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Ultrasensitive electrogenerated chemiluminescent DNA-based biosensing switch for the determination of bleomycin

Yan Li [a,*], Cancan Huang [a], Jianbin Zheng [a,*], Honglan Qi [b]

[a] Institute of Analytical Science, Northwest University, Shaanxi Provincial Key Laboratory of Electroanalytical Chemistry, Xi'an, Shaanxi 710069, China
[b] Key Laboratory of Analytical Chemistry for Life Science of Shaanxi Province, School of Chemistry and Materials Science, Shaanxi Normal University, Xi'an 710062, China

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ABSTRACT

An ultrasensitive electrogenerated chemiluminescent (ECL) DNA-based biosensing switch for the determination of bleomycin (BLM) was developed based on Fe(II) · BLM-mediated hairpin DNA strand cleavage and a structure-switching ECL-dequenching mechanism. A thiolated ss-DNA was used as a substrate for BLMs: one terminus was tethered onto an electrode surface, and the other terminus was labelled with the ECL quencher ferrocene to form a hairpin structure. This thiolated ss-DNA self-assembled on to the tris(2,2′-bipyridine)ruthenium-gold nanoparticle composite modified gold electrode. In the presence of Fe(II) · BLM, the ECL DNA biosensing switch undergoes an irreversible cleavage event that can trigger a significant increase in ECL intensity. The relationship of ECL intensity and the concentration of BLMs was found to be linear in the range of 5 fM – 5000 fM with a detection limit of 2 fM. This work demonstrates that the design of a highly sensitive ECL DNA-based biosensing switch that uses the sequence selectivity of DNA cleavage mediated by the antitumor drug BLM in combination with a chemical quencher, such as ferrocene, to quench ECL signal(s), offers a promising approach for the determination of ultratrace amounts of antitumor drugs.

1. Introduction

Bleomycin (BLM), one of the most potent naturally occurring antitumor drugs, has been used as a chemotherapeutic agent for the clinical treatment of certain cancers, including Hodgkin's disease, non-Hodgkin lymphoma, testicular cancer, and malignant pleural effusions [1–4]. The antitumor activity of BLMs is generally believed to be due to its chemotherapeutic effect, which is mediated by the selective cleavage of either single-stranded (ss) or double-stranded (ds) DNA [5–7] and possibly RNA, as well. The BLM-mediated DNA cleavage mechanism occurs via oxidation of the deoxyribose moiety on DNA. In the presence of Fe(II) and oxygen, BLMs form the binary complex Fe(II) · BLM. When Fe(II) is oxidised to Fe(III), oxygen is reduced to free radicals, which may cause DNA cleavage that can ultimately lead to cell death. Among the various antitumor drugs, BLM is of particular importance due to its advantageously low level of myelosuppression and low level of immunosuppression [8]. However, a dose-limiting side effect of BLM is its renal and lung toxicity, which can cause ruinous pulmonary fibrosis [9]. To achieve the best therapeutic effect and to minimise the toxicity of BLM, considerable effort has been devoted to the development of reliable and sensitive methods for BLM quantification, including high-performance liquid chromatography (HPLC) [10–12], radioimmunoassays (RIAs) [13,14], enzyme immunoassays (EIAs) [15], microbiological assays [16,17], and fluorescence resonance energy transfer (FRET) assays [18]. However, these methods suffer from such drawbacks as the requirement of complicated instruments for HPLC, a hazard to the laboratory technician and to the high expensive of having a rad program and disposing of waste by RIAs, the high cost for EIAs and the tedious experimental procedures required by FRET. Recently, by establishing a simple colorimetric platform with gold nanoparticles as the sensing element, Li et al. achieved a detection limit of 2 nM BLM [19]. With increasingly in-depth investigation(s) regarding the interaction(s) between BLMs and DNA, DNA has, as a result, become a powerful tool for BLM sensing. Yin et al. reported a BLM electrochemical detection assay based on BLM-induced DNA strand scission and they estimated a detection limit of 100 pM BLM [20]. BLM is employed clinically at atypically low dose (~5 μM). To achieve the best therapeutic effect and to minimise the toxicity of BLM, the development of a highly sensitive method for BLM detection is greatly required.

Electrogenerated chemiluminescence (ECL) offers many advantages, such as simplicity, high sensitivity, rapidity and easy controllability. ECL has also been proven to be useful in immunosensing, DNA hybridisation assays and enzymatic biosensors [21–23]. It has been reported that ferrocene (Fc) can efficiently and stably quench the ECL of tris(2,2′-bipyridyl)-ruthenium(II)

* Corresponding authors. Tel.: +86 29 88302077; fax: +86 29 88303448.
E-mail address: yanli@nwu.edu.cn (Y. Li).

