# Electrochemistry of 2′-Anthraquinone-modified Oligonucleotide Immobilized on Gold Surface: Differential Electron Transfer Efficiency between Single and Double Helical Forms

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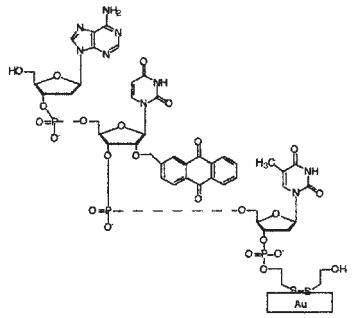
2′-Anthraquinone-modified oligonucleotide (AQ-ODN) possessing a disulfide terminus has been immobilized on gold surface. The AQ-ODN modified electrode exhibited faster electron transferability in double-stranded form than that in single-stranded form, providing a new type of electrochemical DNA sensing device.

Electrochemical probing of a specific DNA sequence on an appropriately modified electrode has been a current subject of intense research. There have been two different approaches one of which has relied on the use of a redox-active DNA intercalator as an affinity ligand of double-helical DNA. A naphthalene diimide intercalator containing two ferrocenyl groups has been shown to possess greater affinity for double-helical DNA than for single-stranded DNA, providing an electrochemical probe for monitoring DNA hybridization.[1] The electrocatalytic signal of methylene blue coupled to $[Fe(CN)_6]^{-3}$ has been used for base-mismatch detection as a probe of charge transport through DNA.[2] The other approach has been conducted by covalent attachment of a redox-active molecule into the specific site of oligonucleotide,[3] providing an electrochemical probe that may be applicable in chip-based assays of DNA.




We have shown a method for incorporation of an anthraquinone group, a redox-active intercalator, via one carbon tether to the 2′-position of DNA.[4] Binding of the anthraquinone-modified oligodeoxynucleotides (AQ-ODN) to their complementary DNA sequences has resulted in the duplexes significantly stabilized by intercalation.[4] The intercalation of anthraquinone moiety has been evident from $^1$H nmr spectral studies.[5] The cyclic voltammetric responses of the anthraquinone moiety bound to ODN were altered upon transfer into hydrophobic core of duplex exhibiting a measurable signal for monitoring hybridization in solution.[6] The attractive feature of our AQ-ODN is that an anthraquinone group can be placed at the designated base-pair pocket in double-helical DNA. It is therefore anticipated that AQ-ODN modified electrode provides a useful device in study of electron transfer though DNA as well as in electrochemical DNA sensing. The present report describes that the AQ-ODN probe chip exhibits faster electron transferability in double-stranded form than in single-stranded form.

Anthraquinone-modified oligonucleotide possessing a disulfide terminus [AQ-ODN-3′-$C_2$-S-S-$C_2$-OH; 5′-dACAU(AQ)GCAGTGTTGAT-3′-$C_2$-S-S-$C_2$-OH, where U(AQ) is 2′-(anthraquinonylmethyl)uridine] has been synthesized according to automated solid-phase techniques, using a silica support containing a protected disulfide linker.[7] Au[111] surfaces onto mica were prepared by a vapor deposition method. Electrodes were then modified by incubation of 0.1–1.0 mM solutions of single- (ss) or double-stranded (ds) AQ-ODN-3′-$C_2$-S-S-$C_2$-OH in 10 mM sodium phoshate and 100 mM NaCl (pH 7) at room temperature for 24 h.[8] As a model monolayer, Au-$SCH_2CH_2OH$ and Au-$SCH_2CH_2OC{=}OAQ$/Au-$SCH_2CH_2OH$ were prepared by use of 2-hydroxyethane disulfide and its monoester derivative with anthraquinone 2-carboxylic acid, respectively. Electrochemical characterization of the modified electrodes was carried out in a three-electrode cell, consisting of the modified Au electrode (ca. 0.2 $cm^2$ geometrical area), a Pt-wire auxiliary electrode, and a Ag/AgCl reference electrode. All measurements were carried out at 22 °C in phosphate buffer (pH 7) containing 10 mM sodium phosphate and 100 mM NaCl that has been thoroughly degassed with nitrogen.

