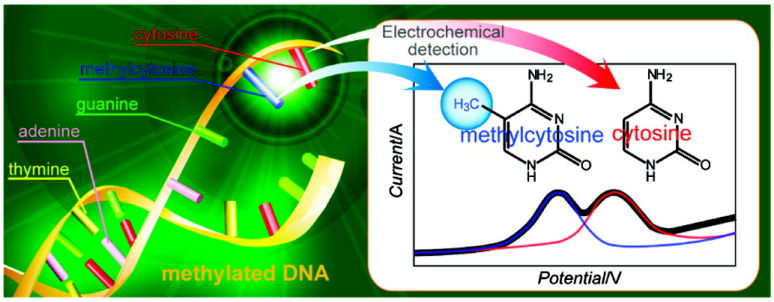
Communication

A Nanocarbon Film Electrode as a Platform for Exploring DNA Methylation

Dai Kato, Naoyuki Sekioka, Akio Ueda, Ryoji Kurita, Shigeru Hirono, Koji Suzuki, and Osamu Niwa

J. Am. Chem. Soc., **2008**, 130 (12), 3716-3717 • DOI: 10.1021/ja710536p



More About This Article

Additional resources and features associated with this article are available within the HTML version:

- Supporting Information
- Links to the 2 articles that cite this article, as of the time of this article download
- Access to high resolution figures
- Links to articles and content related to this article
- Copyright permission to reproduce figures and/or text from this article

View the Full Text HTML

J|A|C|S COMMUNICATIONS

Published on Web 03/04/2008

A Nanocarbon Film Electrode as a Platform for Exploring DNA Methylation

Dai Kato,[†,⊥] Naoyuki Sekioka,[†,‡] Akio Ueda,[†,§] Ryoji Kurita,[†] Shigeru Hirono,[∥] Koji Suzuki,[#,⊥] and Osamu Niwa*[,†,‡,§,⊥]

National Institute of Advanced Industrial Science and Technology, 1-1-1 Higashi, Tsukuba, Ibaraki 305-8566, Japan, University of Tsukuba, 1-1-1 Tenno-dai, Tsukuba, Ibaraki, 305-8571 Japan, Tokyo Institute of Technology, 4259 Nagatsuta-cho, Midori-ku, Yokohama 226-8503, Japan, MES-Afty Corporation, 2-35-2 Hyoe, Hachioji, Tokyo 192-0918, Japan, Keio University, 3-14-1 Kohoku-ku, Yokohama, Kanagawa, 223-8522, Japan, JST-CREST, 4-1-8, Honcho, Kawaguchi, Saitama 332-0012, Japan

Received November 22, 2007; E-mail: niwa.o@aist.go.jp

Epigenetic modification, such as DNA methylation and histone modification, has attracted attention because it plays an important role in the regulation of gene expression without any change in the DNA sequence. Genomic DNA methylation has frequently been observed at C5 of cytosine (C) in the 5′-CG-3′ sequence (CpG). It is predicted that 60−90% of C at CpG islands, which consist of several tens to hundreds of CpG repetitions, occur as a result of acquired methylation. In cancer cells, when promoter CpG islands in tumor suppressor genes including RB and BRCA1 are methylated, their downstream genes are found to be consistently silenced.[1] This is a new carcinogenesis mechanism without any DNA sequence change, which is unlike gene defects or SNPs. Methylcytosine (mC) is now recognized as the fifth DNA base containing heritable information, and therefore an analysis of DNA methylation is essential with respect to future genetic disease diagnosis.

A bisulfite[2−5] or a restriction enzyme[6] is widely used to distinguish both C and mC in DNA. In a bisulfite assay, C is deaminated and then converted to uracil (U), whereas mC remains almost unchanged.[2−5] The restriction enzymes catalyze the scission reaction of the specific base sequence. When the C in the specific sequence is methylated, the scission is inhibited. The use of the above techniques commonly provides a C-positive assay. The bisulfite-based methods are very suitable assays, but they are time-consuming. By contrast, Tanaka et al. have recently reported that an osmium compound can selectively bind mC but not C.[7]

Electrochemical techniques have been widely used for DNA analysis.[8] DNA detection based on direct electrochemical oxidation is the simplest approach.[8−12] If we can oxidize both C and mC in DNA or an oligonucleotide with sufficient potential difference by the voltammetric method, DNA methylation could be distinguished without any treatment as shown in Figure 1a. To achieve this requires electrode materials with a wide potential window and sufficiently high electrode activity.