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($Ru(bpy)_3^{2+}$) at the electrode [24]. Fc shows more efficient quenching of ECL compared with the known quenchers such as phenol and 1, 1-dimethyl-4, 4′-bipyridine dication. Based on this mechanism, several highly sensitive ECL biosensors have been developed [25–28]. For example, our group reported an ECL aptamer-based biosensing method for high sensitivity detection of thrombin employing an aptamer as a molecular recognition element and quenching electrogenerated chemiluminescence of $Ru(bpy)_3^{2+}$ derivative by Fc [25]. The detection limit can be much improved if nanomaterial loaded with $Ru(bpy)_3^{2+}$ derivative tags is used as an ECL label. In recent years, tris(2,2′-bipyridyl)ruthenium(II) ($Ru(bpy)_3^{2+}$)–gold nanoparticles (Ru–GNPs) have been developed as novel biocompatible ECL tag materials, due to their excellent ECL behaviour, chemical stability, easy surface modification and biocompatibility [29–31]. Wang et al. immobilized the complex of $Ru(bpy)_3^{2+}$ and GNPs on the gold electrode surface to form Ru–GNPs ECL film and modified the Fc-labelled molecular beacon on the Ru–GNPs ECL film. The ECL intensity is correlated to the distance which is controlled by the conformation of the Fc-labeled DNA molecular beacon between the Fc and $Ru(bpy)_3^{2+}$ immobilized on the electrode [30]. However, to the best of our knowledge, an ECL DNA-based biosensing switch for the determination of BLMs based on Fe(II) ·BLM-mediated hairpin DNA strand cleavage, and a structure-switching ECL-dequenching mechanism has not been reported in the literature.

The aim of the work presented in this report was to develop a highly sensitive ECL DNA-based biosensing switch for the determination of BLM based on the Fe(II) ·BLMs-mediated DNA strand scission and the dequenching of the ECL of Ru–GNPs by ferrocene. In this paper, the characteristics and the analytical performance of an ECL DNA-based biosensing switch for the determination of BLM were investigated.

2. Experimental

2.1. Chemicals and reagents

Ruthenium(III) chloride hydrate, *N*-hydroxysuccinimide (NHS), $HAuCl_4 \cdot 4H_2O$ (99% w/w), cysteamine ($SH–(CH_2)_2–NH_2$), ferrocene carboxylic acid, 6-mercapto-1-hexanol (MCH) and 1-ethyl-3-[(3-dimethylamino)propyl]carbodiimide (EDC) were purchased from Sigma–Aldrich (USA). Tripropylamine (TPA), 2,2′-bipyridine, and ethylenediamine were obtained from First Reagent Cooperation of Shanghai (Shanghai, China). Bleomycin sulfate (BLM) with an A_2 and B_2 content of up to 95% was obtained from Melone Pharmaceutical Co., Ltd. (Dalian, China). The metal salts ($MgCl_2$, $CaCl_2$, $Cd(NO_3)_2$, $Cu(NO_3)_2$, $Ba(NO_3)_2$, and $FeCl_2 \cdot 4H_2O$), as well as all other reagents, were of analytical grade, purchased from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). Millipore Milli-Q water (18 MΩ cm) was used throughout. A solution of 10 mM phosphate buffered saline (PBS), 0.5 M NaCl, and 0.1 M $NaClO_4$ (pH 8.0) was used as an incubation buffer and washing solution (IW–buffer). The oligonucleotide probe (5′–$SH–(CH_2)_6$–CGCTTTAAAAAAAGCG–$(CH_2)_6–NH_2$-3′) was synthesised and subsequently purified via HPLC by Shenggong Bioengineering Co. Ltd. (Shanghai, China) for the preparation of the ECL DNA-based biosensing switch. The ferrocene acetic acid-labelled oligonucleotide hairpin probes (Fop) were synthesised according to reported literature methods [32].

2.2. Apparatus

ECL measurements were performed using an MPI-A ECL detector (Xi'an Remax Electronics, Xi'an, China). A commercial cylindroid glass cell, which accommodated a conventional three-electrode system consisting of either a biosensing switch or a gold electrode (ϕ=2.0 mm) as the working electrode, a platinum plate as the counter electrode, and an Ag/AgCl (saturated KCl solution) as the reference electrode. ECL emissions were detected with a photomultiplier tube (PMT), which was biased at −900 V, unless otherwise stated.

2.3. Fabrication of an ECL biosensing switch

GNPs were prepared by the reduction of $HAuCl_4$ (1.0×10^{-2}% w/v) with trisodium citrate (1%) according to Grabar et al. [33] and stored at 4 °C. The $Ru(bpy)_3^{2+}$–GNP (Ru–GNP) composites were prepared according to a previously reported method [29,30].

A gold electrode was pre-treated as previously described [34]. The cleaned electrode was thoroughly rinsed with Milli-Q water and immersed in a 0.10 M cysteamine aqueous solution for 2 h at room temperature to form the cysteamine monolayer. Next, the electrode was thoroughly rinsed with Milli-Q water to remove the physically adsorbed cysteamine. A 2 μL aliquot of the suspension of Ru–GNP composite was placed on the cysteamine-derived gold electrode surface [29,30]. The as-prepared electrode was then air-dried at room temperature. After rinsing thoroughly with Milli-Q water, the composite of Ru–GNPs was immobilised on the gold electrode surface.

