

Sensitive electrogenerated chemiluminescence peptide-based biosensor for the determination of troponin I with gold nanoparticles amplification

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Abstract A sensitive electrogenerated chemiluminescence (ECL) peptide-based biosensor was fabricated for the determination of troponin I (TnI) by employing gold nanoparticles as amplification platform. Two specific peptides including peptide1 with a sequence of CFYSHSFHENWPS and peptide2 with a sequence of FYSHSFHENWPSK were employed as capture peptide and report peptide, respectively. The peptide2 was labeled with ruthenium bis(2,2'-bipyridine) (2,2'-bipyridine-4,4'-dicarboxylic acid)-*N*-hydroxysuccinimide ester (Ru(bpy)₂(dc bpy)NHS) at NH₂-containing lysine via acylation reaction and utilized as the ECL probe. Gold nanoparticles were electrodeposited onto gold electrode and used as an amplification platform. The peptide-based biosensor was fabricated by self-assembling peptide1 onto the surface of gold nanoparticles-modified gold electrode through a thiol-containing cysteine at the end of the peptide1. When the biosensor reacted with target TnI, and then incubated with the ECL probe, a strong ECL response was electrochemically generated. The ECL intensity is directly proportional to the logarithm of the concentration of TnI in the range from 1 to 300 pg/mL. The biosensor employing gold nanoparticles as amplification platform shows high sensitivity for the detection of TnI with a detection limit of 0.4 pg/mL ($S/N=3$). Moreover, the biosensor is successfully applied to analysis of TnI in human serum sample. This work demonstrates that the combination of a highly binding peptide with nanoparticle

amplification is a great promising approach for the design of ECL biosensor.

Keywords Electrogenerated chemiluminescence · Gold nanoparticles · Peptide · Troponin I

Introduction

Acute myocardial infarction is a major cause of human death and responsible for one third of deaths in the world. Measurement of cardiac markers is critical in assisting the diagnosis of acute myocardial infarction [1, 2]. Cardiac troponin I (TnI), with a molecular weight of 24 kDa, a part of the troponin complex that is present in cardiac muscle tissues, has been known as a reliable biomarker of cardiac muscle tissue injury and was widely used in the early diagnosis of acute myocardial infarction [3, 4]. The concentration of TnI in blood rises rapidly within 4–6 h after the onset of an acute myocardial infarction and reaches to the maximum at approximately 12 h. After 6–8 days, the TnI level returns to normal, and thus, the concentration of TnI in blood can provide a long diagnostic window for detecting cardiac injury [3]. A variety of methods such as colorimetric [5], electrochemical [2, 3], fluorescent [6, 7], and chemiluminescence [8] methods have been developed to determine TnI. Despite the extensive development of these methods, the major limitation in currently used methods for the determination of TnI assays is low sensitivity at the time of a patient's presentation, owing to a delayed increase in circulating levels of cardiac troponin [2]. Therefore, it is still a critical demand on sensitive and specific methods for the determination of TnI, especially in the point-of-care applications.

Electrogenerated chemiluminescence (also called electrochemiluminescence and abbreviated ECL) method

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has attracted considerable interest due to its high sensitivity, rapidity, easy controllability, and wide dynamic range [9, 10]. Several ECL methods have been developed for the determination of TnI [11–15]. Cui designed ECL immunosensor for the detection of human cardiac troponin I by using luminol [11] and *N*-(aminobutyl)-*N*-(ethylisoluminol) [12]-functionalized gold nanoparticles as labels. Smith developed an ECL immunoassay for detection of rat TnI in serum [13]. The commonly used ECL immunoassays are normally conducted by employing antibodies as molecular recognition elements. However, the antibody drawbacks associated with the production and stability. Short linear binding peptides, which are obtained using phage display, have received considerable interest in protein analysis due to the advantages, such as stable, resistant to harsh environments, and more amenable to engineering at the molecular level than antibodies [16]. Recently, Park et al. reported a new peptide (FYSHSFHENWPS) that selectively bound to TnI with a disassociation constant of the complex in nanomolar level [17]. We developed two homogeneous ECL peptide-based methods for the determination of TnI using this peptide as a molecular recognition element [14, 15]. In previously work [14], one peptide (FYSHSFHENWPSK), as a molecular recognition element, was labeled with ruthenium complex through NH₂-containing lysine on the peptide via acylation reaction and utilized as an ECL probe. In the presence of TnI, a decrease in ECL signal was observed upon the binding event between the ECL probe and target TnI. The binding of small peptide with large target protein results in a sensitive ECL detection of protein compared with homogeneous immunoassay employing antibody as recognition element. However, the detection limit of previously homogeneous ECL method (1.2×10^{-10} g/mL) is limited due to the high background and only one ECL molecule is attached directly to each peptide.

