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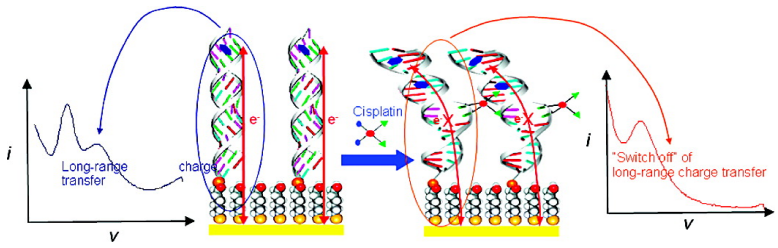


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# The Electrochemical Monitoring of the Perturbation of Charge Transfer through DNA by Cisplatin

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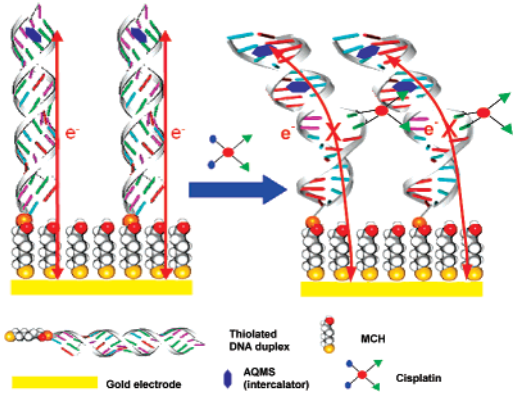
Many anticancer drugs and antibiotics work by targeting DNA in the cell or nucleus. The mode of action is reliant on interfering with the templating function of DNA and hence either affects gene transcription or DNA replication.[1] One way drugs interfere with DNA templating is to induce structural changes in the DNA. Perhaps the best known drug that achieves this is the anticancer drug cisplatin (*cis*-diamminedichloroplatinum(II)).[2,3] Although a variety of binding sites for cisplatin have been identified, the dominant binding mode with therapeutic consequences is believed to occur at 1,2-intrastrand GG sites as the trans platin isomer cannot form 1,2-intrastrand adducts and is therapeutically inactive.[3] X-ray crystallography and NMR studies show the binding of cisplatin causes the bending of DNA duplexes.[3] The bent DNA is then recognized by a number of cellular proteins including the high-mobility group (HMG) domain proteins that has been suggested to mediate the antitumor activity of the drug.[4] The investigation of DNA-drug interactions in biological-like environments is therefore of paramount importance but continues to be a challenge. For example, surface plasmon resonance[5] and quartz crystal microbalance (QCM)[6] allow the detection of drug binding to DNA in real time but confer no information about how the drug binding influences the structure of DNA duplexes.

Herein we report a new biophysical strategy for investigating DNA-drug interactions based on changes to the structure of double stranded DNA (dsDNA) upon drug binding. The strategy exploits the sensitivity of long-range charge transfer through DNA to base-pair stacking. Long-range charge-transfer through double stranded has been shown by Barton and co-workers[7,8] and us[9−11] to be a powerful method of detecting specific sequences of DNA via hybridization. The approach relies on charge transfer to a redox species that has intercalated into the dsDNA base-pair stack. Disruption of the base-pair stack, such as that present when there is a single base-pair mismatch, has been shown to reduce the charge transfer to the redox species.[8−10] Thus the technique has the ability to provide information on the quality of the base-pair stack in DNA duplexes. We have exploited this feature to determine the kinetics of formation of complete DNA duplexes,[11] as have Barton and co-workers to explore protein binding to DNA.[12]

