



Reagent-less electrogenerated chemiluminescence peptide-based biosensor for the determination of prostate-specific antigen

Honglan Qi*, Chen Wang, Xiaoying Qiu, Qiang Gao, Chengxiao Zhang

Key Laboratory of Analytical Chemistry for Life Science of Shaanxi Province, School of Chemistry and Chemical Engineering, Shaanxi Normal University, Xi'an 710062, PR China

ARTICLE INFO

Article history:

Received 21 April 2012

Received in revised form

23 August 2012

Accepted 27 August 2012

Available online 1 September 2012

Keywords:

Electrogenerated chemiluminescence

Biosensor

Peptide

Prostate-specific antigen

ABSTRACT

A novel electrogenerated chemiluminescence peptide-based (ECL-PB) biosensor for highly sensitive and selective determination of prostate-specific antigen (PSA) was developed. A helix peptide (CHSSKLQK) was served as a molecular recognition element and ruthenium bis(2,2'-bipyridine) (2,2'-bipyridine-4,4'-dicarboxylic acid)-N-hydroxysuccinimide ester (Ru(bpy)₂(dc bpy)NHS) was used as an ECL label. The helix peptide was labeled with the ECL label at NH₂-containing lysine and utilized as ECL probe (Ru-peptide). The ECL-PB biosensor was fabricated by immobilizing the ECL probe onto a gold electrode surface via self-assembling technique through a thiol-containing cysteine at the end of the peptide. The principle of ECL measurement is based on the specific proteolytic cleavage event of Ru-peptide on the gold electrode surface in the presence of PSA, resulting in a decrease of ECL signal. The decreased ECL intensity was directly linear to the concentration of PSA in the range from 1.0×10^{-10} g/mL to 8.0×10^{-9} g/mL with a detection limit of 3.8×10^{-11} g/mL. This work demonstrates that the direct transduction of peptide cleavage events into an ECL signal provides a simple and sensitive method for detecting target protein.

© 2012 Elsevier B.V. All rights reserved.

1. Introduction

Sensitive and accurate measurement of disease-related protein is critical to many aspects of modern biochemical and biomedical research. In particular, the clinical measurement of a cancer biomarker shows a great promise for early disease detection and highly reliable prediction [1]. Prostate-specific antigen (PSA), belonging to the human kallikrein family, has been recognized as the premier tumor marker for the detection of early stage prostate cancer and for monitoring the recurrence of the disease after treatment [2,3]. A variety of methods have been developed to determine PSA, such as enzyme-linked immunosorbent assay [4], colorimetric immunoassay [5,6], fluorescence immunoassay [7,8], surface-enhanced Raman scattering immunoassay [9], electrochemical immunoassay [10–12], chemiluminescence [13] and electrogenerated chemiluminescence immunoassay [14,15]. These assays are usually based on immunoassay, in which antibodies are used as molecular recognition elements due to their strong and selective binding. However, the antibody drawbacks associated with their production, stability and manipulation have prompted researchers to seek alternatives and the assay usually requires multiple steps and long incubation time [16].

* Corresponding author. Tel.: +86 29 81530726; fax: +86 29 81530727.
E-mail address: honglanqi@snnu.edu.cn (H. Qi).

Short linear binding peptides obtained using phage display, as substitute antibodies, have several advantages as compared to antibodies, including that peptides can be created synthetically in a reliable and cost-effective manner; peptides are more stable and resistant to harsh environments and peptides are more amenable than antibodies to engineering at the molecular level [16,17]. Several peptide-based biosensors for the detection of protein have been developed, including troponin I [18], prostate-specific antigen (PSA) [19,20], protein kinase [21], HIV [22,23], bacteria [24], etc. The signal transduction approaches were mainly based on optical [18,24] and electrochemical biosensor [19–23]. For example, Zhao et al. reported an electrochemical method to determine the PSA by using the ferrocene-functionalized helix peptide (CHSSKLQK) [19]. The direct transduction of peptide cleavage events into an electrical signal provides a simple, sensitive method for the detection of PSA.

Electrogenerated chemiluminescence (also called electrochemiluminescence and abbreviated ECL), known for high sensitivity, rapidity and easy controllability, has attracted increasing attention in recent years, leading to the development of various ECL sensing approaches in immunosensing [25], DNA hybridization assays [26] and enzymatic biosensors [27]. Although phage display has been widely applied to identify peptides or proteins with specific binding capabilities, the application of peptide in the development of ECL biosensor for detection of PSA has not been published.

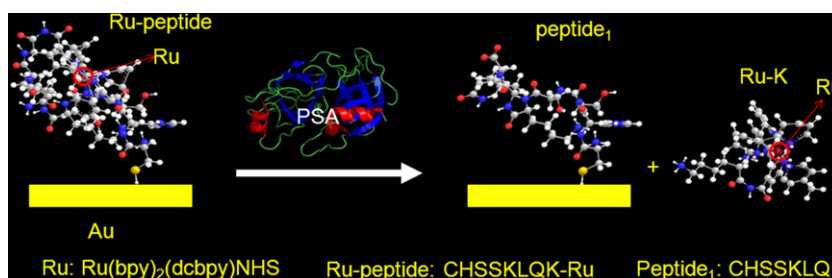


Fig. 1. Schematic diagram of the fabrication of ECL-PB biosensor for the detection of PSA.

