Photoelectrochemical evaluation of pH effect on hole transport through triplex-forming DNA immobilized on a gold electrode†

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We characterized pH effect on hole transport through DNA duplexes possessing a partial triplex-forming region. Direct electrochemical measurement of the current response of photosensitizer-tethered DNA immobilized on a gold electrode revealed that the partial triplex formation under acidic conditions suppressed photocurrent due to hole transport, while dissociation of the triplex into the duplex as occurred upon increasing pH values recovered the photocurrent efficiency. Reversible conversion between duplex and triplex induced upon cyclic alternation of pH values resulted in a rise and fall of photocurrent responses, indicating that pH change may feature in the switching function of hole transport in DNA. These electrochemical behaviors could be correlated to the results obtained in long-range photo-oxidative DNA cleavage experiments, in which DNA cleavage at the hole trapping site beyond the triplex region was significantly suppressed under triplex-forming acidic conditions.

Introduction

Hole transport in DNA is a remarkable characteristic of double helical DNA.[1] Since the phenomenon was first reported, a large number of studies have illustrated that the efficiency of the DNA-mediated hole transport reaction is strongly dependent on the DNA base sequence, conformational dynamics and local flexibility.[1a,d] These reaction characteristics may provide a fundamental basis for possible applications of hole transport to gene analysis of mutation and molecular wires in electronic nano-devices.[2,3]

A pH change is among the potential external triggers for regulation of various functions of biosystems such as biosensors, supramolecules and drugs.[4] The DNA triplex may be a pH-responsive nano-material, in which an oligopyrimidine as the third strand associates with the corresponding DNA duplex *via* Hoogsteen hydrogen bonding under acidic conditions, but dissociates to form the original duplex under basic conditions.[5] Recently, we have displayed the effect of partial triplex formation on hole transport in DNA duplexes by electrochemical measurements.[6] Anthraquinone (AQ)-photosensitized hole transport through DNA was substantially arrested by triplex formation in contrast to the efficient hole transport in DNA duplexes.[7] In this context, change in pH value may be a sensitive tool for the regulation of hole transport in the DNA duplex by pH-dependent triplex formation and dissociation.

In this study, we measured the photocurrent response of a DNA system, in which the DNA duplex–triplex equilibrium in the presence of an oligopyrimidine short strand was regulated with varying pH values, in order to characterize the pH effect on hole transport through a DNA duplex. The photocurrent response of an anthraquinone (AQ) photosensitizer-tethered DNA duplex possessing a partial triplex region revealed that the hole transport was effectively suppressed by triplex formation under acidic conditions (pH 5.5), while being recovered under basic conditions (pH 7.7) favoring dissociation of the triplex to a duplex. Furthermore, cyclic alternation of pH values between 5.5 and 7.7, which induced reversible triplex–duplex conversions, resulted in fall–rise photocurrent responses. These results indicate that the pH change can regulate photoinduced hole transport. In a long-range photo-oxidative DNA cleavage experiment, we also confirmed pH-dependent suppression of hole transport by partial triplex formation.

Results and discussion

Oligodeoxynucleotides (ODNs) listed in Fig. 1 were prepared for evaluation of several properties. The melting behavior of **Triplex I** (ODN 1/ODN 2/ODN 3) was measured by UV spectroscopy in a buffer solution (10 mM sodium cacodylate, 2 mM $MgCl_2$) at a given pH value to confirm the triplex formation. The melting curve of **Triplex I** at pH 5.5 showed two transitions at temperatures of $T_m = 29.5$ and 67.1 °C, as is a typical behavior of the system involving triplex formation of the duplex with a short strand of oligonucleotide (see Fig. S1a†). Thus, the transition at lower T_m is assigned to the dissociation of ODN 3 from **Triplex I**, and the higher T_m corresponds to melting of the resulting duplex into single strands. On the other hand, the lower transition disappeared in the melting curve of **Triplex I** at pH 7.7, indicating that ODN 3 spontaneously dissociated from the triplex under weakly basic conditions. These melting characteristics of **Triplex I** suggest that the triplex formation could be regulated by alternating pH values between 5.5 and 7.7.

