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A dual-functional electrochemical biosensor for the detection of prostate specific antigen and telomerase activity†

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A new dual-functional electrochemical biosensor for the detection of prostate specific antigen (PSA) and telomerase activity was successfully developed based on a sandwich immunobinding format and telomerization assisted hemin–G-quadruplex-based DNAzyme as a biolabel.

Prostate-specific-antigen (PSA) has been regarded as the best cancer biomarker for prostate cancer (CaP)[1] due to its high sensitivity. However it has been shown not to be specific to CaP and is also elevated in other benign conditions of prostate.[2] Because of the low specificity of PSA, up to 75% of men with PSA levels of 2–10 ng mL^{-1} have a negative first biopsy.[3] As the single marker (PSA) detection is not ideal for CaP diagnosis, joint detection of two or multiple biomarkers is urgently needed to maximize information from available data. Telomerase is one of the tumor biomarkers.[4] In most cancer cells, elevated amounts of telomerase could be detected.[5] There is a very strong association between telomerase activity and malignancy in nearly all types of cancer, suggesting that telomerase could be used as a diagnostic marker and a therapeutic target for managing cancer.[6] Therefore, we can expect more accurate results and much progress in clinical diagnosis of CaP if both PSA and telomerase activity could be detected in one assay.[7]

To date, numerous amperometric electrochemical immunosensors have been developed on the basis of multiple labels or spatial resolution.[8] The traditional sandwich-type structure is usually used in these assays.[9] For multiple labeled immunoassays on single electrodes, the steric hindrance and other possible interactions between different labeled secondary antibodies would reduce the reliability and narrow the range for analysis. Whereas for spatial resolution immunoassays based on multi-electrode arrays, multi-channel electrochemical analysis equipment is usually required. Recently, functional DNAzyme, as a burgeoning approach for sensitive electrochemical assays of ions or DNA, has attracted our attention.[10] An interesting example of DNAzyme is a peroxidase-like supramolecular G-quadruplex configuration which consists of hemin and single stranded guanine-rich nucleic acids.[11]

In this work, we combine DNAzyme with the traditional sandwich immunosensing format in one biosensing interface for the detection of two important biomarkers, PSA and telomerase. Based on the different characteristics of the two biomarkers, the designed biosensor could selectively detect the telomerase activity of cells or the concentration of PSA in patient serum samples. As in clinical tests, there are mainly two different testing purposes. One is to test whether the tissue or serum sample is from a cancer or not. And the other purpose is to detect the specific antigen for a specific disease, such as PSA to prostate diseases. The designed dual-functional sensing strategy could just satisfy this demand. It not only provides more information for the diagnosis of prostate diseases from two biomarkers, but also establishes a novel approach which meets the needs of different detection purposes. It could greatly improve the reliability and flexibility in clinical diagnosis.

This dual-functional electrochemical sensing interface for two biomarkers mainly consists of two series-connected reactions on a single electrode. The principle is illustrated in Scheme 1. One part is the sandwich immunoreaction as a conventional detection mode for a target protein, and the other part is the formation of DNAzyme which relies on the telomerization reaction with telomerase. Specifically, PSA, a target protein, was first captured on an Ab_1-coated gold electrode to form a common sandwich immunocomplex with biotinylated Ab_2. Streptavidin was then bound to the biotinylated immunocomplex to act as a bridge for multiplex anchoring of biotinylated single-stranded oligonucleotides (telomerase primer). In the presence of telomerase, telomerization reaction was initiated to form a long single-stranded DNA with TTAGGG repeat units which were continuously added to the 3′-end of the primer in the presence of the nucleotide mixture dNTPs. G-rich sequences could bind to hemin to form the G-quadruplex–hemin DNAzyme,

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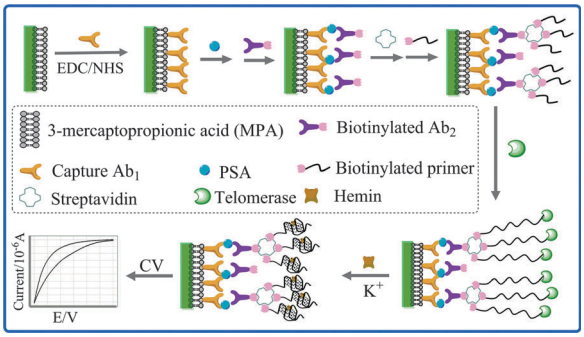

Scheme 1 The process and assay principle of the dual-functional electrochemical biosensor. It mainly consists of two series of connected parts. One is an immunoreaction on the carboxyl activated gold electrode; the other is the formation of G-quadruplex–hemin DNAzyme. See the text for further explanation.

