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Electrogenerated chemiluminescence biosensing method for the discrimination of DNA hydroxymethylation and assay of the β-glucosyltransferase activity

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ABSTRACT

An electrogenerated chemiluminescence (ECL) biosensing method for highly sensitive discrimination of DNA hydroxymethylation and assay of the β-glucosyltransferase (β-GT) activity was developed. The ECL biosensing electrode was fabricated by gold nanoparticles (AuNPs)/Nafion film, and then, tris(2, 2′-ripyridine) dichlororuthenium(II) ($Ru(bpy)_3^{2+}$) was electrostatically adsorbed into the AuNPs/Nafion film, finally, the hydroxymethylated double-stranded DNA (ds-DNA)-tagged with ferrocene was self-assembled onto the surface of the AuNPs. When β-GT and uridine diphosphoglucose (UDP-Glu) were introduced, the hydroxymethylcytosine residues within 5′-CCGG-3′ of ds-DNA on the biosensing electrode were glucosylated. After the glucosylated biosensing electrode was treated by MspI endonuclease, the unglucosylated hydroxymethylcytosine was cleaved, leading to the quencher leaving the electrode, resulting in an increased ECL signal. For the ECL biosensing method, it showed an extremely low detection limit of 0.04 U/mL for β-GT, and offered a good discrimination toward cytosine, 5-methylcytosine, and 5-hydroxymethylcytosine. This work demonstrates that the combination of the enzyme-linkage reactions with the highly sensitive ECL method is a promising strategy for the discrimination of DNA hydroxymethylation, assay of the activity of β-GT, and evaluation of the capability of inhibitors for the β-GT.

1. Introduction

DNA methylation is an important epigenetic modification of genes, which impacts the heritable state of gene expression in both prokaryotes and eukaryotes (Schübeler, 2015). The recent discovery that the ten-eleven translocation (TET) proteins can oxidize 5-methylcytosine (5-mC) to generate 5-hydroxymethylcytosine (5-hmC) has prompted wide interests (Tahiliani et al., 2009; Kriaucionis and Heintz, 2009; Ito et al., 2011). The highest known levels of 5-hmC are found in the brain and in embryonic stem cells (Globisch et al., 2010; Münzel et al., 2010). Recent studies have revealed that 5-hmC may achieve active or passive DNA demethylation, and it has also been implicated in pluripotency, development and disease (Branco et al., 2011). Therefore, the development of analytical technologies is critical for the detection and discrimination of 5-hmC distribution in the context of sequences or genes.

Phage-encoded glucosyltransferases are known to glucosylate 5-hmC, which can be utilized to detect and analyze the 5-hmC as an epigenetic mark in the mammalian epigenome (Terragni et al., 2012). β-glucosyltransferase (β-GT) of the Escherichia coli (E. coli) T4 bacteriophage is an enzyme, or more specifically an inverting glycosyltransferase. T4 Phage β-GT specifically transfers the glucose moiety of uridine diphosphoglucose (UDP-Glu) to the 5-hmC residues in double-stranded DNA, making β-glucosyl-5-hydroxymethylcytosine (5-ghmC) (Sukovich et al., 2015; Song et al., 2011). The role of the enzyme is to protect the infecting viral DNA from the bacteria's restriction enzymes (Borgaro and Zhu, 2013; Booth et al., 2015; Münzel et al., 2011). Glucosylation prevents the virus DNA from being cut-up. Furthermore, glucosylation may help gene expression of the bacteriophage by influencing transcription. Therefore, sensitive determination of the activity of β-GT and fast screening of its inhibitors has attracted significant attention from both biochemical and clinical researchers. Currently, many methods for glycosyltransferases are based on the detection of glycosylated products by using chromatographic, radiochemical, spectrophotometric, or immunological techniques (Wagner and Pesnot, 2010). Although these methods are effective, they mostly suffered from the shortcoming of expensive equipment, laborious treatment, as well as the low sensitivity. Therefore, it is of great

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significance to develop a highly sensitive method for DNA hydroxymethylation discrimination and β-GT activity assays. Interestingly, 5-hmC can be selectively glucosylated by T4 Phage β-GT to form β-glucosyl-5-hydroxymethylcytosine (5-ghmC), which is resistant to the cleaving activities of some methylationinsensitive restriction enzymes, e.g. MspI (Terragni et al., 2012; Sukovich et al., 2015; Kinney et al., 2011; Zhao et al., 2014). Therefore, 5-hmC (in its glucosylated state) but not 5-mC can be retained at short and cleavable sites of restriction endonucleases for the specific genes or sequences.

