Bioconjugate Chemistry

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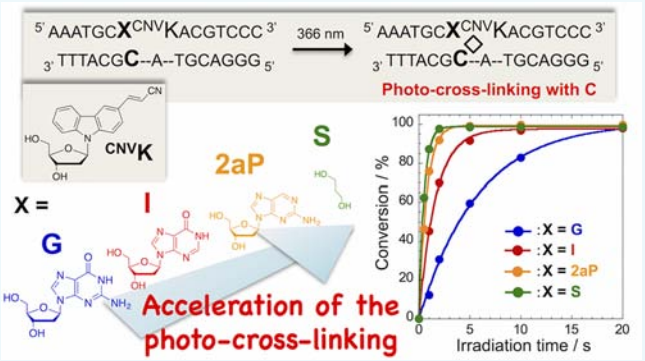
Critical Effect of Base Pairing of Target Pyrimidine on the Interstrand Photo-Cross-Linking of DNA via 3-Cyanovinylcarbazole Nucleoside

Takashi Sakamoto, Minako Ooe, and Kenzo Fujimoto*

School of Materials Science, Japan Advanced Institute of Science and Technology, 1−1 Asahi-dai, Nomi, Ishikawa 923-1292, Japan

Supporting Information

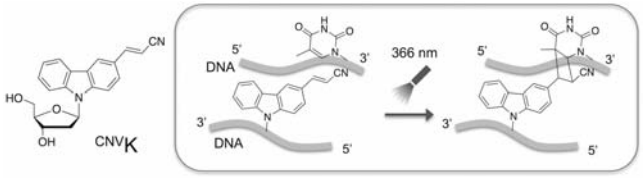
ABSTRACT: To evaluate the effect of base pairing of the target pyrimidine on the interstrand photo-cross-linking reaction of DNA via 3-cyanovinylcarbazole nucleoside (^{CNV}K), a complementary base of target pyrimidine was substituted with noncanonical purine bases or 1,3-propandiol (S). As the decrease of the hydrogen bonds in the base pairing of target C accelerated the photo-cross-linking reaction markedly (3.6- to 7.7-fold), it can be concluded that the number of hydrogen bonds in the base pairing, i.e., the stability of base pairing, of the target pyrimidine plays a critical role in the interstrand photo-cross-linking reaction. In the case of G to S substitution, the highest photoreactivity toward C was observed, whose photoreaction rate constant (k = 2.0 s^{-1}) is comparable to that of ^{CNV}K toward T paired with A (k = 3.5 s^{-1}). This is the most reactive photo-cross-linking reaction toward C in the sequence specific interstrand photo-cross-linking. This might facilitate the design of the photo-cross-linkable oligodeoxyribonucleotides for various target sequences.



INTRODUCTION

The photo-cross-linking technology toward nucleic acids has wide potential for regulating the structures and functions of nucleic acids. Various photo-cross-linkers, e.g., psoralen,[1−4] coumarin,[5] and diazirine,[6,7] which can cross-link with the nucleobase on the complementary strand, have been developed and applied for regulating gene expression[8−11] and RISC functions,[12] and for constructing DNA nanostructures.[13−15] As another class of DNA photo-cross-linker, we previously reported 3-cyanovinylcarbazole nucleoside (^{CNV}K; Scheme 1)

Scheme 1



with an ultrafast photo-cross-linking manner.[16] Since oligodeoxyribonucleotides (ODN(s)) having ^{CNV}K can photo-cross-link with the pyrimidine base in the complementary DNA or RNA possessed at the −1 position of ^{CNV}K via [2 + 2] photocycloaddition reaction within a few seconds of 366 nm irradiation, ^{CNV}K has wide potential for regulating nucleic acid functions without photodamage to cells[17,18] and for constructing DNA-based thermally stable nanostructures.[19−21] In our previous report, we tried to clear the mechanism of the photoreaction from the viewpoint of the structure of the photo-cross-linked product[22] and also the geometry of the vinyl group of ^{CNV}K and the C5−C6 double bond of the target pyrimidine base;[23] however, the effect of the local stability of the base paring of the target pyrimidine has not been evaluated. Most recently, we found that the ^{CNV}K analogue having D-threoninol instead of 2′-deoxyribose has higher photoreactivity compared to ^{CNV}K.[24] As the D-threoninol skeleton has higher flexibility compared to the 2′-deoxyribose skeleton, the photoreactivity of 3-cyanovinylcarbazole might largely depend on the stability and/or the flexibility of the structure around the reaction point, i.e., vinyl group on 3-cyanovinylcarbazole moiety and C5−C6 double bond on the target pyrimidine base.