The elaboration of ECL biosensors is probably one of the most promising ways to solve some of the problems concerning sensitivity, speediness, and stability. And much effort has been devoted to improve the sensitivity of ECL biosensors, such as employment of the nanoparticles-based signal amplification strategy. Nanoparticles including carbon nanotube, metal nanoparticles were employed as the amplification platform for the immobilization of molecular recognition elements [18], such as antibody [19], aptamer [20], carbohydrate [21], or peptide [22]. Gold nanoparticles (GNPs), with unique properties such as their fascinating electrocatalytic activity, large surface area, excellent conductivity, and stability, have been widely used in designing ECL biosensors [23, 24]. Generally, the ECL biosensors utilize GNPs for the modification of the substrate electrodes, which provide large electrode area and also facilitate the electron transfer between the ECL signals and the electrodes, thus affording the possibility of the improvement of ECL

performance. The unique properties of GNPs-modified electrode interfaces lead to novel ECL biosensors with high sensitivity and good stability in immunoassay [25], DNA bioassay [26], and glycan biosensor [27]. In an alternative way, GNPs can work as carriers of conventional ECL signals such as luminol [11] and ruthenium complex [28] and, thus, afford substantial ECL signal amplification. We developed an ECL immunoassay for the determination of human immunoglobulin G at gold nanoparticles-modified paraffin-impregnated graphite electrode [29], an ECL DNA biosensor at gold nanoparticles-modified gold electrode [30] and a signal off aptasensor for the determination of thrombin at gold nanoparticles-modified gold electrode [31] with high sensitivity.

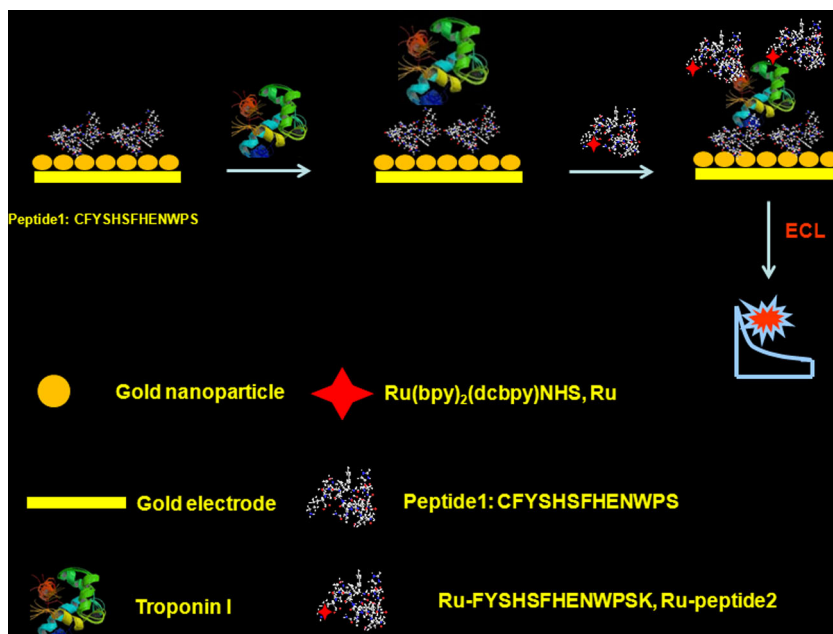
The aim of this work is to develop a highly sensitive ECL peptide-based method for the determination of protein, on basis of the idea of encompassing gold nanoparticles as amplification platform and peptide as molecular recognition element. The principle scheme is demonstrated in Fig. 1. Two specific peptides including peptide1 with a sequence of CFYSHSFHENWPS, in which a thiol-containing cysteine residue was incorporated at the end of the specific peptide to facilitate self-assembly onto the surface of gold, peptide2 with a sequence of FYSHSFHENWPSK, in which a NH₂-containing lysine residue was incorporated at the end of the peptide to covalently couple with ECL signal, were designed according to ref. [17] and employed as capture peptide and report peptide, respectively. The peptide2 was labeled with ruthenium bis(2,2'-bipyridine) (2,2'-bipyridine-4,4'-dicarboxylic acid)-*N*-hydroxysuccinimide ester (Ru(bpy)₂(dcbpy)NHS) via acylation reaction through NH₂-containing lysine at the end of the peptide to form the ECL probe Ru-peptide2. Gold nanoparticles were electrodeposited onto gold electrode and used as an amplification platform. The ECL peptide-based biosensor was fabricated by self-assembling the peptide1 onto a gold nanoparticles-modified gold electrode surface through a thiol-containing cysteine at the end of the peptide1. When the biosensor reacted with target TnI, and then incubated with the ECL probe, a strong ECL response was electrochemically generated. In this paper, the characteristics of the ECL probe and the ECL peptide-based biosensor and the analytical performance for TnI are presented.

