

In vitro study of DNA Adduct 8-OHdG Formation by using Bisphenol A in Calf Thymus DNA and 2'-Deoxyguanosine

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Abstract. The in vitro study of DNA Adduct 8-OHdG Formation due to BisphenolA (BPA) as xenobiotics has been conducted by using calf thymus DNA and 2'deoxyguanosine. The method of study was conducted by incubating calf thymus DNA and 2'dG with compounds trigger to radicals in the variation of pH (7.4 and 8.4), temperature (37°C and 60°C), and BPA concentrations (2 ppm and 10 ppm). To represent the work of CYP 450 enzyme in metabolic process of xenobiotics in the body and the effect of metal presence to the formation of radicals that can lead to 8-OHdG formation, we used iron(II) solution and also fenton reagent (Fe(II) and H₂O₂). The DNA used has 1.8 purity ratio (checked at $\lambda_{260}/\lambda_{280}$ by using Spectrophotometry UV-Vis). The results by using HPLC method showed that BPA could interact with DNA and DNA base (represent as calf thymus and 2'dG) and potentially induced 8-OHdG formation. The presence of iron(II) metal and Fenton reagent also induced the higher 8-OHdG formation. The higher of pH, temperature and concentrations also lead to 8-OHdG formation (ranger between 4 – 70 ppb).

1. Introduction

Plastic is a material that is easy to find in the market, because it is not easily broken and relatively cheaper, making it a popular choice. The most common ingredient for plastic is polycarbonate (PC). Polycarbonate plastic is used for various food and beverage packagings, baby bottles, food packages, medical equipments, dental sealants (thin layer of plastic used to cover the surface of teeth), CD, DVD, spectacle lenses and sports equipments as well as several paper coating (ATM receipts and cash counter receipts) [1]. The main ingredient for making polycarbonate plastic is 2,2-bis (4-hydroxyphenyl) propane, also known as Bisphenol A (BPA). Aside from being the main material for polycarbonate (PC), BPA is also used as an epoxy resin material, mainly as inner coating on metal packaging products that serves to prevent corrosion, such as in food and beverage cans and water supply pipes [2].

BPA is carcinogenic - it induces cancer - because it interacts with DNA and causes what is known as DNA adducts due to mutations in DNA that can trigger continuous, out-of-control cell formation or division [3]. Carcinogenic compounds can trigger oxidative stress and contribute to the formation of reactive oxygen species (ROS). Upon entering the body, carcinogenic compounds will undergo a process called detoxification. It repairs damaged DNA through the mechanism of Excision Base Repair (BER) which can excrete cut-off damaged DNA. The DNA, which is damaged and truncated by BER, is found in the form of 8-hydroxy-2'-deoxyguanosine (8-OHdG) [4].



2. Experimental Method

2.1. Chemicals

Calf thymus DNA, 2'-deoxyguanosine monohydrate, 8-OHdG, aquabidest, Bisphenol A, FeSO₄, H₂O₂, phosphate buffer, acetate buffer, hydrochloric acid, sodium hydroxide, micrococcus nuclease enzyme (MN) and spleen phosphodiesterase (SPDE) enzyme, methanol, DMSO.

2.2 In vitro study of 2'-deoxyguanosine, BPA (2 ppm and 10 ppm), Fe(II), and H₂O₂

100 µL of 2'-deoxyguanosine (600 ppb and 3 ppm) in 0.1 M phosphate buffer solution with pH of 7.4 and 8.4 is added with 100 µL of BPA solution (6 ppm and 30 ppm) and 50 µL of H₂O₂ (12 ppm dan 60 ppm). The sample is then incubated over a varying period of 3 and 9 hours at a varying temperature of 37°C and 60°C.

2.2. In Vitro Studies with Calf Thymus DNA, BPA, Fe(II) and H₂O₂

A total of ± 10 µg (in 100 µL) of calf thymus DNA (100 µg / mL) in 0.1 M of phosphate buffer (pH 7.4 and 8.4) is incubated with 10 µg (in 100 µL) of BPA solution, 249 µg of Fe (II) (in 50 µl) at 37 ° C over a period of 6 hours.

2.3. Hydrolysis of Calf Thymus DNA Enzymatically

Mixture of Calf Thymus DNA and Bisphenol A, the mixture of Calf Thymus DNA, Bisphenol A, FeCl₂ and H₂O₂ and the mixture of Calf Thymus DNA , FeCl₂ and H₂O₂ were centrifuged and the filtrate hydrolyzed using micrococcus nuclease enzyme (MN) and spleenphosphodiesterase (SPDE) enzyme (enzyme ratio 0.02 units: 0.002 unit) with a total enzyme mix of 100 µL. Added with 133 µL sodium succinate 10 mM pH 6 and calcium chloride 5 mM, then incubated for 3 and 9 hours at 37 ° C and 60 ° C, then analyzed using HPLC.

3. Results and Discussion

3.1. Determination of DNA Adduct 8-OHdG with 2'Deoxiguanosine Chemicals

Carcinogenic compounds, can cause DNA damage due to its contribution to the formation of reactive oxygen species (ROS). If the mechanism of DNA repair in the body is slower than the rate of DNA damage, mutations will occur, eventually leading to the onset of cancer [5]. The hydroxyl radical (OH) formed by the ROS mechanism can attack the guanine base in DNA to form an 8-OHdG DNA adduct. 8-OHdG is a DNA damage produced by the addition of hydroxyl radical at C-8 guanine position to the DNA [6].