A 2 μL aliquot of 10 μM Fop was applied to the surface of the Ru–GNPs modified gold electrode and allowed to self-assemble for 12 h at room temperature to form the ECL biosensing switch. The surface density of Fop immobilized was determined quantitatively by the electrochemical method according to Steel's method [35]. The surface density of Fop immobilized on the Ru–GNPs modified gold electrode was estimated to be ca. 1.84×10^{12} molecules/cm^2. The ECL biosensing switch was thoroughly rinsed with 0.10 M PBS (0.10 M NaCl+5 mM NaH_2PO_4+5 mM Na_2HPO_4, pH 7.40) to remove unbound Fop on the surface of the modified electrode, before being immersed in 1 mM MCH for 2 h to block any uncovered surfaces on the electrode. Finally, it was thoroughly rinsed with Milli-Q water. The electrode was sealed to avoid evaporation during the assembly procedure.

2.4. ECL measurement

The fabricated ECL DNA-based biosensing switch was incubated in a single-compartment cell filled with 8 mL of IW–buffer for 1 h to allow the formation of a hairpin structure [20]. The BLM samples were prepared by mixing BLMs with Fe(II) ion in 1:1 M ratio [20]. The ECL DNA-based biosensing switch was immersed in 500 μL of a fixed concentration of Fe(II)·BLM for 30 min followed by thorough washing with IW-buffer to remove any un-reacted Fe(II)·BLM. The biosensing switch was later transferred to an ECL cell. Multiple cyclic voltammetry (CV) of 0.10 M TPA in 0.10 M PBS was conducted between 0 and +1.20 V at 0.1 V/s to achieve an ECL signal. The ECL and CV curves were recorded simultaneously.

3. Results and discussion

3.1. Design and characterisation of ECL DNA-based biosensing switch

A schematic diagram of the ECL DNA-based biosensing switch for the determination of BLM is shown in Fig. 1. Fig. 1 displays the procedure of fabricating the DNA-based biosensing switch for BLM assays and its working principle. The biosensor contains a reported 16-nucleotide hairpin probe [18,36,37] with a thiol group at the 5′ end, which acts as an anchor on the Ru–GNP

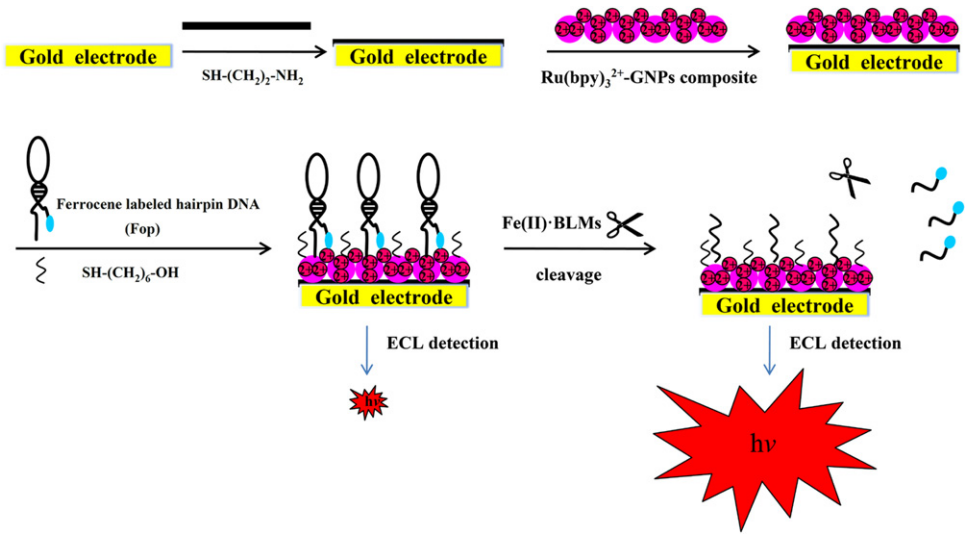

Fig. 1. Schematic diagram of the ECL–DNA biosensing method for the detection of BLM using the quenching of the ECL of tris(2,2′-bipyridine)ruthenium(II) by ferrocene.

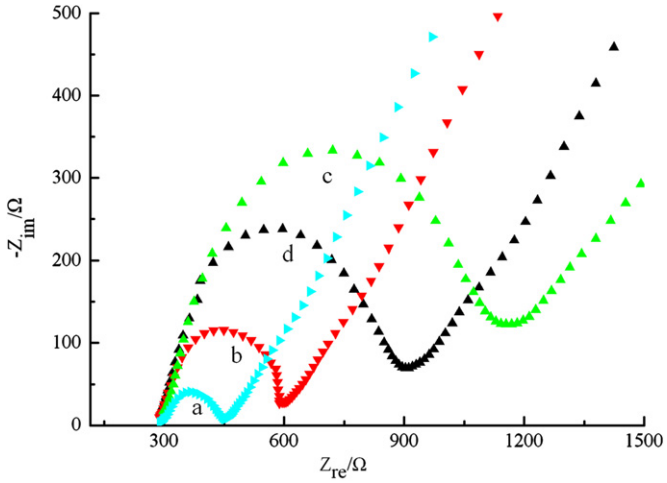

Fig. 2. Nyquist plots obtained in 0.10 M PBS containing 1 mM $Fe(CN)_6^{3-}$/$Fe(CN)_6^{4-}$ and 0.1 M KCl at a bare gold electrode (a), Ru–GNPs electrode (b), Fop-Ru–GNPs electrode (c), and the Fop-Ru–GNPs modified gold electrode in the presence of 100 fM Fe(II) · BLM (d). The biased potential was 0.207 V. The frequency was from 0.1 Hz to 100 kHz, and the amplitude was 5.0 mV.

