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The promotion effect of titania nanoparticles on the direct electrochemistry of lactate dehydrogenase sol–gel modified gold electrode

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ABSTRACT

The promotion effect of titania nanoparticles (nano-TiO_2) on the direct electron transfer between lactate dehydrogenase (LDH) and the silica sol–gel modified gold electrode was investigated by adding nano-TiO_2 (50 nm) in the modification process. This nano-TiO_2–LDH electrode showed a pair of quasi-reversible cyclic voltammetry peaks with the formal potential of 70 mV (vs. SCE). Compared to the previous result of LDH modified electrode with only an irreversible cathodic peak, an anodic peak appeared and the cathodic peak potential shifted to the positive direction on this nano-TiO_2–LDH electrode, which demonstrated that the direct electrochemistry of LDH was enhanced by nano-TiO_2. We supposed that the direct electrochemistry of LDH may be due to the redox reaction of some electroactive amino acids in the LDH molecule. The surface morphologies of electrodes characterized by SEM indicated that LDH was successfully immobilized on the sol–gel matrix and also had some interactions with nano-TiO_2. This electrode can be used as a biosensor for the determination of lactic acid. The calibration range of lactic acid was from 1.0 to 20 μmol L^{-1} and the detection limit was 0.4 μmol L^{-1}. Meanwhile, the small K_m^{app} value (2.2 μmol L^{-1}) suggested that LDH possessed high enzymatic activity and good affinity to lactic acid owing to the promotion effect of nano-TiO_2.

1. Introduction

The direct electrochemistry of enzymes has become a subject of extensive research owing to its great significances in the kinetic studies of biological redox process and the fabrication of third-generation biosensors [1–3]. Nowadays, more and more attentions have focused on this research [4–12]. Common enzyme contains redox center in its molecule to have the direct electrochemistry, and the redox center also acts as the active site for the catalysis on substrates [9–12]. But lactate dehydrogenase (LDH) (EC 1.1.1.27) is different from these kinds of enzyme. The conversion between pyruvate and lactate catalyzed by LDH must be added NADH (or NAD^+) as a cofactor by combining it in the modifying process or into the experimental solutions [13–16]. Considering the key role of LDH in the areas of medicine, biology and environmental assessment [17–19], the study of direct electron transfer (DET) between LDH and electrode will give a fundamental theoretical understanding of LDH and also stimulate its potential application.

However, the DET rates of enzymes are usually prohibitively slow because of the deep burying of the electroactive prosthetic groups, the adsorptive denaturation of enzymes onto electrodes, and the unfavourable orientations at the electrodes [7,8]. Great effort has been made to enhance the DET between enzymes and electrode, for example, the protein-film techniques combined with nanotechnology provide a promising access due to the high electrical conductivity, chemical stability, and significant mechanical strength of nanomaterials. Various kinds of nanomaterials, such as carbon nanotubes [20,21], gold nanoparticles [22,23], titania nanoparticles (nano-TiO_2) [24,25] and clay nanoparticles [26,27] are generally applied to promote the DET. Among these nanomaterials, the nano-TiO_2 has been widely used in solar cell, electronic devices, catalyst supports and immobilization of proteins and enzymes for bioanalytical applications because of its remarkable chemical, electronic, and optical characteristics [25,28–34]. Moreover, the investigation on nano-TiO_2 has also shown that it could be used as a good promoter for the DET of enzymes due to its good biocompatibility, specific binding with

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molecules, high loading and less denaturation of absorbed proteins [24].

In this paper, we study on the promotion effect of nanomaterial on the direct electrochemistry of LDH. Compared with our previous work about the irreversible direct electrochemistry of LDH sol–gel modified electrode [35], we found that the DET of LDH was promoted by immobilizing LDH on the gold electrode by silica sol–gel process with the addition of titania anatase nanoparticles (nano-TiO_2). A series of other promoters, such as carbon nanotubes, C_{60}, gold nanoparticles, nano-Al_2O_3 were also chosen to test their influence on the DET of LDH, but no effect of promotion was found. The surface morphology characterization, the condition optimization and the application of nano-TiO_2–LDH electrode as a lactic acid biosensor were also investigated.

