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Stepwise Regulation of Hole Transport in DNA by Control of Triplex Formation

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Abstract

A functionality for regulating hole transport efficiency is a prerequisite for the utilization of DNA duplexes as nanodevices. Herein, we report the regulation of hole transport in anthraquinone-tethered DNA with dual triplex forming sites. Long-range photooxidation experiments showed that hole transport was effectively suppressed by the formation of triplex at low temperature, while it was recovered by dissociation to the duplex at higher temperature. Variation of temperature induced the formation and dissociation of the third strand at each triplex region individually, leading to the stepwise regulation of hole transport in DNA.

Introduction

There has been an increasing number of studies on long-distance hole transport in DNA, showing that a hole can migrate in a DNA duplex through continuous π-stacking.[1–9] On the basis of this promising hole transport ability of DNA duplexes and the intrinsic fact that DNA forms highly organized structures, DNA is recognized as a potential candidate material for nanoscale molecular devices.[10–18] To utilize DNA duplexes in this way, DNA duplexes need to be equipped with an on/off switching functionality of their hole transport efficiency. Recently, various attempts have been made to modulate the efficiency of hole transport in DNA using modified bases,[19] structural changes of DNA,[20,21] and binding of proteins[22–25] or small molecules[26] to DNA. However, there have been few reports describing reversible on/off regulation of hole transport in DNA duplex.

DNA triplexes are known to be the result of binding a polypyrimidine short strand to a DNA duplex using Hoogsteen hydrogen bonding, the formation of which can be regulated by external triggers such as salt concentration, pH, and temperature.[27–30] Furthermore, experiments on the oxidative cleavage of DNA and the photocurrent measurement of DNA on a gold electrode showed that a DNA triplex region involving a pyrimidine·purine·pyrimidine motif, such as C^+·GC, has a lower hole transport capability because of its cationic environment and dissonant stacking interactions at the duplex-triplex junctions.[31–35]

Following on from these researches on hole transport properties of DNA triplex, we constructed a regulation system of hole transport in DNA by regulating the formation and dissociation of triplex. Figure 1A illustrates the concept of the regulation system, in which hole transport in an anthraquinone (AQ)-tethered duplex[36] is regulated by the thermally-reversible association and dissociation of a short-strand oligodeoxynucleotide (ODN) with duplex DNA to form the triplex. Furthermore, regulation of hole transport by triplex formation and dissociation can be applied to stepwise regulation of hole transport in DNA with dual triplex regions (Figure 1B).

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(Figure 1)

Results and Discussion

Design of AQ-tethered Triplex DNA.

We selected the ODN base sequences so that they form $C^+ \cdot GC$ triplex regions (Figure 2). To evaluate the long-distance hole transport in ODNs with partial triplex structural region, anthraquinone (AQ) chromophore as a photosensitizer for injection of a hole into DNA, was conjugated to the 5'-end of the ODN using a six-carbon linker, according to a previously reported method.[26] Guanine doublets (GG) were arranged on the [32]P-5'-end labeled complementary strands of the AQ-tethered ODNs, because they are an efficient hole trapping site, and thus indicate the occurrence of hole transport by oxidative cleavage.[37]

(Figure 2)

Evaluation of Hole Transport in DNA Possessing a Single Triplex Region.

We initially evaluated the effect of triplex formation on hole transport in DNA duplexes possessing a single triplex region by monitoring oxidative cleavage of G doublets. Long-distance hole transport in **Duplex I** (AQ-ODN 1/ODN 2) and **Triplex I** (AQ-ODN 1/ODN 2/ODN 3) was induced by photosensitization of the AQ chromophore in AQ-ODN 1 in a sodium cacodylate buffer (pH 5.5) at 0 ˚C. Three guanine doublets (GG_1, GG_2, and GG_3) were arranged in the [32]P-5'-end labeled ODN 2 to indicate the occurrence of hole transport. The doublets GG_1 and GG_3 were located adjacent to, and away from the AQ chromophore at the duplex regions, respectively, while GG_2 was in the triplex region of ODN 2. We evaluated the AQ-photosensitized (λ_{ex} = 365 nm) oxidative cleavage of **Duplex I** and **Triplex I** to compare the efficiency of the long-distance hole transport. As shown in Figure 3, GG_1 sites, which were proximal to the AQ chromophore, were cleaved strongly in both

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helices.[38] It is noteworthy that cleavage at the GG_3 doublets in **Triplex I** was reduced to more than

one third of the intensity of **Duplex I**, indicating that partial formation of a triplex can interrupt hole

transport beyond the triplex region. In addition, the cleavage at GG_2 in **Triplex II** was also reduced.

