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A highly sensitive label-free electrochemical aptasensor for interferon-gamma detection based on graphene controlled assembly and nuclease cleavage-assisted target recycling amplification

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ABSTRACT

We report here a highly sensitive and label-free electrochemical aptasensing technology for detection of interferon-gamma (IFN-γ) based on graphene controlled assembly and enzyme cleavage-assisted target recycling amplification strategy. In this work, in the absence of IFN-γ, the graphene could not be assembled onto the 16-mercaptohexadecanoic acid (MHA) modified gold electrode because the IFN-γ binding aptamer was strongly adsorbed on the graphene due to the strong π–π interaction. Thus the electronic transmission was blocked (eT OFF). However, the presence of target IFN-γ and DNase I led to desorption of aptamer from the graphene surface and further cleavage of the aptamer, thereby releasing the IFN-γ. The released IFN-γ could then re-attack other aptamers on the graphene, resulting in the successive release of the aptamers from the graphene. At the same time, the "naked" graphene could be assembled onto the MHA modified gold electrode with hydrophobic interaction and π-conjunction, mediating the electron transfer between the electrode and the electroactive indicator. Then, measurable electrochemical signals were generated (eT ON), which was related to the concentration of the IFN-γ. By taking advantages of graphene and enzyme cleavage-assisted target recycling amplification, the developed label-free electrochemical aptasensing technology showed a linear response to concentration of IFN-γ range from 0.1 to 0.7 pM. The detection limit of IFN-γ was determined to be 0.065 pM. Moreover, this aptasensor shows good selectivity toward the target in the presence of other relevant proteins. Our strategy thus opens new opportunities for label-free and amplified detection of other kinds of proteins.

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1. Introduction

Interferon gamma (IFN-γ), an important cytokine, is predominantly produced by a number of immune cell types including natural killer (NK), natural killer T (NKT) cells, activated $CD4^+$ T cells, and other lymphocytes. IFN-γ is critical for innate and adaptive immunity against viral and intracellular bacterial infections and tumor control (Boehm et al., 1997). Aberrant IFN-γ expression is associated with a number of autoinflammatory and autoimmune diseases. For example, it was reported that IFN-γ is detected in the circulation of patients with autoimmune diseases, such as systemic lupus erythematosus (Valencia et al., 2007). Therefore, the detection and quantification of IFN-γ is important for both immunology research and clinical diagnosis applications (Prussin and Metcalfe, 1995). Antibody-based immunoassay systems can offer high sensitivity for IFN-γ detection. However, shortcomings with their production, stability, and modification limit their extensively application (Suni et al., 1998; Dijksma et al., 2001; Bart et al., 2005; Hermann et al., 2008; Zhu et al., 2009). It is thus highly desired to develop sensitive, selective and simple methods for IFN-γ detection.

Aptamers are single-stranded DNA or RNA sequences artificially selected through systematic evolution of ligands by exponential enrichment (SELEX). They can bind a wide range of specific targets (e.g., small molecules, proteins, amino acids, and even cells) with high specificity and affinity (Ellington and Szostak, 1990; Tuerk and Gold, 1990). As an emerging class of molecules for target recognition, aptamers can provide several advantages over antibodies, such as high selectivity, stability, versatile target binding, easy regeneration capabilities and high resistance against denaturation. There has been increasing interest in using aptamers as alternatives in protein detection. Recently, several aptasensors based on fluorescence (Tuleuova

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et al., 2010), surface plasmon resonance (SPR) (Chang et al., 2012), quartz crystal microbalance (QCM) (Min et al., 2008), electrochemistry (Liu et al., 2010; Zhang et al., 2012; Zhao et al., 2012) have also been constructed for detection of IFN-γ. Among them, electrochemical aptasensor has attracted particular attention because it may offer greater signal stability, sensitivity, simplicity and fast response for IFN-γ detection. But most of them involve complicated labeling, probe immobilization or stripping procedures and thus are unsuitable for practical application. Furthermore, improving the sensitivity of the electrochemical aptasensors for IFN-γ detection in real-world samples is still needed, such as the quantitative determination of IFN-γ in cell culture supernates, serum, plasma, and so on. As the advances in nanotechnology and biotechnology, many kinds of nanomaterials (metal, carbon, semiconductor, magnet) (Rosi and Mirkin, 2005; Xiang et al., 2010; Wang et al., 2011; He et al., 2011; Nie et al., 2009) and bio-amplification strategies (rolling circle amplification (RCA), hybridization chain reaction (HCR), enzyme assisted target amplification strategy) (Zhou et al., 2007; Zhao et al., 2012; Xuan et al., 2012) were widely used in the aptasensors to improve the sensitivity. Especially as reported recently, coupling nanomaterials and bio-amplification strategies advantages showed good application prospects in electrochemical biosensing (Tang et al., 2012; Jie and Yuan, 2012; Xu et al., 2012).

