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A Real-Time QCM-D Approach to Monitoring Mammalian DNA Damage Using DNA Adsorbed to a Polyelectrolyte Surface

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Abstract

We have successfully demonstrated that the quartz crystal microbalance with dissipation monitoring (QCM-D) can be used to monitor real-time damage to genomic mammalian DNA adsorbed to a polyelectrolyte surface. To reveal the capabilities of this technique, we exposed DNA surfaces to quercetin, an agent that has been implicated in causing DNA strand breaks in a Cu(II)-dependent fashion *in vitro*. We show that the QCM-D frequency and dissipation patterns that result from exposure of the DNA surfaces to quercetin/Cu(II) are consistent with the induction of DNA strand scission. We use QCM-D to furthermore demonstrate that this process is dependent on Cu(II) and that the DNA damage induced by quercetin can still be detected if Cu(II) is *in situ* with the DNA surface and not in solution-phase.

Keywords

QCM-D; DNA surfaces; biosensors; DNA damage detection; flavonoids; polyelectrolyte-DNA interaction

Introduction

A variety of biological and chemical methods exist to detect and characterize DNA damage.¹⁻¹² Common biological techniques include gel electrophoresis and the “comet assay” to detect strand breaks,⁵⁻⁹ and regular chemical methods include electrochemical techniques^{1,2,11,12} and chromatographic separation coupled with mass spectrometry.¹⁰ Unfortunately, many of these common biological techniques and chemical methods have limited sensitivity and are generally unable to monitor DNA damage in real-time.

The quartz crystal microbalance with dissipation monitoring (QCM-D) offers excellent advantages over conventional methods for DNA damage detection, allowing for the rapid detection of DNA damage in real time and requiring only small quantities of DNA. QCM-D is a non-invasive gravimetric technique that measures the changes in resonance frequency (ΔF) and dissipation (ΔD) resulting from the deposition of a thin film on a sensitive piezoelectric quartz crystal.¹³ The Sauerbrey equation¹⁴ relates ΔF to the adsorbed mass while ΔD is a measure of the dampening effect of the adsorbed film and provides an indication of the rigidity of the film.^{13,15} Because QCM-D reveals both film rigidity and changes in adsorbate mass simultaneously in real time, the technique is ideal to study DNA films¹⁶. Previous studies have used QCM-D to observe the formation of DNA films and surfaces

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composed of plasmid DNA and short synthetic oligomers¹⁷⁻²², and to monitor enzymatic cleavage of surface-tethered DNA oligomers²³⁻²⁴.

We have recently developed an approach using QCM-D to examine the real-time creation of genomic mammalian DNA surfaces on poly(ethylenimine)-coated SiO₂ crystals, and have used those surfaces to detect intercalation of ethidium bromide, a type of DNA damage.²⁵ In this communication we describe a QCM-D method to use those genomic mammalian DNA surfaces to detect another type of DNA damage, strand scission. Related methods have been reported previously, each with significant differences from our technique. Zhu et al. have recently reported the use of QCM to monitor the hydrolytic cleavage of short, synthetic oligomers tethered to a gold-thiol surface via an avidin-biotin linkage.²⁶ This cleavage was monitored through changes in QCM frequency, which corresponds to changes in adsorbed mass. A piezoelectric quartz crystal impedance method to detect DNA damage has also been reported,³ and that technique focused on the detection of DNA damage to non-mammalian DNA in bulk solutions. Our method differs from previous techniques in two important areas. One, we use the QCM-D dissipation patterns as well as the frequency shifts to detect DNA damage, which allows for the detection of changes in the viscoelastic properties of the DNA film during DNA damage and not merely changes in adsorbed mass. Two, we describe the use of surface-bound genomic mammalian DNA electrostatically adsorbed to a polyelectrolyte surface to detect DNA damage, rather than non-mammalian DNA in bulk solutions. Furthermore, our method does not require the construction of biotin-linked synthetic DNA oligomers in order to create the DNA surface.

