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Multiplexed electrochemical coding of DNA–protein bindings

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ABSTRACT

A simple, sensitive and multiplexed electrochemical sensor for the detection of DNA–protein binding based on the exonuclease protection strategy is described. Two electroactive species, methylene blue (MB)- and ferrocene (Fc)-labeled dsDNA probes are self-assembled on a gold electrode to prepare the sensor surface. The target proteins, vascular endothelial growth factor (VEGF) and estrogen receptor (ERα), bind to the dsDNA probes and protect the probes from digesting by exonuclease III due to the steric hindrance of the bound proteins. These protein-protected, MB/Fc-labeled sequences remaining on the sensor surface display two distinct voltammetric peaks, whose peak potentials (MB: −0.27 V; Fc: +0.27 V) and intensities reflect the identities and amounts of the corresponding target proteins, for simultaneous and multiplexed detection of DNA–protein bindings. The proposed sensor is also selective to the target proteins against other interference molecules. By using labels with distinct voltammetric peaks, the developed method can be easily expanded for simultaneous detection of multiple DNA–protein bindings.

1. Introduction

Many proteins can bind to sequence-specific DNAs to regulate genome expression and maintenance, which plays critical roles in a variety of essential cellular processes such as active gene transcription, DNA replication and repair (Ren et al., 2000; Sen and Baltimore, 1986; Helin, 1998). Transcription factors, the majority of DNA-binding proteins, can control the transcription of genetic information from DNA to mRNA, and the transcription levels sensitively reflect cellular developments and disease states (Latchman, 1997). These DNA-binding proteins can thus serve as biomarkers or targets for disease diagnoses and drug developments (Pandolfi, 2001; Herley, 2002).

Due to the significance of DNA–protein bindings in a variety of biological processes, a number of methods have been developed to monitor this type of interactions. Although the traditional methods, such as electrophoretic mobility shift (Garner and Revzin, 1981), DNase I footprinting (Galas and Schmitz, 1978), enzyme-linked immunosorbent (Renard et al., 2001) and Western blotting assays (Bowen et al., 1980), can offer sensitive detection of DNA–protein bindings, these methods commonly require radioisotope or fluorescent labels and are complicated and time-consuming, which limit their wide applications. Recently, a new exonuclease protection strategy has been coupled with modern biosensing technology to monitor DNA–protein bindings (Wang et al., 2005). This approach relied on the mechanism that the binding of the target protein to a FRET pair-labeled dsDNA protected the dsDNA from digestion by exonuclease III (Exo III) and generated enhanced FRET signals for quantitative protein detection. By using similar strategy, several other exonuclease protection assays for DNA–protein bindings have been demonstrated in connection with fluorescent (Ma et al., 2011; Liu et al., 2013) and colorimetric (Ye et al., 2013; Gao et al., 2013; Ou et al., 2010) transduction means. Despite the technical improvements over the traditional approaches, the reported exonuclease protection assays of DNA–protein bindings still suffer the drawbacks of high cost/false positive responses (fluorescence-based method) and low sensitivities (colorimetry-based method). Besides, the monitoring of DNA–protein bindings in a multiplexed format has been rarely reported. Therefore, the development of low-cost, rapid, sensitive and multiplexed DNA–protein binding assays is highly desired for proteomics, genomics, and biomedicine.

Here, based on electroactive labels with distinct voltammetric signatures, we report on for the first time a multiplexed and sensitive electrochemical approach for the detection of DNA–protein bindings. Electrochemistry has been increasingly used in designing different biosensors due to the obvious advantages of this technique in terms of speed, cost, sensitivity and ease of miniaturization. Analogous to multicolor quantum dots, which can emit fluorescence spectra at different wavelengths upon excitation, some electroactive species can display distinct

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voltammograms in one homogenous electrolyte. This unique voltammetric property of the electroactive species has offered new opportunities for simultaneous and multiplexed electrochemical detection of different types of biomolecules (Liu et al., 2004; Qian et al., 2010; Zhang et al., 2011; Xiang et al., 2011; Wang et al., 2003). Inspired by these useful findings, two types of sequence-specific dsDNA labeled with methylene blue (MB) and ferrocene (Fc), respectively, have been employed as probes to achieve multiplexed DNA–protein bindings by using vascular endothelial growth factor (VEGF) and estrogen receptor (ERα) as the model target proteins. The association of the proteins with the electroactive species-labeled sequence-specific dsDNAs prevents the dsDNAs from digesting by Exo III and the labels exhibit distinct current responses at different potentials, MB at −0.27 V and Fc at +0.27 V, respectively, which indicate the identities and amounts of proteins bound to the dsDNA probes. By coupling mutlivoltammetric labels with the inherent advantages of electrochemical transductions, multiplexed detection of DNA-protein bindings can thus be realized in a simple, rapid and sensitive format.

2. Experimental section

2.1. Reagents and materials

Tris–HCl, 6-mercaptohexanol (MCH), tris-(2-carboxyethyl) phosphine hydrochloride (TCEP), VEGF and ERα were purchased from Sigma-Aldrich (St. Louis, MO, USA). Sodium perchlorate ($NaClO_4$), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), exonuclease III (Exo III) and all oligonucleotides were ordered from Shanghai Sangon Biological Engineering Technology and Services Co., Ltd. (Shanghai, China). The sequences of the oligonucleotides were shown in Table 1. All reagents were analytical grade and solutions were prepared using ultrapure water (specific resistance of 18 MΩ cm).

