# Depurination of dA and dG Induced by 2-bromopropane at the Physiological Condition

**Pritam Thapa, Jyoti Sherchan, Radha Karki, Tae Cheon Jeong, and Eung-Seok Lee\***

*College of Pharmacy, Yeungnam University, Kyongsan 712-749, Korea*

**Abstract** – Depurination, the release of purine bases from nucleosides by hydrolysis of the *N*-glycosidic bond, gives rise to alterations of the cell genome. Although, cells have evolved mechanisms to repair these lesions, unrepaired apurinic sites have been shown to have two biological consequences: lethality and base substitution errors. 2-Bromopropane (2-BP) is used as an intermediate in the synthesis of pharmaceuticals, dyes, and other organics. In addition, 2-BP has been used as a cleaning solvent in electronics industry. But, 2-BP was found to cause reproductive and hematopoietic disorders in local workers exposed to it. We observed massive depurination after incubation of 2'-deoxyadenosine (dA) and 2'-deoxyguanosine (dG) with the excess amount 2-BP at the physiological condition (pH 7.4, 37°C), which were analyzed by HPLC and LC-MS/MS. In addition, time and dose response relationship of depurination in dA and dG induced by 2-BP at the physiological condition were investigated.

**Keywords** □ depurination, 1-bromopropane, 2-bromopropane, time and dose response relationship

## INTRODUCTION

Depurination of nucleic acids, the release of purine bases from nucleosides by hydrolysis of the *N*-glycosidic bond (Fig. 1) gives rise to alterations of the cell genome (Kunkel, 1984; Vousden *et al.*, 1986). The apurinic sites resulting from depurination are quite stable (Lindahl and Andersson, 1972), and cells have evolved mechanisms to repair these lesions (Lindahl, 1982). However, unrepaired apurinic sites have been shown to have two biological consequences: lethality (Drake and Baltz, 1976; Schaaper and Leob, 1981) and base substitution errors (Schaaper and Leob, 1981). Depurination leaves the DNA phosphodiester backbone intact and apurinic sites, which are the excellent candidates for being the causative lesions. They characteristically induce G:C → T:A and A:T → T:A transversions as a result of preferential insertion of adenine residues opposite apuric sites during DNA replication (Loeb, 1985; Schaaper *et al.*, 1983) resulting in base substitution errors. More recently, it has been reported that in early preneoplastic mouse skin, apurinic sites formed by the PAH carcinogen dibenzo[a,l] pyrene (DB[a,l]) undergo error-prone repair to form tumor-intitiating H-*ras* mutations (Chakravarti *et al.*, 2001) by inducing pre-replication repair that is error-prone and forms mismatched heteroduplexes leading to transforming mutations in H-*ras* gene at codon 61 (CAA to CTA) (Chakravarti *et al.*, 2000).
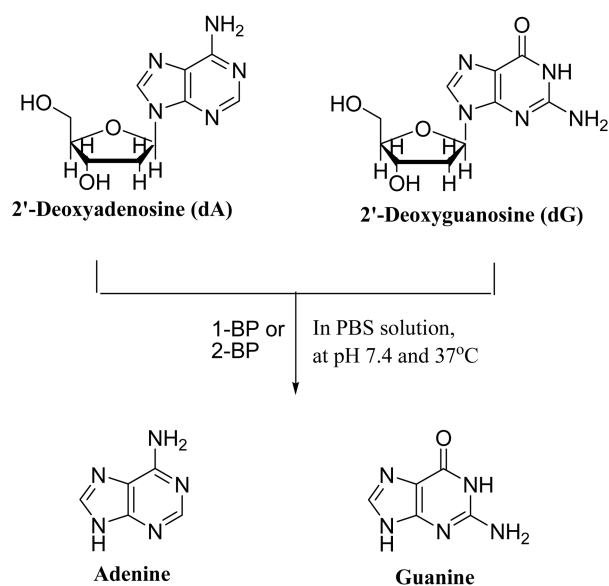


**Fig. 1.** Scheme of depurination, the release of purine bases from dA or dG by hydrolysis of the *N*-glycosidic bond to produce adenine or guanine, respectively.