modified gold electrode, and a ferrocenyl moiety at the 3′ end, which acts as an ECL quencher. This probe can self-assemble on the gold electrode via a thiol-gold linkage and self-hybridise into a hairpin structure with a 6-base-pair (bp) stem and 4- nucleotide (nt) loop with Fc in close proximity to the Ru–GNPs modified gold electrode surface [30]. In the absence of Fe(II) · BLM, a notably weak ECL signal was observed due to the high ECL quenching efficiency of Fc on $Ru(bpy)_3^{2+}$. It is reported that BLMs binding with Fe(II) ions can exhibit sequence DNA strand scission, predominantly at 5′-GC/T-3′ site sequences [18]. When treated with BLMs in the presence of Fe(II) and oxygen, the probe was found to undergo an oxidative transformation, which causes the Fc tag to be selectively cut off and released. These cleaved fragments dissociate into the bulk solution, allowing the ferrocenyl moiety to move away from the gold electrode surface and coincides with the generation of a strong ECL signal.

The processes of preparing the ECL DNA-based biosensing switch and the interaction(s) between the fabricated biosensing switch and BLMs were characterised by electrochemical impedance spectroscopy (EIS). Fig. 2 displays the Nyquist plots of 1 mM $Fe(CN)_6^{3-}$ and 1 mM $Fe(CN)_6^{4-}$ at the biosensing switch in 0.1 M KCl during different stages of the fabrication process. The spectrum for the bare gold electrode exhibited a notably small semicircle at high frequencies (line a) and its electron-transfer resistance (R_{et}) is 148 Ω, indicating a fast electron-transfer process of $[Fe(CN)_6]^{3-/4-}$. In contrast to the bare gold electrode, the spectrum for the Ru–GNP electrode displayed an increased electron-transfer resistance of 318 Ω (line b). When the Fop had self-assembled on the Ru–GNP modified gold electrode, the R_{et} increased to 850 Ω (line c). This increase was attributed to the electrostatic repulsive force between the negatively charged DNA probe monolayer and $[Fe(CN)_6]^{3-/4-}$ [38], which indicated that the Fop was immobilised on the Ru–GNP modified gold electrode. After the Fop-Ru–GNP electrode was incubated in Fe(II) · BLM, the R_{et} greatly decreased to 590 Ω (line d). This may be attributed to the destruction of the stem-loop structure, which releases the Fc tag and allows it to move away from the electrode. The negative charges present on the gold electrode surface decreased, resulting in a decreased Rct. This result indicates that the interaction between the ECL DNA-based biosensing switch and the Fe(II) · BLM complex had occurred.

The fabricated ECL DNA-based biosensing switch was also characterised by ECL. The ECL intensity-potential profiles of the biosensing switches are presented in Fig. 3. As shown in Fig. 3, no ECL signal was measured at the bare gold electrode (line a), whereas immobilisation of Ru–GNPs onto the gold electrode resulted in a strong ECL signal (line b). Comparison of line c with line b reveals that the ECL DNA-based biosensing switch displayed a lower signal (ECL intensity decreased from 1112 to 120), and the ECL intensity was quenched by 98%. This finding is consistent with that of previous work in that the efficiency [24]. Comparison of line c with line d reveals that the ECL DNA-based biosensing switch with an interaction of 5 fM Fe(II) · BLM had a higher signal (ECL intensity increased from 120 to 217). This result indicates that the fabricated ECL DNA-based biosensing switch can respond to target Fe(II) · BLM. When comparing line d with line e, it can be clearly observed that the ECL intensity increases from 217 to 568, due to an elevated Fe(II) · BLM concentration. Thus, the ECL DNA-based biosensing switch may be used to detect target Fe(II) · BLM. A control experiment investigating the effect of only Fe(II) ions or BLMs in the system was performed. The results showed that the introduction of Fe(II) had only a slight effect on the ECL intensity (ECL intensity increased from 120 to 128), even at a high concentration of

