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Electrochemical impedimetric immunosensor for insulin like growth factor-1 using specific monoclonal antibody-nanogold modified electrode

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ABSTRACT

An electrochemical impedimetric immunosensor was developed for ultrasensitive determination of insulin-like growth factor-1 (IGF-1) based on immobilization of a specific monoclonal antibody on gold nanoparticles (GNPs) modified gold electrode. Self-assembly of colloidal gold nanoparticles on the gold electrode was conducted through the thiol groups of 1,6-hexanedithiol (HDT) monolayer as a cross linker. The redox reactions of $[Fe(CN)_6]^{4-}/[Fe(CN)_6]^{3-}$ on the electrode surface was probed for studying the immobilization and determination processes, using cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS). The interaction of antigen with grafted antibody recognition layer was carried out by soaking the modified electrode into antigen solution at 37 °C for 3 h. The immunosensor showed linearity over 1.0–180.0 pg mL^{-1} and the limit of detection was 0.15 pg mL^{-1}. The association constant between IGF-1 and immobilized antibody was calculated to be 9.17×10^{11} M^{-1}. The proposed method is a useful tool for screening picogram amounts of IGF-1 in clinical laboratory as a diagnostic test.

1. Introduction

Insulin-like growth factor 1 (IGF-1), somatomedin C, is mainly secreted by the liver as a result of stimulation by growth hormone (GH). The normal range of IGF-1 is different in various ages and it varies from 1 to 1096 ng mL^{-1}. It is measured as a biomarker to realize the growth abnormalities and to evaluate pituitary function. It has been shown that it plays some roles in the promotion of cell proliferation and the inhibition of apoptosis. IGF-I is involved in regulating neural development after neuronal damage (Russo et al., 2004). Increased serum levels of IGF-1 in children lead to a higher IQ, intelligence quotient (Gunnell et al., 2005). Its deficit can cause hearing loss (Welch and Dawes, 2007) and it also plays an important role in aging (Hammerman, 1987; Darnaudery et al., 2006). IGFs have an important role in cancers (Kooijman et al., 2007; Kleinman et al., 1993; LeRoith and Roberts, 2003) and diabetes (Bach and Rechler, 2009).

The conventional methods for detection of IGF-1 are enzyme-linked immunosorbent assays (ELISAs) (Guidi et al., 2007; Khosravi et al., 1996; Obese et al., 2008) and radioimunoassays (Vega-Rubín de Celis et al., 2004; Breier et al., 1991) that require the use of enzymatic label or apply radioactive materials with detection limits of nano-gram amounts of IGF-1 (e.g. 4.9 ng mL^{-1}). Development of direct, label free immunoassays based on electrochemical techniques have attracted much attention because of their high sensitivity, rapid and precise response, undemanding pretreatment procedures, portability and miniaturization. Impedance spectroscopy is a rapid measurement tool for the analysis if the interfacial properties changes of the modified electrodes upon biorecognition events occur at the modified surfaces. Affinity-binding based impedimetric biosensors are attracting interest, since they are direct and label free electrochemical immunosensors and have many potential advantages with respect to speed, the use of unskilled analysts and the potential development of multi-analyte sensors (Barton et al., 2008; Daniels and Pourmand, 2007; Huang et al., 2008a,b; Wang et al., 2005; Yang et al., 2005).

In these methods the immobilization of biomolecules on the electrode is the most important factor in generating rapid response and fabricating high selective biosensors. The biological species are easily adsorbed on the gold surface; however, the covalent linkage between bulk metal surface and biological entities leads to the reduction of their bioactivity (Liu et al., 2005). Using the gold nanoparticles (GNPs) to modify the surface of metallic gold is a proven way to preserve the bioactivity of biological species (Hong and Kang, 2006; Martin and Mitchell, 1998). Modification of electrode surfaces with self-assembled monolayers (SAMs) of thiols provides a simple way to design modified surfaces that can be further used as functionalized sites to immobilize gold nanoparticles and proteins (Rezaei et al., 2009).

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In this work, an IGF-1 immunosensor was designed through immobilization of IGF-1 specific antibody on to the gold nanoparticles modified gold electrode. Cyclic voltammetry (CV) and electrochemical impedance (EIS) techniques were used to investigate the immobilization of anti-IGF-1 on gold electrode and analysis of IGF-1.

2. Experimental

2.1. Reagents

1,6-Hexadithiol (HDT) was obtained from Acros and hydrogen tetrachloroaurate trihydrate ($HAuCl_4{\cdot}3H_2O$) was purchased from Merck. Potassium ferrocyanide, potassium ferricyanide, sodium citrate and other chemicals were obtained from Merck and millipore water was used throughout.

Monoclonal mouse IGF-1 antibody (Ab40789; Recombinant full length protein (human)) and IGF-1 were purchased from Abcam company and Immunotech company, respectively.

2.2. Instruments

The absorbance spectrum was recorded by means of a diode array UV–vis spectrophotometer (Lightwave, Cambridge,WPA, England). All electrochemical measurements were performed with a potentiostat–galvanostat AutoLab (Echo Chemie, Netherlands) with a conventional three electrode system. Saturated Ag/AgCl (3.0 M KCl) electrode and platinum electrode (Azar electrode co, IRI.2000-E) were used as the reference and auxiliary electrode, respectively. Unmodified or GNPs modified Gold electrodes was applied as working electrode.

