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DNA-Based Biosensor for Comparative Study of Catalytic Effect of Transition Metals on Autoxidation of Sulfite

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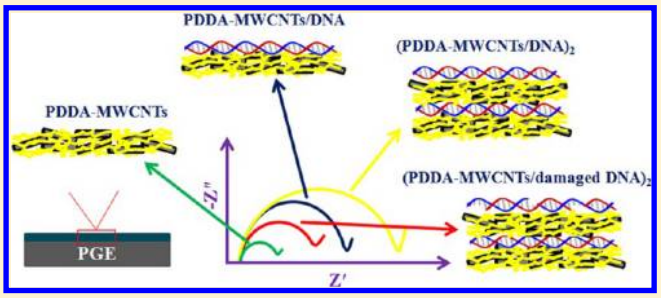
Supporting Information

ABSTRACT: The transition metal-catalyzed oxidation of sulfur(IV) oxides has been known for more than 100 years. However, to the best of the authors' knowledge, no electrochemical quantitative study has yet been carried out to determine its nature. In view of the transition metal catalyzed oxidation of sulfur(IV) oxides, a series of radicals are involved in the overall reaction process whereby the sulfite, in the presence of transition metals, may cause damages to DNA through the generation of these highly reactive species. In the present work, {MWCNTs−PDDA/DNA}$_2$ layer-by-layer (LBL) films were prepared to detect DNA damage induced by radicals generated from sulfite autoxidation using cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS). The change in the peak potential separation (ΔE_p) and charge transfer resistance (R_p) after incubation of the DNA biosensor in the damaging solution for a certain time was used as indicators of DNA damage. It was found that sulfite in the presence of Co(II), Cu(II), Cr(VI), Fe(III), and Mn(II) caused damage to DNA while neither sulfite alone nor metal ions alone did have the same effect. The results suggest that sulfite is rapidly autoxidized in the presence of Co(II), Cu(II), Cr(VI), Fe(III), and Mn(II), producing radicals that cause the DNA damage. These radicals can be ranked in a descending order of their ability to induce DNA damage with sulfite as follows: Fe(III) > Co(II) > Cu(II) > Cr(VI) > Mn(II). The DNA damage induced by sulfite plus Co(II), Cr(VI), and Fe(III) was inhibited by primary alcohols, but they were not when superoxide dismutase (SOD) and *tert*-butyl alcohol were used. Comparison of methods used to determine the minimum concentration of a transition metal for sulfite induced DNA damage revealed that electrochemical impedance spectroscopy and cyclic voltammetry outperformed the quantitative comparison of different reagents.



Sulfur dioxide (SO_2) is a common outdoor air pollutant that exists in aqueous solution at neutral pH as an equilibrium between bisulfite and sulfite ions.[1] Sulfite itself is used as a preservative in foods, beverages, and drugs and is formed in the lung by the hydration of sulfur dioxide.[2] S(IV) exposure can cause such toxic effects as the induction of an acute asthmatic state or adverse genetic effects, most likely by acting as a mutagen or comutagen[3] and a cocarcinogen.[4] At high concentration (1 mol L^{-1}) and at pH 5, sulfite is catalyzed and in isolated DNA, deamination of cytosine to uracil occurs.[5] At lower concentrations, sulfite causes DNA damage by sulfite-generated free radicals.[6] The mechanistic details of sulfite toxicity are not fully understood although several studies have implicated sulfoxy radicals ($SO_3^{\bullet-}$, $SO_4^{\bullet-}$, and $SO_5^{\bullet-}$) as potential oxidants of cell membranes, proteins, and DNA.[7] Generation of these radicals from oxidation of sulfite can be catalyzed by enzymes[8−12] or transition metal complexes[13,14] or even uncatalyzed.[14] Knowledge of the formation of reactive radicals from S(IV) autoxidation, which is enhanced in the presence of some transition metal ions such as cobalt, is of interest in understanding allergic and inflammatory responses to sulfur dioxide exposure.

Kawanishi et al.[15] was the first to report that Co(II) and Cu(II) ions could cause site-specific DNA damage in the presence of S(IV) and postulated the formation of sulfate radicals ($SO_4^{\bullet-}$) as the reactive intermediates responsible for the damaging effect. Shi and Mao[16] showed that $Cr_2O_7^{2-}$, in the presence of sulfite, could induce DNA damage. However, the quantitative nature of the reaction between sulfite and DNA, especially in the presence of transition metals, remains to be clarified.

To the best of our knowledge, there is no report on fabricating electrochemical biosensors in detecting sulfite autoxidation-induced DNA damage. Therefore, designing suitable electrode materials that can provide a foundation for fabricating electrochemical biosensors in detecting sulfite-induced DNA damage and offer an in vitro model to simulate the pathway of DNA damage and protection in living process is highly necessary.