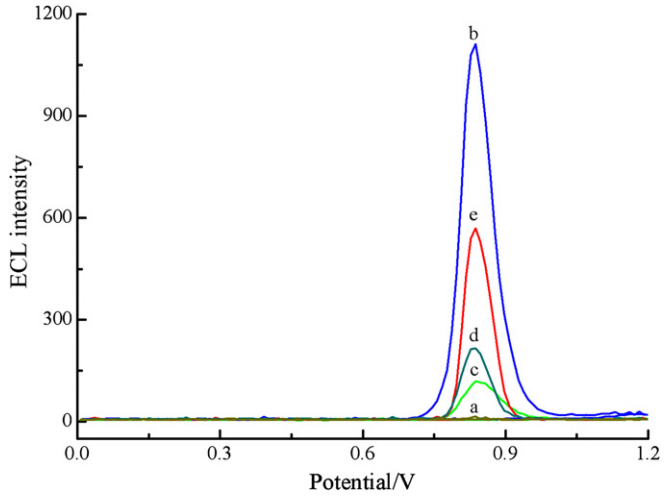

Fig. 3. ECL intensity *vs.* potential profiles for line a: at the bare gold electrode, line b: at Ru–GNPs modified gold electrode, line c: at ECL DNA-based biosensing switch without interaction of Fe(II) ·BLM, line d: at ECL DNA-based biosensing switch with interaction of Fe(II) ·BLM 5 fM, line e: at ECL DNA-based biosensing switch with interaction of Fe(II) ·BLM 500 fM. ECL measurements were performed in 0.10 M PBS containing 0.10 M TPA. Scan rate: 0.1 Vs, Scan range: 0–1.20 V.

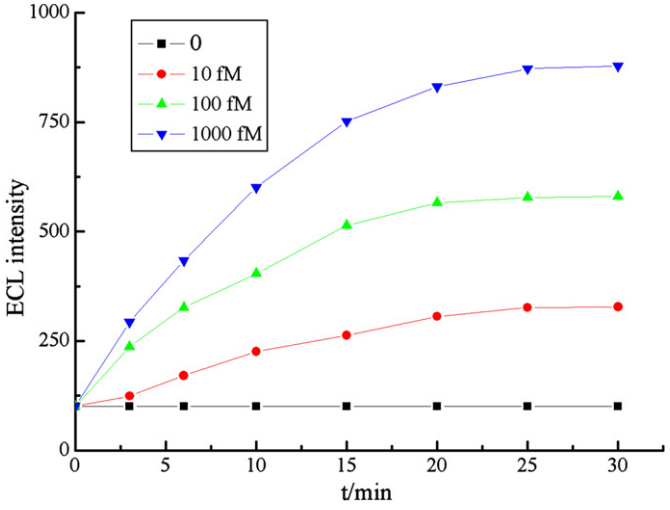

Fig. 4. Dependence of the ECL intensity of the ECL DNA-based biosensing switch on incubation time. The ECL measurement conditions are the same as those in Fig. 3.

10 pM. The ECL intensity of the fabricated biosensing switch was measured after it was incubated in a series of solutions containing only BLM at various concentrations, no significant change in the ECL signal was observed (ECL intensity increased from 120 to 124).

3.2. Incubation time of ECL DNA-based biosensing switch with Fe(II) ·BLM

The incubation time of the ECL DNA-based biosensing switch with solutions containing different concentrations of Fe(II) ·BLM was optimised. Fig. 4 shows the dependence of the ECL intensity on incubation time for three concentrations of Fe(II) ·BLM. From Fig. 4, it can be observed that the ECL intensity increases with increased incubation time, eventually reaching a plateau. Both the slope of the ECL intensity *vs.* the incubation time plot and the time required to reach the maximum ECL intensity were found to be different for the solutions of Fe(II) ·BLM at three different concentration (reach the maximum ECL intensity 30 min for 10 fM Fe(II) ·BLM, 25 min for 100 fM Fe(II) ·BLM and 20 min for 1000 fM Fe(II) ·BLM). It was found that a lower concentration of Fe(II) ·BLM resulted in a lower slope and a longer time required to reach the maximum ECL intensity. It was found that the ECL intensity was able to reach its maximum within 30 min when using an Fe(II) ·BLM testing solution with a concentration as low as 10 fM. Therefore, an Fe(II) ·BLM incubation time of 30 min was chosen. This time is the same as the incubation time reported in the "signal off" by the electrochemical biosensors for Fe(II) ·BLM detection [20].

3.3. Effect of different metal ions on the ECL DNA-based biosensing switch

It is well-known that the BLMs require a reduced transition metal, such as Fe(II) and oxygen, to catalyse DNA lesions that are responsible for tumour necrosis [5]. The effect(s) of different metal ion coordination environments for BLM-mediated DNA cleavage was examined. The BLM samples were prepared by mixing 0.1 pM BLMs with various metal ions in a 1:1 M ratio. The ECL intensity of the fabricated biosensing switch was measured after it had interacted with a mixture of metal ions (Fe^{2+}, Cu^{2+}, Cd^{2+}, Ba^{2+}, Mg^{2+}, or Ca^{2+}) and BLMs. As shown in Fig. 5, only the combination of Fe(II) ·BLM gave any significant ECL intensity, whereas the five other metal ions gave only slight emissions equivalent to that of the blank. These results indicate that the activated Fe(II) ·BLM is able to mediate oxidative signal probe destruction (which irreversibly cleaves the probe at the stem structure to release the Fc tag, resulting in a significant increase in ECL intensity).