We also measured photocurrent responses due to AQ-photosensitized hole transport in **Triplex I** immobilized on a

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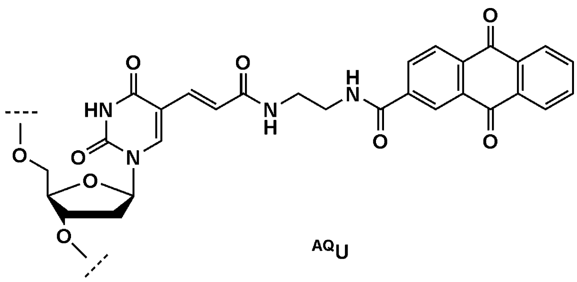
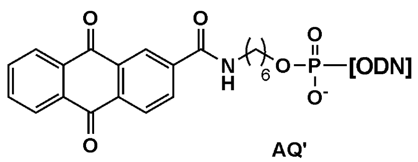
† Electronic supplementary information (ESI) available: UV data for the measurement of melting temperature and gel picture for the long-range oxidative cleavage of Triplex II with pH change. See DOI: 10.1039/b800295a

ODN 1: 5'-TAC *GGA AGG AAG* AGG GAG TC-$(CH_2)_6$-SAu-3'
ODN 2: 3'-A^{AQ}UG *CCT TCC TTC* TCC CTC AG-5'
ODN 3: 5'-*CCT TCC TTC*-3'
Duplex I: ODN 1/ ODN 2
Triplex I: ODN 1/ ODN 2/ODN 3



^{AQ}U

ODN 4: 5'-CTA TCA G_7G_8T *AGA GAA G*TG$_{18}$ G$_{19}$CT AAA-3'
ODN 5: 3'-GAT AGT CCA *TCT CTT C*AC CGA TTT-AQ'-5'
ODN 6: 5'-*TCT CTT C*-3'
Triplex II: ODN 4/ ODN 5/ODN 6



AQ'

Fig. 1 Sequences and structures of oligodeoxynucleotides (ODNs) used in this study. The bases arranged in the triplex region are shown in italics.

gold electrode.[8] The DNA-immobilized gold electrodes (2 mm^2 in area) were prepared by immersing in a thiolated ODN 1 solution, followed by exposure to 1 mM mercaptohexanol to minimize nonspecific adsorption of DNA.[9] The AQ-incorporated ODN 2 and the third strand ODN 3 were then hybridized with the ODN 1 immobilized on the electrode to give electrodes modified with **Triplex I** ($(1.49 \pm 0.18) \times 10^{13}$ DNA cm^{-2}).[10] The photocurrent measurements were carried out using 365 ± 5 nm light at a power density of 13.0 ± 0.3 mW cm^{-2} with an applied potential of 500 mV *versus* SCE. A stable cathodic current appeared immediately upon photoirradiation. As shown in Fig. 2a, photocurrent density (current per electrode area) of **Triplex I** at pH 5.5, where a typical triplex was formed, was 180.0 ± 8.9 nA cm^{-2}. Upon increasing pH value from 5.5 to 7.7, the photocurrent density was enhanced to a substantial extent up to a 1.6-fold increase (280.8 ± 21.9 nA cm^{-2}). When **Triplex I** was re-exposed to solution at pH 5.5 from pH 7.7, the photocurrent density decreased to the original level. This reversible alternation of photocurrent density was synchronized with the cyclic pH change in solution between 5.5 and 7.7. In view of the melting characteristics of **Triplex I**, these results indicate that hole transport through the DNA duplex was effectively altered in the presence of a partial triplex region formed under acidic conditions, but was recovered as a result of dissociation of the short strand ODN 3 from the triplex into the original duplex under basic conditions.[11] To confirm the effect of partial triplex formation on the hole transport, the control experiments with a duplex (**Duplex I**, ODN 1/ODN 2)-modified gold electrode were further performed to measure the photocurrents at a given pH. As shown in Fig. 2b, while all photocurrent densities of **Duplex I** were similar to those of **Triplex I** observed at pH 7.7, the changes in photocurrent response upon cyclic pH alternation were small.[12] These results strongly suggest that partial triplex formation, as can be regulated by pH value, is responsible for the pH-dependent change in photocurrent density. It is also clear from Fig. 2b that the efficiency of hole transport in the present duplex immobilized on the gold electrode is not affected at pH 5.5, while cytosine is known to have a pK_a around 5.5. Even if some bases like cytosine might be protonated at pH 5.5, there seems to be less pH effect on the hole transport process in this particular case. In this relation, we confirmed that the ion strength of solution to be measured does not affect the photocurrent response (Fig. 2c).[13]

