxide, with concomitant reduction of oxygen (Harris and Massey, 1997). As a consequence, the amperometric detection of xanthine was conventionally based on the electrochemical oxidation of the enzymatically generated H_2O_2 and uric acid. Nevertheless, the analytical performance to xanthine is still not satisfactory enough. Due to the fragility of the protein, the sensitivity and operational stability remained markedly lower than those recorded for other oxidase-based biosensors.

* Corresponding authors. Tel.: +86 514 87975590x9519; fax: +86 514 87975244.
E-mail addresses: danshan@yzu.edu.cm (D. Shan), chhgxue@yzu.edu.cn (H. Xue).

0956-5663/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.bios.2008.07.023

To obtain stable and sensitive biosensors, the use of layered double hydroxides (LDHs) is very attractive. LDHs have been demonstrated as promising materials due to their technological importance in catalysis, separation, optics, medical science and nanocomposite materials engineering (Choy et al., 1999; Garcia-Ponce et al., 2000; Crepaldi et al., 2000). LDHs display a layered structure built on a stacking of positive layers ($[M_{1-x}^{II}M_x^{III}(OH)_2]^{x+}$ (De Roy et al., 1992). The electroneutrality of the system can be realized by the presence of exchangeable anions accompanied with water molecules situated in the interlamellar domains. LDHs have been also successfully utilized as the attractive enzyme immobilization matrix in our previous works (Shan et al., 2003a,b, 2004, 2006; Han et al., 2007). Their unusual intercalation properties were exploited for an easy and biocompatible immobilization of enzyme molecules. The procedure was based on a delaminating process of the clay material into dispersed nanoparticles, their mixing with enzyme and the adsorption of both clay nanoparticles and proteins on an electrode surface. To reinforce the resulting inorganic biocoating, glutaraldehyde was added inducing a partial covalent binding between adjacent enzyme molecules (Mousty, 2004). In this present work, XnOx was first immobilized with LDHs via cross-linking procedure by glutaraldehyde. The analytical characteristics of the resulting biosensor for the amperometric detection of xanthine were investigated.

Since allopurinol is a structural isomer of hypoxanthine and acts as an inhibitor for the XnOx activity, the inhibitory effect of allopurinol on the immobilized XnOx was also examined in detail for the further comprehensive study on the XnOx/LDHs-xanthine sensing system.

2. Experimental

2.1. Materials

Xanthine oxidase (EC1.1.3.22, from microbial source, 8.1 U mg^{-1}) was obtained from Sigma. Ferrocenemethanol (MeOHFc) was purchased from Alfa Company. LDHs $Zn_3Al(OH)_8Cl$, denoted [Zn_3–Al–Cl] was synthesized by coprecipitation method developed by De Roy (De Roy et al., 1992; Shan et al., 2006). The colloidal suspension of LDHs was prepared in deionized and decarbonated water. Other aqueous solutions were prepared in deionized distilled water.

2.2. Apparatus

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) was used as reference electrode, and a Pt foil as counter electrode. The working electrode was a platinum disk electrode (diameter 2 mm), polished carefully with 0.05 μm alumina particles on silk followed by rinsing with distilled water and dried in air before use. All measurements were carried out in a thermostated cell, containing phosphate buffer solution. Atomic force microscopy (AFM) images were obtained with a multimode digital instrument (Vecco-DI). SEM photos were realized on a XL-30E apparatus. 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 (2 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. Pt foil was used as the substrate for AFM, SEM and contact angle measurements.

2.3. Construction of biosensor and enzyme immobilization

The colloidal suspension of LDHs (2 mg ml^{-1}) was prepared by dispersing LDHs in deionized and decarbonated water stirring overnight. XnOx was also dissolved in deionized and decarbonated water with concentration of 2 mg ml^{-1}. A defined amount of the aqueous mixture (for instance, containing 26.6 μg XnOx and 13.3 μg LDHs) was spread on the surface of the platinum disk electrode. The coating was dried in air at room temperature. The resulting electrode was then placed in saturated glutaraldehyde vapor for 15 min for the cross-linking of the membrane. The LDHs gel formation was achieved by incubation of the modified electrode into phosphate buffer solution (0.05 M, pH 7.5) for 20 min. The bioelectrode was stored at 4 °C in a refrigerator when not used.

