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Ultrasensitive electrochemical detection of cancer associated biomarker HER3 based on anti-HER3 biosensor

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ABSTRACT

The development of a new impedimetric biosensor for the detection of HER3, based on self-assembled monolayers (SAMs) of 4-aminothiophenol on gold electrodes, is reported. Anti-HER3 was used as a biorecognition element for the first time in an impedimetric biosensor. Cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) techniques were applied to characterize the immobilization process and to detect HER3. To provide the best biosensor response all experimental parameters were optimized. In addition, Kramers–Kronigs transform was also performed on the immobilization and measurement processes successfully. The biosensor had a linear detection range of 0.4–2.4 pg/mL. The chrono-impedance technique to real time monitor the interaction between HER3 and anti-HER3 is also implemented. The biosensor has exhibited good repeatability and reproducibility. To demonstrate the feasibility of the biosensor in practical analysis, the artificial serum samples were experienced.

1. Introduction

The human epidermal growth factor receptor family (HER) consists of four homologous members: EGFR (epidermal growth factor receptor, HER-1 or c-*erb*B-1), HER-2 for which no ligand has been described by now, HER3, and (HER-4). [1] These transmembrane glycoproteins have an extracellular ligand binding domain, a transmembrane region, and an intracellular domain with tyrosine kinase activity [2] though HER3 has little or no tyrosine kinase activity and this is the most distinct difference between it and the other family members [3]. As etiological members of HER family have been related to the progression of some type of human cancers through overexpression or mutational activation, including cancers of the breast, lung, head and neck, brain, and skin [4–6]. HER3 has been shown to be highly expressed in melanomas [6–8], some prostate cancers [9], colorectal cancers [10–14], breast cancers [15–17], ovarian tumors [18], and notably childhood glioma [6,19]. Moreover recent evidence indicated that HER3 responsible for tumor resistance to therapeutic agents targeting EGFR or HER-2 has illuminated its critical role in cancer [20]. Therefore there are interests which have recently been occured by the possibility of utilizing the HER3 as prognostic indicators in some type of carcinomas. Normal physiological levels of HER3 in a person range from 0.06 ng/mL to 2.55 ng/mL. However, in a pathological case, the abnormal values of HER3 should be increased up to 12 ng/mL [21]. Characterizations of HER3 were carried out by immunohistochemistry [2,22], Southern blot [23], enzyme linked immuno assay (ELISA) [24,25], Northern blot [26] for detection of expressed HER3 in blood or tissues. Recently, an electrochemical strategy based on aptamers and nanoprobes was developed for the rapid detection of HER-3 [27]. In the reported study a nanoprobe consisting of aptamers and DNA capped nanocrystals has been designed for the electrochemical detection of HER3.

Affinity-binding based impedimetric biosensors become an efficient method since they are speed, direct and label-free electrochemical immunosensors [28] and due to their affordability and availability, a trend towards the development of impedimetric biosensors appears to be underway. The application of immunosensors for the detection of a wide range of analytes in clinical diagnostics and environmental control is well established. The improvement of hand held devices for point of care detection hold promising and attractive alternatives to existing laboratory-based immunochemical assays [29].

Aim of this study is development of a new impedimetric biosensor, based on the use of anti-HER3 for the determination of HER3 by forming a self-assembled monolayer on gold electrodes. anti-HER3 immobilization steps and measurements were evaluated by cylic voltammetry and electrochemical impedance spectroscopy. In order to investigate interaction betwwen HER3 and anti-HER3 immobilized onto gold surface, a novel impedimetric method called single frequency impedance measurement is also presented.

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2. Materials and methods

2.1. Materials

All reagents were obtained from Sigma-Aldrich (St. Louis, MO, USA). HER3, (human epidermal growth factor receptor family-3) and anti- HER3 were also purchased from Sigma-Aldrich. HER3, anti-HER3, and 0.1% BSA (bovine serum albumin) were prepared in 50 mM, pH 7, phosphate buffer and further dilutions were made using sterile phosphate buffer (pH 7.0, 0.01 M) as well. HER3 and anti-HER3 portions were prepared at certain concentrations and were stored at −20 °C until use. Synthetic serum solution was prepared by using 4.5 mM KCl, 5 mM CaCl2, 1.6 mM MgCl2, 4.7 mM (D+)-glucose, 2.5 mM urea, 0.1% human serum albumin, and 145 mM NaCl. A redox probe solution was prepared in 50 mM, pH 7.0, phosphate buffer which contained 0.1 M KCl, 5 mM $Fe(CN)_6^{4-}$ and 5 mM $Fe(CN)_6^{3-}$.

Gold working electrodes (1.6 mm^2 surface area), Ag/AgCl reference electrode, and Pt counter electrode were obtained from BASi (Warwickshire, UK). Electrochemical experiments were carried out by using a Compactstat with integrated impedance analyzer (Ivium Technologies, Eindhoven, The Netherlands) and Gamry Potentiostate/Galvanostate, Reference 600 (Gamry Instruments, Warminster, USA) interfaced with a PC. All electrochemical experiments were carried out in a Faraday cage (from iBAS, Warwickshire, UK) to block out external static electric fields.