Although triplex formation may cause the suppression of hole transport to GG_2, we could not rule

out the possibility that a reduced degree of cleavage at the GG_2 site in **Triplex I** was the result of

reduced accessibility to oxygen and solvent caused by triplex formation. A photooxidative DNA

cleavage at the G residue occurs via the addition of oxygen and solvent to the G radical cation

intermediate;[39,40] therefore, the cleavage at GG_2 in **Triplex II** may be prevented by steric hindrance

of the triplex-forming strand. In a separate experiment, upon 365 nm photoirradiation of a mixture

of **Duplex I** and mismatched third-strand ODN 4, we detected efficient DNA cleavage at the GG_3

site, which was of a similar magnitude to that observed for **Duplex I** alone (Figure S2). This result

indicates that perfectly matched triplex formation is responsible for the suppression of hole

transport through a DNA duplex.

(Figure 3)

Evaluation of Hole Transport in DNA Possessing Dual Triplex Regions.

We attempted to apply our system to regulate hole transport in DNA possessing dual

triplex-forming regions. We prepared the AQ-tethered 46-mer **Duplex II**, **Triplex II** possessing

7-mer and 15-mer triplex regions, **Triplex III** possessing a 15-mer triplex region, and **Triplex IV**

possessing a 7-mer triplex region (Figure 2). The hole transport at 0 ˚C was evaluated by oxidative

cleavage at three GG sites (GG_4, GG_5, and GG_6), which were arranged to be located at the 3'- and

5'-side of each triplex region. As shown in Figure 4A, photoirradiation of **Duplex II** caused

efficient cleavage at all GG sites, even at the GG_6 doublet, indicating that the hole injected from the

AQ chromophore arrived at the GG_6 site.[38] Addition of the 15-mer ODN 7 to **Duplex II** to form

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Triplex III dramatically reduced cleavage at the GG_6 site, while the GG_4 and GG_5 sites were still cleaved. This result strongly suggests that hole transport between the GG_5 and GG_6 sites was suppressed effectively by the binding of ODN 7 to form a partial triplex, whereas the hole injected by the AQ photosensitizer could still migrate to the GG_5 site. Furthermore, binding of ODN 8, or both ODN 7 and ODN 8, to form **Triplex IV** or **II** evidently reduced oxidative cleavage at GG_5 and GG_6 sites, indicating that the hole transport between GG_4 and GG_6 was suppressed. Thus, hole transport could be properly regulated according to the position of the triplex region. In addition, we evaluated oxidative cleavage of **Triplex II** by photosensitization of 9,10-anthraquinone (Aq) and riboflavin (Ri) which were not linked to the ODN. As shown in Figure 4B, the cleavage pattern at the GG sites in **Triplex II** was similar to that in **Duplex II**. This result indicates that oxygen and solvent can access the guanine radical cations on the GG sites irrespective of triplex formation, resulting in an efficient strand cleavage at every GG site. In view of this, we concluded that the decrease in photosensitized cleavage at the GG_5 and GG_6 sites in the AQ-tethered triplex was almost certainly due to the decrease in hole transport efficiency at the triplex region.

(Figure 4)

Regulation of Hole Transport in DNA Possessing Dual Triplex Regions by Thermal Duplex-Triplex Conversion.

A further attempt was made to regulate hole transport through a DNA possessing dual triplex-forming sites by thermal conversion between the duplex and the triplex. We evaluated AQ-photosensitized oxidative cleavage of **Duplex II** and **Triplex II** to compare the efficiency of hole transport at different reaction temperatures. The reaction temperatures were chosen in the light of the thermal characteristics of **Triplex II** (T_m = 13.6 and 31.1 ˚C for the dissociation of ODN 8 and ODN 7 from **Triplex II**, respectively). The temperature was changed from 0 to 20 ˚C for

