ELECTROCHEMILUMINESCENCE CHIP FOR METHYL-CYTOSINE DETERMINATION IN DNA

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ABSTRACT

This paper reports the first array type chip for DNA methylation assay by electrochemiluminescence (ECL) detection. This was achieved using a small volume (141-nL) sample between two thin film electrodes that enabled us to observe an ECL emission derived from an anti methyl-cytosine antibody with a high signal-to-noise ratio. We performed a single methyl-cytosine measurement in DNA without the conventional bisulfite reaction, PCR amplification or sequencing.

KEYWORDS

Cytosine methylation, Epigenetics, DNA, Electrogenerated Chemiluminescence

INTRODUCTION

DNA methylation is a well-known epigenetic modification mechanism that regulates gene expression and plays crucial roles. Cytosine methylation in CpG islands has received particular attention because it is thought to be involved in controlling genetic expression, including that cancer , genomic imprinting , cellular differentiation and Alzheimer's disease. Methyl-cytosine is now recognized as the fifth DNA base containing heritable information. Therefore, highly sensitive, accurate and quantitative information concerning cytosine methylation in DNA would be valuable with respect to future genetic disease diagnosis.

Two major analytical methods have been reported for cytosine methylation. These methods are hydrolysis and sequencing with a bisulfite salt, and a cleavage assay with methyl-cytosine sensitive (or insensitive) restriction enzymes. A bisulfite based determination method is most often used to distinguish either cytosine or methyl-cytosine in a DNA sequence. Treatment with bisulfite converts cytosine to uracil, while methyl-cytosine remains unaffected. Therefore, information about the methyl-cytosine in DNA can be obtained by combining the bisulfite treatment and a polymerase chain reaction (PCR). A restriction enzyme, which selectively catalyzes a scission of the specific sequence containing methyl-cytosine, can also be used to determine the methylation status in DNA. However, a DNA sample is damaged by the strand scission that occurs during the enzyme reaction, and also by the bisulfite treatment. A non-cleavage assay is more suitable because nonspecific cleavage by the bisulfite and the restriction enzyme is inevitable at present, and this degrades the quantitative performance. Recently, a non-cleavage assay has been reported that was realized by labeling methyl-cytosine with a fluorescent dye via an osmium complex. Unfortunately, thymine is also labeled by the fluorescent dye via osmium tetroxide in addition to methyl-cytosine. This is because the C5-C6 double bond of thymine in DNA is also oxidized by osmium tetroxide. Therefore, the methyl-cytosine assay becomes complicated because we have to combine fluorescence resonance energy transfer (FRET) technology and a fluorescence labeled oligomer to distinguish methyl-cytosine from thymine. Some researchers have reported a non-cleavage assay with an anti-methyl cytosine antibody labeled with fluorescent dye. However, to obtain high sensitivity, they employed a huge and expensive excitation light source such as a Nd-YAG or He-Ne laser, and combined this with a separation technique such as capillary electrophoresis.

Electrogenerated chemiluminescence (ECL) has attracted great interest over the past two decades in relation to converting electrical energy into radiative energy essentially due to its high potential for a wide range of applications including biological analysis. Several researchers have focused on DNA determination by ECL, and some methods using $Ru(bpy)_3^{2+}$ and tripropylamine system have been developed The background level of the ECL based assay is known to be extremely low since no excitation light is used. Therefore, the ECL based DNA assay is considered to achieve a low detection limit more easily and inexpensively than fluorescence detection. For example, DNA hybridization was measured with a luminophore labeled probe DNA. Electrostatic bindings between ruthenium complexes and DNA have been measured as an ECL emission. However, there has been no report regarding the determination of methyl-cytosine in DNA by ECL. This is because selective ECL emission derived solely from methyl-cytosine is impossible using conventional electrostatic binding or hybridization techniques. In this paper, we report the first array type chip for DNA methylation assay by ECL.

EXPERIMENT

Figure 1 shows a photograph and a representation of an ECL chip for DNA methylation measurement. The chip consists of two film electrodes (gold and indium tin oxide (ITO)), separated with an 80-μm spacer. First, a DNA was immobilized on the gold surface (Fig. 2-2), and then an anti methyl-cytosine antibody labeled with tris(2,2-bipyridyl)ruthenium complex was used to recognize cytosine methylation (Fig. 2-3). Finally, ECL was observed with a CCD camera by applying a voltage between the two electrodes for 10 sec after filling the array with tripropylamine (TPA) and covering it with the ITO electrode (Fig. 2-4).