3.4. Performance of ECL DNA-based biosensing switch

3.4.1. Linear range and detection limit

The introduction of Fe(II) ·BLM at different concentrations to the ECL DNA-based biosensing switch resulted in different increases in ECL intensity, which corresponded to the amount of released Fc. Fig. 6 shows the ECL profiles of the biosensing switch after interaction with different concentrations of Fe(II) ·BLM under optimised conditions. Fig. 6A shows that the ECL intensity increases with increasing concentration of Fe(II) ·BLM. The ECL

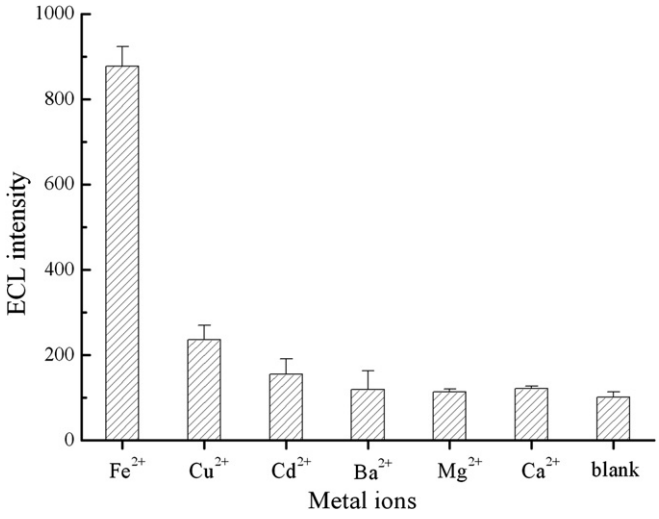

Fig. 5. Effect of different metal ions on the ECL DNA-based biosensing switch. The error bars represent the standard deviation of three independent measurements at each metal ion with equal concentration of the 100 fM BLMs. The ECL conditions are the same as those in Fig. 3.

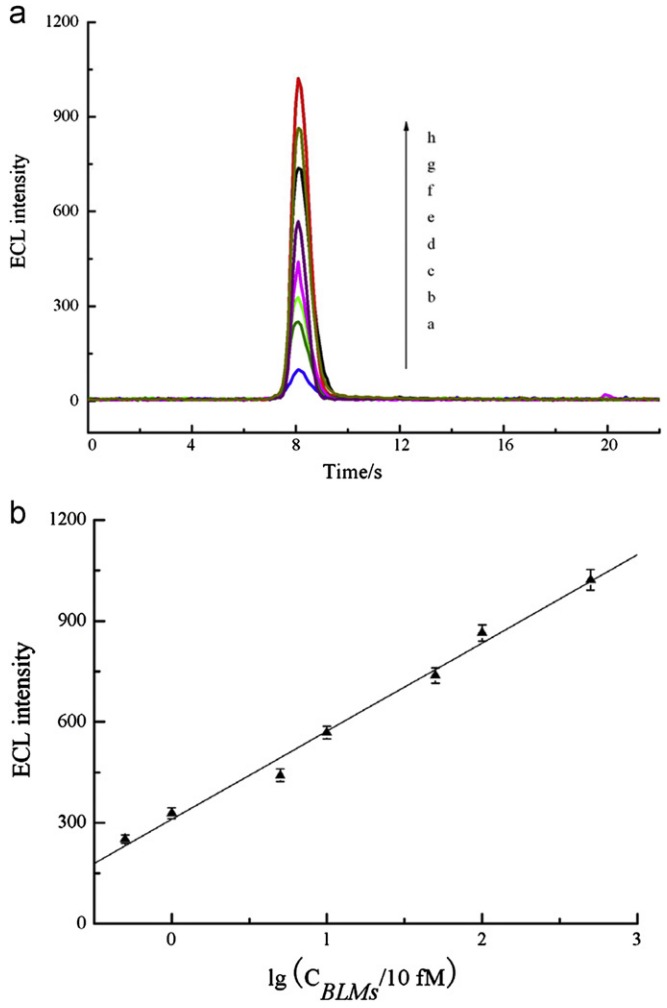

Fig. 6. (A) The ECL profiles of the different concentration of BLMs interaction with ECL DNA-based biosensing switch. The concentration of BLMs: (a) 0, (b) 5 fM, (c) 10 fM, (d) 50 fM, (e) 100 fM, (f) 500 fM, (g) 1000 fM, (h) 5000 fM. (B) Linear relationship between the ECL intensity and the logarithm of the BLMs concentration. The error bars represent the standard deviation of three independent measurements of (A). The ECL conditions are the same as those in Fig. 3.

intensities were logarithmically related to concentrations of Fe(II) · BLM between 5 fM and 5000 fM. The calibration equation was $I=262.0\ \lg C+310.2$ (unit of C is 10 fM) with a correlation coefficient of 0.9952 (Fig. 6B). The detection limit based on mathematical evaluation was 2 fM (S/N=3) though experimental validation of this value was not performed. The relative standard deviation for 0.1 pM Fe(II) · BLM was 5.2% ($n=7$). The detection limit was several orders of magnitude lower than those of HPLC (20 ng/mL of BLMA2) [12], RIA (8 ng/mL of BLM sulfate) [14], bioassay (100 ng/mL of BLM) [17], and fluorescence quenching (30 ng/mL of BLMA5 and 40 ng/mL of BLMA2) [39]. Thus, ECL DNA-based biosensing switches with a high sensitivity and good reproducibility were developed.

