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Using Electrochemical Technique to Detect HER2 Antigen

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Phuc Le Thi Minh
Institute of Biotechnology, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet Str., Cau Giay Dis., Ha Noi, Viet Nam

Phuc Pham Van
Institute of Biotechnology, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet Str., Cau Giay Dis., Ha Noi, Viet Nam

Huan Le Quang
Institute of Biotechnology, Vietnam Academy of Science and Technology, 18 Hoang Quoc Viet Str., Cau Giay Dis., Ha Noi, Viet Nam

ABSTRACT *Early diagnosis of breast cancer is the most effective way to improve the survival rate and increase more chances of patients with breast cancer. In this study, to determine HER2 (human epidermal growth factor receptor 2) antigen, an aptasensor was developed by employing self-assembled of HER2-specific single stranded DNA aptamer on 3-mercaptopropionic acid-modified gold nanoparticles electrode. The surface properties of the gold electrode at each modification step were characterized by changing in cyclic voltammetry and square wave voltammetry responses. The decrease in the peak current was correlation with the concentrations of HER2 in the range of 0.35 ng/ml to 35 ng/ml. The aptasensor was successfully developed to detect HER2 antigen. This study could provide a promising and fast method for early detection of breast cancer.*

1. Introduction

Breast cancer, comprising of 22.9% of all cancer types, is the most common cancer among women worldwide with the incidence and death rates increasing rapidly in recent years [1]. HER2, also known as ErbB2 (avian erythroblastosis oncogene B), is overexpressed 20-30% in breast cancer cell and one of the best specialized biomarkers in the detection of breast cancer [2]. Present studies suggest that soluble fragments of the c-erbB-2 oncogene can be released from the cell surface and become detectable in breast cancer patients [3]. Now although there are many methods to diagnostic c-erbB-2 oncogene such as southern blot, dot blot, in situ hybridization [4, 5] but these methods have low sensitive, complicated, time-consuming, high cost as well as high risk of false positive and false negative. So it is necessary to develop a simple, rapid and effective method for detection HER2.

Recently, aptasensors (aptamer-based biosensors), which combine the high sensitivity of biosensors with the high specificity of aptamers, have been widely used in various fields such as clinical, pharmaceutical, and biochemical [6-8]. Until now some aptasensors for determination of proteins have been reported including electrochemical [9] and optical aptasensors [10, 11]. Among them, electrochemical aptasensors have received much attention due to unique advantages such as low detection limits, small analyte volumes, simple instrumentation, and minimal manipulations [8].

To make an electrochemical aptasensors, immobilization of aptamers on electrode surfaces is very important because it is not only produces a functionalized sensing interface, but also determines the sensitivity and selectivity of the aptasensors [12]. Various methods for the immobilization of aptamers on electrode have been reported, for example, direct absorption [13], covalent coupling [14], avidin-biotin interactions [15], sol-gel entrapment [16], and self-assembled monolayers (SAMs). SAMs, which are spontaneously formed from sulfur-containing compounds in contact with gold surfaces, provide a convenient basis for biomolecule immobilization, which are capable of specific molecular recognition, for sensing applications [17, 18].

This study aimed to make an electrochemical aptasensor for determination of HER2 antigen. The fabrication of electrochemical aptasensor was conducted according to SAMs method. The HER2 specific-ssDNA aptamer molecule was used to immobilization on electrode by modifying its 5' end to have an amine group. Before immobilization amine-terminated aptamer, the surface of gold nanoparticles electrode was pretreated with a self-assembled monolayer of 3-mercaptopropionic acid to produce amide linkage which ensured tight anchoring of the aptamer molecules on the gold surface [19, 20]. The fabrication of the gold electrode was confirmed by cyclic voltammetry (CV) and square wave voltammetry (SWV) responses of surface. Both CV and SWV values are changed substantially, and the relative decrease in the reduction peak current could be tabulated as a quantitative measure of the HER2 concentration. The developed aptasensor could be provided promising way to be used in the early diagnosis of HER2-dependent breast cancer.

