Available online at www.sciencedirect.com
ScienceDirect
Bioelectrochemistry 70 (2007) 228–234
Bioelectrochemistry
www.elsevier.com/locate/bioelechem

DNA biosensor based on the electrochemiluminescence of $Ru(bpy)_3^{2+}$ with DNA-binding intercalators

Jeong-Gun Lee [a], Kyusik Yun [a,*], Guei-Sam Lim [a], Sang Eun Lee [a], Suhyeon Kim [a], Je-Kyun Park [b,*]

[a] Bio Electronics Group, LG Electronics Institute of Technology, 16 Woomyeon-dong, Seocho-gu, Seoul 137–724, South Korea
[b] Department of BioSystems, Korea Advanced Institute of Science and Technology (KAIST), 373–1 Guseong-dong, Yuseong-gu, Daejeon 305–701, South Korea

Received 3 March 2006; received in revised form 8 August 2006; accepted 15 September 2006
Available online 23 September 2006

Abstract

This paper reports a novel detection method for DNA hybridization based on the electrochemiluminescence (ECL) of $Ru(bpy)_3^{2+}$ with a DNA-binding intercalator as a reductant of $Ru(bpy)_3^{3+}$. Some ECL-inducible intercalators have been screened in this study using electrochemical methods combined with a chemiluminescent technique. The double-stranded DNA intercalated by doxorubicin, daunorubicin, or 4′,6-diamidino-2-phenylindole (DAPI) shows a good ECL with $Ru(bpy)_3^{2+}$ at +1.19 V (versus Ag/AgCl), while the non-intercalated single-stranded DNA does not. In order to stabilize the self-assembled DNA molecules during ECL reaction, we constructed the ECL DNA biosensor separating the ECL working electrode with an immobilized DNA probe. A gold electrode array on a plastic plate was assembled with a thru-hole array where oligonucleotide probes were immobilized in the side wall of thru-hole array. The fabricated ECL DNA biosensor was used to detect several pathogens using ECL technique. A good specificity of single point mutations for hepatitis disease was obtained by using the DAPI-intercalated $Ru(bpy)_3^{2+}$ ECL.

Keywords: Electrochemiluminescence (ECL); Tris-(2,2′-bipyridyl)ruthenium(II); DNA-binding intercalator; 4′,6-diamidino-2-phenylindole (DAPI); DNA biosensor; DNA hybridization detection

1. Introduction

Electrochemiluminescence (ECL), which combines chemiluminescence and electrochemistry, continues to impact diverse areas ranging from chemical analysis to the molecular-level understanding of biological processes [1–6]. ECL involves the production of a light near an electrode surface by generating species that can undergo highly energetic electron-transfer reactions with solution-phase reagents [7]. In particular, ECL has several advantages such as inherent high sensitivity, selectivity, wide linear range of chemiluminescence and no requirement for expensive excitation sources [8]. There was much interest in ruthenium complexes, for example, tris-(2,2′-bipyridyl)ruthenium(II) ($Ru(bpy)_3^{2+}$), because they exhibit intrinsic ECL characteristics such as excellent chemical stability, favorable electrochemical properties and relatively prolonged excited state [9]. The ECL reaction of $Ru(bpy)_3^{2+}$ has proven to be a powerful analytical tool that can be useful in detecting a wide range of analytes including oxalate, alkylamines, NADH, hydrazine, amino acids, luminol and a variety of pharmaceutical compounds [10–12]. In addition, the ECL reaction of $Ru(bpy)_3^{2+}$ could be facilitated with a variety of reducing or oxidizing co-reactant, such as tripropylamine [7], peroxyldisulfate [13], and organic acids [14].

The intercalator was first used by Lerman in the 1960s to define a molecule containing a planar aromatic structure which inserts itself between the base pairs of double-stranded DNA [15]. Intercalation of small molecules into DNA is a noncovalent interaction that depends on a number of factors such as planarity, aromaticity, and surface extension of the interaction moiety [16]. Bard and coworkers reported a voltammetric study on the

* Corresponding authors. Yun is to be contacted at Tel.: +82 2 526 4588; fax: +82 2 575 1403. Park, Tel.: +82 42 869 4315; fax: +82 42 869 4310.
E-mail addresses: ykyusik@lge.com (K. Yun), jekyun@kaist.ac.kr (J.-K. Park).

1567-5394/$ - see front matter © 2006 Elsevier B.V. All rights reserved.
doi:10.1016/j.bioelechem.2006.09.003