2.4. Inhibitive measurement procedure

The inhibitory action of allopurinol on the enzymatic activity of XnOx was followed by consideration of the oxidation of xanthine (substrate), catalyzed by XnOx, whose electrochemical oxidation was monitored amperometrically at +0.55 V. The addition of allopurinol causes inhibition of enzyme, consequently decreasing the amount of liberated enzymatic reaction products. The procedure for the evaluation of the effect of the inhibition of enzyme activity as a function of allopurinol concentrations on the biosensor response includes the following steps:

(1) The biosensor was soaked in the phosphate buffer solution for 20–30 min until a stable baseline output signal was reached.
(2) Determine the initial current response (I_1) of the biosensor to xanthine solution of a given concentration.
(3) Determine the current response (I_2) of the biosensor to xanthine solution of the same concentration in the presence of a certain concentration allopurinol.

The degree of inhibition (In %) is then calculated by comparing the current responses before and after addition of inhibitor according to the equation generally used (Tencaliec et al., 2006):

$$\mathrm{In}\,(\%) = \frac{I_1 - I_2}{I_1} \times 100$$

Each experiment was repeated at least three times and a relative standard deviation (R.S.D.) of the output signal was estimated to be not more than 5%. The control experiment for xanthine without the inhibitor was performed after each inhibitor measurement.

3. Results and discussion

3.1. Morphologies of XnOx/LDHs film

The surface morphologies of XnOx/LDHs films were investigated with SEM and AFM. Inset of Fig. 1A shows SEM image of XnOx/LDHs film. Compared with the morphology of pure LDHs reported previously (Shan et al., 2006), the LDHs particles with hexagonal shape are still observable with the enzyme adsorbed on the external surface of the layers. This typical mesoporous structure is bond to favorable to substrate diffusion to reach the immobilized enzyme within the whole LDHs matrix (Shan et al., 2006). However, SEM images can only provide two-dimensional information about XnOx/LDHs film.

AFM has achieved particular relevance as a surface characterization technique, which provides morphological and even mechanical information at the nanometer level. Since the development of tapping operation mode under aqueous environment, AFM has

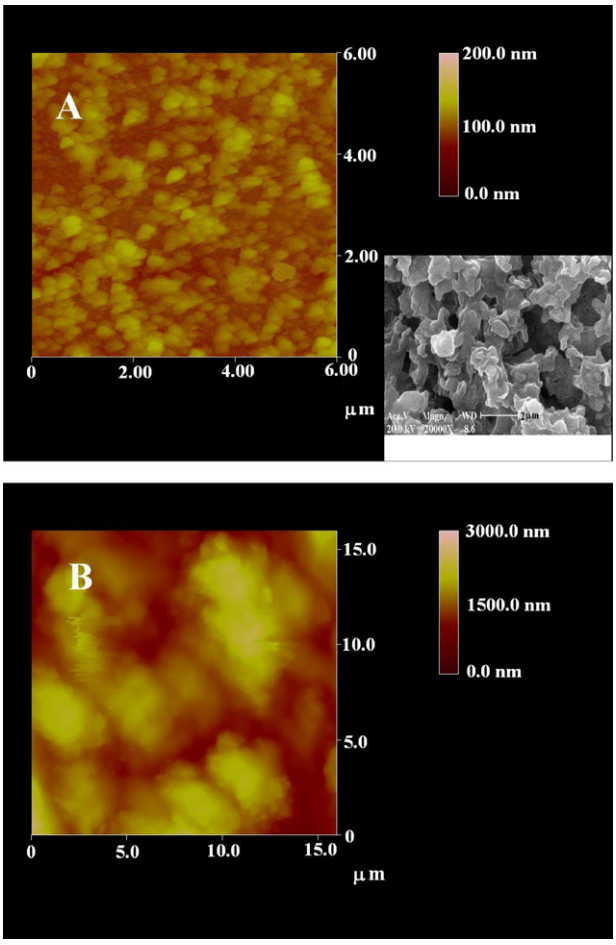

Fig. 1. Tapping mode AFM images of XnOx/LDHs film on a glass side (A) under air environment and (B) under liquid environment. Inset of Fig. 1 (A) displays SEM photograph of XnOx/LDHs (w/w, 2/1) film.