In this study, to evaluate the effect of the base pairing of the target pyrimidine, which is the reactive nucleobase of the interstrand photo-cross-linking reaction of DNA via ^{CNV}K, on the photoreactivity, noncanonical purine bases—inosine (I), 2-aminopurine (2aP) or 2-aminoadenine (2aA), or 1,3-propandiol (S)—were introduced to ODNs having ^{CNV}K at the opposite site of the target pyrimidine base.

RESULTS AND DISCUSSION

To clear the effect of the base pairing of the target cytosine, at first, three duplexes consisting of ODN(XK) (X = G, I, or 2aP) and cODN(C) were irradiated (366 nm) and analyzed by ultra-

Received: June 25, 2015
Revised: July 15, 2015
Published: July 18, 2015

DOI: 10.1021/acs.bioconjchem.5b00352
Bioconjugate Chem. 2015, 26, 1475−1478

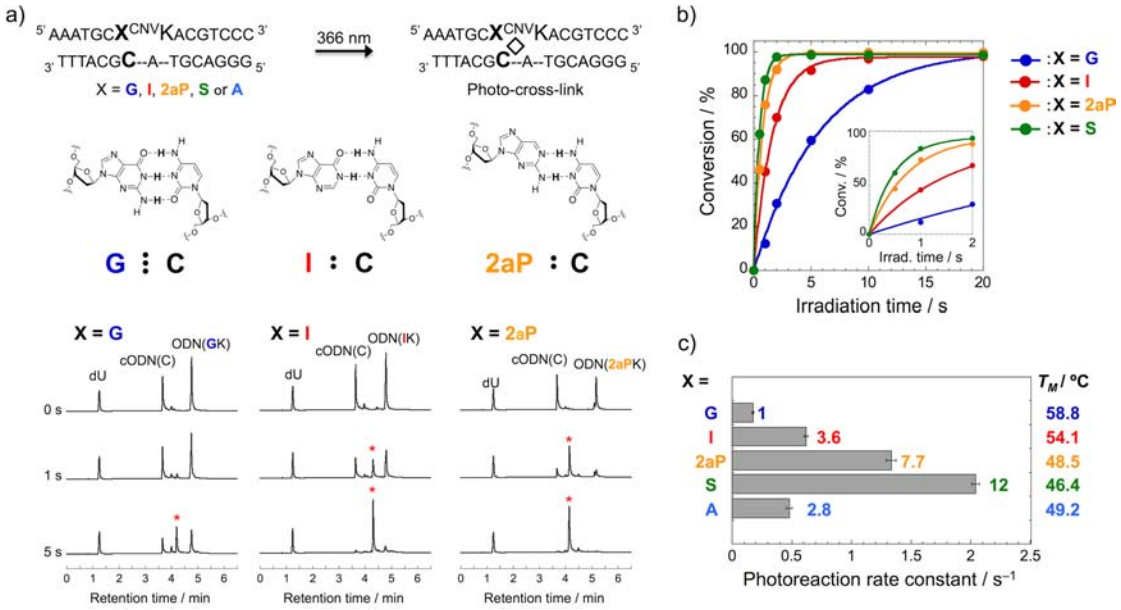


Figure 1. (a) UPLC analysis of the photo-cross-linking reaction of the duplexes consisting of ODN(XK) and cODN(C). [ODN(XK)] = [cODN(C)] = 15 μM, [dU] (internal standard) = 75 μM in 50 mM Na-cacodylate buffer (pH 7.4) containing 100 mM NaCl. Irradiation was performed with an LED light source (366 nm, 1600 mW/cm^2) at 0 °C. Asterisks indicate the photo-cross-linked duplexes. (b) Time course of the photo-cross-linking reactions of the duplexes consisting of ODN(XK) and cODN(C). (c) Photoreaction rate constants and melting temperature (T_M) of the duplexes. The values indicated beside the bars indicate the acceleration ratio compared to the case of X = G. Photoreaction rate constants were estimated from the time course of the photoreactions (part b or Figure S2) with an assumption of first-order reaction kinetics. The values of photoreaction rate constants are listed in Table S2.