2. Materials and methods

2.1. Materials

3-Mercaptopropionic acid (MPA), 2-(N-morpholino) ethanesulfonic acid (MES), N-hydroxy-succinimide (NHS), 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC), $K_3Fe(CN)_6$, $K_4Fe(CN)_6$, Gold (III) chloride hydrate $HAuCl_4$ (99%), H_2SO_4, Bovine Serum Albumine (BSA), PBS, potassium hydroxide (KOH) and hydrogen peroxide (H_2O_2) were purchased from Sigma-Aldrich and used without further purification.

Aptamer NH_2-5'-GCA GCG GTG TGG GGG CAG CGG TGT GGG GGC AGC GGT GTG GGG-3' was purchased from Macrogen, Korea and stored at -20°C before use.

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HER2 antigen: HER2/neu standards-six separate vials of recombinant HER2/neu p105. Standards are calibrated in ng/ml and are labeled with values that are 50-fold greater than the actual vialed dosage was purchased from Siemens Healthcare Diagnostics Inc., USA

Electrochemical measurements including CV and SWV were performed using CPA IOC HH5 computerized polarographic analyser (Institute of Chemistry, Vietnam Academy of Science and Technology, Vietnam).

2.2. Methods

2.2.1. Preparation and modification of gold electrodes

The gold electrode was polished with smooth ab paper, smooth velvet scarf, and then washed with distilled water. After that, the gold electrode was electrochemically activated in 0.5 M H_2SO_4 aqueous solution by cyclic voltammetry (CV) from 0.1 V to 1.8 V at a scan rate of 0.1 Vs^{-1} for 20 cycles with sensibility 5. Subsequently, the electropolished electrodes were surface dried by nitrogen gas and immediately dipped in the 20 mM MPA in ethanol/water (1/3, v/v) deposition solution for 3 h at room temperature, followed by rinsing the residual MPA molecules with ethanol [19]. Then the MPA-modified gold electrode was immersed in 100 mM MES containing 20 mM EDC and 20 mM NHS to activate the carboxyl-terminated group of MPA for another 15 min [21]. Finally, the aptamer was immobilized on the activated MPA SAM electrode by dipping the gold electrode in 2nM aptamer/PBS (10 mM, pH 7.4) solution for 1 h. CV and SWV (square wave voltammetry) of the fabricated electrode were recorded in 5 mM $K_4Fe(CN)_6$, 5 mM $K_3Fe(CN)_6$ and 0.1 M KCl dissociation solution with electrochemical mode from -0.3 V to 0.9 V at a scan rate of 0.1 Vs^{-1} for 10 cycles with sensibility 5 to determine electrochemical signal changes compared to initially gold electrode.

2.2.2. Assessing aptasensor performance

The prepared Au-MPA-Aptamer aptasensor was incubated in 1X BSA solution and then transferred to 10 mM pH 7 PBS containing various concentration of HER2 antigen at room temperature for 15 min each. After washing with 1X PBS to remove the non-attached antigen, CV and SWV were recorded in 5 mM $K_4Fe(CN)_6$, 5 mM $K_3Fe(CN)_6$ and 0.1 M KCl dissociation solution from 0.1 V to 1.8 V at a scan rate of 0.1 Vs^{-1} for 20 cycles with sensibility 5 to determine electrochemical signal changes compared to initially gold electrode.

3. Results and Discussion

3.1. Electropolishing of gold electrode

As shown in figure 1, the cyclic voltammetry on bare gold electrode was repeated when electrochemically polished in 0.5 M H_2SO_4 solution. The cycles tended to reach a stable gold oxide formation and invariant reduction peaks, implying a clean and uniform electrode surface [19, 22]. And prepared gold electrode can be used for subsequent experiments.

