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An Ultrasensitive and Label-free Electrochemical DNA Biosensor for Detection of DNase I Activity

Chen Li [a, b], Xuejuan Chen [a, b], Nan Wang [a, b], Bailin Zhang [a,*]

[a] State Key Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun 130022, P. R. China

[b] University of Science and Technology of China, Hefei 230026, P. R. China

CONTENTS

Materials and reagents

The HPLC-purified DNA reporter probes (5′-SH-$(CH_2)_6$-CGT ACG GAA TTC GCT AGC CCC CCG GCA GGC CAC GGC TTG GGT TGG TCC CAC TGC GCG TGG ATC CGA GCT CCA CGT G-3′) were synthesized by Sangon Biotech Co., Ltd. (Shanghai, China). Deoxyribonuclease I (Worthington Biochemical Corp., USA), tris(2-carboxyethyl)phosphine hydrochloride (TCEP, Alfa Aesar, USA), 6-mercapto-1-hexanol (MCH, Sigma-Aldrich, St. Louis, MO., USA), methylene blue (MB, Fuchen Chemical Reagents Factory, Tianjin, China), and fetal bovine serum (Beijing Dingguo Changsheng Biotech. Co., Ltd, Beijing, China) were all used as purchased without further purification. Other reagents were all of analytical grade and used as received without any further purification. Deionized water (18.2 $M\Omega \cdot cm^{-1}$) from a Milli-Q-water purification system (Millipore) was used throughout.

The DNase I stock solution was prepared with 20 mM Tris–HCl containing 2.5 mM $MgCl_2$, 0.5 mM $CaCl_2$ (pH 7.4) and kept at 4°C. The electrolyte buffer contained 20 mM Tris–HCl and 20 mM NaCl (pH 7.4) and was also used as washing solution.

Apparatus and measurements

All electrochemical measurements were implemented by using a CHI 660A electrochemical workstation (CH Instruments, Inc., Shanghai) at room temperature (RT, 25±2°C). This test system consisted of a modified Au electrode used as the working electrode (Φ 2.0 mm), a saturated calomel electrode (SCE) as reference electrode and a platinum wire as auxiliary electrode. Cyclic voltammetry (CV) and electrochemical impedance spectra (EIS) were performed in 5.0 mM $[Fe(CN)_6]^{3-/4-}$(1:1) containing 0.1 M KCl. The parameters were set to the potential range of −0.2 V to 0.6 V with the rate of 0.05 V s^{-1} and the frequency range of 0.1 Hz to 100 kHz with the amplitude of 5 mV, respectively. Square wave voltammetry (SWV) was performed in Tris–HCl buffer (20 mM, pH 7.4) containing 20 mM NaCl in the potential range of −0.4 V to −0.1 V at frequency of 15 Hz, amplitude of 25 mV, potential increment of 4 mV.

Optimization of experimental conditions

The experimental conditions were optimized for the electrochemical biosensor. The effect of incubation time of DNA probes on the electrode surface was investigated as showed in Fig. S1A. The current signal gradually increased along with the increase of incubation time. When the incubation time reached 6 h, the current reached a platform, indicating the most DNA probes had been immobilized. Thus 6 h of incubation time was applied in the further experiments.

As the redox indicator, the concentration of MB played an important role in the experiment and then the appropriate concentrations of MB were optimized. As showed in Fig. S1B, the electrochemical signal gradually raised following the increase of the concentration of MB until it reached a platform at the concentration of 20 μM. Due to the saturation of the MB on the DNA probes, we chosed 20 μM as the optimal condition in all the experiment.

Fig. S1C and Fig. S1D illustrated the influence of the incubation temperature and reaction pH in the DNase I solution on the SWV peak current of DNA/MCH/Au electrode. The incubation temperature was investigated in the range of 20-60°C and the reaction pH was studied in the range of 5-9. The experimental results indicated that 37°C and pH 7.4 were favorable for this chemical system. Then we finally chose them as the optimal experiment conditions.

Fig. S1E explored the effect of the modified time of MB on SWV current signal of the DNA/MCH/Au electrode. The modified time of MB was investigated in the range of 0.5-6 minutes. When the time get to 5 minutes, the electrochemical signal reached a platform. Then the modified time of MB was 5 minutes as the optimal experiment conditions.

