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Heterogeneous electrochemiluminescence spectrometry of $Ru(bpy)_3^{2+}$ for determination of trace DNA and its application in measurement of gene expression level

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ABSTRACT

In this paper, we reported an ultrasensitive ECL spectrometry for determination of DNA using magnetic streptavidin-coated nanobeads MNBs (SA-MNBs) as the carrier of $Ru(bpy)_3^{2+}$-NHS, where bpy = 2,2′-bipyridyl and NHS = N-hydroxysuccinimide ester, to amplify signal. The SA-MNBs were conjugated to the hybrids consisting of capture DNA, target DNA (t-DNA) and probe DNA immobilized on a substrate, followed by releasing the SA-MNBs and binding a huge number of $Ru(bpy)_3^{2+}$-NHS to the SA-MNBs. The SA-MNBs with $Ru(bpy)_3^{2+}$-NHS were immobilized on an Au film electrode by means of a magnet. In the presence of tri-*n*-propylamine, the ECL spectrum of the $Ru(bpy)_3^{2+}$-NHS at 1.35 V was acquired by using an optical multi-channel analyzer. The maximum emission intensity on the ECL spectrum was used to quantify DNA. Using this method, not only the limit of detection for DNA determination was as low as 1.2×10^{-15} mol/L, but also the ECL spectrum of $Ru(bpy)_3^{2+}$-NHS on the surface of the SA-MNBs was obtained. The ultrasensitive ECL spectrometry could be used to measure gene expression level in cells.

1. Introduction

Electrochemiluminescence (ECL)-based assays are often used to detect trace DNA due to high sensitivity and versatility [1–17]. Tris(2,2′-bipyridyl)ruthenium(II) ($Ru(bpy)_3^{2+}$) is the most extensively studied and used ECL compound due to its high ECL efficiency and high stability in ECL systems [1–3,14]. The $Ru(bpy)_3^{2+}$-based ECL system with a coreactant tri-*n*-propylamine (TPrA) has higher sensitivity than those with other coreactants [18]. Therefore, the $Ru(bpy)_3^{2+}$/TPrA system is commonly used in DNA analysis. Usually, ECL of $Ru(bpy)_3^{2+}$ labeled to target DNA (t-DNA) immobilized a working electrode is measured by a photomultiplier tube (PMT) in the presence of TPrA, when the electrode is held or swept to an appropriate potential. Then, total ECL intensity of $Ru(bpy)_3^{2+}$ is used to quantify t-DNA. However, ECL emission characteristics such as maximum emission wavelength and spectrum profile are not obtained, which are very important for understanding ECL mechanism and reaction process. It is difficult to acquire the ECL spectra using conventional spectrometers that measure a constant light with time, because ECL intensity decreases with time. Some research groups use a series of optical filters with different wavelengths to obtain inaccurate ECL quasi-spectra consisting of several ECL intensity points [19,20]. Moreover, the ECL substances must be photostable during repeatedly applying potential. It is not easy for the trace $Ru(bpy)_3^{2+}$-labeled biomolecules. In this work, we developed a ECL spectrometry to accurately measure ECL spectra of $Ru(bpy)_3^{2+}$ attached to magnetic nanobeads (MNBs) that were labeled to t-DNA. The proposal of the method is shown in Fig. 1. t-DNA is bound to biotinylated capture DNA (B-c-DNA) immobilized on the streptavidin-coated substrate (SA-substrate) through hybridization reaction. Then, biotinylated probe DNA (B-p-DNA) is hybridized with the t-DNA. Next, streptavidin-coated MNBs (SA-MNBs) are conjugated to the B-p-DNA with a ratio of 1:1 via the interaction between streptavidin and biotin (Fig. 1A). The SA-MNBs are released from the substrate to the solution via

Abbreviations: B-p-DNA, biotinylated probe DNA; bpy, 2,2′-bipyridyl; dcbpy, 2,2′-bipyridine-4,4′-dicarboxylic acid; DEPC, diethypyrocarbonate; DMF, N′,N-dimethylformamide; DNase, deoxyribonuclease; dNTP, deoxy-ribonucleoside triphosphate; ECL, electrochemiluminescence; LOD, limit of detection; MNB, magnetic nanobead; mRNA, messenger RNA; NHS, N-hydroxysuccinimide ester; PB, phosphate buffer; PBS, physiological buffer saline; PMT, photomultiplier tube; QD, quantum dot; qRT-PCR, quantitative reverse transcription polymerase chain reaction; RNase, ribonuclease; rRNAsin, RNase-inhibitor; RT, reverse transcription; $Ru(bpy)_3^{2+}$, Tris(2,2′-bipyridyl)ruthenium(II); SA, streptavidin; SA-MNB, streptavidin-coated MNB; SA-substrate, streptavidin-coated substrate; t-DNA, target DNA; TIRFM, total internal reflection fluorescence microscopy; TPrA, tri-*n*-propylamine; TTL buffer, 0.100 mol/L Tris–HCl (pH 8.0), 0.1% Tween-20 and 1 mol/L LiCl.