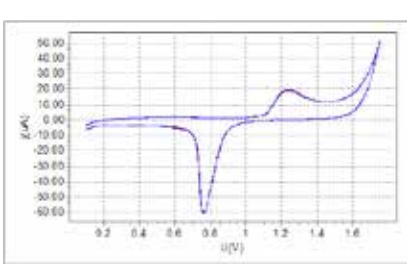

Figure 1. Cyclic voltammetry of bare gold electrode in H_2SO_4 0.5M solution

3.2. Fabrication of the aptasensor

The surface properties of the gold electrode at each modification step were characterized electrochemically. The CV results in 5 mM $K_4Fe(CN)_6$ and 5 mM $K_3Fe(CN)_6$ aqueous solution containing 0.1 M KCl in the range of -0.3 V to 0.9 V with the scan rate of 0.2 Vs^{-1} 1 cycle are shown in Fig. 2.

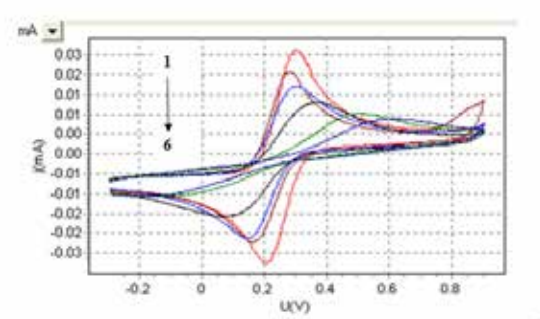

Figure 2. CV values of gold electrode at each modification step. Each curve represented the CV value for bare Au (1), Au-MPA (2), Au-MPA-HER2/NH2 aptamer (3), Au-MPA-HER2/NH2 aptamer-BSA (4) and Au-MPA-HER2/NH2 aptamer-BSA-HER2 antigen (5, 6)

As we known, cyclic voltammetry is a simple, rapid and powerful method for characterizing the electrochemical behavior of analytes that can be electrochemically oxidized or reduced. CV testing showed that the identification and the characterization surface properties of electrode at each step were possible through analysis of current changes at specific potentials.

At each step, CV measurements were carried out three times then their average value is used to evaluate changes in electrochemical signal. More detail data are provided in the table 1 below. In table 1, we recognize that the electrochemical activated bare gold electrode has a couple of well-defined redox wave of $K_3Fe(CN)_6$ at 303 mV and 209 mV (ΔEp = 94 mV, -Ipc = 13.544 µA, Ipa = 17.9 µA) and the redox current of the electrode modified with MPA SAM was decreased significantly to 287.8 mV and 184.7 mV ((ΔEp = 103.2 mV, -Ipc = 11.93 µA, Ipa = 16.1 µA), respectively. The changes mean that the formation of a compact SAM on the gold surface. As is well known, a MPA SAM has a blocking property that could prevent the redox reaction of $K_3Fe(CN)_6$ at the interface [24]. After the immobilization of aptamer, the redox peak currents decreased further, which indicates the aptamer was successfully attached on the electrode surface. The immobilized aptamer acts as an insulator blocking the charge exchange of the $Fe(CN)6^{3-}$ and the gold electrode [19].

Table 1. **Changes in current of gold electrode at each modification step**

	E_{pa}	E_{pc}	ΔE_p	I_{pa}	$-I_{pc}$
Au	303	209	94	17.9	13.544
Au-MPA	287.8	184.7	103.2	16.1	11.93
Au-MPA-Apt	309.03	155.37	153.67	10.59	10.5
Au-MPA-Apt-BSA	358.53	110.06	248.47	8.705	7.82
Au-MPA-Apt-BSA-HER2	539.23	124.26	415.04	4.117	3.36

To illustrate more clearly about obtained results after each step of electrode modification, we were carried out square

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wave voltammetry measurement with parameters follow as: initial potential 0 V, end potential 1 V. SWV result was showed in Fig. 3. We realized that current peak of gold electrode after modified with MPA, aptamer, BSA and HER2 antigen decreased gradually implying the formation of new layer on electrode surface.

