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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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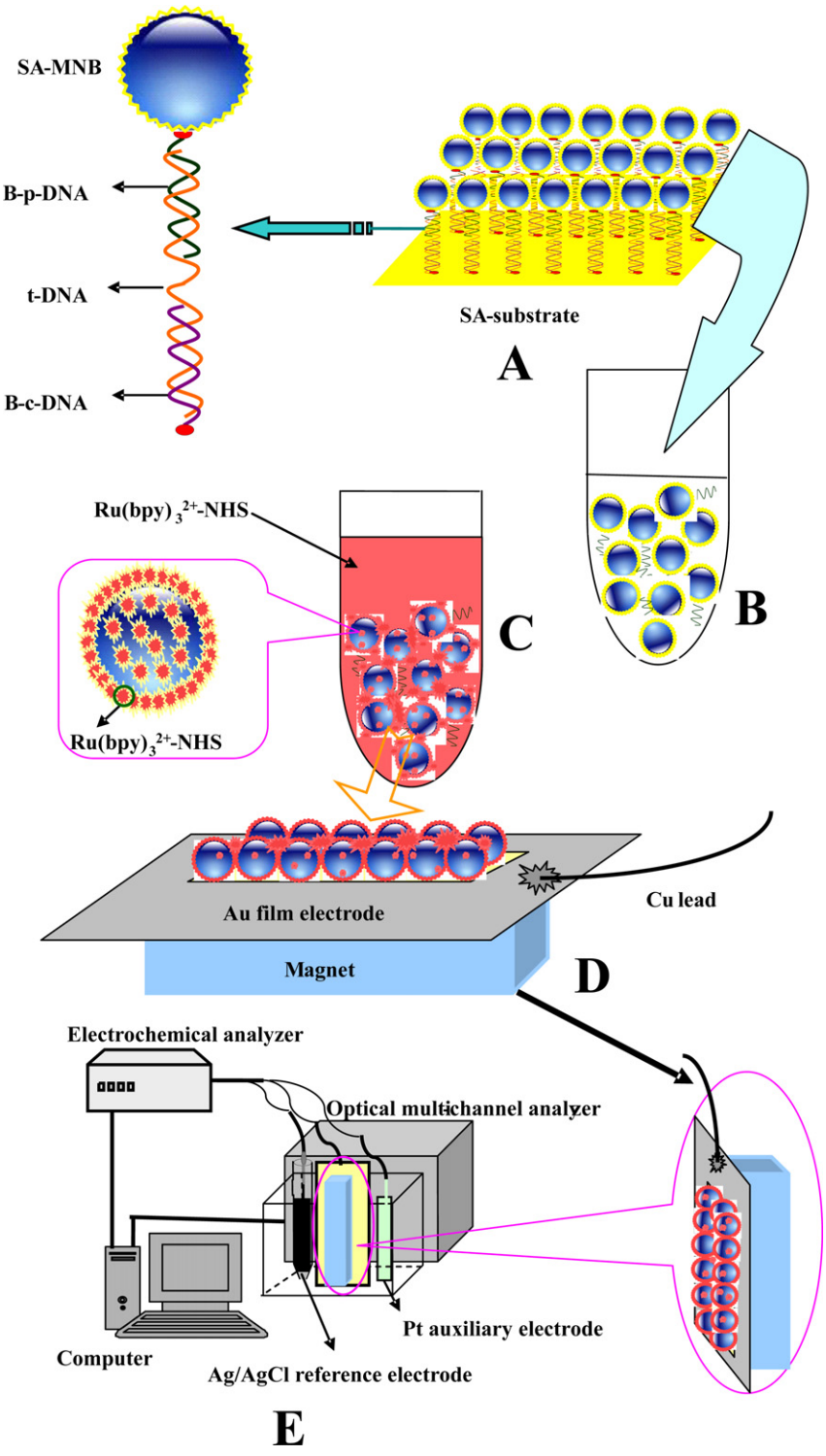

Fig. 1. Schematic representation of the process of ECL spectrometry for determination of DNA by a combination of MNBs attached with $Ru(bpy)_3^{2+}$-NHS as labels and overview of the ECL spectrometric detection system.

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.