Photoelectrochemical Detection of Oxidative DNA Damage Induced by Fenton Reaction with Low Concentration and DNA-Associated Fe²⁺

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The metal ion dependent decomposition of hydrogen peroxide, the so-called Fenton Reaction, yields hydroxyl radicals that can cause oxidative DNA damage both *in vitro* and *in vivo*. We have previously reported a photoelectrochemical sensor for the detection of oxidative DNA damage induced by an Fe²⁺-mediated Fenton Reaction, using a DNA intercalator as a photoelectrochemical signal reporter (Liang, M.; Guo, L.-H. *Environ. Sci. Technol.* **2007**, *41*, 658). The intercalator binds less to the damaged DNA in the sensor film than the native form, resulting in a reduction in the measured photocurrent. In this report, some mechanistic aspects of the sensor were investigated. It was found that Fe²⁺ alone (without the coexistence of H₂O₂) suppressed the photocurrent of the intercalator bound to the DNA film in a pH-dependent manner. Similar pH dependence was observed for the ζ potential of the tin oxide nanoparticle colloid used in the preparation of the semiconductor electrode, leading to the hypothesis that the metal ion binds to the surface oxide groups on the electrode and quenches the photoelectrochemical response. At pH 3, the quenching effect was reduced substantially to permit the detection of DNA damage by as low as 10 μ M Fe²⁺ and 40 μ M H₂O₂, a concentration that is within the physiologically relevant range. It was also found that Fe²⁺ ions associated with the DNA in the sensor film and participated in the DNA damage reaction, a mechanism that has been implicated in previous studies on metal carcinogenesis.

Introduction

Many metals are considered essential trace elements and must be present in low concentrations in the human body for normal cellular function. However, high concentrations of metals in the body are thought to be deleterious. Some transition metals have been confirmed to be human carcinogens or possibly carcinogenic to humans.¹ A number of studies have shown that these metal ions induce their toxic effects primarily through their ability to produce reactive oxygen species. Iron is the most abundant transition metal in biological systems. Fe²⁺ has been found to react with H₂O₂ to produce the extremely reactive hydroxyl radical, the so-called Fenton Reaction. The radical can induce several classes of DNA damage, including single-strand break, double-strand break, abasic sites, and base oxidation.²

Association of iron with DNA has been implicated in a number of studies on DNA damage both *in vivo* and *in vitro*. Multiple-phase dose responses of DNA damage to H₂O₂ in the presence of Fe²⁺ were rationalized by the hypothesis of three types of Fe²⁺ participated in the Fenton reaction,^{3–6} i.e., the ions bound electrostatically to DNA, the ions bound to the DNA bases, and free Fe²⁺. Sequence-specific DNA cleavage by Fe²⁺-mediated Fenton reaction has been observed.^{7,8} Both computer modeling and NMR data support the postulation that the DNA sequence preference arise from the specific coordination geometry of the metal with the DNA bases. Binding of Fe²⁺ and Fe³⁺ with calf thymus DNA was investigated by FTIR and capillary electrophoresis.⁹ It was found that Fe²⁺ bound to DNA through guanine N-7 and the backbone phosphate with binding constants of $K_G = 5.4 \times 10^4 \text{ M}^{-1}$ and $K_P = 2.4 \times 10^4 \text{ M}^{-1}$.

Given the fact that the hydroxyl radical is highly reactive and short-lived, it is very plausible that DNA damage is induced mainly by the radicals generated locally in the Fenton reaction between H₂O₂ and DNA-associated Fe²⁺, which are less prone to scavenging effect. However, there has been no direct experimental evidence to support such mechanism.