been able to provide morphological data of protein deposits without damaging the sample surface (Casero et al., 2006). Therefore, as shown in Fig. 1, we used AFM with tapping mode to determine three-dimensional surface morphologies of XnOx/LDHs films under both air and liquid environment of the same XnOx/LDHs film. The surface morphology of XnOx/LDHs film obtained under air atmosphere with tapping mode depicts distributed grain and a network-like structure (Fig. 1A). The roughness of XnOx/LDHs is 10 nm and the depth is about 110 nm. While, under aqueous environment, the surface morphology displays loose cotton-like structure, with roughness and depth, 365 nm and 1.039 μm, respectively (Fig. 1B). The increased roughness and depth of XnOx/LDHs compared with those obtained under air atmosphere can be ascribed to the swelling properties of LDHs in aqueous environment. The LDHs are swollen; the immobilized enzyme is surrounded by water. Rubio-Retama et al. demonstrated in their work that the behavior of the enzyme immobilized in the swollen gel is similar to the enzyme in solution (Rubio-Retama et al., 2005). So the bioactivity of the immobilized XnOx can be well maintained in this hydrophilic microenvironment.

3.2. Contact angle measurement

The static contact angle measurements give additional information on the physical properties of XnOx/LDHs film. The contact angels of pure LDHs and XnOx/LDHs were measured to be $34.3 \pm 2^\circ$ and $71.1 \pm 3^\circ$, respectively. The inset of Fig. 2 displays the image of the initial droplet on XnOx/LDHs film. The increased value of contact angle of XnOx/LDHs is due to the presence of enzyme molecules exhibiting hydrophobic groups. Both contact angle values are indicative for a hydrophilic surface. It should be noted that both contact angles decrease linearly in 40 s and then achieve the plateau of value about 17° and 25°, for pure LDHs and XnOx/LDHs, respectively (Fig. 2). This phenomenon indicates the high adsorbability to water of XnOx/LDHs.

3.3. Optimization of detection test variables

The pH dependence of the XnOx/LDHs (26.6 μg:13.3 μg) was investigated over the pH range 6–9 in 0.05 M PBS (containing 2 mM EDTA) in the presence of 200 μM xanthine. The optimum response current was found at pH 7.5. This optimum value was situated in the pH range (7–8.5) reported for the free XnOx from microbial source (Xue and Mu, 1995; Balladin et al., 1997; Foppoli et al., 1997; Egwim et al., 2005). This indicates that the immobilization procedure have not altered the optimum pH of XnOx. Therefore, PBS at pH 7.5 was selected as the electrolyte in subsequent experiments.

The dependence of the biosensor on the applied potential for amperometric signal of the XnOx/LDHs (26.6 μg:13.3 μg) sensor was also studied in this work. The biosensor response to 200 μM xanthine increased with increasing applied potential from 0.30 V to 0.55 V and then reached a pseudo-plateau for higher potentials. Taking into account the sensitivity of the biosensor and interference of easily oxidizable compounds present in serum, 0.55 V was selected as the best compromise of applied potential in subsequent experiments.

3.4. Enzyme electrode response characteristics

Fig. 3 shows the typical calibration curve of XnOx/LDHs bioelectrode for the determination of xanthine under the optimal conditions (XnOx/LDHs 26.6 μg: 13.3 μg, pH 7.5, $E = 0.55$ V). The biosensor response to xanthine is linear between 1×10^{-6} M and 2×10^{-4} M. A low detection limit of 0.1 μM xanthine was determined at a signal-to-noise ratio of 3, which is slightly lower than those reported by the conventional determination method of HPLC (3.2 μM) (Czauderna and Kowalczyk, 1997) (0.2 μM) (Cooper et al., 2006), and HPCE (0.5 μM) (Caussé et al., 2007). The xanthine sensitivity is 220 mA M^{-1} cm^{-2} (6.6 mA M^{-1}). This sensitivity for

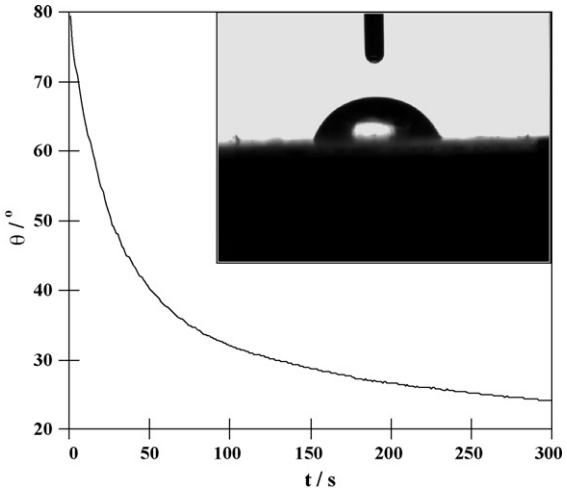

Fig. 2. Static contact angles vs. time. Inset shows the image of the initial droplet on XnOx/LDHs (w/w, 2/1) film.