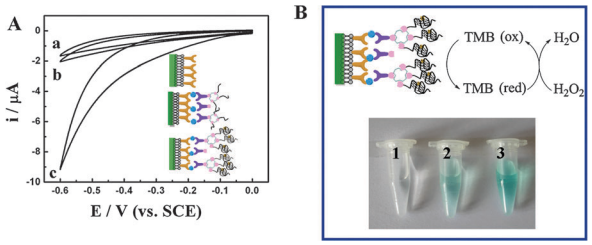

Fig. 1 (A) Cyclic voltammograms (CVs) of Ab_1-modified electrodes in the presence of only telomerase (a), PSA (b) and both PSA and telomerase (c); (B) the photograph of the TMB substrate after treatment with modified electrodes (with both PSA and telomerase) for (1) 0 min, (2) 5 min, (3) 15 min, respectively, at room temperature. Measurements were performed in 10 mM H_2O_2 solution upon interaction with 0.20 mM hemin in 10 mM HEPES (150 mM NaCl, 50 mM KCl, pH 7.2).

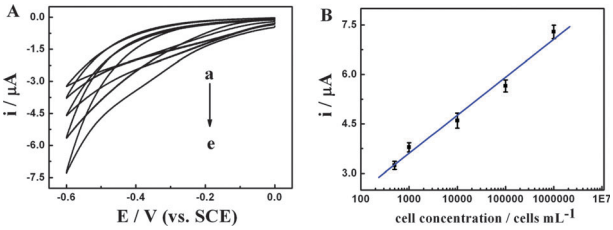

Fig. 2 (A) CVs of the modified electrodes incubated with telomerase extracts from different numbers of LNCaP cells: (a) 5×10^2, (b) 1×10^3, (c) 1×10^4, (d) 1×10^5, (e) 1×10^6 cells per mL. (B) The linear relationship between the current at −0.6 V and logarithmic concentration of LNCaP cells.

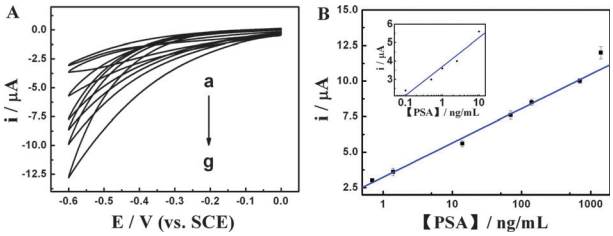

Fig. 3 (A) CVs of the DNAzyme based immunosensors in the presence of different concentrations of PSA from (a) to (g): 0.7, 1.4, 14, 70, 140, 700, 1400 ng mL^{-1}. (B) The linear relationship between the current (at −0.6 V) and PSA concentration. Insets show the calibration plots at a lower concentration range. Other experimental conditions were the same as that shown in Fig. 1A.

which can catalyze the reduction of H_2O_2 and then generate cathodic current for measurements.

The formation of DNAzyme is influenced by the concentration of PSA as well as the telomerase activity. An elevated concentration of PSA and telomerase leads to an increased amount of DNAzyme and a higher cathodic current. On the basis of this characteristic, our sensor thus presents its dual-functional character and provides quantitative measurements for both PSA concentration and telomerase activity as required.

First the feasibility of this strategy was verified *via* electrochemical responses from three different conditions. From Fig. 1A we could find that when any one of the two biomarkers was absent, only a weak current was found (curves a and b). Whereas in the presence of both PSA and telomerase, a greatly increased current was obtained (curve c), indicating the formation of G-quadruplex–hemin DNAzyme which could catalyze the reduction of H_2O_2. Moreover, due to the chromogenic reaction of 3,3′,5,5′-tetramethylbenzidine sulfate (TMB), we could easily observe the catalytic process from the gradually changed solution color by the naked eye (from colorless to blue) (Fig. 1B).

The telomerization reaction was also confirmed using agarose gel electrophoresis (Fig. S1, ESI†). Since the extension process is controlled by the telomerase activity, the reduction current of H_2O_2 is expected to increase with the increased amount of telomerase extracts. We employed a given amount of PSA (1.4 μg mL^{-1}) to observe the telomerase activity of cell samples. The result is shown in Fig. 2A. The results indicated that the current intensities increased gradually with increased number of cancer cells (curves a–e). It means that a higher concentration of cells leads to a higher level of telomerase activity and more extension sections in the experiment. The relationship between the currents and the cell densities is plotted in Fig. 2B. It was found that the current had a linear dependence on the logarithmic number of LNCaP cells in the range of 500–1×10^6 cells per mL. Using this approach, the telomerase activity from as few as 500 cells per mL LNCaP cells could be detected.