The aim of the present work is to develop an ECL peptide-based (ECL-PB) biosensor for the determination of protein. As a model system, PSA is chosen as an analyte and a helix peptide (CHSSKLQK) is taken as molecular recognition element. The principle scheme of the designed ECL-PB biosensor is demonstrated in Fig. 1. The helix peptide with a sequence of CHSSKLQK was labeled with an ECL label, ruthenium bis(2,2'-bipyridine) (2,2'-bipyridine-4,4'-dicarboxylic acid)-N-hydroxysuccinimide ester ($\text{Ru}(\text{bpy})_2(\text{dcbpy})\text{NHS}$, Ru), and immobilized on a gold electrode surface by self-assembling technique through a thiol-containing cysteine incorporated at the end of the peptide. The principle of ECL measurement is based on the specific proteolytic cleavage event of the Ru-peptide on the gold electrode surface in the presence of PSA, resulting in the decrease of the ECL signal of the electrode. In this work, the principle concept of ECL-PB biosensor for determination of PSA was proposed and the fabrication and characterization of the ECL-PB biosensor for PSA were presented. The kinetic studies of peptide with PSA were performed and compared with that of anti-PSA antibody with PSA.

2. Experimental section

2.1. Reagents and apparatus

A peptide (8 mer, CHSSKLQK, MW=929.48) was designed according to Ref. [19] and chemically synthesized and obtained from Sinoasis Pharmaceuticals, Inc. (China). Prostate-specific antigen (PSA, MW=34,000) from human semen was obtained from Fitzgerald Industries International, Inc. (USA). PSA-specific antibody was obtained from Zhongshan Goldenbridge Biotechnology Co. Ltd (China). Ruthenium bis(2,2'-bipyridine) (2,2'-bipyridine-4,4'-dicarboxylic acid)-N-hydroxysuccinimide ester ($\text{Ru}(\text{bpy})_2(\text{dcbpy})\text{NHS}$, Ru) and 6-mercaptohexanol (MCH) was obtained from Sigma-Aldrich (USA). Trypsin was obtained from Beijing BioDee Biotechnology Co. (China). All solutions were prepared with Millipore Milli-Q water (18.2 M Ω cm).

ECL measurements were performed with a MPI-E ECL detector (Xi'an Remax Electronics, China). The experimental set-up for ECL measurement was same as the previous paper [28]. A commercial cylindroid glass cell was used as an ECL cell, which contained a conventional three-electrode system consisting of either a gold electrode (2.0 mm diameter) or a ECL-PB sensor as the working electrode, a platinum plate as the counter electrode, and an Ag/AgCl (saturated KCl) as the reference electrode, respectively. ECL emissions were detected with a photomultiplier tube (PMT) that was biased at -900 V unless otherwise stated.

2.2. Preparation of ECL probes

The ECL probe, ruthenium bis (2,2'-bipyridine) (2,2'-bipyridine-4,4'-dicarboxylic acid)-N-hydroxysuccinimide ester-peptide (Ru-peptide), were synthesized according to literatures with

some modifications [28,29]. Two milligrams of peptide (0.00215 mmol) was dissolved in 10 mL of absolute ethanol. The peptide was labeled by adding a 10-fold molar excess of $\text{Ru}(\text{bpy})_2(\text{dcbpy})\text{NHS}$ to the stirred peptide solution, followed by an overnight incubation at 4 °C. The labeled peptide was purified by gel filtration chromatography on Sephadex G-15 using 50 mM phosphate buffer solution (PBS) containing 0.15 M NaCl (pH 7.2). The concentration of Ru-peptide solution was calculated to be 1.77×10^{-5} M according to the value of UV absorption of $\text{Ru}(\text{bpy})_2(\text{dcbpy})\text{NHS}$ at 457 nm [30,31].

For control, the ECL probe, $\text{Ru}(\text{bpy})_2(\text{dcbpy})\text{NHS}$ labeled antibody ($\text{Ru}(\text{bpy})_2(\text{dcbpy})\text{NHS}$ -antibody), was synthesized as following. A 10-fold molar excess of $\text{Ru}(\text{bpy})_2(\text{dcbpy})\text{NHS}$ was added into 200 μL 1.5×10^{-5} M of stirred antibody solution, followed by an overnight incubation at 4 °C and purification with ultrafiltration centrifugal tube (3000 MWCO) using 0.1 M PBS (pH 7.4). The concentration of $\text{Ru}(\text{bpy})_2(\text{dcbpy})\text{NHS}$ -antibody solution was calculated to be 1.16×10^{-5} M according to the value of UV absorption of $\text{Ru}(\text{bpy})_2(\text{dcbpy})\text{NHS}$ at 457 nm.

2.3. Fabrication of ECL biosensor

The Ru-peptide was immobilized on a cleaned gold electrode surface by self-assembling technique [19]. Prior to the experiment, the gold electrode (2.0 mm diameter) was pretreated according to previously published protocols [28,29]. The self-assembly of Ru-peptide onto the gold electrode surface was performed at 4 °C by dipping the electrode into 10 μM Ru-peptide solution for 30 min. The Ru-peptide modified gold electrode was thoroughly rinsed with ethanol to remove the unbinding ECL probe on the gold electrode. The Ru-peptide modified gold electrode was then immersed in 1 mM mercaptohexanol solution for 30 min. The resulting electrode was washed with water and used as the ECL-PB biosensor.