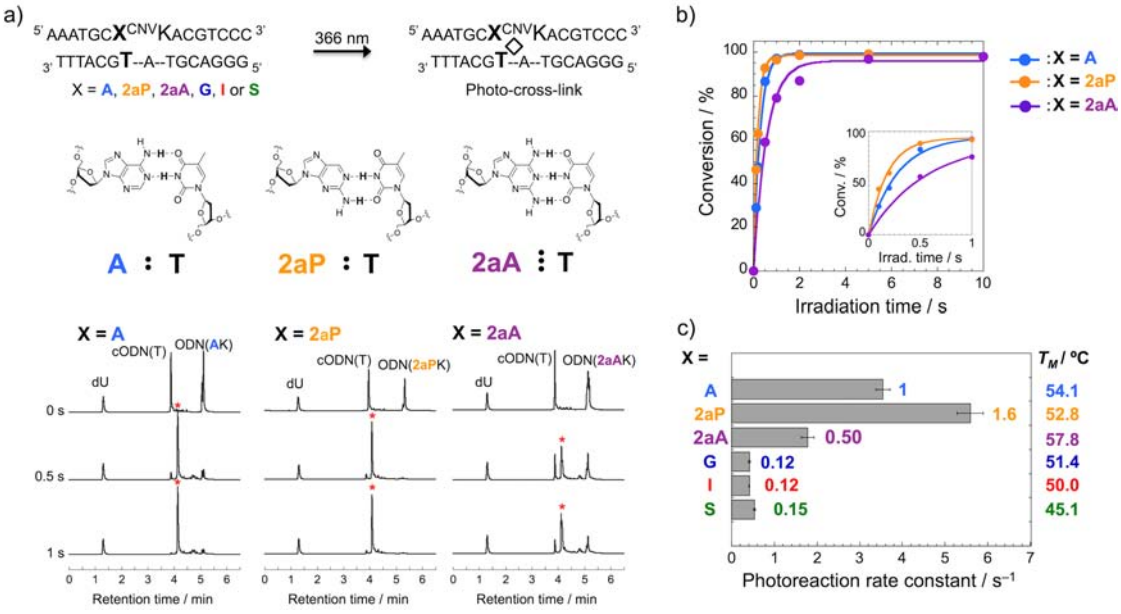


Figure 2. (a) UPLC analysis of the photo-cross-linking reaction of the duplexes consisting of ODN(XK) and cODN(T). [ODN(XK)] = [cODN(T)] = 15 μM, [dU] (internal standard) = 75 μM in 50 mM Na-cacodylate buffer (pH 7.4) containing 100 mM NaCl. Irradiation was performed with an LED light source (366 nm, 1600 mW/cm^2) at 0 °C. Asterisks indicate the photo-cross-linked duplexes. (b) Time course of the photo-cross-linking reactions of the duplexes consisting of ODN(XK) and cODN(T). (c) Photoreaction rate constants and melting temperature (T_M) of the duplexes. The values indicated beside the bars indicate the acceleration ratio compared to the case of X = T. Photoreaction rate constants were estimated from the time course of the photoreactions (part b or Figure S4) with an assumption of first-order reaction kinetics. The values of photoreaction rate constants are listed in Table S3.

high-performance liquid chromatography (UPLC). As shown in Figure 1a, in all three cases, an initial two peaks identical to ODN(XK) and cODN(C) were decreased and a new peak (*) identical to the photo-cross-linked duplexes appeared by the photoirradiation, suggesting that the photo-cross-linking reaction occurred in all three cases. After 5 s irradiation, the initial two peaks completely disappeared in the case of ODN(IK) and ODN(2aPK), although the peaks still remained

DOI: 10.1021/acs.bioconjchem.5b00352
Bioconjugate Chem. 2015, 26, 1475−1478

in the case of ODN(GK), suggesting that the photoreactivity of ^{CNV}K toward C was accelerated by G to I or 2aP substitution.

