Photochemical Site-specific Mutation of 5-Methylcytosine to Thymine

Takashi Matsumura,[1] Masayuki Ogino,[2] Kango Nagayoshi,[1] and Kenzo Fujimoto*[1,2]
[1]*School of Materials Science, Japan Advanced Institute of Science and Technology, Ishikawa 923-1292*
[2]*Japan Science and Technology Agency, Ishikawa 923-1211*

(Received September 3, 2007; CL-070945; E-mail: kenzo@jaist.ac.jp)

In this study, we report that the coupling of 5-methylcytosine with 5-carbamoylvinyl-deoxyuridine-mediated photoligation caused the heat-induced transition from 5-methylcytosine to thymine. Deamination of 5-methylcytosine to thymine proceeded via the formation of cyclobutane dimer.

DNA methylation has attracted interest recently. 5-Methylcytosine appears in genomic DNA, particularly in the CpG sequence. DNA methylation in mammalian cells occurs at the C5-position of cytosine within the CpG dinucleotide. Approximately 70% of CpG are methylated, but neither 5-methylcytosine distribution nor the spatial distribution of the CpG dinucleotide itself is random.[1] 5-Methylcytosine on the DNA plays very important roles in gene silencing and cell type-specific gene expression.[2,3] Genome methylation may specifically change to activate genes that affect the determination of cell fate.[3] Remarkably, 5-methylcytosine on the genome is spontaneously converted to thymine in the deamination reaction.[3]

Sasaki et al. established NO-induced deamination of a target cytidine and 5-methylcytidine.[4] The selectivity and efficiency of NO transfer followed by deamination exhibited in this study were extremely high compared to those of conventional methods using NO gas or other nitrosation agents.[4] We now report on photochemical site-specific mutation of 5-methylcytosine to thymine.

We have recently reported a method for site-specific transition of cytosine to uracil demonstrated via reversible DNA photoligation.[5] In this study, we established a method for site-specific transition of 5-methylcytosine to thymine analogue demonstrated via reversible DNA photoligation. The DNA template-directed reversible photoligation proceeded via [2 + 2] cycloaddition between the double bond of 5-carboxyvinyl-2′-deoxyuridine (^{CV}U) side chain and the C5–C6 double bond of pyrimidine.[6] Photochemical ligation has the benefit of not needing additional reagents and are controllable within space and time by the choice of proper irradiation methods. The deamination of cytosine in cyclobutane pyrimidine dimers was assigned as a key step in this transition based on the reduced half-life of deamination to a matter of hours when the 5,6-bond is saturated compared to the 30,000 year half-life for the deamination of normal monomeric cytosine.[7–14]

5′-d(^{CV}UGCGTG)-3′ (ODN **1**) was prepared, according to standard phosphoramidite chemistry, on a DNA synthesizer using phosphoramidite ^{CV}U.[14] ODN **1** was characterized by the nucleoside composition and MALDI-TOF-MS (ODN **1**: calcd. [(M + H$^+$)] 1879.28, found 1879.23). 5′-d(GAGAG^{m}C)-3′ (ODN **2a**) contains 5-methylcytosine (^{m}C) on the 3′ terminal. Ligation of ODN **1** and **2a** was demonstrated by 366 nm irradiation in the presence of template ODN **3**. The expected ligated 12 mer ODN **4a** was produced in 90% yield as determined by HPLC.

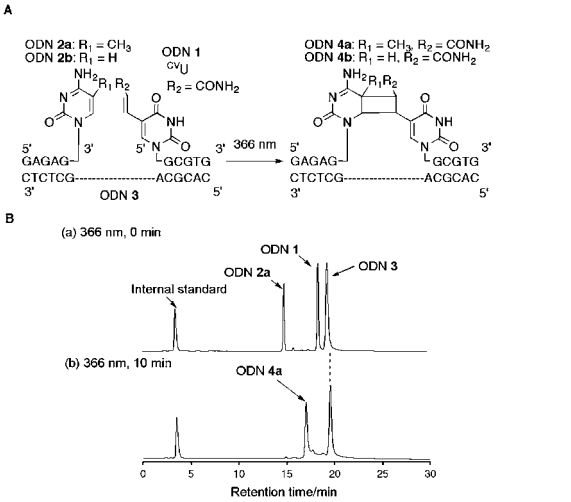

Figure 1. A) Schematical illustration of template-directed photoligation of ODNs via ^{CV}U. B) HPLC analysis of the irradiated ODN **1** and ODN **2a** in the presence of template ODN **3** with 366 nm. (a) Before photoirradiation. (b) Irradiated at 366 nm for 10 min; 90% yield.

