Development of electrochemical detection for methylcytosine and its application

Kazuo Tanaka¹, Kazuki Tainaka¹, Taku Kamei² and Akimitsu Okamoto¹,²

¹Frontier Research System, RIKEN, 2-1 Hirosawa, Wako, Saitama 351-0198, Japan and ²Department of Synthetic Chemistry and Biological Chemistry, Faculty of Engineering, Kyoto University, Kyoto 615-8510, Japan

ABSTRACT

We report electrochemical detection of methylcytosine. We developed a bipyridine ligand possessing an amino linker. This ligand was used for methylcytosine-selective osmium oxidation and subsequent redox labeling. The DNA labeled methylcytosine selectively with anthraquinone showed the current signal through hybridization with a DNA probe fixed on a gold surface.

INTRODUCTION

Hypermethylation at CpG sequences in the promoter region plays a significant role in gene deactivation, and it has been implicated in the pathogenesis of cancers.¹ Therefore, the simple detection method for the cytosine methylation is strongly desired for early diagnosis.

Electrochemical sensors for biomolecules enable the simple and high-throughput assay. For constructing an effective method for a high-throughput detection of methylation in the future, the electrochemical method is one of candidates to be established strongly desired. However, there remains the great difficulty to develop them because there is only a slight difference in the physical properties of cytosine and methylcytosine.

We report a chemical labeling method for methylcytosine using the electroactive molecule, anthraquinone (AQ), which has often been used as an effective redox-active material for electroanalytical chemistry. Methylcytosin-osmium-ligand triad was formed only on methylcytosine via osmium oxidation in the presence of the synthetic ligand 1, which is connected with AQ after the complex formation. In addition, we demonstrated the discrimination between cytosine and methylation with electrochemical signals.

RESULTS AND DISCUSSION

We designed a bipyridine ligand 1, possessing an amino linker (Figure 1). This compound was expected to act as a ligand for an efficient methylcytosine-selective osmium oxidation and also to make possible the postsynthetic modification of the amino group with redox-active molecules. We synthesized 1 and used it for osmium oxidation.²

Osmium oxidation was executed with ODNs (ODN(N), 5'-d(AAAAAGNGAAAA)-3', N = cytosine or methylcytosine), that possessed a single pyrimidine base for evaluating the selectivity of osmium complex between cytosine and methylcytosine in the presence of 1. ODN(N) was added to a mixture of 5 mM potassium osmate, 100 mM potassium hexacyanoferrate(III), and 100 mM the ligand 1 in 100 mM Tris–HCl buffer (pH = 7.7), 1 mM EDTA, and 10% acetonitrile, and then the reaction mixture was incubated at 37 °C for 1 h.² The labeling reaction for resulting osmium-ligand-methylcytosine triad proceeded quantitatively by treating with N-hydroxysuccinimidyl ester of anthraquinonecarboxylic acid at room temperature for 3 h.

A thiolated complementary strand (cODN 5'-d(TTTTTTCGCTTTTT)-SH-3') was immobilized on a quartz crystal microbalance (QCM) surface and questioned with DNA solutions modified with ODN(Me₃-AQ). We monitored the change in mass of the QCM due to adsorption of DNA molecules on the surface. We also used a square wave voltammetry (SWV) for the electrodes modified by ODN(C)/cODN, ODN(Me₃)/cODN and ODN(Me₃-AQ)/cODN. SWV was measured in 1 M NaCl with DNA modified electrode with SCE for the reference electrode and Pt for the counter electrode. Pulse amplitude, 10mV; pulse width 50 ms; frequency 15 Hz.

Figure 1. The synthetic ligand to be labeled with AQ.

Figure 2. Square wave voltammetry (SWV) for the electrodes modified by ODN(C)/cODN, ODN(Me₃)/cODN and ODN(Me₃-AQ)/cODN. SWV was measured in 1 M NaCl with DNA modified electrode with SCE for the reference electrode and Pt for the counter electrode. Pulse amplitude, 10mV; pulse width 50 ms; frequency 15 Hz.
gold electrode using sulfur-gold interaction. Mixed monolayer surfaces containing a thiolated DNA and 6-mercaptop-1-hexanol (MCH) were prepared by immersing a gold electrode (2 mm$^2$ in area) in a 10 μM solution of a thiolated DNA, followed by exposure of the gold surface to an aqueous solution of 1 mM MCH to minimize any non-specific adsorption of the thiolated DNA. By hybridization with the AQ-labeled target DNA, the DNA duplex was assembled on the surface of the gold electrode (5.99 ± 0.52) × 10$^{12}$ DNA cm$^{-2}$).  

Electrochemical measurements on the DNA-modified gold electrode were carried out in 1 M sodium chloride using square wave voltammetry (SWV). SCE was used as a reference electrode. The current responses of the duplexes are shown in Figure 2. For duplexes ODN(C) cODN and ODN(M$_{6}$)-AQ cODN, no significant peak was obtained from SWV measurements. On the other hand, ODN(M$_{6}$)-AQ cODN showed the current signal at −0.7 V, which was the redox potential of AQ versus SCE.

CONCLUSION

In conclusion, we established methylcytosine-selective electrochemical labeling method. The methylcytosine-selective current signal enables us to distinguish 5-methylcytosines from cytosines. Our system would be very useful to an electrochemical epigenotyping system.

REFERENCES


