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# 5-Methylcytosine Selective Photoligation Using Photoresponsive Oligonucleotides Containing Various 5-Vinyl-2′-deoxyuridines Having an Aromatic Group

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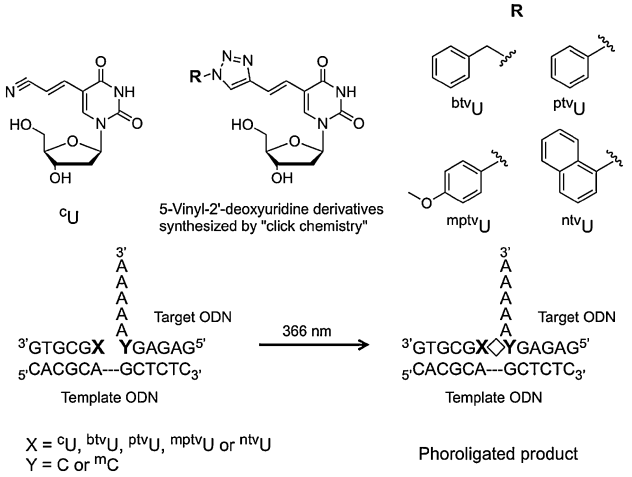
Photoresponsibility of oligonucleotides containing 5-vinyl-2′-deoxyuridine derivatives having an aromatic group and its selectivity for 5-methylcytosine ($^{m}$C) were evaluated. Their high extinction coefficient and hydrophobicity caused $^{m}$C selective photoligation with a 10- to 20-fold higher reaction rate than 5-cyanovinyl-2′-deoxyuridine, previously reported.

Methylation of the cytosine base in genomic DNA plays a role in the epigenetic regulation of gene expression, genomic imprinting, cell differentiation, and tumorigenesis.[1] Technological advancement in DNA methylation analysis is an important and ongoing endeavor of epigenetic research. However, the epigenetic information in genomic DNA is lost upon PCR or subcloning.[2,3] Various methods have been developed for detecting 5-methylcytosine ($^{m}$C) based on chemical and enzymatic concepts.[4] We previously reported on photoresponsive synthetic oligonucleotides (ODN(s)) containing 5-vinyl-2′-deoxyuridine ($^{V}$U) derivatives[5] that can photoligate to target DNA via [2 + 2] photocycloaddition with a pyrimidine base in target DNA. In particular, $^{V}$U derivatives having a hydrophobic group, such as cyano,[6a] cyclohexyl,[6b] and cyclopentyl[6b] were selectively photoligated to ODNs containing $^{m}$C because of the hydrophobic interaction between the 5-methyl group on target $^{m}$C and hydrophobic moiety tethered to $^{V}$U. However, the photoresponsibility of these $^{V}$U derivatives was low, and the photoreaction requires long photoirradiation time, approximately 10 min. Previously, we found that $^{V}$U derivatives having an aromatic group, that could be easily obtained by "click chemistry," could quickly photoligate to a pyrimidine base rather than $^{V}$U because of their high extinction coefficient.[7] As is similar to the case of the cyano, cyclohexyl, and cyclopentyl group, the hydrophobicity of their aromatic group might cause the selective photoreaction for $^{m}$C. In this study, we evaluate the photoresponsibility of $^{V}$U having an aromatic group (Scheme 1) toward $^{m}$C and the selectivity against C.

The ODNs containing 5-vinyl-2′-deoxyuridine derivatives were prepared by cycloaddition reaction between ODN containing 5-ethynylvinyl-2′-deoxyuridine ($^{ev}$U) and appropriate azide compound according to a literature method.[7] Details of the methods for preparation of the photoresponsive ODNs are described in the Supporting Information (Scheme S1[8]).

First, the photoligation reaction was analyzed by ultrahigh-performance liquid chromatography (UPLC). The representative chromatograms, in the case of $^{ntv}$U, are shown in Figure 1, and the time courses of the photoligation reactions using the photoresponsive ODNs are shown in Figure 2. As shown in Figure 1b, the peaks identical to the target ODN($^{m}$C) and




**Scheme 1.** $^{m}$C Selective template directed photoligation using ODNs containing 5-vinyl-2′-deoxyuridine derivatives.

ODN($^{ntv}$U) were decreased, and a new peak identical to the photoligated product (ODN($^{ntv}$U + $^{m}$C)) appeared by UV irradiation, indicating that the ODN($^{ntv}$U) photoligated to target ODN($^{m}$C). On the other hand, the peak identical to the target ODN(C) was also decreased by UV irradiation, where as the decreasing rate of the peak was lower than that of ODN($^{m}$C) (Figures 1a and 2e), indicating that the $^{m}$C selective photoligation occurred by using ODN($^{ntv}$U). A decrease of the peak identical to the template ODN was also observed, suggesting that an unexpected side reaction occurred. In the case of the other photoresponsive ODNs, ODN($^{btv}$U), ODN($^{ptv}$U), and ODN($^{mptv}$U), a similar change in chromatograms were observed (Figures S2–S4[8]).