For control, ECL immunosensor was fabricated with the reduction of *in situ* generated aminophenyl diazonium cation generated from p-phenylenediamine on gold electrode, followed by covalent bonding of $\text{Ru}(\text{bpy})_2(\text{dcbpy})\text{NHS}$ -antibody to the aminophenyl group according to Ref. [32] (see Supporting Information section for the immunosensor fabrication).

2.4. ECL measurement

The ECL-PB biosensor or the ECL immunosensor fabricated was immersed in 100 μL of 10 mM PBS (pH 7.4) containing different concentrations of PSA for 30 min at room temperature. After the reaction, the formed electrode was rinsed thoroughly with 10 mM PBS (pH 7.4) to remove the nonspecific adsorption. The ECL measurement was performed at a constant potential of +0.85 V in 1.0 mL of 0.10 M PBS (pH 7.4) containing 50 mM tripropylamine (TPA) and 0.10 M NaCl. The concentration of PSA was quantified by a decreased ECL intensity ($\Delta I = I_0 - I_s$), where I_s was the ECL intensity of ECL-PB biosensor reacted with PSA and I_0 was

the blank ECL intensity of ECL-PB biosensor. All experiments were carried out at room temperature.

3. Results and discussion

3.1. Characterization of the ECL probe

The ECL probe (Ru-peptide) synthesized according to the process described in the experiment section was characterized by UV–vis spectroscopy and electrogenerated chemiluminescence technique. UV–vis spectra of the Ru-peptide are shown in Fig. 2A. From Fig. 2A, it can be seen that the characteristic peaks appear at 214 nm, 246 nm, 287 nm and 457 nm for the ECL probe (line a), at 214 nm for peptide (line b), at 245 nm, 287 nm and 457 nm for Ru(bpy)₂(dcbpy)NHS (line c). This indicates that Ru(bpy)₂(dcbpy)NHS has been labeled to the peptide.

The ECL probe was also characterized by the ECL method at a gold electrode using a cyclic voltammetry technique. Fig. 2B shows ECL intensity–potential profiles of Ru(bpy)₂(dcbpy)NHS (line a) and Ru-peptide (line b) in 0.10 M PBS (pH 7.4) containing 50 mM TPA. From Fig. 2B, it can be seen that ECL peak of Ru(bpy)₂(dcbpy)NHS appears at 0.83 V and ECL peak of Ru-peptide appears at 0.85 V. The ECL peak potential of Ru-peptide is more positive than that of Ru(bpy)₂(dcbpy)NHS. The ECL intensity of Ru(bpy)₂(dcbpy)NHS was nearly 2-fold higher than that of Ru-peptide at the same concentration. Similar phenomena was observed by Xu for Ru(bpy)₂³⁺ labeled prostate-specific antigen [33]. Xu reported that the ECL intensity of Ru(bpy)₂³⁺ labeled antigen was inversely proportional to molecule's mass and size. Moreover, the diffusion coefficient was related with the molecule's mass and size [34]. Therefore, the decrease of ECL intensity may be ascribed to the increase of the molecular weight and the decreases of diffusion coefficient of Ru-peptide.

3.2. ECL response of the ECL-PB biosensor for PSA

In this work, we employed a helix peptide with a sequence of CHSSKLQK as recognition molecule. Ru(bpy)₂(dcbpy)NHS was covalently labeled onto peptide at NH₂-containing lysine via acylation reaction. Fig. 3 shows that ECL intensity vs time profiles of the ECL-PB biosensor fabricated before (line a) and after incubation in 10 mM PBS (pH 7.4) in the absence of PSA (b), in the presence of 0.5 ng/mL PSA (line c) and in the presence of 5 ng/mL PSA (line d), respectively. Compared line a with line c, ECL-PB biosensor with

interaction of 0.5 ng/mL PSA displayed a small signal. Compared line c with line d, it can be clearly observed that the ECL intensity decreases from 5133 to 3870 as the PSA concentration is elevated. A control experiment performed by incubating the ECL-PB biosensor in 10 mM PBS (pH 7.4) in the absence of PSA found that ECL signal had negligible change (3.1% drop, line b) after 30-min incubation. It was reported that peptide was recognized and cleaved between the glutamine(Q) and lysine (K) position in the presence of target PSA [19,35–37]. This leads to the decrease of the amount of Ru(bpy)₂(dcbpy)NHS on the electrode surface, thus, resulting in the decrease of ECL signal detected. Therefore, the ECL-PB biosensor possibly can be used to detect target PSA. Not only ECL peak height but also peak width were changed after the PB-based biosensor reacted with PSA. Although the integral ECL intensity was more accurate for the quantitative analysis, the ECL peak height was utilized because data handling was simpler and more convenient. As a result, the direct transduction of