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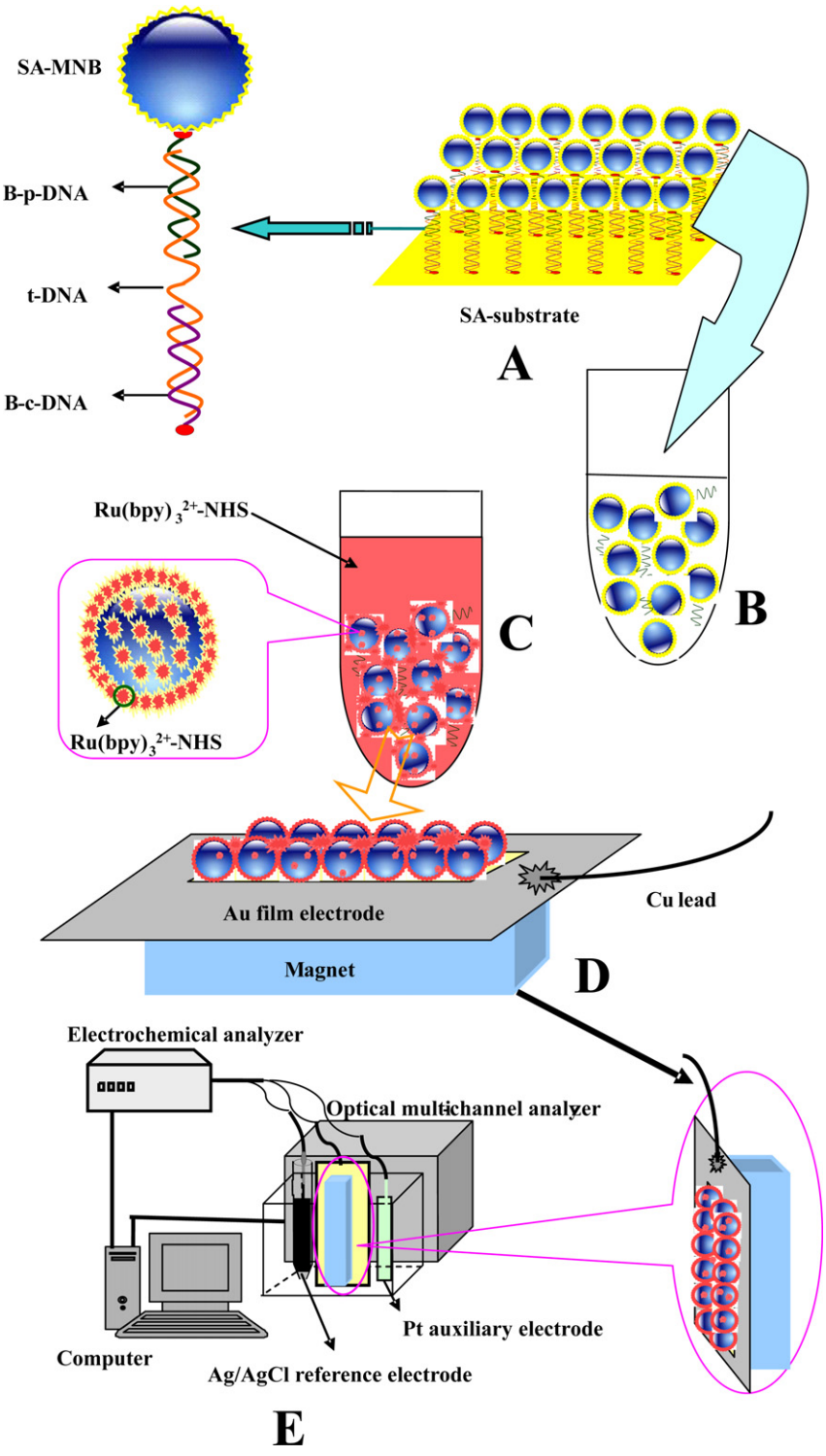

