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DNA Photo-Cross-Linking Using 3-Cyanovinylcarbazole Modified Oligonucleotide with Threoninol Linker

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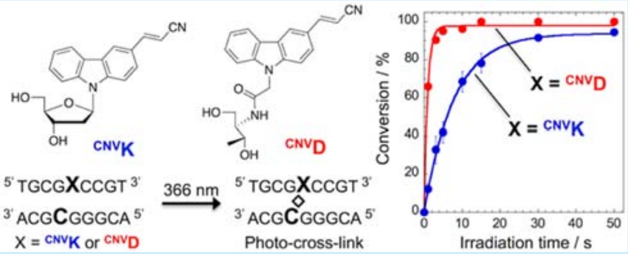
S Supporting Information

ABSTRACT: 3-Cyanovinylcarbazole modified D-threoninol (^{CNV}D) was incorporated in oligodeoxyribonucleotide and tested for a photo-cross-linking reaction with complementary oligodeoxyribonucleotide. The photoreactivity was 1.8- to 8-fold greater than that of 3-cyanovinylcarbazole modified deoxyribose (^{CNV}K) previously reported. From the results of melting analysis and circular dichroism spectroscopy of the duplexes, the relatively flexible structure of ^{CNV}D compared with ^{CNV}K might be advantageous for [2 + 2] photocycloaddition between the cyanovinyl group on the ^{CNV}D and pyrimidine base in the complementary strand.



The photo-cross-linking reaction in nucleic acids has wide potential for photoregulation of the DNA nanostructure or gene expression in cells. Psoralen and its derivatives are well-known DNA photo-cross-linkers that can react with the pyrimidine base in DNA or RNA.[1] The covalent bond formation between the psoralen moiety and the pyrimidine base renders the DNA/DNA or DNA/RNA hybrid irreversible. Therefore, the thermally stable DNA nanostructure[2] and photoregulation of gene expression[3] were achieved using psoralen-modified oligonucleotides.

As another class of DNA and/or RNA photo-cross-linker, we previously reported 3-cyanovinylcarbazole nucleoside (^{CNV}K, Figure 1), which has higher photoreactivity compared with psoralen.[4] Using oligonucleotide having ^{CNV}K, we successfully developed photoreactive triplex forming oligonucleotide,[5] photoreactive antisense oligonucleotide,[6] photoreactive detection probe for DNA[4d] or RNA,[7] and thermally stable DNA nanostructure.[8] To the best of our knowledge, ^{CNV}K is the most reactive DNA/RNA photo-cross-linker at this time; however, the structure of ^{CNV}K is not optimized. In particular, the deoxyribose part of ^{CNV}K has the potential to improve the photoreactivity because the geometry or flexibility of the reaction points, that is, the cyanovinyl group on the ^{CNV}K and C5−C6 carbon on the target pyrimidine base, might be affected largely by the deoxyribose skeleton.

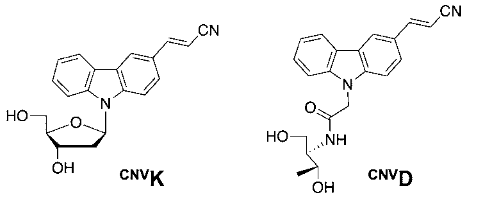
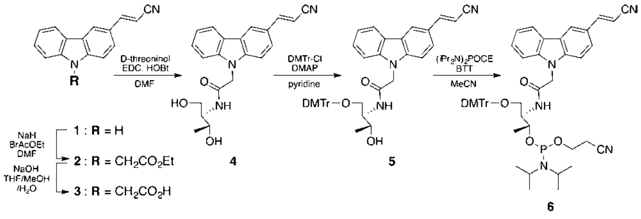


Figure 1.

In this study, 3-cyanovinylcarbazole derivative modified with D-threoninol instead of deoxyribose in the case of ^{CNV}K (Figure 1, CNVD) was synthesized and the photoreactivity of the oligodeoxyribonucleotide (ODN) having ^{CNV}D was investigated.

