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Photosensitized Cleavage of the Thymine Dimer in DNA via Carbazole Nucleoside

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

Keyword: photocleavage, thymine dimer, photosensitizer, DNA

1. Introduction

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).

2. Experimental

2.1. Materials

The reagents for the DNA synthesizer such as A, G, C, T-β-cyanoethyl phosphoramidite, and CPG support were purchased from Glen Research.

9-Carbazole-1'-β-deoxyriboside-3',5'-di-(*p*-toluoyl)ester **(2)**

To a solution of potassium hydroxide (0.99 g, 3.87 mmol) and Tris[2-(2-methoxyethoxy) ethyl]amine (39.0 mg, 0.12 mmol) in CH_3CN (200 mL) was added carbazole (**1**) (1.00 g, 5.98 mmol) at room temperature and the reaction mixture was stirred at room temperature for 20 min. To this

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reaction mixture was added chlorosugar (2.93 g, 6.16 mmol) at room temperature and the reaction mixture was stirred at room temperature for 20 min. The reaction mixture was monitored by TLC (hexane/AcOEt, 4:1), which showed the absence of starting material. After the reaction mixture was evaporated, the residue was chromatographed on a silica gel using hexane/AcOEt (4:1, v/v) as eluent to give **2** (1.84 g, 59%) as a white powder. ^{1}H NMR ($CDCl_3$) δ 8.03-8.06 (m, 2H), 8.04 (d, 2H, J = 8.2 Hz), 7.99 (d, 2H, J = 8.2 Hz), 7.62-7.65 (m, 2H), 7.20-7.30 (m, 8H), 6.72 (dd, 1H, J = 9.3, 5.8 Hz), 5.80-5.84 (m, 1H), 4.87 (dd, 1H, J = 12.1, 3.0 Hz), 4.80 (dd, 1H, J = 12.1, 3.3 Hz), 4.54-4.57 (m, 1H), 3.16-3.24 (m, 1H), 2.45-2.48 (m, 1H), 2.44 (s, 3H), 2.43 (s, 3H), HRMS (MALDI): calcd. for $C_{33}H_{30}NO_5$ [$(M+H)^+$] 520.2124, found 520.2174.

9-Carbazole-1'-β-deoxyriboside **(3)**

To a solution of **2** (1.83 g, 3.53 mmol) in CH_3OH (180 mL) was added 0.5 M methanolic $NaOCH_3$ (21.2 mL, 10.6 mmol) at room temperature and the reaction mixture was stirred at room temperature for 2 h. The reaction mixture was monitored by TLC ($CHCl_3/CH_3OH$, 9:1), which showed the absence of starting material. After the reaction mixture was evaporated, the residue was chromatographed on a silica gel using $CHCl_3/CH_3OH$ (9:1, v/v) as eluent to give **3** (0.85 g, 86%) as a white powder. ^{1}H NMR ($CDCl_3$) δ 8.06 (d, 2H, J = 8.0 Hz), 7.54 (d, 2H, J = 8.2 Hz), 7.42 (dt, 2H, J = 8.2, 1.1 Hz), 7.20-7.27 (m, 2H), 6.66 (t-like, 1H, J = 7.4 Hz), 4.74-4.77 (m, 1H), 3.97-4.02 (m, 3H), 2.99 (dt, 1H, J = 14.0, 8.2 Hz), 2.26 (ddd, 1H, J = 14.0, 7.0, 3.6 Hz), 2.08 (brs, 1H), 1.99 (brs, 1H), HRMS (MALDI): calcd. for $C_{17}H_{18}NO_3$ [$(M+H)^+$] 284.1286, found 284.1191.

