

Electrochemical Evaluation of Alternating Duplex–Triplex Conversion Effect on the Anthraquinone-Photoinjected Hole Transport through DNA Duplex Immobilized on a Gold Electrode

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The hole transport property of duplex DNA has been intensively studied and is thereby recognized to occur over a 20 nm range under certain conditions.^{1,2} DNA is thus expected to be a promising molecular material for constructing bioelectronic nanodevices, coupled with its characteristic ability for the self-assembly into various modes of higher-order structures dependent on the base sequences. For achieving this goal, minute regulation of the transport property of duplex DNA is considered as one of the key nanotechnologies.

The association of oligopyrimidine (OPD) short strands to the DNA duplex by Hoogsteen hydrogen bonding has been shown to generate DNA triplexes.³ These DNA triplexes, producing a cationic environment and altered stacking interactions of DNA bases at the duplex–triplex junctions, have been presumed to arrest the hole transport,⁴ in accord with experimental evidence that photooxidative cleavage at remotely located guanine residues of DNA strands bearing several types of sensitizers is considerably suppressed by the triplex formation. However, the effect of triplex formation on the hole transport is still a controversial issue because it has been studied with experiments based on photosensitized DNA cleavage and not by direct electrochemical measurements of current response from DNA triplexes relative to duplexes.

In this study, an electrochemical approach⁵ using photocurrent measurements was taken to characterize effects of the formation of a partial triplex with OPD short strands on the hole transport through a DNA duplex.⁶ We designed a 20-mer oligodeoxynucleotide (ODN) with an anthraquinone (AQ) photosensitizer linked to the 3'-end and hybridized it to a complementary ODN immobilized at the thiol-modified 3'-end on a gold electrode to produce **Duplex 1** (Figure 1a). Injection of holes (radical cations) to the AQ-linked end of **Duplex 1** was performed by AQ-photosensitized one-electron oxidation.^{1d,7} The resulting photocurrents due to hole transport were measured in the absence and presence of three types of OPD short strands that were designed to associate with **Duplex 1** at specified structural regions to form **Triplexes 1–3** (Figure 1a). Alternating duplex–triplex conversion was accomplished by regulating the temperatures around the characteristic melting temperatures of **Triplex 1**.

After the spontaneous formation of a monolayer consisting of a thiolated ODN single strand on the surface of a gold electrode, the electrode was immersed in a solution of 6-mercapto-1-hexanol (MCH) to avoid possible nonspecific adsorption of the thiolated ODN.⁸ The formation of **Duplex 1** was achieved by hybridization of the thiolated ODN single strand immobilized on the gold electrode with the AQ-linked complementary ODN. In a representa-

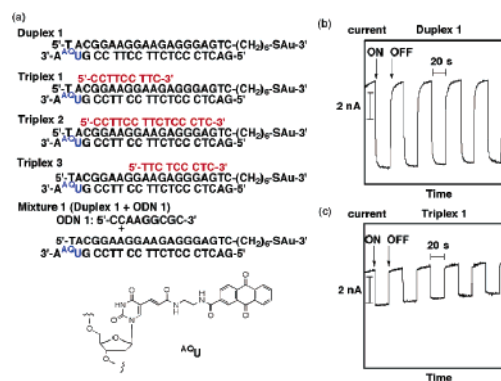


Figure 1. (a) Sequences and structures of ODNs used in this study. (b) Photocurrent response of **Duplex 1** immobilized on a gold electrode, as observed at an applied potential of 500 mV vs SCE upon photoirradiation with 365 ± 5 nm light (13.0 mW cm^{-2}) at 15°C . (c) Photocurrent response of **Triplex 1** under the same conditions.

tive experiment, hybridization of a 9-mer OPD strand (5'-CCTTCC TTC-3') to the AQ-linked **Duplex 1** at 4°C formed **Triplex 1**⁹ (Figure 1a) on the surface of the electrode [$(1.49 \pm 0.18) \times 10^{13}$ DNA cm^{-2}].