Fig. 1. Schematic representation of the process of ECL spectrometry for determination of DNA by a combination of MNBs attached with $Ru(bpy)_3^{2+}$-NHS as labels and overview of the ECL spectrometric detection system.

dehybridization between B-p-DNA and t-DNA using a urea solution. The SA-MNBs are transferred to a vessel (Fig. 1B). Then the SA-MNBs are conjugated with $Ru(bpy)_2(dcbpy)NHS$ ($Ru(bpy)_3^{2+}$-NHS), where bpy = 2,2′-bipyridyl, dcbpy = 2,2′-bipyridine-4,4′-dicarboxylic acid, NHS = N-hydroxysuccinimide ester (Fig. 1C). The SA-MNBs with $Ru(bpy)_3^{2+}$-NHS are immobilized on an Au electrode using a magnet (Fig. 1D). Finally, in the presence of TPrA, ECL spectrum of the $Ru(bpy)_3^{2+}$-NHS on the SA-MNBs is acquired by applying a constant potential to the Au electrode using an optical multi-channel analyzer (Fig. 1E). The limit of detection (LOD) of the ECL spectrometry for determination of the t-DNA was as low as 1.2×10^{-15} mol/L. The ECL spectrometry was used to measure beta-2-microglobulin (β2M) gene expression level in human breast cancer cells.

2. Experimental

2.1. Materials

Reagents, solutions and $Ru(bpy)_3^{2+}$-NHS synthesis are given in Supplemental Information. The phosphate buffered saline (PBS) consisted of 0.15 mol/L NaCl, 7.6×10^{-3} mol/L NaH_2PO_4 and 2.4×10^{-3} mol/L Na_2HPO_4 (pH 7.4). TTL buffer consisted of 0.100 mol/L Tris–HCl (pH 8.0), 0.1% Tween-20 and 1 mol/L LiCl.

2.2. Apparatus

The experimental setup for ECL spectrometry is shown in Fig. 1. Measurements of ECL spectra were carried out using a CHI832 electrochemical analyzer (CH Instruments, Austin, TX, USA) coupled with an optical multi-channel analyzer (SpectraPro300i, Acton Research Co., Acton, MA, USA). A cuboid electrochemical cell fabricated using glass plates with an Au film working electrode, an Ag/AgCl reference electrode and a Pt auxiliary electrode were used in this work. The Au film electrode was made by cutting a recordable compact disc (Kodak, USA) into a plate of 3 cm × 1 cm. After stripping the polymeric protection layer from the Au plate by immersing it into concentrated HNO_3 for 15 min, the Au plate was washed with distilled water and ethanol, respectively and then dried. A coiled end of a 0.5-mm-diameter, ~6-cm-long orthogonal copper lead was glued to one end of the Au plate with silver epoxy. The ensemble was cured for 30 min at 150 °C in an oven. Epoxy resin was applied to the junction between the Au plate and the copper lead in order to protect the electrical junction. An electrode area of 15 mm × 1.5 mm was fabricated by covering epoxy resin onto the Au plate. The same electrochemical analyzer and the electrochemical cell with the three electrodes were used to measure ECL. A PMT (model CR114, Beijing Hamamatsu, Beijing, China) biased at −1000 V controlled by a high-voltage power supply (model CC171, Beijing Hamamatsu, Beijing, China) was used to measure the ECL intensity.

The total internal reflection fluorescence microscopy (TIRFM) system described in our previous work [21] was used to accomplish TIRFM measurements of single quantum dot (QD)-labeled t-DNA molecules. An inverted microscope equipped with a 60× oil-immersion objective, a halogen lamp and a CCD camera (DP70, Olympus, Tokyo, Japan) was used to acquire the bright-field images of SA-MNBs bound to the substrate.

2.3. Fabrication of DNA hybrids on streptavidin-coated substrate

After washing with PBS, the wells of the streptavidin-coated microtiter plates (S6940, 96 wells) from Sigma (St. Louis, MO, USA) were used as the reactors. B-c-DNA was conjugated to the SA-substrate of the well by incubating 10 μL of 50 μmol/L B-c-DNA solution for 2 h at room temterature with frequent gentle shaking. The residual B-c-DNA was washed away with PBS and 0.5 mol/L NaCl. After 100 μL of t-DNA solution and 60 μL of 0.5 mol/L NaCl were added, the solution was incubated for 4 h in a constant-humidity chamber. The substrate was washed with PBS and 0.5 mol/L NaCl. Then, 10 μL of 10 μmol/L B-p-DNA solution and 50 μL of 0.5 mol/L NaCl were added, the solution was incubated for 4 h in the constant-humidity chamber. The substrate was washed with PBS.