It is well established that the surface density of Au-SR bonds can be coulometrically estimated.[3b,9] Hartwich et al. have shown the surface density of the Au-$SCH_2CH_2NH_2$ monolayer to be $6.5 \times 10^{14}$ thiols $cm^{-2}$ and a nearest-neighbor distance of 4.5 Å, based on the required charges ($400\,\mu C\,cm^{-2}$) for oxidative deposition of the thiolate from the surface.[3b] In a similar way, we have estimated the surface density of Au-SR in the Au-S-$CH_2CH_2OH$ and in the Au-$SCH_2CH_2OC{=}OAQ$/Au-$SCH_2CH_2OH$ to be $6 \times 10^{14}$ and $3.5 \times 10^{13}$ thiols $cm^{-2}$, respectively. Surface coverage of ss and ds AQ-ODN were then evaluated to be $5.5 \times 10^{12}$ and $4 \times 10^{12}$ molecules $cm^{-2}$. The value of surface coverage for ds AQ-ODN is close to that for a modified ODN containing a redox-active molecule at the terminal base.[3b]

Figure 1 shows the cyclic voltammetry of ss and ds AQ-ODN modified electrodes. The AQ group of both ss and ds ODN at the electrode surface exhibited the symmetrical peaks and provided $E_{1/2}$ of −0.46 V (vs Ag/AgCl) for ss and −0.43 V for ds. This observation is comparable to that the $E_{1/2}$ of the AQ was positively shifted (0.02–0.03 V) upon binding of AQ-ODN to DNA in solution.[6] A plot of cathodic peak current (*ipc*) vs scan rate ($v$) is linear, thus confirming that the observed CV responses of ss and ds AQ-ODN are derived from the surface confined redox-active molecule. It has been known that the dependence of the separation of the electroreduction and electrooxidation peaks ($\Delta$Ep) on the CV scan rate ($v$) is a good indicator for electron transfer rate between the redox center and the electrode.[3b,10]

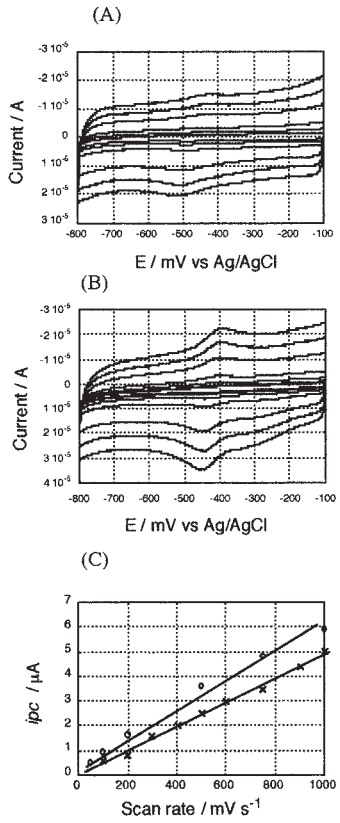



**Figure 1.** Panel A: Cyclic voltammetry of single-stranded AQ-ODN modified electrode. Measurements were carried out at 22 °C in 10 mM sodium phosphate buffer (pH 7) containing 100 m M NaCl at a scan rate of 100, 200, 300, 500, 750, and 1000 mV $s^{-1}$. Panel B: Cyclic voltammetry of double-standed AQ-ODNA modified electrode under the same conditions as Panel A. Scan rate = 30, 50, 100, 200, 500, 750, and 1000 mV $s^{-1}$. Panel C: Plot of *ipc* vs scan rate. × = double-stranded, ◯ = single-stranded.

Figure 2 shows the dependence of ΔEp on the rate $v$ for ss and ds AQ-ODN. With increase in the scan rate, ΔEp for ss AQ-ODN becomes wider than that for ds AQ-ODN. This result strongly indicates that the AQ-ODN modified electrode exhibited faster electron transferability in double-stranded form than that in single-stranded form.[11]

In summary, we have demonstrated that anthraquinone-oligonucleotides immobilized on gold surface have potentially useful properties as an electrochemical probe of DNA. Further researches are in progress to evaluate full properties of the present DNA chip in distance/driving force dependent electron transfer though DNA as well as in mismatch base detection.