We initially conducted comparative photoelectrochemical measurements for **Duplex 1** on the gold electrode and its modification into **Triplex 1** possessing a 9-mer partial triplex region in 10 mM sodium cacodylate buffer (pH 5.5), using 365 ± 5 nm UV light at a power density of $13.0 \pm 0.3 \text{ mW cm}^{-2}$ and an applied potential of 500 mV vs SCE. Figure 1b and c shows representative cathodic photocurrent responses, which appeared immediately upon irradiation of a common AQ photosensitizer linked to both **Duplex 1** and **Triplex 1**. It is striking that the photocurrent response of **Triplex 1** was reduced by half relative to the response of **Duplex 1**; the photocurrent densities (current per electrode area) observed for **Duplex 1** and **Triplex 1** were 296 ± 26 and $167 \pm 11 \text{ nA cm}^{-2}$, respectively. By reference to the background photocurrent density ($141 \pm 36 \text{ nA cm}^{-2}$) observed upon similar UV irradiation ($365 \pm 5 \text{ nm}$; $13.0 \pm 0.3 \text{ mW cm}^{-2}$) of a control duplex with the same base sequences as in **Duplex 1** but not the linked AQ photosensitizer, the partial triplex structural region could substantially arrest the transport of the hole that was photochemically injected and migrated through the DNA duplex on the electrode.

To obtain further structural insight into the effects of triplex formation on the hole transport through a DNA duplex, we measured the photocurrent densities for **Triplex 2** possessing a triplex region formed by the association with a longer 15-mer OPD (see Figures 1a and 2a). **Triplex 2** further decreased the photocurrent density ($143 \pm 12 \text{ nA cm}^{-2}$) relative to that of **Triplex 1**,

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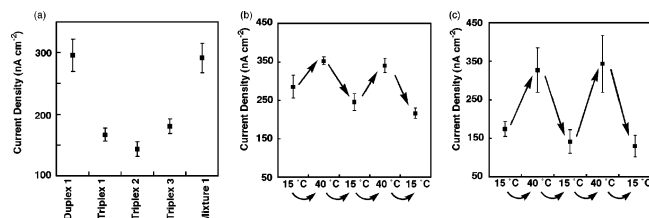


Figure 2. (a) Effect of hybridization on photocurrent density, as evaluated for various AQ-linked ODNs immobilized on gold electrodes at an applied potential of 500 mV vs SCE. Solutions of the AQ-linked ODNs in 10 mM sodium cacodylate buffer (pH 5.5) containing 2 mM MgCl_2 were photoirradiated (365 ± 5 nm light at 13.0 ± 0.3 mW cm^{-2}) at 15°C . Each error bar represents the SE calculated from 10 experimental results that were collected using different freshly prepared gold electrodes. (b and c) Reversible regulation of the photocurrent response for **Duplex 1** immobilized on a gold electrode by alternating duplex–triplex conversion, evaluated at an applied potential of 500 mV vs SCE in the absence (b) and presence (c) of a 9-mer OPD short strand, upon photoirradiation (365 ± 5 nm light at 13.0 ± 0.3 mW cm^{-2}) at a temperature cycle between 15 and 40°C . Each error bar represents the SE calculated from five experimental results that were collected using different freshly prepared gold electrodes.

clearly indicating that the efficiency of photoinjected hole transport through DNA triplexes is reduced as the length of third strand increases. The photocurrent response was also confirmed to be slightly affected by the position of triplex. Thus, **Triplex 1**, with the partial triplex-forming region close to AQ at the hole photo-injection end, suppressed hole transport more efficiently than did **Triplex 3** with a triplex remote from AQ but near the electrode. It is most likely that the hole injection by photoexcited AQ may be more effectively retarded by the triplex formation occurring more closely to AQ. In a control experiment, we also measured the photocurrent density for a mixture of **Duplex 1** and a noncomplementary third strand **ODN 1 (Mixture 1; Figure 1a)**. As predicted, **Mixture 1** could not suppress the photocurrent response of **Duplex 1**, strongly indicating that the formation of perfectly matched triplex is responsible for the retardation of hole transport through the DNA duplex immobilized on the electrode. Similar suppression effects, caused by the formation of a partial triplex, on the photocurrent was confirmed for a duplex–triplex couple with different base sequence.¹⁰