2.4. Binding SA-MNBs to the DNA hybrids on SA-substrate, releasing the SA-MNBs and conjugating $Ru(bpy)_3^{2+}$-NHS to the SA-MNBs

Ten μL of SA-MNB suspension (3.32×10^{11} beads/mL) was washed five times with 400 μL of TTL buffer, to remove surfactants. Then, 100 μL of PBS was added. Ten μL of the SA-MNB suspension in PBS was added into the well with the hybrids consisting of B-c-DNA, t-DNA and B-p-DNA on the substrate and incubated for 2 h. The substrate was washed with PBS. Then, 200 μL of 50% (w/w) urea solution was added. After 10 min, the urea solution containing released SA-MNBs was transferred to a vessel. The SA-MNBs were magnetically separated, followed by washing the SA-MNBs with PBS. Then, 64 μL of 1.93×10^{-2} mol/L $Ru(bpy)_3^{2+}$-NHS, 10 μL of N′,N-dimethylformamide (DMF) and 826 μL of 0.1 mol/L phosphate buffer (pH 9.2) were added and the solution was incubated for 8 h. The resultant $Ru(bpy)_3^{2+}$-NHS-MNBs were magnetically separated and washed with PBS under a magnetic field. Next, 20 μL of PBS was added for ECL or ECL spectrometric experiment.

2.5. ECL and ECL spectrometric measurements

First, the Au film electrode was treated by performing ten repetitive cyclic scans between 0 and 1.5 V at 100 mV/s in a 0.5 mol/L H_2SO_4. After rinsing with tap water, the electrode was washed with doubly distilled water. Subsequently, 20 μL of $Ru(bpy)_3^{2+}$-NHS-MNBs solution was dropped on the Au electrode, followed by drying them at room temperature. A magnet was pasted to the back of the electrode by using adhesive tape to immobilize the $Ru(bpy)_3^{2+}$-NHS-MNBs on the electrode. The Au electrode with $Ru(bpy)_3^{2+}$-NHS-MNBs was inserted into the electrochemical cell containing PB-TPrA (0.15 mol/L phosphate buffer (PB) (pH 7.5) containing 0.1 mol/L KNO_3, and 0.10 mol/L TPrA) and faced the observation window of the PMT or the optical multi-channel analyzer. In ECL measurements, the Au electrode and the output end of the PMT were connected to the first and second working electrode ports of the electrochemical analyzer, respectively. The curve of ECL intensity (I_{ECL}) versus potential (E) was recorded by scanning the potential from 0.5 to 1.5 V. In ECL spectrometric experiments, the ECL spectrum was recorded by applying a constant potential of 1.35 V to the Au electrode for 5 s. All experiments were carried out in a dark environment.

2.6. Measurement of β2M gene expression level in cells

The total RNA was obtained from cells using Unizol (Biostar Co., Ltd., Shanghai, China) according to the manufacturer's instructions. Then, genomic DNA was digested using 1 U of ribonuclease (RNase)-free deoxyribonuclease (DNase) and 1 U of RNase-inhibitor (rRNAsin). After, RNase activity was blocked by incubating for 30 min at 37 °C and DNase was inactivated by incubating for 5 min at 75 °C, 1 μL of 1×10^{-4} mol/L reverse transcription (RT) oligo dT primer was added to 5 μL of the RNA sample. The solution was incubated for 5 min at 70 °C, followed by cooling on ice. In order to synthesize double-stranded RNA–DNA hybrids consisting of mRNAs and corresponding complementary DNAs, the solution was mixed with 14 μL of Master Mix consisting of 4 μL of RT buffer, 2 μL of 10 mmol/L deoxy-ribonucleoside triphosphate (dNTP) mixture, 0.5 μL of rRNAsin, 1 μL of M-MLV reverse transcriptase and 6.5 μL of RNase-free H_2O, and incubated for 1 h at 42 °C. After the reaction was terminated by incubating for a 5 min at 70 °C, the mRNAs in the RNA–DNA hybrids were digested using 1 μL of RNase H (2 U) by incubating for 5 min at 37 °C. Then, the RNase H was inactivated by incubating for 5 min at 95 °C. The solution was mixed with 10 μL of isopropyl alcohol for 5–10 min and then centrifuged for 5 min at 8000 *g* at 4 °C. After the supernatant was discarded and dried at room temperature, the deposition was dissolved in 100 μL of diethypyrocarbonate (DEPC) treated water at 55–60 °C. After dilution, the complementary DNA corresponding to β2M mRNA was determined by using the ECL spectrometry coupled with MNBs as the

