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Ultrafast Reversible Photo-Cross-Linking Reaction: Toward in Situ DNA Manipulation

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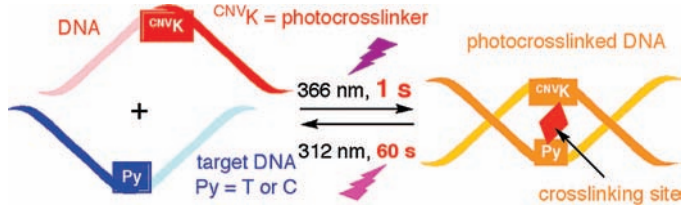
ABSTRACT



We describe a novel ultrafast reversible DNA interstrand photo-cross-linking reaction via 3-cyanovinylcarbazole nucleoside (^{CNV}K). Oligodeoxynucleotide (ODN) containing ^{CNV}K can be photo-cross-linked by irradiation at 366 nm for 1 s, and the photo-cross-linked ODN can be split by irradiation at 312 nm for 60 s.

The cross-linking reaction has widely been used to stabilize complexes with DNA by a covalent-bond formation. Biotechnological approaches based on the cross-linking reactions have been also used in inhibiting gene expression,[1] appreciating DNA damage,[2] and investigating RNA structures.[3] Psoralen and its analogues have enjoyed remarkable successes as T-selective photo-cross-linkers, applicable to a wide variety of in vitro as well as in vivo studies.[4] However, the photo-cross-linking reaction from the use of psoralens limits the choice of the target DNA, as an efficient cross-linking reaction requires a TpA step.[5] Additionally, the photo-cross-linked DNAs, via a [2 + 2] cycloaddition between psoralen and thymine base, were regenerated to the parent DNA by 254 nm irradiation resulting in fatal damage to normal DNA, due to the formation of pyrimidine dimer.[6] To overcome such sequence dependence and DNA damage, researchers have been seeking alternative types of photo-cross-linkers with high reactivity. We have also been studying artificial DNA bases as a tool for the photochemical DNA cross-linking method.[7] By using the carbazole nucleoside incorporated into DNA, we have observed the repair of a thymine dimer and have used the same as a tool for photochemical DNA

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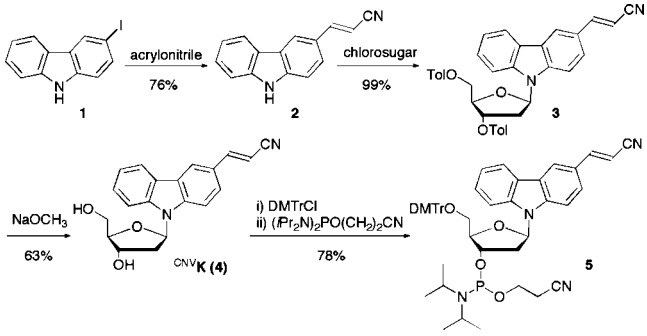
manipulation.[8] Herein, we report the development of a novel ultrafast interstrand photo-cross-linking reaction via 3-cyanovinylcarbazole nucleoside (^{CNV}K) in duplex DNA. We have demonstrated that the modified oligodeoxynucleotide (ODN) containing ^{CNV}K was reversibly photo-cross-linked with an adjacent pyrimidine base via UV irradiation at two different wavelengths.

The synthesis of the phosphoramidite of ^{CNV}K is outlined in Scheme 1. Compound **1** was prepared according to a method reported in literature.[9] Compound **2** was synthesized from **1** and acylonitrile, while compound **3** was synthesized from **2** and Hoffer's α-chlorosugar. The deprotection of **3** with sodium methoxide afforded **4** (^{CNV}K). Compound **4** was dimethoxytritylated and converted into nucleoside phosphoramidite **5**. The various modified ODNs, ODN(X^{CNV}KY) (X, Y = A, G, C, or T), were prepared, according to standard phosphoramidite chemistry, on a DNA synthesizer using phosphoramidite **5**. ODNs containing ^{CNV}K were characterized by MALDI-TOF-MS.