In the case of all photoresponsive ODNs having aromatic moiety, the peaks identical to the target ODN(C) and ODN($^{m}$C) were both quickly decreased by UV irradiation compared to the case of ODN($^{c}$U) (Figure 2), indicating that the photoresponsibility of these ODNs was higher than that of ODN($^{c}$U). The rate and the selectivity of the photoligation reaction for the target ODNs are listed in Table 1. The results indicate that all photoresponsive ODNs having aromatic moiety have 10- to 20-fold higher reactivity for target ODN(C) and target ODN($^{m}$C) compared with ODN($^{c}$U). As the extinction coefficient of these photoresponsive ODNs is higher than that of ODN($^{c}$U),[7] these results are reasonable. These photoresponsive ODNs selectively photoligated to the target ODN($^{m}$C), although the selectivity was 2- to 3-fold lower than that of ODN($^{c}$U). It seems that the high photoresponsibility of these photoresponsive ODNs reduces the

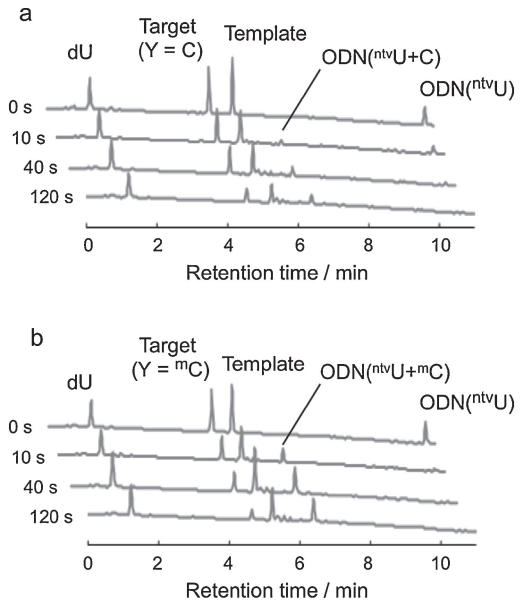



**Figure 1.** UPLC analysis of $^{m}$C selective photoligation reaction using ODN($^{ntv}$U). a) The mixture of ODN($^{ntv}$U) (10 μM), target ODN(C) (10 μM), and template ODN (11 μM) was irradiated (366 nm) at 0 °C and then subjected to a UPLC analysis at presented time periods. b) The mixture of ODN($^{ntv}$U) (10 μM), target ODN($^{m}$C) (10 μM), and template ODN (11 μM) was irradiated (366 nm) at 0 °C and then subjected to a UPLC analysis at presented time periods.

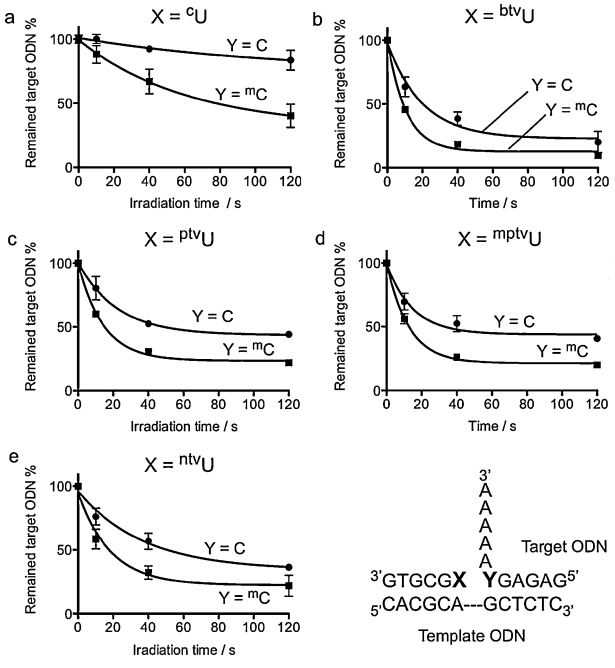



**Figure 2.** Time course of the photoligation reaction using ODN($^{c}$U) (a), ODN($^{btv}$U) (b), ODN($^{ptv}$U) (c), ODN($^{mptv}$U) (d), and ODN($^{ntv}$U) (e). Error bars represent standard deviation of triplicate determinations in a single experiment.