Recent years have witnessed a fast growth in the design of electrochemical sensors based on nanomaterials due to their special physical and chemical properties such as increased surface area, mass transport, and catalysis.[17] One method for

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noncovalently immobilizing biomolecules on nanomaterials is to entrap them in a polymer such as polyethyleneimine,[18] Nafion,[19] chitosan,[20] or poly(diallydimethlammonium chloride).[21] The coating polymers not only prevent the aggregation of nanomaterials but also provide abundant positions for functionalization with second biomolecules. In this study, poly(diallydimethlammonium chloride), PDDA, was used as a dispersant of MWCNTs. PDDA is a water-soluble, quaternary ammonium, cationic polyelectrolyte that usually acts as a positively charged colloid when dissolved in aqueous solutions.[22] Positively charged PDDA molecules are easily coated on the negatively charged surface of the MWCNTs by electrostatic interaction. The PDDA molecules can combine considerably well with DNA to form DNA films because it is a strong linear cationic polyelectrolyte.[22] MWCNTs not only display unique electron transfer properties that induce the conductivity of PDDA and improve electron transfer characteristics, but also they increase the amount of PDDA deposited on the electrode as well.

$\{MWCNTs-PDDA/DNA\}_n$ layer by layer (LBL) films were assembled on a pencil graphite (PG) electrode, where DNA stood for natural double-stranded DNA, and the DNA damage induced by radicals generated from the SO_3^{2-} in the presence of some transition metals was detected by electrochemical impedance spectroscopy (EIS) and cyclic voltammetry (CV) using $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$. Compared to other electrodes, PGEs have such advantages as cost effectiveness, commercial availability, and disposability.[23,24] Furthermore, there are many functional groups on the PG surface which lead to better absorption of more biomolecules, nanoparticles, and polymers on the PG electrode. They can also catalyze the oxidation/reduction of the analyte.[25,26]

Damage to DNA is a critical event not only in the initiation[27] but also in the promotion phase of carcinogenesis.[28−30] In the promotion phase, the role of free radicals has been emphasized. However, the possibility of participation of sulfur oxyradicals produced by sulfite plus metal ions in the promotion phase may be considered. Further research is necessary to clarify whether sulfite plus metal-induced DNA damage can occur in the cells. In the present work, autoxidation of sulfite is used as a model reaction. There are many similar radical chain reactions whose mechanisms may be studied with the proposed biosensor using the EIS technique. This biosensor is capable of comparing effects of reagents that increase or decrease the rate of radical chain reactions. Also, by studying the effect of inhibitors on the potency of species in DNA damage, the type of radicals involved in the radical chain reactions can be achieved. Understanding the DNA damage caused by molecules or ions using the proposed voltammetric and impedimetric techniques for in situ generation of reactive intermediates is a complementary tool for the study of these biomolecular radical chain reaction mechanisms. Understanding how hazardous compounds interact with DNA may aid in explaining the differences in reactivity between similar moieties.

EXPERIMENTAL SECTION

Materials. Sodium sulfite, *tert*-buthyl alcohol, $CoCl_2$, $CuC1_2$, $MnC1_2$, $FeC1_3$, $NiC1_2$, $ZnC1_2$, $K_2Cr_2O_7$, and $CdC1_2$ were purchased from Merck (Darmstadt, Germany). A stock solution of 0.1 mol L^{-1} sodium sulfite was made up fresh when required. Superoxide dismutase (SOD), DNA sodium salt of salmon testes (dsDNA, catalog no. D1626), and poly(diallyldimethylammonium chloride) (PDDA, low molecular weight) were purchased from Sigma (St. Louis, MO) and used as received. Absolute ethanol was purchased from Bidestan Co. (Tehran, Iran). Single-stranded DNA (ssDNA) was produced by heating dsDNA solution to 100 °C for 10 min and then rapidly cooling it in an ice bath.[31] All other chemicals were of reagent grade. Air saturated solutions were employed in most of the experiments.

Apparatus. Electrochemical and impedimetric measurements were performed with an Autolab PGSTAT 12, potentiostat/galvanostat in a conventional three-electrode electrochemical cell using pencil graphite (PG, 0.7 mm diameter, Pentel Co., Ltd., Japan) as the working electrode, platinum wire as an auxiliary electrode, and an Ag/AgCl reference electrode in 3.0 mol L^{-1} KCl aqueous media. A standard one-compartment three-electrode cell of 10 mL capacity and a renewable pencil graphite electrode (PGE) was used in all experiments (as described in Erdem et al).[32] A Noki pencil was used as a holder for the pencil graphite leads. Electrical contact with the lead was obtained by soldering a metallic wire to the metallic part. The pencil was held vertically with 12 mm of the lead being extruded outside (9 mm of which was immersed in the solution). All electroanalytical measurements were performed at room temperature. A pH meter, Metrohm (model 827), with a glass electrode (filled with saturated KCl) was used for pH measurements.

Functionalization and Purification of MWCNTs. Multiwall carbon nanotubes (MWCNTs) were purified and functionalized as described elsewhere.[33] A mass of 120 mg of MWCNTs was stirred in 10 mL of a 3 mol L^{-1} nitric acid solution for 20 h. The solid product was collected on a filter paper and washed several times with pure water until the filtrate solution was neutral (pH 7.0). The functionalized MWCNTs obtained were then dried in the oven at 80 °C for 24 h. Nitric acid usually causes significant destruction to carbon nanotubes and introduces −COOH groups at the ends or at the sidewall defects of the nanotube structure.