Table 1 8-OHdG Concentration (ppb) in the variation of reaction

Variation of reaction, Incubation time 3 hours, BPA	Concentration 8-OHdG (ppb)			
	Temperature 37° C		Temperature 60° C	
	pH 7,4	pH 8,4	pH 7,4	pH 8,4
2 ppm dG + BPA + Fe(II) + H2O2	10.849	12.950	11.219	12.953

In the reaction of 2'-deoxyguanosine with BPA 2 ppm for 3 hours, the variation of pH and temperature was conducted. The results showed the increasion of 8-OHdG concentration as the increase of pH and temperature. The highest amount of 8-OHdG was obtained at temperature 60°C and pH 8.4 as much as 12.953 ppb.

3.2. Result of 8-OHdG DNA Adduct Formation with Calf thymus DNA

3.2.1. Incubation of Calf thymus DNA with BPA

The formation of DNA adducts from incubation with this HPLC-analyzed BPA compound detects the formation of 8 OHdG at retention time of 9.386 (Fig. 3.1) with a concentration of 8 OHdG of 2.580 ppb, whereas at retention time 6,817 it's the peak of deoxyguanosine (dG). This result is based on dG standard and 8 OHdG which was previously analyzed under similar equipment condition.

The formation of 8 OHdG DNA Adduct in the sample indicates that BPA may contribute to the reactive oxygen species (ROS) that are hydroxyl radicals which bind with deoxyguanosine to form 8 OHdG [7].

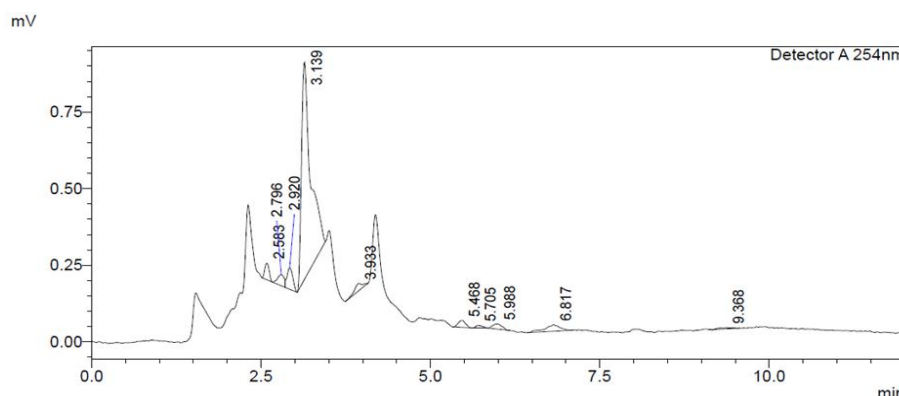


Figure 1 HPLC chromatogram on the result of hydrolysis between the calf thymus DNA and BPA

3.2.2. Incubation of Calf thymus DNA with BPA, Fe(II) and H2O2

Test result is the formation of 8 OHdG at retention time of 10.132. HPLC chromatogram of incubated calf thymus DNA at pH 7.4 and 37°C temperature shows 8-2 OHdG yield of 7.272 ppb, whereas peak deoxyguanosine appears at retention time of 7.030 (Fig. 3.2)

Fenton reagents (Fe (II) metal and H₂O₂) in this variation are used to determine the effect of fenton reagents in the formation of 8 OHdG. Based on the results, the amount of 8 OHdG is high. The reactive oxygen species produced from this fenton reagent bound to dG forms 8-OHdG through the following mechanism:



In the human body, the fenton reaction takes place through the mechanism of P450 cytochrome [8]. The mechanism of the fenton reaction itself is the reduction of hydrogen peroxide by transitional metal ions, producing reactive hydroxyl radicals and oxidized metal ions [9].

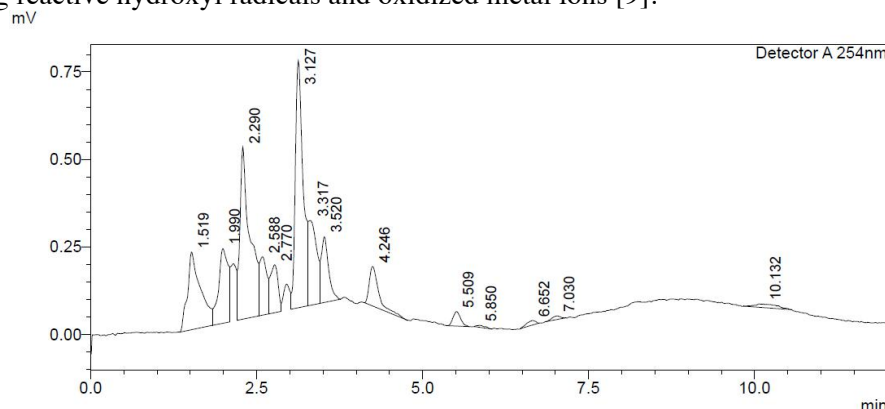


Figure 2 HPLC chromatogram on the result of hydrolysis between the calf thymus DNA and BPA, Fe(II) and H₂O₂

4. Conclusion

Most of the samples have an 8-OHdG yield trend that will increase as the temperature, pH, concentration increase and incubation time become longer. The reaction between dG and BPA may increase the concentration of 8-OHdG when in the presence of fenton reagents (Fe (II) and H₂O₂ in the reaction. Incubation of calf thymus DNA with free radical contributor compound that is BPA with addition of fenton reagent can produce 8 OHdG equal to 7.272 ppb.

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