2. Experimental

2.1. Reagents

L-Lactic dehydrogenase solution (from Bovine Heart, 549 Unit mg^{-1}) was purchased from Sigma (St. Louis, MO, USA). Sodium silicate ($Na_2SiO_3{\cdot}9H_2O$, Sinopharm Chemical Reagent Co., Ltd., China) was used to prepare the silica sol–gel. A 0.1% PVA stock solution was prepared by polyvinyl alcohol (Shanghai Chemical Reagent Plant imported from Japan). Nano-TiO_2 (Anatase type of titania, 50 nm, Taishan Nanomaterials Ltd., China) was used for modification as a kind of promoter. A NAD^+ solution was prepared by dissolving β-nicotinamide adenine dinucleotide (Shanghai Bio. Life Science & Technology Co., Ltd., China) in twice distilled water. L-Lactic acid stock solution was prepared by dissolving L-lactic acid (Sinopharm Chemical Reagent Co., Ltd., China) in twice distilled water. A 0.03 mol L^{-1} phosphate buffer solution (PBS) was prepared by Na_2HPO_4 and KH_2PO_4. All the chemicals were of analytical grade and were used without further purification. Water was twice distilled with a quartz apparatus.

2.2. Apparatus

BAS 100B electrochemical workstation (Bioanalytical Systems Inc., West Lafayette, IN, USA) and CHI660B Electrochemical workstation (CH Instruments Inc., USA) were employed for the electrochemical measurement. A three-electrode voltammetric system was consisted by an LDH-modified gold electrode as work electrode, a platinum counter electrode and a saturated calomel reference electrode (SCE). All the experiments were carried out at 37 °C and in pH 7.0 PBS buffers deaerated by N_2 except the especial explanations. Scanning electron microscopy (SEM) measurement was carried out by a JSM-5610LV Scanning Electron Microscope (JEOL Ltd., Japan).

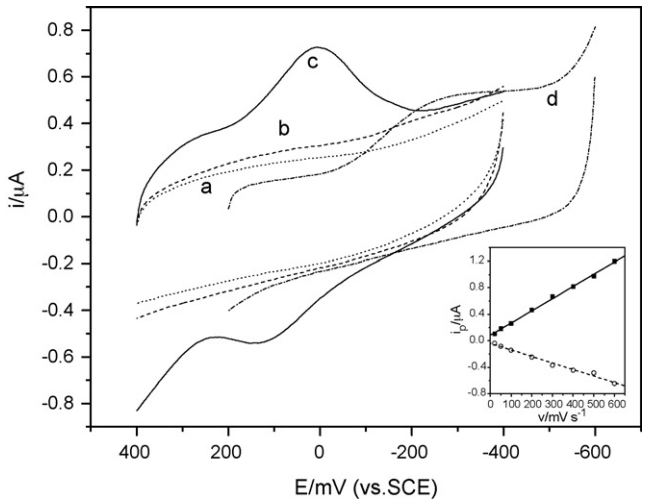

Fig. 1. Cyclic voltammograms of (a) bare Au electrode (. . .); (b) sol–gel/PVA/TiO_2/Au electrode (– – –); (c) sol–gel/PVA/TiO_2–LDH/Au electrode (—); (d) sol–gel/PVA–LDH/Au electrode (–·–·) at 100 mV s^{-1} in 0.03 mol L^{-1} Na_2HPO_4–KH_2PO_4 (pH 7.0, 37 °C). Inset: plot of peak current vs. scan rate. Scan rate (mV s^{-1}): 20, 50, 100, 200, 300, 400, 500 and 600. (■) Cathodic peak current and (○) anodic peak current.

2.3. Preparation of the nano-TiO_2–LDH electrode

The preparation of silica colloidal sol was as previously described [35–37]. The LDH modified electrode was prepared according to the steps thereinafter. The gold electrode (2 mm in diameter, CH Instruments Inc., USA) was first polished with sand paper followed by 1.0, 0.3, 0.05 μm alumina slurry, then sonicated in ethanol and twice distilled water for 15 min, respectively. The cleaned electrode was dipped into the freshly prepared *Piranha* solution (H_2SO_4:30% H_2O_2 = 3:1) for 2 min and pretreated electrochemically in 0.5 mol L^{-1} H_2SO_4 by cyclic voltammetry in the potential range of −0.4 to 1.5 V at a potential scan rate of 100 mV s^{-1} to obtain a steady state cyclic voltammogram of a clear and reproducible gold surface. The nano-TiO_2 solution used as promoters was made by dispersing the TiO_2 nanoparticles in twice distilled water in the magnetic stirrer. Then, the solution for modification was prepared by mixing nano-TiO_2 solution with silica colloidal sol, 0.1% PVA solution, LDH solution (the ratio of volume was 2:5:2:7). Finally, the gold electrode was dipped into the mixed solution for 4–7 h at 4 °C in the refrigerator, then dried by N_2 for the electrochemical measurements.