Scheme 1. Synthesis of the Phosphoramidite of 3-Cyanovinylcarbazole Nucleoside (**4**, ^{CNV}K)



We determined the feasibility of the interstrand photo-cross-linking via ODN containing ^{CNV}K as shown in Figure 1. When ODN(A^{CNV}K) (5′-d(TGCA^{CNV}KCCGT)-3′) and ODN(GT) (5′-d(ACGGGTGCA)-3′) were irradiated at 366 nm for 1 s, HPLC showed the appearance of a peak relating to ODN(A^{CNV}K-GT) in 97% yield along with the disappearance of ODN(A^{CNV}K) and ODN(GT) peaks (Figure 2).[10]

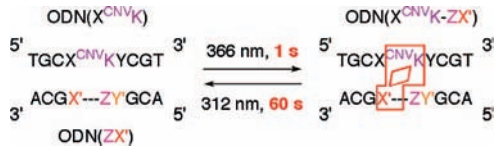


Figure 1. Schematic illustration of photo-cross-linking of ODNs with ^{CNV}K. X, X′, Y, Y′, or Z = A, G, C, or T.

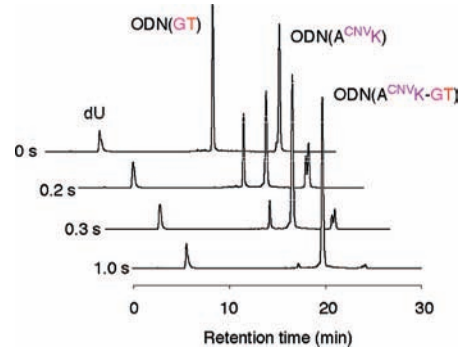


Figure 2. HPLC analysis of the irradiated ODN(A^{CNV}K) in the presence of ODN(GT). 2′-Deoxyuridine (dU) was used as an internal standard.

MALDI-TOF-MS indicates that the isolated ODN(A^{CNV}K-GT) obtained from HPLC purification was a photo-cross-linked product of ODN(A^{CNV}K) and ODN(GT) (calcd 5546.76 for $[M + H]^+$, found 5546.69). The enzymatic digestion of isolated ODN(A^{CNV}K-GT) showed the formation of dCyd, dGuo, dThd, and dAdo in a ratio of 5:6:2:3, together with ^{CNV}K<>T photoadduct, which was confirmed by MALDI-TOF-MS (calcd 599.2118 for $[M + Na]^+$, found 599.2033). On the other hand, when ODN(G^{CNV}K) (5′-d(TGCG^{CNV}KCCGT)-3′) and ODN(GC) (5′-d(ACGGGCGCA)-3′) were used in photo-cross-linking, the cytosine base reacted with photoexcited ^{CNV}K to produce a photo-cross-linked product ODN(G^{CNV}K-GC) efficiently.[11] As shown in Figure 3, the photo-cross-linking reaction between ODN (A^{CNV}K) and ODN(GT) was finished by 366 nm irradiation for only 1 s.

To examine the effect of sequence contexts around the photo-cross-linking site, we constructed 64 sequences of ODNs, ODN(Y′ZX′) (X′, Y′, Z = A, G, C, or T), in the opposite strand. The photo-cross-linking yield obtained by photoirradiation at 366 nm for 1 s was determined by HPLC and UPLC analysis. Among the various combinations of base pairs between ^{CNV}K and natural bases Z (Z = A, G, C, or T), we observed that ODNs containing ^{CNV}K produced a

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(10) The reaction mixture (total volume 30 μL) containing ODN(A^{CNV}K) and ODN(GT) (each 20 μM, strand concn) in 50 mM sodium cacodylate buffer (pH 7.0) and 100 mM sodium chloride was irradiated with a UV-LED (366 ± 15 nm light at 1600 mW/cm^2) at a distance of 1.5 cm at 0 °C for 1 s. After irradiation, the progress of the photoreaction was monitored by HPLC. The yield was calculated on the basis of ODN(GT). Quantum yield of the formation of photo-cross-linked product was measured at 366 nm, based on the disappearance of ODN(GT) by employing valerophenone as an actinometer. The formation of ODN(A^{CNV}K-GT): $\Phi = 0.251$.