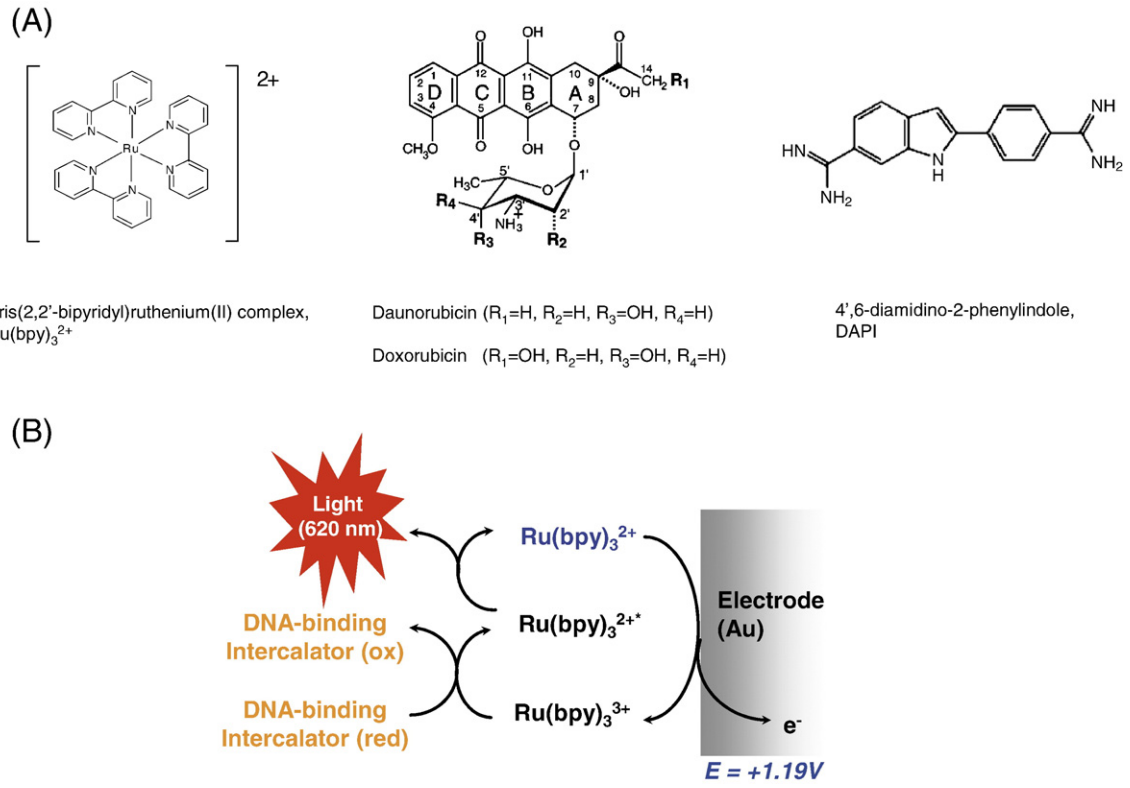

Fig. 1. (A) Structure of the tris-(2,2′-bipyridyl)ruthenium(II) complex, daunorubicin, doxorubicin, and 4′,6-diamidino-2-phenylindole. (B) Proposed ECL mechanism of $Ru(bpy)_3^{2+}$ with a DNA-binding intercalator.

interaction of an intercalator with DNA [2,17]. Tris-(1,10-phenathroline)cobalt complex was interacted with DNA through hydrophobic interactions with the interior of DNA molecules [17]. The electrochemical properties of intercalators as hybridization indicators were also investigated to select optimum intercalators for the DNA sensor using voltammetric methods [18]. Millan and coworkers reported a sequence-selective biosensor using a DNA modified electrode and $Co(bpy)_3^{3+}$ as a hybridization indicator instead of labeled probes [19]. Until now, anthracycline antibiotics, of which daunorubicin (DNR) and doxorubicin (DOX) are the parent compounds, have been widely used in cancer chemotherapy. These compounds are known as DNA intercalators and are important models for understanding how small molecules interact with DNA in a sequence-specific manner [20,21]. In addition, the trypanocide, 4′,6-diamidino-2-phenylindole (DAPI) has been widely used as a DNA intercalator with a high-affinity site for the minor groove region [22]. However, to the best of our knowledge, these DNA intercalators have not been used in ECL applications for DNA hybridization detection. The chemical structures of the ECL related compounds are shown in Fig. 1A.

In this paper, we have developed a highly sensitive $Ru(bpy)_3^{2+}$ based ECL method using DNR, DOX, or DAPI as a co-reductant in aqueous solution from various pathogenic DNA samples. Fig. 1B shows the detection scheme for DNA hybridization using $Ru(bpy)_3^{2+}$ ECL with a DNA intercalator as a reductant of $Ru(bpy)_3^{3+}$. In this case, DNA intercalators act as a reducing agent like tripropylamine in oxidative-reduction pathway for $Ru(bpy)_3^{2+}$ ECL [10]. When a voltage of about +1.19 V (versus Ag/AgCl) is applied to the working electrode, $Ru(bpy)_3^{2+}$ is oxidized to $Ru(bpy)_3^{3+}$ and consequently converted into its excited form, $Ru(bpy)_3^{2+}*$, by redox reaction with an intercalator near the electrode. Then the compound generates an orange-colored light of about 620 nm when it returns to the ground state, $Ru(bpy)_3^{2+}$. The amount of light generated at this point is proportional to the amount of intercalator. Thus, the measured amount of light is directly correlated to the amount of target DNA contained in a sample because the concentration of intercalator is proportional to the concentration of the target DNA after DNA hybridization. We have studied the characteristics of ECL reaction with some intercalators to apply DNA hybridization detection. In addition, we optimize the conditions for pathogen detection based on the ECL DNA biosensor with thru-hole array, which consists of gold walls of 16 thru-holes that are self-assembled by specific oligonucleotide probes. By separating the working electrode of ECL from the self-assembled DNA layer, we were able to improve signal sensitivity and stability of the immobilized DNA molecules during ECL reaction.

