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Tetrahedral DNA probe coupling with hybridization chain reaction for competitive thrombin aptasensor

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ABSTRACT

A novel competitive aptasensor for thrombin detection is developed by using a tetrahedral DNA (T-DNA) probe and hybridization chain reaction (HCR) signal amplification. Sulfur and nitrogen co-doped reduced graphene oxide (SN-rGO) is firstly prepared by a simple reflux method and used for supporting substrate of biosensor. Then, T-DNA probe is modified on the electrode by Au-S bond and a competition is happened between target thrombin and the complementary DNA (cDNA) of aptamer. The aptamer binding to thrombin forms an aptamer-target conjugate and make the cDNA remained, and subsequently hybridizes with the vertical domain of T-DNA. Finally, the cDNAs trigger HCR, which results in a great current response by the catalysis of horseradish peroxidase to the hydrogen peroxide + hydroquinone system. For thrombin detection, the proposed biosensor shows a wide linearity range of 10^{-13}–10^{-8} M and a low detection limit of 11.6 fM (S/N = 3), which is hopeful to apply in biotechnology and clinical diagnosis.

1. Introduction

Protein is a crucial ingredient which makes up whole cell and tissue of human (Li et al., 2017). The vital parts of body all need proteins' participation, so the detection of protein fleetly and accurately in disease diagnosis is particularly important with the increasing importance of precision medicine (Lv et al., 2017; Cardoso et al., 2017). Thrombin, a serine protease produced from inactive zymogen prothrombin through a sequence of enzyme fission processes in humans (Du et al., 2016), is the dominating proteinase in the blood coagulation system, possessing many procedures in phlogosis and tissue repair at the vascular wall (Ye et al., 2017). The concentration of thrombin in blood is different in various conditions. It is not present in blood under normal conditions. However, it can reach low-micromolar concentrations during the coagulation process, and low levels (low nM) of thrombin generated early in hemostasis are also important to the overall process (Shuman and Majerus, 1976). Out of the hemostatic process, circulating thrombin has been detected at high-picomolar range in blood of patients suffering from diseases known to be associated with coagulation abnormalities (Bichler et al., 1996). Hence, it would be remarkably ponderable to fabricate a rapid and sensitive pathway for detecting thrombin.

The traditional methods of monitoring thrombin are enzyme-linked immunosorbent assay (Pelzer et al., 1988), radioimmunoassay (Shuman et al., 1976) and so on. However, antibodies used in these methods are expensive, hard to operate, need more time and manpower, and the preparation and preservation of antibodies are difficult. The aptamer-based sensing platform draws increasing attention of researchers in recent years (Nguyen et al., 2017; Jeddi et al., 2017). Aptamer is a single-stranded oligonucleotide, which is entirely designed from random-sequence DNA or RNA libraries in an extracorporeal choice procedure that can bundle many types of target molecules with affinity and specificity (Ellington et al., 1990; Hou et al., 2017), such as small inorganic (Tan et al., 2017), organic substances (Li et al., 2016), protein (Yang et al., 2017) and cell (Chen et al., 2016a). Most of all, aptamer-based sensing strategies exert visible merits compared with traditional methods, including low cost, simple synthesis, good chemical stability and close binding force, which make aptamers are extensively applied in the fabrication of multifarious biosensor (Ha et al., 2017; Taghdisi et al., 2016). For human thrombin, there is an aptamer with 29 bases DNA oligonucleotide which can bind to the heparin-binding exosite of thrombin (Zhao et al., 2015). In this circumstance, it's appropriate to fabricate detecting methods for thrombin that utilizes the firm binding force of the thrombin and its corresponding aptamer. Electrochemical method is an acknowledged and befitting technique for fast, sensitive and selective detection of thrombin (Zhao et al., 2017). The primary difficulty is the grasp of density and orientation of molecules at the electrode surface. In order to solve this problem, a tetrahedral DNA

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nanostructure (T-DNA) based holder has been developed, which is consist of 4 single-stranded DNAs (Lin et al., 2016). This kind of stereo structure can exactly control the assembled probe DNA on the electrode interface and rise above the spatial effect of a conventional single-stranded probe to increase accessibility and reactivity, and prevent the entrance of spacer molecules (Poturnayová et al., 2015; Huang et al., 2015; Miao et al., 2015).