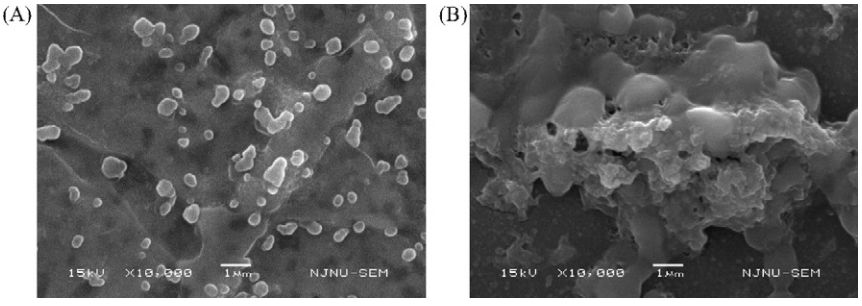

Fig. 2. SEM view with the same magnification: (A) sol–gel/PVA/TiO_2 film; (B) sol–gel/PVA/TiO_2–LDH film.

3. Results and discussion

3.1. The promotion of nano-TiO_2 on the direct electron transfer between LDH and electrode

The promotion effect of nano-TiO_2 on the direct electrochemistry of LDH sol–gel modified gold electrode can be seen in Fig. 1. There are no redox peak at the bare Au electrode and sol–gel/PVA/TiO_2/Au electrode. Meanwhile, a pair of voltammetric peaks with a formal potential ($E^{\circ\prime}$) of 70 mV (vs. SCE) is observed at the sol–gel/PVA/TiO_2–LDH/Au electrode, reflecting the direct electrochemistry of LDH (Fig. 1, curve c). The current ratio of the cathodic peak current to the anodic one (I_{pc}/I_{pc} = 1.87 at 100 mV s^{-1}) and the separation between the anodic and cathodic peak potentials (E_{pa}–E_{pc} = 160 mV at 100 mV s^{-1}) indicated that the electrode process of LDH was quasi-reversible on the gold electrode. There were several evidences that indicated the promotion effect of nano-TiO_2 on the DET between LDH and electrode (Fig. 1, curves c and d [35]): (1) the positive shift of cathodic peak potential (E_{pc} = −10 mV) compared with the result of LDH modified electrode without nano-TiO_2 (E_{pc} = −200 mV); (2) the quasi-reversible electrode process compared with the irreversible electrode process without nano-TiO_2; (3) the increased cathodic peak current (I_{pc} = 300 nA) compared with the value of 200 nA for I_{pc} without nano-TiO_2. On the other hand, the cathodic and anodic peak currents exhibited a linear relationship with the scan rate in the range of 20–600 mV s^{-1} as shown in inset of Fig. 1. This suggested that the electrochemical reaction of LDH immobilized in silica sol–gel film was typical of surface-controlled process which was in accordance with the reports on the direct electrochemistry of other enzymes [36,38,39]. The pure TiO_2 modified electrode, pure LDH modified electrode and TiO_2–LDH modified electrode are also characterized by CV and no obvious redox peaks were found (figures are not shown).

3.2. Characterization of the surface morphology of nano-TiO_2–LDH electrode

The surface morphologies of different electrodes were characterized by SEM (as shown in Fig. 2) since the morphology was also related to the performance of electrode. The top view of silica sol–gel membrane displays a three-dimensional uniform structure and the titania nanoparticles attached on the sol–gel matrix aggregate into a spherical structure, which is similar to the results of other reports [40–43] (Fig. 2A). When LDH is entrapped in the sol–gel matrix, an agglomerate crystal structure of LDH is observed from Fig. 2B. The disappearance of spherical titania nanoparticles on the sol–gel/PVA/TiO_2–LDH film suggests that there exists interaction between LDH and TiO_2. The comparison of the two films also indicated that LDH has successfully immobilized in the sol–gel matrix [44,45].

3.3. Condition optimization and stability of the nano-TiO_2–LDH electrode

The experimental conditions were optimized by investigating the cathodic current response in the cyclic voltammograms of different modified electrodes. A maximum response occurred at 37 °C and pH 7.0. The optimal concentrations of LDH and nano-TiO_2 were found to be 400–650 Unit mL^{-1} and 2–20 mg mL^{-1}, and 7 h was chosen as the modifying time (as shown in Fig. 3). The reproducibility of the nano-TiO_2–LDH electrode is good with a relative standard derivation of 5.6% (n = 8) by measuring the cathodic peak current which is similar to the results reported by other group [21,46,47].