To evaluate quantitatively the acceleration effect, the time course of the photoreactions were plotted and the photoreaction rate constants were estimated by nonlinear least-squares curve-fitting (Figure 1b). As shown in Figure 1c, the photoreaction rate constraints of ODN(IK) and ODN(2aPK) were 3.6- and 7.7-fold larger than that of ODN(GK), respectively, suggesting that the number of hydrogen bonds between the base pair largely affected the photoreactivity of ^{CNV}K, and the relatively weak base pairing of target C was favorable for the photo-cross-linking reaction. Surprisingly, 12-fold acceleration of the photoreactivity was observed in the case of ODN(SK). Together with the result of the case of ODN(IK) and (2aPK), it is strongly suggested that the restricted motion of C in the double-stranded DNA caused by base pairing with an opposite nucleobase suppressed the photo-cross-linking reaction of ^{CNV}K and C. In the case of ODN(AK), which forms a mismatched base pair with target C, 2.8-fold acceleration was observed, suggesting that the perturbation of the structure around target C might increase the flexibility of target C and enhance the accessibility of the vinyl moiety of ^{CNV}K toward the C5−C6 double bond of target C. As the melting temperature (T_M) of the duplexes decreased upon substitution of G with I, 2aP, or S, it is also confirmed that the number of hydrogen bonds between target C and the opposite base decreased clearly by the substitution.

Next, to clear the base pairing effect on the photo-cross-linking reaction toward T, the A possessed at the opposite position of target T was substituted with 2aP or 2aA, which forms a base pair with T via two or three hydrogen bonds, respectively, (ODN(2aPK) and ODN(2aAK)). UPLC analysis after the 1 s of photoirradiation (Figure 2a) clearly shows that the initial peaks identical to unreacted ODN(XK) and cODN(T) completely disappeared in the case of ODN(AK) and ODN(2aPK), although the peaks still remained in the case of ODN(2aAK). Indeed, the photoreaction rate constant of ODN(2aAK) was 50% lower than that of ODN(AK) (Figure 2c). This suggests that the increase in the number of the hydrogen bonds, which increases the stability of the base pairing and decreases the flexibility of the target pyrimidine, suppresses the photoreactivity toward T, same as in the case of C. As the T_M of the duplex in the case of X = 2aA was higher than that in the case of X = A, it also confirmed that the number of hydrogen bonds between target T and the opposite base was clearly increased by the substitution. Contrary to the case of the photo-cross-linking with C, the substitution of S suppresses the reactivity toward target T. It seems that when the target T paired with A, the geometry of the C5−C6 double bond on the T and the vinyl group on ^{CNV}K was favorable for the photoreaction, and the flexible structure caused by the substitution of A to S disrupted the favorable geometry.

As shown in Table S2 (Supporting Information), the largest photoreaction rate constant for cross-linking with C (k = 2.0 s^{-1}) was observed in the case of S possessed at the opposite site of target C. Since the value is comparable to the photoreaction rate constant toward T paired with A (k = 3.5 s^{-1}, Table S3), degrees of freedom for designing photoreactive ODN sequence having ^{CNV}K is increased. This might strongly contribute to further application of this interstrand photo-cross-linking technology. When we take into account that the ^{CNV}K based interstrand photo-cross-linking reaction is now being applied for various biotechnologies, such as ribosome display microarray,[25] construction of nanostructured DNAs,[20] and as the surface primer for next-generation genome sequencing,[26] the findings described here will provide basic and valuable knowledge for applying the photoreaction for various biotechnologies.

In conclusion, the photoreactivity of ^{CNV}K in double-stranded DNA depended largely on the nucleobase paired with the target pyrimidine. The photoreactivity was increased with the decrease of hydrogen bonds in the base pairing of the target pyrimidine, suggesting strongly that the local stability and/or flexibility of the target pyrimidine largely affected the photoreactivity of ^{CNV}K. The accelerated reactivity toward C might contribute largely to facilitating the design of photoreactive ODNs having ^{CNV}K.

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures, characterization of ODNs, UPLC chromatograms, photoreaction rate constants, UV melting curves. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.bioconjchem.5b00352.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: kenzo@jaist.ac.jp. Tel & Fax: +81-761-51-1671.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This study was supported by JSPS KAKENHI Grant Number 26288077 (Grant-in-Aid for Scientific Research (B)), 22220001 (Grant-in-Aid for Scientific Research (S)) and MEXT KAKENHI Grant Number 24104003 (Grant-in-Aid for Scientific Research on Innovative Areas (Molecular Robotics)) to K.F.

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DOI: 10.1021/acs.bioconjchem.5b00352
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