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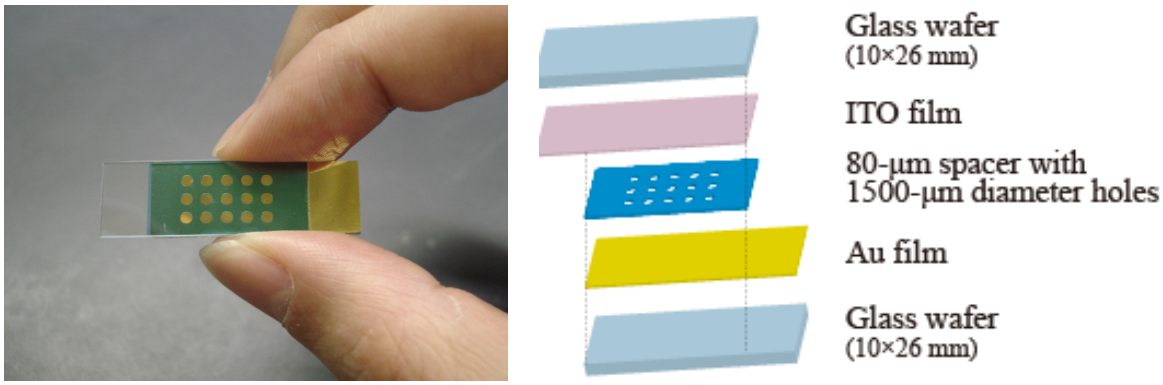

Figure 1 Photograph (left) and representation (right) of ECL chip for DNA methylation analysis.

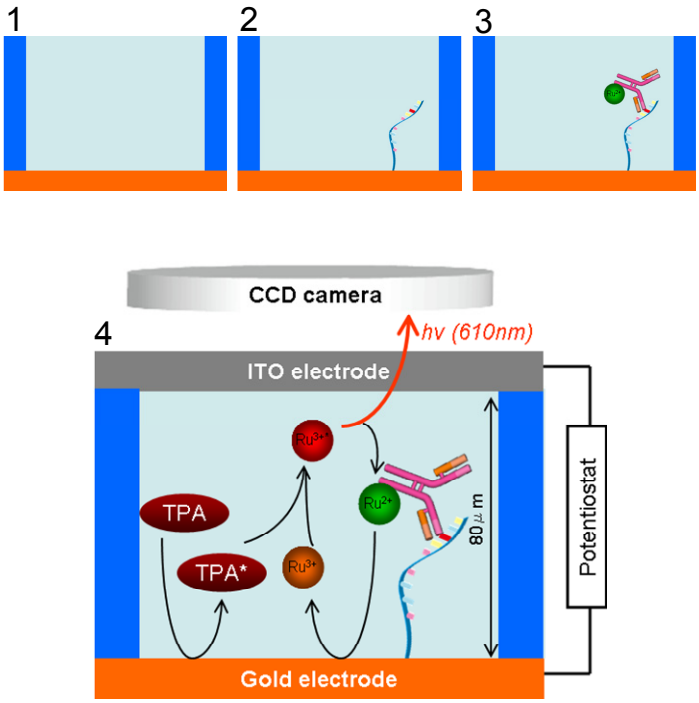

Figure 2 DNA methylation procedure. 1) Preparation of gold film electrode with a spacer. 2) DNA immobilization on gold surface. 3) Immunoreaction with anti methyl-cytosine antibody labeled with tris(2,2-bipyridyl)ruthenium. 4) Cover with transparent ITO electrode for ECL observation.

RESULTS and Discussion

Figure 3 shows the variation in the ECL image when the voltage is varied. The brightest ECL was observed at 2400 mV, which indicates that the reaction of the TPA radical with oxidized tris(2,2-bipyridyl)ruthenium was optimum at this voltage. Figure 4 shows ECL images for methyl-cytosine containing DNA. The DNA sequence is 5'-CTTTTTCTTTTTGTCCTTTTTAGGCTCTGT-3' (the underlined cytosine is methylated), which is part of the MGMT promoter. The ECL intensity clearly increased as the antibody concentration increased. Figure 5 compares ECL images for methylated and non-methylated DNA. The brighter ECL image was clearly obtained with the single-cytosine methylated DNA.

This highly sensitive single methylation measurement is realized due to the 100-% oxidation of the ruthenium complexes and the subsequent chemical reaction with TPA radicals in a short CCD exposure time (10 sec). This is because the micro-well depth (distance between two electrodes) is only 80 μm, which is much shorter than the diffusion distance in 10 sec (around 140 μm). Very recently, we reported an ECL-based DNA methylation assay [2]. Since the immunoreactions were performed on a conventional microtiter plate, the resulting immunoassay fluids were injected repeatedly into a separate electrochemical cell to observe the ECL. In this study, all the

immunoreactions and detection were performed on an arrayed micro-well with a gold electrode for electrogeneration, therefore arrayed ECL emissions can be immediately and simultaneously obtained with a small volume.