5'-*O*-(4,4'-dimethoxytrityl)-9-carbazole-1'-β-deoxyriboside **(4)**

To a solution of **3** (0.84 g, 2.97 mmol) in pyridine (4.0 mL) was added a solution of 4,4'-dimethoxytrityl chloride (1.20 g, 3.56 mmol) and 4-(dimethylamino)pyridine (73.0 mg, 0.59 mmol) in pyridine (6.0 mL) at room temperature and the reaction mixture was stirred at room temperature for 15 h. After the reaction mixture was evaporated, the residue was chromatographed on a silica gel using $CHCl_3/CH_3OH$ (99:1, v/v) as eluent to give **4** (0.62 g, 36%) as a yellow powder. ^{1}H NMR ($CDCl_3$) δ 8.02-8.05 (m, 2H), 7.61-7.64 (m, 2H), 7.47-7.50 (m, 2H), 7.37 (dd, 4H, J = 8.8, 1.9 Hz), 7.19-7.30 (m, 5H), 6.81 (dd, 4H, J = 8.8, 1.9 Hz), 6.63 (dd, 1H, J = 8.2, 6.6 Hz), 4.73-4.76 (m, 1H), 4.02-4.07 (m, 1H), 3.77 (s, 3H), 3.76 (s, 3H), 3.54-3.56 (m, 2H), 2.92 (dt, 1H, J = 14.0, 8.2 Hz), 2.19 (ddd, 1H, J = 14.0, 6.6, 3.0 Hz), 2.05 (d, 1H, J = 3.3 Hz), HRMS (MALDI): calcd. for $C_{38}H_{36}NO_5$ [$(M+H)^+$] 586.2593, found 586.2594.

5'-*O*-(4,4'-dimethoxytrityl)-9-carbazole-1'-β-deoxyriboside-3'-*O*-(cyanoethoxy-*N,N*-diisopropylamino)phosphoramidite **(5)**

Compound **4** (0.20 g, 0.34 mmol) in a sealed bottle was dissolved in CH_3CN and coevaporated three times in *vacuo*. After substituted with argon, 2-cyanoethyl *N,N,N',N'*-tetraisopropylphosphorodi-amidite (108 μL, 0.34 mmol) in CH_3CN (1.5 mL) and 0.45 M 1*H*-tetrazole in CH_3CN (0.76 □L, 0.34 mmol) were added, and the reaction mixture was stirred at room temperature for 2 h. Then the reaction mixture was extracted with AcOEt, which was washed with a saturated aqueous solution of $NaHCO_3$, and water. The organic layer was collected, dried over anhydrous sodium sulfate, filtered, and evaporated to dryness under reduced pressure. Then, the crude product **5** (0.26 g, quant.) in a sealed bottle was dissolved in CH_3CN and coevaporated three times, and was used for automated DNA synthesizer without further purification.

2.2. Synthesis of oligodeoxynucleotide from **5**

Oligodeoxynucleotide (ODN) sequences were synthesized by the conventional phosphoramidite method by using an Applied Biosystems 3400 DNA synthesizer. The coupling efficiency was monitored with a trityl monitor. The coupling efficiency of crude mixture of **5** was 97% yield. The coupling time of crude mixture of **5** was 999 sec. They were deprotected by incubation with 28% ammonia for 8 h at 55 °C and were purified on a Chemcobond 5-ODS-H column (10 × 150 mm) by reverse phase HPLC; elution was with 0.05 M ammonium formate containing 3-25% CH_3CN, linear gradient (30 min) at a flow rate of 3.0 mL/min. Preparation of oligonucleotides was confirmed by MALDI-TOF-MS analysis.

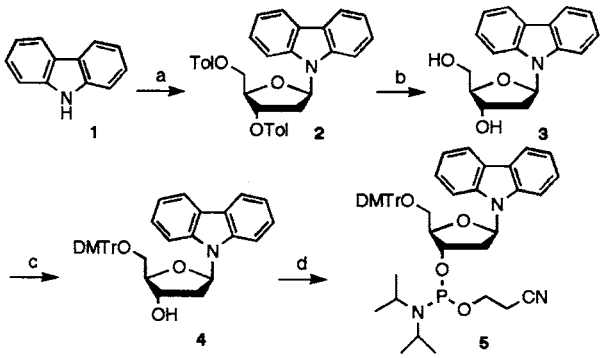
2.3. Measurement

^{1}H NMR spectra were measured with Varian Gemini 300 (300 MHz) spectrometer. Coupling constant (J value) are reported in hertz. The chemical shifts are expressed in ppm downfield from tetramethylsilane, using residual chloroform