^{CNV}D (Compound **4**) was successfully synthesized according to Scheme 1 with a total yield of 80% (details are described in the Supporting Information). After 5′ DMTr protection and phosphoramidite derivatization with the general method, ^{CNV}D was incorporated in ODN (9 mer) with an automated DNA synthesizer. 3-Cyanovinylcarbazole derivative that has L-threoninol linker (^{CNV}L) was also synthesized with the same procedures shown in Scheme 1 (see the Supporting Information) and ODNs having ^{CNV}L were also prepared. The coupling yields of ^{CNV}D and ^{CNV}L in the ODN synthesis were up to 95%.

Scheme 1



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First, the photoreactivity of $^{\text{CNV}}$D in double-stranded DNA was investigated by ultra-high-performance liquid chromatography (UPLC). As shown in Figure 2b, ODN having $^{\text{CNV}}$D (ODN(AD); 5′TGCA$^{\text{CNV}}$DCCGT3′) and cODN having T at the −1 position of $^{\text{CNV}}$D (cODN(GT); 5′ACGGGTGCA3′) were clearly decreased by 366 nm irradiation and a new peak (*) identical to the photodimer consisting of ODN(AD) and cODN(GT) (5572.06 calcd. for [(M+H)$^+$], found 5571.37) appeared. These results suggest strongly that dimerization through the photo-cross-linking reaction between ODN(AD) and cODN(GT) occurred. The same phenomena were also observed in the case of ODN(GD) (5′TGCG$^{\text{CNV}}$DCCGT3′) and cODN having C at the −1 position of $^{\text{CNV}}$D (cODN(GC); 5′ACGGGCGCA3′) (Figure 2c), although the photoreactivity was lower than that in the case of ODN(AD)/cODN(GT). These results suggest that the pyrimidine base at the −1 position of $^{\text{CNV}}$D on the complementary strand is the reaction site of $^{\text{CNV}}$D. Indeed, the enzymatic digestion of ODN(AD)/cODN(GT) photodimer with nuclease and alkaline phosphatase treatment yielded nucleoside dimer consisting of $^{\text{CNV}}$D and dT (628.24 calcd. for [(M+H)$^+$], found 628.99) (Supporting Information Figure S1).

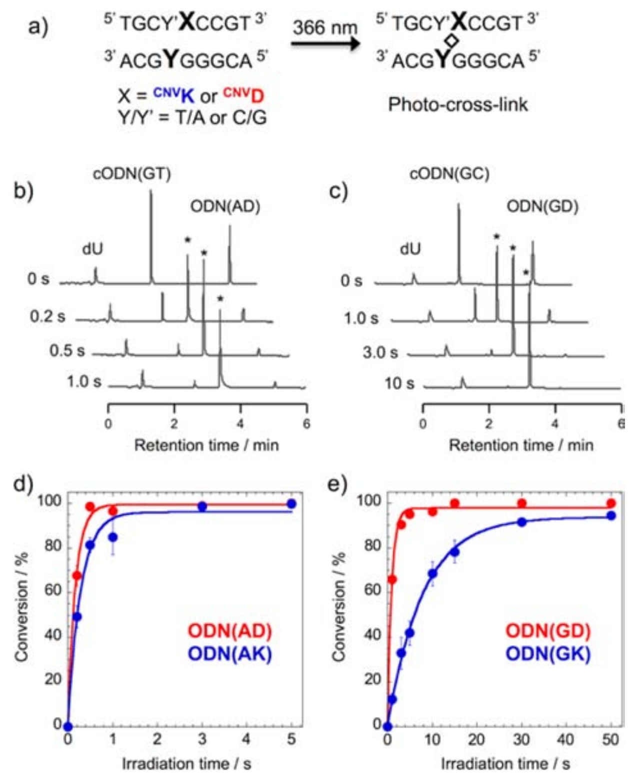

Figure 2. UPLC analysis of the photo-cross-linking reaction of $^{\text{CNV}}$D in oligonucleotide duplexes. (a) Schematic drawing of the photo-cross-linking reaction. UPLC chromatograms of (b) ODN(AD)/cODN(GT) and (c) ODN(GD)/cODN(GC) duplexes after the indicated time period of 366 nm photoirradiation and the time course of the photo-cross-linking reaction of (d) ODN(AX)/cODN(GT) and (e) ODN(GX)/cODN(GC) duplexes. [duplex] = 15 μM in 50 mM Na-Cacodylate buffer (pH 7.4) containing 100 mM NaCl and 50 μM dU (as an internal standard). Photoirradiation (366 nm, 1280 mW/cm^2) was performed at 0 °C.

