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# Syntheses of anthraquinone capped hairpin DNAs and electrochemical redox responses from their self-assembled monolayers on gold electrode

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**Abstract**—An anthraquinone (AQ) based DNA linker and hairpin-forming DNAs linked by the AQ linker with variable A–T base pairs were synthesized for the investigation of electron transfer through double helical DNA (DNA-ET) in self-assembled monolayers (SAMs). The spectroscopic analysis of absorption spectra indicated that the AQ of the hairpin DNA stacked with adjacent A–T base pair. Electrochemical redox response due to the AQ was observed from the hairpin DNA immobilized on gold electrode, thus the hairpin DNA is suitable for the investigation of DNA-ET in SAMs.


Electron transfer through double helical DNA (DNA-ET) in self-assembled monolayers (SAMs) on solid surface should find several applications in materials science and biotechnology. DNA-ET in SAMs has been investigated by using a redox active molecule or a photosensitizer as a probe in electrochemical- or photoinduced process.[1–8] It has been found that the efficiency of DNA-ET is greatly affected by the interactions of the probes and DNA bases. Especially, when the probe interacts with DNA in a π-stacked bridge, the electron transfer can be effectively initialized. Thus, the design of a probe that attached to DNA is particularly important for efficient DNA-ET.

Hairpin forming DNA capped by a probe as a bridge could be one of the useful systems to investigate DNA-ET in SAMs, because the probe can be stacked with DNA base-pair rigidly, hairpin DNA forms a stable double helix, the stoichiometry of the probe:DNA is definitely 1:1, and the probe is placed on the surface of SAMs. Lewis and co-workers have reported that hairpin DNA capped by an aromatic chromophore such as stilbene, naphthalene, and phenanthrene adopts B-form structure in which the linker chromophore is parallel to the adjacent base pair. Hence, they use the chromophore-capped hairpin DNA for the investigation of photoinduced electron transfer process between the chromophore and nucleobase in solution.[9]

An anthraquinone (AQ) derivative is an intercalative redox active molecule and has been used in electrochemical redox reporting of DNA,[6a–c,7,10,11] as well as in photosensitized DNA oxidations.[8,12] However, AQ end-capped hairpin DNA system has not yet been reported. Herein we describe the synthesis of an AQ based DNA linker that can act as an end-cap for DNA hairpin structures. Electrochemical redox responses were observed from the SAM of AQ end-capped hairpin DNA on gold electrode.

The objective of synthetic work is AQ derivative **1** shown in Scheme 1 to link two complementary DNAs by automated synthesis. The preparation of **1** began with the anthraquinone-2,6-disulfonic acid disodium salt **2**. By reaction with $PCl_5$, **2** was converted to disulfonyl dichloride **3**, which was used in subsequent reaction without purification.[13] Disulfonyl chloride **3** was allowed to react with 3-amino-1-propanol to give **4** in dichloromethane containing triethylamine in 58% yield

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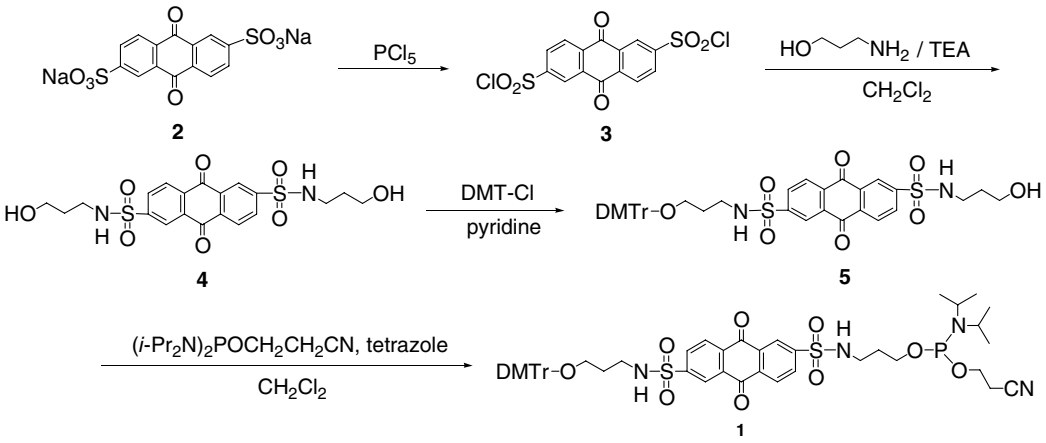
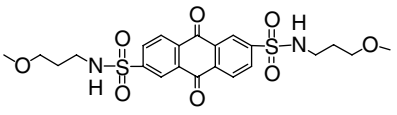