(δ = 7.24 in ^{1}H NMR) as an internal standard. Mass spectra were recorded on a Voyager-DE PRO-SF, Applied Biosystems. UV spectra of DNA (3.0 μM) were taken in 50 mM sodium cacodylate buffer (pH 7.0) and 100 mM sodium chloride using a Beckman Coulter DU800 UV/VIS spectrophotometer. In T_m measurements of the duplex, sigmoidal curves on the change of A_{260} were obtained, and the T_m value was calculated from the first part of the curve. Fluorescence spectra of DNA (200 μM) were taken in 50 mM sodium cacodylate buffer (pH 7.0) and 100 mM sodium chloride using a JASCO FP-6500 spectrofluorometer. After irradiation, the progress of photoreaction was monitored by HPLC on a Chemcobond 5C18 ODS column (4.6 × 150 mm, elution with a gradient of 50 mM $HCOONH_4/CH_3CN$ (94:6 to 87:13 over 30 min, to 70:30 over 10 min) at a flow rate 1.0 mL/min). The experiments were carried out on a Beckman P/ACE System MDQ (Beckman Coulter, Fullerton, CA) equipped with an UV absorbance detector. Separations were performed at an applied voltage of 20 kV and at a temperature of 30 °C. ODNs were detected by monitoring their absorbance at 254 nm.

3. Results and Discussion

3.1. Synthesis of oligodeoxynucleotide



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) $(iPr_2N)_2PO(CH_2)_2CN$, 1*H*-tetrazole, CH_3CN, rt, 2 h, quant.

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

3.2. Photochemical repair of a thymine dimer

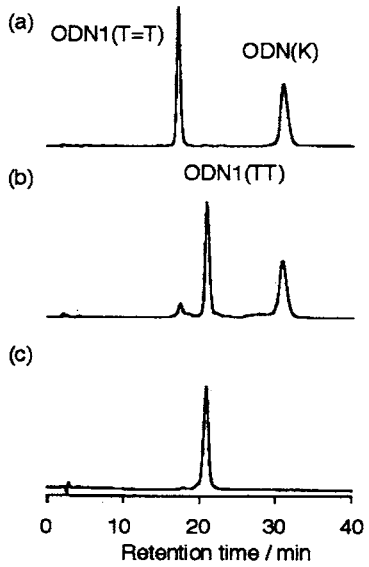


Figure 1. HPLC analysis of the irradiated ODN(K) in the presence of ODN1(T=T): (a) before irradiation; (b) irradiated at 365 nm for 30 min; (c) non-irradiated authentic ODN1(TT).

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

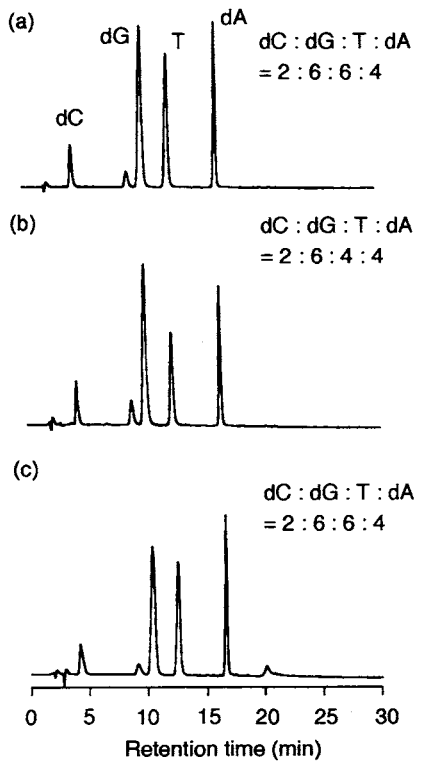


Figure 2. HPLC analysis of the enzymatic digestion: (a) authentic ODN1(TT); (b) ODN1(T=T); (c) isolated ODN1(TT).

