Effect of Cr(VI) and bisphenol A on the formation of 8-hydroxy-2’deoxyguanosine DNA adduct from 2’-deoxyguanosine through a Fenton-like reaction

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Abstract. In this research, we study the effect of the addition of bisphenol A (BPA) and Cr(VI) on the formation of the DNA adduct, 8-hydroxy-2’-deoxyguanosine (8-OHdG). The formation of 8-OHdG, which is monitored using reversed phase high performance liquid chromatography (HPLC) with a Ultraviolet-Visible (UV-Vis) detector at 254 nm, can be achieved through the reaction of 2’-deoxyguanosine (dG) with BPA in the presence of Fenton-like reagents and vitamin C. The reaction is performed under different conditions, including several pH (7.4 and 8.4), temperatures (37 °C and 60 °C), and incubation times (7 and 12 h). To detect 8-OHdG, the minimum concentration was set as the limit of detection (LOD) value of 5.19 ppb, and the minimum concentration for its quantification was set as the limit of quantification (LOQ) value of 17.29 ppb. The results of this study indicate that the concentration of 8-OHdG increases upon addition of BPA and Cr(VI), and most of the concentration values obtained from the analysis are greater than the LOD value. The addition of Fenton-like reagent and vitamin C causes an increase in the concentration of 8-OHdG. For most samples, the conditions that cause the highest 8-OHdG concentration are a pH of 7.4, a temperature of 60 °C, and an incubation time of 12 h.

Keywords: BPA, Cr(VI), DNA adduct, 8-OHdG, Fenton-like reaction

1. Introduction
Bisphenol A (BPA: 4,4’-dihydroxy-2,2-diphenylpropane) is an extensively produced chemical that is normally used in goods for packaging foodstuffs, such as baby bottles, reusable plastic bottles, plates, cups, heat-resistant plates, and storage containers, and in food and beverage cans [1]. BPA is renowned to negatively impact the biological environment because it can trigger cellular oxidative stress, which, in turn, activates an inflammatory pathway that eventually leads to the formation of tumor cells [2].

However, Cr(VI) compounds, which is carcinogen to humans in accordance to classification by International Agency for Research on Cancer (IARC), are extensively observed in the environment, particularly in leather textiles, exhaust from cars or drainage, and cigarette smoke [3].

This research was conducted to analyze the influence of BPA during the formation of the deoxyribonucleic acid (DNA) adduct, 8-hydroxy-2’-deoxyguanosine (8-OHdG), which is a biomarker of the oxidative damage to DNA due to the exposure to chemical carcinogen. The formation of 8-OHdG was performed in vitro conducting a Fenton-like reaction of 2’-deoxyguanosine-monohydrate (dG) with BPA, Cr(VI), H₂O, and vitamin C, and the effect of the temperature, pH, and incubation time was evaluated.
2. Materials and methods

2.1. Materials
To perform the high performance liquid chromatography (HPLC) analysis, a Hitachi Primaide HPLC instrument equipped with a YMC-TriartC18/S-5um/12 nm, a 250 × 4.6 mm reversed phase column, and a 254-nm UV detector was used. For the sample preparation, vials, a centrifugation instrument, centrifugation tubes, a pH meter, micropipettes, a spray bottle, a weighing bottle, and a scale were used. Bisphenol A (BPA), 2′-deoxyguanosine monohydrate (dG), and 8-hydroxy-2′-deoxyguanosine standard (8-OHdG) were purchased from Sigma Aldrich. Vitamin C, Cr(VI), hydrogen peroxide, KH₂PO₄ buffer (with pH of 7.4 and 8.4), Na-acetate buffers, Na-phosphate buffers, and LC-gradient grade methanol were purchased from Merck.

2.2. Methods
Upon incubation of 2′-deoxyguanosine (6 ppm) with BPA (60 ppm) under different pH (7.4 and 8.4), temperatures (37 °C and 60 °C), and incubation times (7 and 12 h), the formation of 8-OHdG was detected by HPLC analysis. To perform the analysis, the samples were first centrifuged for 15 min and then decanted. 20 μL of the decanted samples were injected into a reversed-phase HPLC column with a UV detector at a 254-nm wavelength. A solution of methanol and a 10 mmol/L sodium phosphate buffer (pH 6.7) in a ratio of 85:15 was used as the mobile phase (eluent) at a flow rate of 1 mL/min. The results of the measurements were compared with the 8-OHdG standard calibration curve. The quantification of 8-OHdG in the samples was calculated using the regression equation of y=81.18x−326.33 was obtained from the 8-OHdG standard calibration curve at various concentrations (10, 30, 50, 80, and 100 ppb with coefficient of correlation 0.998 [4]. Furthermore, similar tests with addition of Cr(VI) (120 ppm), vitamin C (60 ppm), H₂O₂ (120 ppm), and other combinations of compounds were performed.

