Biosensors and Bioelectronics 70 (2015) 366–371

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: www.elsevier.com/locate/bios

Site-specific immunochemical methylation assessment from genome DNA utilizing a conformational difference between looped-out target and stacked-in nontarget methylcytosines

Ryoji Kurita*, Hiroyuki Yanagisawa, Kyoko Yoshioka, Osamu Niwa

National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba Central 6, 1-1-1 Higashi, Tsukuba, Ibaraki, 305-8566, Japan

ARTICLE INFO

Article history:
Received 26 December 2014
Received in revised form
23 March 2015
Accepted 24 March 2015
Available online 28 March 2015

Keywords:
DNA methylation
Immunoassay
Surface plasmon resonance
Epigenetics

ABSTRACT

We report the sequence-selective immunochemical discrimination of methylcytosine from genomic DNA that we achieved by utilizing selective antibody binding to a looped-out methylcytosine in a bulge region and without using bisulfite treatment, a methylation-sensitive restriction enzyme, or PCR. First, we investigated the affinity of an anti-methylcytosine antibody for methylcytosine in full match, bulge, mismatch and abasic sites using surface plasmon resonance measurement and a microtiter plate to explore the differences as regards antibody binding to the target methylcytosine. The highest affinity, which was comparable to that in a single strand of DNA, was observed in the bulge region. In particular, no affinity was observed in a full match site. This is because there is no interaction such as hydrogen bond or π–π stacking for the bulged methylcytosine, thus enabling only the target in the bulge to be looped out. Methylated and unmethylated genomic DNA were blended to form a model DNA with which to assess the methylation ratio at a specific site. Fragmented DNA was hybridized with a biotinylated probe DNA, which has a sequence capable of forming a single base bulge at the target. The probe design is simple because it consists solely of the elimination of guanine paired with the target cytosine from a full match sequence. As a result, we successfully obtained a linear relationship ($r^2=0.9962$) between the immunoassay signal and the methylation ratio of a specific site within 4 h.

1. Introduction

The methylation of the 5′ carbon of cytosine in DNA is an epigenetic modification that regulates gene expression and plays crucial roles in embryonic development (Yoder et al., 1997). Cytosine methylation at CpG islands has received particular attention because it is thought to be involved in controlling genetic expression (Doerfler, 1984), including that in cancer (Jones, 1996), genomic imprinting (Monk, 1995), cellular differentiation and Alzheimer's disease (Ledoux et al., 1994). 5-methylcytosine (^{m}C) is now recognized as the fifth DNA base containing heritable information.

Bisulfite-based sequencing is the gold standard for methylation analysis (Cokus et al., 2008; Frommer et al., 1992; Herman et al., 1996). However, DNA degradation occurs during the time-consuming bisulfite treatment owing to oxidative damage, namely depurination, under the required acidic and thermal conditions. Only 0.1% of the original DNA is reported to remain after 16 h of bisulfite treatment (Tanaka and Okamoto, 2007). Moreover, the bisulfite treatment makes the analyte DNA thymine rich since unmethylated cytosine is converted to thymine, and this complicates the design of specific probes for PCR amplification. Otherwise, methylation-sensitive restriction enzyme-based methods have been used for the site-specific detection of DNA methylation (Rush and Plass, 2002; Xiong and Laird, 1997), because certain very important CpG sites were recently reported that affect gene regulation with a single methylation or that are closely related to diseases (Fuerst et al., 2012; Ling et al., 2013; Nanduri et al., 2012; Nile et al., 2008) Unfortunately, these techniques may be biased and limited because they depend on the available methylation-sensitive restriction enzyme. Therefore, new methylcytosine assays that do not require bisulfite treatment or a methylation-sensitive restriction enzyme have been investigated (Bareyt and Carell, 2008; Dadmehr et al., 2014; Geng et al., 2014; Tanaka et al., 2007a; Yu et al., 2010; Zhu et al., 2015). For example, methods have been reported for labeling methylcytosine with methyl-CpG binding proteins (Acevedo et al., 2011; Yu et al., 2010), osmium complex and vanadium complex (Bareyt and Carell, 2008; Tanaka et al., 2007a; Tanaka et al., 2007b). Unfortunately, thymine is

* Corresponding author.
E-mail address: r.kurita@aist.go.jp (R. Kurita).

http://dx.doi.org/10.1016/j.bios.2015.03.061
0956-5663/

labeled via OsO_4 as well as methylcytosine. Therefore, this complicates the distinction of methylcytosine from thymine by OsO_4 labeling. Mixtures of V_2O_5 or $NaIO_4$ and LiBr were used in an anaerobic condition to distinguish methylcytosine from both cytosine and thymine, followed by a hot piperidine treatment and electrophoretic analysis.

We have briefly reported that an anti-methylcytosine antibody can recognize methylcytosine in a mismatch region, but cannot recognize methylcytosine in a full match duplex with a synthesized oligo-DNA (Kurita and Niwa, 2012). This implies the possibility of the site-specific discrimination of the methylation status of genome DNA by utilizing the affinity difference between mismatched and paired methylcytosines. However, the number of methylcytosines in the full match region is much greater than the number of target methylcytosines in genome DNA. Therefore, an appropriate pretreatment and a large kinetic difference between the two DNA structures are required for the recognition of a target methylcytosine in a specific genome DNA sequence. The conformation of methylcytosine located in various structures should be studied to understand the effect of the DNA structures on sequence-selective methylcytosine detection.