2. Materials and methods

2.1. Materials

Tris-(2,2′-bipyridyl)dichloro-ruthenium(II) hexahydrate ($Ru(bpy)_3^{2+}$), daunomycin hydrochloride, doxorubicin, 4′,6-diamidino-2-phenylindole (DAPI), hoechst 33342, hoechst 33258, netropsin, distamycin A, ethidium bromide, acridin orange were purchased from Sigma-Aldrich Co. (St. Louis, MO). To monitor

Table 1
DNA Probes for pathogen detection using the ECL technique

Probe name	Target organism	DNA sequence	T_m (°C)	GC%
A1	Hepatitis A virus	5′-SHC6-GTCCCTCTTGGAAGTCCATGGT-3′	77.27	54.55
B1	Hepatitis B virus	5′-SHC6-TACCACAGAGTCTAGACTCGTG-3′	72.15	50.00
QC3	Hepatitis C virus	5′-SHC6-ACTCGCAAGCACCCTATCAGGC-3′	78.50	59.09
GQC3	Hepatitis C virus	5′-SHC6-ACTCGCAAGCAGCCTATCAGGC-3′	78.13	59.09
AQC3	Hepatitis C virus	5′-SHC6-ACTCGCAAGCAACCTATCAGGC-3′	76.15	54.55
RQC3	Hepatitis C virus	5′-SHC6-GCCTGATAGGGTGCTTGCGAGT-3′	78.50	59.09

ECL of intercalated oligomer, the RQC3 (Operon Biotechnologies, AL) oligonucleotide, 5′-GCC TGA TAG GGT GCT TGC GAG T-3′ identified in the hepatitis C virus (HCV), was prepared. All oligonucleotide probes used in the experiment are listed in Table 1. Glycerin (Ducksan Chemical Co., Ltd., Korea) was added in the sample solution at a concentration of 3% (V/V) in order to prevent the droplet from vaporizing. All other reagents were of reagent grade. All solutions were prepared with purified water through a Mill-Q system (Millipore, Bedford, MA).

2.2. ECL screening for DNA-binding intercalators

All electrochemical experiments for ECL-inducible intercalator screening were carried out with an EG&G Princeton Applied Research 273 potentiostat (Princeton, NJ) interfaced with a computer through a peripheral interface card (GPIB, National Instruments, TX). A three-electrode system consisting of a circular gold working electrode (1.6 mm diameter) fabricated by the sputtering method, a wire-type flexible Ag/AgCl reference electrode (Super-dri-ref; World Precision Instruments, FL), and a platinum wire as the auxiliary electrode were selected. Cyclic voltammetry (CV) experiments were carried out in a polystyrene curvet of 1 mL at room temperature. Prior to the measurement, all working electrodes were cleaned by cycling 10 times between −1.5 and +1.5 V (versus Ag/AgCl) with a scan rate of 50 mV s^{-1} in concentrated sulfuric acid solution.

ECL intensities against various intercalators were measured through the vertical side of the curvet with a photomultiplier tube (PMT, model H7421–50; Hamamatsu Photonics, Japan). The emitted light from the reaction reaches the PMT window through a 7.2 mm aperture. To find an optimum ECL operating voltage, ECL intensities of various intercalators were simultaneously measured at the light-shielded chamber during CV experiments. The ECL intensities obtained are given in arbitrary unit (a.u.). The outputs from the PMT and potentiostat were fed into a data acquisition card (DAQ 6062E; National Instruments), and the data acquisition and display were programmed using a LabVIEW program (National Instruments). Unless otherwise noted, each intercalator solution was incubated with 1 mM $Ru(bpy)_3^{2+}$ solution in phosphate buffered saline (PBS) buffer (pH 7.4) for 15 min. A mixture solution containing $Ru(bpy)_3^{2+}$ with intercalator was scanned from +0.8 to +1.3 V (versus Ag/AgCl) with a scan rate of 50 mV s^{-1} in the CV experiments.

2.3. Fabrication of the ECL DNA biosensor

In order to stabilize the self-assembled DNA molecules, we exploited the ECL DNA biosensor with the ECL working electrode and thru-hole array for DNA immobilization (Fig. 2A). For the ECL reaction, in addition to the 16 electrode array, the counter electrode was fabricated by the gold wire on a PEEK plate (1 in.×1 in., ChonWoo Tech, Korea). The reference electrode (Ag/AgCl) was fabricated by the silver wire and reacted with 100 mM NaCl solution for 1 min chlorination. Meanwhile, the 16 thru-hole array was fabricated on the printed circuit board