**Scheme 1.**

after recrystallization with ethanol.[14] One of the hydroxyl group of **4** was protected with 4,4′-dimethoxytrityl chloride (DMT-Cl) to give DMT-AQ-sulfonic amide **5** in 44% yield after purification by silica gel column chromatography.[15] DMT protected sulfonic amide **5** was converted to **1** in the reaction with 2-cyanoethyl tetraisopropylphosphoro-diamidite in dichloromethane containing tetrazole. Purification with silica gel column chromatography gave **1** in 51% yield for use in automated synthesis.[16]

All the DNAs shown in Chart 1 were prepared by conventional phosphoramidite chemistry using an automated DNA synthesizer. After the recommended workup, they were purified by the reversed-phase HPLC. The characterization of the DNAs was carried out by absorption spectra and thermal denaturation. In the absorption spectra of **6**–**8**, a short-wavelength absorption band around 260 nm attributed to the nucleobases and a long-wavelength absorption band at 327 nm attributed to AQ were observed. The intensity of the short-wavelength band around 260 nm increases and the band maximum shifts to shorter wavelength with increase of the number of A–T base pairs. The band maximums the long wavelength absorption for **6**–**8** appears at the same wavelength.

| Sequences | $T_m$ / °C |
|---|---|
| **6**: 5'-$dA_8$ -**AQ**- $T_8$-3'-$(CH_2)_3$-SS-$(CH_2)_3$-OH | 56.6 |
| **7**: 5'-$dA_{12}$ -**AQ**- $T_{12}$-3'-$(CH_2)_3$-SS-$(CH_2)_3$-OH | 63.6 |
| **8**: 5'-$dA_{20}$ -**AQ**- $T_{20}$-3'-$(CH_2)_3$-SS-$(CH_2)_3$-OH | 65.7 |
| **9**: 5'-$dA_{20}$-3' / 5'-**AQ**-$dT_{20}$-3'-$(CH_2)_3$-SS-$(CH_2)_3$-OH | 46.0 |
| **10**: 5'-$dA_{20}$-3' / 5'-$dT_{20}$-3'-$(CH_2)_3$-SS-$(CH_2)_3$-OH | 42.9 |



**AQ**

**Chart 1.** Sequences AQ modified DNAs.

Helical stability for **6**–**8** was measured by thermal denaturation experiments monitored by absorbance changes at 260 nm. The DNAs **6**–**8** exhibited single-phase transition profiles at 260 nm and they have melting temperatures greater than 55 °C as shown in Figure 1. We additionally synthesized 20mer double stranded DNAs, one is 5′-AQ modified duplex **9**, another is unmodified duplex **10**, to comparison with **6**–**8**. These double stranded DNAs also exhibited single-phase transition profiles, whereas their melting temperatures are lower than those of **6**–**8**. The melting temperatures ($T_m$) obtained from the first derivatives of the transition profiles for the DNAs are summarized in Chart 1. Figure 2 shows the temperature dependence of the UV spectrum of **8**. On elevating temperature above $T_m$ (65.7 °C), the band maximum at 327 nm shifted to shorter wavelength and exhibited hyperchromic effect. These results indicate that **6**–**8** form an AQ end-capped hairpin structures and the AQ moiety π-stacked with a neighboring nucleobase pair in the hairpin below melting temperature. It is known that double stranded poly (A–T) forms relatively rigid and linear structure as A-tract.[17] Furthermore, the stable B-form structures of hairpin forming poly (A–T)s

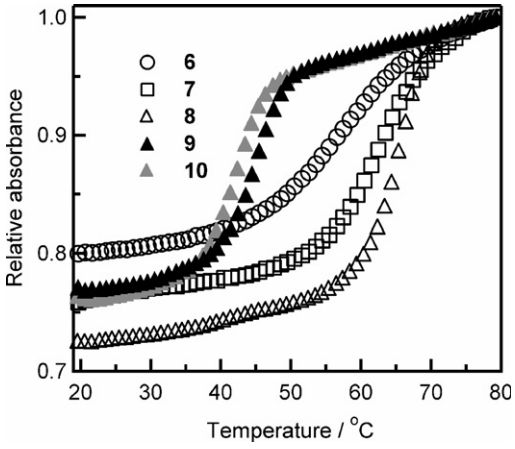



**Figure 1.** Melting profiles of AQ-modified DNAs monitored at 260 nm in a buffer containing 0.01 M $NaHPO_4$ and 0.1 M NaCl adjusted to pH 7.0.