Film Assembly. The surface of the PGE was pretreated by applying +1.40 V for 60 s in 0.5 mol L^{-1} acetate buffer (pH 4.8). An aqueous solution of 1.0 mg mL^{-1} PDDA was initially prepared with 0.5 mol L^{-1} NaCl. Then, 1.0 mg of MWCNTs were dispersed into 1.0 mL of PDDA solution. The mixture was sonicated for 3 h to obtain a homogeneous black suspension, which was sonicated for 15 min immediately before preparing the film. To assemble the $\{MWCNTs-PDDA/DNA\}_n$ layer by layer films, the PGE was alternately immersed into MWCNTs−PDDA solution for 30 min and into the DNA solution (1.0 mg mL^{-1}, in 0.02 mol L^{-1} tris buffers at pH 7.0 containing 0.5 mol L^{-1} NaCl and 1.0 mmol L^{-1} EDTA) for 10 min with intermediate water washing and nitrogen stream drying until the desired number of bilayers (n) was reached.

Procedure. Prior to measurements, the dry DNA modified electrode was pretreated as an original biosensor by immersing into the 0.1 mol L^{-1} phosphate buffer solution (PBS), pH 7.0, for 5 min under stirring. Depending on the type of electrolyte used for DNA damage detection, the CV signal of $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ and EIS of DNA modified electrode were obtained with optimum supporting electrolytes typically recommended in the literature for the individual detection procedures described below. To detect the effect of sulfite ions on DNA, the same sensor was incubated in the air saturated sulfite solution in the presence or absence of transition metals in 0.1 mol L^{-1} PBS for a given time (30 min, 2 h, or 5 h) under stirring and was rinsed with water. Then, the voltammetric and

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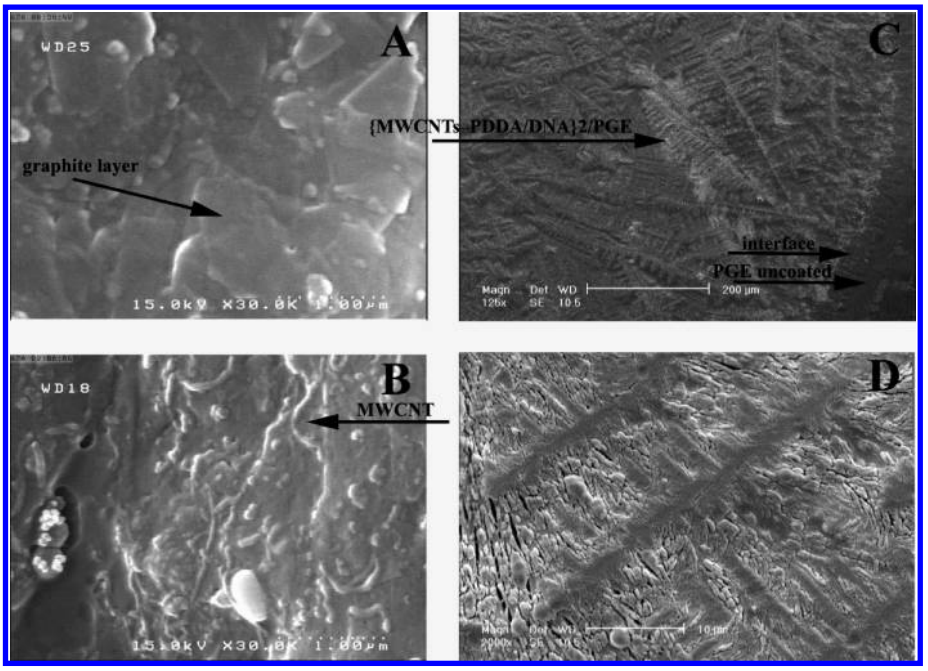

Figure 1. SEM image of (A) bare PGE, (B) MWCNTs−PDDA/PGE, and (C and D) {MWCNTs−PDDA/DNA}$_2$/PGE with different magnitudes.

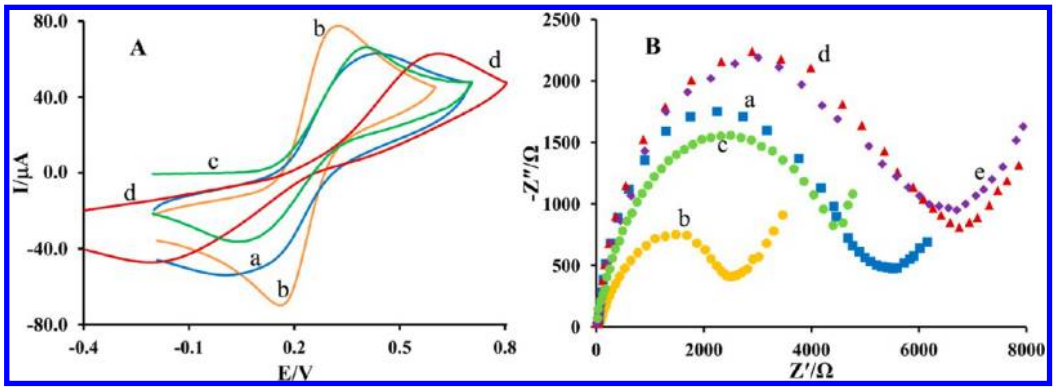

Figure 2. CVs (A) and impedance spectra (B) of (a) a bare PGE, (b) MWCNTs−PDDA/PGE, (c) {MWCNTs−PDDA/DNA}/PGE, and (d) {MWCNTs−PDDA/DNA}$_2$/PGE in 5.0 mmol L^{-1} $Fe(CN)_6^{3-/4-}$ containing 0.10 mol L^{-1} KCl. (e) Impedance spectra for {MWCNTs−PDDA/DNA}$_3$/PGE.

impedimetric response was obtained again in the appropriate supporting electrolyte.