Experimental

Reagents and apparatus

Two peptides chemically synthesized, including peptide1 with a sequence of CFYSHSFHENWPS (13 mer, MW=1,640.77), peptide2 with a sequence of FYSHSFHENWPSK (13 mer, MW=1,665.80), were designed according to ref. [17] and purchased from Sinoasis Pharmaceuticals, Inc

Fig. 1 Schematic representation of the ECL peptide-based biosensor for the determination of TnI



(China). Cardiac troponin I (TnI, human heart) was obtained from Abcam, Inc. (Cambridge, UK). Mercaptohexanol (MCH), Bis(2,2'-bipyridine)-4,4'-dicarboxybipyridine-ruthenium di(*N*-succinimidyl ester) bis(hexafluorophosphate) ($\text{Ru}(\text{bpy})_2(\text{dcbpy-NHS})(\text{PF}_6)_2$, abbreviated as Ru) and HAuCl_4 were obtained from Sigma Aldrich (USA). Human alpha-fetoprotein (AFP) and prostate specific antigen (PSA) were obtained from Fitzgerald Industries International, Inc. (USA). Human immunoglobulin G (IgG) and bovine serum albumin (BSA) were obtained from Beijing Biosynthesis Biotechnology Co., Ltd. (China). Albumin chicken egg protein was obtained from Sino-American Biotechnology Co., Ltd. (China).

The serum samples were provided by Xianyang Central Hospital (China). Phosphate buffered saline (PBS) (0.1 M) consisted of 0.1 M NaH_2PO_4 , 0.1 M Na_2HPO_4 , and 0.1 M KCl (pH 7.4). The other reagents used in this work were of analytical grade and directly used without additional purification. Millipore Milli-Q water (18.2 M Ω cm) was used to prepare all solutions.

ECL measurements were performed with a MPI-A ECL detector (Xi'an Remax Electronics, China). A commercial cylindroid glass cell was used as an ECL cell, which contained a conventional three-electrode system that consisted of a gold electrode (2.0 mm diameter) as the working electrode, a platinum wire as the counter electrode, and an Ag/AgCl (saturated KCl) as the reference electrode. ECL emissions were detected with a photomultiplier tube (PMT) that was biased at -900 V unless otherwise stated. Electrochemical experiments were performed with a CHI 660 electrochemical workstation (Chenhua Instruments Co., China).