disappearance of ODN1(T=T) (Figure 1). The reaction mixture (total volume 30 μL) containing ODN(K) and ODN1(T=T) (each 20 μM, strand concn) in 50 mM sodium cacodylate buffer (pH 7.0) and 100 mM sodium chloride was irradiated with 150 W short arc metal halide lamp (1000 mW/cm^2) equipped with 365 nm UV filter at 0 °C for 30 min. The yield was calculated based on ODN1(T=T). MALDI-TOF-MS indicated that isolated ODN1(TT) obtained from HPLC purification was a repaired product of ODN1(T=T) (calcd. 5570.69 for $[M + H]^+$, found 5570.70). The isolated ODN1(TT) was digested with AP and P1 nuclease at 37 °C for 4 h. As shown in Figure 2, 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 (Φ = 0.014) at 365 nm, based on the disappearance of ODN1(T=T) by employing valerophenone as an actinometer [10]. 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, 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) as shown in Figure 3.

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).

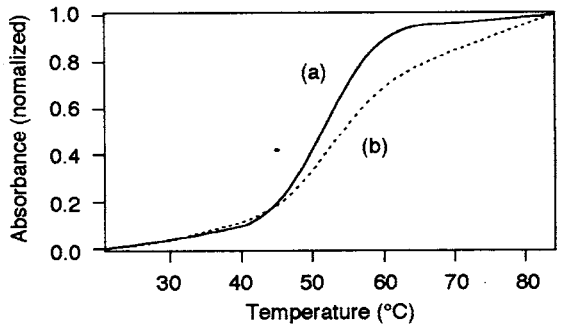


Figure 3. Melting curves: (a) ODN1(TT)/ODN(KK); (b) ODN1(T=T)/ODN(KK).

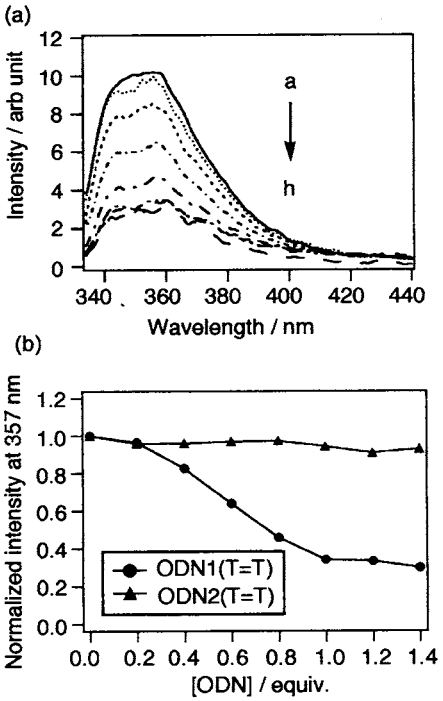


Figure 4. (a) Fluorescence spectra of ODN(K) excited at 330 nm in the absence/presence of ODN1(T=T) of varying concentrations: a) 0, b) 0.2, c) 0.4, d) 0.6, e) 0.8, f) 1.0, g) 1.2, h) 1.4 equiv. (b) fluorescence quenching of ODN(K) with ODN1(T=T) or ODN2(T=T).

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 4a,

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=TATACAGA GAG)-3′, was used as a quencher, the fluorescence of ODN(K) was scarcely quenched (Figure 4b). 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.

3.3. Catalytic repair of a thymine dimer

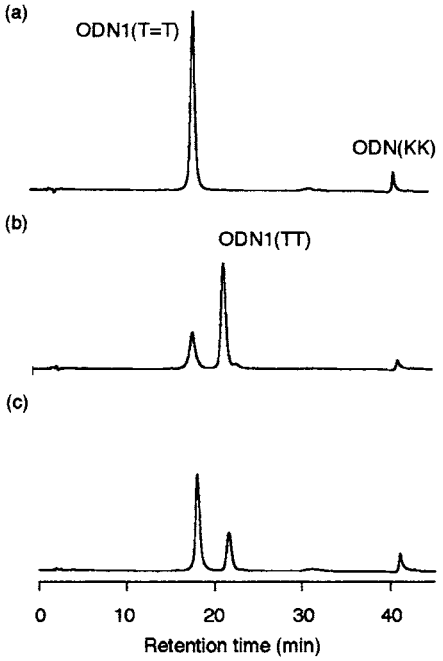


Figure 5. HPLC analysis of the irradiated ODN(KK) in the presence of ODN1(T=T): (a) before irradiation; (b) irradiated at 365 nm for 12 h, at 25 °C; (c) irradiated at 365 nm for 12 h, at 0 °C.