dissociation of ODN 8 from **Triplex II**, and then the sample was heated to 40 ˚C for further dissociation of ODN 7. The resulting duplex was cooled to 20 ˚C once again for binding of ODN 7 to form a partial triplex. The photoirradiation was conducted at each temperature to evaluate the stepwise regulation of hole transport in DNA. Figure 5A shows a representative gel picture, and Figure 5B shows the normalized increment of cleavage intensity at the GG_6 site. Compared with the extent of cleavage at the GG_4 site in **Triplex II** (lane 7 in Figure 5A), a weak cleavage at the GG_5 and GG_6 sites in **Triplex II** was observed at 0 ˚C, indicating that hole transport was effectively suppressed by binding of both ODN 7 and ODN 8. Raising the reaction temperature from 0 to 20 ˚C induced a marked increase in cleavage at the GG_5 sites in **Triplex II** (lane 8), but cleavage at GG_6 was still suppressed (Figure 5B). In contrast, we confirmed that cleavage at the GG_6 site in **Duplex II** increased at 20 ˚C (lanes 3) because of the temperature effect on long-range hole transport through the DNA duplex, consistent with previous reports.[34,35,41] These results suggest that the hole injected from the AQ chromophore in **Triplex II** at 20 ˚C could arrive at the GG_5 sites as a result of the dissociation of ODN 8, whereas hole transport between the GG_5 and GG_6 sites was still suppressed by the binding of ODN 7 to form a partial triplex. Further raising the reaction temperature to 40 ˚C increased DNA cleavage at the GG_6 site in **Triplex II** (lane 9 in Figure 5A and Figure 5B),[17] indicating that the dissociation of ODN 7 to form a duplex allows for hole transport to the GG_6 site. The inverse of this behavior was observed on lowering the reaction temperature from 40 to 20 ˚C, showing a resuppression of DNA cleavage at the GG_6 site in **Triplex II** (lane 10 in Figure 5A and Figure 5B). Thus, the rebinding of ODN 7 suppresses hole transport between the GG_5 and GG_6 sites. From these evidences, we conclude that hole transport in DNA possessing triplex regions at dual sites can be regulated individually using a change in temperature.

(Figure 5)

Conclusion

We have demonstrated that reversible structural changes of DNA duplex into triplex are effective for regulating hole transport in DNA. AQ-photosensitized long-distance hole transport in DNA was suppressed to a large extent by triplex formation and was recovered by dissociation of the third strand. This regulation system can be expanded to a stepwise regulation of hole transport in DNA possessing dual triplex-forming sites. Thermal conversion of the duplex and triplex at each triplex-forming site resulted in a site-selective regulation of hole transport in DNA. Such a simple method for the regulation of hole transport would be applicable to on/off switching of DNA hole transport. Further investigations into the on/off regulation of hole transport in higher-order DNA structures are in progress.

Supplementary data

The data includes the autoradiogram of gel electrophoresis for the mixture of Duplex I and ODN 4 after photoirradiation and quantified cleavage data.

Experimental

General. Gel electrophoresis was carried out on a Gibco BRL Model S2 apparatus. $[\gamma\text{-}^{32}\text{P}]$ATP (6000 Ci/mmol) was obtained from Amersham Bioscience. Unmodified DNA oligomers were purchased from Invitrogen. Anthraquinone tethered ODN (AQ-ODN 1 and AQ-ODN 4) was synthesized by the previously reported method.[26] All aqueous solutions were prepared using purified water (YAMATO, WR600A).

T_m **Measurement.** 2.5 μM Solutions of the appropriate ODNs in the presence of MgCl$_2$ in 10 mM sodium cacodylate buffer (pH 5.5) were prepared to determine the melting temperatures (T_m) of duplex and triplex. Melting curves were obtained by monitoring variation of the UV absorbance at 260 nm with elevating temperatures from 2 ˚C to 90 ˚C at a rate of 1 ˚C/min.

Preparation of 5'-^{32}P-End-Labeled ODNs. ODNs (400 pmol strand concentration) were labeled by phosphorylation with 4 μL of $[\gamma\text{-}^{32}\text{P}]$ATP and 4 μL of T$_4$ polynucleotide kinase using standard procedures. The 5'-end-labeled ODNs were recovered by ethanol precipitation, further purified by 15% nondenaturing gel electrophoresis, and then isolated by the crush and soak method.