Graphene is a single-atom-thick and two-dimensional carbon material. It becomes one of the popular nanomaterials for its outstanding properties since its first isolation in 2004. Due to its excellent properties such as excellent electronic transport properties, high surface area, low cost, etc., graphene is also an ideal material for electrochemistry (Pumera, 2009; Pumera et al., 2010; Chen et al., 2010). By coupling with bio-amplification strategies, graphene has attracted much attention in highly sensitive electrochemical sensing (Tang et al., 2011; Chen et al., 2011; Jiang et al., 2012). However, in these reported works, graphene was directly deposited or collected on the electrode surface to enhance the surface area and increase the conductivity of the electrode. Recently, self-assembled monolayer (SAM) films in the electrochemical fields have triggered enormous interest because of their potential applications in sensor fabrication (Brust et al., 1998; Horswell et al., 2003; Su et al., 2006). When the electrode surface is modified with alkyl chain, inhibition of electron transfer is observed. If well conductive material such as metal nanoparticles or carbon nanomaterials, was assembled on the alkyl chain modified electrode through covalently or noncovalently bonding, fast electron transfer to aqueous redox couples will take place. Based on this property, carbon nanotubes controlled assembled electrode have been well developed to fabricate signal amplification sensor (Wu et al., 2009; Guo et al., 2011; Nie et al., 2012), which was chiefly based on the different absorption ability between "naked" carbon nanotubes and ssDNA wrapped carbon nanotubes onto the alkyl chain modified electrode. As far as we know, although graphene-SAM film electrode were also reported and studied (Yang et al., 2010; Xie et al., 2010), the reports about using the graphene-SAM film electrode in biosensing application were relatively few (Li et al., 2012). Herein, we demonstrated a label-free and highly sensitive electrochemical aptasensor for indirect detection of IFN-γ in a homogeneous solution phase with controlled assemble of graphene on the MHA/SAM modified gold electrode and DNase I assisted target recycling. In our protocol, graphene prevented DNA from nuclease cleavage firstly, owing to the steric-hindrance effect, single-stranded aptamer are bound to graphene surface through hydrophobic and π-stacking interactions between the nucleobases and graphene to form graphene–aptamer composites. When the aptamer combined with the target, DNase I could cleave the free aptamer and release the target for another attack. Likewise, a small amount of target IFN-γ could efficiently induce the release of a large number of the aptamers from the graphene surface; more "naked" graphene could then be assembled on the electrode. Graphene acts not only as an indirect signal reporter but also as a "signal amplifier" to enhance the electrochemical response. Furthermore, the emergence of nuclease cleavage provides exciting new possibilities for achieving higher sensitivity than the 1:1 binding ratio assays (Beyer and Simmel, 2006; Yang and Mierzejewski, 2010; Shukla et al., 2010; Pu et al., 2011). So the dual amplification strategies ensure the high sensitivity of the assay. The detection limit of IFN-γ was determined to be 0.065 pM. This label-free signal-amplified electrochemical biosensor for IFN-γ detection in homogeneous solution with good sensitivity and selectivity has good potential for practical applications.

2. Experimental

2.1. Reagents and materials

Oligonucleotides designed in this study were synthesized by Sangon Biotechnol. Co. Ltd. (Shanghai, China), which were purified by HPLC and confirmed by mass spectrometry. The sequence of IFN-γ aptamer is 5′-GGGGTTGGTTGTGTTGGGTGTTGTGT-3′. The IFN-γ aptamer stock solution was obtained by dissolving synthesized IFN-γ aptamer in Tris–HCl buffer solutions (pH 7.4). DNase I and 1x reaction buffer(10 mM Tris–HCl, 2.5 mM $MgCl_2$, 0.5 mM $CaCl_2$, pH 7.6) were purchased from New England Biolabs (Beijing), Ltd. (Recombinant DNase I: 1000 U/mL). Graphene oxide was purchased from Xianfeng Nano Co. Ltd. (Nanjing, China). IFN-γ, 3-(N-morpholino) propanesulfonic acid (MOPS), and $AgNO_3$ were obtained from Beijing Dingguo Biotechnol. Co. Ltd. (Beijing, China). 90% 16-mercaptohexadecanoic acid (MHA) and ferrocenecarboxylic acid (FcCOOH) were purchased from Sigma Aldrich Chemical Co. Ltd. All other reagents were of analytical grade and were used without further purification. All solutions were prepared and diluted using ultrapure water (18.2 MΩ cm) produced by the Millipore Milli-Q system.

2.2. Instrumentation

Electrochemical measurements were performed at room temperature using a CHI660A electrochemical workstation (Shanghai Chenhua Instrument Corporation, China). A conventional three-electrode cell was employed, which involved a gold working electrode of a diameter of 2 mm, a platinum wire counter electrode, and a saturated calomel reference electrode (SCE). All potentials in this paper were reported with respect to SCE. The Atomic force microscopy (AFM) imaging was performed using the tapping mode in open air on a multimode AFM (SPI3800N-SPA400, Seiko Instrument).

2.3. Preparation of graphene

Graphenes were prepared by the chemical reduction of graphene oxide with hydrazine according to the literature (Hummers and Offeman, 1958; Guo et al., 2010). Briefly, 20.0 mL of the homogeneous graphene oxide dispersion (0.5 mg/mL) was mixed with 200.0 μL of ammonia solution, followed by the addition of 30 μL of hydrazine solution. After being vigorously stirred for a few minutes, the vial was put in a water bath (60 °C) for 3.5 h. Then, a stable black dispersion was obtained. The black dispersion was subsequently filtered with a nylon membrane (0.22 μm) to obtain graphenes that can be redispersed readily in water upon ultrasonication. The AFM imaging results demonstrated that the size of the obtained graphenes was about in range of 100–500 nm.

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