In this study, HER2-specific single strand DNA aptamer was modified its 5' end with an amine then the amine-terminated aptamer was immobilized on the gold electrode surface covered with a self-assembled monolayer (SAM) of 3-mercaptopropionic acid. The carboxyl groups of MPA reacted with the 5'-end amine groups of the aptamers to produce peptide bonds [20]. And these bonds make anchoring of the aptamer molecules on the gold surface more sustainable. Next BSA was used to block free position on Au/MPA/aptamer electrode surface. It helps the interaction between aptamer and HER2 antigen became more specific

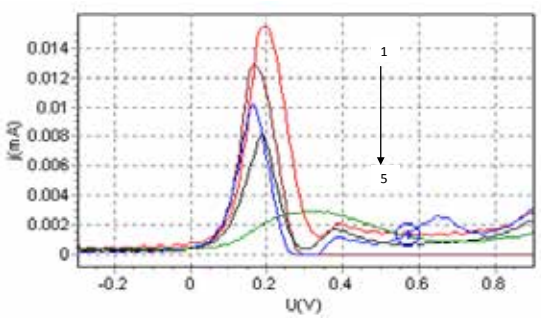

Figure 3. SWV values of gold electrode at each modification step. Each curve represented the CV value for bare Au (1), Au-MPA (2), Au-MPA-HER2/NH2 aptamer (3), Au-MPA-HER2/NH2 aptamer-BSA (4) and Au-MPA-HER2/NH2 aptamer-BSA-HER2 antigen (5)

A gold/MPA/aptamer HER2-NH2/BSA electrode was incubated in PBS solution containing different concentrations of HER2 antigen (from 0.35 ng/ml to 35 ng/ml) to get the corresponding CV, SWV responses. As shown in Fig. 4a and Fig. 4b, CV and SWV values decreased slightly proportional to increase of HER2 antigen concentration. This implies prepared aptasensor can be able to detect HER2 antigen in the solution.

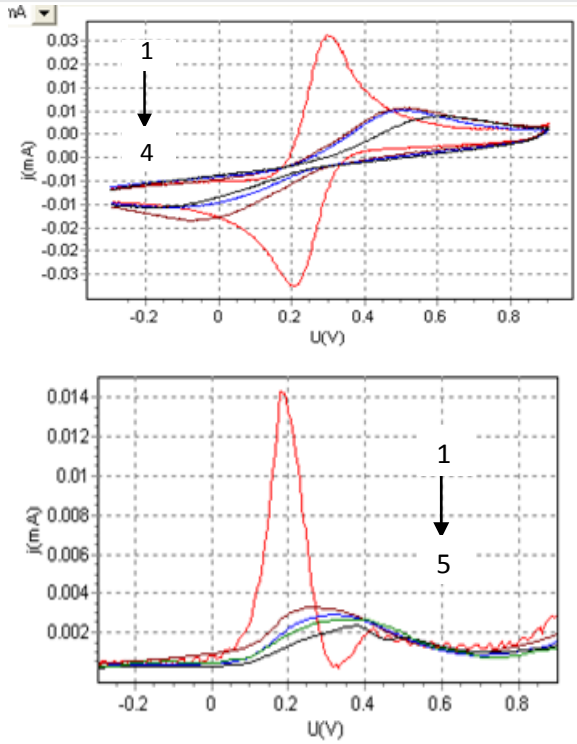

Figure 4. CV and SWV values of Au electrode and Au/MPA/Aptamer HER2-NH2/BSA/HER2 antigen at different concentrations. Each curve represented CV and SWV values for bare Au (1), Au/MPA/Aptamer HER2-NH2/BSA/HER2 antigen 0.35 ng/ml (2), Au/MPA/Aptamer HER2-NH2/BSA/HER2 antigen 3.5 ng/ml (3), Au/MPA/Aptamer HER2-NH2/BSA/HER2 antigen 35 ng/ml (4, 5)

4. Conclusion

We were successful developed an electrochemical aptasensor to determine HER2 antigen. This aptasensor could be detected HER2 in PBS in a concentration range from 0.35 ng/ml to 35 ng/ml.

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