General Procedures for Photoinduced Cleavage of ^{32}P-5'-End-Labeled ODN Hybridized with AQ-ODN. Triplex samples were prepared in the presence of 2 mM MgCl$_2$ in sodium cacodylate buffer (pH 5.5) by hybridizing 1 μM of AQ-ODN 1 or 5 with a mixture of their cold and radiolabeled complementary strand (1 μM) and 5 μM of the third strand (ODN 3 for **Triplex I**, ODN 7 for **Triplex III**, ODN 8 for **Triplex IV**, and both ODN 7 and ODN 8 for **Triplex II**). Duplex formation was achieved by heating the sample at 90 ˚C for 5 min and slowly cooling to room temperature. Triplex formation was achieved by cooling at 4 ˚C overnight after addition of the third strand to the duplex samples. The ^{32}P-5'-end-labeled duplex and triplex were irradiated at 365

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nm at given temperatures. In the experiment of regulation of hole transport, the reaction vessels were alternately transferred between different reaction temperatures. After irradiation, all reaction mixtures were precipitated with addition of 10 μL of herring sperm DNA (1 mg/mL), 10 μL of 3 M sodium acetate and 800 μL of ethanol. The precipitated DNA was washed with 100 μL of 80% cold ethanol and dried *in vacuo*. The resulting DNA was resolved in 50 μL of 10% piperidine (v/v), heated at 90 ˚C for 20 min and then concentrated. The radioactivity of the samples was assayed using an Aloka 1000 liquid scintillation counter, and the dried DNA pellets were resuspended in 80% formamide loading buffer (a solution of 80% formamide (v/v), 1 mM EDTA, 0.1% xylene cyanol and 0.1% bromophenol blue). All reactions, along with the Maxam-Gilbert G+A sequencing reactions were heat-denatured at 90 ˚C for 3 min and quickly chilled on ice. The samples (1–2 μL, 2–5 $\times$ 10^3 cpm) were loaded onto 15% of polyacrylamide/7 M urea sequencing gels, electrophoresed at 1900 V for 60 min, transferred to a cassette and then stored at –80 ˚C with Fuji X-ray film (RX-U). The gels were analyzed by autoradiography with the ATTO densitograph software library (version 3.0). The intensity of the spots resulting from piperidine treatment was determined by volume integration.

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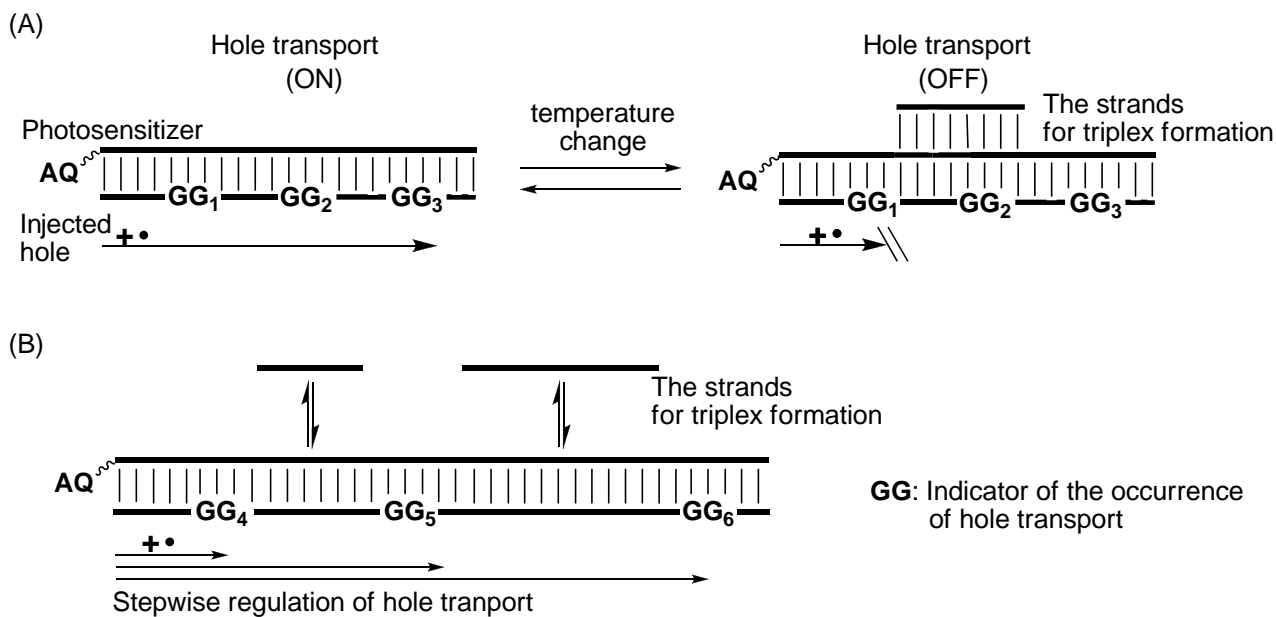(41) Núñez, M. E.; Hall, D. B.; Barton, J. K. *Chem. Biol.* **1999**, *6*, 85.