3. Methods

3.1. Preparation of the self-assembled monolayer of 4-aminothiophenol

Before the use the gold electrodes were first polished with 0.05 μm alumina powder and then washed with ultrapure water. Following that, the electrodes were ultrasonically washed in absolute ethanol for 2 min to remove alumina residues. Then the electrodes were immersed in Piranha (H_2O_2/H_2SO_4, 30/70, v/v) solution for 3 min. Following that the electrodes were washed with ultra pure water. For the next step, the surfaces of the electrodes were dried by a pure argon stream. This polishing and cleaning procedure was repeated before every electrode preparation step. The clean gold electrodes were immediately immersed into 4-aminothiophenol solution (0.1 M, in pure ethanol) for 16 h. After this period, they were rinsed with ethanol and gently dried with an argon stream.

3.2. Anti-HER3 immobilization procedure on SAM of 4-ATP

For anti-HER3 immobilization, the electrodes modified with 4-ATP (Au/4-ATP) were immersed into 2.5% glutaraldehyde solution for 15 min in a dark surrounding to activate the amino ends of the 4-aminothiophenol. Later, the gold electrodes were washed with ultra-pure water gently, and were then dried by a pure argon stream again. Then 10 μl of anti-HER3 portion was applied to the active electrodes surface by a pipette. The electrodes were allowed to incubate for an hour in moisture and dark medium. After that electrodes were washed and dried as noted above. Finally 10 μl BSA solution (0.1%) was dropped onto the electrodes to block the active ends on the surface. The bare electrodes and the modified electrodes were indicated as Au, Au/4ATP, Au/4ATP/anti-HER3, and Au/4ATP/anti-HER3/BSA.

3.3. Measurement procedure

Cyclic voltammetry was used to characterize the layer-by-layer formation of the biosensors. The potential was varied between 0 and 500 mV (step size, 20 mV; scan rate, 50 mV/s) in the presence of 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 with 10 mV of amplitude was applied to the electrode over the formal potential of the redox couple (0.18 V). The redox couple used for impedance studies was the same as that used in cyclic voltammetry. Impedance spectra were collected in the frequency range between 10000 and 0.05 Hz.

After each of the anti-HER3 biosensors was assembled, the biosensor surface was used to interact with the HER3 solution. The standard solution of HER3 was injected over the biosensor surface by a micropipette. For each time measurement, the injection volume was 5 μL. The response value was read after one hour incubation in a moisture and dark medium. After this incubation period, the biosensor was gently immersed into the ultrapure water 20 times to remove physically adsorbed HER3 molecules. Finally, the biosensor was again put into the cell containing the $Fe(CN)_6^{4-/3-}$ redox probe solution and the electrochemical measurements were carried out as described previously.

4. Results and discussion

4.1. Anti-HER3 immobilization by SAM of 4-aminothiophenol

The use of SAMs has shown to provide molecular level control over the immobilization of several types of biomolecules [30].

Formation of organized monolayers of alkanethiols on gold surfaces was first discovered by Nuzzo and Allara [31]. Since then SAMs have been widely used in many different applications for surface modifications. SAMs can also be integrated with several molecular and cellular processes such as protein interactions [32,33]. Moreover they have been used for constructing molecular switching [34,35], biosensors [36–38] and micro/macrroarrays [39,40]. In fact the success of SAMs in several technological fields is caused by important advantages of gold itself. For example; gold is an inert metal, consequently oxidation could not easily occured on gold surfaces. Moreover the gold–sulfur interaction is a strong and a specific chemical process that allows the formation of self-assembled monolayers carrying other functional groups. Most importantly preparation of SAMs is a quite simple process which does not require sophisticated and high-priced equipment or extensive experience to be performed successfully.

This is the first biosensor system for determination of HER3 by SAMs modified gold electrode and using anti-HER3 as a biorecognition component. The changes occured on the surfaces of modified gold electrodes by formation of SAM and anti-HER3 immobization were investigated using CV and EIS. In the experiments, the redox couple $Fe(CN)_6^{3-/4-}$ was preferred for use as an electrochemical probe because $Fe(CN)_6^{3-/4-}$ was more sensitive to surface modifications, such as binding of HER3 to its antibody. Electrochemical impedance spectra were significantly affected by modifications on the electrode surface. Electrochemical impedance spectroscopy consists resistive and capacitive elements besides Warburg element and is a powerful method for analyzing the complex electrical resistance of a system. EIS is also sensitive to surface characteristic and changes of bulk properties, moreover it is especially well-suited to the detection of binding events on the transducer surface in biosensor fields, therefore it is a valuable and progressive technique [41].

A well-defined characteristic voltammogram of the redox couple could be observed on the bare gold electrode (Fig. 1A).