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Layer-by-layer assembly sensitive electrochemical sensor for selectively probing L-histidine based on molecular imprinting sol–gel at functionalized indium tin oxide electrode

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ABSTRACT

A novel sensitive and selective imprinted electrochemical sensor was successfully constructed for the direct detection of L-histidine by combination of a molecular imprinting film and multi-walled carbon nanotubes (MWNTs). The sensor was fabricated onto an indium tin oxide (ITO) electrode via stepwise modification of MWNTs and a thin film of molecularly imprinted polymers (MIPs) via sol–gel technology. The introduced MWNTs exhibited noticeable enhancement on the sensitivity of the MIPs sensor. Meanwhile, the molecularly imprinted film displayed high sensitivity and excellent selectivity for the target molecule L-histidine. The proposed imprinted sensor was characterized by using scanning electron microscope (SEM) and electrochemical methods involving cyclic voltammetry (CV), differential pulse voltammetry (DPV) and amperometric *i–t* curve. A linear ranging from 2.0 μmol L^{-1} to 1.0 mmol L^{-1} for the detection of L-histidine was observed with the detection limit of 5.8×10^{-9} mol L^{-1} for $S/N = 3$. This imprinted electrochemical sensor was successfully employed to detect L-histidine in human blood serum.

1. Introduction

L-Histidine (2-amino-3-(4-imidazolyl) propanoic acid), a neurotransmitter, is an essential bioactive amino-acid component of many proteins. The chiral recognition and quantitative assay of L-histidine isomer is associated with the diagnosis of L-histidine metabolism disorders, particularly 'histedinemia' at elevated levels (Kovach and Meyerhoff, 1982; VanGelder et al., 1987; Rao et al., 1993) in physiological fluids. Therefore selective and sensitive detection of L-histidine is preciously important for clinical applications. Several technologies including fluorescence with capillary electrophoresis (Zhang and Sun, 2004) mass-spectrometry (Miyagi and Nakazawa, 2008), chromatography (Ruta et al., 2007), and spectroscopy (Lim et al., 2008) have been used for L-histidine detection. However, large sample volumes, expensive instrumentation, lack of reproducibility and selectivity, and eco-unfriendly solvents were serious concerns associated with these techniques.

Over the past two decades, molecularly imprinted polymers (MIPs) have attracted broad interest from scientists engaged in sensor development (Umpleby et al., 2004; Alexander et al., 2006; Holthoff and Bright, 2007). This attention can be explained by the potential advantages of using the MIPs in place of natural receptors and enzymes such as superior stability, low cost and easy preparation. The high selectivity and affinity of the MIPs for the template make them as ideal recognition element for sensors, and considerable research efforts are constantly devoted to develop MIP-based chemical sensors (Kindschy and Alocilja, 2004; Dickert and Hayden, 2000). However, most of these biomimetic sensors reported are based on mass accumulation in the MIPs coating such as quartz crystal microbalances (Dickert et al., 2004; Hayden et al., 2006), surface plasmon resonance devices (Li and Husson, 2006), field-effect devices (Hedborg et al., 1993), and conductometry (Sergeyeva et al., 1999). It is surprising that scarce report on the design of electrochemical sensor for analyte detect through its reaction on the supported substrate. Since the development of sol–gel technology, the imprinted polymers are of growing interest for their potential applications as thin films for electrochemical sensor devices (Marx and Liron, 2001). Briefly, an inorganic framework is formed around a suitable template via non-covalently/covalently interaction among functional monomers and the template in sol–gel process.