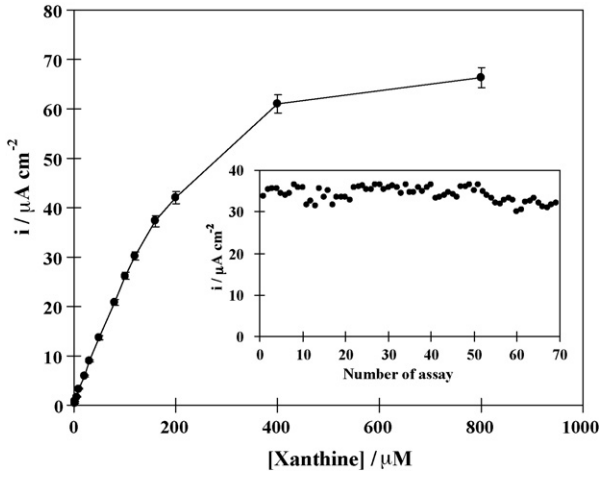

Fig. 3. Calibration curve of XnOx/LDHs (w/w, 26.6 μg/13.3 μg) electrode for xanthine, under the optimum conditions (0.05 M PBS (pH 7.5) containing 2 mM EDTA, E_{app} = 0.55 V, at 35 °C). Insets show the operational stability of the biosensor to 200 μM xanthine.

xanthine is much higher (about 27 times) than that of xanthine biosensor constructed via supramolecular associations (Villalonga et al., 2007), and higher than those based on conducting polymer, poly-TTCA (5.35 mA M^{-1}) (Rahman et al., 2007) and XnOx immobilized in polypyrrole/Au-colloid (5.01 mA M^{-1}) (Liu et al., 2004). The apparent Michaelis–Menten constant (K_M^{app}), calculated from Lineweaver–Burk equation, is 263 μM. Such drastic improvement in biosensor performance reflects the expected beneficial effect of the biocompatible LDHs matrix on the activity of the entrapped XnOx. In contrast, the organic polymers constitute a hydrophobic host matrix while the supramolecular assemblies require the chemical modification of the XnOx molecules. In addition, LDHs-enzyme coatings exhibit a higher permeability than conventional electropolymerized films (Cosnier et al., 2006). As previously reported for cyanine, tetraborate or As(V) anion, a possible accumulation of anionic species can occur within LDHs matrix (Cosnier et al., 2006; Shan et al., 2004; De Melo et al., 2002). This phenomenon may facilitate the electrochemical oxidation of the enzymatically generated uric acid and hence improve the biosensor sensitivity.

The biosensor reproducibility was tested with eight different electrodes. The R.S.D. to xanthine determination was 4.5%. The operational stability of the XnOx/LDHs electrode was examined. A reproducible current response with a R.S.D. of 5.0% was observed in 70 successive assays of 200 μM xanthine (inset of Fig. 3). The long-term stability of the XnOx/LDHs electrode stored at 4 °C was investigated by recording periodically its current response to 200 μM xanthine. The biosensor exhibited excellent stability. No significance loss of activity of the immobilized enzyme was observed during 15 days; it still retained about 80% of its original response after 52 days of storage. This biosensor stability is superior to XnOx-CMC-CD immobilized on ADA-Au electrode constructed via supramolecular associations (21 days) (Villalonga et al., 2007). Good long-term stability of the XnOx/LDHs-modified electrode can be attributed to the enzyme immobilization matrix LDHs, which provided a friendly hydrophilic microenvironment for the immobilized XnOx.

The influence of the presence of easily oxidizable endogeneous and exogeneous interferents at their physiological normal levels on the biosensor response to xanthine (200 μM) was also examined. Their interferences were estimated as a percentage of increase in the biosensor signal recorded in the presence of interferents, compared to the steady-state response to 200 μM xanthine alone. Ascorbic acid (AA) (0.1 mM) and uric acid (0.1 mM) are the major interferents leading to 25% and 30% interference, respectively, due to a combination of their high concentration and low oxidization potential.