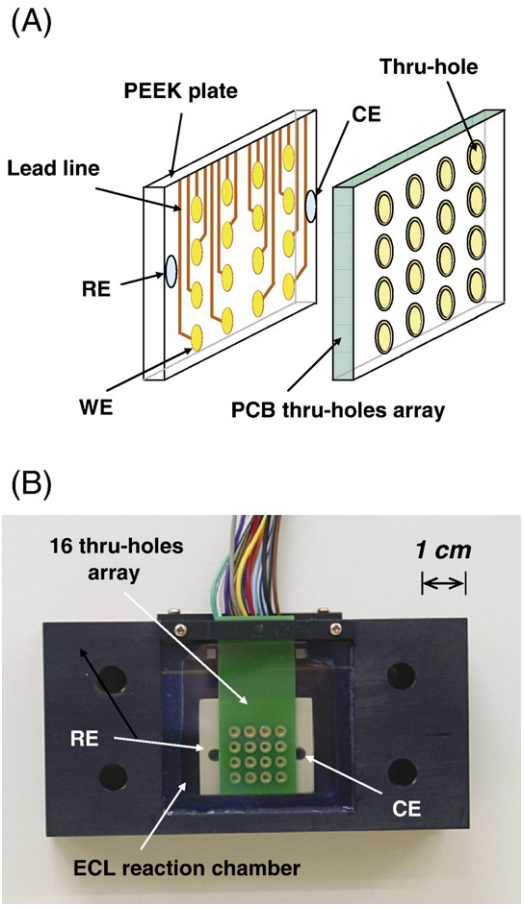

Fig. 2. The ECL DNA sensor with 16 thru-hole array. (A) Schematic diagram of the ECL DNA sensor. WE: gold working electrode; RE: Ag/AgCl reference electrode; CE: counter electrode. (B) A photograph of the ECL DNA biosensor assembled with a thru-hole array in the ECL reaction chamber. The cover of ECL reaction chamber is opened.

(PCB), which consisted of thru-holes with walls inside coated with electroless gold plating and used for DNA immobilization. The diameter of each working electrode and the thru-hole was 1.0 and 1.5 mm, respectively. The thru-hole array was designed to prevent interference from the electric field of other electrodes and to focus luminescence from each electrode by separated holes. The ECL DNA biosensor assembled with PCB thru-hole array at the ECL reaction chamber is shown in Fig. 2B. The ECL DNA biosensor was measured with a fully automated ECL detection system (LG-Elite, Korea), which consists of the XYZ-stage (Hanra Precision Eng., Korea), its controller and charge coupled detector (CCD; PCO imaging, Germany). In order to control the distance between working electrode arrays and the thru-hole arrays, the precise position of the PMT detector (Hamamatsu Photonics) was controlled by the XYZ-stage. To generate an electric field for the ECL reaction, a programmable multiple potentiostat (Analog Research System, Korea) was designed.

2.4. Pathogen detection using ECL technique

Pathogenic virus detection based on the DAPI-intercalated $Ru(bpy)_3^{2+}$ ECL was performed using the ECL DNA biosensor. As shown in Table 1, the probes sequences (22-mer) were listed for hepatitis viruses. Each oligonucleotide primer for specific hepatitis diseases was synthesized by conventional phosphoramidite synthesis (Operon Biotechnologies). The 5′ end of all probes was labeled with SH-C6 in order to increase the accessibility in hybridization reactions and immobilization on the gold surface fabricated on the inside walls of the PCB thru-hole arrays. Unless otherwise noted, 10 μM of capture probes were immobilized by a self-assembly technique on the cylindrical gold surface of the thru-hole arrays in PBS buffer (pH 7.4) for 2 h. Then, the ECL DNA biosensor was washed at room temperature to remove non-adsorbed probe, each for 1 min in a washing buffer I (5× SSC; 750 mM sodium chloride, 75 mM sodium citrate, pH 7.0).

The 60 μl of 1 μM target DNA (RQC3) was injected into the ECL reaction chamber and the hybridization was performed at 40 °C for 1 h. After DNA hybridization, the ECL DNA biosensor was immersed into an intercalator solution (PBS of pH 7.4) for 30 min at room temperature under a dark condition. Then, the intercalated ECL DNA biosensor was washed at room temperature to remove non-specific probe, each for 3 min in washing buffer I, followed by washing buffer II (1× SSC) for 1 min, and was sufficiently dried with air. The ECL DNA biosensor was measured at the 1 mM $Ru(bpy)_3^{2+}$ solution in PBS buffer (pH 7.4) with applied potential of 1.19 V (versus Ag/AgCl).

3. Results and discussion

3.1. Intercalator screening for ECL reaction

Some intercalators inducing ECL have been screened using electrochemical methods combined with a chemiluminescent technique. To select a DNA-binding intercalator used for Ru $(bpy)_3^{2+}$ ECL reaction, cyclic voltammetry (CV) and ECL detection experiments were simultaneously conducted at the light-shield ECL reaction chamber. ECL intensities were measured with various intercalators while a mixture solution containing 1 mM $Ru(bpy)_3^{2+}$ with an intercalator (1 mM) was scanned from +0.8 to +1.3 V (versus Ag/AgCl) with a scan rate of 50 mV s^{-1} in the CV experiments. As shown in Fig. 3A, doxorubicin (DOX), daunorubicin (DNR), and 4′,6-diamidino-2-phenylindole (DAPI) display different voltammograms, but they represent a good ECL in $Ru(bpy)_3^{2+}$ solution. It seems that the intercalators used in this experiment show different electrochemical behavior with $Ru(bpy)_3^{3+}$ as the reductant. DOX and DNR are closely related in structure with anthracycline differing only in functional groups at the C9 position in ring A (Fig. 1A). However, the molecular structure of DAPI is entirely different from that of DOX or DNR, and includes amino groups. DAPI is a fluorescent stain that binds strongly to DNA. Since DAPI passes through an intact cell membrane, it is

