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Hybridization chain reaction and target recycling enhanced tumor necrosis factor alpha aptasensor with host-guest interaction for signal probe collection

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ABSTRACT

In this paper, we have proposed a new hybridization chain reaction (HCR) and target recycling enhanced tumor necrosis factor alpha (TNF-α) aptasensor with host-guest interaction for signal probe collection. HCR induced DNA nanowires can load substantial methylene blue (MB) on electrode surface, in the presence of target TNF-α and RecJf exonuclease, the TNF-α firstly bound with corresponding TNF-α binding aptamer (S2, which used for TNF-α recognition and HCR initiation) and thus resulted in the dissociation of MB intercalated DNA nanowires from electrode into solution, while the RecJf exonuclease further made the recycling of TNF-α to obtain more dissociated DNA nanowires. With the assistant of duplex-specific nuclease (DSN), the dissociated DNA nanowires with substantial intercalated MB in solution released the free MB, which then selectively captured by the cucurbituril 7 (CB)/nano gold@chitosan functionalized electrode via host-guest interaction to produce the electrochemical signal, so the electrochemical signal would be changed by TNF-α. By tracing the electrochemical signal of adsorbed MB, our aptasensor can exhibit high sensitivity for TNF-α detection with a wide linear range from 0.001 ng/mL to 100 ng/mL and an extremely low detection limit of 0.5 pg/mL, which can also easily distinguish TNF-α in the complex samples with high specificity, providing a great potential in clinical applications in the future.

1. Introduction

Pro-inflammatory cytokine tumor necrosis factor alpha (TNF-α) is a 157-amino acid long polypeptide with an apparent molecular weight of 51 kDa when exists as a trimer [1,2]. It mediates a variety of cell functions, including the stimulation of nitric oxide (NO) production which has been related to oxidative stress and diseases such as stroke, diabetes, severe meningococcemia, rheumatoid arthritis, and chronic inflammation [3–6]. Research has shown that TNF-α is not produced by normal cells, but rather to be induced by invasive stimuli in the setting of both endoplastic and infectious disease. Therefore, the development of sensitive methods for detection of TNF-α is particularly important for biomedical research and clinical diagnosis. Traditional methods such as enzyme-linked immunosorbent assays (ELISA) and radioimmunoassays (RIA) are restricted with the detection limit and thus difficult to realize the sensitive TNF-α detection owing to its low level in human fluidics [7,8]. Moreover, these assays rely on antibody pairs for creation of sandwich immunoassays, requiring expensive reagents and multiple washing steps to develop the signal. Recently, electrochemical aptamer-based sensor (aptasensor) as a new kind of biosensor has attracted considerable attention due to their high sensitivity, low cost and simple device portability.

For electrochemical aptasensor, signal amplification is a very effective way to obtain a low detection limit and high sensitivity. In this regard, up to now, various signal amplification strategies including enzyme-free and –dependent techniques have been designed and developed. For example, hybridization chain reaction (HCR) is an isothermal enzyme-free amplification technique by which the initiator can trigger hybridization and thus carry out the polymerization of oligonucleotides into long nicked dsDNA polymers spontaneously under mild conditions, which avoids the need of rigorous conditions to keep the protein-enzyme activity [9–12]. Importantly, obtained products from HCR are DNA double helices that are highly ordered. Substantial signaling probes can be attached on these helices with precisely controlled density and may be beneficial for the final amplification efficiency [13,14]. Therefore, HCR displays great potential in signal amplification. Moreover, target recycling carried out by enzymes such as nicking enzymes, DNAzymes and exonucleases has also been

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Table 1
Sequence of synthesized oligonucleotides used in this work.

Oligonucleotide	Sequence (from 5′ to 3′)
Capture DNA (S1)	SH-$(CH_2)_6$-TTT TCG TTC GCT TGG AAA GAC CTT ATC
TNF-α binding aptamer (S2)	GCG GCC GAT AAG GTC TTT CCA AGC GAA CGA AAA
Auxiliary probe 1 (H1)	TAC GTG GCT TGG ACC GAC CGA ATT AAC GAT A
Auxiliary probe 2 (H2)	GTC CAA GCC ACG TAT ATC GTT AAT TCG GTC G

proven as the effective signal amplification [15–17]. With the usage of catalytic nuclease in fabricated aptasensor, further recycling and reuse of the target will be performed that significantly amplifies the detection signal. Based on the observation mentioned above, two favorable strategies, including HCR and exonuclease-catalyzed target recycling, were employed for amplifying the signal to construct an electrochemical based TNF-α aptasensor in this paper.