In the current study we present our QCM-D method of DNA damage detection by demonstrating its ability to detect strand scission induced by quercetin, a common flavonoid. Flavonoids have been hailed for their antioxidant and anti-carcinogenic capabilities²⁷⁻²⁹, but previous research has indicated that some flavonoids may exhibit mutagenic and carcinogenic properties.^{1,2,4-7} Quercetin specifically has been mentioned to possess mutagenic properties in a number of studies.^{1,2,4-6} Several of these studies have used common biological techniques, such as gel electrophoresis and the “comet-assay”, to characterize the ability of quercetin to induce strand scission in both plasmid and mammalian DNA.^{5,6,8,9} They have also demonstrated that this ability is dependent upon the presence of Cu(II)^{5,6}, a common biological trace element that has been shown to have an affinity for DNA^{30,31}, and which is presumed to help maintain DNA structure.^{32,33} Electrochemical approaches have also been applied to demonstrate that quercetin can induce damage to mammalian DNA *in vitro*.^{1,2} From such experiments, a mechanism by which the quercetin-Cu(II) system is thought to induce DNA damage has been proposed and this mechanism involves the generation of a quercetin free radical upon oxidation by Cu(II) and the subsequent formation of a DNA-Cu(I)-oxygen complex.⁵ While gel electrophoresis studies can provide evidence of DNA strand breakage, they are unable to detect DNA damage in real time. To that end, we have applied our QCM-D method for DNA damage detection to monitor the Cu(II)-dependent strand scission induced by quercetin.

Experimental Section

Materials

Poly(ethylenimine) (MW = 25,000 g mol⁻¹, mixture of linear and branched chains), quercetin, CuSO₄, and calf thymus DNA sodium salt were obtained from Aldrich and were used as received. PEI solutions were prepared at 10 mM (using the molecular weight of the monomer repeat unit) with ultra-pure water (resistivity > 18 MΩcm) at pH 7. DNA solutions were prepared at 100μg/mL also using ultra-pure water. Quercetin and CuSO₄ solutions were prepared in either ultra-pure water (QCM-D Experiment) or 10mM Tris-HCl (pH 7.4) (Gel Electrophoresis Experiment) to the concentrations described.

Gel Electrophoresis

pRS414 plasmid harvested from *E. Coli* was kindly provided by Dr. Tina Negritto. pRS414 DNA was made to a concentration of 0.015 µg/µL in 10mM Tris-HCl (pH 7.4) and was incubated at 23°C for 1hr in the presence of CuSO₄, Quercetin, CuSO₄ + Quercetin, or no additive. 10 µL aliquots of each sample were then run on a 0.8% agarose gel and visualized with ethidium bromide.

QCM-D

Frequency and dissipation data of the formation of DNA and PEI surfaces were obtained using a quartz crystal microbalance with dissipation (QCM-D) monitoring (E4, Q-Sense, Gothenburg, Sweden). The QCM-D sensor consisted of an AT-cut piezoelectric quartz crystal disk, coated with an Au electrode (100 nm thick) on the back and an active surface layer of SiO₂ (~50 nm thick). The QCM-D sensor crystal (14 mm × 0.3 mm, active area of 1 cm²) operated at a frequency of 4.95 MHz ± 50 kHz. The crystals were optically polished with a surface roughness of less than 3 nm (root mean square). The active side was in contact with the aqueous solutions of PEI, DNA, or quercetin. The crystal was mounted in a flow cell with a total volume of 40 µL. The 5th overtone values were used for frequency and dissipation measurements. At this overtone, the frequency values were obtained with an accuracy of ± 2 Hz or less and the dissipation factor with an accuracy of ± 2 × 10⁻⁶ or less. *F* and *D* values were measured relative to a baseline obtained in ultra-pure water. We have previously described the details of QCM-D data acquisition and the creation of dsDNA surfaces elsewhere.^{25,34}

Before each run, the SiO₂-coated quartz crystals were decontaminated by UV/Ozone treatment for 5-10 minutes, soaking in 2% Hellmanex® solution (Hellma, Co) for 30 minutes, and UV/Ozone treatment again for 5-10 minutes, with the final two steps followed by an ultra-pure water rinse and drying with N₂. Ultra-pure water was also flushed through the QCM-D flow cells until a stable baseline was achieved. During the experiments, solutions of PEI, DNA, quercetin, and CuSO₄ were passed through the QCM-D chambers as indicated. These steps were considered to be complete when *F* and *D* stopped changing with time. The cells were also rinsed with ultra-pure water between PEI and DNA depositions and as indicated during exposure to quercetin or CuSO₄. These water rinses were performed, in part, to ensure that the observed changes in *F* and *D* were not due to changes in the solutions to which the surfaces were being exposed. If the different solutions were causing changes in *F* and *D*, then a sharp shift would be observed upon water rinsing as the system re-equilibrated. In no case were the observed changes in *F* and *D* found to be due to differences in solutions. The QCM-D data collected during these water rinses also permitted detection of any leaching of DNA or PEI from the crystal surface. These effects were found to be negligible, and the leaching data of DNA surfaces is seen more clearly elsewhere.²⁵ The flow rate for each experiment was 100 µL/min, and all experiments were conducted at 23°C.