Similarly, the sensitivity and quantitative range for PSA were studied with different PSA concentrations based on the developed protocol (using telomerase extracts from 10^6 LNCaP cells as a constant). As expected, the current increased with the increasing PSA levels in the sample solution (Fig. 3A). A linear dependence between the peak currents and the logarithm of PSA levels was obtained in a wide range from 0.14 ng mL^{-1} to 1400 ng mL^{-1}. The calibration plot at a lower concentration range is presented in the inset of Fig. 3B. The linear regression equation is i (μA) = $1.57 \times \log c + 3.46$, where i is the current intensity at a potential of −0.6 V and c is the PSA concentration (ng mL^{-1}). The limit of detection (LOD) was 0.14 ng mL^{-1} (a signal-to-noise ratio of 3). The reproducibility expressed in terms of the relative standard deviation (RSD) was about 7.5% (n = 3) at a PSA concentration of 1.4 ng mL^{-1}. As the concentration of PSA in human serum at 4–10 ng mL^{-1} is a danger zone, and the normal levels of PSA are below 4 ng mL^{-1}, the LOD is low enough to meet the needs of clinical applications.

Table 1 Comparison of the detection results of PSA concentrations from the designed electrochemical biosensor (EC) with those from chemiluminescence (CL) immunoassay

Clinical sample	EC, ng mL^{-1}	CL, ng mL^{-1}	Deviation, %
1	0.38	0.4	5.0
2	0.67	0.68	1.5
3	3.18	3.59	11.4
4	0.83	0.86	3.5
5	1.27	1.24	2.4
6	2.29	2.61	12.3
7	0.83	0.87	4.6
8	5.75	5.65	1.8

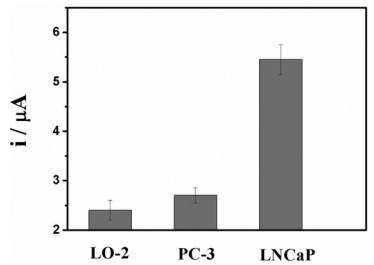

Fig. 4 Responses of CVs of Ab_1-modified electrodes which were treated with cell solutions and cells extracts of LO-2, PC-3 and LNCaP in their exponential phase of growth, respectively.

Finally, to demonstrate the potential clinical applications of this biosensing approach for PSA detection, 8 human serum samples from patients with various PSA levels were measured *via* these DNAzyme-based electrochemical immunosensors and the results were compared with the clinical data obtained from chemiluminescence immunoassays. Table 1 presents the estimated concentrations of PSA in above samples from both methods. It was shown that the as-prepared electrochemical immunosensor displayed a deviation (absolute value) range of 1.5% to 12.3% from those data obtained from a chemiluminescence method. These results illustrated that the as-prepared immunosensor for PSA was comparable to the classical method in the clinical assay and could be considered as an optional approach for determination of PSA in clinical diagnostics.

Moreover, three different cell lines (LO-2, PC-3 and LNCaP) with different conditions of PSA and telomerase activity were employed to further substantiate the validity of the dual-marker detection approach. Data are shown in Fig. 4. LO-2 is a human normal liver cell line, in which both PSA and telomerase are absent, while PC-3 and LNCaP are two kinds of prostate cancer cell lines derived from the left supraclavicular lymph node and bone of human prostate carcinoma, respectively. PSA is present in the LNCaP cell line, while it is absent in the PC-3 cell line. Both cell lines are telomerase positive. From Fig. 4, we could find that an obviously increased current was obtained only when the modified electrode was treated with LNCaP cells solution and the extracts while the control LO-2 and PC-3 cell lines did not show that change. This result not only further verified the feasibility of the designed strategy but also showed the usefulness of this method in diagnosis of prostate cancers. Because the resulting signals were obtained only in samples with two biomarkers, it could provide a more accurate assay to find whether the samples were from CaP or even to differentiate CaP derived from different tissues.

In summary, a versatile sensing method was developed for dual-functional detection of the PSA antigen and telomerase activity based on the series-connected reactions on a single electrode. The reported method employed the amplification technique of the multiplex binding of the biotin–streptavidin system and the hemin–G-quadruplex DNAzyme. The method could optionally detect two important biomarkers on clinical demand. It could be easily used to detect telomerase activity of the given cells to find the cancerous state, as well as the PSA antigen in serum with a satisfactory sensitivity to prostate diseases. The method does not require different labels on detecting antibodies and avoids complex operations or interactions from side-by-side immunoreactions. The proposed immunosensor could also be applied for the detection of other antigens by simply changing the respective antibodies. Therefore the proposed dual-functional sensing strategy would provide a smart dual-biomarker assay and more precise clinical diagnostics.

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