To obtain further insight into the effect of pH-dependent triplex formation on hole transport in DNA, we determined a correlation between photosensitized hole transport and subsequent piperidine-induced cleavage of **Triplex II** (ODN 4/ODN 5/ODN 6) consisting of AQ-tethered 24 mer ODN 5, ^{32}P-labeled complementary ODN 4, and the third strand ODN 6 (Fig. 1).[14]

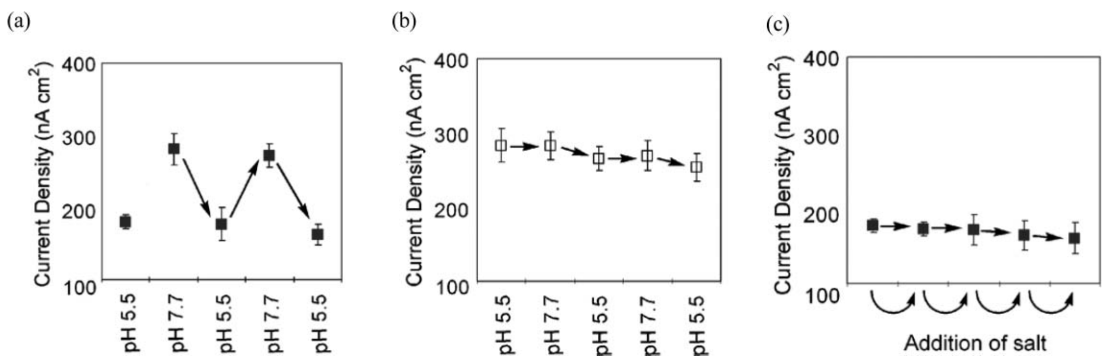


Fig. 2 (a, b) Reversible regulation of the photocurrent response by alternating duplex–triplex conversion at a pH cycle, as evaluated at an applied potential of 500 mV *vs.* SCE. **Duplex I** (ODN 1/ODN 2) immobilized on the gold electrode was photoirradiated (365 ± 5 nm light at 13.0 ± 0.3 mW cm^{-2}) at 15 °C in 10 mM sodium cacodylate buffer containing 2 mM $MgCl_2$ upon cyclic alternation of pH condition between 5.5 and 7.7 in the presence (a) and absence (b) of the third strand (ODN 3), respectively. The pH change was achieved by addition of an appropriate amount of 5 M NaOH or 5 M HCl aqueous solutions. (c) Effect of ion strength on the photocurrent density of **Triplex I** immobilized on a gold electrode. To the sodium cacodylate buffer solution (pH 5.5), 5 M NaCl aqueous solution was added each time instead of the addition of 5 M NaOH or HCl solutions. The photocurrent responses of **Triplex I** were measured in a similar manner. Each error bar represents SE calculated from five experimental results that were collected using freshly prepared different gold electrodes.

