Photoinduced repair of a thymine dimer in DNA via carbazole nucleoside

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ABSTRACT

We report the photoinduced 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 photoinduced repair of a thymine dimer.

INTRODUCTION

Considerable environmental damage to DNA is caused by the formation of UV-induced photoslesions. 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 flavin 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, which both produce a light-induced electron from the DNA strand.$^4$ Carbazole derivatives have strongly hydrophobic surfaces and have been used as electron donors.$^5$ Deoxyribosides of carbazole were used as probes to detect nucleic acid hybridization.$^6$ 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.$^7$ We now report on the photoinduced repair of a thymine dimer incorporated in a DNA duplex via K.

RESULTS AND DISCUSSION

The nucleoside phosphoramidite of K was prepared according to a method reported in the literature.$^8$ The assignment of β-stereochemistry at C1’ for K was based on COSY and NOESY spectra, which showed a cross-peak between H1’ and H4’. The modified ODN containing K, ODN 1, ODN 2, and ODN 3, were prepared, according to the standard phosphoramidite chemistry, on a DNA synthesizer using phosphoramidite of K (Fig. 1a). ODNs containing K were characterized by the nucleoside composition and MALDI–TOF–MS. The synthesized ODNs are summarized in Table 1. Thymine dimer formation in synthetic ODNs was performed photochemically according to a method reported in literature.$^9$

![Fig. 1 (a) Structure of carbazole nucleoside, K. (b) Schematic illustration of the photoinduced repair of a thymine dimer.](https://example.com/fig1.jpg)

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We determined the feasibility of the photochemical repair of a thymine dimer in DNA via ODN containing K. When ODN 1 was irradiated at 365 nm for 30 min in the presence of ODN 4 (Fig. 1b), we observed the appearance of a peak of ODN 5 in 92% yield as determined by HPLC with the disappearance of ODN 4 (Fig. 2). MALDI–TOF–MS indicated that isolated ODN 5 obtained from HPLC purification was a repaired product of ODN 4 (calcd.
Enzymatic digestion of isolated ODN 5 showed the formation of dC, dG, dT, and dA in a ratio of 2:6:6:4 (Fig. 3). The quantum yield for the photochemical repair by using ODN 1 was estimated (Φ = 0.014) at 365 nm, based on the disappearance of ODN 4 by employing valerophenone as an actinometer.\(^\text{10}\) When ODN 2 or ODN 3 was used in the repair of a thymine dimer, we observed the appearance of a peak of ODN 5 in 94, 93% yields, respectively. The thermal stability of the duplex between ODN containing K and ODN 4 was investigated by monitoring the melting temperature (T\(_m\)). The T\(_m\) value (53.0°C) of ODN 1 and ODN 4 was lower than that of ODN 2 and ODN 5 (56.9°C), whereas the T\(_m\) value (53.5°C) of ODN 2 and ODN 4 was higher than that of ODN 2 and ODN 5 (51.7°C). The T\(_m\) value (53.6°C) of ODN 3 and ODN 4 was equal to that of ODN 3 and ODN 5.

To elucidate the electron transfer phenomena from ODN 1 to ODN 4, fluorescence quenching of ODN 1 with ODN 4 was performed in a 50 mM sodium cacodylate buffer (pH 7.0) and 100 mM sodium chloride at a strand concentration of 200 μM. The fluorescence of ODN 1 was quenched efficiently by ODN 4. On the other hand, when ODN composed of mismatch bases, ODN 6, was used as a quencher, the fluorescence of ODN 1 was scarcely quenched. Furthermore, when ODN 6 was used in repair, the repaired product of ODN 6 was scarcely observed. When ODN 4 was irradiated at 365 nm in the presence of complementary ODN, ODN 7, the repaired product of ODN 4 was scarcely observed. From these results, ODN 1 can promote the repair of thymine dimer incorporated in a DNA duplex by electron transfer from carbazole.

**CONCLUSION**

We demonstrated the photoinduced 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 repairs through reductive photoinduced electron transfer. ODN containing K can be used for the photoinduced repair of a thymine dimer and has the potential to allow spectroscopic investigation of electron transfer in DNA.

**REFERENCES**