Despite the increasing application of molecularly imprinted polymers in analytical chemistry, the construction of a sensitive biomimetic sensor remains still challenging. As a sensing material, diffusion of the analytes across the MIPs film needs to be acceler-

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ated to obtain a quick response (Xu et al., 2009). Furthermore, in order to effectively convert the binding signals from the molecular recognition to detectable electrical signals, the proper design of the signal transducer still remains challenging to enhance sensitivity of this sensing system (Walcarius et al., 2005). Recently, the expanding availability of a variety of new materials with unique properties at the nanoscale dimension, e.g. multi-walled carbon nanotubes (MWNTs) (Wang, 2005; Zong et al., 2007; Choong et al., 2009; Iijima, 1991), have attracted widespread attention in their utilization for enhancing the sensitivity of the electrochemical detection. For example, Rahimi et al. (2010) reported on ionic-liquid/NH_2-MWNTs as a highly sensitive nano-composite for catalase direct electrochemistry.

The aim of this paper is to construct a highly sensitive and excellent selectivity biomimetic sensor. Aminopropyltriethoxysilane (APTES) was self-assembled on a cleaned hydroxyl-functionalized indium tin oxide (ITO) electrode, on which amino-functionalized layer was formed, obtaining Si-ITO electrode (Gao et al., 2007). Then, the MWNTs, which represent an important group of nanomaterials with attractive geometrical, mechanical, electronic and chemical properties, were grafted on Si-ITO electrode. Finally, L-histidine-imprinted sol solution (involving TEOS, MTMOS and PTMOS) was electrodeposited onto the modified surface, and the MIP/sol/MWNTs/Si-ITO electrode was obtained. Due to the inherent specificity of the MIPs, a novel MWNTs functionalized MIPs electrode was fabricated to detect L-histidine with high sensitivity and selectivity. The function of MWNTs and the performance of sensor with respect to sensitive, linear range, and selectivity of the MIPs sensor towards L-histidine were investigated in detail.

2. Experimental

2.1. Reagents

MWNTs were obtained from Shenzhen Carbon Nanotechnologies Co. Ltd. L-Histidine, D-histidine, L-phenylalanine, L-proline, D,L-methionine and L-norleucin were obtained from Sigma (USA). Tetraethylorthosilicate (98%, TEOS), phenyltrimethoxysilane (99%, PTMOS), 3-aminopropyltriethoxysilane (98%, APTES) and methyltrimethoxysilane (99%, MTMOS) were purchased from Aldrich (USA). Anhydrous ethanol, sulfoxide chloride, chloroform, hydrochloric acid, hydronitric acid, anhydrous tetrahydrofuran, potassium ferricyanide ($K_3[Fe(CN)_6]$), potassium ferrocyanide ($K_4[Fe(CN)_6]$), potassium dihydrogen phosphate (KH_2PO_4), and dipotassium hydrogen phosphate (K_2HPO_4) were purchased from Changsha Chemical Co. (Hunan, China). All other reagents were of analytical grade and were used without further purification. Double distilled water was used in all experiments.

Phosphate buffer solution (PBS, 0.2 mol L^{-1}) of pH 7.0 was used as the background electrolyte. The probing solution was prepared by using the mixture of 10.0 mmol L^{-1} $K_3[Fe(CN)_6]$ and $K_4[Fe(CN)_6]$ (1:1), containing 0.2 mol L^{-1} PBS (pH 7.0).

2.2. Apparatus

The electrochemical measurements were carried out on a CHI660B electrochemical workstation (Shanghai Chenhua Apparatus Co.). All experiments were performed at room temperature with a conventional three-electrode system comprising a home-made ITO working electrode, a platinum wire auxiliary electrode and a saturated calomel reference electrode (SCE). All potentials were relative to the reference electrode. The ITO glass (surface resistance <10 Ω, Laibao Electrical Co., Shenzhen, China) was cut into 4.0 cm (length) × 2.0 cm (width) × 0.5 cm (height) and used as the working electrode. The ITO glass was pretreated by soldering a metallic wire to the metallic part, then attaching with resin to receive the effective electrode area of 1.0 cm^2.