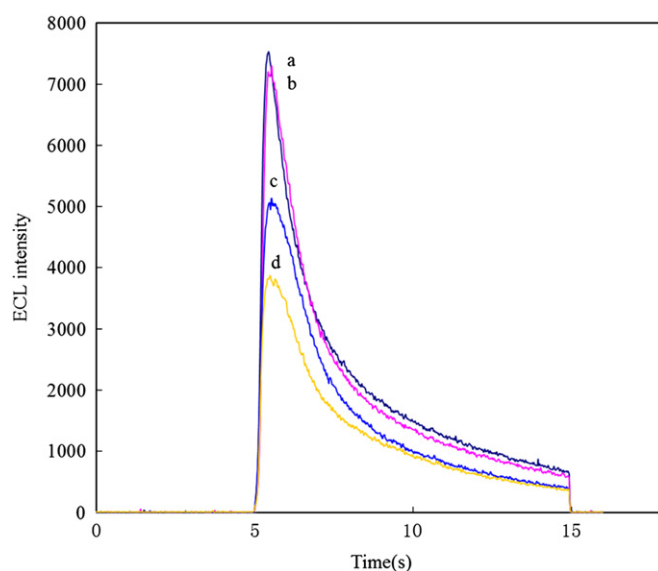


Fig. 3. ECL intensity vs time profiles of the ECL-PB biosensor fabricated before (line a) and after incubation in 10 mM PBS (pH 7.4) in the absence of PSA (b), in the presence of 0.5 ng/mL PSA (line c) and in the presence of 5 ng/mL PSA (line d). Cleavage time, 30 min; detection solution, 0.10 M PBS (pH 7.4) containing 50 mM TPA; applied potential 0.85 V.

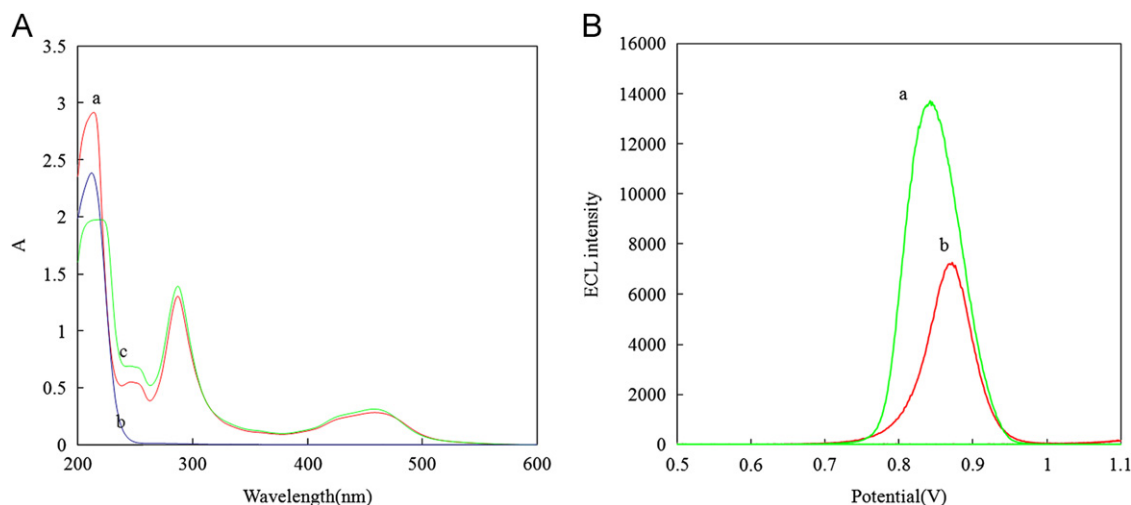


Fig. 2. (A) UV–vis spectra of Ru-peptide (a, red line), peptide (b, blue line) and Ru(bpy)₂(dcbpy)NHS (c, green line). (B) ECL intensity vs potential profiles of Ru(bpy)₂(dcbpy)NHS (line a) and Ru-peptide (line b) in 0.10 M PBS (pH 7.4) containing 50 mM TPA. Scan rate 50 mV/s. (For interpretation of references to color in this figure legend, the reader is referred to the web version of this article.)

peptide cleavage events into an ECL signal can provide a simple, sensitive route to detect the concentration of PSA.

3.3. Optimization of conditions

The applied potential to ECL-PB biosensor designed is an important parameter because it is related to the sensitivity of the ECL-PB biosensor. The dependence of the ECL intensity of the ECL-PB biosensor on applied potential was checked. The results are shown in Fig. 4A. From Fig. 4A, it can be seen that when the electrode potential is positive than 0.70 V, an obvious ECL appears, attributed to the oxidation of TPA at modified electrode [38]. Experimentally, +0.90 V was found to be the optimal potential (Fig. 4A). A negative potential shift of 0.25 V from the reversible redox potential of $\text{Ru}(\text{bpy})_3^{2+}$ (1.15 V vs SCE) [39] in solution could result from the effect of the electrode material [29]. The decrease in ECL intensity at a potential higher than +0.9 V remains unclear but could be related to the desorption of Ru-peptide and the oxidation of water that produces extra amounts of oxygen, resulting in the consumption of TPA radicals needed for the generation of the $\text{Ru}(\text{bpy})_2(\text{dcbpy})\text{NHS}^*$ species [40,41]. It was also found that the reproducibility of the ECL-PB biosensors was dwindled at +0.9 V. This is attributed to the oxidative desorption of thiol of the ECL probe on the gold electrode at the higher potentials [42,43]. It was lucky enough to obtain a much highly enhanced ECL value at +0.85 V. Therefore, the constant potential of +0.85 V was chosen in following experiments.