Preparation of ECL probes

The ECL probe, $\text{Ru}(\text{bpy})_2(\text{dcbpy-NHS})(\text{PF}_6)_2$ labeled peptide2 (Ru-peptide2), were synthesized according to literatures with some modifications [14, 15]. Briefly, 1 mg of the peptide2 (0.0006 mol) was dissolved in 0.5 mL of 0.1 M phosphate buffer (PB) consisted of 0.1 M NaH_2PO_4 and 0.1 M Na_2HPO_4 (pH 7.4). Then, a 25 μL 0.02 M $\text{Ru}(\text{bpy})_2(\text{dcbpy-NHS})(\text{PF}_6)_2$ was added into 0.5 mL 10-diluted peptide2 solution under stirring, followed by an overnight incubation. The Ru-peptide2 was purified by dialysis for 12 h at 4 $^\circ\text{C}$ using MD34-2 Da molecular weight cutoff membrane with 0.1 M PBS (pH 7.4). The concentration of Ru-peptide2 solution was estimated to be 1.5×10^{-5} M, on the basis of UV absorbance of $\text{Ru}(\text{bpy})_2(\text{dcbpy-NHS})(\text{PF}_6)_2$ at 457 nm [32, 33].

Fabrication of the ECL peptide-based biosensor

The biosensor was fabricated by two steps including an electrochemical deposition step and an immobilization step. The procedure for the deposition of gold nanoparticles onto gold electrode was adapted from the ref. [34]. Prior to the experiment, the gold electrode was polished with 0.3 μm alumina slurry and then ultra-sonicated in water for 5 min. The polished gold electrode was immersed in 0.1 M HClO_4 containing 0.1 % HAuCl_4 , which was degassed with N_2 stream for at least 20 min before the electrochemical deposition. Gold nanoparticles were electrodeposited on the gold electrode by holding a constant potential of +1.1 V (vs. Ag/AgCl, sat. KCl) for 60 s and then a constant potential of

–0.1 V for 5 min in 0.1 M HClO₄ containing 0.1 % HAuCl₄ to form gold nanoparticles-modified gold electrode (GNPs/Au electrode).

The peptide1 (CFYSHSFHENWPS) was immobilized onto the surface of GNPs/Au electrode by dipping the electrode into 11.3 μM peptide1 solution for 2 h at 4 °C and rinsing with 10 mM PB (pH 7.4) to remove the unbinding peptide1. The peptide-modified GNPs/Au electrode was then immersed in 1 mM mercaptohexanol solution for 30 min to block the uncovered surface of the electrode. The resulting electrode (peptide1/GNPs/Au electrode) was washed with water and used as the ECL peptide-based biosensor.

ECL measurement

The ECL peptide-based biosensor fabricated was immersed in 100 μL 10 mM PBS (pH 7.4) containing different concentrations of TnI for 1 h at room temperature. Then, the resulting ECL peptide-based biosensor was dipped into 100 μL of 1.5 μM ECL probe for 1 h at room temperature. After each incubation, the biosensor was rinsed thoroughly with 10 mM PBS (pH 7.4) to remove adsorption components. The ECL measurement was performed at a constant potential of +0.90 V in 2.0 mL of 0.10 M PBS (pH 7.4) containing 50 mM tripropylamine (TPA). The concentration of TnI was quantified by an increased ECL intensity ($\Delta I = I_S - I_0$), where I_S was the ECL intensity of ECL peptide-based biosensor reacted with TnI and I_0 was the blank ECL intensity of ECL peptide-based biosensor. All experiments were carried out at room temperature.

Results and discussion

Characterization of the ECL probe

The ECL probe Ru-peptide2 synthesized was characterized by UV–vis spectroscopy and ECL. Figure 2a shows UV–vis spectra of the peptide2, Ru(bpy)₂(dcbpy)NHS and Ru-peptide2. The characteristic peaks of peptide2 appear at 263 nm (line a) and the characteristic peaks of Ru(bpy)₂(dcbpy)NHS appear at 201, 276, and 457 nm (line b). The characteristic absorption peaks of Ru-peptide2 appear at 207, 276, and 457 nm (line c), corresponding to the characteristic peaks of Ru(bpy)₂(dcbpy)NHS at 450, 276, 207 nm and peptide at 263 nm, respectively. This indicates that Ru(bpy)₂(dcbpy)NHS is labeled to the peptide2.