Figure 1. The protocol for reversible regulation of hole transport in DNA with a single triplex region (A), and dual triplex regions (B). The hole transport is suppressed by triplex formation and efficiently recovered by dissociation of triplex into duplex, as regulated by temperature change.

ODN 1: 5'-TTTAGCC A*TTCC TTCT*ACC TTGAT-3'

ODN 2: 3'-AAATC**GG₁**T*AAGG₂AAGA*T**GG₃**AACTA-5'

ODN 3:   5'-*TTCC   TTCT*-3'

ODN 4:   5'-CCGCGCCA-3'

ODN 5: 5'-TTTAGCC A*CTTCTCT*ACC TGATAGG*TCTCTCTCTCTCT*ACC AGC-3'

ODN 6: 3'-AAATC**GG₄**T*GAAGAGA*T**GG₅**ACTATCC*AGAGAGAGAGAGAGA*T**GG₆**TCG-5'

ODN 7:   3'-*TCTCTCTCTCTCT*-5'

ODN 8:   3'-*CTTCTCT*-5'
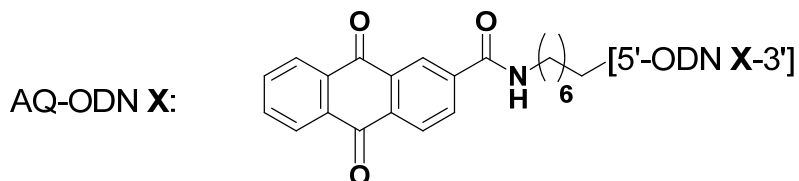
AQ-ODN **X**:

Duplex I: AQ-ODN 1/ODN 2   **Duplex II**: AQ-ODN 5/ODN 6

Triplex I: AQ-ODN 1/ODN 2/ODN 3   **Triplex II**: AQ-ODN 5/ODN 6/ODN 7/ODN 8

                                  Triplex III: AQ-ODN 5/ODN 6/ODN 7

                                  Triplex IV: AQ-ODN 5/ODN 6/ODN 8

Figure 2. Sequences and structure of the oligodeoxynucleotides (ODNs) used in this study. The bases arranged in the triplex region are shown in italics.
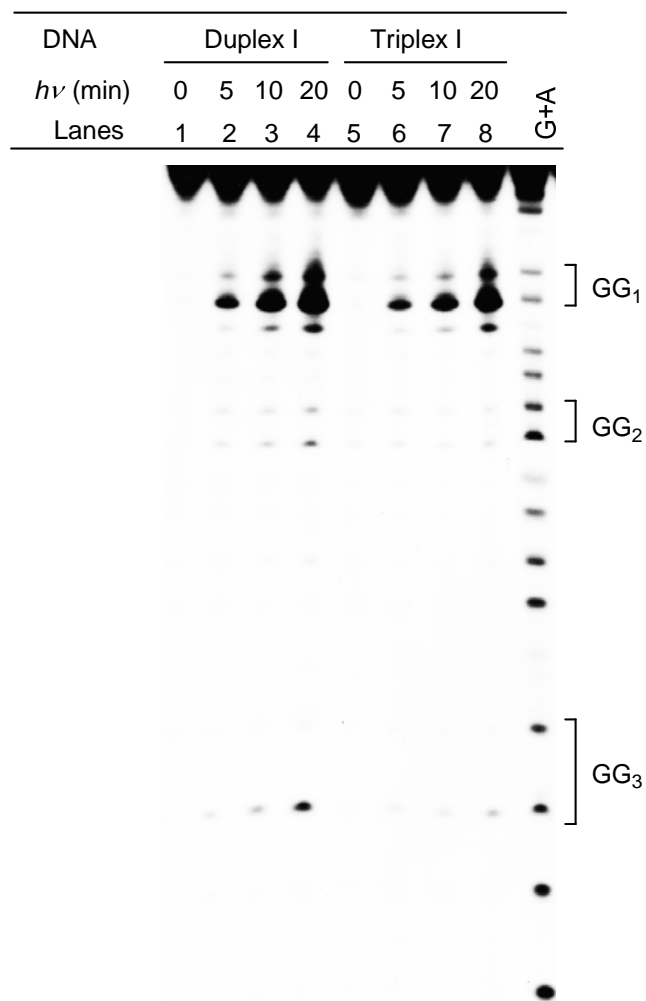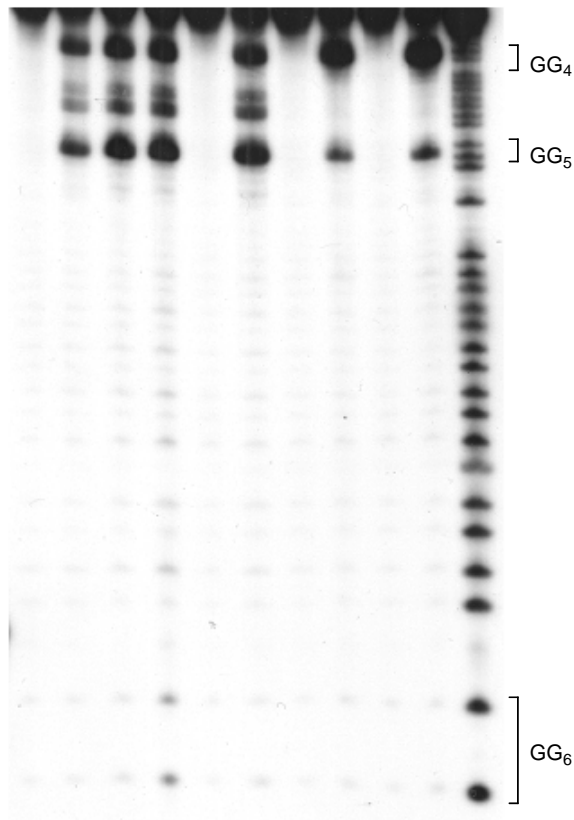
Figure 3. An autoradiogram of a denaturing sequencing gel for the **Duplex I** (AQ-ODN 1/^{32}P-end labeled ODN 2, lanes 1-4) and the the **Triplex I** (AQ-ODN 1/^{32}P-end labeled ODN 2/ODN 3, lanes 5-8) after photooxidation. The samples were photoirradiated at 365 nm in the presence of 2 mM MgCl$_2$ in sodium cacodylate buffer (pH 5.5) at 0 ˚C for 0 (lanes 1 and 5), 5 (lanes 2 and 6), 10 (lanes 3 and 7) and 20 min (lanes 4 and 8) followed by hot piperidine treatment (90 ˚C, 20 min). The lane labeled G + A is Maxam-Gilbert sequencing lane.