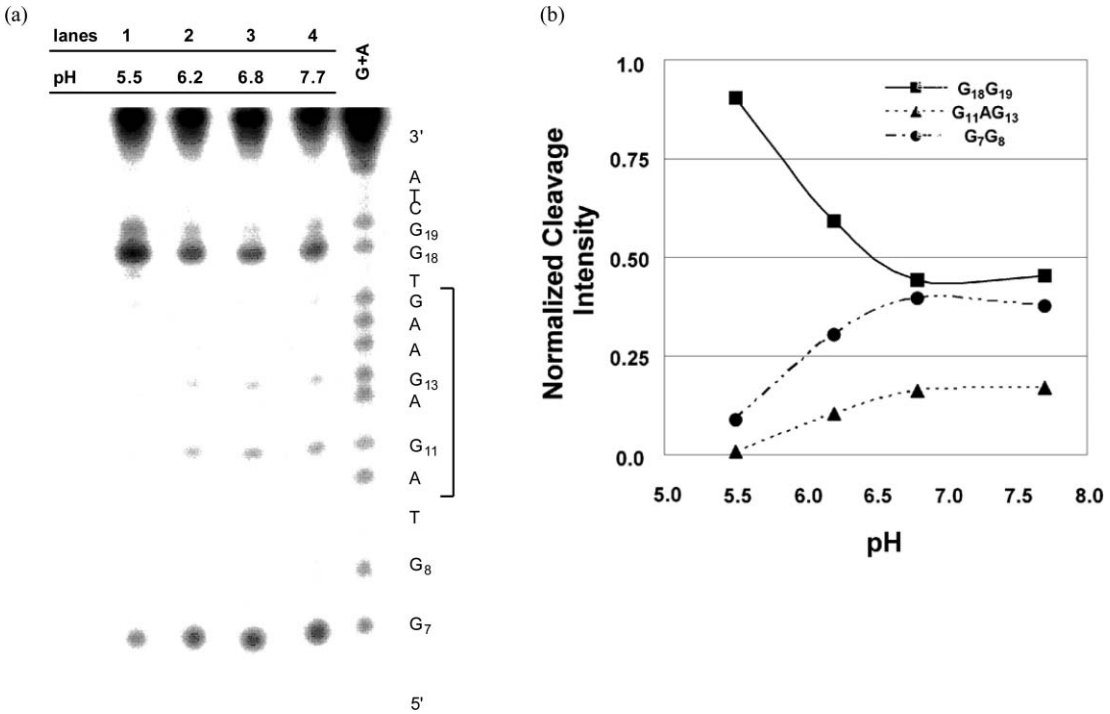


Fig. 3 (a) Autoradiogram of a denaturing gel electrophoresis for ^{32}P-5′-end labeled ODN 4 after photooxidation of **Triplex II** (ODN 4/ODN 5/ODN 6). Each sample in 10 mM sodium cacodylate containing 2 mM $MgCl_2$ was photoirradiated ($\lambda_{ex} = 365$ nm) at 0 °C, followed by hot piperidine treatment. Lanes 1–4, photoirradiation of **Triplex II** for 30 min at pH 5.5, 6.2, 6.8 and 7.7, respectively. The lane labeled G + A is a Maxam–Gilbert sequencing lane. The designed triplex-forming site is indicated by a bracket. (b) The normalized intensity of photooxidative cleavage in Fig. 3a as a function of pH value: (■) $G_{18}G_{19}$, (▲) $G_{11}AG_{13}$ and (●) G_7G_8 in ODN 4.

ODN 4 in **Triplex II** contained two 5′-GG-3′ sites of $G_{18}G_{19}$ and G_7G_8 across the triplex-forming region along with $G_{11}AG_{13}$ in the interior of the triplex-forming region, as internal indicators for the photoinduced hole transport.[15] Fig. 3a is a representative gel picture indicating the result of photoirradiation at pH 5.5, 6.2, 6.8 and 7.7, and Fig. 3b graphically shows quantitative comparisons of the normalized amounts of oxidative cleavage at $G_{18}G_{19}$, $G_{11}AG_{13}$ and G_7G_8 as a function of pH value.[16] Under neutral and basic conditions at pH > 6.8, where ODN 6 favors dissociation from **Triplex II** to form the corresponding duplex (**Duplex II**, ODN 4/ODN 5),[17] the DNA cleavage occurred in larger amounts at $G_{18}G_{19}$ and G_7G_8, while in smaller amounts at $G_{11}AG_{13}$. This DNA cleavage profile implicates that the photoinjected hole could be distributed over the base sequences inserted between the indicator sites of $G_{18}G_{19}$ and G_7G_8. In contrast, under acidic conditions at pH < 6.8, the DNA cleavage at $G_{18}G_{19}$ as located adjacent to the AQ-photosensitizer occurred to a greater extent relative to those at $G_{11}AG_{13}$ and G_7G_8, which located in the interior of and beyond the triplex-forming region, respectively. These results strongly suggest that the photoinjected hole was mainly trapped at $G_{18}G_{19}$ under acidic conditions, where ODN 6 associates with the triplex-forming region of **Duplex II** into **Triplex II** and thereby suppresses the long-range hole transport over the triplex-forming region. The long-range photo-oxidative cleavage experiments are consistent with the results obtained from the separate photocurrent measurements. Thus, a conclusion may be drawn that the duplex–triplex conversion induced by pH alternation can regulate hole transport through the DNA duplex.[18]