Figure 2b shows ECL intensity–potential profiles of Ru(bpy)₂(dcbpy-NHS)(PF₆)₂ (line a) and Ru-peptide2 (line b) in 0.10 M PBS containing 50 mM TPA. From Fig. 2b, it can be seen that both ECL peaks appear at near +900 mV, which is similar with that (+900 mV) in ref. [35],

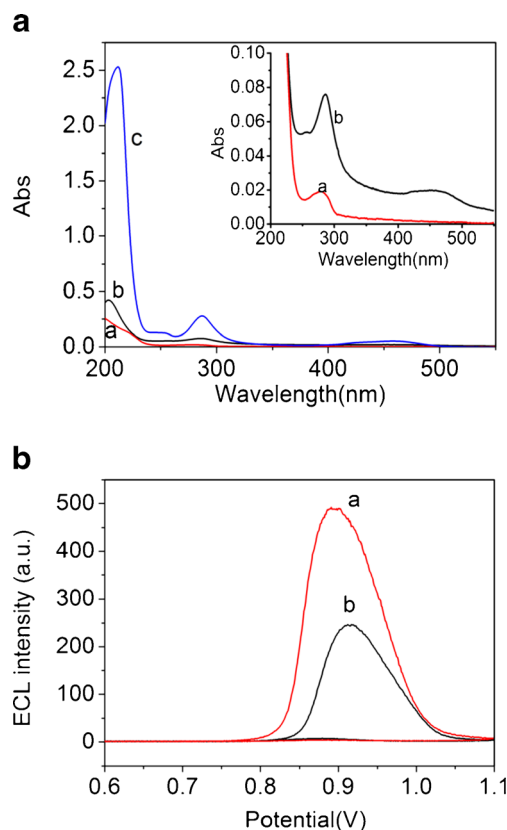


Fig. 2 **a** UV–vis spectra of peptide (a), Ru(bpy)₂(dcbpy-NHS)(PF₆)₂ (b) and Ru-peptide2 (c). **b** ECL intensity-potential profiles of 1.5×10^{-7} M Ru(bpy)₂(dcbpy-NHS)(PF₆)₂ (a) and 1.5×10^{-7} M Ru-peptide2 (b) in 0.10 M PBS (pH 7.4) containing 50 mM TPA. Scan rate, 50 mV/s

indicating that the ECL behavior of Ru-peptide2 is similar to that of Ru(bpy)₂(dcbpy)NHS and Ru(bpy)₂(dcbpy)NHS is attached to peptide2.

Feasibility of ECL peptide-based biosensor for the determination of TnI

The fabrication process of the peptide-based biosensor was characterized by cyclic voltammetry in the presence of the ferri/ferrocyanide redox couple as redox probe (see Fig. S1 A in supporting information). As expected, K₃[Fe(CN)₆]/K₄[Fe(CN)₆] showed the reversible behavior on a bare gold electrode and on a gold nanoparticles-modified electrode with a peak-to-peak separation ΔE_p of 76 mV (Fig. S1 A, line a–b). After gold nanoparticles were electrodeposited onto gold electrode, the peak current increased from 23.89 to 29.21 μA (Fig. S1 A, line b), ascribing to the increase of electrode surface area, which is confirmed by the CV results in 0.1 M H₂SO₄ (Fig. S1 B). The immobilization of peptide1 on the surface of gold nanoparticles-modified gold electrode led to a significant increase in the peak-to-peak separation ($\Delta E_p = 143$ mV) (Fig. S1 A, line c) and decrease of peak current (21.22 μA), indicating that peptide1 is self-assembled on the

electrode. This is mainly attributed to the fact that the peptide1 modified on the surface of the electrode prohibits the mass transfer of $[\text{Fe}(\text{CN})_6]^{3-/4-}$ from the solution to the surface of electrode. After reacted with TnI and Ru-peptide2, the peak-to-peak separation further increased ($\Delta E_p=196$ mV) and the peak current decreased to $17.22 \mu\text{A}$ (Fig. S1A, line d). This indicates that the peptide is self-assembled onto the electrode surface and the sandwich conjugates is formed onto the surface electrode.

Figure 3a shows the ECL intensity vs potential profiles of the ECL peptide-based biosensor reacted with 1.0×10^{-11} and 1.0×10^{-10} g/mL TnI, respectively. Compared line a and line b, it can be seen that the ECL peptide-based biosensor shows a low ECL signal (line a, 1,048) while the ECL peptide-based biosensor reacted with 1.0×10^{-11} g/mL TnI displays a higher ECL signal (line b, 4,955) in 0.10 M PBS (pH 7.4) containing 50 mM TPA. Compared line b with line c, it can be clearly observed that the ECL peak intensity increases from 4,955 to 8,648 as the concentration of TnI is elevated from 1.0×10^{-11}

to 1.0×10^{-10} g/mL. The results indicate that the ECL method is feasible for the determination of TnI.