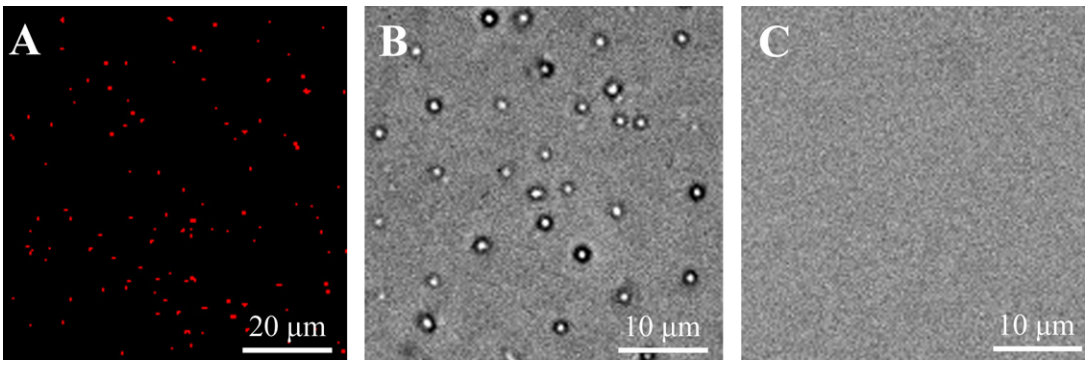

Fig. 2. (A) Fluorescence image of single QD-labeled DNA hybrids on the substrate and bright-field images of SA-MNBs conjugated to the DNA hybrids on the substrate (B) before and (C) after dehybridization using 50% (w/w) urea solution. The DNA hybrids on the substrate were fabricated using 5.0×10^{-13} mol/L t-DNA.

carrier of $Ru(bpy)_3^{2+}$ species according to the procedure described above.

3. Results and discussion

3.1. Conjugatiing SA-MNBs to the DNA hybrids on SA-substrate

DNA–DNA sandwich hybridization is the most commonly used technique for DNA analysis. In this work, two hybridization steps (the primary hybridization between B-c-DNA on the substrate and t-DNA, and the secondary hybridization between t-DNA and B-p-DNA) were carried out. First, B-c-DNA was conjugated onto the SA-substrate via the streptavidin/biotin interaction. In order to cover the binding sites on the surface of the SA-substrate with B-c-DNA, 500 pmol of B-c-DNA were used to react with 300 pmol binding sites of biotin as reported by the manufacture's instructions [22]. In this method, since one SA-MNB is bound to one DNA hybrid containing t-DNA on the substrate, the distance between two adjacent hybrids captured on the substrate should be larger than that between two adjacent SA-MNBs. The substrate of 32 mm^2 can be occupied by 3×10^8 SA-MNBs with a 350-nm diameter. Therefore, when t-DNA is lower than 5×10^{-16} mol, this condition can be satisfied. In our experiments, the amount of t-DNA lower than 1.0×10^{-17} mol (1.0×10^{-13} mol/L in 100 μL) was used. In this case, if all t-DNA molecules are captured on the substrate, the average distance between two hybrids is larger than the diameter of one SA-MNB. This could be demonstrated by the single-molecule fluorescence experiment. Fig. 2A shows the fluorescence image of single QD-labeled t-DNA molecules on the substrate for 5.0×10^{-13} mol/L t-DNA acquired by TIRFM. In the image, each bright spot is corresponding to a single t-DNA molecule. The distance between two bright spots is larger than 1.5 μm. Therefore, when the concentrations of t-DNA are lower than 5.0×10^{-13} mol/L, one t-DNA molecule can bring one SA-MNB onto the substrate. After formation of the hybrids of t-DNA and B-c-DNA on the substrate, the B-p-DNA was bound to the hybrids via the hybridization reaction between t-DNA and B-p-DNA by incubating the hybrids on the substrate with a large number of B-p-DNA. In order to enhance the stability of the DNA hybrids on the substrate, the high concentration of NaCl (0.5 mol/L) was used as the hybridization buffer during hybridization reaction. At the same time, the unbound sites on the surface of the SA-substrate were blocked with B-p-DNA. Conjugation of SA-MNBs to the B-p-DNA of the DNA hybrids on the substrate was through the interaction between streptavidin and biotin. Fig. 2B shows the bright-field image of the SA-MNBs conjugated to the substrate. In the image, each visible SA-MNB is corresponding to a single t-DNA molecule. It can be found that the distance between two SA-MNBs is larger than 2.3 μm.