We studied the effect of cleavage time on the ECL signal of ECL-PB biosensor in the presence of PSA and found that the ECL signal decreased with the increase of the incubation time. Therefore, the dependence of the ECL intensity on cleavage time, that is, the time of immersing the ECL-PB biosensors in the PSA solution, was investigated to obtain the optimum cleavage time. When a series of the ECL-PB biosensors fabricated were immersed in 5 ng/mL PSA solution for different times, the ECL signals were recorded in 0.10 M PBS containing 50 mM TPA at a constant potential of +0.85 V. The results showed that the ECL intensity decreased with the increase of cleavage time from 5 to 30 min and then became stable after 30 min (as shown in Fig. 4B, a). The decreased ECL intensity reached maximum value and kept stable when the cleavage time was up to 30 min (as shown in Fig. 4B, b). This suggests that 30 min is enough for the system to reach

equilibrium. Therefore, 30 min was chosen as cleavage time in the following experiments.

3.4. Performance of ECL-PB biosensors

3.4.1. Linear range and detection limit

The quantitative behavior of the ECL-PB biosensor fabricated was assessed according to the protocol described in ECL measurement. Fig. 5A shows the ECL profiles of the ECL-PB biosensors after interacting with different concentrations of PSA under the optimized conditions. From Fig. 5A, it can be seen that the ECL intensity decreases with an increase of the concentration of PSA. The decreased ECL intensity had a linear relationship with the concentration of PSA in the range from 1.0×10^{-10} g/mL to 8.0×10^{-9} g/mL. The linear regression equation was $\Delta I = 2038 \lg C + 2612$ (unit of C is 10^{-9} g/mL) and the correlation coefficient was 0.9591. The detection limit (DL) was 0.038 ng/mL (3σ) [44]. The detection limit is comparable with that reported in Refs. (enzyme-linked immunosorbent assay (1×10^{-10} g/mL [4]), colorimetric immunoassay (1×10^{-12} g/mL [5]), fluorescence immunoassay (1.0×10^{-12} g/mL (34fM) [7] and 6×10^{-10} g/mL [8]), electrochemical immunoassay (1×10^{-10} g/mL [10] and 7.6×10^{-10} g/mL [11]) and electrochemical peptide-based assay (2×10^{-10} g/mL [19])). DL of the ECL-PB biosensor was slightly larger than that of 4×10^{-11} g/mL PSA on ECL immunoassay by employing carbon nanotube forests and $[\text{Ru}(\text{bpy})_3]^{2+}$ -doped silica nanoparticles [14] and of 1×10^{-12} g/mL PSA on ECL immunosensor array [15]. Since age-adjusted cutoff values for the normal range are <2.5, <3.5, <4.5, and <5.5 ng/mL for men aged 40–50, 51–60, 61–70, and 71–80 years, respectively, the ECL-PB biosensor DL is still well below the normal serum levels of PSA, and the ECL-PB biosensor presents a clinically viable concentration range [45].

In order to illustrate the good recognition molecular ability, a control immunosensor was fabricated with the reduction of *in situ* generated aminophenyl diazonium cation generated from p-phenylenediamine on gold electrode, followed by covalent bonding of antibody to the aminophenyl group. When the immunosensor reacted with target PSA, an immunocomplex of antibody-PSA was formed and a decrease of ECL intensity was observed. Fig. 5B shows the ECL profiles of the ECL immunosensor after interaction with different concentrations of PSA. The results showed that the decreased ECL intensity of immunosensor had a linear relationship with the increase of concentration of PSA in the range from 1.0×10^{-9} g/mL to 4.0×10^{-8} g/mL. The regression equation was

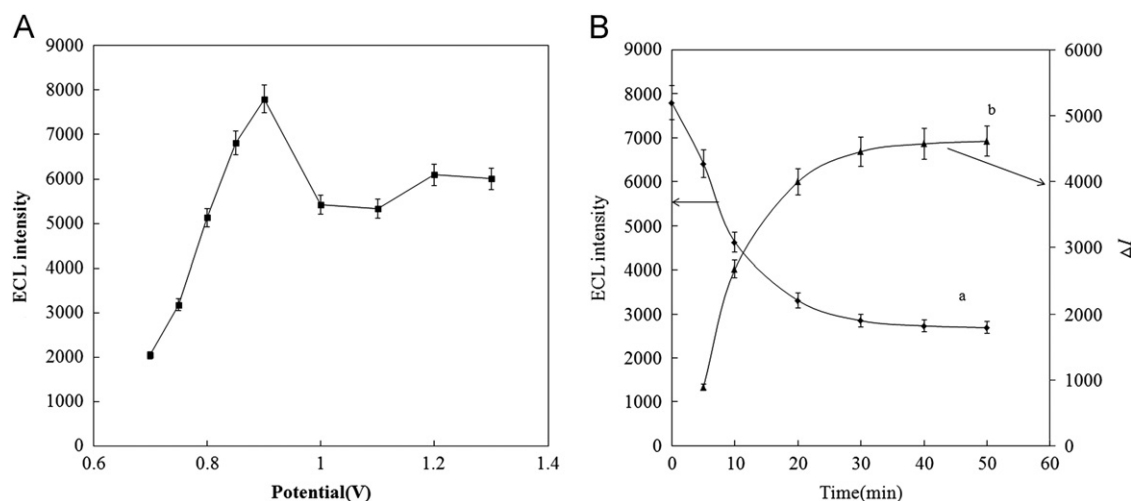


Fig. 4. (A) Dependence of the ECL intensity of the ECL-PB biosensor on applied potential obtained in 0.10 M PBS (pH 7.4) containing 50 mM TPA. (B) Dependence of the ECL intensity of ECL-PB biosensor (a) and the decreased ECL intensity (b) with interaction of 5 ng/mL PSA on cleavage time. The ECL measurement conditions are same as Fig. 3.