2.3. Preparation of gold nanoparticles

Colloidal gold nanoparticles were prepared by the sodium citrate reduction method (Lu et al., 2002). All glassware used in the following procedures was thoroughly cleaned with freshly prepared aqua regia (HCl:HNO_3, 3:1), washed with ultrapure water, and dried prior to use. In brief, 1.75 mL of 1% sodium citrate solution was added into 50 mL of solution containing 0.01% of $HAuCl_4{\cdot}3H_2O$ while refluxing with vigorous stirring. A color change from pale yellow to bright red was seen. Boiling was continued for 10 min, followed by continued stirring until the solution reached room temperature.

The strong absorbance at 519 nm (UV–vis spectrum) confirmed the characteristic of mono-dispersed colloidal gold (Slot and Geuze, 1985). The prepared gold nanoparticles were stored in a dark bottle at 4 °C until further use.

2.4. Immobilization of gold nanoparticles on gold electrode

The gold disk electrode (2.0 mm in diameter, Azar electrode co. IRI.2000-E) was polished to a mirror-finished with slurry alumina 1.0, 0.3 and 0.05 μm, respectively. Then the electrode was rinsed with redistilled water and cleaned ultrasonically in a 1:1 mixture of ethanol:water for 5 min. To remove any possible surface contaminant, the polished electrodes were immersed in a piranha solution (3:1, sulfuric acid:hydrogen peroxide) for 1 min and then rinsed with distilled water and ethanol. Piranha solutions are strong oxidizers used to remove organic residues from substrates. As such, piranha solutions are extremely corrosive, reactive, and potentially explosive, therefore the handling of piranha solutions requires special protection equipment. The gold electrode was then subjected to potential cycling from 0.0 to 1.5 V with the scan rate of 50 mV s^{-1} in a 0.2 M aqueous $HClO_4$ until cyclic voltammograms did not change any more, and the final potential was 0.0 V vs. Ag/AgCl.

The cleaned gold electrode was modified by immersion in a 20.0 mM ethanolic solution of 1,6-hexanedithiol containing 1.0 M of $LiClO_4$ for 7 h. Then the electrode was thoroughly rinsed with pure ethanol and water to remove physically adsorbed dithiols. Afterwards, the electrode was dipped in a solution of colloidal gold nanoparticles for 44 h and kept in refrigerator to obtain the GNPs modified gold electrode (GNPs/HDT/gold electrode).

2.5. Antibody coupling

GNPs prepared by chemical reduction of the gold metal salt in the presence of citrate anions as a stabilizer (which binds to their surface to impart high stability and rich linking chemistry and provide the desired charge and solubility properties) have a negative surface charge as a consequence of a weakly bound citrate coating. Therefore, as these particles are immobilized on the surface of gold electrode make the electrode to be negatively charged.

In order to facilitate the antibody immobilization on the negatively charged colloidal gold surface (Liz-Marzan et al., 1996), this process was carried out in acidic media. 30.0 μL of 20.0 μg mL^{-1} monoclonal anti IGF-1 solution (0.1 M KH_2PO_4, 0.1 M KCl, pH = 4.4) was placed onto the GNPs/HDT/gold electrode surface and the modified electrode was kept in high humidity condition for 2 h at room temperature. Finally, 1.0% bovine serum albumin (BSA) solution was added to block the non-specific reactive sites, and subsequently rinsed with 0.1 M KH_2PO_4, 0.1 M KCl (pH = 4.4,) solution to eliminate possible unstable adsorbed antibodies from the electrode surface. This electrode was used for determination of IGF-1 during the experiment.

2.6. Electrochemical measurements

Cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) were performed in a solution containing 0.1 M KH_2PO_4, 0.1 M KCl (pH = 4.4) and 1.0 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$. In CVs, potential was cycled from −0.2 to 0.8 V, with scan rate of 50 mV s^{-1}. The EIS measurements were recorded within the frequency range of 0.01 Hz to 100 kHz at 0.23 V.

2.7. Insulin like growth factor-1 detection

30.0 μL of different concentrations of IGF-1 solution (0.1 M KH_2PO_4, 0.1 M KCl, pH = 4.4) were placed on the surface of antibody modified electrode, kept under desiccator for 3 h, and then rinsed with 0.1 M KH_2PO_4, 0.1 M KCl (pH = 4.4) solution to remove unbound antigen.

3. Results and discussion

3.1. Electrochemical characterization of prepared electrodes

3.1.1. Cyclic voltammetry

Cyclic voltammetry of electroactive species, $Fe(CN)_6^{4-/3-}$, is a valuable tool for testing the kinetic barrier of the interface. Fig. 1 shows the CV responses of $Fe(CN)_6^{4-/3-}$ at bare gold electrode, dithiol modified electrode, gold nanoparticle modified electrode, and antibody immobilized electrode in the absence and presence of antigen. As can be seen, $Fe(CN)_6^{4-/3-}$ shows a reversible one-electron redox waves at bare gold electrode (Fig. 1(a)) with a peak–peak separation of 71.4 mV at 50.0 mV s^{-1}. After modifying the electrode with 1,6-hexanedithiol, the peak related to $Fe(CN)_6^{4-/3-}$ redox reaction disappears (Fig. 1(b)). 1,6-Hexanedithiol layer acts as the inert electron and mass transfer blocking layer, and thus hinders the diffusion of $Fe(CN)_6^{4-/3-}$ towards the electrode surface. On the contrary, when GNPs were attached to the electrode surface, the voltammetric response of