Cyclic Voltammetry of $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$. The cyclic voltammogram of 5.0 mmol L^{-1} $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ in 0.1 mol L^{-1} KCl was recorded using a scan rate of 100 mV s^{-1}. The difference in the anodic to cathodic peak potential separation was evaluated.

Electrochemical Impedance Spectroscopy. Measurements were carried out in the presence of 5.0 mmol L^{-1} $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ as a redox probe in 0.1 mol L^{-1} KCl at a polarization potential of 0.20 V in the frequency range of 0.005−10^5 Hz and with an amplitude of 10 mV.

RESULTS AND DISCUSSION

Characteristics of {MWCNTs−PDDA/DNA}$_n$−PGE. A preliminary chemical modification of the PGE surface was used both to enhance electrode conductivity and to form an interface with sites for DNA immobilization. The films of the electrode modifier were formed via the adsorption method represented by an accumulation of species without any faradaic process. The surface of the PGE became functionalized upon electrochemical treament.[34] Such pretreatments are believed to result in an increase in surface quinone and other functional groups, which can then catalyze the oxidation/reduction of the analyte. Thus, MWCNTs−PDDA nanocomposite with positive charges could be adsorbed onto the PG surface by both electrostatic and hydrophobic interactions. Negatively charged DNA and positively charged MWCNTs−PDDA nanocomposite in solution could then be alternately adsorbed onto the PG surface by the electrostatic attraction between them, forming {MWCNTs−PDDA/DNA}$_n$ LBL films. The surface topographies of the stepwise immunosensor fabrication processes were investigated using SEM (Figure 1). Figure 1A displays the SEM image of the bare PGE. The graphite layers can be seen clearly in this figure. The MWCNTs were well dispersed in the PDDA solution (Figure 1B). When DNA was immobilized on the surface of MWCNTs−PDDA, the surface morphology was changed (Figure 1C) and the clear MWCNTs images became dim. Coated and uncoated surfaces of the PGE and the interface between the PGE and the solution can be clearly seen in Figure 1C. A higher magnification SEM image of {MWCNTs−PDDA/DNA}$_2$−PGE is shown in Figure 1D.

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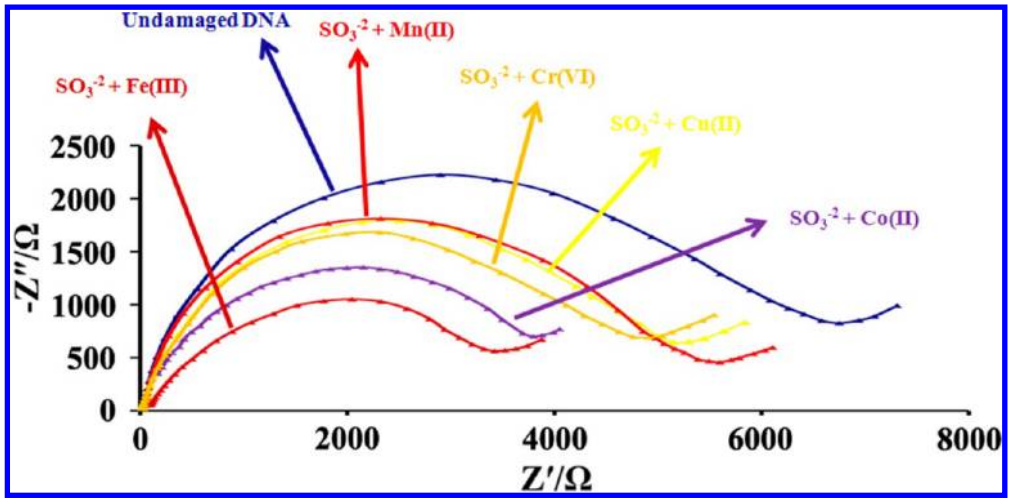

Figure 3. Impedance spectra of {MWCNTs−PDDA/DNA}$_2$/PGE in 5.0 mmol L^{-1} Fe(CN)$_6^{3-/4-}$ containing 0.1 mol L^{-1} KCl. {MWCNTs−PDDA/DNA}$_2$/PGE was incubated in a sulfite solution in the presence or absence of different transition metals in 0.1 mol L^{-1} PBS (pH 7.0) with a stirring time of 2 h.

This result demonstrates that PDDA can be used as a good matrix for MWCNTs and DNA, with PDDA acting as a polymer backbone to yield stable and homogeneous thin films.