3.4.2. *Selectivity of the ECL DNA-based biosensing switch*

The selectivity of the ECL DNA-based biosensing switch was evaluated by the detection of three antitumor drugs (daunorubicin, mitomycin and dactinomycin) at the biosensing switch. The ECL intensity of the proposed ECL-DNA biosensing switch was examined after immersing the ECL–DNA biosensing switch in either 100 fM BLM, 100 fM daunorubicin, mitomycin or dactinomycin according to the protocol described in 2.3 *ECL measurement*.

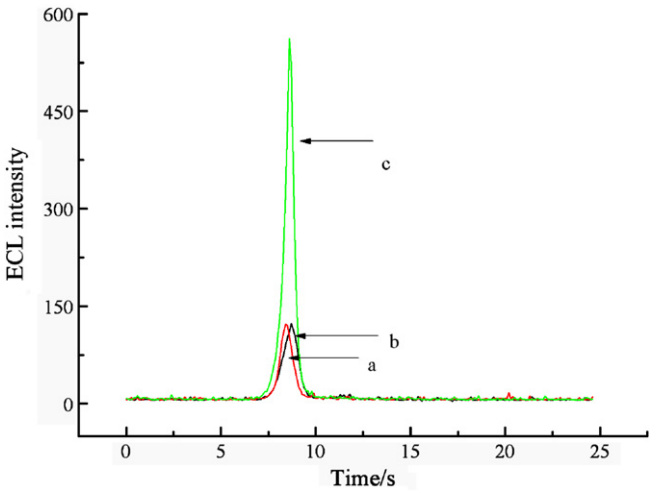

Fig. 7. ECL responses of the ECL DNA-based biosensing switch in a complex sample matrix of 50% serum. (a) Blank, (b) 50% serum, and (c) 50% serum with 100 fM Fe(II) · BLM. The ECL conditions are the same as those in Fig. 3.

The results showed a significant increase in ECL intensity (ECL intensity increased from 108 to 565) induced by the binding of the hairpin DNA probe and Fe(II) · BLM, compared to the other three antitumor drugs. This finding indicates that the ECL–DNA biosensing method has good selectivity for the discrimination of BLM from other antitumor drugs.

3.4.3. *Serum sample detection*

Fig. 7 shows the potential applicability of the ECL DNA-based biosensing switch in a complex sample matrix of 50% serum diluted 1:1 with IW–buffer. The biosensing switch fabricated was immersed for 30 min in either 500 μL of 50% serum or in 500 μL of 50% serum containing 100 fM Fe(II) · BLM. Fig. 7 shows that the ECL intensity obtained in the 50% serum was close to that obtained in the blank solution. The ECL intensity increased from 111 to 562 in the 50% serum containing 100 fM Fe(II) · BLM (a similar change in ECL intensity from 101 to 568 was observed in 100 fM Fe(II) · BLM, as shown in Fig. 6). The relative standard deviation for the 50% serum containing 0.1 pM Fe(II) · BLM was 5.8% ($n=7$). These results suggest that the proposed biosensing switch for BLM determination may be used for real-life sample analysis.

4. Conclusions

A ECL DNA-based biosensing switch for the highly sensitive detection of an antitumor drug, bleomycin, has been developed, which employs a hairpin DNA as a molecular reorganisation element and ferrocene for quenching the ECL of tris(2,2′-bipyridine)ruthenium. This work demonstrates that the sequence selectivity of DNA cleavage mediated by Fe(II) · BLM in combination with a highly sensitive ECL technique can be used to quantify BLMs. The ECL DNA-based biosensing switch designed in this study offers several advantages, including a fast response, high selectivity and efficient sensitivity due to nanomaterials loaded with tris(2,2′-bipyridine)ruthenium. Moreover, the proposed biosensing switch provides a promising strategy for the determination of ultratrace antitumor drugs in clinical samples.