Electrogenerated chemiluminescence (ECL) is a method of generating light by using electrochemical reactions to produce highly reactive species at the surface of an electrode that can produce excited states in energetic electron transfer reactions (Liu and Bard, 2008). ECL has received considerable attention during the past several decades owing to its inherent features, such as low background, high sensitivity, good reproducibility, and selectivity (Miao, 2008; Sun et al., 2010). ECL has been widely used in many chemical and biochemical related applications, including immunoassay, DNA damage detection, DNA methylation detection, cancer cells detection, food and water testing, as well as for biowarfare agent or explosive material detection (Dennany et al., 2004; Miao, 2008; Li et al., 2012; Yang et al., 2013; Nie et al., 2013; Liu et al., 2015a, 2015b; Jiang et al., 2015). Despite these advantages, ECL biosensing methods for the discrimination of DNA hydroxymethylation and assay of the β-GT activity have not been reported. The aim of present work is to develop a highly sensitive ECL biosensing method for the discrimination of DNA hydroxymethylation and assay of the β-GT. A schematic diagram of the fabrication process of ECL biosensing electrode for the discrimination of DNA hydroxymethylation and assay of β-GT activity is shown in Fig. 1. In this paper, the fabrication of the ECL biosensing electrode and the analytical performance for the discrimination of DNA hydroxymethylation and assay of the β-GT were presented.

2. Experimental

2.1. Reagents and apparatus

Tris(2, 2′-bipyridine) dichlororuthenium(II) hexahydrate ($Ru(bpy)_3^{2+}$), tris(2-carboxyethyl) phosphinehydrochloride (TCEP, 98%), dithiothreitol (DTT), 6-mercapto-1-hexanol (MCH), Nafion (perfluorinated ion-exchangeresin, 5 wt% solution in a mixture of lower aliphatic alcohols and water) and chloroauric acid ($HAuCl_4$) were obtained from Sigma-Aldrich (USA). 0.1 M phosphate buffered saline (PBS) consisted of 0.1 M NaH_2PO_4, 0.1 M Na_2HPO_4 and

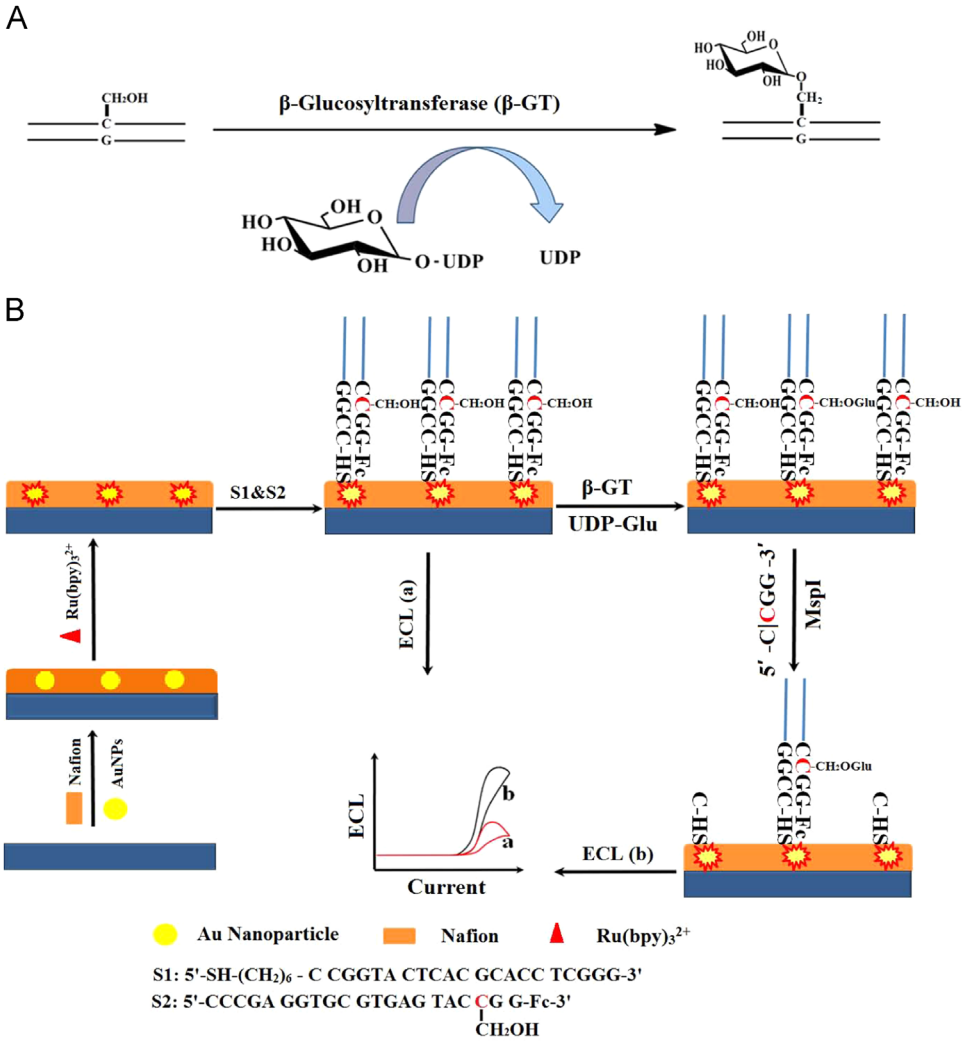

Fig. 1. Schematic diagram of the ECL biosensing method for the detection of DNA hydroxymethylation and assay of β-glucosyltransferase activity.