Figure 2A shows the cyclic voltammograms of 5.0 mmol L^{-1} $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ containing 0.1 mol L^{-1} KCl on the surface of different electrodes. As can be seen, a bare PGE has a pair of well-defined voltammetric peaks with a cathodic peak potential (E_{pc}) of 0.40 V and an anodic peak potential (E_{pa}) of 0.058 V. The peak-to-peak separation (ΔE_p) is 342 mV. The peak current increased dramatically after coating with MWCNTs−PDDA nanocomposites. Therefore, the MWCNTs−PDDA nanocomposite modified electrode may be claimed to promote electron transfer reactions better than the bare PG electrode does. Furthermore, the MWCNTs surface had a great promotion upon the formation of the positively charged PDDA film. Because dsDNA is negatively charged, {MWCNTs−PDDA/DNA}$_n$ films block the electron transfer of $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$, and the redox peak currents might have gradually decreased with the growth of the bilayer number (n) when this was accompanied by the increase in the peak-to-peak separation. These trends could be seen in Figure 2A. Both the peak currents and peak-to-peak separation slightly changed when the bilayer number was larger than 2.

Figure 2B presents the Nyquist plots of different electrodes in 5.0 mmol L^{-1} $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ (1:1) containing 0.10 mol L^{-1} KCl. The diameter of the semicircle (R_p, the charge transfer resistance) decreased dramatically after modification of PGE by MWCNTs−PDDA nanocomposites. It was found that the impedance of the electrode drastically decreased in the presence of MWCNTs−PDDA nanocomposite. It may be the nanocomposite of MWCNTs and PDDA that promoted the electron exchange between $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ and the electrode. When the MWCNTs−PDDA/dsDNA bilayers were assembled, the semicircle portions became larger at higher frequencies, indicating that the formation of the negatively charged DNA layer on the electrode surface. The diameter of the semicircle part increased significantly for n = 2 and then increased only slightly as the bilayer number was larger than 2, indicating that the(MWCNTs−PDDA/dsDNA)$_n$ films hade been successfully assembled layer-by-layer on the surface of PGE when n = 2. Therefore, the n = 2 preparation was used for the preparation of the ds-DNA−modified PGE in all the subsequent experiments. The modified PGEs thus obtained are designated as {MWCNTs−PDDA/DNA}$_2$−PGE.

DNA Damage by Sulfite Plus Metal Ions. The voltammetric and impedimetric detection of DNA damage has been accomplished using an electroactive compound, i.e., $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ complex. It is suggested that the surface-attached dsDNA could slow down the rate of electron transfer due to the electrostatic repulsion between the negatively charged dsDNA and Fe(CN)$_6^{3-/4-}$. This phenomenon increases the charge transfer resistance (R_p) and the peak potential separation (ΔE_p) of $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ complex (Figure 2). The damage of dsDNA by damaging reagents decreases the charge transfer rate by reducing the negative charge on the electrode surface. The diminished negative charge facilitates the conversion of the ferri-/ferrocyanide redox couple. Therefore, the change of the surface-attached dsDNA after incubation in the damaging solution was indicated by a decrease in R_p and ΔE_p of $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ complex due to an increase in the electrochemical reversibility of the redox couple.[35,36]

Cyclic voltammetry and EIS were used to examine whether sulfite caused DNA damage in the presence of different metal ions. The effect of sulfite on the DNA layer at the electrode surface was evaluated after the incubation of the biosensor in an air-saturated sulfite solution both in the presence and absence of transition metals. To detect the influence of sulfite on DNA, the dry {MWCNTs−PDDA/DNA}$_2$−PGE as an original biosensor was immersed into 0.1 mol L^{-1} PBS (pH 7.0) for 5 min under stirring. Then, the CV and EIS signals of the {MWCNTs−PDDA/DNA}$_2$−PGE were obtained in 5.0 mmol L^{-1} $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ containing 0.10 mol L^{-1} KCl (pH, 7.0). The same DNA-biosensor was incubated in the sulfite solution both in the absence and presence of the transition metals (10.0 μmol L^{-1}) in 0.1 mol L^{-1} PBS (pH 7.0) for a given time (30 min, 2 h, and 5 h) under stirring. The DNA-biosensor was subsequently rinsed with water. Then, the voltammetric and impedimetric responses were obtained in 5.0 mmol L^{-1} $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ containing 0.10 mol L^{-1} KCl in 0.1 mol L^{-1} PBS (pH 7.0). The results showed no significant change in the peak potential separation (ΔE_p) of $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ complex or the charge transfer