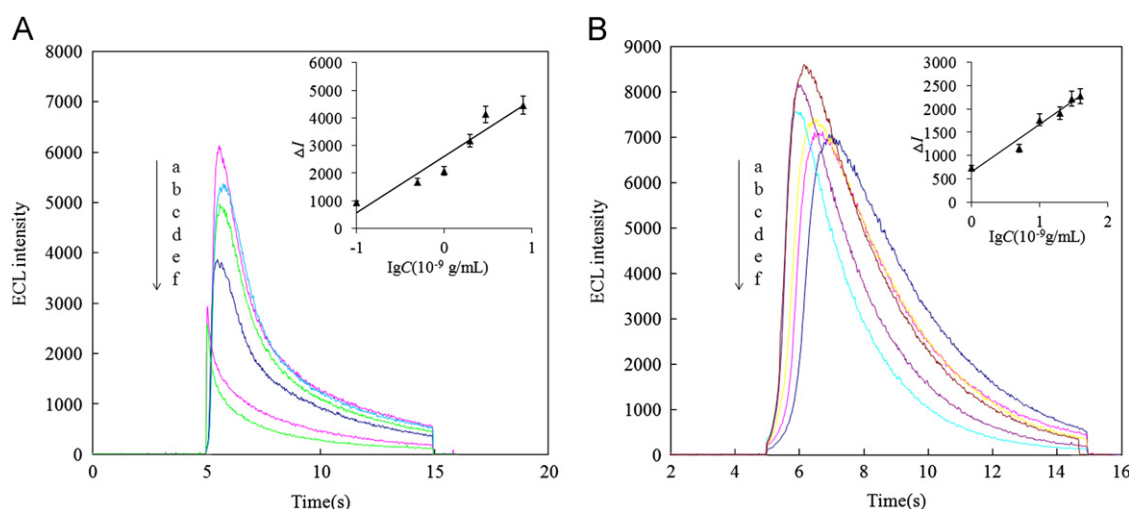


Fig. 5. (A) ECL responses of the ECL-PB biosensors with interaction of different concentrations of PSA. (a) 1.0×10^{-10} g/mL; (b) 5.0×10^{-10} g/mL; (c) 1.0×10^{-9} g/mL; (d) 2.0×10^{-9} g/mL; (e) 5.0×10^{-9} g/mL and (f) 8.0×10^{-9} g/mL. (B) ECL responses of the ECL immunosensor biosensors with interaction of different concentrations of PSA. (a) 1.0×10^{-8} g/mL; (b) 5.0×10^{-8} g/mL; (c) 1.0×10^{-8} g/mL; (d) 2.0×10^{-8} g/mL; (e) 3.0×10^{-8} g/mL and (f) 4.0×10^{-8} g/mL. The ECL measurement conditions are same as Fig. 3.

$\Delta I = 1009 \lg C + 655.2$ (unit of C is 10^{-9} g/mL) and the correlation coefficient was 0.9823. The detection limit was 0.33 ng/mL, which was 10 fold higher than that of at ECL-PB biosensor. The slope at the ECL-PB biosensor was 2 fold higher than that at the ECL immunosensor. Therefore, a high sensitivity of the ECL-PB biosensor developed was obtained.

The precision was estimated for 1 ng/mL PSA with 6 ECL-PB biosensors and yielded reproducible results with a relative standard deviation (RSD) of 3.3%. The day-to-day prepared ECL-PB biosensors showed good reproducibility with RSD of 2.3% ($n=6$). Therefore, a good reproducibility of the ECL-PB biosensors developed was obtained.

3.4.2. Selectivity, stability and application

The evaluation of the selectivity of ECL-PB biosensor fabricated was performed by examining trypsin which belongs to proteinases and the mixture of trypsin and PSA (as shown in Fig. S-1). The ECL intensity of the proposed ECL-PB biosensors was examined after immersing the ECL-PB biosensors in 5 ng/mL PSA, 50 ng/mL of trypsin, and the mixture of trypsin and PSA according to the protocol described in ECL measurement, respectively. A significant decrease induced by the interaction of the ECL-PB biosensor with PSA and mixture (62.4% for PSA, 63.8% for mixture) was observed compared with that of trypsin (5.8% for trypsin), indicating that the developed strategy has a sufficient selectivity and PSA could be unequivocally identified. This may be explained by the fact that the peptide with the sequence of CHSSKLQK could be cleaved by PSA specifically while trypsin showed lower selectivity of cleavage activity.

