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Electrochemical biosensor based on self-assembled monolayers modified with gold nanoparticles for detection of HER-3

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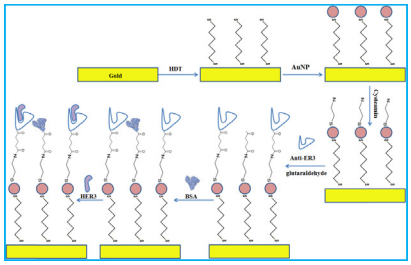
HIGHLIGHTS

- Single frequency impedance technique was firstly used for characterization of interaction between HER-3 and anti-HER-3.
- Anti-HER-3 antibody was firstly utilized in an immunosensor as a bioreceptor.
- The biosensor exhibits high analytical performance with a linear range 0.2–1.4 pg mL^{-1}.
- Kramers–Kronig transform was successfully performed on the experimental impedance data.

GRAPHICAL ABSTRACT



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ABSTRACT

We have developed a new immunological biosensor for ultrasensitive quantification of human epidermal growth factor receptor-3(HER-3). In order to construct the biosensor, the gold electrode surface was layered with, hexanedithiol, gold nanoparticles, and cysteamine, respectively. Anti-HER-3 antibody was covalently attached to cysteamine by glutaraldehyde and used as a bioreceptor in a biosensor system for the first time by this study. Surface characterization was obtained by means of electrochemical impedance spectroscopy and voltammetry. The proposed biosensor showed a good analytical performance for the detection of HER-3 ranging from 0.2 to 1.4 pg mL^{-1}. Kramers–Kronig transform was performed on the experimental impedance data. Moreover, in an immunosensor system, the single frequency impedance technique was firstly used for characterization of interaction between HER-3 and anti-HER-3. Finally the presented biosensor was applied to artificial serum samples spiked with HER-3.

1. Introduction

The human epidermal growth factor receptor (HER) family plays a key role in regulation of mammalian cell survival, proliferation, adhesion, and differentiation. This HER family of receptor tyrosine kinases comprises four structurally related transmembrane receptors: HER-1 (EGFR or c-erbB-1), HER-2 (HER-2neu or c-erb-B-2), HER-3 (c-erb-B-3), and HER-4 (c-erb-B-4). All members of the family have an extracellular ligand binding domain, a transmembrane domain, and a cytoplasmic tyrosine kinase domain, which for HER-3 is nonfunctioning [1–5]. HER-3 protein exists in normal human adult and fetal tissues, including the breast, and has also been shown to be expressed at both the mRNA and protein levels in a number of tumor cell lines and primary tumor material [6,7]. Normal HER-3 level in a healthy person ranges from 0.06 ng mL^{-1} to 2.55 ng mL^{-1}. Moreover, in a risk of cancer, the abnormal levels of HER-3 should be increased up to 12 ng mL^{-1}. [8].

HER-3 protein has been found to be over expressed in a range of tumors including those of the breast [9] and non-small-cell lung

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carcinoma [10]. Since determination of c-erbB-3 in blood or tissues is essential, many methods such as immunohistochemical [11], enzyme linked immuno assay (ELISA) [12], and Western blot [13] have been reported to detect c-erbB-3.

Recently, electrochemical impedance spectroscopy (EIS) was used effectively for identification of membranes, biosensor characterization and fabrication with the help of cyclic voltammetry [14–17]. In fact, like electrical resistance, electrochemical impedance can be conceived as a measure of the ability of a circuit to resist the flow of electrical current, however unlike electrical resistance, electrochemical impedance is dependent of frequency and usually measured by applying an AC voltage to an electrochemical cell and then measuring the current through the cell [18–20]. However, in this study a novel impedance method has been applied to the immunosensor proposed based on gold nanoparticles. "Single frequency impedance" was performed to reveal binding the characteristics between HER-3 and anti-HER-3 attached to the biosensor surface. In electrochemistry, in order to discover surfaces of working electrodes, several methods such as scanning electrochemical microscopy, scanning tunneling microscopy, and scanning reference electrodes should be operated. However, using these methods to potential researchers is commonly time consuming and requires expensive equipments. Moreover scanning electron microscope (SEM) and atomic force microscope (AFM) should also be used for surface characterization of the biosensors. As is well known, both of these equipments are extremely expensive and in any laboratory SEM/AFM should not be provided. Consequently, as a valuable alternative way, measurements can be performed across the electrode at a single frequency to create an image of the electrode or, alternatively, performed at a given location to create a complete spectrum. In other words, single frequency impedance measurements can be performed to monitor the changes in the surfaces of the biosensors.