In light of the above effect of triplex formation, further attempts were made to regulate hole transport through a DNA duplex¹¹ on the gold electrode by thermal conversion between **Duplex 1** and **Triplex 1** in the presence of 9-mer OPD short strand (see Figure 2b and c). In view of the melting temperature (T_m) of 29.5°C assigned to the dissociation of **Triplex 1** into **Duplex 1**,⁹ we measured the varying photocurrent responses arising from a **Duplex 1**–**Triplex 1** structural conversion on the electrode upon cyclic alternation of temperatures between 15 and 40°C . Consistent with the previously reported temperature effect on the long-distance hole transport through a DNA duplex,¹² the intrinsic current density for **Duplex 1** was enhanced to a relatively lesser extent by elevating the temperature from 15 to 40°C . In contrast to this intrinsic hole transport property of **Duplex 1**, the photocurrent density for **Triplex 1** was excessively suppressed at 15°C relative to that of **Duplex 1**, but was recovered to the intrinsic level of **Duplex 1** by alternative photoirradiation at 40°C . In view of the melting characteristics of **Triplex 1**, the hole transport through **Duplex 1** could be effectively retarded as a result of association with the third OPD short strand to form **Triplex 1** at 15°C and was recovered along with dissociation of the OPD short strand from **Triplex 1** to regenerate **Duplex 1** at 40°C . These results demonstrate that an alternating duplex–triplex conversion, which can be reversibly regulated by

temperature changes around the T_m value characteristic of the triplex, may be featured in the switching function of the hole transport efficiency of a DNA duplex immobilized on a gold electrode.

In summary, we evaluated the effects of partial triplex formation on the hole transport by direct measurement of the photocurrent generated in an AQ-linked DNA duplex immobilized on a gold electrode. The partial triplex structural region in the DNA duplex, formed by the association with a third OPD short strand, was demonstrated to substantially arrest the transport of an AQ-photoinjected hole through the duplex. This is consistent with the previous conclusion drawn from a photoinduced DNA cleavage assay. More remarkable was that photoinjected hole transport efficiency could be reversibly regulated by thermally induced alternating duplex–triplex conversion. Further exploration of on/off switching methods for the DNA conductivity is in progress.

Supporting Information Available: Experimental data on the photoelectrochemical assays, UV and CD spectra of the related DNA samples (PDF) are available. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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- (9) The formation of **Triplex 1** in aqueous solution was confirmed by the measurements of thermal denaturation profiles (Figure S1) and CD spectra (Figure S2). The melting curve of **Triplex 1** showed two transitions at the temperatures at $T_m = 29.5$ and 67.1°C . The transition at lower T_m is assigned to the dissociation of a third 9-mer OPD strand from **Triplex 1** to form **Duplex 1**, and the higher T_m corresponds to the denaturation of **Duplex 1** into single strands. In the CD spectra, the negative band at ca. 210 nm characteristic of the triplex was observed at 15°C , which disappeared clearly at elevated temperatures (Figure S2 and see ref 4a). The UV thermal dissociation spectra of the other triplexes were also consistent with the assignment of triplex structures.
- (10) Photocurrent densities of a triplex (5'-TAGAGAGGGAGAGGG-(CH₂)₆-SAu-3'/5'-CCCTCTCCCTCTC^{AQ}UA-3'/5'-TCTCCCTCT-3') and the corresponding duplex were evaluated to be 151 ± 19 and 311 ± 31 nA cm^{-2} , respectively.
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