3.2. Releasing the SA-MNBs from the DNA hybids on substrate and conjugating $Ru(bpy)_3^{2+}$-NHS to the SA-MNBs

Urea solution can be used to release SA-MNBs from the substrate through dehybridization [23]. In this step, the base pairs between t-DNA and B-p-DNA as well as the base pairs between t-DNA and B-c-DNA are dehybridized. Fig. 2C shows the bright-field image of the substrate after releasing SA-MNBs. The visible SA-MNBs conjugated to the hybrids on the substrate disappeared, indicating that SA-MNBs were completely released from the substrate. The released SA-MNBs could be conjugated with $Ru(bpy)_3^{2+}$-NHS through the interaction between the amino group of SA and the NHS of $Ru(bpy)_3^{2+}$-NHS.

3.3. ECL spectra of $Ru(bpy)_3^{2+}$, $Ru(bpy)_3^{2+}$-NHS and $Ru(bpy)_3^{2+}$-NHS-MNBs in PB-KNO_3 with and without TPrA

Since the maximum ECL intensity of the $Ru(bpy)_3^{2+}$ at the Au electrode in PB-TPrA was at 1.35 V (Supplemental Information Fig. S1), in the ECL spectrometric measurements, the potential was applied to the Au electrode for 5 s to record the ECL spectra. Fig. 3 depicts the spectra of the optical multi-channel analyzer (curve 1), PB-KNO_3 solution consisting of 0.15 mol/L PB (pH 7.5) and 0.1 mol/L KNO_3 (curve 2), PB-TPrA solution consisting of PB-KNO_3 and 0.10 mol/L TPrA (curve 3), and 3.0×10^{-8} mol/L $Ru(bpy)_3^{2+}$ in PB-KNO_3 (curve 4). It is shown that: (1) the optical analyzer has a detectable noise; (2) the spectrum of the optical analyzer and the ECL spectrum of PB-TPrA solution are identical, indicating that no detectable ECL from TPrA is observed. Thus, the background noise in PB-TPrA is mainly from that of the optical analyzer; (3) $Ru(bpy)_3^{2+}$ yields a peak-shape signal at >550 nm with a maximum ECL wavelength of 630 nm; and (4) the ECL spectrum for $Ru(bpy)_3^{2+}$ as low as 3.0×10^{-8} mol/L in the absence of TPrA can be measured.

When $Ru(bpy)_3^{2+}$-NHS-MNBs were immobilized on the Au electrode by means of a magnet and a suitable positive potential was applied to the electrode, the $Ru(bpy)_3^{2+}$-NHS molecules could generate ECL. The ECL spectra of 3.0×10^{-8} mol/L $Ru(bpy)_3^{2+}$ (curve 5), 5.0×10^{-8} mol/L $Ru(bpy)_3^{2+}$-NHS (curve 6) and 5.0×10^6 MNBs/cm^2 $Ru(bpy)_3^{2+}$-NHS-MNBs immobilized on the Au electrode (curve 7) in PB-TPrA are also shown in Fig. 3. In the presence of TPrA, ECL intensity of $Ru(bpy)_3^{2+}$ was greatly increased. The maximum emission wavelength (λ_m) of $Ru(bpy)_3^{2+}$ was 630 nm. By comparing the spectrum of $Ru(bpy)_3^{2+}$, a 40-nm red shift was observed on the spectra of $Ru(bpy)_3^{2+}$-NHS in solution and $Ru(bpy)_3^{2+}$-NHS-MNBs on the electrode. The same λ_m (670 nm) for the both indicates that the ECL mechanism of $Ru(bpy)_3^{2+}$-NHS on the $Ru(bpy)_3^{2+}$-NHS-MNBs is the same as that in the solution.