3.5. Inhibitory effect of allupurinol

Allopurinol is a specific potent inhibitor for XnOx and can be clinically used for gout treatment (Hsieh et al., 2007). Thus, for the further comprehensive study on the XnOx/LDHs-xanthine sensing system, we evaluated the inhibitory effect by allopurinol.

The electrochemical behavior of newly constructed XnOx/LDHs electrode was tested in a MeOHFc solution (0.1 mM). In the potential range of 0–0.4 V, there are well-defined anodic and cathodic peaks (ΔE_p = 78 mV, at the scan rate of 10 mV s^{-1}) with the mean potential $E_{1/2} = (E_{pa} + E_{pc})/2$ at 0.194 V (curve a in Fig. 4). It corresponds to the reversible one electron oxidation of MeOHFc to $MeOHFc^+$ (Shan et al., 2007a). A significant increase of the anodic current was observed after 200 μM xanthine being introduced to the buffer (curve b in Fig. 4). MeOHFc was electrochemically oxidized to $MeOHFc^+$ but readily reduced by enzymatic reaction in the presence of xanthine in solution, giving rise to a typical catalytic curve shape. Upon the addition of allopurinol to the test solution containing xanthine, the catalytic current decreased dramatically (curve c in Fig. 4). This phenomenon is indicative of the effective inhibitory of the immobilized XnOx by allopurinol.

The reversibility of inhibiting action was tested and the corresponding experimental results were summarized in inset A of Fig. 4. The current response of the XnOx/LDHs bioelectrode to 200 μM xanthine was 1.32 μA with a rapid response time less than 8 s. While, the presence of 100 μM allopurinol induced a strong inhibitory effect on the biosensor response and the response to 200 μM xanthine decreased to 413 nA, the response time was pro-

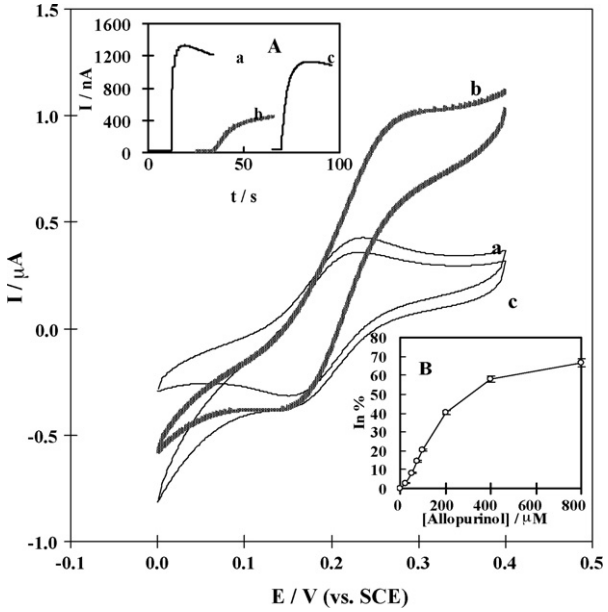

Fig. 4. Cyclic voltammogram of Pt electrode modified by XnOx/LDHs (w/w, 26.6 μg/13.3 μg) bioelectrode in 0.05 M PBS (pH 7.5) containing 0.1 mM ferrocenemethanol (a) in the absence (b) in the presence of 200 μM xanthine, (c) in the presence of 200 μM xanthine and 0.1 mM allopurinol (ν = 10 mV s^{-1}, A = 0.03 cm^{-2}). Inset A: current–time response of XnOx/LDHs (w/w, 26.6 μg/13.3 μg) bioelectrode to 200 μM xanthine (a), 200 μM xanthine+0.1 mM allopurinol (b) and another response of electrode to 200 μM xanthine after communicating with allopurinol and rinsing with PBS simple (c) in PBS with pH 7.5 at 25 °C. Applied potential, 0.55 V. Inset B: inhibition calibration curve for allopurinol determination with XnOx/LDHs (w/w, 26.6 μg/13.3 μg) bioelectrode, the concentration of xanthine fixed as 200 μM.