For the sake of obtaining a low detection limit for the thrombin sensor, a variety of signal amplification techniques are induced. Hybridization chain reaction (HCR) is an isothermal method for signal amplification without the participation of any bio-enzymes or thermal cycler (Dirks et al., 2004; Liu et al., 2016). Hence, many limitations of relevant enzymatic methods like pinpoint control of pH, temperature and buffer media can be avoided, leading to a widespread application of HCR for constructing the sensing platform. In addition, r using nanomaterials for signal amplification is another suitable element because they commonly possess big specific area to immobilize more signal molecules at the electrode interface, and is favorable to speed up the electron transfer (Wang et al., 2017; Singh et al., 2016). Graphene oxide (GO), its particular electronical, physical, and chemical performances have made it a hopeful option for various application in biosensor (Huang et al., 2016, 2014). Substitutive adulteration with ethnic sulfur and nitrogen atoms into the carbon net can adjust the chemical performances, produce new vivid sites, and markedly facilitate the catalytic activity of GO (Duan et al., 2015). Additionally, co-doping by two elements with different electronegativities can induce a distinctive electron allocation and then issue in a synergistic effect (Chen et al., 2016b).

Au nanoparticles (AuNPs) are mostly recommended owing to the fact that they can greatly increase the current response of the modified sensor with a good conductive ability and immobilization of biomolecular by Au–S bond (Shuai et al., 2016), and have been widely used to construct aptamer sensors (Shuai et al., 2017; Kuang et al., 2016). The aforementioned standpoints have motivated the present assay. In this work, we prepare S, N co-doped reduced graphene oxide (SN-rGO) as electrode supporting substrate by an effective and simple reflux method. Then, gold nanoparticles (AuNPs) are electrodeposited and T-DNA probe is modified on the electrode by Au–S bond. A competitive aptasensor for the sensitive detecting thrombin is constructed by combining SN-rGO, T-DNA probe and HCR signal amplification. Besides, a complementary DNA (cDNA) is introduced which is competed with target thrombin of aptamers. By using the developed assay, thrombin can be detected with a low detection limit of 11.6 fM, which is significantly lower than other aptasensor. The results can be attributed to the good conductivity of SN-rGO, stable structure of T-DNA probe and HCR signal amplification strategy.

2. Experimental

2.1. Experimental reagent and instrument

Graphite powder, $(NH_4)_2S_2O_8$, $KMnO_4$, thiocarbamide, glycol, carbamide and Na_2S were purchased from Sigma Aldrich (St. Louis, MO). Thrombin, carcinoembryonic antigen (CEA), bovine serum albumin (BSA), platelet derived growth factor-BB (PDGF-BB) and hydroquinone (HQ) were obtained from Shanghai Xueman Biotechnology Co. Ltd (China). The DNA sequences were acquired from Shanghai Sangon Biological Engineering Technology Co. Ltd (Shanghai, China). All DNA sequences were synthesized by using standard phosphoramidite chemistry and purified using reversed phase HPLC. The sequences of synthetic DNA are shown in Table 1.

Cyclic Voltammetry (CV) and differential pulse voltammetry (DPV) were measured on an EC550 electrochemical workstation (Wuhan, Gaoss Union, China) and electrochemical impedance spectroscopy (EIS) working curves were recorded on a RST5200F electrochemical workstation (Zhengzhou Shi Rui Si Instrument, China). A conventional three-electrode system was used which made up of a glassy carbon electrode (GCE) working electrode, an Hg/Hg_2Cl_2 reference electrode and a platinum wire auxiliary electrode. The as-prepared materials were characterized by a Hitachi S-4800 scanning electron microscope (SEM, Tokyo, Japan), a JEM 2100 transmission electron microscope (TEM, JEOL, Tokyo, Japan) and a K-ALPHA 0.5EV X-ray Photoelectron Spectrometer (XPS, Thermo Fisher Scientific, UK.). Moreover, energy dispersive spectrum (EDS) analysis was attached to SEM for exploring elementary component.