To compare the photoreactivity of $^{\text{CNV}}$D with $^{\text{CNV}}$K, time courses of the photo-cross-linking reaction toward T or C were determined. As shown in Figure 2d and e, the photo-cross-linking rates of $^{\text{CNV}}$D toward T and C were both higher than that of $^{\text{CNV}}$K. The first-order reaction rate constants of the photo-cross-linking reaction (Table 1) toward T and C were 1.8- and 8-fold higher than that of $^{\text{CNV}}$K, respectively, suggesting that a relatively flexible structure of $^{\text{CNV}}$D compared with $^{\text{CNV}}$K is suitable for the photo-cross-linking reaction. Contrary to the case of $^{\text{CNV}}$D, $^{\text{CNV}}$L, which is the stereoisomer of $^{\text{CNV}}$D, has lower photoreactivity compared with $^{\text{CNV}}$K (Table 1, Supporting Information Figure S2), suggesting that the enhanced photoreactivity observed in the case of $^{\text{CNV}}$D relies not only on the flexibility, but also on the geometry of the reaction point. As the photoreactivity of 3-cyanovinylcarbazole toward C was dramatically enhanced by the use of the D-threoninol skeleton, the reaction selectivity toward T was decreased compared to $^{\text{CNV}}$K. This facilitates design of the photo-cross-linkable ODN for various target sequences. In the case of the use of complementary oligoribonucleotide (ORN), the reaction rate constant of the photo-cross-linking reaction toward U was 2.8-fold higher than that of $^{\text{CNV}}$K (Table 1, Supporting Information Figure S3), suggesting that the same enhancement effect of $^{\text{CNV}}$D was caused not only for DNA, but also for RNA. Occurrence of the photosplitting reaction of the photodimer consisting of ODN(AD) and cODN(GT) was clearly evident by 312 nm irradiation with the same reaction rate in the case of $^{\text{CNV}}$K (Supporting Information Figure S4). This suggests that the difference in the linker molecule having 3-cyanovinylcarbazole does not affect the photosplitting reaction, and that the quick photosplitting reaction might be applicable for photochemical DNA/RNA editing without any photodamage of target DNA or RNA similarly to $^{\text{CNV}}$K.[9]

Table 1. Reaction Rate Constants of the Photo-Cross-Linking Reaction

duplex	k (s^{-1})[a]			
	X =	D	K	L
ODN(AX)/cODN(GT)		5.9	3.5	2.5
ODN(GX)/cODN(GC)		1.1	0.13	–
ODN(AX)/cORN(GT)		4.4	1.6	–

[a] The values were estimated from the time courses shown in Figure 2d,e and Supporting Information Figure S2 with the assumption of first-order reaction kinetics.

