

Supporting Information

Dual-functional electrochemical biosensor for the detection of prostate specific antigen and telomerase activity

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Experimental Section

Chemicals and Materials

3-[(3-Cholamidopropyl) dimethylammonio]-1-propanesulfonic acid (CHAPS), phenylmethylsulfonyl fluoride (PMSF), ethylene glycol bis(-aminoethyl ether)-*N,N,N',N'*-tetraacetic acid (EGTA), glycerol, and Tween 20 were purchased from Biosharp Biotechnology. 3-mercaptopropionic acid (MPA), Bovine serum albumin (BSA), imidazole, N-(3-dimethylaminopropyl)-*N'*-ethyl-carbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS) were purchased from Sigma-Aldrich. EDC and NHS were dissolved in water immediately before use. Human prostate-specific antigen (PSA), two mouse antihuman total PSA monoclonal antibodies (primary capture antibody, Ab₁ and biotin linked secondary detection antibody, Biotin-Ab₂) were received from Linc-Bio Science Co., Ltd. All other reagents were of analytical reagent grade. All the water used in the work was RNase-free.

DNA primer, 5'-Biotin-TTTTTAATCCGTCGAGCAGAGTT-3' was synthesized by Sangon Biotech (Shanghai) Co., Ltd. and purified using HPLC method. The deoxynucleotide solution mixture (dNTPs) was purchased from TaKaRa Bio Inc. (Dalian, China). 3, 3', 5, 5'-tetramethylbenzidine sulfate (TMB) and diethyl pyrocarbonate (DEPC) were obtained from KeyGEN Biotech. Co., Ltd. (Nanjing).

Human normal liver cell line (LO-2), two kinds of prostate cancer cell lines (LNCaP cell line in which PSA was positive; PC-3 cell line in which PSA was negative) were obtained from KeyGEN Biotech. Co., Ltd. (Nanjing). LNCaP cell line was derived from left supraclavicular lymph node of human prostate carcinoma; PC-3 cell line was derived from bone of human prostate carcinoma.

Cells were cultured in RPMI 1640 medium (GIBCO) supplemented with fetal bovine serum (10%) (FBS, GIBCO), penicillin (80 µg mL⁻¹), and streptomycin (100 µg mL⁻¹) at 37°C in a humid atmosphere containing 5% CO₂. After 48 h, the cells were collected and separated from the medium by centrifugation at 1500 rpm for 5 min and then washed twice with sterile phosphate buffer saline (PBS) (pH 7.4). The sediment was resuspended in PBS to obtain a

homogeneous cell suspension at a certain concentration. The cell number was determined using a Petroff Hausser cell counter (USA). Human serum samples from patients with various PSA levels and the clinical data of the chemiluminescence immunoassays for these serum samples were obtained from Jiangsu cancer hospital (Nanjing, China).

Preparation of Telomerase Extract from LNCaP Cells

The telomerase was extracted by the CHAPS method.¹ Cells were collected in the exponential phase of growth and separated from the medium by centrifugation at 1500 rpm for 5 min and then washed twice with PBS (pH 7.4). The sediment was resuspended in PBS to obtain a homogeneous cell suspension at a certain concentration. The pellet was washed twice with ice-cold PBS, and then, the pellet was resuspended in 200 μL of ice cold lysis buffer (0.5% CHAPS, 10 mM Tris-HCl, pH 7.5, 1 mM MgCl_2 , 1 mM EGTA, 0.1 mM PMSF, 10% glycerol) by shaking at least three times and kept on ice for 30 min. The mixture was centrifuged at 16 000 rpm for 20 min at 4 $^\circ\text{C}$, and the supernatant was collected carefully. The resulting extract was used immediately or flashily frozen in low temperature refrigerator at -80°C . For control experiments, telomerase extracts were heat treated by incubating at 90°C for 10 min prior to the assay to inactivate telomerase.

Sandwich Immunoreaction on gold electrode

Gold disk electrodes (diameter of 2.0 mm) were polished with 0.05 μm alumina powder prior to use and then cleaned ultrasonically in acetone and water for 3 min. They were then electrochemically cleaned by a series of oxidation and reduction cycles in 0.5 M H_2SO_4 .