The strategy for detecting the interaction of cisplatin with DNA is depicted in Scheme 1. Clean gold electrodes were placed in a high ionic solution (1 M phosphate buffer, pH 4.5) containing thiolated synthetic DNA duplex of 20 base-pairs (prehybridized in 10 mM Trizma buffer, 1 M NaCl, pH 7.0) for 1 h to give a self-assembled monolayer of a DNA duplex tethered with a mercaptohexyl linker at the 3′ end. The sequence used (see Supporting Information for the DNA sequence, ***P1***, Geneworks Inc., Sydney, NSW, Australia) possessed four GG sites. The DNA modified electrode was subsequently immersed in 6-mercaptohexanol (MCH) to cover any bare gold so as to minimize any nonspecific adsorption

***Scheme 1.*** The Electrode Interface That Exploits Long-Range Charge Transfer to Probe Structural Changes in DNA Duplexes Induced by DNA-Drug Interactions




of cisplatin to the electrode surface. The redox label used was 2-anthraquinonemonosulfonic acid (AQMS) which has a reversible two electron, two proton redox couple between the quinone and hydroquinone. We have shown previously that long-range charge transfer through the dsDNA could be detected electrochemically by incubating the electrode in a 25 $\mu$M AQMS solutions (0.05 M phosphate buffer, 50 mM NaCl) for 10 min with stirring followed by performing Osteryoung square-wave voltammetry (OSWV) in the AQMS solution (Figure 1a).[9] The larger peak at −550 mV was attributed to the nonspecific access of AQMS to the electrode surface and the peak at −400 mV was due to long-range charge transfer induced by intercalation of AQMS into DNA duplex.[9] The −400 mV peak after background subtraction of the −550mV peak is shown in Figure 1b. Exposure of the DNA modified surface to 1 $\mu$M cisplatin solution containing a 25 $\mu$M AQMS, 0.05 M phosphate buffer and 50 mM NaCl for 10 min resulted in complete suppression of the peak at −400 mV and some diminution of the peak at −550 mV.

The "switching off" of the long-range charge-transfer peak could be due to (i) removal of DNA duplex from the surface, (ii) the denaturation of the DNA duplex, resulting in a single-stranded DNA modified gold surface such that long-range charge transfer cannot occur, or (iii) the perturbation of base-pair stacking in the DNA duplex upon exposure to the cisplatin molecules. Chronocoulometry using rutheniumhexaammine ($Ru(NH_3)^{3+}$) molecules[13] to determine the amount of surface-bound DNA before and after exposure to cisplatin showed there was no change in the amount of surface-bound DNA. The constancy in the amount of surface-bound DNA duplexes rules out the first two possibilities and provides strong evidence that the suppression of the −400 mV peak comes from the perturbation of DNA base-pair stacking in duplex by cisplatin molecules. The diminution of the nonspecific peak at −550 mV we speculate is due to the bent conformation of the DNA−cisplatin complexes restricting access of the AQMS to the electrode surface.

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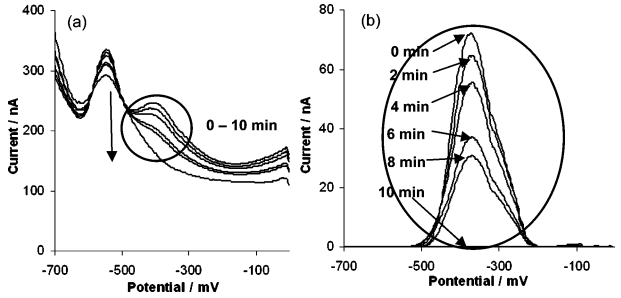


***Figure 1.*** (a) Raw and (b) background subtracted OSW voltammograms of the DNA modified electrode performed in the 0.05 M phosphate buffer (pH 7.0), containing 50 mM NaCl, 25 $\mu$M AQMS intercalator solution after different periods of exposure (2 min interval) to 1 $\mu$M cisplatin. The step is 4 mV, with pulse amplitude of 25 mV and frequency of 10 Hz.