Conclusions

In summary, we characterized the pH effect on hole transport through an AQ-linked DNA duplex possessing a partial triplex-forming region. The photocurrent responses due to hole transport through the DNA duplex immobilized on a gold electrode were clearly decreased under acidic conditions, where formation of a partial triplex structure occurred to effectively suppress the hole transport. Under basic conditions, on the other hand, the triplex dissociated into the corresponding duplex and a short strand to enhance the hole transport and thereby the photocurrent response. Additionally, we confirmed that the photocurrent characteristics can be correlated to the long-range photo-oxidative DNA cleavage results, which showed that the DNA cleavage at the hole trapping sites beyond the triplex-forming region was efficiently suppressed by partial triplex formation under acidic conditions. This property of DNA triplexes would provide a useful functionality of on/off regulation of the hole transport upon cyclic alternation of pH values. Such a regulation method will facilitate a possible application of DNA duplexes to potential nano-scale devices.

Experimental

Material

Modified oligodeoxynucleotides (ODN 1, ODN 2 and ODN 5) were synthesized by a previously reported method.[6,8,14] In the measurement of photocurrent with amperometry, the reagents for DNA synthesis were purchased from Glen Research. ODNs, T4

polynucleotide kinase, and γ-[^{32}P]-ATP (10 mCi ml^{-1}) were used as received from Invitrogen, NIPPON GENE (10 units per μL), and Amersham Bioscience, respectively. All aqueous solutions were prepared using purified water (YAMATO, WR600A).

Instrumentation

UV absortion spectra were recorded on a Jasco V-530. Photoirradiation at 365 nm for DNA cleavage experiments was carried out using an ULTRA-VIOLET PRODUCTS NTFL-40 transilluminator, and a 100 W UV lamp (ASAHI SPECTRA, LAX101) with monochromatic excitation light through a 365 ± 5 nm band pass filter (ASAHI SPECTRA, ϕ 25 mm) was used for the measurement of photocurrent responses. A GIBCO BRL Model S2 sequencing gel electrophoresis apparatus was used for PAGE. The gels were analyzed by densitometry with an ATTO Lane Analyzer (version 3). The photocurrent was measured in a three-electrode arrangement (ALS, model 660B). Mass spectrometry of oligodeoxynucleotides (ODNs) was performed with a MALDI-TOF MS (Perseptive Voager Elite, acceleration voltage 21 kV, negative mode) with 2′,3′,4′-trihydroxyacetophenone as a matrix, using T_8 ([M − H]$^-$ 2370.61), T_{17} ([M − H]$^-$ 5108.37) and T_{27} ([M − H]$^-$ 8150.33) as the internal standards. Reversed-phase HPLC was performed with a Shimadzu 10A or HITACHI D-7000 HPLC system. Sample solutions were injected on a reversed phase column (Inertial ODS-3, GL Science Inc, ϕ 4.6 mm × 150 mm).

DNA characterization

Each ODN synthesized in this study was characterized by MALDI-TOF MS: 5′-TACGGAAGGAAGAGGGAGTC-$(CH_2)_6$-S-S-$(CH_2)_6$-T)-3′, *m/z* 6911.92 (calcd for [M − H]$^-$ 6911.71); 5′-GACTCCCTCTTCCTTCCG^{AQ}UA-3′ *m/z* 6286.60 (calcd for [M − H]$^-$ 6286.16); 5′-AQ′-TTTAGCCACTTCTCT-ACCTGATAG-3′, *m/z* 7665.51 (calcd for [M − H]$^-$ 7666.05).

T_m Measurement

2.5 μM solutions of the appropriate ODNs in a reaction buffer (10 mM sodium cacodylate buffer at pH 5.5, 6.8 or 7.7 and 2 mM $MgCl_2$) were prepared to determine the melting temperatures (T_m) of duplex and triplex. Duplex formation was achieved by heating the sample at 90 °C for 5 min and slowly cooling to room temperature. Furthermore, a partial triplex formation was achieved by cooling at 4 °C for 12 h after addition of a third strand to the duplex sample. 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 per min.