(11) MALDI-TOF-MS: calcd 5547.74 for ODN(G^{CNV}K-GC) $[(M + H)^+]$, found 5547.88; calcd 584.2121 for ^{CNV}K<>C photoadduct $[(M + Na)^+]$, found 584.2102. The yield was calculated on the basis of ODN(GC).

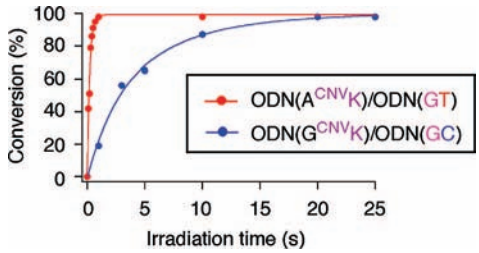

Figure 3. Time-course of photo-cross-linking reaction with ODN(A^{CNV}K) (red) and ODN(G^{CNV}K) (blue).

photo-cross-linked product efficiently in each base pair (Figure 4A, 4B). Importantly, adenine and guanine bases on the photo-cross-linking site are inactive toward photo-cross-linking reaction with ODNs containing ^{CNV}K (see Supporting Information). Therefore, we can determine the difference between the pyrimidine and purine bases of the target DNAs using a photo-cross-linking reaction.

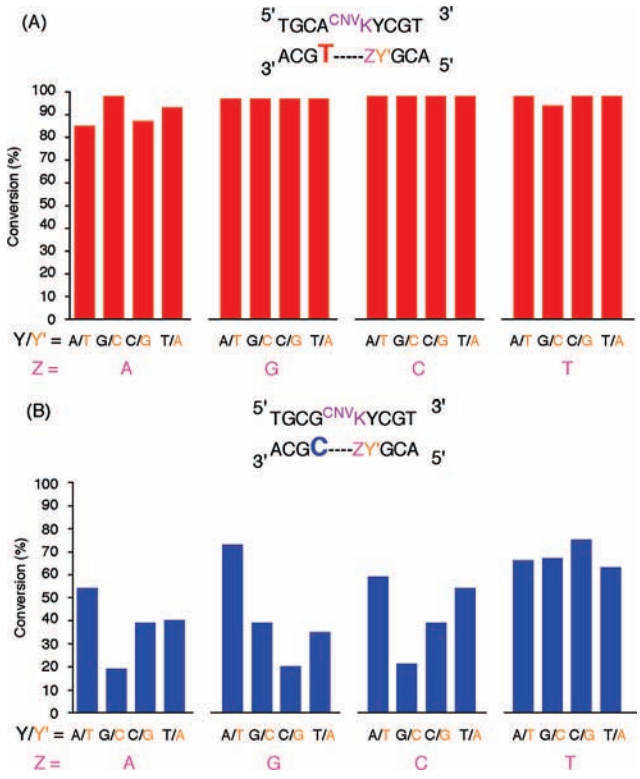

Figure 4. Photo-cross-linking yield obtained by photoirradiation at 366 nm for 1 s by using ODNs containing ^{CNV}K. Y, Y′, Z = A, G, C, or T. (A) Photoreaction with T base, (B) photoreaction with C base.

