Cyclic Voltammetric Responses in Hybrid Formation of 2'-Anthraquinone-Modified Oligonucleotide with DNA

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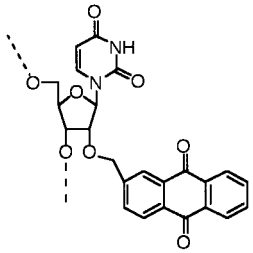
Electrochemical properties of oligonucleotides possessing an anthraquinonylmethyl group at 2'-sugar residue have been investigated. The anthraquinone oligomers exhibited measurable changes in cyclic voltammeric responses upon hybridization with DNA.

Probing of a specific DNA sequence by an electrochemical method has been a current subject of intense research. One possible approach has relied on the redox active molecules that act as affinity ligands of double-helical DNA. A ferrocenyl naphtyldimide intercalator has been shown to possess greater affinity for double-helical DNA than for single-stranded DNA, thus providing an electrochemical mediator for monitoring DNA hybridization.[1] Methylene Blue and daunomycin have been used in 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 both in solution-based and chip-based assays of DNA.

1 : dACAU(2'AQ)GCAGTGTTGAT

U(2'AQ) =



We have developed a method for incorporation of anthraquinone as a redox active intercalator via a short tether into the 2'-position of oligonucleotides.[4] The anthraquinone-modified oligonucleotides bind to DNA in a sequence specific manner to afford duplexes that are significantly stabilized by intercalation. The intercalation of the anthraquinone moiety has been evident from ^{1}H NMR spectral measurements.[5] The attractive features of our oligonucleotide probes are that an anthraquinone group can be placed at the designated base-pair pocket in double-helical DNA. It is therefore expected that redox properties of the anthraquinone moiety may be altered upon transfer into hydrophobic core of duplex to give a measurable signal for monitoring hybridization. In the present work, we have focused on investigation of electrochemical properties of an anthraquinone-modified oligonucleotide and its duplex in solution. The important findings are that hybridization of the anthraquinone probe to DNA causes measurable changes in cyclic voltammetric (CV) responses.

Electrochemical properties of anthraquinone-modified oligonucleotide 15-mer **1**[6] were measured by a conventional three-electrode cell, consisting of a glassy carbon electrode (5 mm diameter, ca. 0.2 cm^2 geometrical area) as a working electrode, a Pt-wire auxiliary electrode, and a Ag/AgCl reference electrode. All measurements were carried out at 22 °C for modified-oligomer (67 μM) in phosphate buffer (pH 7.0) containing 100 mM NaCl and 10 mM sodium phosphate that has been thoroughly degassed with nitrogen.

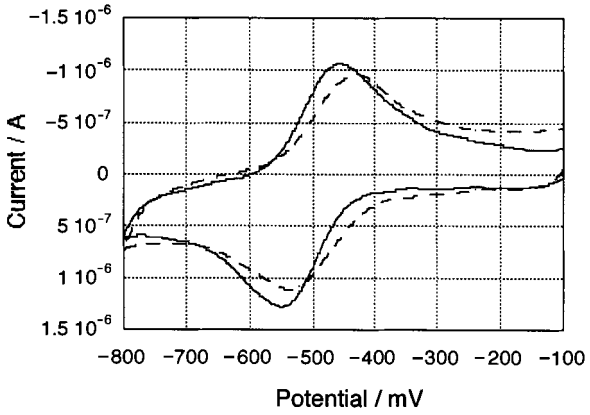


Figure 1. Cyclic voltammograms of anthraquinone-modified oligonucleotide **1** (solid line) and its duplex (dashed line).

Figure 1 shows the cyclic voltammograms of oligomer **1** and its duplex formed with complementary DNA. The single-stranded oligomer exhibited the symmetrical peaks ($E_{1/2}$, –502 mV) that are due to reversible redox reaction corresponding to those of anthraquinone. The difference voltage (ΔE) between cathodic peak and anodic peak were varied from 70–90 mV at scan rate of 10–200 mV/s, wider than that of an ideal value (30 mV), indicating that the redox reactions involve slow electron transfer process between the anthraquinone and electrode. Upon binding of the modified-oligomer to DNA, the anthraquinone moiety is transferred to the base-pair pocket of DNA duplex[4,5] that should differentiate the environment around the anthraquinone. With this transfer, both cathodic peak and anodic peak were positively shifted and small reduction of peak current intensity was also observed.[7] Figure 2 indicates the $E_{1/2}$ dependence on the concentration of target DNA. With increase in DNA concentration up to equivalent amount of the modified oligomer, the observed change was almost proportional to the amount of DNA added. Little or no further change was observed when excess DNA was added to the duplex of 1:1 strand ratio. Cyclic voltammetric responses of oligomer **1** have not been altered in the presence of non-complementary DNA. In contrast to the present observations, oligonucleotides con-