longed to 20 s. Then, the XnOx/LDHs bioelectrode was taken out and rinsed with PBS for 10 min. The response current to xanthine with the same concentration was finally investigated. The data indicated the biosensor response to 200 μM xanthine retain 85% of original response. This behavior suggests that the inhibition of allopurinol to XnOx is "quasi-reversible" and be quite different to those reported for free XnOx (irreversible) (Pacher et al., 2006; Galbusera et al., 2006). Allopurinol can be rapidly oxidized by XnOx to its active metabolite oxypurinol, which also inhibits XnOx (Pacher et al., 2006). Generally, allopurinol and xanthine bind to the same oxidized Mo^{VI}; oxypurinol binds to the reduced Mo^{IV}. The reduced XnOx–oxypurinol complex then slowly rearranges into a tightly bound inhibitory complex. Oxypurinol dissociate slowly from XnOx–oxypurinol complex, which induces the irreversible inhibition (Galbusera et al., 2006). However, the quasi-reversible behavior in our work might due to the high and specific sensitivity of the immobilized XnOx to xanthine. Xanthine can occupy the enzyme active site dominantly, which is not favorable for the oxidation of allopurinol by the immobilized XnOx and furthermore the amount of oxypurinol was reduced.

A typical inhibition calibration curve for the determination of allopurinol is shown in inset B of Fig. 4. XnOx inhibition increased significantly with the addition of allopurinol, which decreased the production of uric acid by inhibiting the action of XnOx. A maximum inhibition of about 70% was obtained for 0.8 mM allopurinol. The linear range of allopurinol concentration is obtained up to 200 μM. IC_{50}, i.e. the concentration of the inhibitor corresponding to 50% of the inhibition signal was calculated to be 310 μM from the data of inset B. This value is quite larger than that reported IC_{50} value of 0.2–50 μM for free XnOx (Pacher et al., 2006). It indicates that the affinity to allopurinol of XnOx/LDHs is not strong. These results corroborate our hypothesis mentioned above.

3.6. Effect of temperature on the inhibition of allopurinol

The effect of temperature on the biosensor was one of the most important parameter that affected analytical performance of the biosensor. At the applied potential of +0.55 V, the influence of temperature on the response currents of the XnOx/LDHs to 200 μM xanthine solution containing 100 μM allopurinol is shown in curve b of Fig. 5. In the temperature range 10–45 °C, the response current of biosensor increased with the increment of temperature and the maximum current was obtained at 40 °C. While, in the absence of allopurinol, the maximum response current appears at about 60 °C (curve a of Fig. 5). The temperature of 60 °C for the optimal bioactivity is higher than the previous reports (Kirgöz et al., 2004; Rahman et al., 2007). The improved thermal stability of immobilized XnOx can be attributed to the microenvironment of LDHs-gel on the platinum electrode surface, which can protect the secondary structure of protein against denaturation by thermal treatment process. Thus, XnOx in microenvironment of LDHs-gel at platinum electrode has better thermal stability. The Arrhenius plot for xanthine biosensor without inhibition shows two clearly different regions intersect at 25 °C (curve a of inset). The region corresponding to the low temperatures presents the greater activation energy 21.6 kJ mol^{-1} (R^2 = 0.9918), whereas the activation energy at high temperature was 8.2 kJ mol^{-1} (R^2 = 0.943). Such Arrhenius plot have already been reported for other enzyme electrodes and have been ascribed to changes in the medium surrounding the enzyme (Cosnier et al., 2001; Rubio-Retama et al., 2005; Shan et al., 2007b; López et al., 2007). In the presence of allopurinol, the apparent activation energy of the biosensor is slight higher (30.5 kJ mol^{-1}) (R^2 = 0.9923) and the activity of immobilized XnOx was inhibited, which might result in the shift of the maximum response from 60 °C to 40 °C.

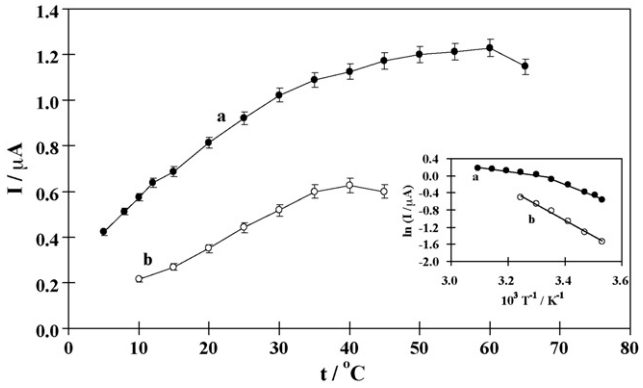

Fig. 5. The effect of temperature on the biosensor response current of the biosensor to 200 μM xanthine (a), 200 μM xanthine + 100 μM allopurinol (b) in 0.05 M PBS (pH 7.5) containing 2 mM EDTA, E_{app} = 0.55 V. Inset shows the relationship between in i and T^{-1}.