In order to illustrate the function of gold nanoparticles, another kind of the peptide-based biosensor was fabricated by self-assembling peptide1 onto bare gold electrode surface through a thiol-containing cysteine at the end of the peptide1 and evaluated according to the protocol described in experimental section. Figure 3b shows the ECL intensity-potential profiles of the ECL peptide-based biosensor employing gold electrode as platform for the determination of TnI. The ECL peptide-based biosensor shows a low ECL signal (line a, 697). The ECL intensity were 2,779 (line b) and 4,576 (line c) after reacting with 1.0×10^{-11} and 1.0×10^{-10} g/mL TnI, respectively. Comparing Fig. 3a, b, the ECL intensity at the peptide1/GNPs/Au electrode was 1.5–1.9 times higher than that obtained at a peptide1/Au electrodes at same condition. The packing density of the peptide1 on the different electrodes surface is estimated on basis of the electrode surface area and the amount of peptide1. The amount of peptide1 was related with the charge associated with the electrode desorption reaction arising from the one-electron reduction of peptide1 layer on gold surface according Faraday law [36]. The peptide densities at the bare electrode and gold nanoparticles-modified electrode were 3.58×10^{-10} and 11.2×10^{-10} molecules. cm^{-2} , respectively, corresponding the effective electrode area of 0.037 mm^2 for bare gold electrode and 0.05 mm^2 for gold nanoparticles-modified gold electrode (as seen in supporting information Figure S2). The packing density of the peptide1 on gold nanoparticles-modified electrode was 3.1-folds larger than that of the bare gold electrode. In summer, gold nanoparticles not only facilitate the electron transfer at the electrode interface and catalyze the ECL process of ruthenium complex/TPA system [25, 37], but also increase the interface area of the electrode to capture more molecular recognition element for recognition of targets, and then immobilize numerous signal-generating molecules. The signal enhancement of the gold nanoparticles for the ECL peptide-based biosensor designed is evident.

Optimization of conditions

The applied potential is an important parameter because it decides the sensitivity of the ECL peptide-based biosensors. The dependence of the ECL intensity of the ECL peptide-based biosensor on applied potential was checked for 1.0×10^{-10} g/mL TnI. Figure 4a shows that the ECL intensity increases when the applied potential is raised from +0.8 to +0.9 V and reaches a maximum at +0.9 V. Therefore, the constant potential of +0.9 V was chosen in following experiments.

Figure 4b shows the effect of binding time between the peptide1 and TnI on the ECL intensity. The ECL intensity sharply increases with increasing binding time from 20 to 60 min and reaches a maximum at about 60 min. When further