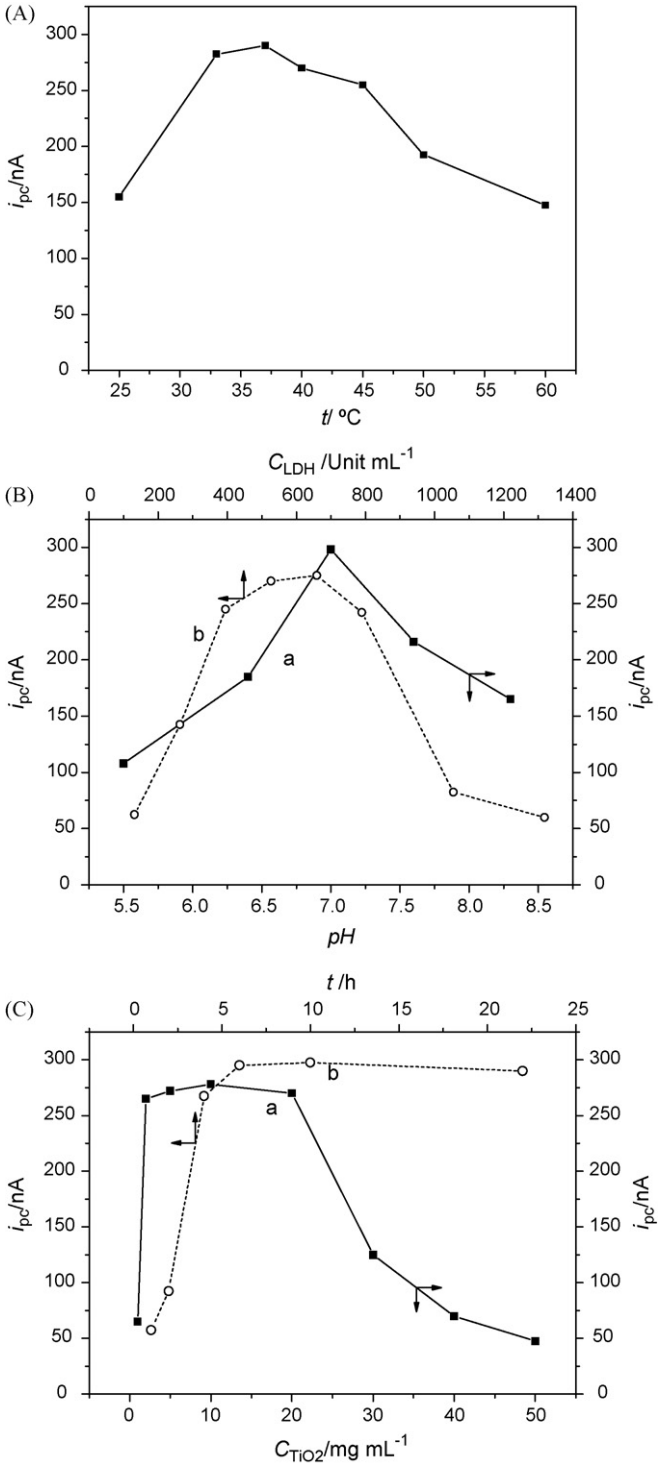

Fig. 3. Optimization of the experimental parameters for preparation of the LDH-modified electrode: (A) effect of temperature on I_{pc}; (B) effect of (a) pH (—) and (b) concentration of LDH (– – –) on I_{pc}; (C) effect of (a) concentration of TiO_2 (—) and (b) modifying time (– – –) on I_{pc}. The other conditions are the same as Fig. 1.

The storage stability of the LDH-modified electrode was also tested. When the enzyme electrode was not in use, it can be stored at 4 °C in refrigerator. The signal of the direct electrochemistry retained about 92% of its initial current response after a week and about 86% after a month.