2.2. Preparation of S, N co-doped reduced graphene oxide

GO was prepared by modified Hummer's method (Hummers et al., 1958). The SN-rGO was prepared according to reference (Bag et al., 2015). GO was first dispersed in 1 mg mL^{-1} glycol solution. 3 mM thiocarbamide's glycol solution was then added into the dispersion serving as dopant of S and N. Subsequently, the mixture was transferred to a round-bottom flask and kept at 180 °C for 3 h. After cooling to room temperature, the mixture was centrifuged and washed with DI water and ethanol, and then dried in vacuum drying oven at 60 °C for 10 h. The S-rGO and N-rGO were prepared with the similar process except that by using carbamide and Na_2S as dopants.

2.3. Preparation of tetrahedral DNA probe

The GCE was polished with 0.3 and 0.05 aluminium oxide slurry to obtain a mirror surface, followed by swashing with ethanol and ultrapure water to remove remaining impurities, and then dried by nitrogen. An 8 μL SN-rGO suspension (1 mg mL^{-1}) was dropped on GCE and dried in air to form a homogeneous membrane. After that, AuNPs were immobilized on the GCE by electrodeposition method in 0.1% $HAuCl_4$ with 0.1 M KNO_3 to obtain AuNPs/SN-rGO/GCE. The modification of tetrahedral DNA probe (T-DNA) on AuNPs/SN-rGO/GCE was carried out as follows: four ssDNAs (Tetrahedron A, B, C, D) were respective dissolved in 10 mM Tris-HCl buffer solution (10 mM TCEP, 50 mM $MgCl_2$, pH 8.0) to form a concentration of 4 μM. The four ssDNAs were mixed and heated at 95 °C for 2 min with the final concentration of 1 μM. After the mixture cooled to 4 °C in ice bath, T-DNA was formed and then 8 μL solution was applied on AuNPs/SN-rGO/GCE and incubated at 4 °C over night. The obtained T-DNA/AuNPs/SN- rGO/GCE was dipped in 4 μL MCH (0.5 mM) to eliminate the non-specificity adsorption of electrode surface and rinsed carefully before use.

2.4. Preparation of competitive aptasensor

2 μL aptamer (0.5 μM) was mixed with 5 μL thrombin and then incubated at 30 °C for 120 min. Subsequently, 2 μL cDNA (0.5 μM) was added and kept at 30 °C for 60 min. After that, the mixture was dropped on the T-DNA/AuNPs/SN-rGO/GCE and incubated at 30 °C for another 60 min. After thoroughly swashing, the electrode was treated with the mixture of H1 and H2 at room temperature for different time. Soon afterwards, 8 μL avidin-HRP (10 μg mL^{-1}) was covered on the electrode and incubated for 30 min at room temperature. In the end, the obtained biosensor was washed entirely with 10 mM PBS (pH 7.0).

2.5. Electrochemical measurement

Cyclic voltammetry (CV) was tested in 1.0 mM $[Fe(CN)_6]^{3/4-}$ solution including 0.1 M KCl with a potential range from − 0.2V to 0.6 V and the scan rate of 100 mV s^{-1}. The electrodeposition experiment was carried out in 0.1% $HAuCl_4$ solution containing 0.1 M KNO_3 with a initial potential of − 0.2 V. Electrochemical impedance spectroscopy (EIS) was carried out in a 10 mL aqueous solution including 1.0 mM $[Fe(CN)_6]^{3-/4-}$ and 0.1 M KCl at a potential of 0.2 V over the frequency range from 0.1 Hz to 100 kHz. Differential pulse voltammetry (DPV) was executed in 10 mL PBS buffer containing HQ and H_2O_2 with the