3.7. Kinetic study of XnOx inhibitory by allopurinol

It is well known that the preparation procedure of a sensor affects the substrate as well as inhibitor kinetics of an enzyme. Thus, in this present work, the kinetic study of the inhibitory for allopurinol to the XnOx-based biosensor was also carried out. The inhibition mechanism can be studied by examining the relationship between the response of the sensor to the inhibitor and to the substrate concentration. The calibration curves to xanthine for the XnOx-based bioelectrode, recorded in the absence and in the presence of the investigated inhibitors allopurinol, were interpreted using the Lineweaver–Burk plots (Fig. 6). Finally, the parameters describing the inhibition process were estimated using I_{max} values and K_M^{app} values. The maximum current has practically the same value, irrespective of inhibitors presence or absence, while the K_M^{app} value increased linearly with increase in inhibitor concentration

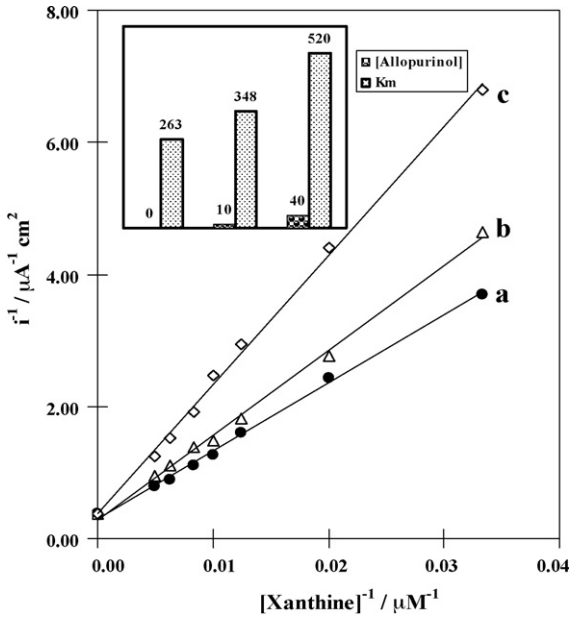

Fig. 6. Lineweaver–Burk plots for xanthine without (a) and with 10 μM (b) 40 μM (c) allopurinol. Inset shows the relation between K_M^{app} and [allopurinol].

(inset of Fig. 6). It was concluded that allopurinol exerted a competitive inhibition, which can compete with xanthine for the active site of XnOx to form the XnOx–inhibitor complex.

4. Conclusion

This paper describes the development of a new amperometric biosensor for monitoring xanthine. XnOx was immobilized in anionic caly, LDHs, followed by the crossing-linking procedure via glutaraldehyde. The advantages of the XnOx/LDHs such as swelling property, high porosity, thermal stability and anionic exchange ability induced the highly developed analytical performance to xanthine. The applicability of the XnOx biosensor as assay of allopurinol was tested based on catalytic inhibition principle. Due to the specificity to xanthine, the inhibition system is quasi-reversible and of a competitive type. The affinity between the immobilized enzyme and inhibitor is poor.

But broader and more in-depth research for in vivo application still needs to be carried out in this respect in future work.

Acknowledgements

The authors are grateful to the financial supports of National Natural Science Foundation of China (Grant Nos. 20505014 and 20773108), the Key Project of Chinese Ministry of Education (No. 207041), and the project sponsored for D. Shan by the scientific research foundation for the returned overseas Chinese scholars, state education ministry.