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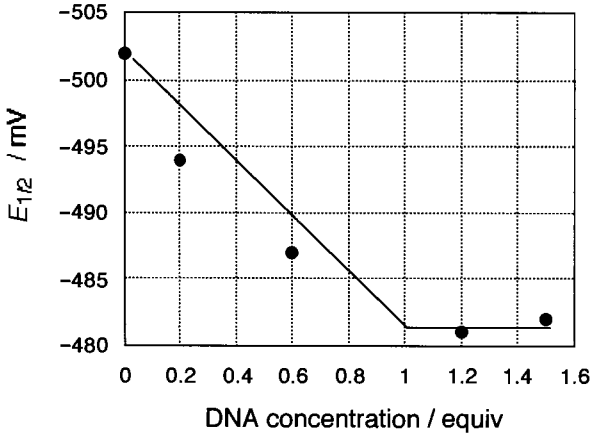
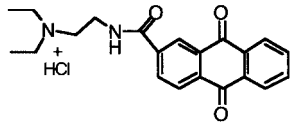


Figure 2. $E_{1/2}$ dependence on the concentration of target DNA. Conditions for the measurements were the same as Figure 1.

taining an anthraquinone group via a long tether exhibited different CV responses.[3b] Owing to the long tether, intercalation of the anthraquinone may not be appreciated, thus having resulted in difficulty to measure CV changes upon hybridization with DNA.[3b] It is therefore concluded that our anthraquinone-modified probe is an electrochemical indicator useful for monitoring DNA hybridization in solution.

2 :



In order to confirm that the observed CV responses are due to anthraquinone intercalation, cyclic voltammetry was carried out for cationic anthraquinone derivative **2** that has been known to intercalate into double-helical DNA.[8] The compound **2** (0.25 mM) in the buffer solution exhibited CV curves whose shape is similar to the anthraquinone-modified oligonucleotide. Upon binding to double-helical DNA (~1.25 mM), positive shift of the $E_{1/2}$ (~20 mV) was indeed observed.

In the present experiments, the redox process of the anthraquinone moiety (AQ) involves the following reaction (eq 1, K is equilibrium constant for this reaction). The voltammetric E for the AQ/AQH_2 couples can be defined as eq 2.

$$AQ + 2H^+ + 2e^- \overset{K}{\rightleftharpoons} AQH_2 \quad \text{(eq 1)}$$

$$E = E_0 - (RT/nF) \ln K \quad \text{(eq 2)}$$

Thus decrease in the K should result in positive shift of $E_{1/2}$. Since the interior of double-stranded DNA is known to be hydrophobic, we speculate that the increased activity of H^+ in the hydrophbic pocket may be responsible for the change in redox equilibrium of the AQ. The reduction of the peak current intensity observed upon AQ intercalation is due to lowered diffusion of the AQ-modified oligonucleotide by hybridization with complementary DNA. Further studies should be necessary to clarify the mechanisms of the CV responses observed for AQ-modified oligonucleotide upon hybridization.[9]

In summary, we have demonstrated that anthraquinone-modified oligonucleotides have useful properties as an electrochemical probe of DNA. Research efforts are in progress to explore the potential of the anthraquinone oligonucleotide as a probe in chip-based assays of DNA using modified electrode.

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References and Notes

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6 Duplex of **1** with complementary DNA shows T_m of 60 °C, while corresponding unmodified duplex possesses T_m of 54 °C at total strand concentration of 4.3×10^{-5} M in the same buffer used for the CV measurements.

7 Similar CV responses have been observed for doubly modified-oligomer, 5'-dACAU(2'AQ)GCAGU(2'-AQ)GTTGAT, upon binding to DNA. The resulting duplex exhibits T_m of 64 °C under the identical conditions as described in ref 6.

8 D. T. Breslin and G. B. Schuster *J. Am. Chem. Soc.*, **118**, 2311 (1996). Binding constant of compound **2** was determined to be 15.7×10^5 M^{-1}.

9 Positive shift of $E_{1/2}$ and reduction of peak current intensity have been observed for the metal complexes containing intercalative ligands upon interaction with double-helical DNA. See, M. T. Carter, M. Rodriguez, and A. J. Bard, *J. Am. Chem. Soc.*, **111**, 8901(1989).