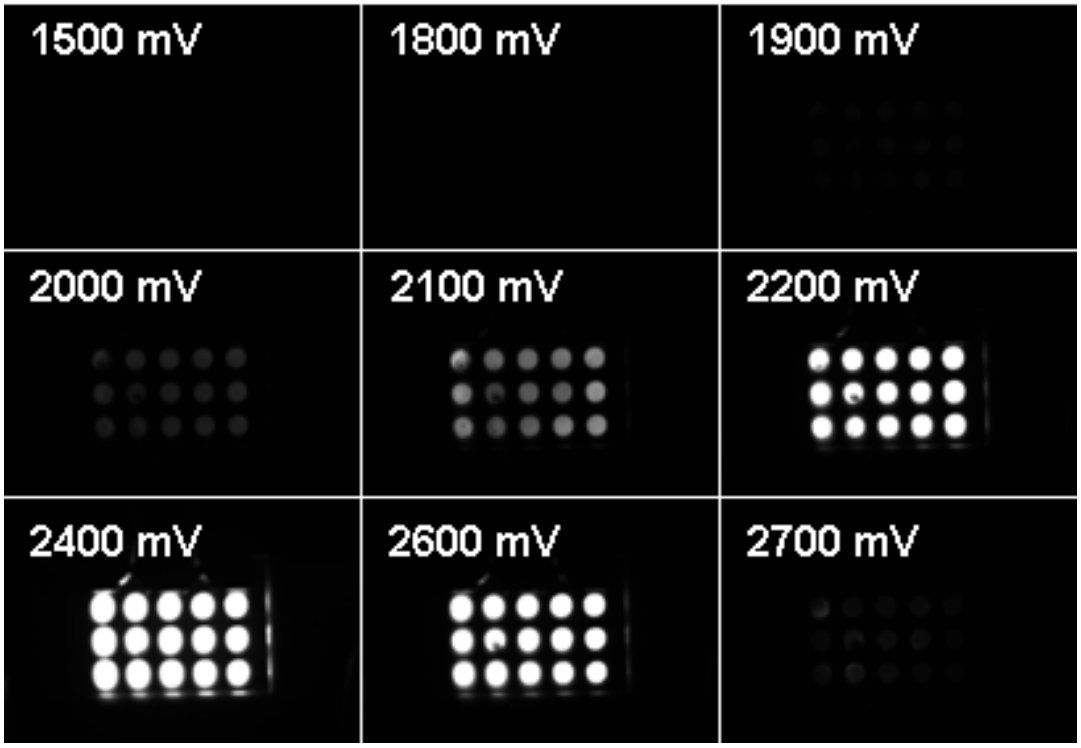

Figure 3 ECL images when varying applied potential.

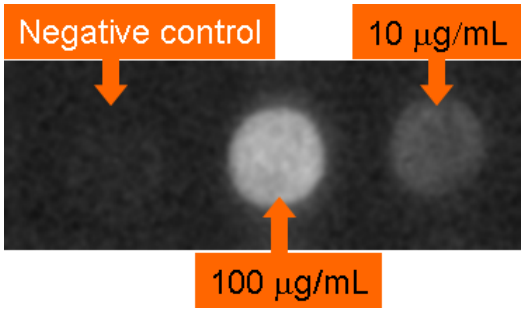

Figure 4. ECL images of methyl-cytosine containing DNA detected with a 100 or 10 μg/mL anti methyl-cytosine antibody solution.

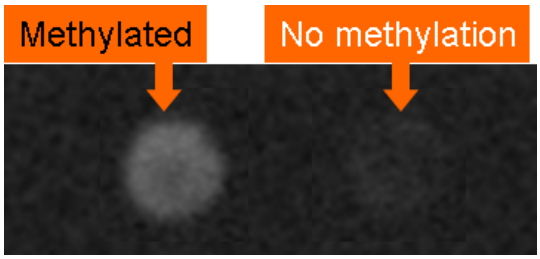

Figure 5. ECL images of DNA containing single methylated cytosine and no methylated cytosine.

REFERENCES

[1] Ryoji Kurita et al, Determination of DNA Methylation using Electrochemiluminescence with Surface Accumulable Coreactant, *Analytical Chemistry*, 84 (2012) 1799-1803.

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