There are currently a number of cell-based assays as well as biochemical and chemical analytical techniques for the detection of DNA damage and the assessment of genetic toxicity. It is recommended that the genetic toxicity screening test should include three cell-based assays for gene mutation in bacteria, gene mutation in mammalian cells, and chromosomal aberration in mammalian cells, respectively.¹⁰ DNA damage products have been identified and quantified by a wide range of analytic techniques, such as single-cell gel electrophoresis, ³²P-postlabeling, immunoassay, gas chromatography/mass spectrometry, high-performance liquid chromatography, and electrochemical and electrochemiluminescent sensors.^{11–16} We have developed a photoelectrochemical sensor for the detection of DNA damage by Fe²⁺ and styrene oxide.¹⁷ The sensor was assembled by depositing a layer of calf-thymus DNA on a tin oxide nanoparticle electrode. A DNA intercalator, Ru(bpy)₂(dppz)²⁺ (bpy = 2,2'-bipyridine, dppz = dipyrido[3,2-*a*:2',3'-*c*]phenazine), was employed as the photoelectrochemical signal reporter. When the sensor was exposed to a solution containing 1 mM Fe²⁺ and 4 mM H₂O₂, the DNA on the sensor surface was damaged by the Fenton reaction, resulting in less binding with Ru(bpy)₂(dppz)²⁺ and consequently lower signal than the native DNA (Scheme 1). However, the concentration of the two Fenton reagents was significantly higher than what would be encountered *in vivo*. In this report, some mechanistic aspects of the sensor were investigated in an effort to improve the sensitivity

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SCHEME 1: Illustration of Experimental Procedure:
(1) Preparation of DNA Film Electrode, (2) DNA Damage Reaction, (3) Binding of Signal Molecule, and (4) Photocurrent Measurement

of the sensor. After optimization, the sensor was able to detect DNA damage induced by as low as 10 μM Fe^{2+} and 40 μM H_2O_2 . In addition, the sensor configuration provided a unique tool to investigate the role of DNA-bound Fe^{2+} in Fenton reaction and DNA damage. The results demonstrate explicitly that Fe^{2+} ions associated with DNA induced DNA damage in the presence of H_2O_2 .

Experimental Methods

Poly-(diallyldimethyl ammonium chloride) (PDDA) and double stranded calf thymus DNA (ds-DNA, 13K base pairs) were from Sigma-Aldrich (St. Louis, MO). Iron sulfate heptahydrate was from Acros (Geel, Belgium). Fifteen percent tin(IV) oxide, as a colloidal dispersion of 15 nm particles, was obtained from Alfa Aesar (Ward Hill, MA), and so was hydrogen peroxide. All other chemicals and solvents were of analytical grade. $\text{Ru}(\text{bpy})_2(\text{dppz})(\text{BF}_4)_2$ was synthesized according to the published procedure.^{18,19} Solutions were prepared in high-purity water from a Millipore Milli-Q (Biocel) water purification system. Tin-doped indium oxide conductive glass was supplied by Weiguang Corp (Shenzhen, People's Republic of China).

Zeta potential of the SnO_2 nanoparticle colloid was measured on a Zetasizer 2000 ζ potential analyzer (Malvern Corp, United Kingdom). The purchased SnO_2 colloid was diluted to 0.07% in water for the measurement. The pH of the colloid was adjusted with HCl.

The preparation of SnO_2 nanoparticle electrodes and the fabrication of the DNA films on the SnO_2 electrode was the same as previously described.^{17,20} The concentrations of PDDA and DNA solution for film deposition were 2 and 0.5 mg/mL, respectively. The DNA-modified electrode was denoted as $\text{SnO}_2/\text{PDDA}/\text{DNA}$. The DNA film on the electrode was damaged by exposing to $\text{Fe}^{2+}/\text{H}_2\text{O}_2$ in water at 37 $^\circ\text{C}$ with vortex (200rpm) for 1 h or for a time period as specified.

The photocurrent was measured on a CHI 630A electrochemical analyzer (CH Instruments, Austin, TX) using a Pt flag counter electrode, a Ag/AgCl (3M KCl) reference electrode, and a bias voltage of +0.1 V. The excitation light source was a blue light emitting diode (Lamp Inc., Shenzhen, People's Republic of China) with an illumination area of 0.2 cm^2 . A DNA film electrode with either intact or damaged DNA was first reacted with 50 μM $\text{Ru}(\text{bpy})_2(\text{dppz})^{2+}$ for 30 min in order for the signal reporter to bind to the DNA film. After the reaction, the unbound metal complex was washed off by water. Photocurrent was then measured by placing the electrode in 30 mM oxalate buffer, pH 5.8.