Recently, we developed a nanocarbon film consisting of nanocrystalline sp^2 and sp^3 mixed bonds formed by employing the electron cyclotron resonance (ECR) sputtering method. The film structure is quite different from heterogeneous BDD or amorphous diamond-like carbon.[13] The ECR nanocarbon film electrode has excellent properties including an atomically flat surface, a wide electrochemical potential window, and little surface fouling while maintaining relatively high electrode activity for various biomolecules.[14−15] These properties allow us to obtain direct quantitative measurements of both C and mC bases of oligonucleotides by measuring the potential peak differences caused by C methylation. This enables us to construct a simple, rapid, and low cost assay for DNA methylation. Here we describe the quantitative nonlabel electrochemical detection of both C and mC in oligonucleotides using ECR nanocarbon film electrodes.

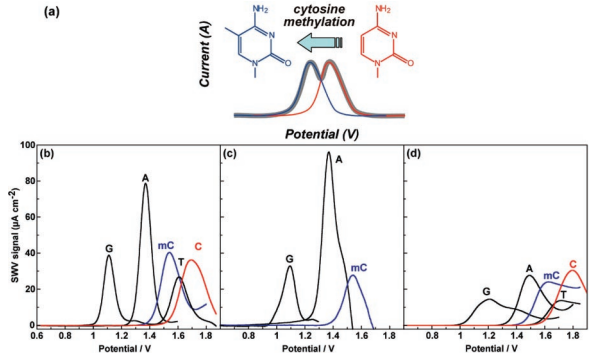


Figure 1. Schematic of electrochemical methylation detection method for single strand oligonucleotides (a) and background-subtracted SWVs of 100 μM of nucleosides at ECR nanocarbon film (b), GC (c), and BDD (d) electrodes, respectively, measured in 50 mM pH 5.0 acetate buffer. Acetate buffer (pH 5.0) is the most appropriate for obtaining well-defined peaks with high sensitivity.[12] Amplitude = 25 mV, ΔE = 5 mV, f = 10 Hz.

We used an ECR nanocarbon film electrode with optimized electrochemical properties by changing the ion acceleration voltage during sputtering and electrochemical pretreatment, respectively, depending on the analytes. A film with an $sp^2/(sp^2 + sp^3)$ ratio of 0.6 has a sufficiently wide potential window with high electrode activity and stability against fouling caused by the biomolecule oxidation.[14−15] Figure 1b−d show background-subtracted square wave voltammograms (SWVs) for each nucleoside. The SWV at the ECR nanocarbon film electrode exhibited all the nucleoside oxidation currents at individual peak potentials. The difference between the oxidation peaks in C and mC was also detectable. The mC peak was about 130−150 mV smaller than that of C (Figure 1b). In the same way, we found that T methylated at C5 of U also exhibited smaller oxidation peaks than U (not shown). As regards the pyrimidine bases, which are methylated at C5, their C5−C6 double bonds appear to be easily oxidized[7] because of the electron donor property of the methyl group. The peak potentials of guanosine, adenosine, and 5-methylcytidine oxidation at the ECR nanocarbon film electrode were almost the same as those at the GC electrode. However, the oxidation peaks of thymidine and cytidine could not be observed at the GC electrode since there was a large background current resulting from the narrow potential window that overlapped the thymidine and cytidine oxidation peaks (Figure 1c). A highly oriented pyrolytic graphite (HOPG) electrode also exhibited similar results to those obtained with the GC electrode (Supporting information). The SWV at the BDD electrode also showed the oxidation peaks of all the bases after the electrode

[†] National Institute of Advanced Industrial Science and Technology.
[‡] University of Tsukuba.
[§] Tokyo Institute of Technology.
[∥] MES-Afty Corporation.
[#] Keio University.
[⊥] JST-CREST.