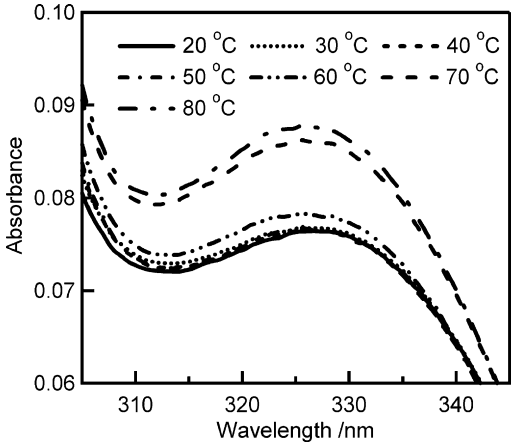



**Figure 2.** Temperature dependence of the AQ absorption band of **8** in a buffer containing 0.01 M $NaHPO_4$ and 0.1 M NaCl adjusted to pH 7.0.

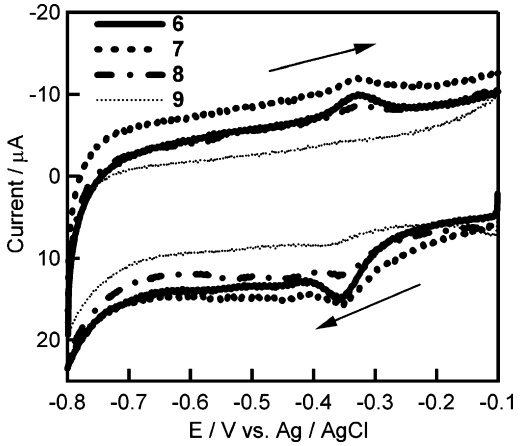



**Figure 3.** Cyclic voltammograms of DNA modified electrodes with 500 mV $s^{-1}$ of scan rate. The observations were carried out in a buffer containing 0.01 M $NaHPO_4$ and 0.1 M NaCl adjusted to pH 7.0.

bridged by a short chain linker containing a chromophore have been evaluate by means of crystal structures, CD spectra, and molecular modeling.[9,18] Thus the AQ capped hairpin-DNAs in the present system should take A-tract structures.

The self-assembled monolayers derived from **6**–**8** on gold electrode were prepared by deposition of the aqueous buffer (pH 7.0, containing 0.01 M $NaHPO_4$, 0.1 M NaCl, and 0.01 M $MgCl_2$) solution of the hairpin DNA (1 mM) onto Au (1 1 1)/slide glass (deposition area was 0.2 $cm^2$) for overnight with subsequent deposition of the buffer solution of 3-mercapto-1-propanol (2.5 mM) for 2 h. The surface conditions of the monolayers were confirmed by cyclic voltammetry in 1 mM $K_4[Fe(CN)_6]$ and 1 M $KNO_3$ aqueous solution.[19] The total amounts of the duplexes on the gold substrate were measured in pH 7.4, 0.01 M tris(hydoroxymethyl)aminomethane hydrochloride aqueous buffer containing 50 μM $Ru(NH_3)_6^{+3}$, which is electrostatically trapped for phosphate backbone of oligonucleotide and electrochemically quantified by chronocoulometry,[20] and determined to be 2.0–4.5 pmol/$cm^2$.[19,21] These values are consistent with those for DNA monolayers on gold surface reported previously.[20]

In the cyclic voltammograms of the hairpin DNAs modified electrode, the reduction peak (−350 to −400 mV) and oxidation peak (−300 to −350 mV) due to AQ were observed in a pH 7 buffer containing 0.1 M NaCl and 0.01 M $NaHPO_4$.[19] Irrespective of DNA length, the hairpin DNA modified electrode exhibited the similar redox peak due to AQ moiety (Fig. 3). The cyclic voltammetry of **9** immobilized on gold electrode was also carried out, however electrochemical response due to AQ moiety was barely observed (Fig. 3). This is indicative that the AQ capped hairpin structure is capable of inducing the efficient electron transfer through DNA base π-stacking.

The redox peak current increased with increase in CV scan rates. Semi-linear correlation between the peak current and scan rates was obtained. These results indicate that the observed redox signal was due to the surface-bound redox species. The plots of peak splitting $\Delta E_{cp}(\Delta E_{cp} = E_{cp} - E_{1/2})$ was analyzed as a function of log(scan rate) to determine ET rate constants according to Laviorn's equation (Fig. 4).[22] The ET rate constants for the hairpin DNA modified gold electrodes estimated from the plots were about 50 $s^{-1}$. The electron transfer rate constant is independent of the DNA length. This result is consistent with that reported by Barton and co-workers previously.[6a]

In summary, we synthesized AQ based DNA linker forming AQ end-capped hairpin DNA. The AQ moiety in the hairpin DNA stacked with an adjacent base pair. We demonstrated that the hairpin DNA is useful for the studies of DNA-ET. Further studies of DNA-ET in SAMs with AQ end-capped hairpin DNA containing other bases are in progress in our laboratory.