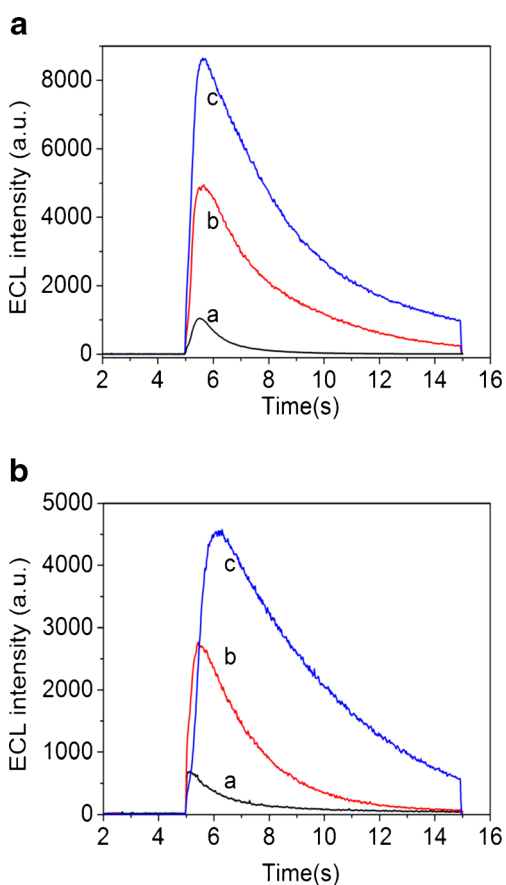


Fig. 3 ECL intensity-potential profiles of the ECL peptide-based biosensor fabricated on GNPs-modified gold electrode (**a**) and the ECL peptide-based biosensor fabricated on bare gold electrode (**b**) in 0.10 M PBS (pH 7.4) containing 50 mM TPA at a constant potential of 0.90 V, before (*a*) and after reaction with 1.0×10^{-11} g/mL TnI (*b*) and 1.0×10^{-10} g/mL TnI (*c*), respectively

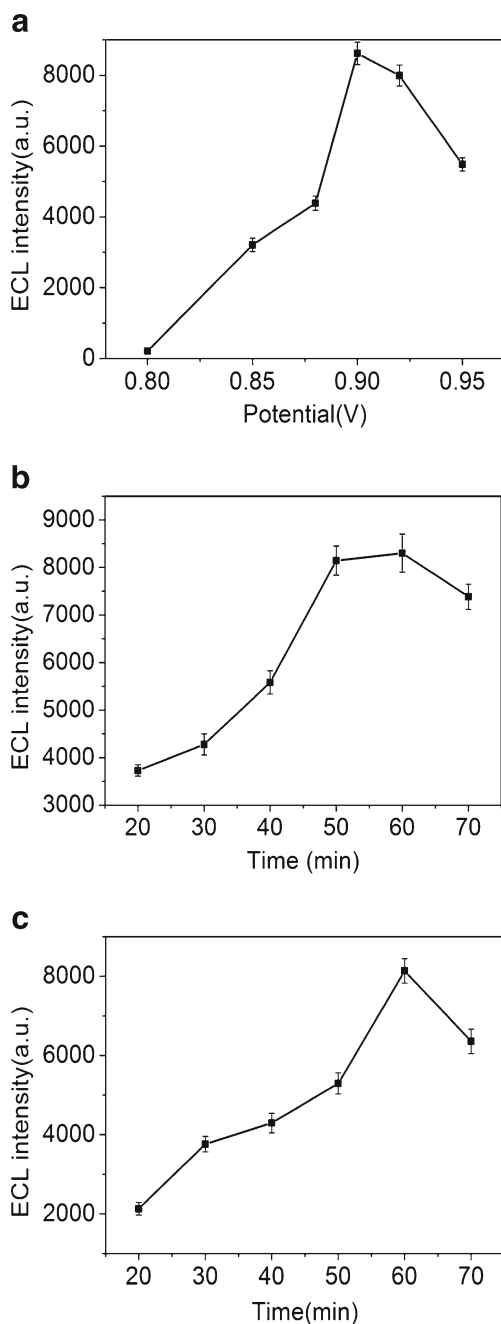


Fig. 4 **a** Effect of applied potential on the ECL intensity; **b** effect of binding time between peptide1 and 1.0×10^{-10} g/mL TnI on the ECL intensity. **c** Effect of binding time between 1.0×10^{-10} g/mL TnI and Ru-peptide2 on the ECL intensity. In 0.10 M PBS (pH 7.4) containing 50 mM TPA

increasing the binding time, the ECL intensity decreases slightly. This is attributed to steric and electrostatic hindrance arising from the more tightly packed TnI monolayer. Figure 4c demonstrates the effect of the binding time between TnI and the Ru-peptide2 on the ECL intensity. The results showed that the ECL intensity sharply increased with increasing binding time from 20 to 60 min, and then, reached a maximum at 60 min. Considering both of the binding time between