2.3. Synthesis of MWNTs-COCl

MWNTs-COCl was prepared by the amidation reaction similar to that described by Kan et al. (2008). A total of crude MWNTs (0.5 g) was added to 60.0 mL of HNO_3 under sonication for 10 min. Then the mixture was stirred under 85 °C for 16 h. Cooled to room temperature, the mixture was filtered through a 0.22 μm polycarbonate membrane and washed with distilled water for several times until the pH of the filtrate was neutral. The filtered solid was dried with N_2, obtaining carboxylic acid-functionalized MWNTs (MWNTs-COOH). Subsequently, the MWNTs-COOH (0.4 g) was suspended in the mixture of 10.0 mL of sulfoxide chloride ($SOCl_2$) and 30.0 mL chloroform at 60 °C for 24 h under reflux. The solid was washed by tetrahydrofuran (THF) for several times to remove the excess $SOCl_2$ and dried with N_2 to obtain MWNTs-COCl.

2.4. Preparation of MIPs/sol/MWNTs/Si-ITO electrode

The fabrication protocol of the imprinted ITO sensor was presented in Fig. 1. Firstly, the ITO electrode was cleaned by using acetone, ethanol and water in succession. Dried under N_2, the ITO electrode was immersed into 1.0 mol L^{-1} HCl for 10 min. After washing thoroughly with water, the ITO electrode was immersed into a mixing solution of 1:1:5 (v/v/v) $H_2O_2/NH_4OH/H_2O$ for 1 h to obtain a hydroxyl (–OH) grafted ITO electrode. Then, the hydroxyl (–OH) grafted ITO electrode was rinsed with water and dried under N_2. Amino group was coupled onto the ITO electrode by immersion in APTES (1 wt%, in ethanol) for 12 h at room temperature. After that, the ITO electrode was washed thoroughly with ethanol to remove the physically adsorbed APTES molecules and dried under N_2. Finally, the ITO electrode was immersed in MWNTs-COCl ethanol solution for 12 h to introduce MWNTs by layer-by-layer assembly method and dried with N_2. Thus, the MWNTs functionalized ITO electrode (MWNTs/Si-ITO) was obtained.

A selective and sensitive MIPs film towards L-histidine was modified on the MWNTs/Si-ITO electrode by sol–gel technology. The mixing sol consisting of 3.0 mL (13.5 mmol) of TEOS, 3.0 mL of ethanol, 230 μL (1.2 mmol) of PTMOS, 180 μL (1.2 mmol) of MTMOS, 1.0 mL of H_2O, and 0.1 mL of concentrated HCl was stirred at room temperature for 2 h. Then 2.0 mL of this sol was mixed with 0.2 mL of 0.1 mol L^{-1} L-histidine aqueous solution, and was used as imprinted sol. Additionally, the left original sol without L-histidine was used as non-imprinted polymers (NIPs) sol. Finally, the imprinted or non-imprinted sol was electrodeposited onto the modified ITO electrode surface by using cyclic voltammetry from −0.8 V to +0.9 V with the scan rate of 50 mV s^{-1} and left to dry overnight at room temperature. Thus the MIPs/sol/MWNTs/Si-ITO and NIPs/sol/MWNTs/Si-ITO electrode were obtained.

The interaction principle between the MIPs film and L-histidine during the extraction and rebinding process was shown in the inset of Fig. 1. As noted, reversible interactions, including hydrogen-bond with nitrogen heteroatom, and electrostatic interaction between the template molecule L-histidine and the insoluble polymer network were formed in the modified MIPs film. The doped L-histidine was extracted from the MIPs/sol/MWNTs/Si-ITO electrode by repetitive immersion in 0.2 mol L^{-1} phosphate buffer solution (PBS, pH 7.0). Thus, L-histidine could interact selectively with the MIPs/sol/MWNTs/Si-ITO electrode. The incubation was carried out in 0.2 mol L^{-1} phosphate buffer solution (PBS, pH 7.0) containing 1.0 mmol L^{-1} L-histidine.

The surface morphologies of the modified ITO electrodes were analyzed by JSM-6700F scanning electron microscope (SEM, Japan).