Figure 1. (A) Plot of anodic photocurrent of $\text{Ru}(\text{bpy})_2(\text{dppz})^{2+}$ bound to $\text{SnO}_2/\text{PDDA}/\text{DNA}$ electrode after the DNA film was exposed to 1 mM Fe^{2+} at different pH values. Inset is typical photocurrent response curve. (B) ζ potential of 15 nm tin oxide colloid diluted in water as a function of pH.

Results and Discussion

As already described in the introduction, the photoelectrochemical sensor we reported before was able to detect DNA damage in 1 mM $\text{Fe}^{2+}/4$ mM H_2O_2 Fenton reagents. The photocurrent of $\text{Ru}(\text{bpy})_2(\text{dppz})^{2+}$ bound to the damaged DNA film was reduced remarkably as compared with the native DNA. To make the detection relevant to the *in vivo* situation, the concentration of the two reagents must be lowered to low micromolarity, which is the typical concentration inside living cells.^{21,22} In the process of improving the sensor sensitivity, it was found that exposure of the DNA electrode to the metal ion alone (without H_2O_2) suppressed the measured photocurrent in a pH-dependent manner (Figure 1a). The photocurrent was reduced to background after the $\text{SnO}_2/\text{PDDA}/\text{DNA}$ electrode was exposed to 1 mM Fe^{2+} at pH 4.0 or higher, while at pH below 2.0 the signal did not change significantly as compared with the nonexposed electrode. Since photocurrent was measured in an oxalate solution after the DNA electrode was exposed to Fe^{2+} and rinsed, the detrimental effect must be caused by the metal ions adsorbed on the sensor surface. In a control experiment, a bare SnO_2 electrode was first exposed to 1 mM Fe^{2+} at pH 5.3, followed by the assembly of DNA film, binding of $\text{Ru}(\text{bpy})_2(\text{dppz})^{2+}$, and photocurrent measurement. Signal reduction was also observed, suggesting the detrimental effect arose from the Fe^{2+} ions adsorbed directly on the semiconductor surface. In the study of photodegradation of organic dyes by TiO_2 nanoparticles under visible irradiation, it was found that some transition metal ions such as Cu^{2+} and Fe^{3+} having suitable redox potentials altered the photoinduced electron-transfer pathway and markedly depressed the dye degradation efficiency.²³ The reduction potential of $\text{Fe}^{3+}/\text{Fe}^{2+}$, $\text{Ru}^{3+}/\text{Ru}^{2+}$, and $\text{Ru}^{3+}/\text{Ru}^{2+*}$ is 0.77, 1.62, and -0.38 V vs normal hydrogen electrode, respectively.²⁴ At pH 3, the reduction potential of the conduction band and valence band of SnO_2 nanoparticle is 0.3 and 3.9 V, respectively.²⁵ According to the illustration shown in Scheme 2, it can be speculated that in our work the Fe^{2+} ions adsorbed on the SnO_2 surface can scavenge the photoex-

SCHEME 2: Illustration of Possible Quenching Mechanism of $\text{Ru}(\text{bpy})_2(\text{dppz})^{2+}$ Photocurrent by Surface-Adsorbed Fe^{2+} (Potentials Are Not Drawn to Scale)

cited electrons of the ruthenium complex and reduce its photocurrent.

The observed pH dependence suggests there are some charged groups on the SnO_2 surface that are involved in the interaction with Fe^{2+} . To investigate the issue further, the ζ potential of the SnO_2 nanoparticle colloid used for the fabrication of the electrode was measured at various pH solutions. As shown in Figure 1b, the ζ potential displays a pH dependence very similar to the one observed in the photocurrent measurement. From the graph, the PZC (point of zero charge) of SnO_2 is estimated to be pH 3.2, which is slightly lower than the value of pH 4.0 reported in the literature.²⁶ The discrepancy may be caused by the presence of some dispersing agents in the colloid we purchased. At pH above 3.2, the ζ potential is negative. It is well known that there exist hydroxyl groups on the surface of many metal oxides that may interact with metal ions by either electrostatic attraction or coordination. The strong correlation of pH dependence between photocurrent, ζ potential, as well as the PZC value supports the notion that Fe^{2+} ions can adsorb on SnO_2 electrode through its surface hydroxyl groups.

