Catalytic Repair of a Thymine Dimer in DNA via Carbazole Nucleoside

Yoshinaga Yoshimura and Kenzo Fujimoto*
School of Materials Science, Japan Advanced Institute of Science and Technology and PRESTO, Japan Science and Technology Agency, 1-1 Ashahidai, Nomi 923-1292

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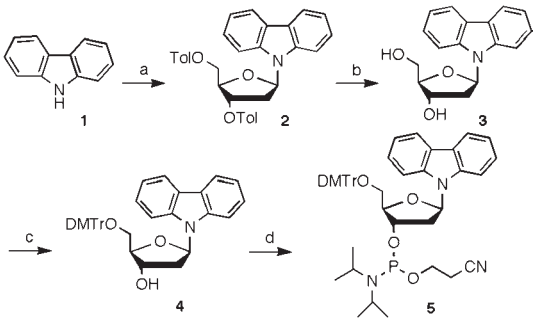
We report the catalytic repair of a thymine dimer incorporated in a DNA duplex via oligodeoxynucleotide (ODN) containing carbazole nucleoside (K). The occurrence of an electron transfer between K and thymine dimer is evidenced by fluorescence quenching measurements. K acts as a good electron donor for the catalytic repair of a thymine dimer.

Considerable environmental damage to DNA is caused by the formation of UV-induced photolesions, which can be both mutagenic and carcinogenic. UV irradiation of cells induces a [2 + 2] cycloaddition of pyrimidines located above each other in the DNA double strand.[1] DNA photolyase selectively recognizes the thymine dimer in DNA single and double strands and repairs it by photoinduced electron transfer, using reduced flavine coenzyme as the electron donor.[2] By using a DNA assay consisting of an artificial DNA base with a flavine structure as the electron donor, Carell et al. could show that the thymine dimer in DNA repairs through reductive photoinduced electron transfer.[3] Barton et al. have observed the repair of a thymine dimer as its radical cations with a rhodium intercalator or a naphthalene diimide intercalator, which both remove a light-induced electron from the DNA strand.[4] Although a photolyase mimic composed of small-molecule recognition units was used to achieve catalytic repair of a thymine dimer,[5] the catalytic repair of a thymine dimer incorporated in a DNA duplex has not been extensively investigated. The catalytic repair is convenient and practical from a medicinal perspective. Carbazole derivatives have strongly hydrophobic surfaces and have been used as electron donors.[6] Deoxyribosides of carbazole have been incorporated into oligodeoxynucleotide (ODN) and were used as probes to detect nucleic acid hybridization.[7] These properties of carbazole derivatives are expected to be exploited as electron donors to study the repair of a thymine dimer in DNA. We have been studying artificial DNA bases as a tool for photochemical DNA manipulations.[8] We now report on the catalytic repair of a thymine dimer incorporated in a DNA duplex via carbazole nucleoside (K).

The synthesis of the phosphoramidite of K is outlined in Scheme 1. Compound **2** was synthesized from carbazole **1** and Hoffer's α-chlorosugar. Deprotection of **2** with sodium methoxide afforded **3** (K) in improved yield as compared with the method reported in the literature.[7] Compound **3** was dimethoxytritylated, and converted into the nucleoside phosphoramidite **5**. The assignments of β-stereochemistry at C1′ for **3** was based on COSY and NOESY spectra, which showed a cross-peak between H1′ and H4′. The modified ODN containing K, ODN(K) 5′-d(ACTGTCACGCKTCACAT)-3′, ODN(KK) 5′-d(ACTGTCACGCKKTCACAT)-3′, ODN(KA) 5′-d(ACTGTCACGCKATCACAT)-3′, were prepared, according to the standard phosphoramidite chemistry, on a DNA synthesizer using phosphoramidite



Scheme 1. Reagents and conditions: (a) KOH, TDA-1, chlorosugar, CH_3CN, rt, 20 min, 59%; (b) $NaOCH_3$, CH_3OH, rt, 2 h, 86%; (c) 4,4′-dimethoxytrityl chloride, DMAP, pyridine, rt, 15 h, 36%; (d) $(i\text{-}Pr_2N)_2PO(CH_2)_2CN$, 1*H*-tetrazole, CH_3CN, rt, 2 h, quant.

5. ODNs containing K were characterized by the nucleoside composition and MALDI-TOF-MS.[9] Thymine dimer formation in synthetic ODNs was performed photochemically according to a method reported in the literature.[10]

We determined the feasibility of the photochemical repair of a thymine dimer in DNA via ODN containing K. When ODN(K) was irradiated at 365 nm for 30 min in the presence of ODN1(T=T) 5′-d(ATGTGAT=TGCGTGACAGT)-3′, we observed the appearance of a peak of ODN1(TT) 5′-d(ATGTGATTGCGTGACAGT)-3′ in 92% yield as determined by HPLC with the disappearance of ODN1(T=T) (Figure 1).[11,12] MALDI-TOF-MS indicated that isolated ODN1(TT) obtained from HPLC purification was a repaired product of ODN1(T=T) (m/z: calcd 5570.69 for $[M + H]^+$, found 5570.70). The isolated ODN1(TT) was digested with AP and P1 nuclease at 37 °C for

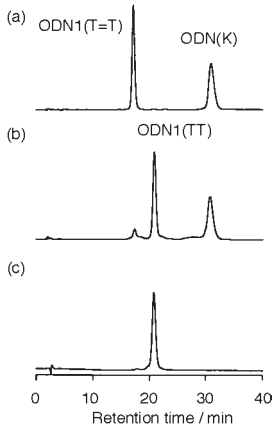


Figure 1. HPLC analysis of the irradiated ODN(K) in the presence of ODN1(T=T): (a) before irradiation; (b) irradiated at 365 nm for 30 min; (c) nonirradiated authentic ODN1(TT).