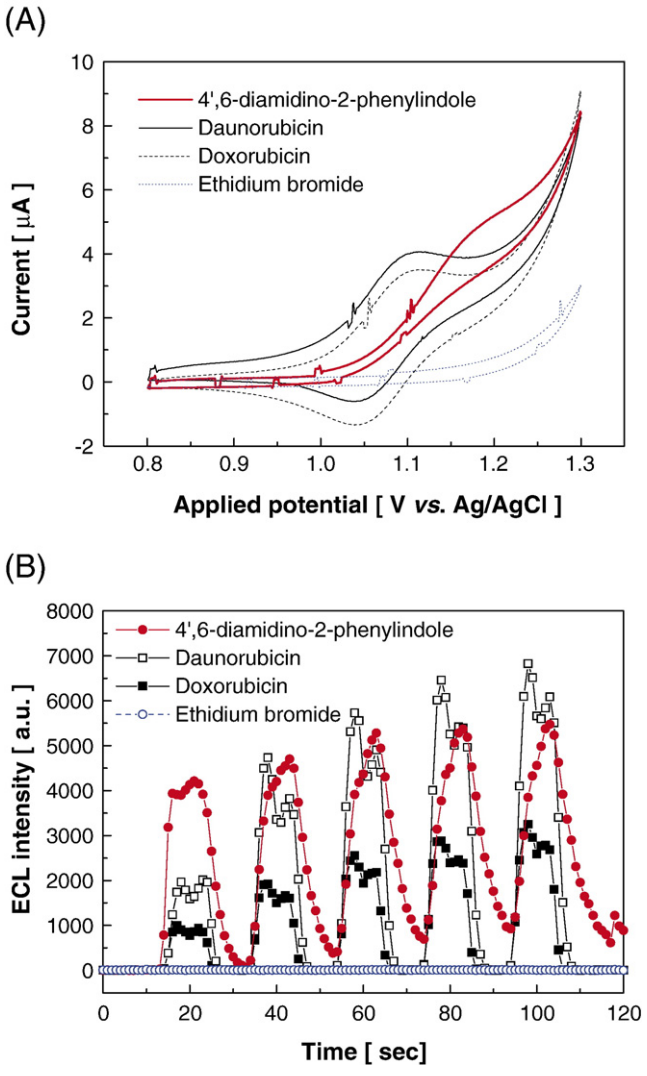

Fig. 3. (A) Cyclic voltammograms of various DNA-binding intercalators. A mixture solution containing 1 mM $Ru(bpy)_3^{2+}$ with each intercalator (1 mM) was scanned from +0.8 to +1.3 V with a scan rate of 50 mV s^{-1}. Steady-state voltammograms were obtained after five cycles of scan. (B) The ECL intensities observed were simultaneously measured during the CV experiments and given in arbitrary unit (a.u.).

used to stain live and fixed cells. These crucial differences in molecular structure resulted in the unique trend of the resulting currents of DAPI as shown in Fig. 3A. Accordingly, the ECL intensities of intercalators were shown the differences in ECL observed between DOX, DNR, and DAPI. In addition, the repetitive ECL signals were represented according to the oxidation peak potential during the CV experiments. We only observed the ECL signals when the oxidation potential of Ru $(bpy)_3^{2+}$ was applied. As shown in Fig. 3B, ECL intensity reached a plateau value after the $Ru(bpy)_3^{2+}$ oxidation potential (about 1.19 V). This trend was repeated every 20 s during the CV experiments with a scan rate 50 mV s^{-1}. However, no other intercalators including hoechst 33342, hoechst 33258, netropsin, distamycin A, ethidium bromide, and acridin orange showed the voltammetric signal as well as any ECL intensity. The ECL data of these compounds were nearly negligible and cyclic voltammograms showed a similar pattern to that of ethidium bromide. This fact indicates that there are no interactions between $Ru(bpy)_3^{3+}$ and some intercalators.

To find an optimum condition for the ECL reaction using a DNA-binding intercalator as a reductant of $Ru(bpy)_3^{3+}$, we have checked the intercalator concentration and pH by measuring the ECL intensity of the sample solution in the ECL reaction chamber. As shown in Fig. 4, the ECL intensity of sample solution with an intercalator was increased very rapidly above 10^{-5} M intercalator to reach the maximum ECL intensity at the 10^{-3} M. Therefore, in order to maximize the ECL intensity in DNA sensor applications, we fixed the 1 mM concentration of intercalator for the subsequent DNA hybridization experiments. Here, the ECL intensity-concentration profile for DOX is not conformable to one of DAPI and DNR. The reason for this difference is due to the low efficiency of the reaction between $Ru(bpy)_3^{3+}$ and DOX. The optimum pH by Tris buffer and PBS buffer was 9.0 and 7.4, respectively. However, the ECL intensity in Tris buffer was greater than that in the PBS buffer (Fig. 5). These results indicate that the pH of Tris buffer have a strong influence on the ECL reactions. This is due to the amine components of Tris buffer [12,23]. Actually, this amine component interferes with precise pathogen detection due to increased background signal. Thus, we used the PBS buffer of pH 7.4 for the subsequent ECL experiments.