Figure 4. Autoradiogram of a denaturing gel electrophoresis for **Duplex II**, **Triplex II**, **Triplex III**, and **Triplex IV** after photooxidation. (A) The **Duplex II** (lanes 1-4), **Triplex II** (lanes 9, 10), **Triplex III** (lanes 5, 6) and **Triplex IV** (lanes 7, 8) were photoirradiated at 365 nm in the presence of 2 mM $MgCl_2$ in sodium cacodylate buffer (pH 5.5) at 0 °C for 0 (lanes 1, 5, 7, and 9), 40 min (lanes 2), 80 min (lanes 3) and 120 min (lanes 4, 6, 8, and 10) followed by hot piperidine treatment (90 °C, 20 min). The lane labeled G + A is Maxam-Gilbert sequencing lane. (B) **Duplex II** (lanes 1-4) and **Triplex II** (lanes 5-8) were photoirradiated in the presence or absence of 9,10-anthraquinone (Aq: 1 μM) or riboflavin (Ri: 4.8 μM) in sodium cacodylate buffer (pH 5.5) containing 2 mM $MgCl_2$ at 0 °C for 120 min. After photoirradiation, the samples were treated with piperidine at 90 °C for 20 min. Lane 1 and 5, dark control in the presence of Aq; lane 2 and 6, irradiation in the absence of photosensitizers; lane 3 and 7, irradiation in the presence of Aq; lane 4 and 8, irradiation in the presence of Ri. The lane labeled G + A is Maxam-Gilbert sequencing lane.
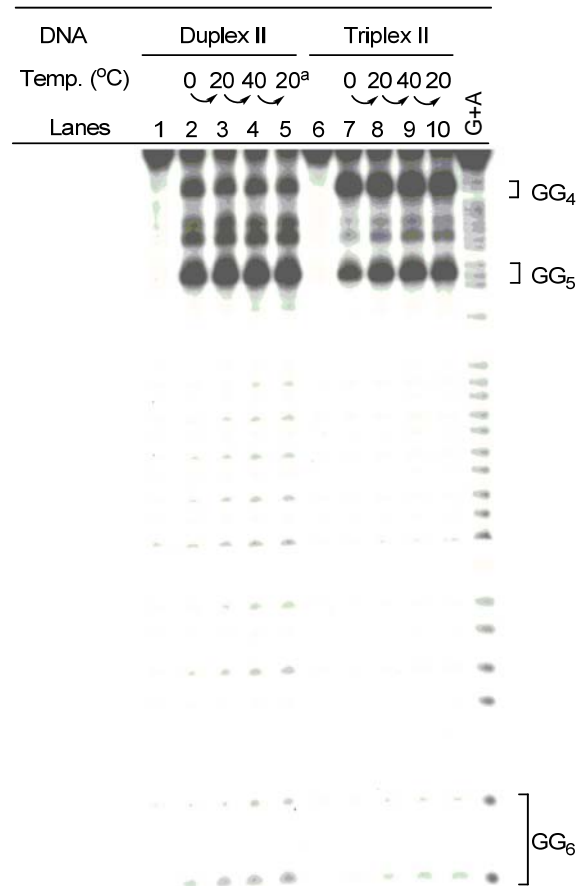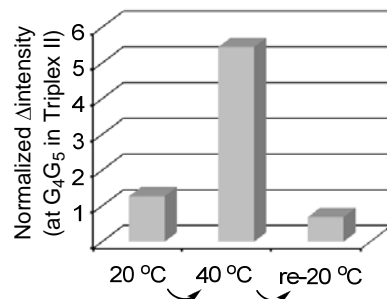
Figure 5. (A) Autoradiogram of a denaturing gel electrophoresis for ^{32}P-5'-end labeled ODN 6 after photooxidation of the **Duplex II** (AQ-ODN 5/ODN 6, lanes 1-5) and **Triplex II** (AQ-ODN 5/ODN 6/ODN 7/ODN 8, lanes 6-10). The reaction vessels containing each DNA strands were transferred into the water baths maintained at 0 (lanes 2 and 7), 20 (lanes 3 and 8) and 40 $^{\circ}$C (lanes 4 and 9) in sequence. Then, the vessels were transferred into the water bath at 20 $^{\circ}$C (lanes 5 and 10), again. The samples were irradiated at 365 nm for 5 min at each temperature. After photoirradiation, small aliquots were removed to analyze photooxidation of helices by hot piperidine treatment. Lanes 1, dark control; lane 2 and 7, irradiation at 0 $^{\circ}$C; lane 3 and 8, irradiation of the sample at 0 $^{\circ}$C and then 20 $^{\circ}$C; lane 4 and 9, irradiation of the sample at 0 and 20 $^{\circ}$C and then 40 $^{\circ}$C.; lane 5 and 10, irradiation of the sample at 0, 20, and 40 $^{\circ}$C and then 20 $^{\circ}$C. (B) The increments of cleavage intensity at GG_6 based on densitometry analysis of Figure 5A. Each Δintensity were obtained as follows. ΔIntensity at GG_6 (20 $^{\circ}$C) = cleavage intensity at GG_6 obtained from lane 8 in Figure 5A (20 $^{\circ}$C) – intensity obtained from lane 7 (0 $^{\circ}$C). Δintensity at GG_6 (40 $^{\circ}$C) = intensity obtained from lane 9 (40 $^{\circ}$C) – intensity obtained from lane 8 (20 $^{\circ}$C). Δintensity at GG_6 (re-20 $^{\circ}$C) = intensity obtained from lane 10 (re-cooled, 20 $^{\circ}$C) – intensity obtained from lane 9 (40 $^{\circ}$C).