*Corresponding author
Tel: +82-53-810-2827,    Fax: +82-53-810-4654
E-mail: eslee@yu.ac.kr

It has been reported by Hazardous Substances Data Bank (HSDB) that 2-bromopropane (2-BP) is used as an intermediate in the synthesis of pharmaceuticals, dyes, and other organics. 2-BP (CAS No. 75-26-3) has been used as a cleaning solvent in electronics industry in order to replace chlorofluorocarbons (CFCs) and 1,1,1-trichloroethane (Kim *et al.*, 1996; Kim *et al.*, 1999; Park *et al.*, 1997; Ichihara *et al.*, 1999). But in 1995, an outbreak of reproductive and hematopoietic disorders occurred in male and female workers exposed to the solvent containing 2-BP as a major ingredient in an electronics factory in South Korea (Kim *et al.*, 1996; Park *et al.*, 1997; Takeuchi *et al.*, 1997), which caused the Korean Ministry of Labor to establish the threshold limit value (TLV) for 2-BP in the workplace as 1 ppm. Owing to the toxicity of 2-BP, there has been a growing tendency to use 1-bromopropane (1-BP) as an alternative cleaning solvent to 2-BP. However, 1-BP has a depressing action on the central nervous system (CNS) (Patty, 1962) and is reported to cause irritation to the skin and eyes of mice (Sax, 1968). But the details of 1-BP toxicity have not been studied well. Presently, 1-BP is still used in the work place despite insufficient information regarding its toxicity (Sekiguchi *et al.*, 2002).

We have reported that formation of $N^7$-guanine adduct ($N^7$-isopropyl guanine) in DNA by 2-BP would be one of the mechanism for its toxicity (Zhao *et al.*, 2002). Our research group continued to investigate formation of adducts on nucleosides moiety induced by 1- and 2-bromopropane. However, we observed the massive depurination after incubation of dA and dG with the excess amount (512 equivalent) of 2-BP at the physiological condition (pH 7.4, 37ºC) for 48 h, which were analyzed by HPLC and LC-MS/MS. In addition, time and dose response relationship of depurination in dA and dG induced by 2-BP at the physiological condition were investigated.

## MATERIALS AND METHODS

### Materials

1-Bromopropane (99%), 2-bromopropane (99%), 2'-deoxyadenosine (dA, 99-100%), 2'-deoxyguanosine hydrate (dG, 99%), adenine, guanine, 5-fluorouracil (99%), 5-fluorouridine, phosphate buffered saline (PBS, pH 7.4) and ammonium acetate (99.995+ %) were purchased from Sigma Aldrich Co. (ST. Louis, MO). HPLC grade acetonitrile and methanol was purchased from World Science, Korea.

### Depurination reaction

Each nucleoside (dA or dG, 1 mg) was dissolved in 1 ml of phosphate buffered saline solution (PBS) in 5 ml vial. Ten μl of 5-fluorouridine (5 mg in 1 ml of PBS) for dA and twenty μl of 5-fluorouracil (10 mg in 1 ml of PBS) for dG was added as an internal standard. It was incubated with excess amount (512 equivalents) of 1-BP or 2-BP at the physiological condition for 48 h. It was analyzed by HPLC and LC-MS/MS. All the reactions were repeated for three times.

### Time response reaction

Each nucleoside (dA or dG, 1 mg) was dissolved in 1 ml of PBS in ten separate 5 ml vials, respectively. Ten μl of 5-fluorouridine (5 mg in 1 ml of PBS) for dA and twenty μl of 5-fluorouracil (10 mg in 1 ml of PBS) for dG was added as an internal standard. They were incubated with excess amount (512 equivalent) of 2-BP at the physiological condition. Ten μl of sample in each vial were taken after time interval of 3 h, respectively and analyzed by HPLC and LC/MS/MS. All the experiments were repeated for three times.

### Dose response reaction

Each nucleoside (dA or dG, 1 mg) was dissolved in 1 ml of PBS in ten separate 5 ml vials, respectively. Ten μl of 5-fluorouridine (5 mg in 1 ml of PBS) for dA and twenty μl of 5-fluorouracil (10 mg in 1 ml of PBS) for dG was added as an internal standard. They were incubated with different amount (0, 2, 4, 8, 16, 32, 64, 128, 256 and 512 equivalents) of 2-BP at the physiological condition for a time period (24 h) at which 100% depurination occurred during time response reaction. Ten μl of sample in each vial was taken, respectively and analyzed by HPLC and LC/MS/MS. All the experiments were repeated for three times.