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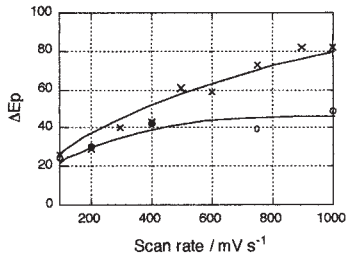


**Figure 2.** Dependence of ΔEp on scan rate. ◯ = double-stranded, × = single-stranded.

**References and Notes**

1 a) S. Takenaka, K. Yamashita, M. Takagi, Y. Uto, and H. Kondo, *Anal. Chem.*, **72**, 1334 (2000). b) K. Yamashita, M. Takagi, H. Kondo, and S. Takenaka, *Chem. Lett.*, **2000**, 1038. c) S. Takenaka, Y. Uto, M. Takagi, and H. Kondo, *Chem. Lett.*, **1998**, 989.

2 a) E. M. Boon, D. M. Ceres, T. G. Drummond, M. G. Hill, and J. K. Barton, *Nat. Biotechnol.*, **18**, 1096 (2000). b) S. O. Kelly and J. K. Barton, *Bioconjugate Chem.*, **8**, 31 (1997).

3 a) J. J. Rack, E. S. Krider, and T. J. Meade, *J. Am. Chem. Soc.*, **122**, 6287 (2000). b) G. Hartwich, D. J. Caruana, T. de Lumley-Woodyear, Y. Wu, C. N. Campbell, and A. Heller, *J. Am. Chem. Soc.*, **121**, 10803 (1999). c) N. A. Whittemore, A. N. Mullenix, G. B. Inamati, M. Manoharan, P. D. Cook, A. A. Tuinman, D. C. Baker, and J. Q. Chambers, *Bioconjugate Chem.*, **10**, 261 (1999). d) T. Ihara, M. Nakayama, M. Murata, K. Nakano, and M. Maeda, *Chem. Commun.*, **1997**, 1609. e) T. Ihara, Y. Matuo, S. Takenaka, and M. Takagi, *Nucleic Acids Res.*, **24**, 4273 (1996).

4 a) K. Yamana, Y. Nishijima, T. Ikeda, T. Gokota, H. Nakano, O. Sangen, and T. Shimidzu, *Bioconjugate Chem.*, **1**, 319 (1990). b) K. Yamana, T. Mitsui, J. Yoshioka, T. Isuno, and H. Nakano, *Bioconjugate Chem.*, **7**, 715 (1996).

5 H. M. Deshmukh, S. P. Joglekar, and A. D. Broom, *Bioconjugate Chem.*, **6**, 578 (1995).

6 K. Yamana, S. Kumamoto, H. Nakano, Y. Matsuo, and Y. Sugie, *Chem. Lett.*, **2001**, 1132.

7 DMT-$OCH_2CH_2S$-$SCH_2CH_2O$-modified silica support was prepared by condensation of DMT-$OCH_2CH_2S$-$SCH_2CH_2OH$ with $HOOCCH_2CH_2CONHCH_2CH_2CH_2$-derivatized silica. Disulfide terminated AQ-ODN on a support was deprotected by a usual procedure using conc. ammonium hydroxide and then purified by 20% DPAGE (Ref. 4b).

8 A solution of disulfide terminated AQ-ODN and fully complementary DNA 15-mer in 10 mM sodium phosphate and 100 mM NaCl was annealed at 90 °C for 5 min and then gradually cooled. The resulting solution was used for preparation of the ds AQ-ODN modified electrode.

9 C. A. Widrig, C. Chung, and M. D. Porter, *J. Electroanal. Chem.*, **310**, 335 (1991).

10 E. Laviron, *J. Electroanal. Chem.*, **101**, 19 (1979).

11 The electron transfer rate constant of surface confined redox molecules can be obtained from the following equation (Ref. 3b and 10).

$$\log k_0 = \alpha \log(1-\alpha) + (1-\alpha)\log\alpha - \log(RT/nFv) - \alpha(1-\alpha)nF\Delta Ep/2.3RT$$

where, $k_0$ = electron transfer rate, $v$ = scan rate

By using this equation, double-stranded AQ-ODN provides faster electron transfer rate constant (15.0 $s^{-1}$) than that (9.5 $s^{-1}$) of single-stranded AQ-ODN.