Results and Discussion

In order to confirm that quercetin can induce DNA strand breaks in the presence of Cu(II), we conducted a gel electrophoresis experiment with plasmid DNA harvested from *E. coli*. Figure 1 demonstrates that the mostly super-coiled plasmid DNA was converted to predominantly linear and open circular forms when in the presence of 0.1 mM quercetin and 0.1 mM CuSO₄, consistent with strand scission.^{5,6,9,35}

We then applied our QCM-D method for DNA damage detection to observe quercetin-induced strand scission in real-time on a mammalian DNA surface. DNA surfaces were created and monitored using QCM-D as previously described.²⁵ Heat-denatured calf thymus DNA was deposited on a poly(ethylenimine) (PEI) surface adsorbed to a SiO₂-coated quartz crystal and

then hybridization between the surface-bound ssDNA and complementary strands in solution was allowed to occur, forming a mostly dsDNA surface (Figure 2A).^{*} These DNA surfaces were then exposed to quercetin, Cu(II), or both and ΔF and ΔD were monitored in order to determine the DNA damage induced (Figures 2,3).

To demonstrate the ability of our DNA surfaces to detect potential mutagens through QCM-D, and to support the observation that the DNA-damaging ability of quercetin is dependent upon the presence of Cu(II)³, we exposed our DNA surface to a solution of 0.1mM quercetin and 0.1mM CuSO₄ in ultra-pure water, after generating a stable baseline in 0.1mM CuSO₄ alone (Figure 2B). Upon the addition of CuSO₄ alone, there was an initial drop in frequency, a small increase, and then a leveling off, resulting in ~ 1.5 Hz net decrease in ΔF and no significant change in ΔD . The shift in ΔF corresponds to a mass deposition of ~ 25 ng/cm² using the Sauerbrey equation¹⁴, and can be explained by the adsorption and subsequent chelation of the positively charged Cu(II) ions to the negatively charged DNA surface. When the surface was then exposed to the quercetin/Cu(II) solution, a dramatic increase in both frequency and dissipation was observed over ~ 250 minutes of exposure. The increase in frequency indicates a removal of mass from the DNA surfaces and the large increase in dissipation indicates a marked decrease in the rigidity of the adsorbed film. When DNA surfaces with or without adsorbed Cu(II) were rinsed with H₂O or 0.1mM CuSO₄ for extended periods of time, similar shifts in ΔF and ΔD were not observed (data not shown).

From the gel electrophoresis data (see Fig. 1), we concluded that the quercetin-Cu(II) system specifically causes strand scission in DNA. That mechanism is consistent with the observed QCM-D frequency and dissipation shifts in response to quercetin-Cu(II) exposure in Figure 2. As the backbone of the side-attached surface-bound DNA is continually nicked, the DNA surface begins to corrode, releasing small sections of DNA from the surface and allowing partially surface-bound DNA fragments to extend into solution, resulting in a much looser and less compact DNA film. Furthermore, ΔF and ΔD never reach equilibrium and continue to increase during the final water rinse. It is presumed that the quercetin-Cu(II) system will continue to induce strand scission while DNA still exists on the surface and after enough damage has been induced to the DNA surface, any liquid flowing over the surface will provide sufficient shear stress to disassemble the DNA film.

To verify that the damage induced by quercetin to the DNA surfaces was dependent upon Cu (II), we exposed a DNA surface to 0.1mM quercetin alone and then to a solution of 0.1mM quercetin and 0.1mM Cu(II) (Figure 3A). No significant change in either ΔF or ΔD was observed during the quercetin exposure alone, confirming that quercetin by itself could not induce DNA damage. During exposure to quercetin/Cu(II) there was an initial decrease in frequency and a subsequent increase. This indicates a competition between the binding of the Cu(II) and the destruction induced by quercetin/Cu(II). The Cu(II) binding is expected to be quick, causing the initial frequency decrease, and the slower DNA damage effect is revealed after the DNA surface is saturated with Cu(II) ions, explaining the subsequent frequency increase. As with the previous experiment, an increase in ΔD , indicating the destruction of the DNA surface, was observed during the quercetin/Cu(II) exposure.