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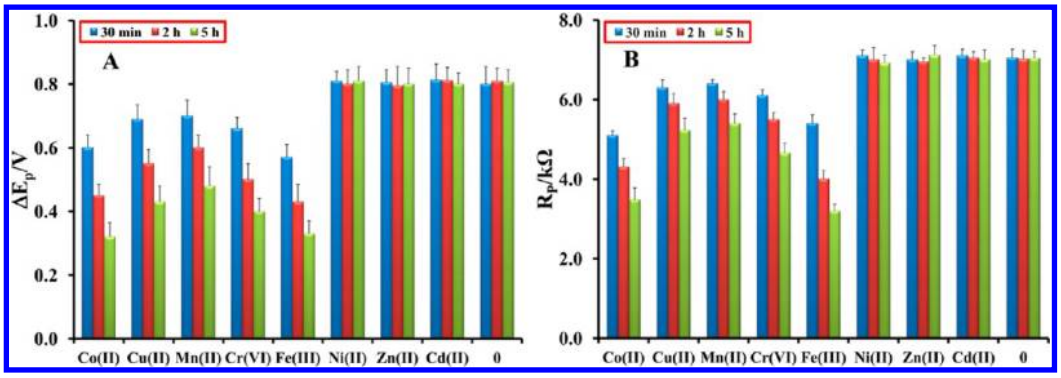

Figure 4. Dependence of the {MWCNTs–PDDA/DNA}$_2$/PGE response on different transition metals. {MWCNTs–PDDA/DNA}$_2$/PGE was incubated in the sulfite solution in 0.1 mol L^{-1} PBS (pH 7.0) for 30 min (left bars), 2 h (middle bars), and 5 h (right bars) under stirring. (A) Anodic to cathodic peak potential separation, ΔE_p, in CV for 5.0 mmol L^{-1} $Fe(CN)_6^{3-/4-}$ containing 0.1 mol L^{-1} PBS (pH 7.0) with scan rate of 50 mV s^{-1}. (B) Charge transfer resistance (R_p) of {MWCNTs–PDDA/DNA}$_2$/PGE in 5.0 mmol L^{-1} $Fe(CN)_6^{3-/4-}$ containing 0.1 mol L^{-1} PBS (pH 7.0) and 0.1 mol L^{-1} KCl.

resistance (R_p) with sulfite alone (Figure S-1 in the Supporting Information), indicating that sulfite itself is not a DNA damaging agent. Co(II), Cu(II), Cr(VI), Fe(III), and Mn(II) induced the decrease in R_p (Figures 3 and 4B) and the change in ΔE_p (Figure 4A), whereas Ni(II), Zn(II), and Cd(II) showed little or no effect under the same conditions (Figure 4). It may be claimed, therefore, that Co(II), Cu(II), Cr(VI), Fe(III), and Mn(II) induced damage to DNA in the presence of sulfite. Metal ions alone induced little or no significant change in the voltammetric and impedimetric parameters and did not induce any DNA damage (figure not shown). When ranked in terms of their ability to induce DNA damage with sulfite, the following order was obtained: Fe(III) > Co(II) > Cu(II) > Cr(VI) > Mn(II).

The dependence of sulfite concentration (in the range of 0.0–2000.0 μmol L^{-1}) on the voltammetric and impedimetric signals after incubation (2 h) of the DNA-biosensor in the sulfite solution plus the metal ions was studied (Figure 5). The results showed that the value of R_p decreased with increasing sulfite concentration. As shown in Figure 5, the rate of these changes is much more for Fe(III) and Co(II). The results also suggest that sulfite is rapidly autoxidized in the presence of Fe(III) and Co(II), causing DNA damage.

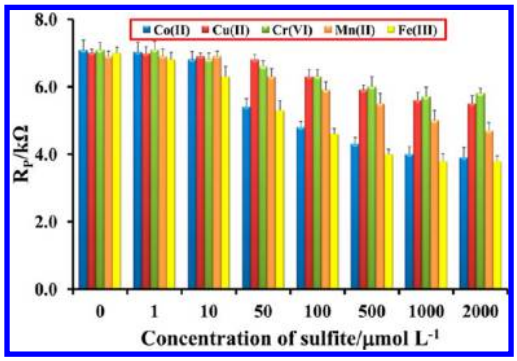

Figure 5. The dependence of the R_p of {MWCNTs–PDDA/DNA}$_2$/PGE on the sulfite concentration in 5.0 mmol L^{-1} $Fe(CN)_6^{3-/4-}$, 0.1 mol L^{-1} PBS (pH 7.0), and 0.1 mol L^{-1} KCl. {MWCNTs–PDDA/DNA}$_2$/PGE was incubated in the sulfite solution in the presence of different transition metals (Co(II), first bars; Cu(II), 2nd bars; Cr(VI), 3rd bars; Mn(II), 4th bars; and Fe(III), 5th bars) in 0.1 mol L^{-1} PBS (pH 7.0) with a stirring time of 2 h.

Figure 6 shows the effect of the transition metal concentrations on R_p and the oxidation of the DNA

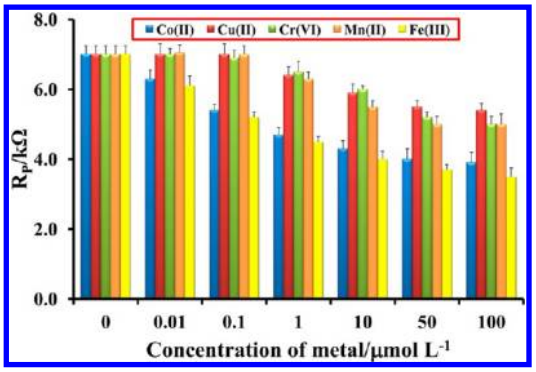

Figure 6. Dependence of the R_p of {MWCNTs–PDDA/DNA}$_2$/PGE on the transition metal concentration in 5.0 mmol L^{-1} $Fe(CN)_6^{3-/4-}$, 0.1 mol L^{-1} PBS (pH 7.0), and 0.1 mol L^{-1} KCl. {MWCNTs–PDDA/DNA}$_2$/PGE was incubated in the sulfite solution in the presence of different transition metals (Co(II), first bars; Cu(II), 2nd bars; Cr(VI), 3rd bars; Mn(II), 4th bars; and Fe(III), 5th bars) in 0.1 mol L^{-1} PBS, pH 7.0, with a stirring time of 2 h.