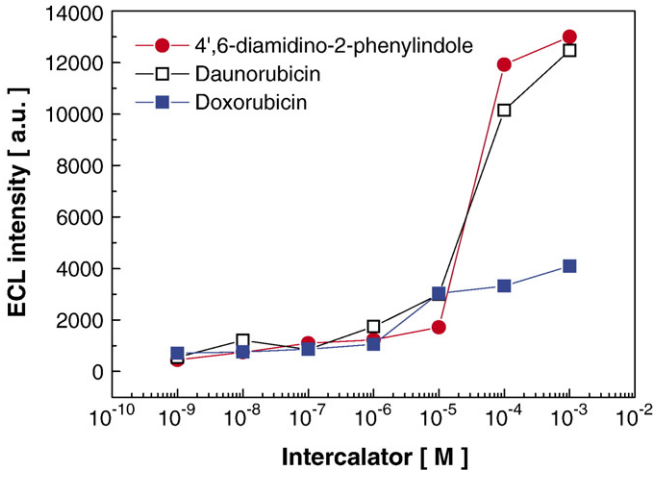

Fig. 4. ECL intensity profiles for various intercalator concentrations in 1 mM Ru $(bpy)_3^{2+}$ solution. The ECL intensities were measured at 80 s after the potential of 1.19 V was applied to a working electrode versus Ag/AgCl.

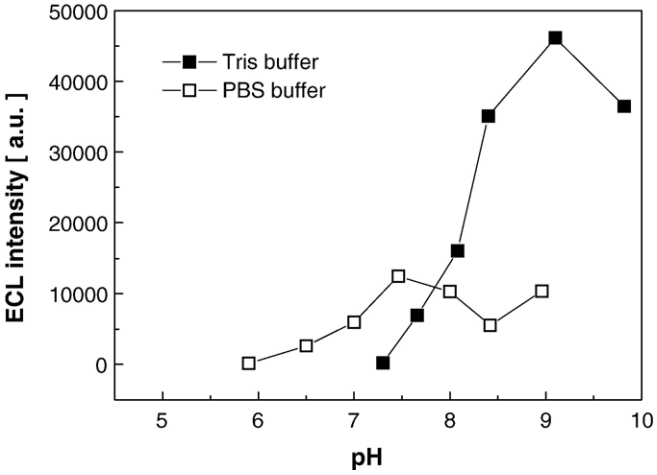

Fig. 5. Effect of pH on $Ru(bpy)_3^{2+}$ ECL. The ECL intensities of daunorubicin were measured at 80 s after the potential of 1.19 V was applied to a working electrode versus Ag/AgCl. Daunorubicin (1 mM) was dissolved in Tris buffer (25 mM) or PBS buffer with 1 mM $Ru(bpy)_3^{2+}$.

3.2. ECL detection after DNA hybridization

To see the feasibility of the ECL detection system in detecting pathogens, we investigated the ECL intensity changes with some intercalators after DNA hybridization. We observed an ECL reaction with intercalation of 1 mM of DOX, DNR, or DAPI after hybridization, and obtained a detection of 1 μM of hepatitis C virus (RQC3). The use of DOX or DNR as an intercalator was not economic due to the high cost of anthracycline DNA-binding

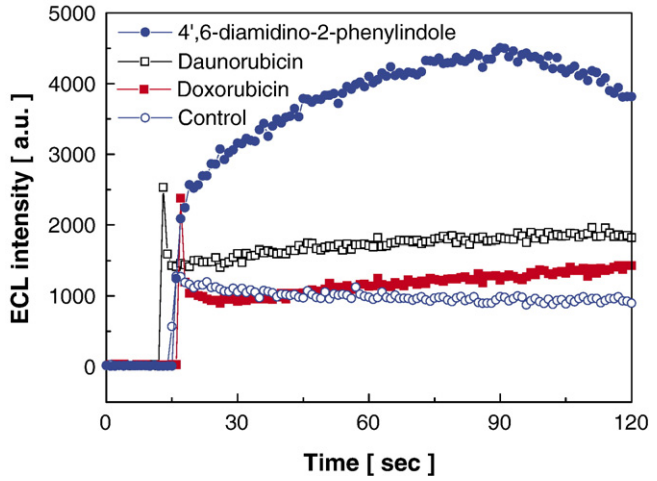

Fig. 6. Comparison of the ECL intensities in doxorubicin, daunorubicin, and 4′,6-diamidino-2-phenylindole (DAPI)-intercalated $Ru(bpy)_3^{2+}$ ECL. After DNA hybridization, each intercalator (1 mM) was reacted for 30 min and the ECL intensities in the ECL DNA biosensor were measured after the potential of 1.19 V was applied to a working electrode versus Ag/AgCl. Control is the non-intercalated ECL signal of a single-stranded DNA in 1 mM $Ru(bpy)_3^{2+}$.