Fig. S1F illustrated the influence of the incubation time in the DNase I solution on the SWV peak current of DNA/MCH/Au electrode. In Fig. S1E, the current signal gradually increased along with the increase of incubation time. When the incubation time reached 1 h, the current reached a platform. Thus 1 h of incubation time in the DNase I solution was applied in the further experiments.

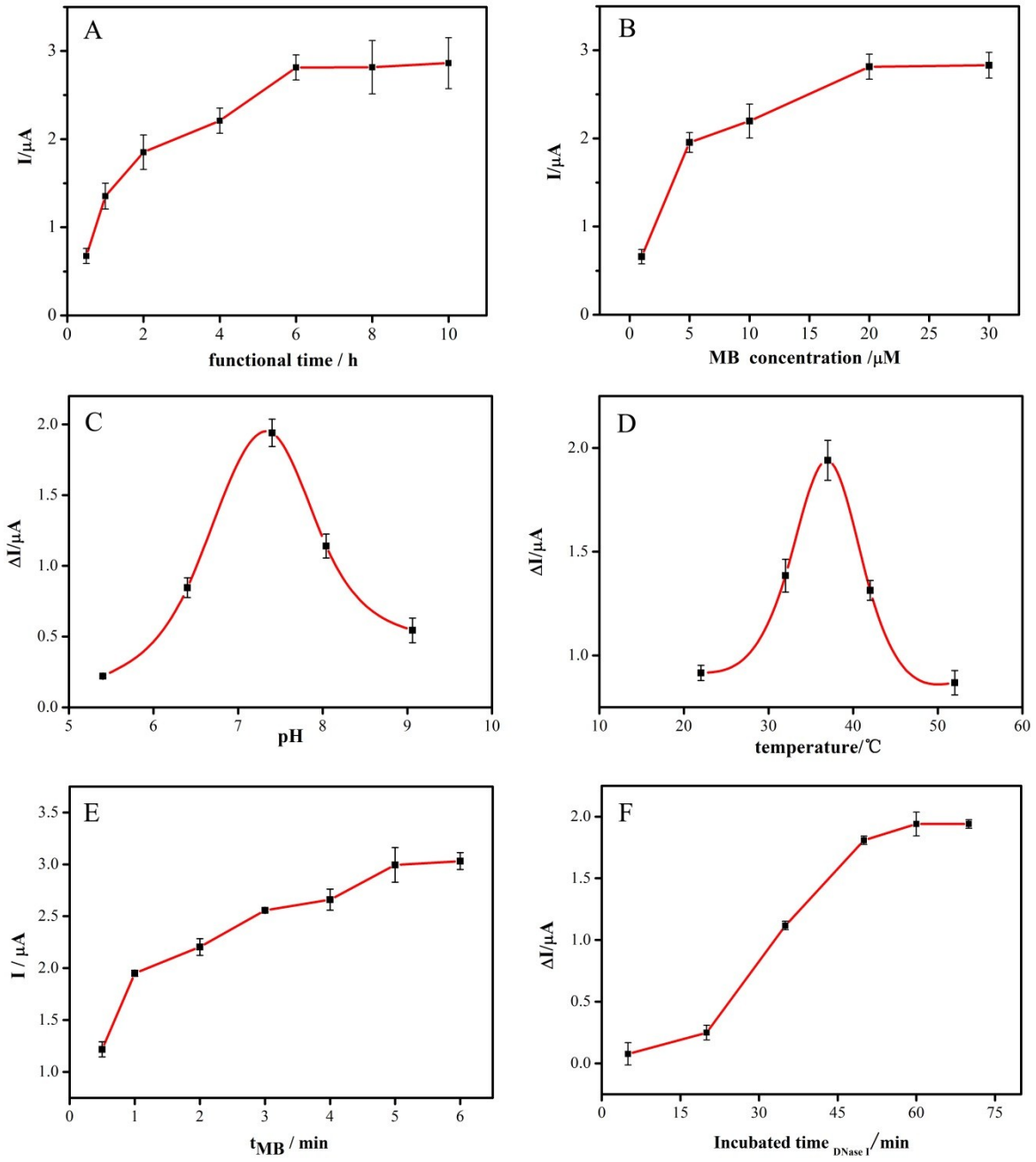

Fig. S1. Optimization of the incubation conditions. (A) Effect of the modified time of DNA probes on SWV current signal of the DNA/Au electrode, (B) Effect of concentrations of MB on SWV current signal of DNA/MCH/Au electrode, (C) Effect of the incubation temperature in the DNase I solution on differential SWV current signal of DNA/MCH/Au electrode, (D) Effect of the reaction pH in the DNase I solution on differential SWV current signal of DNA/MCH/Au electrode, (E) Effect of the modified time of MB on SWV current signal of the DNA/MCH/Au electrode, (F) Effect of the incubation time in the DNase I solution on differential SWV current signal of DNA/MCH/Au electrode. The error bars represent the standard deviations of three repetitive experiments.