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References

[1] H. Umezawa, in: J.M. Cassady, J.D. Douros (Eds.), Anticancer agents based on natural product models, Academic Press, New York, 1980, pp. 147–166.
[2] S.M. Hecht, in: W.O. Foye (Ed.), Cancer chemotherapeutic agents, American Chemical Society, Washington, DC, 1995, pp. 369–388.
[3] J.S. Lazo, B.A. Chabner, B.A. Chabner, D.L. Longo, Cancer Chemotherapy and Biotherapy; Principles and Practice, Lippincott–Raven Publishers, Philadelphia, 1996, pp. 379–394.
[4] U. Galm, M.H. Hager, S.G. Van Lanen, J. Ju, J.S. Thorson, B. Shen, Chem. Rev. 105 (2005) 739.
[5] C.A. Claussen, E.C. Long, Chem. Rev. 99 (1999) 2797.
[6] R.J. Steighner, L.F. Povirk, Proc. Natl. Acad. Sci. U.S.A 87 (1990) 8350.
[7] L.F. Povirk, Y.H. Han, R.J. Steighner, Biochemistry 28 (1989) 5808.
[8] G. Tisman, V. Herbert, L.T. Go, L. Brenner, Blood 41 (1973) 721.
[9] S. Sleijfer, Chest 120 (2) (2001) 617.
[10] R.P. Klett, J.P. Chovan, I.H. Danse, J. Chromatogr. 310 (1984) 361.
[11] Aszalos, J. Crawford, P. Vollmer, N. Kantor, T. Alexander, J. Pharm. Sci. 70 (1981) 878.
[12] G.K. Shiu, T.J. Goehl, W.H. Pitlick, J. Pharm. Sci. 68 (1979) 232.
[13] S.T. Crooke, F. Luft, A. Broughton, J. Strong, K. Casson, L. Einhorn, Cancer 39 (1977) 1430.
[14] J.D. Teale, J.M. Clough, V. Marks, Br. J. Cancer 35 (1977) 822.
[15] K. Fujiwara, M. Isobe, H. Saikusa, H. Nakamura, T. Kitagawa, S. Takahashi, Cancer Treat. Rep. 67 (1983) 363.
[16] T. Onuma, J.F. Holland, H. Masuda, J.A. Waligunda, G.A. Goldberg, Cancer 33 (1974) 1230.
[17] R.F. Pittillo, C. Woolley, L.S. Rice, Appl. Environ. Microbiol. 22 (1971) 564.
[18] Q. Ma, Y. Akiyama, Z. Xu, K. Konishi, S.M. Hecht, J. Am. Chem. Soc. 131 (2009) 2013.
[19] F. Li, Y. Feng, C. Zhao, B. Tang, Biosens. Bioelectron. 26 (2011) 4628.
[20] B.C. Yin, D. Wu, B.C. Ye, Anal. Chem. 82 (2010) 8272.
[21] W.J. Miao, Chem. Rev. 108 (2008) 2506.
[22] L. Hu, G. Xu, Chem. Soc. Rev. 39 (8) (2010) 3275.
[23] Y. Li, H. Qi, Q. Gao, C. Zhang, Biosens. Bioelectron. 26 (2011) 2733.
[24] W. Cao, J.P. Ferrance, J. Demas, J.P. Landers, J. Am. Chem. Soc. 128 (23) (2006) 7572.
[25] Y. Li, H.L. Qi, Y.G. Peng, Q. Gao, C.X. Zhang, Electrochem. Commun. 10 (2008) 1322.
[26] X.Y. Wang, P. Dong, W. Yun, Y. Xu, P.G. He, Y.Z. Fang, Biosens. Bioelectron. 24 (2009) 3288.
[27] L. Chen, Q. Cai, F. Luo, X. Chen, X. Zhu, B. Qiu, Z. Lin, G. Chen, Chem. Commun. 46 (41) (2010) 7751.
[28] S.J. Ye, H.X. Li, W. Cao, Biosens. Bioelectron. 26 (2011) 2215.
[29] X.P. Sun, Y. Du, S.J. Dong, E.K. Wang, Anal. Chem. 77 (2005) 8166.
[30] X.Y. Wang, W. Yun, P. Dong, J.M. Zhou, P.G. He, Y.Z. Fang, Langmuir 24 (2008) 2200.
[31] J. Zhang, P.P. Chen, X.Y. Wu, J.H. Chen, L.J. Xu, G.N. Chen, F.F. Fu, Biosens. Bioelectron. 26 (2011) 2645.
[32] S. Takenaka, Y. Uto, H. Kondo, T. Ihara, M. Takagi, Anal. Biochem. 218 (1994) 436.
[33] K.C. Grabar, R.G. Freeman, M.B. Hommer, M.J. Natan, Anal. Chem. 67 (1995) 735.
[34] J. Zhang, H.L. Qi, Y. Li, J. Yang, Q. Gao, C.X. Zhang, Anal. Chem. 80 (2008) 2888.
[35] A.B. Steel, T.M. Herne, M.J. Tarlov, Anal. Chem. 70 (1998) 4670.
[36] S. Hashimoto, B. Wang, S.M. Hecht, J. Am. Chem. Soc. 123 (2001) 7437.
[37] Y. Akiyama, Q. Ma, E. Edgar, A. Laikhter, S.M. Hecht, Org. Lett. 10 (2008) 2127.
[38] E. Radi, J.L.A. Sanchez, E. Baldrich, C.K. O'Sullivan, Anal. Chem. 77 (2005) 6320.
[39] J. Liu, Z. Liu, X. Hu, L. Kong, S. Liu, Luminescence 23 (2008) 1.