Although the adsorption of 1 mM Fe^{2+} on the semiconductor surface was eliminated at pH below 2.0, its Fenton reaction with H_2O_2 was also prohibited at these low pH values, as evident from the loss of change in photocurrent after the $\text{SnO}_2/\text{PDDA}/\text{DNA}$ sensor was exposed to the Fenton reagents. As a compromise, pH 3.0 was selected. At this pH, the deleterious effect of Fe^{2+} on photocurrent was completely eliminated as long as its concentration was kept at 100 μM or less, and the Fenton reaction could still proceed well (Figure 2). In addition, reduction in photocurrent of the damaged DNA film in the sensor decreased as the concentration of the two Fenton reagents (Fe^{2+} and H_2O_2) was lowered. The lowest concentration the photoelectrochemical sensor detected was 10 μM $\text{Fe}^{2+}/40$ μM H_2O_2 . The metal ion and H_2O_2 did not show such effect individually, indicating that the observed drop in photocurrent was induced by the Fenton reaction between the two reagents. It has been reported that the concentration of Fe^{2+} inside living cells is in the low micromolar range.^{21,22} Therefore, the photoelectrochemical DNA sensor is now capable of detecting DNA damage at physiologically relevant concentrations.

As mentioned in the Introduction, association of iron with DNA and its participation in Fenton reaction and DNA damage has been implicated in a number of studies.^{3–8} However, there has been no direct experimental evidence to support such hypothesis. The surface reaction mode in our photoelectrochemical sensor provided a unique opportunity to investigate

Figure 2. Anodic photocurrent response of $\text{Ru}(\text{bpy})_2(\text{dppz})^{2+}$ bound to $\text{SnO}_2/\text{PDDA}/\text{DNA}$ electrodes after the DNA film was exposed to (a) water, (b) 400 μM H_2O_2 , (c) 100 μM FeSO_4 , pH 3.0, (d) 10 μM $\text{FeSO}_4/40$ μM H_2O_2 , pH 3.0, (e) 20 μM $\text{FeSO}_4/80$ μM H_2O_2 , pH 3.0, and (f) 100 μM $\text{FeSO}_4/400$ μM H_2O_2 , pH 3.0. Excitation light was switched on and off as indicated.

Figure 3. Anodic photocurrent response of $\text{Ru}(\text{bpy})_2(\text{dppz})^{2+}$ bound to $\text{SnO}_2/\text{PDDA}/\text{ds-DNA}$ electrode after the DNA film was exposed to (a) water for 2 h, (b) 400 μM H_2O_2 for 2 h, (c) 100 μM Fe^{2+} for 1 h, water rinse, then 400 μM H_2O_2 for 1 h, (d) 100 μM Fe^{2+} for 1 h, 5 mM EDTA rinse, then 400 μM H_2O_2 for 1 h. Excitation light was switched on and off as indicated.

the role of DNA-associated metals in the Fenton reaction. In the experiment, the $\text{SnO}_2/\text{PDDA}/\text{DNA}$ sensor was assembled as usual and then exposed to 100 μM Fe^{2+} . After rinse with water, the sensor was reacted with 400 μM H_2O_2 . Finally, the DNA film was allowed to bind to $\text{Ru}(\text{bpy})_2(\text{dppz})^{2+}$, and its photocurrent was measured. As can be seen in Figure 3, the measured signal was reduced by one-third as compared with the blank control (water only) and H_2O_2 control (H_2O_2 only). Since the sensor was rinsed after Fe^{2+} exposure, the result suggests the metal bound to the sensor film and participated in the Fenton reaction during the subsequent exposure to H_2O_2 . In another experiment, the sensor was incubated in an EDTA solution immediately after its reaction with Fe^{2+} , followed by the same treatment with H_2O_2 and $\text{Ru}(\text{bpy})_2(\text{dppz})^{2+}$. The photocurrent was essentially the same as the blank control. EDTA is a very strong chelator for Fe^{2+} . Apparently, the chelator had removed the metal ion from the sensor film and thus eliminated the subsequent Fenton reaction with H_2O_2 .

