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A novel protein analytical method based on hybrid-aptamer and DNA-arrayed electrodes

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ABSTRACT

A novel protein assay method based on a DNA array was developed, in which human immunoglobulin E (hIgE) and its DNA aptamer were used as an analytical model. The target protein hIgE was captured by the aptamer in homogeneous solution and then the resulting hIgE-aptamer complex was hybridized onto probes self-assembled on the DNA array. Measured by electrochemical impedance spectroscopy (EIS), the charge transfer resistance (Rct) of electrodes before and after hybridization was compared. To test the selectivity of the method, four different probes with one to three mismatched bases were immobilized on respective electrodes. The results showed that the complex could be hybridized and detected out on the electrodes modified with the fully complementary sequences. In addition, the DNA array could be employed to analyze multiple samples selectively with the matched aptamer.

1. Introduction

DNA chips had expanded their applications in many fields and the techniques for fabricating and detecting DNA chips are becoming self-contained such that the more target analytes including proteins and carbohydrates are involved [1,2]. In recent years, DNA-directed immobilization (DDI) methods [3] have been applied in addressing assembly of proteins [4] on DNA chips. However, key steps that link DNA tails with antibodies need complex steps including gently reaction and troublesome purification.

Compared to antibodies, aptamers are more stable with considerable affinity and facile synthesis [5]. Many biosensors were prepared by combining aptamers with various detection techniques [6–8], in most of which aptamers were directly immobilized onto chips [9,10]. However, aptamers might suffer from the lying or astriction on the surface of chips such that the binding efficiency was minified [11].

Here we present a new technique combining DNA chips with aptamers named as DNA-directed immobilization of aptamer (DDIOA) method, in which aptamers recognizes the target protein in homogeneous solution and then hybridizes onto the DNA chips. Free motion in homogeneous solutions makes the aptamers bind target proteins with right configuration. In addition, the DNA array could be employed in protein analysis remaining multiplex designed modes. In our experiments, hIgE and its DNA aptamer [12] were used as a mode to be assayed on a DNA electrode array. Label-free electrochemical impedance spectroscopy (EIS) was employed to measure the surface condition changed according to hybridization and specific binding.

2. Materials and methods

2.1. Materials

All oligonucleotides and aptamers were customized from Sangon Co. (Shanghai, China). Immunoglobulin E purified from human plasma was purchased from US Biological Inc. (Swampscott, MA). Other chemicals were of analytical reagent grade. All samples and buffers were prepared using deionized water obtained from a Milli-Q water purification system.

2.2. Design of aptamers and probe sequences

All the capture probes were modified with thiol at 5′ end for their self-assembled on gold electrodes. T20 was employed to hybridize with Apt1. For array analysis, Apt2 was used to bind target hIgE and the probe complementary to it was P1. The probes with 1–3 mismatch bases comparing to P1 were named P2, P3, P4, respectively. Forenamed sequences were listed below:

T20: 5′-HS-$(CH_2)_6$-TTT TTT TTT TTT TTT TTT TT-3′
P1: 5′-HS-$(CH_2)_6$-AGG ACA CGT TTT TGC TCC-3′
P2: 5′-HS-$(CH_2)_6$-AGG ACA CGT TT***G*** TGC TCC-3′
P3: 5′-HS-$(CH_2)_6$-AGG ACA CGT ***G***T***G*** TGC TCC-3′

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P4: 5′-HS-$(CH_2)_6$-AGG ACA CGT ***GGG*** TGC TCC-3′
Apt1: 5′-GGG GCA CGT TTA TCC GTC CCT CCT AGT GGC GTG CCC CAA AAA AAA AAA AAA AAA AAA-3′
Apt2: 5′-GGG GCA CGT TTA TCC GTC CCT CCT AGT GGC GTG CCC CGG AGC AAA AAC GTG TCC T-3′
Apt3: 5′-GGG GCA CGT TTA TCC GTC CCT CCT AGT GGC GTG CCC CGG AGC ACC CAC GTG TCC T-3′

2.3. Preparation of gold arrayed electrodes modified with probes

The gold electrode array was prepared on glass slides by vapor deposition with thickness of 200 nm and 5-nm-thick chromium was between photolithographic gold film and glass. The diameter of each working electrode was 1.5 mm. The chip was always immersed in a piranha solution for 30 min and rinsed carefully with deionized water and ethanol.

After being dried under a stream of nitrogen, custom-built hydrophobic port seals were attached onto the chip for immobilization of probes by enclosing electrodes with matched microwells (Fig. 1A). 1 μM thiolated probe was mixed with 10 μM 3-mercapto-1-propanol and the resulting solution was transferred into the relative microwells to incubate overnight. Then the chips were washed with PBS for three times after immobilization.