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 [11]. 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. The reaction mixture (total volume 30 μL) containing ODN(KK) (2.0 μM, strand concn) in the presence of ODN1(T=T) (20 μM, strand concn) in 50 mM sodium cacodylate buffer (pH 7.0) and 100 mM sodium chloride was irradiated with 25 W transilluminator (5.7 mW/cm^2) equipped with 365 nm UV filter at 0 or 25 °C for 12 h (Figure 5). These results showed that for all three cases at 25 °C adequate amounts of turnover were observed (Figure 6). 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 turnovers even at 0 °C.

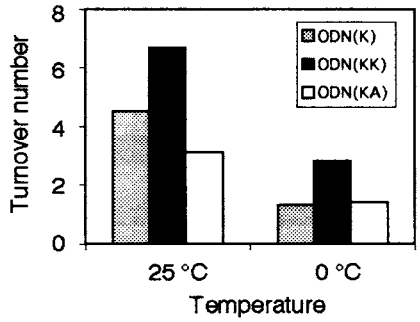


Figure 6. Effect of temperature on turnover for three ODNs containing K.

3.4. Kinetic analysis in DNA hybridization

Kinetic analysis on the binding of ODN1(T=T) or ODN1(TT) to ODN(KK) would provide important information for examining the catalytic repair of a thymine dimer. A biotin-labeled ODN(KK) (5'-d(ACTGTCACGCKKTCACAT)-biotin-3') was immobilized on the surface of the sensor chip by binding with streptavidin according to the procedure recommended by Biacore [12]. The SPR response of the biotin-labeled ODN(KK) was about 1200 RU; a value indicating that about 1.2 ng/mm^2 of the ODN had been bound, i.e., 1000 RU corresponds approximately to 1.0 ng/mm^2 of biomolecules on the sensor chip surface. To determine the kinetic parameters involved in the interactions between target ODN and immobilized ODN(KK), the target ODN were injected into the system and allowed to flow over the immobilized ODN(KK). This was performed at 20 °C at a 30 μl/min flow rate for 3 min. After hybridization, the DNA-DNA duplex was washed with 10 mM HCl alone at a flow rate of 30 μl/min for 1 min to initiate dissociation. The resultant SPR signal corresponded to the changes of the refractive index near the surface of the sensor chip due to the

binding of the target ODN to the immobilized ODN(KK), and a decreasing SPR signal was observed during the dissociation of the target ODN from the formed duplex on the sensor chip surface. The theoretical background and the basic method for determining the kinetic parameters using the procedure recommended by Biacore were previously reported. Figure 7 shows a sensorgram of the interactions between the immobilized ODN(KK) and target ODN. The value of the association rate constant (k_a) for ODN1(T=T) was 2.70×10^4 $M^{-1}s^{-1}$, and the value of the dissociation rate constant (k_d) for ODN1(T=T) was 2.31×10^{-4} s^{-1}. On the other hand, the value of the association rate constant (k_a) for ODN1(TT) was 2.56×10^4 $M^{-1}s^{-1}$, and the value of the dissociation rate constant (k_d) for ODN1(TT) was 1.63×10^{-4} s^{-1}. Interestingly, the former ODN had a k_a value 1.2 times larger. This result indicates that the duplex of ODN 1(TT) and ODN(KK) was less thermodynamically stable than the duplex of ODN 1(T=T) and ODN(KK).

