<table>
<thead>
<tr>
<th>Title</th>
<th>Site-specific transition of cytosine to uracil via reversible DNA photoligation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Author(s)</td>
<td>Fujimoto, Kenzo; Matsuda, Shigeo; Yoshimura, Yoshinaga; Matsumura, Takashi; Hayashi, Masayuki; Saito, Isao</td>
</tr>
<tr>
<td>Citation</td>
<td>Chemical Communications, 2006(30): 3223-3225</td>
</tr>
<tr>
<td>Issue Date</td>
<td>2006</td>
</tr>
<tr>
<td>Type</td>
<td>Journal Article</td>
</tr>
<tr>
<td>Text version</td>
<td>author</td>
</tr>
<tr>
<td>URL</td>
<td><a href="http://hdl.handle.net/10119/7905">http://hdl.handle.net/10119/7905</a></td>
</tr>
<tr>
<td>Description</td>
<td></td>
</tr>
</tbody>
</table>
Site-specific Transition of Cytosine to Uracil via Reversible DNA Photoligation

Kenzo Fujimoto,* † Shigeo Matsuda, Yoshinaga Yoshimura, Takashi Matsumura, Masayuki Hayashi and Isao Saito

We report that deamination coupled with 5-carboxyvinyldeoxuryridine-mediated photobranching causes the heat-induced transition of cytosine to uracil with high efficiency without any side reaction.

The development of a technique for artificial site-specific mutation will be a useful genetic tool for preparing non-native proteins. The mutation of nucleic acid bases is also caused by intrinsic factors such as sunlight and ultraviolet light.1 In living cells, transition of C is also caused by the formation of pyrimidine dimer in the 5'-CT-3', 5'-TC-3' and 5'-CC-3' sequence by UV irradiation coupled with enzymatic repair.2,3 The deamination of cytosine in cyclobutane pyrimidine dimers was assigned as a key step in these transitions 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 years' half-life for the deamination of normal monomeric cytosine. While many kinetic and biological studies on such deamination have already been reported,5–13 a method for C to U transition based on this deamination process with site-specificity has not been reported.

We have recently reported a template-directed DNA photoligation mediated by 5-vinyldeoxuryridine (V) via [2+2] cycloaddition between the 3' base and terminal C.14 In fact, the rapid deamination of the photoligated dimer in an enzymatic digestion process has been also observed in this study.15 We have also reported the photochemical synthesis of branched oligodeoxynucleotides (ODNs) via 5-carboxyvinyldeoxuryridine (CVU).14 We therefore report here the artificial site-specific transition from cytosine to uracil mediated by reversible DNA photoligation. The efficient, selective transition of cytosine to uracil was achieved by photochemical branched ODN formation at the target cytosine and the successive photosplitting of the branched ODN after heat treatment.

CVU-containing ODN was synthesized according to standard phosphoramidite chemistry on a DNA synthesizer using phosphoramidite of methyl ester of CVU coupled with the post-modification procedure after ODN synthesis (Scheme 1). CVU containing ODN at 356 nm while addressing the CVU-containing hairpin template ODN introduced the branch structure at the target cytosine via [2+2] cycloaddition between the CVU base and the target cytosine. The deamination of the target cytosine was promoted site-specifically with heat treatment to produce a uracil analogue in ligated ODN. After heat treatment, uracil-containing ODN was produced by photosplitting at 302-nm irradiation. The uracil base produced in ODN was removed with uracil DNA glycosylase (UDG), and the resulting abasic site was converted into strand breaks with piperidine treatment, which was detected by PAGE analysis (Fig. 1).16 When 32P-5'-end-labeled 11mer 5'-
The rate constant for deamination at 37 °C was similar to that at 1.8 °C.

Cytosine analogue in denatured DNA to protons and hydroxyl ions. The energy for deamination is likely attributed not only to a gain in its activation energy but also to the increased accessibility of the cytosine analogue in denatured DNA to protons and hydroxyl ions. The rate constant for deamination at 37 °C was similar to that at 1.8 °C.

This acceleration in the rate constant for deamination at 37 °C is likely attributed not only to a gain in its activation energy but also to the increased accessibility of the cytosine analogue in denatured DNA to protons and hydroxyl ions.

Fig. 2 Autoradiogram of denaturing polyacrylamide gel electrophoresis of site-selective cytosine to uracil mutation of a C<>U photoadduct by heating at 90 °C and photosplitting at 302 nm. This method for the transition of cytosine to uracil proceeded effectively at every step. Uracil formation was also directly confirmed by the HPLC analysis of enzymatic digestion of isolated ligated 17mer after heat treatment.