### Calculation for depurination ratio in nucleosides

Depurination ratio (DR%) was calculated on the basis of the decreased amount of the nucleosides in percentage by comparing the integration value of the nucleosides in HPLC and LC/MS/MS using the formula below:

$$\text{Depurination ratio(\%)} = \frac{\dfrac{A_o}{IS_o} - \dfrac{A_t}{IS_t}}{\dfrac{A_o}{IS_o}} \times 100\%$$

where '$A_o$' is the initial amount of nucleoside; '$A_t$' is the amount of nucleoside after time, t ; '$IS_o$' is the initial amount of internal standard and '$IS_t$' is the amount of internal standard after time, t.

## Apparatus

HPLC analysis were performed using two Shimadzu LC-10AT pumps gradient-controlled HPLC system equipped with Shimadzu photo diode array detector (Model SPD-M10A) and dual channel UV detection at 280 nm. Analytes were eluted with a 4.6×250 mm, 5 μm Waters XTerra® $C_{18}$ reverse phase analytical column using the following HPLC condition: Isocratic elution with 4% acetonitrile in water with 50 mM ammonium formate for dA incubation, or 5% acetonitrile in water with 50 mM ammonium formate for dG incubation, at pH 6.9 for 20 minutes at a flow rate of 1 ml/min, and 10 μl injection volume.

ESI LC/MS analyses were performed with a Finnigan LCQ Advantage® LC-MS/MS spectrometry utilizing Xcalibur® program. The samples were analyzed using 2.1×150 mm, 3.5 μm Waters XTerra® $C_{18}$ reverse phase analytical column using the following LC condition: Isocratic elution with 3% acetonitrile in water with 50 mM ammonium formate at pH 6.9 for 20 minutes at a flow rate of 0.18 ml/min, and 2 μl injection volume. The mass spectrometer was operated in the positive polarity mode with ESI source type. Capillary voltage was controlled at 10 V and 270ºC, and nitrogen was used as the sheath gas.

## Statistical analysis

All the reactions were performed at least three times (n≥3). The mean value ± standard error (SE) was determined for each test. Student's t-test was used to compare statistical significance of data. The significant values at either P<0.05(*) or P<0.01(**) were represented by asterisks.

## RESULTS

## Analysis of deadenylation of dA induced by 1-BP or 2-BP by HPLC

Fig. 2 shows the HPLC chromatograms under the chromatographic condition described in material and methods for the analysis of deadenylation of dA induced by 1-BP or 2-BP.

In Fig. 2, chromatogram 1 indicated chromatogram of authentic adenine at retention time of 6.20 min, and chromatogram 2 indicated chromatogram of the mixture of dA and 5-fuorouridine utilized as an internal standard at retention times of 11.40 min and 4.69 min, respectively. Adenine, dA and 5-fuorouridine were well separated from the biological background under the described chromatographic condition. Chromatogram 3 indicated chromatogram of the mixture after
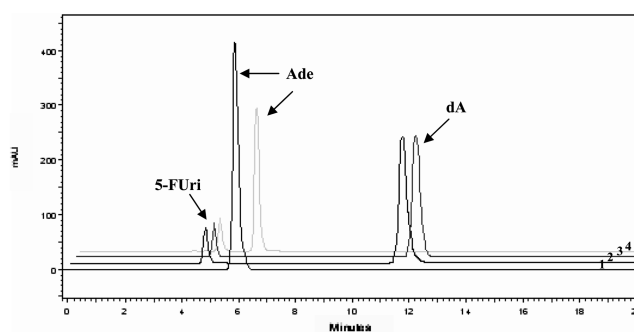


**Fig. 2.** HPLC chromatogram of (1) authentic adenine (Ade), (2) dA + 5-fluorouridine (5-FUri), (3) dA + 5-FUri + 1-BP (48 h) and (4) dA + 5-FUri + 2-BP (48 h). Retention time for 5-FUri, Ade and dA were 4.69, 6.20 and 11.40 min respectively under the HPLC condition mentioned in materials and methods.

incubation of dA and excess amount (512 equivalent) of 1-BP for 48 h at the physiological condition, which informed almost no change in amount of dA and no production of adenine at that condition. Chromatogram 4 indicated chromatogram of the mixture after incubation of dA and excess amount (512 equivalent) of 2-BP for 48 h at the physiological condition, which indicated the peak of retention time at 11.40 min which is corresponding to dA was completely disappeared and a peak of retention time at 6.20 min corresponding to adenine was newly appeared. The results indicated that complete deadenylation was occurred when dA was incubated with excess amount of 2-BP for 48 h. It was not observed the change of amount of 5-fuorouridine during incubation of dA with 1-BP or 2-BP, which indicated the concentration of 5-fuorouridine was consistently well maintained and 5-fuorouridine was not affected by 1-BP or 2-BP.