HPLC shows that peaks of ODN **1** and **2a** disappear and a new peak of a linked ODN (ODN **4a**: calcd. [(M + H$^+$)] 3734.54, found 3734.56) appears (Figure 1).[15] After the isolated ODN **4a** was heat-treated at 90 °C for 6 h, ^{m}C-containing ODN **4a** deaminated to T. HPLC shows that the peak of ODN **4a** disappears and a new peak of a deaminated ODN **5a** appears (Figure 2). After deamination, quantitative photosplitting was achieved by 312 nm irradiation for 10 min. The peak of ODN **5a** transformed from ^{m}C to T disappears and ODN **6a**, ODN **1**, and ODN **1′** appear (Figure 3). ODN **6a** was completely hydrolyzed to produce the nucleosides. ODN **1′** is the cis isomer of ^{CV}U.

To confirm the transition of ^{m}C to T, ODN **2a** and ODN **6a** were subjected to enzymatic digestion with nuclease P1 and alkalinephosphatase at 37 °C for 4 h. HPLC analysis of the hydrolysates showed peaks of T or d^{m}C together with dG and dA (Figure 4).[15]

To evaluate the effects of the methyl group, we compared the conversion yields of the deamination reaction of d^{m}C and dC on photoligated ODN at 90 °C. The deamination of d^{m}C was slow compared to dC, which indicated that the methyl group protects against attack by water at C4 of the 5-methylcytosine (Figure 5).

To investigate the generality of sequence discrimination, we constructed photoligation with a one-mismatch template 5′-d(CACGCAGCTATC)-3′(ODN **7**), a two-mismatch template 5′-d(CACGCAGCCCCC)-3′(ODN **8**), or a three-mismatch template 5′-d(CACGCAGCCACC)-3′(ODN **9**). Results showed that

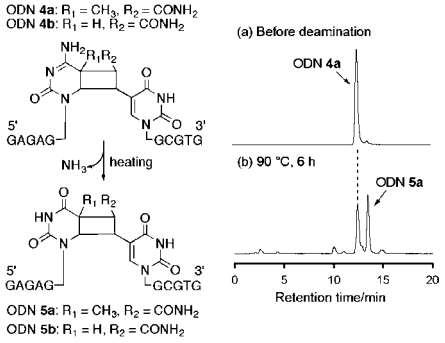

Figure 2. HPLC profile for the deamination of isolated photoligated ODN **4a** by incubation at 90 °C. (a) Before heat treatment, (b) heat treatment at 90 °C for 6 h; 45% yield.

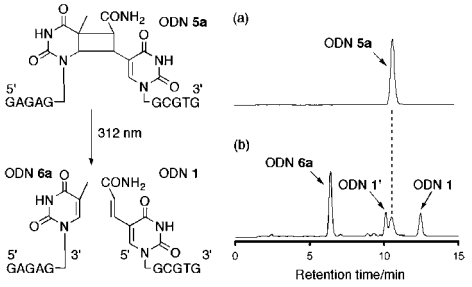

Figure 3. HPLC analysis for the photosplitting of isolated ODN **5a**. (a) Before photoirradiation, (b) 312 nm photoirradiation for 10 min; 78% yield.

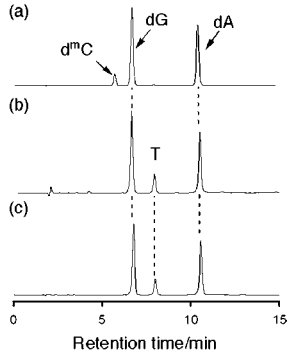

Figure 4. HPLC analysis of enzymatic digestion: (a) ODN **2a**, (b) ODN **6a**, (c) authentic sample ODN (5′-GAGAGT-3′).

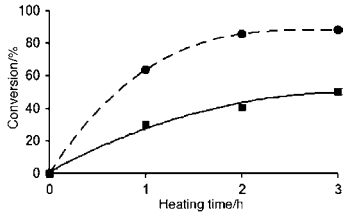

Figure 5. Comparison of deamination rates with ODN **4a** (filled squares) versus ODN **4b** (filled circles).

a single mismatch yielded very little photoligated product (Table 1). It was induced that the photoligation has high sequence selectivity. This sequence selectivity of DNA photoligation was investigated by monitoring the melting temperature (T_m). The T_m value of the ODN **1**·ODN **2** and complementary

Table 1. Conversion yield of photoligation with various template

	Mismatch ODN[a]	Photoligation yields/%[b]
ODN **3**	5′-d(CACGCAGCTCTC)-3′	90
ODN **7**	5′-d(CACGCAGCTATC)-3′	0
ODN **8**	5′-d(CACGCAGCCCCC)-3′	0
ODN **9**	5′-d(CACGCAGCCACC)-3′	0

[a]Underlined characters indicate a mismatched base. [b]Photoligation at 366 nm for 15 min at 25 °C.

