Communications to the Editor

Direct Evidence for Preassociation Preceding Covalent Binding in the Reaction of *cis*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ with Surface Immobilized Oligonucleotides

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Herein it is demonstrated that Quartz crystal microbalance (QCM) electrodes modified with single stranded oligonucleotides can be used to study preassociation (i.e., electrostatic binding) prior to covalent binding between the immobilized oligonucleotides and a mononuclear Pt(II) complex. Cisplatin (*cis*-[Pt-$((NH_3)_2(Cl)_2]$) is a widely used anticancer drug.[1] Hydrolysis of the Pt−Cl bonds of cisplatin occurs in vivo producing *cis*-[Pt-$(NH_3)_2(Cl)(H_2O)]^+$ (**1**) and *cis*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ (**2**). DNA, the drug's primary target, is likely first attacked by **1** while **2** has been used as a convenient route to simple preparation of the bifunctional adducts which are the final products of DNA platination. Coordination occurs largely at the N7 positions of guanine (G) and adenine (A), with adjacent Gs being the most common binding sites. The N3 position of cytosine (C) is platinated less frequently, and thymine (T) is essentially inert at neutral and acidic pH.[2] It has been proposed by Lippard and co-workers[3] that the DNA binding rates of such Pt(II)-based antitumor agents are facilitated through weak interactions resulting in preassociation between the platinum complexes and oligonucleotides. Specifically, directed diffusion of weakly associated platinum complex along the phosphorthioate-modified oligonucleotide backbone was invoked to explain increased platination rates with increasing strand length.[3] Importantly, the QCM data reported here strongly support this kinetic model by showing direct, real-time detection of a weak preassociation between **2** and the oligonucleotides prior to covalent binding.

Gold QCM electrodes coated with a thin layer of electrodeposited silver[4] (three monolayers) were modified with oligonucleotides functionalized at the 5′ end with a disulfide linking group (HO-$(CH_2)_6$-SS-$(CH_2)_6$-5′-DNA). This surface is subsequently reacted with mercapto-hexanol to reduce interactions between the oligonucleotide backbone and the electrode surface. This scheme mimics the well-characterized surface modification chemistry reported by Tarlov and co-workers for immobilizing oligonucleotides on bare gold surfaces.[5] The thin layer of electrodeposited silver used here prevents adsorption of **2** to electrode surfaces modified with only mercapto-hexanol (Figure 2: Control).[6] Decreased electrode capacitance measured by linear scan voltammetry indicates deposition of both the disulfide functionalized oligonucleotide and mercapto-hexanol on the silver coated gold QCM electrodes used here (Supporting Information). Figure 1 shows the idealized model of the surface architecture. The oligonucleotide sequence is: 5′-GGGAAGGATGGCG-CACGCTG-3′, containing suitable GG sites for bifunctional covalent attachment of platinum and which has been previously used in binding studies.[7] Interactions between the platinum complex and the hydroxyl terminal groups of the mercapto-hexanol portion of the surface are not detected at the concentrations used for this study (Figure 2).

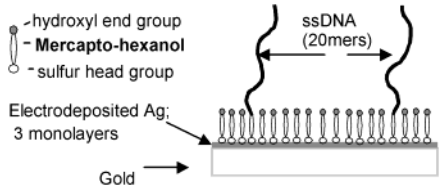

Figure 1. Idealized structure of the modified electrode.

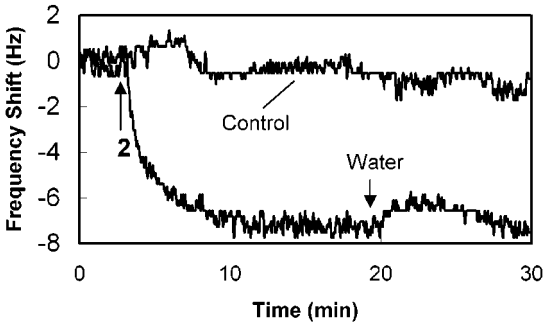

Figure 2. QCM frequency responses for exposure of *cis*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ (0.25 mM, estimated pH 4.2) to a mercapto-hexanol-modified electrode (control experiment) and to an oligonucleotide-modified electrode.

A wall-jet flow cell (see Supporting Information for conditions) is used to expose the QCM electrode surfaces to aqueous platinum complex solution. After a stable baseline frequency is observed with water flowing through the cell, the flow is changed to aqueous platinum complex solution and back to water. Arrows in the Figures indicate the approximate times of changing the flow.

Representative data for exposure of **2** to an oligonulceotide-modified electrode is shown in Figure 2. The reproducibility between different electrodes is good and allows comparisons of platination rates under varied conditions. The increase in mass indicated by the decrease in QCM resonant frequency (8 ± 1 ng; 1.13 Hz/ng) is due to covalent binding of **2** to the immobilized oligonucleotides. Upon changing the flow back to water (at ca. 19 min), no removal of **2** is observed, indicating that the platinum

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(6) Mercapto-hexanol monolayers formed on bare gold QCM electrodes and on gold QCM electrodes coated with 1 monolayer of electrodeposited silver show irreversible adsorption of the platinum complex (Supporting Information).
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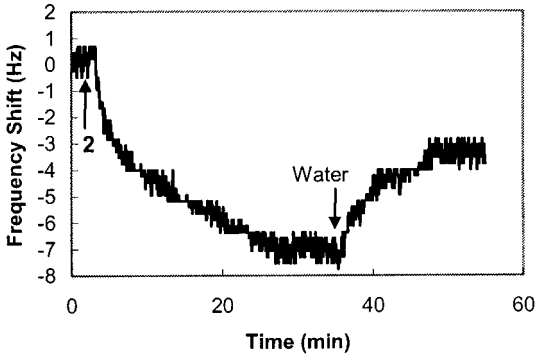

Figure 3. QCM frequency shift for exposure of *cis*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ (0.10 mM, estimated pH 5.4) to an oligonucleotide-modified electrode and for changing the flow back to water.