To examine the influence of the photo-cross-linking reaction on thermal stability, the melting temperature (T_m) of the duplex ODN(A^{CNV}K)/ODN(GT) or ODN(A^{CNV}K-GT) was determined by UV-monitored thermal denaturation. The duplex ODN(A^{CNV}K)/ODN(GT) showed a melting temperature of 36.4 °C, whereas ODN(A^{CNV}K-GT) melted at 67.9 °C (see Supporting Information). An example of this behavior has been seen for photo-cross-linked ODNs by the ODN-containing *p*-carbamoylvinyl phenol nucleoside.[7] The duplex ODN(G^{CNV}K)/ODN(GC) showed a melting temperature of 40.4 °C, whereas ODN(G^{CNV}K-GC) melted at 75.8 °C. Thus, photo-cross-linking increased T_m of ODN, a dramatic stabilization of the duplex form.

To confirm the photoreversibility of the photo-cross-linked product, irradiation of the photo-cross-linked ODN(A^{CNV}K-GT) at 312 nm was examined. The rapid disappearance of ODN(A^{CNV}K-GT) was observed by 312 nm irradiation for 60 s to revert to two ODNs (Figure 5),[12] while the reverse

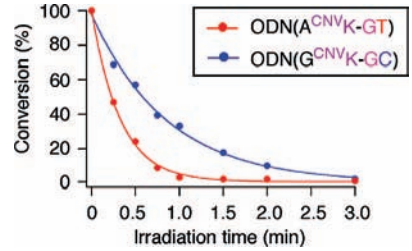

Figure 5. Time-course of the photosplitting reaction with ODN (A^{CNV}K-GT) (red) and ODN(G^{CNV}K-GC) (blue).

photoreaction produced only ODN(A^{CNV}K) and ODN(GT) without any byproducts. When the photo-cross-linked ODN (G^{CNV}K-GC) was used in reverse photoreaction, the rapid disappearance of ODN(G^{CNV}K-GC) was observed by 312 nm irradiation for 3 min to revert to two ODNs. Therefore, we succeeded in the reverse reaction by irradiation at 312 nm, resulting in no damage to normal DNA.

We examined molecular modeling studies of the duplexes of ODN(A^{CNV}K) and ODN(GT). The vinyl group of ^{CNV}K is stacked on the C5−C6 double bond of the T base of ODN(GT) (see Supporting Information). The molecular weight of ODN(A^{CNV}K-GT) was equal to the sum of the molecular weights of ODN(A^{CNV}K) and ODN(GT). As judged from the molecular modeling, the photoreversibility, and UV spectrum of ^{CNV}K<>T photoadduct, there is a strong suggestion that the photo-cross-linking proceeded via [2 + 2] cycloaddition between the double bond of ^{CNV}K and the C5−C6 double bond of thymine, giving rise to the formation of a cyclobutane structure.[13]

In conclusion, we have demonstrated that a modified ODN-containing ^{CNV}K can be cross-linked by irradiating at 366 nm with an adjacent pyrimidine base in a [2 + 2] manner. The photo-cross-linked ODNs were reverted to the

(12) A solution (total volume 60 mL) containing ODN(A^{CNV}K-GT) (20 μM, strand concn) in CH_3CN/H_2O (1:1) containing urea (2 M) was irradiated with 15 W transilluminator (312 nm) at 25 °C for 60 s. After irradiation, the progress of the photoreaction was monitored by HPLC. The yield was calculated on the basis of ODN(A^{CNV}K-GT).

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original ODN by 312 nm irradiation. The photo-cross-linking reaction of ODN containing ^{CNV}K is ultrafast (with an irradiation time of only 1 s) and clean. The photo-cross-linking reaction would require no added reagents to carry out the reaction and would be controllable with space and time by the choice of proper irradiation methods. The reactivity, selectivity, and reversibility of the novel photo-cross-linker will be beneficial to the in situ DNA manipulation applicable to the inhibition of gene expression and fluorescent labeling of DNA.

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Supporting Information Available: Synthetic procedures for ^{CNV}K-containing ODN and detailed experimental data of the reversible photo-cross-linking reaction. This material is available free of charge via the Internet at http://pubs.acs.org.

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