For antibody immobilization, electrodes were first subjected to 3 mM MPA in water for 30 min to obtain $-\text{COOH}$ on the surface of gold electrode. Then the $-\text{COOH}$ modified electrode was immersed in 1 mL freshly prepared Imidazole-HCl buffer containing 20 mg EDC and 10 mg NHS for 1 h at room temperature. Then, 10 μL of 20 $\mu\text{g mL}^{-1}$ Ab_1 was dropped onto the electrode surface, allowing it to react for 16 h to get the Ab_1 -immobilized gold electrode. The washed Ab_1 -gold electrode was incubated in 3% BSA for 30 min at 37 $^\circ\text{C}$ to block the excess active groups on the surface, followed by washing, and used for PSA detection. 10 μL of various concentration of target PSA was dropped onto the surface of the Ab_1 -gold electrode and incubated for 2 h at 37 $^\circ\text{C}$. Then the reaction solution was removed and the electrode was rinsed with pure water and dried again with nitrogen. Following this, 10 μL of biotin- Ab_2 was dropped on the electrode and incubated at 37 $^\circ\text{C}$ for 2 h. The modified electrode was then washed thrice and used for the following operation.

Immobilization of Telomerase primer and telomerization reaction

After the sandwich immunoreaction, the electrodes were incubated with 10 μL of 2 $\mu\text{g mL}^{-1}$ streptavidin solution at 37 $^\circ\text{C}$ for 1.5 h. After washing with PBS, 10 μL of 1 μM biotinylated primer DNA was added to each electrode and the electrodes were incubated at 37 $^\circ\text{C}$ for 1.5 h. The telomerization reaction was initiated by immersing the electrodes into 100 μL of reaction buffer (50 mM Tris-HCl, pH 7.5, 4 mM MgCl_2 , 1 mM EGTA, 50 mM KCl, 0.05% Tween 20) containing telomerase extract and 0.2 mM dNTPs mixture at 30 $^\circ\text{C}$.

Formation of DNAzymes on the electrodes

After washing with PBS, the modified electrode was suspended into a 0.2 mM hemin solution, and reacted for 30 min at room temperature to form the hemin/G-quadruplex (i.e. DNAzyme) complex.

Gel Electrophoresis

The telomerase primer (5'-AATCCGTCGAGCAGAGTT-3'), and the products by telomerization reaction with telomerase extracts were characterized by 4% agarose gel electrophoresis. Tris-Acetic acid-EDTA (TAE) was used as the separation buffer. Electrophoresis was carried out at 13 V/cm for 0.5 h at room temperature. The gels were stained with ethidium bromide (EB, 1.0 $\mu\text{g/ml}$) for 0.5 h. The visualization and photography were performed using a digital camera under UV illumination.

Electrochemical measurement

A standard three electrode configuration including the modified electrode as the working electrode, a platinum wire as the counter electrode and a saturated calomel electrode (SCE) as the reference electrode was used for the measurements. The resulting electrode was placed in a glass cell containing 10 mM H_2O_2 in HEPES buffer solution (10 mM, pH 7.2), and connected to a RST5000 workstation (Risetest Instruments Co. Ltd., Suzhou, China) for measurements. The HEPES buffer solution was bubbled thoroughly with nitrogen for 15 min before the measurements. Cyclic voltammograms (CVs) were recorded by cycling the potential between -0.6 V to 0 V at a scan rate of 10 mV s^{-1} , and the current responses at -0.6 V were chosen as the analytical signals.

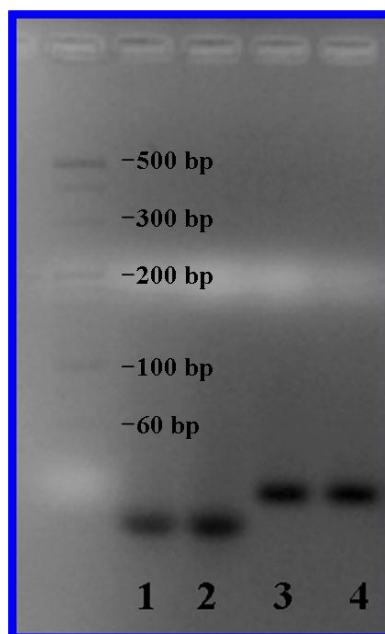


Fig. S1. Agarose gel electrophoresis experiments. lane 1: Telomerase primer incubated with buffer; lane 2: with addition of heat-inactivated control; lane 3,4: Telomerase primer incubated with telomerase extracts.

As shown in Figure S1, due to the increased molecular weight for the product from telomerase-catalyzed elongation, samples with telomerase extract derived from 10^6 LNCaP

cells (lanes 3, 4) exhibited strong fluorescent bands which lagged behind the TS primers incubated with buffer (lane 1) or with addition of heat-inactivated control (lane 2).

References

1 M. A. Piatyszek, N. W. Kim, S. L. Weinrich, K. Hiyama, E. Hiyama, W. E. Wright and J. W. Shay, *Methods Cell Sci.*, 1995, **17**, 1.