In recent years, host–guest molecular recognition has been extensively applied in bioanalysis [18,19] due to its high selectivity, outstanding stability and bioorthogonal structure [20,21]. Usually, the host is a kind of molecule that contains a large-volume cavity, which could load various guest molecules without an additional catalyst because of its hydrophobic internal cavity [22–24]. For example, β-cyclodextrin, a cyclic oligomer of glucose composed of seven glucose units and a hydrophobic cavity at the center of its molecular arrangement [25,26], can specifically recognize the redox probe ferrocene [27,28]. Cucurbituril 7 (CB), a pumpkin-shaped polymacrocycle with seven glycouril units strapped together by pairs of bridging methylene groups between nitrogen atoms [29], can recognize the redox probes such as methylene blue (MB) and thionine [30,31]. Herein, based on the superior properties for host-guest interaction, we proposed a HCR and target recycling enhanced electrochemical aptasensor to sensitively detect TNF-α with host-guest interaction for signal probe collection. HCR induced DNA nanowires can load substantial MB on electrode surface, in the presence of target TNF-α and RecJf exonuclease, the TNF-α firstly bound with corresponding TNF-α binding aptamer (S2) and thus resulted in the dissociation of MB intercalated DNA nanowires from electrode into solution, while the RecJf exonuclease further made the recycling of TNF-α to obtain more dissociated DNA nanowires. With the assistant of duplex-specific nuclease (DSN), the dissociated DNA nanowires with substantial intercalated MB in solution released the free MB, which then selectively captured by the CB/nano gold@chitosan functionalized electrode via host-guest interaction to produce the electrochemical signal for quantitatively detection of target. The proposed approach skillfully combined the amplification strategies with host-guest interaction, exhibiting excellent performance in the electrochemical detection of TNF-α with ultrahigh sensitivity and specificity.

2. Experiment

2.1. Chemicals and material

Tetrachloroauric acid ($HAuCl_4$), hexanethiol (96%, HT), cucurbituril 7 (CB), methylene blue (MB), chitosan and single-walled carbon nanohorns (SWCNHs) were bought from Sigma Chemical Co. (St. Louis, MO, USA). Duplex-specific nuclease (DSN) was purchased from Evrogen Joint Stock Company (Moscow, Russia). RecJf exonuclease was obtained from New England Biolabs (Beijing) Ltd. (Beijing, China). All the oligonucleotides used here were synthesized and purified by HPLC by Shanghai Shenggong Biotechnology Co. (Shanghai, China) and used without further purification (see Table 1). Aptamer stock solutions were obtained by dissolving oligonucleotides in 20 mM Tris-HCl buffer (pH 7.4) containing 140 mM NaCl, 5 mM KCl and 1 mM $MgCl_2$. 0.1 M phosphate buffered solutions (PBS, pH 7.0) containing 10 mM KH_2PO_4, 10 mM Na_2HPO_4 and 2 mM $MgCl_2$ was employed throughout the experiment. All other chemicals used in this investigation were of analytical grade unless mentioned otherwise. Double distilled water was used throughout this study.

2.2. Instrumentation

The scanning electron micrographs were obtained with scanning electron microscope (SEM, S-4800, Hitachi Instrument, Japan). X-ray photoelectron spectroscopy (XPS) measurements were performed with a VG Scientific ESCALAB 250 spectrometer, using Al Ka X-ray (1486.6 eV) as the light source (Thermofisher, England). Electrochemical measurements, including cyclic voltammetry (CV), differential pulse voltammograms (DPV) and electrochemical impedance spectroscopy (EIS) were performed on a CHI660D electrochemical workstation (Shanghai Chenhua Instrument, China) using a conventional three-electrode system with a modified glassy carbon electrode as working electrode (GCE, $\Phi = 4$ mm), a platinum wire as auxiliary electrode, and a saturated calomel electrode (SCE) as reference electrode. EIS and CV of the electrode fabrication were carried out in 0.1 M PBS containing 5.0 mM $[Fe(CN)_6]^{3-/4-}$ (pH 7.0). DPV was performed in 0.1 M PBS (pH 7.0) containing 10 mM KH_2PO_4, 10 mM Na_2HPO_4 and 2 mM $MgCl_2$.

2.3. Fabrication of the aptasensor

With the sequential use of 0.3, and 0.05 μm alumina powder, a bare GCE was polished until a mirror-like surface emerged and cleaned thoroughly before use. After that, 8 μL of 0.5 mg/mL SWCNHs solution that dispersed in N,N-Dimethylformamide (DMF) was added onto the electrode and dried. Then, the SWCNHs modified electrode was immersed into $HAuCl_4$ solution for electrochemical deposition at a potential of −0.2 V for 30 s. After air-drying and rinsed with water, 20 μL mixture containing 2.0 μM S1 and 2.0 μM S2 was attached to the depAu/SWCNHs/GCE for 16 h at room temperature. After that, 20 μL of 1.0 mM HT was dripped onto the electrode surface for 40 min to eliminate the nonspecific binding effect and block the active groups that are left. Then, in order to obtain long DNA nanowires with substantial MB on electrode surface, the modified electrode was subjected to incubation with the 20 μL mixture containing 2.0 μM H1, 2.0 μM H2 and 0.5 mM MB for 2 h. After washing, the resulting electrode nanowires/HT/S1 + S2/depAu/SWCNHs/GCE electrode was stored at 4 °C when not used.

2.4. Preparation of CB/nano gold@chitosan/GCE detection electrode

Analogously, a bare GCE was polished until a mirror-like surface emerged and cleaned thoroughly before use. First of all, 5.0 μL of nano gold@chitosan solution (1.0 mg/mL) that was dispersed in chitosan (0.5 *wt%*) was added onto the electrode and dried. Then, 10 μL 1 mM CB was attached to the electrode surface for 1 h at room temperature to finally obtain the CB/nano gold@chitosan/GCE detection electrode.

2.5. Electrochemical detection of TNF-α

The prepared nanowires/HT/S1 + S2/depAu/SWCNHs/GCE electrode was subjected to 30 μL of TNF-α with varying concentrations containing 0.15 U/μL of RecJf exonuclease and incubated for 40 min at 37 °C. After that, the 30 μL mixture solution was collected and diluted to 60 μL. Finally, the CB/nano gold@chitosan/GCE was