To clarify why the enhanced photoreactivity was observed in the case of $^{\text{CNV}}$D, at first, molecular modeling of the duplexes consisting of ODN(GX) and cODN(GC) were performed (Supporting Information Figure S5). Similar to the case of azobenzene reported by Asanuma's group,[10] we expected that the 3-cyanovinylcarbazole moiety was held on a different part of the duplex depending on the stereoisomers of threoninol; however, there is no significant difference between three duplexes having $^{\text{CNV}}$K, $^{\text{CNV}}$D, and $^{\text{CNV}}$L. It seems that the relatively flexible linker between threoninol and 3-cyanovinylcarbazole compared to the case of azobenzene contributes to the decrease of the stereoisomeric effect of the threoninol skeleton. Next, we measured the absorbance spectra of ODN(AK) and ODN(AD), and compared the absorbance around photoirradiation wavelength (Supporting Information Figure S6). Although a slight red shift (around 360 nm) was observed in the case of ODN(AD), there is no significant difference in the absorbance around 360 nm, suggesting that the molecular extinction efficiency was not the reason for the enhanced photoreactivity of ^{CNV}D. Finally, we performed thermodynamic analysis of the duplex consisting of ODN(AD) and cODN(GT) before the photo-cross-linking reaction. As shown in Figure 3a, the melting transition of the ODN(AD)/cODN(GT) duplex was sharper than that of the ODN(AK)/cODN(GT) duplex, suggesting that there are entropic differences between the hybridization of each duplex. Δ*S* through ODN(AD)/cODN(GT) hybridization was smaller than that of ODN(AK)/cODN(GT) (Table 2), suggesting that a relatively flexible structure of ^{CNV}D suppresses the entropic loss through the hybridization. Interestingly, the melting profiles at 345 nm, that is, the absorbance region of carbazole moiety, were different between these two duplexes. In the case of the ODN(AD)/cODN(GT) duplex, a sigmoidal curve was clearly observed although such a clear curve was not observed in the case of the ODN(AK)/cODN(GT) duplex (Figure 3b), suggesting that the 3-cyanovinylcarbazole moiety in the ODN(AD)/cODN(GT) duplex was smoothly intercalated to the base stacking structure of the duplex rather than that in the case of the ODN(AK)/cODN(GT) duplex. The difference observed in the circular dichroism spectra of these duplexes (Figure 3d) supports the structural difference predicted from the melting profile of the duplexes. In the case of the ODN(AL)/cODN(GT) duplex, the melting profile at 345 nm was similar to the case of the ODN(AD)/cODN(GT) duplex (Supporting Information Figure S7), although the reactivity of ^{CNV}L was lower than that of ^{CNV}D, indicating that the enhanced photoreactivity of ^{CNV}D cannot be explained with only the smooth stacking of 3-cyanovinylcarbazole moiety. This suggests that the different stereochemical configurations between ^{CNV}D and ^{CNV}L also affect the photoreactivity of 3-cyanovinylcarbazole moiety in double-stranded DNA.

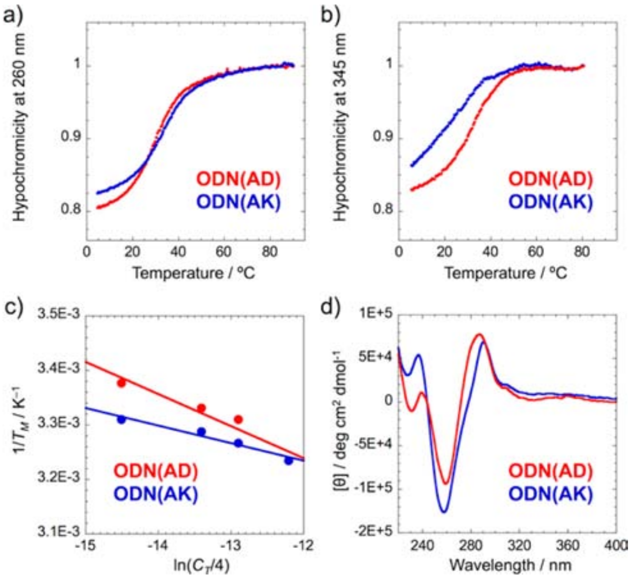

Figure 3. Thermodynamic and structural analysis of the duplex consisting of ODN(AD) and cODN(GT). Melting curves of ODN(AD)/cODN(GT) (red) and ODN(AK)/cODN(GT) (blue) duplex monitored at 260 nm (a) and 345 nm (b). (c) $1/T_M$ vs $\ln(C_T/4)$ plots of ODN(AD)/cODN(GT) (red) and ODN(AK)/cODN(GT) (blue) duplex. (d) Circular dichroism spectra of ODN(AD)/cODN(GT) (red) and ODN(AK)/cODN(GT) (blue) duplex. [duplex] = 5 μM (for a) or 15 μM (for b and d) in 50 mM Na-Cacodylate buffer (pH 7.4) containing 100 mM NaCl.