From previous work in which we exposed the DNA surfaces to Cu(II) (data not shown), we noticed that Cu(II) appeared to not be completely removed upon rinsing the surfaces with H₂O for short periods of time. Previous research has proposed an affinity of Cu(II) for DNA^{30,31,33}, and we surmise that the Cu(II) remaining after water rinsing is chelated by the

^{*}We do not expect that complete hybridization will occur between each surface-bound ssDNA strand and its complementary strand in solution. Instead, partial hybridization between multiple strands is a more likely model. This idea is corroborated in other work we have performed in which the hybridization efficiency of our DNA surfaces was observed to be greater than 100%.²⁵

DNA surface. Therefore, we investigated whether quercetin-induced DNA damage would still occur if the Cu(II) ions were not pre-mixed with the quercetin solution, but were *in situ* with the DNA surface. After exposing a DNA surface to 0.1mM CuSO₄ as before, we rinsed the surface with H₂O, removing most of the non-chelated Cu(II) ions, and then exposed the surface to 0.1mM quercetin without the addition of Cu(II) (Figure 3B). As in the experiments with quercetin and Cu(II) together in solution, we observed an increase in frequency and a large increase in dissipation, confirming that the quercetin/Cu(II)-induced DNA damage could still occur when Cu(II) was *in situ* with the DNA surface.

Conclusions

Using genomic mammalian DNA adsorbed to a polyelectrolyte surface, we have shown that QCM-D can be used to detect quercetin-induced DNA damage in real time by monitoring changes in QCM frequency and dissipation. Consistent with gel electrophoresis experiments, we have demonstrated that the DNA damage detected by QCM-D is dependent on the presence of Cu(II) with quercetin. We have also shown that the damage will occur whether Cu(II) is in solution with quercetin or *in situ* with the DNA surface. We are currently using this same method to examine other compounds whose mutagenic properties have not been fully characterized.

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Figure 1.

Gel electrophoresis data demonstrating the copper-dependent DNA damage induced by quercetin. pRS414 plasmid DNA (0.015 μ g/ μ L) was incubated at 23°C for 1hr in the presence of the indicated additives: Lane 1, no additive; Lane 2, 0.1mM CuSO₄; Lane 3, 0.1mM quercetin; Lanes 4 and 5, 0.1mM quercetin and 0.1mM CuSO₄. Aliquots of each sample were run on a 0.8% agarose gel and visualized with ethidium bromide. Super-coiled, open circular, and linear forms of the plasmid DNA are represented schematically and their corresponding bands are indicated. This identification of the band patterns is consistent with previous research.^{6,9}

Figure 2.

QCM-D fifth overtone frequency and dissipation shifts of (A) the creation of a calf thymus dsDNA surface and (B) its subsequent exposure to 0.1 mM CuSO_4 then 0.1 mM quercetin with 0.1 mM CuSO_4 . (A) shows the creation of a mostly dsDNA surface by allowing ssDNA to hybridize with its complementary strand on a PEI surface as previously described.²⁵ Significant shifts in ΔF and ΔD are labeled and we have discussed elsewhere the significance of these shifts as well as the lag time observed between the ssDNA deposition and hybridization.²⁵ ΔF for the total DNA surface corresponds to a calculated areal mass of 170 ng/cm^2 and a density of 1.70 g/cm^3 using the Sauerbrey equation with an estimated thickness of 1.0 nm from ellipsometric thickness data (not shown). (B) shows the exposure of that same DNA surface to Cu(II) and then quercetin/Cu(II). Gray shading indicates an ultra-pure water rinse.

Figure 3.

QCM-D fifth overtone frequency and dissipation shifts of DNA surfaces exposed to (A) 0.1mM quercetin then 0.1mM quercetin with 0.1mM CuSO_4 or (B) 0.1mM CuSO_4 , an ultra-pure water rinse, and then 0.1mM quercetin alone. Each figure represents a zoom on the data collected after the formation of the respective DNA surfaces. Periods of exposure of the DNA surface to CuSO_4 and/or quercetin are labeled. Gray shading indicates an ultra-pure water rinse.