The long-term stability of the ECL-PB biosensors is an important issue for the development and practical implementation for the detection of PSA. Therefore, the storage stability of the fabricated ECL biosensors was investigated. It was found that the response of ECL-PB biosensors to 5.0 ng/mL PSA did not significantly change when stored in 10 mM PBS (pH 7.4) at 4 °C over 10 days and the signal only decreased about 6.4%. Therefore, a stability of the ECL-PB biosensors fabricated was obtained.

The proposed method was also implemented in the analysis of control human serum. A series of samples were prepared by adding different concentrations PSA to human serum, obtained from Hospital of Shaanxi normal university. The results are shown

in Table S-1. As can be seen from Table S-1, average recovery determination of prostate-specific antigen is 97.2%, indicating a feasibility of possibility in clinical application.

3.4.3. Kinetic studies of immobilized peptide interacted with PSA

In order to understand the fundamental of cleavage event and the performance of designed biosensors, the dissociation constant of the immobilized peptide with PSA (K_d) and the association constant of the immobilized antibody with PSA (K_b) were determined by the ECL method according to Refs. [41,46–49]. For the ECL-PB biosensor, we supposed that the cleavage reaction consists 2 steps [42]. The first step is represented by the binding of PSA to the Ru-peptide immobilized on the gold electrode surface, to form a Ru-peptide-PSA complex. In second step, the Ru-peptide is cleaved by PSA, following releasing one product (Ru-K) in solution and remaining peptide₁ (CHSSKLQ) on the electrode. According to the results as shown in Fig. S-2, an apparent K_d between immobilized peptide and PSA of 3.5 ng/mL (1.0×10^{-10} M) was obtained. For comparison, the association constant of the immobilized antibody with PSA was evaluated according to Refs. [41,48,49]. Fig. S-3 shows the correlation between the decreased ECL intensity and the PSA concentrations. According to the results in Fig. S-3, K_b between immobilized antibody and PSA of 0.24 ng/mL^{-1} ($8.0 \times 10^9 \text{ M}^{-1}$) are obtained ($K_d = 4.3 \text{ ng/mL}$ ($1.3 \times 10^{-10} \text{ M}$)), which is similar with that of Ref. [50], $K_d = 0.1$ and 0.4 nM . Both the low dissociation constants indicate that high affinity of peptide/antibody with PSA.

4. Conclusion

In conclusion, a sensitive and selective reagent-less ECL peptide-based biosensor for the detection of PSA has been developed. A peptide was used as a molecular recognition element and ruthenium complex was used as an ECL label. High affinity of peptide with PSA results in a sensitive detection of PSA with a detection limit of 3.8×10^{-11} g/mL. This work demonstrates that the direct transduction of peptide cleavage events into an ECL signal provides a simple, reagent-less, rapid, and sensitive method for disease biomarker assay.

Acknowledgments

We gratefully acknowledge the financial supports from the National Natural Science Foundation of China (Nos. 21027007 and 20805028) and the Fundamental Research Funds for the Central Universities (No. GK201002027).

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at <http://dx.doi.org/10.1016/j.talanta.2012.08.037>.