Inhibition effects on the DNase I activity

The functionalization of DNase I depends on the divalent ions, while the DNase I activity depends on the Ca^{2+} and is activated by Mg^{2+} and Mn^{2+}. When the system contains Mg^{2+} ion, DNase I can randomly cleave the DNA strands at the arbitrary sites. Ethylenediamine tetraacetic acid (EDTA) is a widely used chelating agent that binds strongly to divalent ions. The presence of EDTA would deplete divalent ions in the solution, and then inhibit DNase I activity[1]. In the experiment, we investigated the inhibition effect of EDTA on DNase I activity. As shown in Fig. 4, treated with a serial of different diluted concentrations of DNase I in buffers with or without EDTA, the SWV results of DNA/MCH/Au electrode were measured in a buffer solution. In the absence of EDTA, the differential SWV signals were obtained in the change of gradient. In contrast, in the presence of EDTA, the differential signals were almost invariant at about zero. Apparently, the activity of DNase I was completely inhibited by EDTA.

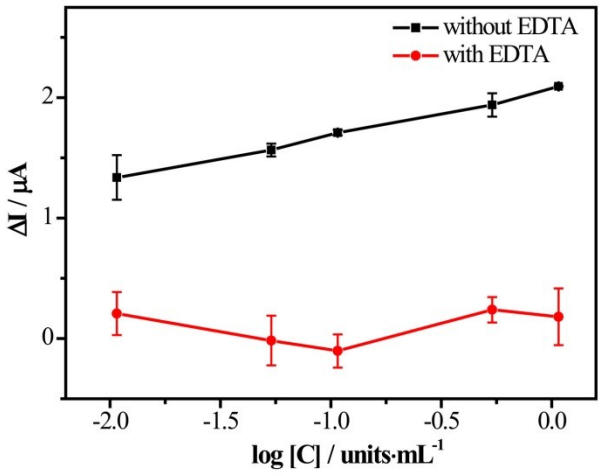

Fig. S2. The differential SWV signal plots of MB on the DNA/MCH/Au electrode incubated with different concentrations DNase I solution for 1 h with (black curve) or without (red curve) EDTA in 20 mM Tris–HCl buffer containing 20 mM NaCl. The error bars represent the standard deviations of three repetitive experiments.

Different analytical methods for detection of DNase I

Table S1 Different analytical methods for detection of DNase I

No.	Sensing Method	Detection Limit (units·mL^{-1})	Linear Range (units·mL^{-1})	Reference
1	A graphene-based real-time fluorescent assay	1.75	1.75–70	[2]
2	Potentiometric sensing and oxidative damage of single-stranded DNA using a polycation-sensitive membrane electrode	0.45	1–10	[3]
3	Enzyme detection by using tailor-made nanoparticles	Not given	0.1–3	[4]
4	FluorescentassaybasedonDNA-templatedsilvernanocluster/grapheneoxidenanocomposite	0.1	0–100	[5]
5	A label-free fluorescent assay based on DNA-templated silver nanocluster/graphene oxide nanocomposite	0.1	0–10	[6]
6	Electrochemical detection using FcODN modified electrode (Au-S linkage)	0.1	0.1–10	[7]
7	Electrochemical detection using FcODN modified electrode (cytosine oligonucleotide)	0.01	0.01–1	[8]
8	Simultaneous fluorescence imaging in living cells with chimeric oligonucleotide probes	0.04	0.04–0.5	[9]
9	Immunochemical assay in body fluids	0.005	0.005–0.1	[10]
10	Homogeneous immunochemical assay on the lateral flow strip	Not given	9.8×10^{-4}–0.125	[11]
11	An ultrasensitive and label-free DNA biosensor using electrochemical method	0.001	0.01-500	This work

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