2.2. Multiplexed sensing protocol

First of all, 5 μL of 100 μM SH-VEGF, SH-ERE and MB-ERE DNA were separately incubated with 5 μL of 10 mM TCEP for 60 min at room temperature to reduce the disulfide bonds in the thiolated-DNA and the MB-moiety of the MB-ERE DNA as well. Then, the SH-VEGF (2.0 μM) and SH-ERE (2.0 μM) were separately hybridized with their redox-tag-conjugated corresponding complementary DNA (2.0 μM) to form dsDNA (SH-VEGF/MB-VEGF and SH-ERE/Fc-ERE) in the annealing buffer (10 mM Tris–HCl, 100 mM NaCl, 50 mM EDTA, pH 7.4). The mixture was heated to 95 °C for 10 min and cooled down to room temperature slowly.

The gold electrodes (3 mm in diameter, CH Instruments Inc., Shanghai, China) were first soaked in a fresh warm piranha solution (volume of concentrated H_2SO_4: volume of 30% H_2O_2 =3:1) for 30 min. After rinsing thoroughly with water, the electrodes were polished with 0.3 and 0.05 μm aluminum slurry and sonicated sequentially in distilled water, ethanol and distilled water for 5 min each. Then, the electrodes were electrochemically cleaned in 0.5 M H_2SO_4 with potential scanning from 0.2 to 1.6 V until remarkable voltammetric peaks were obtained. This was followed by sonication again and drying with nitrogen. Next, 10 μL of the mixture of SH-VEGF/MB-VEGF (1 μM) and SH-ERE/Fc-ERE (1 μM) was cast onto the pretreated electrode and incubated overnight at room temperature in humidity. The electrode surface was rinsed with water and blocked with 1 mM MCH for 2 h.

The electrode was then washed with 10 mM Tris–HCl buffer (10 mM Tris–HCl, 100 mM NaCl, 50 mM EDTA, pH 7.4), and the target proteins (10 μL) at various concentrations were added and incubated with the electrode for 30 min. After washing with Tris–HCl buffer, 10 U of Exo III was incubated with the electrode for 60 min at 37 °C, followed by another washing step with Tris–HCl buffer. Finally, the modified electrode was transferred to an electrochemical cell for measurement.

Table 1
Oligonucleotides used in this work.

Oligonucleotide	Sequence
SH-VEGF	5′-CCAGA*GGGCAGGGTGCTC*TTGCAGATTTTTT-$(CH_2)_6$-SH-3′
MB-VEGF	5′-MB-ATCTGCAA*GAGCACCCTGCCC*TCTGG-3′
VEGF-FAM	5′-FAM- ATCTGCAA*GAGCACCCTGCCC*TCTGG-3′
SH-ERE	5′-GATCA*GGTCACTGTGACC*TGACTTTGTTTTT-$(CH_2)_6$-SH-3′
Fc-ERE	5′-Fc-CAAAGCTA*GGTCACAGTGACC*TGATC-3′
ERE-FAM	5′-FAM- CAAAGCTA*GGTCACAGTGACC*TGATC-3′

The italic parts indicate the protein binding sites.

2.3. Electrochemical measurements

Cyclic voltammetry (CV) and square wave voltammetry (SWV) were performed on a CHI 621D electrochemical workstation (CH Instruments, Shanghai, China). A conventional three-electrode configuration was used, with the modified gold working electrode, an Ag/AgCl (3 M KCl) reference electrode, and a platinum wire counter electrode. SWV measurements were performed in HEPES/$NaClO_4$ buffer (10 mM HEPES and 0.5 M $NaClO_4$, pH 7.0) by scanning the potential from −0.50 V to +0.5 V with a step potential of 4 mV, a frequency of 60 Hz and amplitude of 50 mV. Data processing was made by using the “linear baseline correction” function of the CHI 621D software. Values with error bars represent the standard deviations of three parallel samples at each target concentration.

2.4. Gel electrophoresis

Ten microlitre sample solution mixed with 2 μL 6 × loading buffer was analyzed in 16% nondenaturing polyacrylamide gel. The electrophoresis was conducted in 1 × TBE (pH 8.2) at 100 V constant voltage for 60 min. The gel was visualized under UV light and photographed with a Single Lens Reflex (SLR) camera (Canon 550D).

3. Results and discussion

The principle of our exonuclease protection-based, multiplexed electrochemical coding of DNA–protein bindings is illustrated in Scheme 1. The protein-binding, sequence-specific dsDNAs (the colored parts indicate the protein binding sites) are designed in such a way that these dsDNAs have 3′-blunt termini at one end and 3′-protruding termini at the other end. Exo III can catalyze the stepwise removal of mononucleotides from the 3′-hydroxyl termini of dsDNA with 3′-blunt or recessed termini and is inactive on the 3′-protruding termini. The dsDNA probes can thus be degraded by Exo III only from the 3′-blunt termini. The sensing surface is constructed by the self-assembly of a mixed monolayer of thiol-modified, MB/Fc-labeled dsDNA probes and MCH on the gold electrode. In the absence of the target DNA-binding proteins, Exo III catalytically digests the surface-immobilized dsDNA probes from the 3′-blunt termini and the MB/Fc labels can thus be effectively removed from the sensing surface upon extensive washing, leading to highly minimized background current responses. On the contrary, the bindings between the target proteins and the dsDNAs protect the MB/Fc-labeled sequences from digesting by Exo III due to the large steric hindrance effect of the bound proteins. The protected MB/Fc labels remain intact on the