2.4. Reaction and detection of hIgE

HIgE was mixed with the aptamer of the certain concentration firstly, followed immediately by adding the resulting mixture into reaction chamber enclosed by frames. For array analysis, the frames for single electrodes were removed and bigger frames was stuck onto the chips to form reaction chambers, each of which enclosed four electrodes patterned with different probes. After the binding and hybridization were carried out for 30 min, the reaction solutions were taken away and the chip was washed by PBS for three times.

2.5. Measurement of Faraday impedance

AutoLab PGSTAT302 electrochemical workstation (Eco Chemie, Netherlands) was employed to measure Faraday impedance of gold electrode arrays. A two-electrode system consisted of the gold working electrode and a standard Ag/AgCl electrode, which served both as the counter and reference electrode. Washing away all reaction solution in the frame by PBS buffer, 5 mM $[Fe(CN)_6]^{3-/4-}$ in PBS was added and the impedance was measured by software control. The frequency range investigated was from 100 kHz to 1 Hz at the formal potential of 250 mV using an alternate voltage of 5 mV.

3. Results and discussion

3.1. Characterizing feasibility of the method

In DDI method, the linkage of proteins and DNA needs complex reaction as well as purification. In addition, the synthesis of DNA–antibody conjugates might impair the activity of the protein even by mild linking process because the chemical reaction is not site-selective [4]. In our protocol, we linked the DNA and the protein by biological recognition remaining the native character of the biomolecules. Thus aptamer as a bifunctional linkage could transform detection of aptamer–protein recognition into that of hybridization. In addition, hybridization based on the liner structure of DNA is easier than recognition between protein and surface-tied aptamer relying on the secondary structure.

HIgE and its aptamer as a model were used to validate the bound of protein and aptamers, while poly(A)20-poly(T)20 as a complementary recognition pair to testify the bound of aptamers and DNA electrodes. As shown in Fig. 1B, hIgE was mixed with

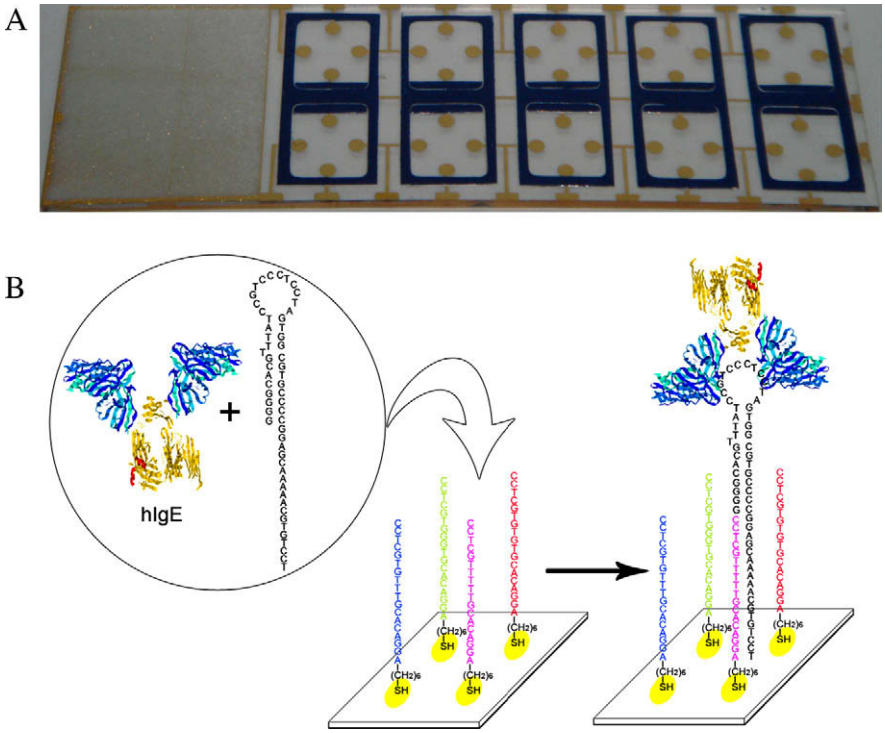

Fig. 1. (A) Photo of gold electrode array. The chip was divided into separated microwells by hydrophobic port seals. (B) Scheme of DNA-directed immobilization of hIgE-aptamer complex onto a DNA-arrayed electrode. HIgE bound its DNA aptamer, followed by hybridization of the resulting complex with capture probe on the array by tagged sequence.