Immobilization of thiolated ODN 1 on a gold electrode

A gold electrode, 2 mm^2 in area, was used for this study. Prior to ODN immobilization, the electrode was soaked in boiling 2 M potassium hydroxide for 3 hours and washed with deionized water. Following this treatment, the electrode was further soaked in concentrated nitric acid for 1 hour and washed with deionized water. For chemisorption of ODN 1, a 1 μL solution of 5 μM thiolated ODN 1 was placed on a gold electrode turned upside-down and the opening of the electrode vessel was then stuffed with a rubber stopper to avoid evaporation of the solvent. After leaving the assembly for 2 hours at room temperature, a 1 μL solution of 1 mM 6-mercaptohexanol in deionized water was placed on a gold electrode for masking of the gold surface and the opening of the electrode vessel was stuffed with a rubber stopper, again. After leaving the assembly for 1 hour at room temperature, the electrode was carefully washed with a small amount of deionized water. For the duplex formation with thiolated ODN 1 immobilized on a gold electrode, 1 μL of 10 μM AQ-linked ODN 2 in aqueous solution was placed on a gold electrode turned upside-down and the opening of the electrode vessel was then stuffed with a rubber stopper. The assembly (**Duplex I**) was left for 2 hours at 4 °C prior to photoelectrochemical measurement. For the triplex formation with the third strand (ODN 3), 1 μL of 20 μM ODN 3 in 10 mM sodium cacodylate buffer (pH 5.5) containing 2 mM $MgCl_2$ was placed on a duplex-modified gold electrode turned upside-down and the opening of the electrode vessel was then stuffed with a rubber stopper. The assembly (**Triplex I**) was left for 12 hours at 4 °C prior to photoelectrochemical measurement.

Photoelectrochemical measurement

Photocurrents were measured for solutions in 10 mM sodium cacodylate buffer (pH 5.5) containing 2 mM $MgCl_2$ in a one-compartment Pyrex cell at an applied potential of 500 mV *vs.* SCE under illumination by a 100 W UV lamp with monochromatic excitation light through a 365 ± 5 nm band pass filter. The cell for photocurrent measurement consisted of a three-electrode arrangement (ALS, model 660B) of a modified Au working electrode (electrode area, 2 mm^2), a platinum counter electrode and an SCE reference electrode. The measurements of triplex samples were performed in 10 mM sodium cacodylate buffer containing 2 mM $MgCl_2$ and 2 μM ODN 3 at 15 °C. The duplex–triplex conversion was accomplished by cyclic alteration of pH value between 5.5 and 7.7, which was achieved by addition of an appropriate amount of 5 M NaOH or 5M HCl to buffer solution. The solution was kept at 15 °C for 15 min before each photocurrent measurement.

Preparation of 5′-^{32}P-end-labeled ODN 4

ODN 4 (400 pmol strand concentration) were labeled by phosphorylation with 4 μL of [γ-^{32}P] ATP and 4 μL of T_4 polynucleotide kinase using standard procedures.[19] The 5′-end-labeled ODN 4 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 Triplex II

Triplex samples were prepared by hybridizing a mixture of cold and radiolabeled ODN 4 (1 μM) with 1 μM of ODN 5 and 1 μM of ODN 6 in sodium cacodylate buffer containing 2 mM $MgCl_2$ at a given pH value. The ^{32}P-5′-end-labeled **Triplex II** were irradiated at 365 nm at 0 °C under given pH conditions. After photoirradiation, all reaction mixtures were precipitated with addition of 10 μL of herring sperm DNA (1 mg mL^{-1}), 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 × 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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17 The spontaneous dissociation of **Triplex II** into the corresponding **Duplex II** at pH 6.8 was confirmed by the measurements of thermal denaturation profiles (Fig. S1†). The melting curve of **Triplex II** at pH 6.8 showed only a single transition, which is assigned to dissociation of the duplex (*i.e.* **Duplex II**) into single strands, while two transitions as in the typical behavior of the triplex were observed at pH 5.5.

18 We also confirmed the recovery of hole transport efficiency in DNA by increasing pH value in the long-range photo-oxidative DNA cleavage experiments. Thus, the cleavage at G_7G_8 in **Triplex II** was enhanced by increasing the pH value from 5.5 to 6.8 (see Fig. S2†).

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