ODN **3** was 26.3 °C, and when mismatched complementary strands ODN **7**, ODN **8**, and ODN **9** were 22.8 °C, not determined (nd), and nd, respectively. As a result, we considered that the photoligation reaction is mainly promoted by thermal stability.

In summary, site-specific transition of 5-methylcytosine to thymine was induced by deamination without additional reagents. Our deamination method using reversible DNA photoligation has high sequence selectivity and efficiency at the target cytosine compared with other methods for converting cytosine analogue to thymine analogue using enzymatic or chemical procedures. The present site-specific C to U and ^{m}C to T transition can be widely used for the site-specific mutation of DNA. This method may also find application to performance analysis on a protein that uses the mutated protein. A variety of mutant proteins have already been made based on developments in genetic engineering which has contributed to performance analysis. This method will be important when a new functional protein is created. Future work will be aimed at testing other substrates such as ^{m}C-containing sequences at the middle of DNA by using photochemical synthesis of branched DNA.

References and Notes

1 K. D. Robertson, A. P. Wolffe, *Nat. Rev. Genet.* **2000**, *1*, 11.
2 M. Biel, V. Wascholowski, A. Giannis. *Angew. Chem., Int. Ed.* **2005**, *44*, 3186.
3 M. Nakao, *Gene* **2001**, *278*, 25.
4 M. M. Ali, M. R. Alam, T. Kawasaki, S. Nakayama, F. Nagatsugi, S. Sasaki. *J. Am. Chem. Soc.* **2004**, *126*, 8864.
5 K. Fujimoto, S. Matsuda, Y. Yoshimura, T. Matsumura, M. Hayashi, I. Saito, *Chem. Commun.* **2006**, 3223.
6 a) K. Fujimoto, S. Matsuda, N. Takahashi, I. Saito, *J. Am. Chem. Soc.* **2000**, *122*, 5646. b) Y. Saito, Y. Miyauchi, K. Fujimoto, *Tetrahedron Lett.* **2005**, *46*, 97. c) M. Ogino, Y. Yoshimura, A. Nakazawa, I. Saito, K. Fujimoto, *Org. Lett.* **2005**, *7*, 2853. d) S. Ogasawara, K. Fujimoto, *ChemBioChem* **2005**, *6*, 1756. e) Y. Yoshimura, Y. Noguchi, H. Sato, K. Fujimoto, *ChemBioChem* **2006**, *7*, 598.
7 T. Lindahl, B. Nyberg, *Biochemistry* **1974**, *13*, 3405.
8 R. Y. H. Wang, K. C. Kuo, C. W. Gehrek, L. H. Huang, M. Ehrlich, *Biochim. Biophys. Acta* **1982**, *697*, 371.
9 a) L. A. Frederico, T. A. Kunkel, B. R. Shaw, *Biochemistry* **1990**, *29*, 2532. b) L. A. Frederico, T. A. Kunkel, B. R. Shaw, *Biochemistry* **1993**, *32*, 6523. c) H. Chen, B. R. Shaw, *Biochemistry* **1993**, *32*, 3535. d) H. Chen, B. R. Shaw, *Biochemistry* **1994**, *33*, 4121. e) W. Peng, B. R. Shaw, *Biochemistry* **1996**, *35*, 10172.
10 a) I. Tessman, *Photochem. Photobiol.* **1992**, *55*, 80S. b) I. Tessman, *Photochem. Photobiol.* **1992**, *55*, 83S. c) I. Tessman, M. A. Kennedy, S. K. Liu, *J. Mol. Biol.* **1994**, *235*, 807.
11 T. Douki, J. Cadet, *J. Photochem. Photobiol. B* **1992**, *15*, 199.
12 D. G. Lemaire, B. P. Ruzsicska, *Biochemistry* **1993**, *32*, 2525.
13 Y. Barak, O. Cohen-Fix, Z. Livneh, *J. Biol. Chem.* **1995**, *270*, 24174.
14 Y. Tu, R. Dammann, G. P. Pfeifer, *J. Mol. Biol.* **1998**, *284*, 297.
15 Supporting Information is available electronically on the CSJ-Journal web site; http://www.csj.jp/journals/chem-lett/.

Published on the web (Advance View) December 15, 2007; doi:10.1246/cl.2008.94