(damaging). For this purpose, the DNA-biosensor was incubated in 5.0 mmol L^{-1} sulfite solution in the presence of the transition metals with different concentrations in 0.1 mol L^{-1} PBS (pH 7.0) for 2 h under stirring. After rinsing the biosensor with water, the impedimetric response was obtained in 5.0 mmol L^{-1} $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ containing 0.1 mol L^{-1} KCl. A change in R_p was observed when the concentrations of Fe(III) and/or Co(II) were 0.01 μmol L^{-1}. No significant change was observed in R_p with sulfite plus 0.01 μmol L^{-1} Cu(II), Mn(II), and Cr(VI), showing that sulfite, in the presence of 0.01 μmol L^{-1} of these transition metals, is not a DNA damaging agent. A noteworthy change in R_p was observed when the concentrations of Cu(II), Mn(II), and Cr(VI) were 1.0 μmol L^{-1}. This shows that the minimum concentrations of Cu(II), Mn(II), and Cr(VI) for sulfite induced DNA damage is approximately 100 times higher than those of Fe(III) and Co(II).

Table 1 compares the results of the analysis of sulfite induced DNA damage in different water matrixes containing 0.50 mmol L^{-1} SO_3^{2-} after 2 h incubation of the {MWCNTs–PDDA/DNA}$_2$–PGE. The results show that although sulfite itself did

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Table 1. Effect of Different Water Matrixes on the Charge Transfer Resistance of {MWCNTs−PDDA/DNA}$_2$ Film after 2 h Incubation in Samples Containing 0.5 mmol L^{-1} SO_3^{2-}

	drinking water	distilled water	double distilled water	deionized water
R_p	3.21 ± 0.20	6.43 ± 0.12	6.94 ± 0.15	7.03 ± 0.12

not cause DNA damage in deionized and doubly distilled water, it could induce DNA damage in drinking and distilled water. Ordering the inducing effects on sulfite-dependent DNA damage yields: drinking water ≫ distilled water. The order was consistent with the presence of the transition metals cations in these matrixes. We masked the contribution to the reaction from trace metal impurities by complexing them with an effective chelating agent. EDTA and 1,10-phenanthroline were chosen in studying the sulfite oxidation since they are most effective at a high pH range, where they act as a sexadentate ligand occupying all coordination sites of the complexed metal ions. It is also important to note that the two chelating agents used are not capable of acting as an oxidation inhibitor in the classic sense of terminating free-radical chains. Addition of complexing agents such as EDTA or 1,10-phenanthroline, in order to mask possibly present metal ions, reduced the DNA damage rate (Table S-1 in the Supporting Information).

To investigate whether superoxide or hydrogen peroxide are involved in the reaction of DNA with sulfite plus transition metals, the effect of superoxide dismutase (SOD) was examined. The results showed that R_p would not change after the addition of SOD to sulfite solution containing Co(II), Cu(II), Fe(III), and Cr(VI), whereas it changed for a solution containing Mn(II) (Table 2). As a result, DNA damage induced by sulfite plus Co(II), Cu(II), Fe(III), and Cr(VI) was not affected by SOD, whereas DNA damage induced by sulfite plus Mn(II) was inhibited by SOD (Table 2). SOD failed to decompose reactive species generated by sulfite plus Co(II), Cu(II), Fe(III), and Cr(VI), indicating that the reactive species were neither hydrogen peroxide nor superoxide. The mechanism for Mn(II) catalyzed autoxidation of sulfite seems to be different from that for the Co(II), Fe(III), Cu(II), or Cr(VI) catalyzed oxidation.