10.1021/ja710536p CCC: $40.75

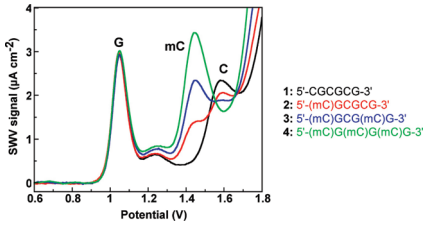


Figure 2. Background subtracted SWVs of 3 μM of CpG oligonucleotides (**1**−**4**) at ECR nanocarbon film electrode in 50 mM pH 5.0 acetate buffer containing 0.3 M $NaNO_3$. The SWV conditions are the same as those in Figure 1.

surface had been anodically oxidized (Supporting information). This treatment provided the BDD electrode with a wider potential window[10−11,16] that was sufficient for detecting the oxidation of thymidine and cytidine. However, the peak potentials were 90−120 mV higher, and the current responses, smaller and much broader than those obtained at the ECR nanocarbon film and GC electrodes (Figure 1d), indicating lower electrochemical activity for each base at the pretreated BDD electrode. This suggests that the electrochemical responses of DNA bases depend on the sp^2-carbon content of the electrode surfaces since polar aromatic analytes such as catechols are strongly adsorbed on sp^2-carbon electrodes owing to $\pi-\pi$ interactions.[16−19] The slow kinetics for these DNA bases at the pretreated BDD electrode is mainly due to weak adsorption on the sp^3 diamond surface despite it having little sp^2-carbon content.[16,18−19] ECR nanocarbon film contains a large sp^2-carbon content of 0.6, which induces high electrode activity as with the GC electrode, while maintaining a wide potential window as with the BDD electrode. This property results from the homogeneous and stable structure consisting solely of nanocrystalline sp^2- and sp^3-carbon hybrids that allowed electrochemical responses for all the bases that were superior to those of the GC or BDD electrodes. The detection limits for all the bases at the ECR nanocarbon electrode were one order lower than that at the GC electrode; namely they were 0.1 μM for guanosine, adenosine, methylcytidine, and thymidine and 1 μM for cytidine.

We measured the short oligonucleotides that constitute a CpG unit with different numbers of mC by using the ECR nanocarbon film electrode. Figure 2 shows background-subtracted SWVs of non-methylated oligonucleotide **1** (5′-CGCGCG-3′) and its methylated oligonucleotides **2**−**4**. We could quantitatively differentiate the voltammograms for these oligonucleotides because the current responses coincided precisely with the base content of each oligonucleotide at the ECR nanocarbon electrode. Moreover, the oxidation currents of both mC and C in the oligonucleotides could be simultaneously determined, revealing the potential for the C methylation ratio in oligonucleotides to be roughly quantified from the heights of the two currents. The SWV of **2** was also measured in the same way with the GC and BDD electrodes (Supporting information). However, with these measurements, it was not possible to distinguish each oxidation current more clearly because of the large background current at the GC electrode and because of the low electrochemical activity at the BDD electrode (despite its wide potential window). This indicates that it is difficult to detect DNA methylation in the low concentration region using GC and BDD electrodes. Figure 3a shows the background-subtracted SWVs of oligonucleotides **5** (5′-TTA(mC)GC-3′) and **6** (5′-TTA(mC)G-(mC)-3′), whose methylation statuses are different. As regards these oligonucleotides that include other bases, especially T, the mC and C peaks obtained from the voltammograms overlapped even when using the ECR nanocarbon film electrode. However, we could achieve excellent discrimination of single C methylation in oligonucleotides by the subtraction of the two voltammograms, without

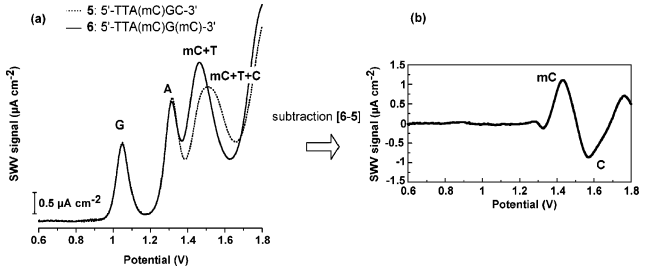


Figure 3. Background subtracted SWVs of 3 μM of oligonucleotide **5** and **6** (a) and the subtraction curve (b) at ECR nanocarbon film electrode in 50 mM pH 5.0 acetate buffer containing 0.3 M $NaNO_3$. The SWV conditions are the same as those in Figure 1.

any current height change for G and A (Figure 3b). It is noteworthy that this method can provide simple DNA methylation detection for a particular sequence without any bisulfite or labeling processes. This subtraction method can also be employed by correcting the peak height of other bases such as G when the two sample concentrations are different.