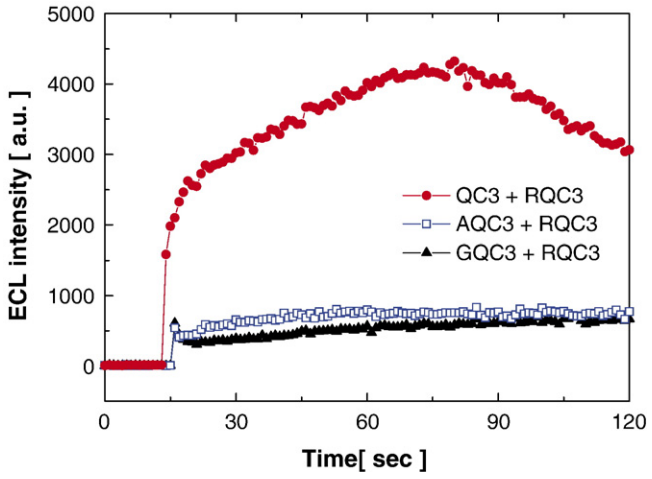

Fig. 7. Specificity of single point mutation in a mixed system using the DAPI-intercalated $Ru(bpy)_3^{2+}$ ECL technique. Three types of capture probe were prepared by self-assembly technique on the gold surface of three thru-holes in the ECL DNA biosensor, respectively, and each was hybridized with the target DNA (RQC3). The other conditions were the same as that of Fig. 6.

drugs and relatively low signal-to-noise (S/N) ratio of ECL intensity. Unlike the DOX- and DNR-intercalated ECL, the ECL intensity of the DAPI-intercalated ECL reaction shows the maximum value after 90 s and gradually decays as time passes, indicating the irreversible characteristics of DAPI as a reducing agent of $Ru(bpy)_3^{3+}$ (Fig. 6). However, the signal sensitivity of DAPI was two times greater than those of DOX- and DNR-intercalated ECL. This effect can be explained as follows. Due to the small molecular size of DAPI and ability to bind well to the minor groove of DNA even at lower concentrations, DAPI is very useful as a reducing agent in a reaction with $Ru(bpy)_3^{3+}$. In contrast to DAPI, DNR and DOX showed less efficiency in oxidation reactions with $Ru(bpy)_3^{3+}$ because of their large anthracycline structure. An efficient ECL reaction with intercalation of DAPI after DNA hybridization could mainly be attributed to the numerous amine groups attached to polar ends of DAPI. Therefore, DAPI was selected in the following experiments as a DNA-binding intercalator for the ECL DNA biosensor measurement because DAPI has relatively low cost and a good S/N ratio (4.55) of ECL reaction at the point of maximum ECL intensity.

We also investigated the sensitivity of the DAPI-intercalated $Ru(bpy)_3^{2+}$ ECL. Three types of capture probe (A1, B1, QC3) were prepared by self-assembly technique on the gold surface of three thru-holes in the ECL DNA biosensor, respectively. The spot intensity of DNA was checked after hybridization for 1 h at 40 °C, intercalation with 1 mM of DAPI for 15 min, and washing step. After hybridization with the target DNA for hepatitis C virus (RQC3), it was clearly shown that the nucleic acids of the target virus (HCV) were detected by the ECL DNA biosensor (data not shown). The significant signal levels for hepatitis C virus oligomer (QC3) was shown after hybridization for 1 h at 40 °C, and the S/N ratio of 2.69 versus control (A1 and B1) at 80 s was obtained without cross hybridization. This indicates that the DAPI-intercalated double-stranded DNA was effectively used for $Ru(bpy)_3^{2+}$ ECL reaction. From the results, the ECL DNA biosensor could be successfully performed for pathogen detection related to hepatitis diseases.

In addition, we tried to check the detection specificity of a single point mutation in a mixed system that has pathogens as well as host cells. We used hepatitis C virus as a model for viral pathogen. To find the hepatitis C virus in multiple samples, hepatitis C virus oligomer (QC3-C6SH) was immobilized on a gold surface by self-assembly technique. Each of the single point mutations of the oligomer such as GQC3-C6SH and AQC3-C6SH was also designed and applied. Detailed sequences were listed in Table 1. Significant signal levels for hepatitis C virus oligomer (QC3) after hybridization for 1 h at 40 °C were obtained to give a S/N ratio of 7.41 without cross hybridization (Fig. 7). This observation suggests that the pathogen detection with the DAPI-intercalated ECL technique can be applied for single nucleotide polymorphism (SNP) analysis.

4. Conclusion

We have developed a thru-hole type DNA biosensor based on the $Ru(bpy)_3^{2+}$ ECL with a DNA-binding intercalator. This paper specifically described the use of DNA intercalators, including DOX, DNR, and DAPI, as reductants of $Ru(bpy)_3^{2+}$ to detect DNA hybridization. Among the various intercalators, we observed an efficient ECL reaction with intercalation of DAPI after DNA hybridization. Main requirements for efficient intercalator to reduce ruthenium (III) complex are high specificity of the intercalation for dsDNA and the presence of amino groups in structure. These requirements for DNA-binding intercalators could help ECL reactions with ruthenium (III) complex be more efficient. We also determined the single nucleotide polymorphism (SNP) for pathogen detection by the DAPI-intercalated ECL system. The application of this detection scheme for DNA biosensor could be useful in developing a compact DNA diagnostic system without a laser detection system and expensive fluorescence dye. In summary, the PCB thru-hole DNA biosensor has potential as an efficient pathogen detection method well-suited for disposable application.

Acknowledgments

This work was supported by the Ministry of Commerce, Industry and Energy (MOCIE) and LG Electronics Inc., Korea.