M13mp2C141 ds DNA exposed to UV at 37 °C, pH 7.4. This suggests the similarity of solvent exposure originating in local structural and conformational changes between C<>U dimer-containing ODN in duplex and C<>C dimer-containing ODN in duplex. The effect of pH for deamination was also examined at 37 °C. However no difference was observed in its rate constant for deamination at pH 5.0, pH 7.0 and pH 9.0. This indicates that the protonation of N3 of the cytosine analogue and attack by water or hydroxyl at C4 of the cytosine analogue in this addition elimination pathway of deamination might also play an important role in the kinetics of deamination. These results indicate that the cytosine residue in the C<>CV U photoadduct was easily transformed to uracil residue at higher temperatures.

Fig. 3 Autoradiogram of denaturing polyacrylamide gel electrophoresis of site-specific cytosine to the uracil mutation of C<>U-containing ODN 6 (7 μM) and 2P-5’-end-labeled ODN 5 (1 μM) in a sodium cacodylate buffer (50 mM, pH 7.0) containing NaCl (100 mM). Lane 1, before reaction; lane 2, ODN 5 + ODN 6, irradiation at 366 nm, 3 h on ice; lane 3, incubation at 90 °C for 2 h of lane 3; lane 4, irradiation at 302 nm, 1 h at 40 °C of lane 3; lane 5, UDG treatment at 37 °C for 1 h followed by incubation with piperidine at 90 °C for 20 min of lane 4; lane 6, irradiation at 302 nm, 1 h of lane 2; lane 7, UDG treatment followed by piperidine treatment of lane 6 under the same conditions as lane 5; lane 8, treatment of ODN 8 with UDG and piperidine under the same conditions as lane 5; lane 9, incubation with piperidine at 90 °C for 20 min of lane 8.

We further demonstrate that deamination coupled with C<>U-mediated photobranching can be carried out when ODN containing two consecutive cytosines is used as a target sequence. When 2P-5’-end-labeled 20mer 5’-d(TGGCTACGAGCCAACAACAA) (ODN 5)” and 27mer 5’-d(CUGCGTGTTTTCACGCCAAGCGTCA GCCA) (ODN 6) were irradiated at 366 nm for 3 h, the expected ligated 47mer ODN 7 was produced in 80% yield as determined by densitometric assay of PAGE (Fig. 3, lane 3). After heating at 90 °C for 2 h (Fig. 3, lane 3), quantitative photosplitting was achieved by 302-nm irradiation for 1 h (Fig. 3, lane 4). A cleavage band was observed by piperidine treatment after enzymatic base excision using UDG after photosplitting (Fig. 3, lane 5). The mobility of this band was equal to the band that was produced after strand cleavage at the abasic sites produced by UDG treatment of authentic deoxyuridine-containing ODN 8 (Fig. 3, lane 8). This cleaved band was not observed when heating of photoligated ODN 7 at 90 °C was not carried out (Fig. 3, lane 6) or when piperidine treatment was carried out.
out without UDG treatment after photosplitting (Fig. 3, lane 9). These results clearly indicated that the cleaved band seen in lane 5 was caused by the transformation from cytosine to uracil at the target site via deamination of C<>U photoadduct by heating at 90 °C and phototolysis at 302 nm. It was also found that an efficient transformation from cytosine to uracil occurred at the target cytosine at eleventh position with site-selectivity. These deamination at C at eleventh position and C at twelfth position were 88% and 12% yield, respectively.

The heat-induced deamination of the cytosine residue of a cyclobutane dimer followed by splitting of the photolyase7,8,12 or photoreactivation6,11 has thus far been known as a method for converting cytosine to uracil. However, these methods are not site-specific. Our deamination method using reversible DNA photoligation has high sequence selectivity and efficiency at the target cytosine compared with other methods for converting cytosine to uracil using enzymatic or chemical procedures.5,7,11

In summary, we have demonstrated that deamination coupled with CVU-mediated photobranching causes the heat-induced transition of cytosine to uracil with high efficiency without any side reaction. The present site-specific C to U transformation can be widely used for the site-specific mutation of DNA. This method may also find application in mutation not only for DNA but also for messenger RNA.

Kenzo Fujimoto, a,b* Shigeo Matsuda, *Yoshinaga Yoshimura, a Takashi Matsumura, a Masayuki Hayashi a and Isao Saito a
aSchool of Materials Science, Japan Advanced Institute of Science and Technology, Ishikawa 923-1292, Japan. Fax: +81 761 51 1671; Tel: +81 761 51 1671; E-mail: kenzo@jaist.ac.jp
bPRESTO, Japan Science and Technology Agency, Kawaguchi 332-0012, Japan
*Current address: Department of Chemistry, The Scripps Research Institute, La Jolla, CA 92037, USA
bDepartment of Synthetic Chemistry and Biological Chemistry, Faculty of Engineering, Kyoto University, Kyoto 606-8501, Japan
aCurrent address: NEWCAT Institute, School of Engineering, Nihon University, Koriyama 963-8642, Japan

Notes and references

5 T. Lindahl and B. Nyberg, Biochemistry, 1974, 13, 3405.