References

- [1] J. Lin, H. Ju, *Biosensors Bioelectron.* 20 (2005) 1461–1470.
- [2] E.P. Diamandis, *Clin. Chem.* 46 (2000) 896–900.
- [3] S.F. Kingsmore, *Nat. Rev. Drug Discovery* 5 (2006) 310–321.
- [4] B. Acevedo, Y. Perera, M. Ruiz, G. Rojas, J. Benítez, M. Ayala, J. Gavilondo, *Clin. Chim. Acta* 317 (2002) 55–63.
- [5] C. Cao, X. Li, J. Lee, S.J. Sim, *Biosensors Bioelectron.* 24 (2009) 1292–1297.
- [6] F. Qu, T. Li, M. Yang, *Biosensors Bioelectron.* 26 (2011) 3927–3931.
- [7] Y. Wang, A. Brunson, U. Jonas, J. Dostalek, W. Knoll, *Anal. Chem.* 81 (2009) 9625–9632.
- [8] T. Kokko, T. Liljenbäck, M.T. Peltola, L. Kokko, T. Soukka, *Anal. Chem.* 80 (2008) 9763–9768.
- [9] D.S. Grubisha, R.J. Lipert, H.Y. Park, J. Driskell, M.D. Porter, *Anal. Chem.* 75 (2003) 5936–5943.
- [10] S.S. Zhang, P. Du, F. Li, *Talanta* 72 (2007) 1487–1493.
- [11] B. Qu, X. Chu, G.L. Shen, R.Q. Yu, *Talanta* 76 (2008) 785–790.
- [12] Y. Wu, S. Liu, L. He, *Analyst* 136 (2011) 2558–2563.
- [13] Y. Zheng, H. Chen, X.P. Liu, J.H. Jiang, Y. Luo, G.L. Shen, R.Q. Yu, *Talanta* 77 (2008) 809–814.
- [14] N. Sardesai, S. Pan, J. Rusling, *Chem. Commun.* 33 (2009) 4968–4970.
- [15] N.P. Sardesai, J.C. Barron, J.F. Rusling, *Anal. Chem.* 83 (2011) 6698–6703.
- [16] S.S. Iqbal, M.W. Mayo, J.G. Bruno, B.V. Bronk, C.A. Batt, J.P. Chambers, *Biosensors Bioelectron.* 15 (2000) 549–578.
- [17] V.A. Petrenko, V.J. Vodyanoy, *J. Microbiol. Methods* 53 (2003) 253–262.
- [18] J.P. Park, D.M. Crotek, S. Banta, *Biotechnol. Bioeng.* 105 (2010) 678–686.
- [19] N. Zhao, Y.Q. He, X. Mao, Y.H. Sun, X.B. Zhang, C.Z. Li, Y.H. Lin, G.D. Liu, *Electrochem. Commun.* 12 (2010) 471–474.
- [20] M.A. Roberts, S.O. Kelley, *J. Am. Chem. Soc.* 129 (2007) 11356–11357.
- [21] K. Kerman, H. Song, J.S. Duncan, D.W. Litchfield, H.B. Kraatz, *Anal. Chem.* 80 (2008) 9395–9401.
- [22] J.Y. Gerasimov, R.Y. Lai, *Chem. Commun.* 46 (2010) 395–397.
- [23] K. Kerman, H.B. Kraatz, *Analyst* 134 (2009) 2400–2404.
- [24] N.V. Kulagina, M.E. Lassman, F.S. Ligler, C.R. Taitt, *Anal. Chem.* 77 (2005) 6504–6508.
- [25] H.L. Qi, C.X. Zhang, *Anal. Chim. Acta* 501 (2004) 31–35.
- [26] H. Wang, C.X. Zhang, Y. Li, H.L. Qi, *Anal. Chim. Acta* 575 (2006) 205–211.
- [27] W. Miao, *Chem. Rev.* 108 (2008) 2506–2553.
- [28] Y. Li, H. Qi, Y. Peng, J. Yang, C. Zhang, *Electrochem. Commun.* 9 (2007) 2571–2575.
- [29] W.J. Miao, A.J. Bard, *Anal. Chem.* 75 (2003) 5825–5834.
- [30] T. Shimizu, T. Iyoda, K. Izaki, *J. Phys. Chem.* 89 (1985) 642–645.
- [31] J. Zhang, H. Qi, Y. Li, J. Yang, Q. Gao, C. Zhang, *Anal. Chem.* 80 (2008) 2888–2894.
- [32] S. Boland, F. Barrie're, D. Leech, *Langmuir* 24 (2008) 6351–6358.
- [33] X.H.N. Xu, R.B. Jeffers, J. Gao, B. Logan, *Analyst* 126 (2001) 1285–1292.
- [34] A.J. Bard, L.R. Faulkner, *Electrochemical Methods: Fundamentals and Applications*, 2nd ed., John Wiley & Sons, INC., 2000.
- [35] P. Niemela, J. Lovgren, M. Karp, H. Lilja, K. Pettersson, *Clin. Chem.* 48 (2002) 1257–1264.
- [36] G.S. Coombs, R.C. Bergstrom, J.L. Pellequer, S.I. Baker, M. Navre, M.M. Smith, J.A. Tainer, E.L. Madison, D.R. Corey, *Chem. Biol.* 5 (1998) 475–488.
- [37] S.R. Denmeade, W. Lou, J. Lovgren, J. Maim, H. Lilja, J.T. Isaacs, *Cancer Res.* 57 (1997) 4924–4930.
- [38] Y.B. Zu, A.J. Bard, *Anal. Chem.* 73 (2001) 3960–3964.
- [39] Y.B. Zu, A.J. Bard, *Anal. Chem.* 72 (2000) 3223–3232.
- [40] H. Zheng, Y. Zu, *J. Phys. Chem. B* 109 (2005) 12049–12053.
- [41] B. Sun, H. Qi, F. Ma, Q. Gao, C. Zhang, W. Miao, *Anal. Chem.* 82 (2010) 5046–5052.
- [42] C. Bertolino, M. MacSweeney, J. Tobin, B. O'Neill, M.M. Sheehan, S. Coluccia, H. Berney, *Biosensors Bioelectron.* 21 (2005) 565–573.
- [43] A. Heller, *Faraday Discuss.* 116 (2000) 1–13.
- [44] H. Kaiser, *Pure Appl. Chem.* 34 (1973) 35–62.
- [45] G.J. Kelloff, D.S. Coffey, B.A. Chabner, A.P. Dicker, K.Z. Guyton, P.D. Nisen, H.R. Soule, A.V. D'Amico, *Clin. Cancer Res.* 10 (2004) 3927–3933.
- [46] F. Ma, B. Sun, H. Qi, H. Zhang, Q. Gao, C. Zhang, *Anal. Chim. Acta* 683 (2011) 234–241.
- [47] C. Haenel, M. Satzger, D.D. Ducata, R. Ostendorp, B. Brocks, *Anal. Biochem.* 339 (2005) 182–184.
- [48] I. Langmuir, *J. Am. Chem. Soc.* 38 (1916) 2221–2295.
- [49] I. Langmuir, *J. Am. Chem. Soc.* 40 (1918) 1361–1402.
- [50] R. Abraham, S. Buxbaum, J. Link, R. Smith, C. Venti, M. Darsley, *J. Mol. Recogn.* 9 (1996) 456–461.