Table 2. Thermodynamic Parameters of the Duplexes Consisting of ODN(AX) and cODN(GT)[a]

X	$\Delta\Delta G^\circ_{37}$ kcal/mol	ΔH° kcal/mol	ΔS° cal/mol·K	T_M °C
D	−7.3	−33.8	−85.6	29
K	−7.5	−63.6	−181.1	34

[a] Melting temperatures (T_M) were estimated from Figure 3a and thermodynamic parameters were evaluated from Figure 3c.

To check the effects of surrounding nucleobases on the photo-cross-linking reaction of ODNs having ^{CNV}D, duplexes having all variations of base pair at the −1, +1 position of ^{CNV}D and also four different nucleobases on the site opposite to ^{CNV}D in cODN were prepared, and the conversion after 1 s of 366 nm irradiation was evaluated by UPLC. As shown in Figure 4, only in the case of T or C possessed at X′ on the cODN strand among 64 combinations of the DNA sequence did the photo-cross-linking reaction occur, suggesting that the photoreaction has the selectivity for pyrimidine bases. Some differences in the reactivity were observed among these duplexes, but there are no clear tendencies dependent on the surrounding base pairs. These results suggest that some unclear effects, such as local duplex stability and local electrostatic environment around the 3-cyanovinylcarbazole moiety, still remain on this photo-cross-linking reaction.

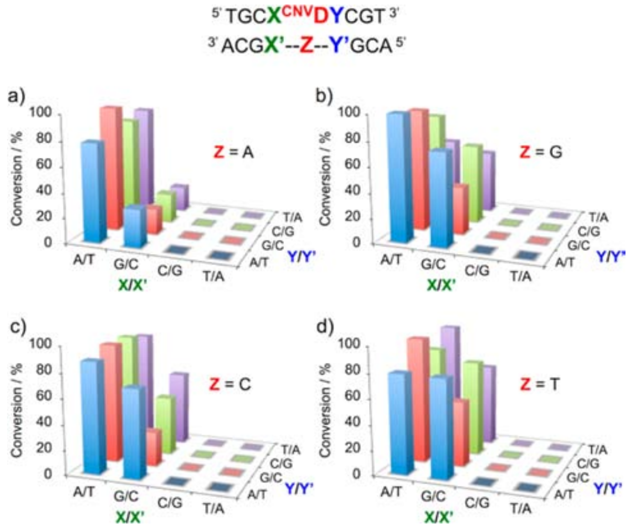

Figure 4. Effect of surrounding nucleobases of ^{CNV}D on its photoreactivity in the DNA double strand. [duplex] = 15 μM in 50 mM Na-Cacodylate buffer (pH 7.4) containing 100 mM NaCl and 50 μM dU (as an internal standard). Photoirradiation (366 nm, 1280 mW/cm^2, 1 s) was performed at 0 °C. Conversion was analyzed by UPLC.

In this study, we successfully demonstrated that the ^{CNV}D whose structure is flexible compared to ^{CNV}K has greater photoreactivity for the pyrimidine base at the −1 position in the cODN strand. The photoreactivity was 1.8- (for T), 8- (for C), and 2.8-fold (for U in RNA strand) greater than that of ^{CNV}K. The high photoreactivity of ^{CNV}D can spread the applicability of the DNA photo-cross-linking reaction in nanotechnology and biotechnology because the photodamage on nucleic acids or cells is reduced. In particular, the improved photoreactivity observed toward C might enable use of photochemical DNA/RNA editing[9] in cells with lower phototoxic effect.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental procedures and a list of oligonucleotide sequences; HPLC analysis of cross-linked ODN after enzymatic digestion; UPLC chromatograms of photo-cross-linking reaction of ODN(AL) and cODN(GT); UPLC chromatograms of photo-cross-linking reaction of ODN(AX) and cORN(GU); UPLC chromatograms of photosplitting reaction of ODN(AX) and cODN(GT); UV melting curve and CD spectra of the duplex consisting of ODN(AL) and cODN(GT). This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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