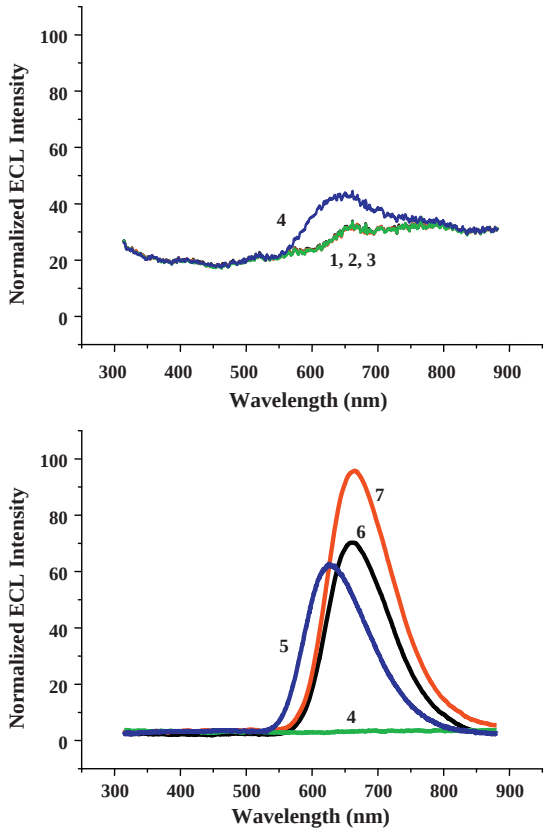

Fig. 3. Spectra of (1) optical multi-channel analyzer, (2) 0.15 mol/L PB (pH 7.5) and 0.1 mol/L KNO_3 (PB-KNO_3), (3) 0.10 mol/L TPrA in PB-KNO_3 and (4) 3.0×10^{-8} mol/L $Ru(bpy)_3^{2+}$ in PB-KNO_3, (5) 3.0×10^{-8} mol/L $Ru(bpy)_3^{2+}$ in PB-TPrA, (6) 5.0×10^{-8} mol/L $Ru(bpy)_3^{2+}$-NHS in PB-TPrA and (7) 5.0×10^6 MNBs/cm^2 $Ru(bpy)_3^{2+}$-NHS-MNBs immobilized on the Au electrode in PB-TPrA. A potential of 1.35 V was applied at the Au electrode for 5 s.

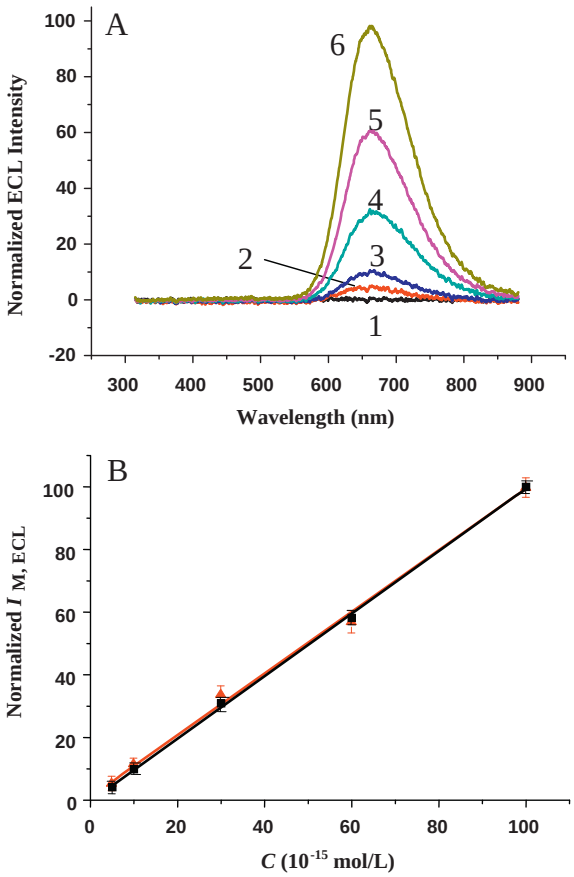

Fig. 4. (A) Spectra of $Ru(bpy)_3^{2+}$-NHS-MNBs immobilized on the Au electrode in PB-TPrA for different concentrations of t-DNA (10^{-15} mol/L): (1) 0, (2) 5.0, (3) 10, (4) 30, (5) 60 and (6) 100 by applying a potential of 1.35 V to the Au electrode for 20 s and (B) relationship between $I_{M,ECL}$ on the spectra and the concentration of t-DNA (black line) and $DNA_{\beta 2M}$ (red line) ($n = 3$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.4. Quantification of t-DNA by ECL spectrometry