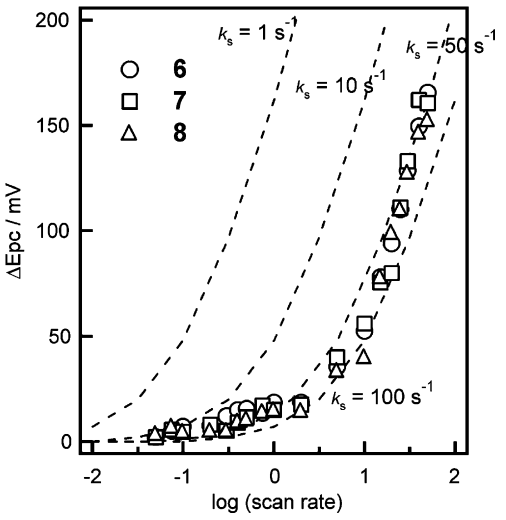



**Figure 4.** Plots of $\Delta E_{cp}(\Delta E_{cp} = E_{cp} - E_{1/2})$ versus log (scan rate) of hairpin DNA modified gold electrodes.

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13. The mixture of **2** (5 g, 12 mmol) and $PCl_5$ (12.4 g, 60 mmol) was heated at 180 °C for overnight with stirring. After cooling to room temperature, water and crushed ice were added to the reaction mixture to decompose unreacted $PCl_5$. Product **3** was collected and washed with water and then dried under reduced pressure.
14. To a suspension of **3** (2 g, 5 mmol) in dichloromethane (30 mL) containing triethylamine (2 mL) was added a solution of 3-amino-1-propanol (3.4 g, 23 mmol) in dichloromethane (20 mL) under argon atmosphere at 0 °C. The solution was stirred for 1 h at 0 °C and then warmed up to room temperature with stirring. The solution was acidified with dilute HCl. The precipitate was collected and washed with several portions of ethanol. Recrystallization with ethanol gave pure product **4** in 58% yield (1.4 g). TLC (10:1, $CH_2Cl_2$–MeOH, v/v), $R_f$ 0.41; $^1H$ NMR (DMSO-$d_6$): 1.52 (m, 4H, $CH_2CH_2CH_2$), 2.84 (m, 4H, $NHCH_2$), 4.42 (t, 2H, OH), 8.05 (t, 2H, NH), 8.27 (d, 2H, Ar), 8.42 (d, 2H, Ar), 8.70 (s, 2H, Ar).
15. To a solution of **4** (1 g, 2 mmol), which was dried by coevaporation with pyridine three times, in dry pyridine (40 mL) was added a solution of 4,4′-dimethoxytrityl chloride (0.77 g, 2.2 mmol) in dry pyridine (20 mL). After stirring for 6 h at room temperature, the solution was concentrated to near dryness. The residual material was dissolved in dichloromethane (150 mL) and then washed with water. The organic phase was dried over $Na_2SO_4$ and evaporated to near dryness. Product **5** was purified with silica gel column chromatography with dichloromethane containing methanol (9:1, $CH_2Cl_2$–MeOH, v/v) in 44% yield (0.72 g). TLC (9:1, $CH_2Cl_2$–MeOH, v/v), $R_f$ 0.37; $^1H$ NMR (DMSO-$d_6$): 1.51 (m, 2H, $CH_2CH_2CH_2$), 1.63 (m, 2H, $CH_2CH_2CH_2$), 2.84 (m, 2H, $NHCH_2$), 2.91 (m, 2H, $NHCH_2$), 4.41 (t, 1H, OH), 6.80–7.26 (m, 13H, Ar of DMT), 8.01 (t, 2H, NH), 8.24–8.67 (m, 6H, Ar of AQ).
16. 2-Cyanoethyl-*N*,*N*,*N*′*N*′-tetraisopropylphosphateamidite (0.13 ml, 0.38 mmol) was added to a solution of **5** (0.2 g, 0.24 mmol) and tetrazole (4.5 mg, 0.6 mmol) in dry dichloromethane (1 mL). The solution was stirred for 2 h at room temperature and then dichloromethane (5 mL) was added to the solution. The solution was washed with 10% $NaHCO_3$ aqueous solution, dried over $Na_2SO_4$, and then evaporated to near dryness. Product **1** was purified by silica gel column chromatography with dichloromethane containing ethyl acetate and triethylamine (45:45:10, $CH_2Cl_2$–AcOEt–$Et_3N$, v/v) in 51% yield (0.12 g). TLC (45:45:10, $CH_2Cl_2$–AcOEt–$Et_3N$, v/v), $R_f$ 0.69.
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