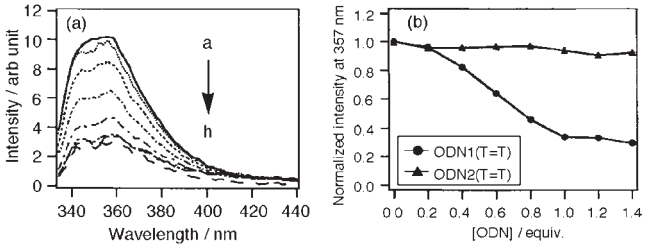


Figure 2. (a) Fluorescence spectra of ODN(K) excited at 330 nm in the absence/presence of ODN1(T=T) of varying concentrations: a) 0, b) 0.2, c) 0.4, d) 0.6, e) 0.8, f) 1.0, g) 1.2, and h) 1.4 equiv. (b) Fluorescence quenching of ODN(K) with ODN1(T=T) or ODN2(T=T).

4 h. Enzymatic digestion of isolated ODN1(TT) showed the formation of dC, dG, dT, and dA in a ratio of 2:6:6:4. The quantum yield for the photochemical repair by using ODN(K) was estimated ($\Phi = 0.014$) at 365 nm, based on the disappearance of ODN1(T=T) by employing valerophenone as an actinometer.[13] When ODN(KK) or ODN(KA) was used in the repair of a thymine dimer, we observed the appearance of a peak of ODN1(TT) in 94 and 93% yields, respectively. The thermal stability of the duplex between ODN containing K and ODN1(T=T) was investigated by monitoring the melting temperature (T_m). The T_m value (53.0 °C) of ODN(K) and ODN1(T=T) was lower than that of ODN(K) and ODN1(TT) (56.9 °C), whereas the T_m value (53.5 °C) of ODN(KK) and ODN1(T=T) was higher than that of ODN(KK) and ODN1(TT) (51.7 °C). The T_m value (53.6 °C) of ODN(KA) and ODN1(T=T) was equal to that of ODN(KA) and ODN1(TT).

To elucidate the electron-transfer phenomena from ODN(K) to ODN1(T=T), fluorescence quenching of ODN(K) with ODN1(T=T) was performed in a 50 mM sodium cacodylate buffer (pH 7.0) and 100 mM sodium chloride at a strand concentration of 200 μM. As shown in Figure 2a, the fluorescence of ODN(K) was quenched efficiently by ODN1(T=T). On the other hand, when ODN composed of mismatch bases, ODN2(T=T) 5′-d(GCACAGT=TATACAGAGAG)-3′, was used as a quencher, the fluorescence of ODN(K) was scarcely quenched (Figure 2b). Furthermore, when ODN2(T=T) was used in repair, the repaired product of ODN2(T=T) was scarcely observed. When ODN1(T=T) was irradiated at 365 nm in the presence of complementary ODN 5′-d(ACTGTCACGCAATCACAT)-3′, the repaired product of ODN1(T=T) was scarcely observed. From these results, ODN(K) can promote the repair of thymine dimer incorporated in a DNA duplex by electron transfer from carbazole.

We determined the feasibility of the catalytic repair of a thymine dimer in DNA via the modified ODN containing K. To measure turnover with ODNs containing K, we compared the number of equivalents of yield to moles of ODN containing K.[14] When ODN containing K was irradiated at 365 nm for 12 h in the presence of ODN1(T=T), we observed the number of turnovers by HPLC analysis.[15] These results showed that for all three cases at 25 °C adequate amounts of turnover were observed (Figure 3). In particular, the case of ODN(KK) was the most efficient of all, yielding 6.7 turnovers. The temperature is expected to have significant effects, both on the yield of repair and on turnover efficiency. To test for such effects, we carried out catalytic repairs at 0 °C. For most cases, we observed the

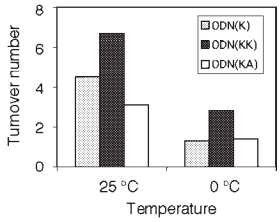


Figure 3. Effect of temperature on turnover for three ODNs containing K.

turnovers even at 0 °C.

In conclusion, we demonstrated the catalytic repair of a thymine dimer in DNA via ODN containing K. When ODN containing K was photoirradiated in the presence of ODN containing thymine dimer, the thymine dimer in DNA was catalytically repaired through reductive photoinduced electron transfer. ODN containing K can be used for the catalytic repair of a thymine dimer and has the potential to allow spectroscopic investigation of electron transfer in DNA.

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