References

[1] J. Gonzales-Velasco, I. Rubinstein, R.J. Crutchley, A.P.B. Lever, A.J. Bard, Electrogenerated chemiluminescence. 42. Electrochemistry and electrogenerated chemiluminescence of the tris(2,2′-bipyrazine)ruthenium(II) system, Inorg. Chem. 22 (1983) 822–825.
[2] X.-H. Xu, A.J. Bard, Immobilization and hybridization of DNA on an aluminum(III) alkanebisphosphonate thin-film with electrogenerated chemiluminescent detection, J. Am. Chem. Soc. 117 (1995) 2627–2631.
[3] K.A. Fahnrich, M. Pravda, G.G. Guibault, Recent applications of electrogenerated chemiluminescence in chemical analysis, Talanta 54 (2001) 531–539.

[4] C. Marquette, B. Leca, L.J. Blum, Electrogenerated chemiluminescence of luminol for oxidase-based fiber-optic biosensor, Luminescence 16 (2001) 159–165.
[5] M. Yang, C. Liu, K. Qian, P. He, Y. Fang, Study on the electrochemiluminescence behavior of ABEI and its application in DNA hybridization analysis, Analyst 127 (2002) 1267–1271.
[6] M.-L. Calvo-Muñoz, A. Dupont-Filliard, M. Billon, S. Guillerez, G. Bidan, C. Marquette, L. Blum, Detection of DNA hybridization by ABEI electrochemiluminescence in DNA-chip compatible assembly, Bioelectrochemistry 66 (2005) 139–143.
[7] J.K. Leland, M.J. Powell, Electrogenerated chemiluminescence. An oxidative-reduction type ECL reaction sequence using tripropyl amine, J. Electrochem. Soc. 137 (1990) 3127–3131.
[8] C. Bertolino, M. MacSweeney, J. Tobin, B. O'Neill, M.M. Sheehan, S. Coluccia, H. Berney, A monolithic silicon based integrated signal generation and detection system for monitoring DNA hybridization, Biosens. Bioelectron. 21 (2005) 565–573.
[9] X. Guobao, D. Shaojun, Electrochemiluminescent detection of chlorpromazine by selective preconcentration at a lauric acid-modified carbon paste electrode using tris(2,2′-bipyridine)ruthenium(II), Anal. Chem. 72 (2000) 5308–5312.
[10] W.-Y. Lee, Tris (2,2′-bipyridyl)ruthenium(II) electrogenerated chemiluminescence in analytical science, Mikrochim. Acta 127 (1997) 19–37.
[11] A.W. Knight, A review of recent trends in analytical applications of electrogenerated chemiluminescence, Trends Anal. Chem. 18 (1999) 47–62.
[12] M.M. Richter, Electrochemiluminescence (ECL), Chem. Rev. 104 (2004) 3003–3036.
[13] D. Ege, W.G. Becker, A.J. Bard, Electrogenerated chemiluminescent determination of $Ru(bpy)_3^{2+}$ at low levels, Anal. Chem. 56 (1984) 2413–2417.
[14] I. Rubinstein, C.R. Martin, A.J. Bard, Electrogenerated chemiluminescent determination of oxalate, Anal. Chem. 54 (1983) 1580–1582.
[15] L.S. Lerman, Structural considerations in the interaction of DNA and acridines, J. Mol. Biol. 3 (1961) 18–30.
[16] M. Cusumano, M.L. Di Pietro, A. Giannetto, Stacking surface effect in the DNA intercalation of some polypyridine platinum(II) complexes, Inorg. Chem. 38 (1999) 1754–1758.
[17] M.T. Carter, A.J. Bard, Electrochemical investigations of the metal chelates with DNA. 3. Electrogenerated chemiluminescent investigation of the interaction of tris(1,10-phenanthroline)ruthenium(II) with DNA, Bioconjug. Chem. 1 (1990) 257–263.
[18] K. Hashimoto, K. Ito, Y. Ishimori, Novel DNA sensor for electrochemical gene detection, Anal. Chim. Acta 286 (1994) 219–224.
[19] K.M. Millan, A.S.R. Saraullo, S.R. Mikkelsen, Voltammetric DNA biosensor for cystic fibrosis based on a modified carbon paste electrode, Anal. Chem. 66 (1994) 2943–2948.
[20] F. Leng, R. Savkur, I. Fokt, T. Przewloka, W. Priebe, J.B. Chaires, Base specific and regioselective chemical cross-linking of daunorubicin to DNA, J. Am. Chem. Soc. 118 (1996) 4731–4738.
[21] T. Kuwabara, T. Noda, H. Ohtake, T. Ohtake, S. Toyama, Y. Ikariyama, Classification of DNA-binding mode of antitumor and antiviral agents by the electrochemiluminescence of ruthenium complex, Anal. Biochem. 314 (2003) 30–37.
[22] M. Kubista, B. Åkerman, B. Nordén, Characterization of interaction between DNA and 4′,6-diamidino-2-phenylindole by optical spectroscopy, Biochemistry 26 (1987) 4545–4553.
[23] K. Uchikura, M. Kirisawa, A. Sugii, Electrochemiluminescence detection of primary amines using tris(bipyridine)ruthenium(III) after derivatization with divinylsulfone, Anal. Sci. 9 (1993) 121–123.