3. Results and discussion

3.1. Optimization of the HPLC conditions
In the optimum HPLC conditions, the peaks corresponding to standard dG at a pH of 7.4 and standard dG at a pH of 8.4 were observed at a retention time of 6,007 min, whereas that corresponding to 8-OHdG was detected at 7,473 min. The chromatogram profiles for standard dG at a pH of 7.4, dG at a pH of 8.4, and 8-OHdG can be observed in figure 1.

Figure 1. Standard chromatograms of (a) dG at a pH of 7.4 (6 ppm), (b) dG at a pH of 8.4 (6 ppm), and (c) 8-OHdG (500 ppb).
3.2. The effect of BPA addition

The metabolic conversion of BPA into a metabolite that can be bound to DNA is expected to be catalyzed by the microsomal cytochrome P450 [5]. Figure 2 depicts the effect of BPA incubation with 2’-dG on 8-OHdG concentration. For most of the samples, an increase in the concentration of 8-OHdG was observed upon the addition of BPA, which indicates the role of BPA as a prooxidant.

3.3. The effect of Cr(VI) and H₂O₂

Cr(VI) compounds can induce DNA damage because they produce several kinds of reactive intermediates and reactive oxygen species (ROS) in cells [6]. For most of the samples that were analyzed in this study, the addition of Cr(VI) was observed to cause an increase in the concentration of 8-OHdG (figure 3), and the highest 8-OHdG concentration of 30.32 ppb was obtained at a temperature of 60 °C, an incubation time of 12 h, and a pH of 7.4 in the presence of BPA and Cr(VI).

Hydrogen peroxide (H₂O₂) is also classified as ROS [7]. Although H₂O₂ is a stable compound, it can produce highly reactive hydroxyl radicals when it reacts with transition metals [7]. Figure 4 depicts the effect of adding H₂O₂ and Cr(VI) as Fenton reagents to the reaction. For most of the samples, a significant increase was observed in the 8-OHdG concentrations with the addition of Fenton-like reagents.

The interaction of vitamin C with metals is known to contribute to the oxidative damage through the production of hydroxyl radicals [8]. Thus, vitamin C can reduce Cr(VI) and interfere with the Fenton-like reaction, leading to the formation of hydroxyl radicals [9]. The effect of vitamin C on the
Figure 4. The effect of the addition of Fenton-like reagents on 8-OHdG concentration.

Figure 5. The effect of Fenton-like reagents and vitamin C on the 8-OHdG concentration.

Figure 6. Effect of pH on the 8-OHdG concentration at 60 °C with a 12 h incubation time.

The final concentration of 8-OHdG was also investigated in this study, and the results are depicted in figure 5. As can be observed, all the samples contained 8-OHdG in concentrations that were greater than the LOQ value in the presence of Fenton-like reagents and vitamin C. In some cases, the samples exhibited higher 8-OHdG concentration after the addition of these compounds.
3.4. The effect of pH and temperature

Figure 6 depicts the effect of pH on the 8-OHdG concentration at 60 °C with an incubation time of 12 h. Most of the samples exhibited higher 8-OHdG concentration at a pH of 7.4 than that observed at a pH of 8.4. This is in accordance with a previous study conducted by Shi et al. [9] in which the production of free radicals due to the reduction of Cr(VI) by vitamin C, which can damage the double-stranded DNA, was demonstrated to occur at a physiological pH.

The temperature of the reaction also had an effect on the final concentration of 8-OHdG. Figure 7 displays the results that were obtained at a pH of 7.4 with an incubation time of 12 h at 60 °C and 37 °C. In most of the cases, higher 8-OHdG concentrations were obtained at 60 °C.

In this study, the effect of the incubation time was also evaluated. For most of the samples, higher 8-OHdG concentrations were obtained with 12 h of incubation time as compared with that observed at an incubation time of 7 h (Figure 8).

4. Conclusions

The formation of the biomarker 8-OHdG from 2'-deoxyguanosine was evaluated in the presence of BPA, Cr(VI), H₂O₂, vitamin C, and a combination of these compounds under different reaction conditions. Most of the samples exhibited 8-OHdG concentrations that were greater than that observed in the LOD and LOQ values. It was observed that higher 8-OHdG concentrations were obtained upon
the addition of BPA, Cr(VI), Fenton-like reagent, and vitamin C at a pH of 7.4, a temperature of 60 °C, and an incubation time of 12 h.

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