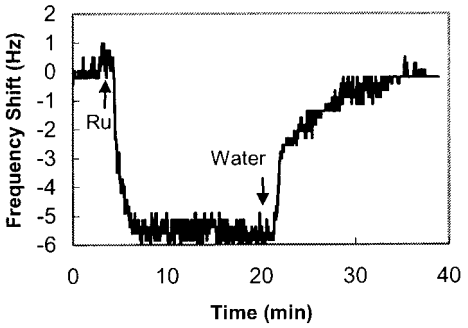

Figure 4. QCM frequency shift for exposure of the oligonucleotide-modified electrode to 0.25 mM $[Ru(NH_3)_6]^{3+}$ and for changing the flow back to water.

complex is irreversibly bound to the immobilized oligonucleotides. The mass increase is consistent with an oligonucleotide surface coverage of ca. $(1.6 \pm 0.2) \times 10^{-11}$ mol/cm^2, assuming complete electrostatic compensation (10 Pt(II) complexes per 20mer oligonucleotide; -19 charge).

At lower concentrations, QCM data also provide evidence for weak preassociation of **2** prior to covalent coordination. Figure 3 shows the QCM response for exposure of a 0.10 mM solution of **2** to an oligonucleotide-modified electrode. The QCM frequency shift upon changing the flow back to water is also shown. The total mass increase indicated by the first frequency plateau ($\sim$7 ng at 28 min) is approximately equal to that observed for exposure of the oligonucleotide to the higher concentration (0.25 mM) of **2** (Figure 2). Upon changing the flow back to water, however, an increase in QCM frequency indicates that about half of the platinum complex is removed from the electrode surface. This suggests that the covalent reaction is incomplete. Longer exposure times (1 h) at this concentration result in complete covalent binding (i.e., no removal of **2** upon changing the flow back to water). Some variation in the time required for complete preassociation (frequency plateau) is observed at this concentration. Nevertheless, this result is consistent with preassociation between **2** and the immobilized oligonucleotides prior to covalent binding.

The frequency shift profile shown in Figure 2 is also likely a response to preassociation with covalent binding occurring at a slower rate. The kinetics of preassociation of **2** appear to be biphasic with an initial fast phase followed by a slower phase. The data in Figure 2 suggest that the average rate of preassociation from $t = 0$ to $t = 1.3$ min is 7×10^{-8} mol/s·cm^2 ($t = 0$ corresponds to 3 min in Figure 2). While the average rate of preassociation from $t = 2.2$ to $t = 7$ min is 8×10^{-9} mol/s·cm^2.

To further examine the evidence for preassociation, **2** was exposed to a poly (T_{25})-modified electrode. The QCM data show a mass increase (e.g., 12 ng). As expected, **2** is completely removed from the electrode surface upon changing the flow back to water because it only binds through electrostatic interactions (Supporting Information). Control experiments were also conducted using ruthenium hexammine, $[Ru(NH_3)_6]^{3+}$ (Figure 4). This inert cationic complex, chosen because it binds only electrostatically to DNA,[5e] similar to the much studied $[Co(NH_3)_6]^{3+}$ and $[RuCl(NH_3)_5]^{2+}$[8] binds reversibly to QCM electrodes modified with the oligonucleotide. That is, the frequency shift is also reversed upon changing the flow to water. These control experiments strongly suggest that the reversal observed for **2** is due to preassociation.

The mass increase of ca. 5 ng (frequency plateau at 8 min in Figure 4) for electrostatic binding of $[Ru(NH_3)_6]^{3+}$ is in agreement with the oligonucleotide surface coverage estimated from the platinum binding data and the binding site size (i.e., two base)[5e] for $[Ru(NH_3)_6]^{3+}$ on single-stranded oligonulceotides. Tarlov and co-workers[5e] have shown good agreement between metal cation binding constants for immobilized oligonucleotides and solution-phase DNA.

This system permits direct, real time detection of interactions between platinum complexes and surface immobilized oligonucleotides. The data verify weak preassociation between *cis*-$[Pt(NH_3)_2(H_2O)_2]^{2+}$ and the oligonucleotides before covalent attachment. Recently, preassociation has been observed in HSQC [^{1}H,^{15}N] NMR studies of DNA and polynuclear platinum complexes.[9] The QCM technique will be useful in understanding how such electrostatic interactions may contribute to measured rates of covalent binding of platinum complexes in general and how they affect or dictate sequence specificity of the adducts formed. This method should also be useful for characterizing the recognition of platinum−DNA adducts by biomolecules believed to be involved in DNA repair.[1]

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Supporting Information Available: Figure S1 capacitance data for electrode modification and discussion of mercapto-hexanol monolayers on silver, Figures S2 and S3 of the flow cell, QCM mass sensitivity and calibration, Figure S4 of a poly (T_{25}) control experiment, synthesis of **2** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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