In a further experiment, the location of Fe^{2+} bound to the sensor film was investigated. As described in the Experimental section, the sensor film was fabricated by layer-by-layer assembly of PDDA and DNA on a tin oxide electrode. The sensor was exposed to 100 μM Fe^{2+} at different stages during the film assembly process, as illustrated below. The arrow sign indicates the stage where the sensor was exposed to the metal ion. After DNA was deposited on the electrode, the sensor was incubated in H_2O_2 and then $\text{Ru}(\text{bpy})_2(\text{dppz})^{2+}$. As shown in Figure 4, exposure of either SnO_2 or SnO_2/PDDA to a solution of 100 μM Fe^{2+} did not induce any change in photocurrent.

Figure 4. Photocurrent of $\text{Ru}(\text{bpy})_2(\text{dppz})^{2+}$ bound to the $\text{SnO}_2/\text{PDDA}/\text{DNA}$ electrode after the film was exposed to $100 \mu\text{M}$ Fe^{2+} , pH 3.0 (filled column), or exposed first to $100 \mu\text{M}$ FeSO_4 , pH 3.0, then to $400 \mu\text{M}$ H_2O_2 after the DNA layer was deposited (empty column). The electrode was exposed to Fe^{2+} at different stages during the film assembly process. (1) Bare SnO_2 exposed to Fe^{2+} , (2) SnO_2/PDDA exposed to Fe^{2+} , (3) $\text{SnO}_2/\text{PDDA}/\text{DNA}$ exposed to Fe^{2+} .

Figure 5. Anodic photocurrent response of $\text{Ru}(\text{bpy})_2(\text{dppz})^{2+}$ bound to $\text{SnO}_2/\text{PDDA}/\text{ds-DNA}$ electrode after the DNA film was exposed to $100 \mu\text{M}$ Fe^{2+} for 1 h, water rinse and then in (a) $0 \mu\text{M}$, (b) $40 \mu\text{M}$, (c) $400 \mu\text{M}$, and (d) 4 mM H_2O_2 for 1 h. Excitation light was switched on and off as indicated.

However, as already described above, when the DNA modified electrode ($\text{SnO}_2/\text{PDDA}/\text{DNA}$) was reacted with the metal ion first and then reacted with H_2O_2 , the signal dropped substantially. The combined data in Figures 3 and 4 revealed very clearly that Fe^{2+} ions were associated with the DNA molecules in the sensor film and engaged in the Fenton reaction and DNA damage.

A solution of $10 \mu\text{M}$ Fe^{2+} did not produce detectable signal change after its association with DNA film. Therefore, adsorption isotherm with DNA is not available.

The dependence of photocurrent on H_2O_2 concentration was examined and is shown in Figure 5. It seems there is an optimal H_2O_2 concentration for DNA-associated Fe^{2+} adsorbed from $100 \mu\text{M}$ solution.

Conclusions

In conclusion, the previously developed photoelectrochemical sensor for Fe^{2+} -mediated oxidative DNA damage was investi-

gated in details. It was found that Fe^{2+} alone suppressed the measured photocurrent signal in a pH-dependent manner. On the basis of the ζ potential measurement of the tin oxide nanoparticle colloid, the detrimental effect was attributed to the adsorption of the metal ion on the semiconductor surface and its quenching effect on the photoexcited signaling molecule. At pH 3, the deleterious effect was reduced substantially to permit the detection of DNA damage by as low as $10 \mu\text{M}$ Fe^{2+} and $40 \mu\text{M}$ H_2O_2 , a concentration that is within the physiologically relevant range. Taking the advantage of the surface reaction mode of the sensor, Fe^{2+} association with DNA in the sensor film and its participation in the subsequent Fenton reaction with H_2O_2 and DNA damage was demonstrated explicitly, a mechanism that has been implicated in previous studies on metal carcinogenesis.

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