**Table 1.** Time to reach the 50% conversion and the selectivity for the target ODN($^{m}$C)

| Photoresponsive ODNs | Target ODN(C) /s | Target ODN($^{m}$C) /s | Selectivity C/$^{m}$C |
|---|---|---|---|
| ODN($^{c}$U) | 480 | 78 | 6.2 |
| ODN($^{btv}$U) | 20 | 9 | 2.2 |
| ODN($^{ptv}$U) | 40 | 15 | 2.7 |
| ODN($^{mptv}$U) | 25 | 14 | 2.5 |
| ODN($^{ntv}$U) | 48 | 16 | 3.0 |

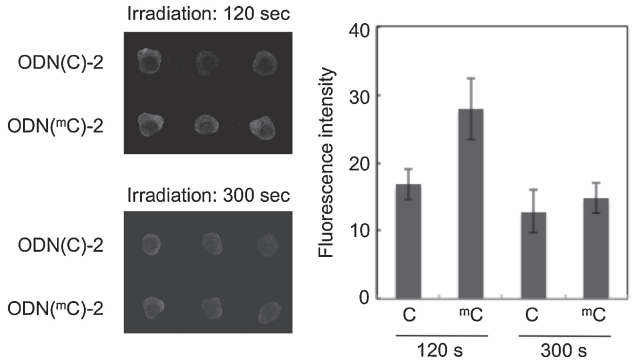



**Figure 3.** $^{m}$C Detection using ODN($^{ntv}$U)-2 immobilized microarray. ODN($^{ntv}$U)-2: $^{ntv}$**UGACGTGTATCG**CATTGG–SSSS–$C_6$–$NH_2$ (S: hexaethyleneglycol linker), target ODN(C)-2 and ODN($^{m}$C)-2: Cy3-GCCCCAGCTGCTCACCATCGCTA-TCTGAGCAGCGCTCAtggtgggggcagYGCCTCACAACCT-CCGTCATGTGCTGTGACTGCTTGTAGATGGCCATGGC (Y = C or $^{m}$C), template ODN-2: **CGATACACGTCA**-gctgcccccacca. Bold and small characters indicate the complementary nucleotides for ODN($^{ntv}$U) and target ODN, respectively. Error bars represent standard deviation of fluorescence intensity of three spots in the fluorescence image.

selectivity. The regulation of the photoresponsibility of these ODNs by the optimization of the conditions, such as salt concentration, reaction temperature, and irradiation energy, may enable more selective photoligation for ODN($^{m}$C). Indeed the selectivity of ODN($^{mptv}$U) was clearly improved at 25 °C (Figure S5[8]). It was concluded that photoresponsive ODNs having aromatic moiety could quickly photoligate to ODN($^{m}$C) with a $^{m}$C selective manner.

To evaluate the feasibility of the photoresponsive ODNs for the oligonucleotide microarray-based $^{m}$C detection, ODN($^{ntv}$U)-2 was immobilized on aldehyde-coated glass slides, and the mixture of template ODN-2 and the Cy3-labeled target ODN(C)-2 or ODN($^{m}$C)-2 were added. After the UV irradiation and washing, Cy3 fluorescence on the slides was quantified by a microarray scanner. As shown in Figure 3, fluorescent spots appeared in both cases of the target ODN by the 120 s irradiation. In the case of target ODN($^{m}$C), however, the fluorescence intensity of the spots was higher than that in the case of target ODN(C)-2, indicating that the $^{m}$C selective photoligation also occurred on the glass surface and that the ODN($^{m}$C)-2 was clearly detected by this method. In the case of

300 s irradiation, the fluorescence intensity of the spots was not dependent on the target having $^{m}$C, indicating that the long irradiation time caused the decrease of the selectivity of the photoligation to $^{m}$C. The decrease of the selectivity was also observed in UPLC analysis (Figure 2e), indicating that the photoligation on the glass surface occurs in a similar manner in the case of the photoligation in a solution phase. Decrease of the fluorescence intensity was observed with the elongation of photoirradiation time, suggesting that the photobleaching of the Cy3 fluorophore tethered with target ODN occurred.

In conclusion, we examined $^{m}$C selective photoligation using 5-vinyl-2′-deoxyuridine derivatives having aromatic moiety. These derivatives have higher photoresponsibility compared with $^{c}$U, although the selectivity of the photoligation for $^{m}$C was lower than that of $^{c}$U. As the 5-vinyl-2′-deoxyuridine derivatives are easily obtained by the click-based postmodification method, it is expected that more effective photoresponsive ODN will be explored using this method.

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8 Supporting Information is available electronically on the CSJ-Journal Web site, http://www.csj.jp/journals/chem-lett/index.html.


  