References

Amigo, J.M., Coello, J., Maspoch, S., 2005. Anal. Bioanal. Chem. 382, 1380–1388.
Balladin, D.A., Narinesingh, D., Stoute, V.A., Ngo, T.T., 1997. Appl. Biochem. Biotechnol. 62, 317–328.
Casero, E., de Quesada, A.M.G., Jin, J., Quintana, M.C., Pariente, F., Abruña, H.D., Vázquez, L., Lorenzo, E., 2006. Anal. Chem. 78, 530–537.
Caussé, E., Pradelles, A., Dirat, B., Negre-Salvayre, A., Salvayre, R., Couderc, F., 2007. Electrophoresis 28, 381–387.
Chen, G., Chu, Q., Zhang, L., Ye, J., 2002. Anal. Chim. Acta 457, 225–233.
Choy, J.H., Kwak, S.Y., Park, J.S., Jeong, Y.J., Portier, J., 1999. J. Am. Chem. Soc. 121, 1399–1400.
Cooper, N., Khosravan, R., Erdmann, C., Fiene, J., Lee, J.W., 2006. J. Chromatogr. B 837, 1–10.
Cosnier, S., Szunerits, S., Marks, R.S., Novoa, A., Puech, L., Perez, E., Rio-Lattes, I., 2001. Talanta 55, 889–897.
Cosnier, S., Mousty, C., Cui, X., Yang, X., Dong, S., 2006. Anal. Chem. 78, 4985–4989.
Crepaldi, E.L., Pavan, P.C., Valim, J.B., 2000. J. Mater. Chem. 10, 1337–1348.
Czauderna, M., Kowalczyk, J., 1997. J. Chromatogr. B 704, 89–98.
De Melo, J.V., Cosnier, S., Mousty, C., Martelet, C., Jaffrezic-Renault, N., 2002. Anal. Chem. 74, 4037–4043.
De Roy, A., Forano, C., El Malki, K., Besse, J.P., 1992. In: Occelli, M.L., Robson, H.E. (Eds.), Expanded Clays and Other Microporous Solids. Expanded Clays and Other Microporous Solids. Van Nostrand Reinhold, New York, pp. 108–169.
Egwim, E.C., Vunchi, M.A., Egwin, P.O., 2005. Biokemistri 17, 1–6.
Foppoli, C., Coccia, R., Cini, C., Rosei, M.A., 1997. Biochim. Biophys. Acta 1334, 200–206.
Galbusera, C., Orth, P., Fedida, D., Spector, T., 2006. Biochem. Pharmacol. 71, 1747–1752.
Garcia-Ponce, A.L., Prevot, V., Casal, B., Ruiz-Hitzky, E., 2000. N. J. Chem. 24, 119–126.
Han, E., Shan, D., Xue, H., Cosnier, S., 2007. Biomacromolecules 8, 971–975.
Harris, C.M., Massey, V., 1997. J. Biol. Chem. 272, 22512–122512.
Hsieh, J.F., Wu, S.H., Yang, Y.L., Choong, K.F., Chen, S.T., 2007. Bioorgan. Med. Chem. 15, 3450–3456.
Kirgöz, Ü.A., Timur, S., Wang, J., Telefoncu, A., 2004. Electrochem. Commun. 6, 913–916.
Liu, Y., Nie, L., Tao, W., Yao, S., 2004. Electroanalysis 16, 1271–1278.
López, M.S., Pérez, J.P.H., López-Cabarcos, E., López-Ruiz, B., 2007. Electroanalysis 19, 370–378.
Metzler, D.E., 1997. Biochemistry: The Chemical Reactions of Living Cells. Academic Press, New York, p. 882.
Mousty, C., 2004. Appl. Clay Sci. 27, 159–177.
Pacher, P., Nivorozhkin, A., Szabó, C., 2006. Pharmacol. Rev. 58, 87–114.
Rahman, M.A., Won, M.S., Shim, Y.B., 2007. Electroanalysis 19, 631–637.
Rubio-Retama, J., López-Cabarcos, E., López-Ruiz, B., 2005. Talanta 68, 99–107.
Shan, D., Cosnier, S., Mousty, C., 2003a. Anal. Chem. 75, 3872–3879.
Shan, D., Cosnier, S., Mousty, C., 2003b. Anal. Lett. 36, 909–922.
Shan, D., Mousty, C., Cosnier, S., 2004. Anal. Chem. 76, 178–183.
Shan, D., Yao, W.J., Xue, H.G., 2006. Electroanalysis 18, 1485–1491.
Shan, D., Yao, W., Xue, H.G., 2007a. Biosens. Bioelectron. 23, 432–437.
Shan, D., Zhu, M., Xue, H., Cosnier, S., 2007b. Biosens. Bioelectron. 22, 1612–1617.
Tencaliec, A.M., Litchi, S., Magearu, V., Mascini, M., 2006. Talanta 69, 365-.
Villalonga, R., Matos, M., Cao, R., 2007. Electrochem. Commun. 9, 454–458.
Xue, H., Mu, S., 1995. J. Electroanal. Chem. 397, 241–247.
Zhao, J., O'Daly, J.P., Henkens, R.W., 1996. Biosens. Bioelectron. 11, 493–502.