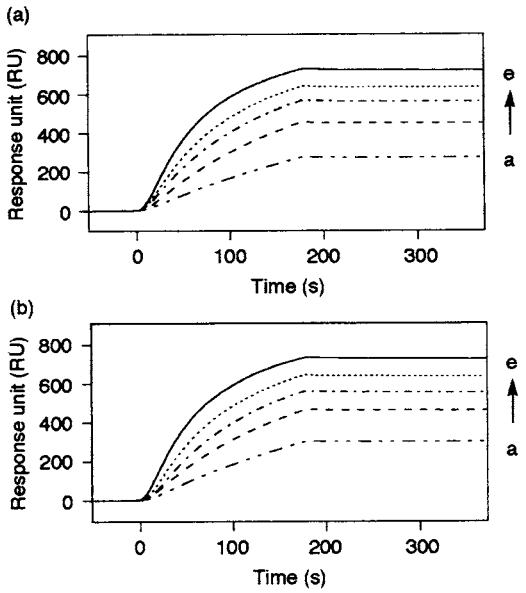


Figure 7. SPR sensorgram for B-ODN(KK): (a) ODN1(T=T); (b) ODN1(TT). The concentrations of ODN1(T=T) or ODN1(TT) are a) 100, b) 200, c) 300, d) 400, and e) 600 nM.

4. Conclusion

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 catalytically repairs 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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References

1. a) J.-S. Taylor, *Acc. Chem. Res.*, **27** (1994), 76. b) P. F. Heelis, R. F. Hartman, S. D. Rose, *Chem. Soc. Rev.*, *24* (1995), 289.
2. a) T. P. Begley, *Acc. Chem. Res.*, **27** (1994), 394. b) A. Sancar, *Chem. Rev.*, **103** (2003), 2203.
3. a) S. Breeger, U. Hennecke, T. Carell, *J. Am. Chem. Soc.*, **126** (2004), 1302. b) C. Haas, K. Kräling, M. Cichon, N. Rahe, T. Carell, *Angew. Chem. Int. Ed.*, **43** (2004), 1842.
4. a) P. J. Dandliker, R. E. Holmlin, J. K. Barton, *Science*, **275** (1997), 1465. b) D. A. Vicic, D. T. Odom, M. E. Núñez, D. A. Gianolio, L. W. McMaughlin, J. K. Barton, *J. Am. Chem. Soc.*, **122** (2000), 8603.
5. O. Wiest, C. B. Harrison, N. J. Saettel, R. Cibulka, M. Sax, B. König, *J. Org. Chem.*, **69** (2004), 8183.
6. a) B. Zelent, G. Durocher, *J. Org. Chem.*, **46** (1981), 1496. b) L.-P. Zhang, B. Chen, L.-Z. Wu, C.-H. Tung, H. Cao, Y. Tanimoto, *Chem. Eur. J.*, **9** (2003), 2763.
7. R. K. Pandey, S. Tripathi, K. Misra, *Nucleosides & Nucleotides*, **17** (1998), 1937.
8. a) K. Fujimoto, S. Matsuda, N. Takahashi, I. Saito, *J. Am. Chem. Soc.*, **122** (2000), 5646. b) S. Ogasawara, K. Fujimoto, *Angew. Chem., Int. Ed.*, **45** (2006), 4512. c) M. Ogino, K. Fujimoto, *Angew. Chem., Int. Ed.*, **45** (2006), 7223. d) Y. Yoshimura, Y. Noguchi, K, Fujimoto. *Org. Biomol. Chem.*, **5** (2007), 139. e) T. Ami, G. Ozaki, Y. Yoshimura, K. Fujimoto, *Chem. Lett.*, **37** (2008), 134.
9. P. J. Dandlinker, M. E. Núñez, J. K. Barton, *Biochemistry*, **37** (1998), 6491.
10. P. J. Wagner, M. J. Thomas, E. Harris, *J. Am. Chem. Soc.*, **98** (1976), 7675.
11. H. Abe, E. T. Kool, *J. Am. Chem. Soc.*, **126** (2004), 13980.
12. J.-P. Jost, O. Munch, T. Andersson, *Nucleic Acids Res.*, **15** (1991), 731.