DNA structures containing mismatches have already been analyzed using biophysical techniques such as NMR (Kalnik et al., 1989,1990; Nikonowicz et al., 1989), electro paramagnetic resonance (EPR) (Cekan and Sigurdsson, 2012), X-ray crystallography (Joshuator et al., 1988) and FRET (Gohlke et al., 1994). However, crystallographic analysis, for example, indicated that a mismatched base had a looped-out structure, while an NMR study of the same sequence showed that the base was stacked in the duplex. It is important to perform a kinetic assessment under biological conditions because it has been suggested that a mismatch base is in a conformational equilibrium between looped-out and stacked-in states (Baase et al., 2009; Cekan and Sigurdsson, 2012).

In this paper, we report an immunochemical approach for studying the conformation of mismatched methylcytosines in DNA that employs a kinetic assessment with surface plasmon resonance (SPR) (Scarano et al., 2010; Su et al., 2005). We reveal that the methylcytosine at a single base bulge is predominantly in a looped-out state, and we also consider the results of a melting curve analysis. This makes it possible to undertake a sequence-selective immunochemical CpG methylation analysis of genome DNA on a conventional microtiter plate with a bulge-inducing probe DNA, as summarized in Fig. 1.

2. Experimental section

2.1. Analyte and probe DNA designs

The analyte DNA sequence is shown in Table 1S and is part of the MGMT promoter gene. The sequences of Analyte-1–4 were the same, however the methylation region (underlined in Table 1S)

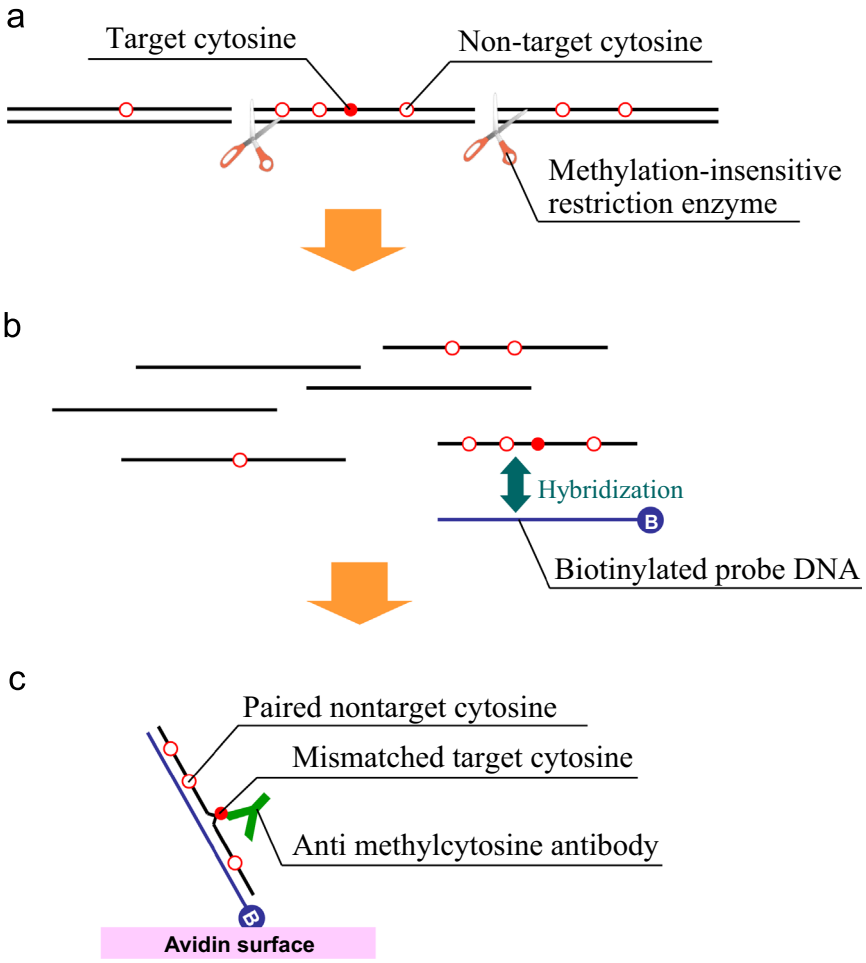

Fig. 1. Schematic procedure for sequence-selective methylcytosine determination. (a) Sample DNA is fragmented by a methylation-insensitive restriction enzyme. (b) Biotinylated probe DNA is added to the fragmented DNA. The mixture is denatured by heating it, and then cooled for hybridization. The biotinylated probe DNA has an almost complementary sequence for hybridizing the sequence of interest, and for forming a mismatch region at the target cytosine. (c) A biotinylated duplex is collected on an avidin surface, and then an immunoassay is performed with an anti-methylcytosine antibody.