In the present method, one MNB with a large number of $Ru(bpy)_3^{2+}$-NHS corresponds to one t-DNA molecule. Therefore, a large amplification factor of $Ru(bpy)_3^{2+}$-NHS for each t-DNA molecule can be achieved. We used the DNA with a sequence of 5′-GGA TTA TTG TTA AAT ATT GAT AAG GAT-3′ to serve as the model t-DNA. The sequences of the corresponding B-c-DNA and B-p-DNA were 5′-TAA CAA TAA TCC-T_{20}-3′-biotin and biotin-5′-T_{20}-ATC CTT ATC AAT ATT-3′, respectively, the sequences of which were complementary to the combined sequence of the t-DNA. B-c-DNA and B-p-DNA had a sequence of 20 thymines at the biotin end acting as a spacer to reduce the steric hindrance between both DNA and the surface-confined streptavidin interaction. The maximum of emission intensity ($I_{M,ECL}$) on the ECL spectra of $Ru(bpy)_3^{2+}$-NHS-MNBs immobilized on the Au electrode could be used to quantify t-DNA. Fig. 4A shows the spectra of $Ru(bpy)_3^{2+}$-NHS-MNBs obtained from different concentrations of t-DNA. The relationship between $I_{M,ECL}$ and the concentration of t-DNA in the range of 5.0×10^{-15} to 1.0×10^{-13} mol/L t-DNA is displayed in Fig. 4B. A straight line with a slope of 1.04 and a correlation coefficient of 0.9986 ($n = 3$) in the concentration range was obtained. The LOD was 1.2×10^{-15} mol/L according to LOD = $3\sigma/S$ where σ and S are the standard deviation for a series of nine measurements of the blank solution and the slop of the calibration curve. The LOD was lower than that using Au nanoparticles (Au-NPs) as the carrier of $Ru(bpy)_3^{2+}$ (4×10^{-11} mol/L) [15], because the amount of $Ru(bpy)_3^{2+}$ species on the surface of one SA-MNB is much more than that on the surface of one Au-NP. In order to explore the applicability of the method, the selectivity experiment of the ECL spectrometry for determination of t-DNA was carried out by comparing the $I_{M,ECL}$ value for 1.0×10^{-13} mol/L t-DNA, single base mismatched DNA and a mixture consisting of five random noncomplementary DNAs with the same concentration. The $I_{M,ECL}$ values detected from the single base mismatched DNA and the noncomplementary DNA mixture was 4.8% and 6.5% of that obtained from the t-DNA, demonstrating the high selectivity of the ECL spectrometry.

3.5. Measurement of β2M gene expression level in human breast cancer cells

β2M gene expression level in cells could be measured through quantification of complementary DNA ($DNA_{\beta 2M}$) corresponding to β2M mRNA. The $DNA_{\beta 2M}$ was obtained by synthesizing the double-stranded RNA–DNA hybrids consisting of total RNA from the cell extracts and corresponding complementary DNAs through cellular RNA templates using reverse transcription and then by digesting the RNAs in the RNA–DNA hybrids using ribonuclease. The $DNA_{\beta 2M}$ as the t-DNA was detected using the ECL spectrometry. The linear dynamic range (5.0×10^{-15} to 1.0×10^{-13} mol/L) for the $DNA_{\beta 2M}$ was the same as that for the model t-DNA used above (Fig. 4). From the cell number in sample solution and the detected average $DNA_{\beta 2M}$ concentration ($n = 3$), the average molecule number of β2M mRNA in a cell was 2.0×10^4, which was in agreement with the value (1.8×10^4) provided by School

of Life Sciences, Shandong University, China using real-time quantitative reverse transcription polymerase chain reaction (qRT-PCR) technique.

4. Conclusions

When an optical multi-channel analyzer serves as the detector in ECL measurements, ECL spectra can be easily obtained. The ECL spectrometry can measure ECL emission characteristics such as maximum emission wavelength and spectrum profile. When the ECL spectrometry coupled with MNBs as the carrier of a large number of $Ru(bpy)_3^{2+}$ species is used, the sensitivity of ECL spectrometry for DNA analysis is highly enhanced. The sensitive ECL spectrometry can be used to measure gene expression level in cells. The ECL spectrometry-based strategy for determination of trace DNA proposed here can also be applied in other formats, e.g., immunoassay.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.talanta.2011.12.056.

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