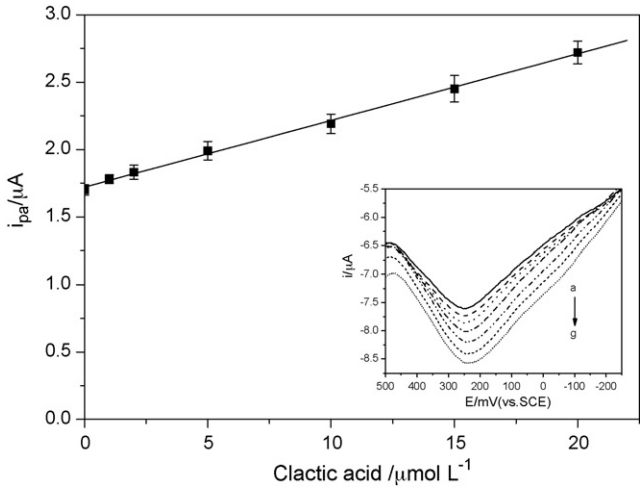

Fig. 4. The calibration curve of the biosensor for lactic acid determination. Inset: differential pulse voltammograms of sol–gel/PVA/TiO_2–LDH/Au electrode in 0.03 mol L^{-1} Na_2HPO_4–KH_2PO_4 (pH 7.0, 37 °C) in the presence of 1.0 mmol L^{-1} NAD^+ and lactic acid with different concentrations (μmol L^{-1}): (a) 0, (b) 1.0, (c) 2.0, (d) 5.0, (e) 10, (f) 15 and (g) 20. Scan rate: 100 mV s^{-1}.

3.4. Application of the nano-TiO_2–LDH electrode as a sensitive lactic acid biosensor with lower detection limit and smaller Michaelis–Menten constant

The electrocatalytic behavior of nano-TiO_2–LDH electrode for lactic acid was also tested according to the method described in previous report [35]. The changes of differential pulse voltammograms were monitored in the 1.0 mmol L^{-1} NAD^+ solution by the additions of lactic acid (inset of Fig. 4). The reaction of lactic acid catalyzed by LDH is: lactate + $NAD^+ \overset{LDH}{\rightleftharpoons}$ pyruvate + NADH + H^+. From the corresponding relationship between the regenerated NADH and the consumed lactic acid according to the reaction formula, the increased anodic current response of NADH was used to represent the increasing lactic acid concentration. The calibration range for determination of lactic acid was from 1.0 to 20 μmol L^{-1} (see Fig. 4). The linear calibration equation was I (μA) = 1.72 + 0.0493 C (μmol L^{-1}) with a correlation coefficient of 0.9988. The detection limit calculated at signal-to-noise ratio of 3 was 0.4 μmol L^{-1} which was lower than the result of 2.0 μmol L^{-1} by only immobilizing LDH on the electrode [35]. The relative standard derivation was 7.5% for five successive assays. The calibration curve also showed the characteristic of the Michaelis–Menten kinetic mechanism. The apparent Michaelis–Menten constant (K_m^{app}), can be determined from the electrochemical version of the Lineweaver–Burk equation [48]: $1/I_{ss} = 1/I_{max} + K_m^{app}/I_{max}c$, where I_{ss} is the steady-state current after the addition of substrate, c is the bulk concentration of the substrate and I_{max} is the maximum current measured under saturated substrate condition. The K_m^{app} was calculated as 2.2 μmol L^{-1} by analysis of the slope and intercept of $1/I_{ss}$ vs. $1/c$ plot. This value is lower than the result of 6.4 μmol L^{-1} without nano-TiO_2. The smaller K_m^{app} value means that the immobilized LDH in sol–gel/PVA/TiO_2 film possesses higher enzymatic activity and higher affinity to lactic acid [20,49,50] which was attributed to the addition of nano-TiO_2. The sensor retained about 85% of its initial catalytic response on lactic acid after intermittent use after 1 month.

4. Conclusion

In the current work, the nano-TiO_2–LDH electrode was prepared by immobilizing LDH on the silica sol–gel modified gold electrode with the addition of nano-TiO_2. Due to the promoting effect of nano-TiO_2, the direct electron transfer between LDH and electrode was enhanced and a quasi-reversible direct electrochemistry of LDH on the gold electrode was discovered in the optimal conditions of physiological situations (pH 7.0, 37 °C). It is difficult to conclude which group is definitely served for the redox action of LDH because of its complex structure. Considering that LDH is composed by units of the polypeptide which may have some amino acids residues, we supposed that the direct electrochemistry of nano-TiO_2–LDH electrode may be due to the redox reaction of some electroactive amino acids [51,52]. Based on this method, a new biosensor of lactic acid was constructed, which shows a stable and sensitive response of lactic acid. This nano-TiO_2–LDH electrode could also be anticipated to apply to the mechanistic studies in biological systems by constructing the small and convenient biosensors.

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