Self-assembly process is the spontaneous organization of substances into specific metal surfaces. Self assembled monolayer (SAM) of different substances have frequently utilized for development biosensors, microarrays, biochips, and molecular switches [21]. There are several advantages for the use of SAMs as a platform for immobilization of biomolecules such as easy formation of SAMs, providing suitable surface for biomolecule immobilization, flexibility to design the head group of SAM with various functional ends, and small amount of biomolecule is needed for immobilization on SAM [22].

In the current study, anti-HER-3 antibody was used as a biorecognition element for the first time to quantify HER-3. Self-assembly of hexanedithiol on a gold electrode was successfully performed and evaluated. Gold nanoparticles were used to enhance the surface area of a SAM modified electrode. Cysteamine monolayers were self-assembled on the gold nanoparticles. For immobilization of anti-HER-3, glutaraldehyde was used as a crosslinking agent. Immobilization steps were monitored by EIS and CV. For analyzing EIS data it fitted to an equivalent circuit model. Certain important parameters were optimized to obtain the best biosensor results. Finally, artificial serum samples spiked with HER-3 were analyzed by the biosensor.

2. Experimental

2.1. Materials and instrumentation

All reagents were purchased from Sigma–Aldrich (St. Louis, MO, USA) and were of analytical purity unless stated otherwise. HER-3 and anti-HER-3 were purchased from Sigma–Aldrich (St. Louis, MO, USA). For all dilutions, sterile phosphate buffers (pH 7, 0.01 M) were used. Anti-HER-3 and HER-3 portions were prepared at certain concentrations and were stored at −20 °C until use. Artificial serum solution was prepared using 4.5 mM KCl, 5 mM $CaCl_2$, 4.7 mM D(+)-glucose, 2.5 mM urea, 0.1% bovine serum albumin, and 145 mM NaCl. A three-electrode system, consisting of a gold working electrode (with a surface area of 2.01 mm^2), an Ag/AgCl (saturated KCl) reference electrode and a Pt counter electrode, was fabricated in a 10 mL electrochemical cell (all the electrodes were obtained from iBAS, Warwickshire, UK). Electrochemical experiments were performed using a Gamry Potentiostat/Galvanostat, Reference 600 (Gamry Instruments, Warminster, USA) interfaced with a PC via an EChem Analyst containing physical electrochemistry, pulse voltammetry, and electrochemical impedance spectroscopy software (Gamry Instruments, Warminster, USA).

2.2. Preparation of the self-assembled monolayer of hexanedithiol

Firstly, the surfaces of the Au electrodes were polished with 0.05 μm alumina and then washed ultrasonically in ethanol for 5 min to remove alumina particles. Then the electrodes were immersed in piranha (H_2O_2/H_2SO_4, 1/3 v/v) solution for 3 min. Afterwards, the electrodes were washed by immersion in ultrapure water ten times. For the next step, the surfaces of the electrodes were dried in a pure argon stream. This polishing and cleaning procedure was repeated before every electrode preparation step. The clean gold electrodes were immersed in a hexanedithiol solution (0.1 M, in pure ethanol) for 24 h. After this period, they were rinsed with ethanol and carefully dried with an argon gas.

2.3. Preparation of gold nanoparticles

2.4 mL of 40 mM sodium boron hydride ($NaBH_4$) was added to 50 mL 200 ppm $HAuCl_4$. Finally, gold electrodes were immersed in this solution for 24 h in a dark ambient. At the end of this step, the electrodes were flushed with ultra-pure water and dried with argon gas gently.

2.4. Covalent immobilization of anti-HER-3 on a modified gold electrode

Thereinafter, the electrodes were immediately immersed in a cysteamine solution (0.5 M in pure ethanol) and left overnight in the dark. After this period, they were rinsed with ethanol and gently dried in an argon stream. For the activation of amino ends, 5 μL 5% glutaraldehyde solution and 5 μL anti-HER-3 (5 μg μL^{-1}) were applied to the electrodes surfaces modified with cysteamine (Au/Cys) by a pipette. The electrodes were incubated for an hour in a moisture medium. Finally the electrodes were immersed in ultrapure water to remove physically adsorbed anti-HER-3 molecules. After washing the surfaces of the electrodes, 10 μL BSA solutions (1%) were dripped onto the electrode to block the active ends of the surfaces and this continued for an hour in a humid environment.

2.5. Electrochemical measurements

Cyclic voltammetry was used to characterize SAM formation on the bare gold electrodes, and the modified electrodes with different layers. The potential was varied between 0 and 500 mV (step size: 20 mV, scan rate: 50 mV s^{-1}) in the presence of a 5 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (1:1) solution which served as a redox probe containing 0.1 M KCl. For electrochemical impedance studies, an alternating wave of 10 mV amplitude was applied to the electrode over the formal potential of the redox couple (0 V). The redox couple used for the impedance studies was the same as cyclic