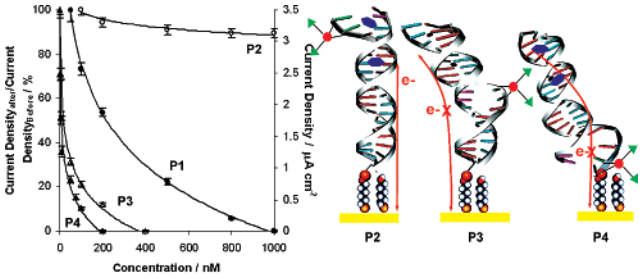


***Figure 2.*** Plots of the attenuation in current (relative to before exposure to cisplatin) with concentrations of cisplatin for sequences ***P1***, ***P2***, ***P3***, and ***P4*** modified surfaces. The bending of DNA backbone at the GG sites is also schematically represented.

Figure 1b illustrates the charge-transfer strategy can be used to monitor the structural changes to DNA upon cisplatin binding in real time. The time required to observe the DNA current changes induced by cisplatin was 10 min at 1 $\mu$M. This time scale is similar to that observed by Wang et al.[6] for the kinetics, measured using a QCM, of binding of cisplatin to a DNA recognition interface fabricated in the same way as described here.[14] Similar timescales for cisplatin binding are also observed by NMR studies of dsDNA in solution.[15] As the long-range charge-transfer signal approach is dependent on perturbation of base-pair stacking in dsDNA while the QCM measurement is dependent on the time of binding of the cisplatin to the DNA, the similarity in the time scales of the different processes supports the proposition that the disruption of base-pair stacking is a rapid process compared with the kinetics of cisplatin binding to DNA.[3]

The dependence of the suppression of the long-range charge-transfer current with the concentration of cisplatin is shown in Figure 2 (see curve ***P1***). The impact of the binding of cisplatin to DNA modified surfaces on the current signal was reproducible, with a relative standard deviation of 4.5% to 6.5% over the concentration range of the calibration curve (for five independently prepared electrodes). The lowest concentration of cisplatin to have a measurable effect on the electrode modified with the ***P1*** sequence of DNA was 50 nM. The current density attributed to long-range charge transfer is completely suppressed for cisplatin concentrations above 1000 nM. This cisplatin concentration dependent experiment provides further evidence that the diminution in current of the −400 mV peak is attributed to the disruption of DNA base-pair stacking induced by cisplatin.

To gain a better understanding of the impact of cisplatin binding on base-pair stacking, and thereby "switching off" long-range charge transfer, DNA duplex modified surfaces with the adjacent GG bases located at the distal (***P2***), in the middle (***P3***), and at the proximal (***P4***) end of the surface-bound DNA duplexes were explored. Chronocoulometry showed that in all cases the amount of dsDNA immobilized onto the electrode surface was identical. The calibration curves obtained from using these DNA modified surfaces are shown in Figure 2. Complete suppression of the −400 mV peak was observed using both the ***P3*** and ***P4*** modified surfaces after cisplatin exposure of 400 and 200 nM, respectively. However, when the GG sites are located at the distal end of the immobilized DNA (***P2*** modified surface), only 10% diminution in current density was observed even after exposure to cisplatin concentration as high as 1000 nM. The complete suppression of the shoulder peak only when the GG bases are located either at the proximal end or in the middle of the immobilized DNA duplex supports previous postulation that the AQMS molecules intercalate above the middle of DNA duplex.[9]

In summary, we have shown that a long-range charge transfer approach can be used to probe cisplatin-induced DNA perturbations, which are attributed to conformation changes of DNA duplexes, in real time in a solution environment. The same approach could be applied to other DNA binding drugs and provide information regarding possible structural changes to the DNA as well as the kinetics of these processes.

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**Supporting Information Available:** DNA sequences for ***P1***, ***P2***, ***P3***, and ***P4*** modified surfaces, control experiment of electrochemical detection of DNA hybridization via the long-range charge transfer approach, chronocoulometry plot of ***P1*** modified surface prior to and after exposure to cisplatin.

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