In conclusion, we successfully used ECR nanocarbon film to develop a simple electrochemical DNA methylation analysis technique. This film allowed us to perform both C- and mC-positive assays solely by using the electrochemical oxidation of oligonucleotides without bisulfite or labeling processes. We will develop this film to achieve a more sophisticated DNA methylation electroanalysis technique with a focus to extending it to various sequences including longer DNA fragments or between double and single strand DNA. ECR nanocarbon film can also be applied to various DNA sensor platforms combined with enzymatic or separation technologies because this film electrode can measure all the bases.

Supporting Information Available: SWVs of each nucleoside at the HOPG electrode and of oligonucleotide **2** at GC and BDD electrodes. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Ushijima, T. *Nat. Rev. Cancer* **2005**, *5*, 223−231.
(2) Debono, M.; Molloy, R. M.; Bauer, D.; Iizuka, T.; Schaffner, K.; Jeger, O. *J. Am. Chem. Soc.* **1970**, *92*, 422−424.
(3) Frommer, M.; McDonald, L. E.; Millar, D. S.; Collis, C. M.; Watt, F.; Grigg, G. W.; Molloy, P. L.; Paul, C. L. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 1827−1831.
(4) Herman, J. G.; Graff, J. R.; Myöhänen, S.; Nelkin, B. D.; Baylin, S. B. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 9821−9826.
(5) Chen, R. Z.; Pettersson, U.; Beard, C.; Jackson-Grusby, L.; Jaenisch, R. *Nature* **1998**, *395*, 89−93.
(6) Toyota, M.; Ho, C.; Ahuja, N.; Jair, K. W.; Li, Q.; Ohe-Toyota, M.; Baylin, S. B.; Issa, J. P. J. *Cancer Res.* **1999**, *59*, 2307−2312.
(7) Tanaka, K.; Tainaka, K.; Kamei, T.; Okamoto, A. *J. Am. Chem. Soc.* **2007**, *129*, 5612−5620.
(8) Drummond, T. G.; Hill, M. G.; Barton, J. K. *Nat. Biotechnol.* **2003**, *21*, 1192−1199.
(9) Oliveira-Brett, A. M.; Piedade, J. A. P.; Silva, L. A.; Diculescu, V. C. *Anal. Biochem.* **2004**, *332*, 321−329.
(10) Ivandini, T. A.; Sarada, B. V.; Rao, T. N.; Fujishima, A. *Analyst* **2003**, *128*, 924−929.
(11) Ivandini, T. A.; Honda, K.; Rao, T. N.; Fujishima, A.; Einaga, Y. *Talanta* **2007**, *71*, 648−655.
(12) Prado, C.; Flechsig, G. U.; Grundler, P.; Foord, J. S.; Marken, F.; Compton, R. G. *Analyst* **2002**, *127*, 329−332.
(13) Hirono, S.; Umemura, S.; Kaneko, R. *Appl. Phys. Lett.* **2002**, *80*, 425−427.
(14) Niwa, O.; Jia, J.; Sato, Y.; Kato, D.; Kurita, R.; Maruyama, K.; Suzuki, K.; Hirono, S. *J. Am. Chem. Soc.* **2006**, *128*, 7144−7145.
(15) Jia, J. B.; Kato, D.; Kurita, R.; Sato, Y.; Maruyama, K.; Suzuki, K.; Hirono, S.; Ando, T.; Niwa, O. *Anal. Chem.* **2007**, *79*, 98−105.
(16) Duo, I.; Fujishima, A.; Comninellis, C. H. *Electrochem. Commun.* **2003**, *5*, 695−700.
(17) Chen, P.; McCreery, R. L. *Anal. Chem.* **1996**, *68*, 3958−3965.
(18) Show, Y.; Witek, M. A.; Sonthalia, P.; Swain, G. M. *Chem. Mater.* **2003**, *15*, 879−888.
(19) Bennett, J. A.; Wang, J. A.: Show, Y.; Swain, G. M. *J. Electrochem. Soc.* **2004**, *151*, E306−E313.

JA710536P