As Yang pointed out,[37] superoxide is involved in the chain reaction of Mn(II) catalyzed autoxidation of sulfite. However, superoxide does not play an important role in Co(II), Fe(III), Cu(II), or Cr(VI) catalyzed autoxidation of sulfite. In addition, our study showed that SOD did not inhibit DNA cleavage induced by sulfite plus Co(II), Fe(III), Cu(II), or Cr(VI), whereas alcohols were able to inhibit it. Table 2 shows the effects of alcohols on R_p and DNA damage induced by sulfite plus transition metals. Ethanol showed the inhibitory effect on DNA damage induced by sulfite plus Co(II), Fe(III), and Cr(VI). *tert*-Butyl alcohol did not change R_p, neither did it inhibit DNA damage (Table 2). The effects of alcohols on DNA damage induced by sulfite plus Cu(II) were completely different from those induced by sulfite plus Co(II), Fe(III), and Cr(VI). Ethanol accelerated DNA damage induced by sulfite plus Cu(II) by the decrease in R_p, whereas *tert*-butyl alcohol did not accelerate it but increased the R_p value (Table 2). As shown in Table 2, both alcohols did not cause any change in R_p or in the subsequent DNA damage induced by sulfite plus Mn(II). The experiments with alcohols show that intermediates in these reactions (except Mn(II) catalyzed oxidation of sulfite) can be scavenged by the alcohols. It has been previously shown that alcohols are good scavengers of the sulfite radical.[38] These results suggest that, rather than the oxygen free radicals, the SO_3^- radical or the active species derived from the SO_3^- radical are the ones that contribute to the DNA damage induced by sulfite plus Co(II), Fe(III), Cr(VI), and Cu(II). These differences in the effects of alcohols on DNA damage led us to the idea that active sulfur species (the sulfite radical, SO_3^-, the sulfate radical, SO_4^-, the peroxomonosulfate radical, SO_5^-, and the hydrogen peroxomonosulfate anion, HSO_5^-) cause the DNA damage in the case of sulfite plus Co(II), Fe(III), and Cr(VI) and that they are different from those involved in the case of sulfite plus Cu(II). On the other hand, SOD inhibited DNA cleavage induced by sulfite plus Mn(II), suggesting oxygen radical participation in Mn(II) dependent DNA damage.

In another experiment, after 2 h reaction with sulfite solution plus Co(II), the {MWCNTs−PDDA/DNA}$_2$−PGE was immersed into a 5.0 mmol L^{-1} ethanol solution for 2 h, followed by EIS scanning. The values for R_p before and after immersion of the electrode into the alcohol solution were found to be identical. This indicated that although ethanol inhibited DNA cleavage induced by sulfite plus metal ions, it did not have any effects on the oxidative DNA. In order to test whether ethanol itself affects and/or dissolves the unoxidized DNA at the surface, the {MWCNTs−PDDA/DNA}$_2$−PGE was immersed into a 5.0 mmol L^{-1} ethanol solution for 2 h and EIS scanning was performed before and after immersion. The values for R_p before and after exposure of the {MWCNTs−PDDA/DNA}$_2$−PGE to ethanol were 7.01 ± 0.21 and 6.95 ± 0.14, respectively. This result indicated that ethanol did not have any effect on the unoxidized DNA at the surface.

The results show that electrochemical impedance spectroscopy and cyclic voltammetry are the best tools for quantitative comparison of different reagents for DNA damage. The present results are consistent with those reported in the literature.[15,16]

Table 2. Inhibitory Effect of Ethanol, *t*-BuOH, and SOD on the Charge Transfer Resistance of {MWCNTs−PDDA/DNA}$_2$ Film and DNA Damage after 2 h Incubation in Different Reagents

	ΔR_p[a,b] (kΩ)		
reagent	25 mmol L^{-1} EtOH	25 mmol L^{-1} t-BuOH	0.5 mg mL^{-1} SOD
0.50 mmol L^{-1} SO_3^{2-} + 10 μmol L^{-1} Co^{2+}	1.42 ± 0.36	−0.08 ± 0.33	0.09 ± 0.29
0.50 mmol L^{-1} SO_3^{2-} + 10 μmol L^{-1} Cu^{2+}	−0.51 ± 0.29	0.93 ± 0.32	0.14 ± 0.33
0.50 mmol L^{-1} SO_3^{2-} + 10 μmol L^{-1} Mn^{2+}	0.03 ± 0.27	−0.04 ± 0.32	0.89 ± 0.27
0.50 mmol L^{-1} SO_3^{2-} + 10 μmol L^{-1} Cr^{6+}	1.24 ± 0.34	−0.15 ± 0.38	0.16 ± 0.35
0.50 mmol L^{-1} SO_3^{2-} + 10 μmol L^{-1} Fe^{3+}	2.33 ± 0.35	0.10 ± 0.26	0.24 ± 0.23

[a] $\Delta R_p = R_p$(with inhibitor) − R_p(without inhibitor). [b] Data expressed as the mean ± the standard deviation (n = 3)

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CONCLUSIONS

A DNA-based biosensor was fabricated for the first time for the electrochemical study of transition metal-catalyzed oxidation of sulfite. The experimental results obtained in this study suggest that DNA damage is highly affected by the concentration of the transition metals, the incubation time, and the sulfite concentration. It was also found that the intermediates responsible for the DNA damage are not the same for different transition metals. Finally, the lowest concentrations of transition metals for sulfite induced DNA damage were also compared. This work not only provides a foundation for fabricating electrochemical biosensors in detecting sulfite induced DNA damage but also offers an in vitro model to simulate the pathway of DNA damage and protection in living organisms. Another facet of the present study was the finding that the influence of different reagents on the reaction rate of DNA damage could be studied by electrochemical methods. Understanding of DNA damage by molecules or ions for in situ generation of reactive intermediates is a complementary tool for the study of the biomolecular interaction and radical chain reaction mechanisms.

ASSOCIATED CONTENT

Supporting Information

Additional information as noted in text. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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