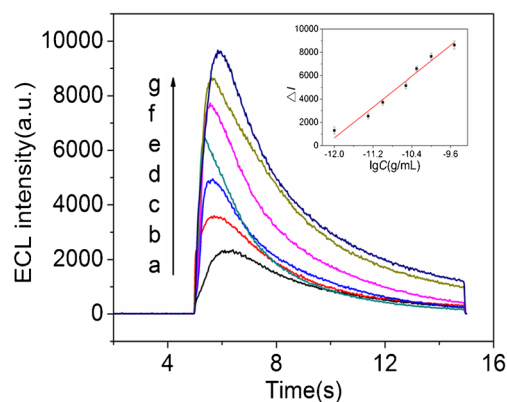


Fig. 5 ECL intensity vs time profiles for different concentrations of TnI in 0.10 M PBS (pH=7.4) containing 50 mM TPA: **a** 1.0×10^{-12} g/mL, **b** 5.0×10^{-12} g/mL, **c** 1.0×10^{-11} g/mL, **d** 3.0×10^{-11} g/mL, **e** 5.0×10^{-11} g/mL, **f** 1.0×10^{-10} g/mL, **g** 3.0×10^{-10} g/mL. *Insert*, calibration curve of TnI. Experimental conditions, applied potential, 0.90 V; binding time, 60 min; 0.10 M PBS (pH 7.4) containing 50 mM TPA

peptide1 and TnI with the binding time of Ru-peptide2 and TnI, we chosen 60 min as binding time for both of incubation process in following experiments.

Analytical performance of ECL peptide-based biosensor

The quantitative behavior of the ECL peptide-based biosensor fabricated was assessed under the optimized conditions. Figure 5 shows the ECL intensity vs time profiles of the ECL peptide-based biosensors for the determination of TnI. The ECL intensity increases with an increase of the concentration of TnI. The increased ECL intensity has a linear relationship with the logarithm of the concentration of TnI in the range from 1.0×10^{-12} to 3.0×10^{-10} g/mL. The linear regression equation is $\Delta I = 3,303 \lg C + 40,305$ (unit of C is in gram per milliliter) and the correlation coefficient was 0.9662. The relative standard derivation for 1.0×10^{-11} g/mL TnI was 2.8 %. The detection limit (DL) is 0.4 pg/mL, which is 300-fold lower than that obtained by homogenous ECL method using Ru-labeled peptide [14] and 3-fold lower than that obtained by homogenous ECL method using Ru-

Table 1 Analytical results of TnI in clinical serum samples

Sample number	CL method (ng/mL) ^a	This method ($n=3$, $P=0.9$, ng/mL) ^b	Relative error (%)
1	5.09	4.85 ± 0.70	-4.7
2	2.94	2.95 ± 0.06	0.4
3	1.02	1.01 ± 0.01	-1.0

^a The CL results of TnI in serum samples from clinical reports provided by Xianyang Central Hospital.

^b Confidence interval

encapsulated liposomes labeled peptide as the ECL probe in our previous report [15]. The employment of gold nanoparticles as amplification platform and peptide as molecular recognition element to improve the sensitivity is evident.

The evaluation of the selectivity of the ECL peptide-based biosensor was performed by examining 1.0×10^{-10} g/mL (8.3×10^{-11} M) TnI or 1.5×10^{-7} M other proteins including AFP, PSA, albumin chicken egg protein and IgG, respectively. A significant increase in ECL intensity (72.5 %) by the interaction with TnI was observed (as shown in Fig. S3). On the other hand, very slight increases in ECL intensity were found for the other tested proteins including AFP (7.2 %), PSA (6.3 %), albumin chicken egg protein (8.4 %), and IgG (7.9 %), respectively. This indicates that the developed strategy has good selectivity for TnI.

Sample analysis

To evaluate a potential application of the ECL peptide-based biosensor, serum samples obtained from Xianyang Central Hospital were assayed using the proposed method in this work. The results are presented in Table 1. The obtained results show an acceptable agreement with the data provided by Xianyang Central Hospital (China) using a standard chemiluminescence (CL) method with an Abbott Immunoanalyzer (Abbott AxSYM, i1000, USA), no statistical significance (P value=0.9) is obtained between the result using the ECL method in this work and that CL method, therefore, signifying the feasibility of the ECL method in clinical sample.

Conclusion

In this work, we fabricated a high sensitive electrogenerated chemiluminescence peptide-based biosensor for the detection of TnI. Great signal amplification was achieved with an extremely low detection limit of 0.4 pg/mL attributed to the combination of gold nanoparticles as amplification platform and peptide as molecular recognition element. The strategy presented in this study could be easily extended to the detection of other biomarkers.

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