## Analysis of deguanylation of dG induced by 1-BP or 2-BP by HPLC

Fig. 3 shows the HPLC chromatograms under the chromatographic condition described in material and methods for the analysis of deguanylation of dG induced by 1-BP or 2-BP.

In Fig. 3, chromatogram 1 indicated chromatogram of authentic guanine at retention time of 4.82 min, and chromatogram 2 indicated chromatogram of the mixture of dG and 5-fuorouracil utilized as an internal standard at retention times of 8.38 min and 4.08 min, respectively. Guanine, dG and 5-fuorouracil were well separated from the biological background under the described chromatographic condition. Chromatogram 3 indicated chromatogram of the mixture after incubation of dG and excess amount (512 equivalent) of 1-BP for 48 h at the physio-
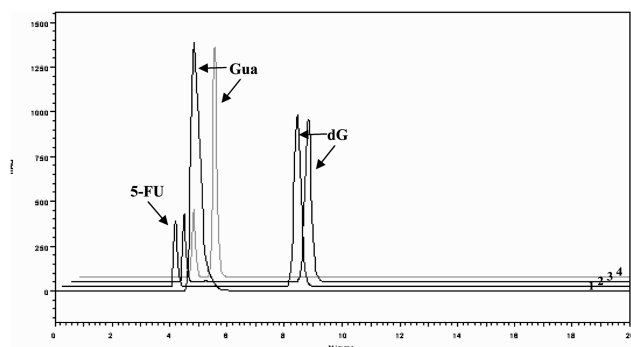
**Fig. 3.** HPLC chromatogram of (1) authentic guanine (Gua), (2) dG + 5-fluorouracil (5-FU), (3) dG + 5-FU + 1-BP (48 h) and (4) dG + 5-FU + 2-BP (48 h). Retention time for 5-FU, Gua and dG were 4.08, 4.82 and 8.38 min respectively under the HPLC condition mentioned in materials and methods.

**Table I.** Depurination by 1-BP or 2-BP with dA and dG at the physiological condition for 48 h

| Haloalkanes | DR (%) in dA | DR (%) in dG |
|---|---|---|
| 1-BP | 1.37 | 1.54 |
| 2-BP | 100.00 | 100.00 |

1 mg of nucleoside (dA or dG) was dissolved in 1 ml phosphate buffered saline solution (PBS) in 5 ml vial. 5-fluorouridine, 10 ml (5 mg in 1 ml of PBS) was added as an internal standard. It was incubated with excess amount (512 equivalents) of 1-BP or 2-BP at the physiological condition for 48 h.

logical condition, which informed almost no change in amount of dG and no production of guanine at that condition. Chromatogram 4 indicated chromatogram of the mixture after incubation of dG and excess amount (512 equivalent) of 2-BP for 48 h at the physiological condition, which indicated the peak of retention time at 8.38 min which is corresponding to dG was completely disappeared and a peak of retention time at 4.82 min corresponding to guanine was newly appeared. The results indicated that complete deguanylation was occurred when dG was incubated with excess amount of 2-BP for 48 h. It was not observed the change of amount of 5-fuorouracil during incubation of dG with 1-BP or 2-BP, which indicated the concentration of 5-fuorouracil was consistently well maintained and 5-fuorouracil was not affected by 1-BP or 2-BP. From the HPLC

analysis, it was found that 100% depurination occurred in dA and dG by 2-BP at the physiological condition for 48 hr. However almost no depurination was observed by 1-BP with the depurination ratio less than 1.6% compared to the reaction by 2-BP, which was summarized in Table I.

**Analysis of time response depurination of dA and dG induced by 2-BP**

Fig. 4 shows time response curves of depurination rate of dA or dG induced by 2-BP according to time. Fig. 4(a) indicated time response curve of deadenylation after incubation of dA and 512 dose equivalent of 2-BP at the physiological condition at a time interval of 3 h. Deadenylation begin to occur at 6 h, and drastically increase until 18 h in time dependent manner. Complete deadenylation was occurred at 21 h. Fig. 4(b) indicated time response curve of deguanylation after incubation of dG and 512 dose equivalent of 2-BP at the physiological condition at a time interval of 3 h. Deguanylation begin to occur at 3 h, and drastically increase until 15 h in time dependent manner. Complete deadenylation was occurred at 18 h. Compared to
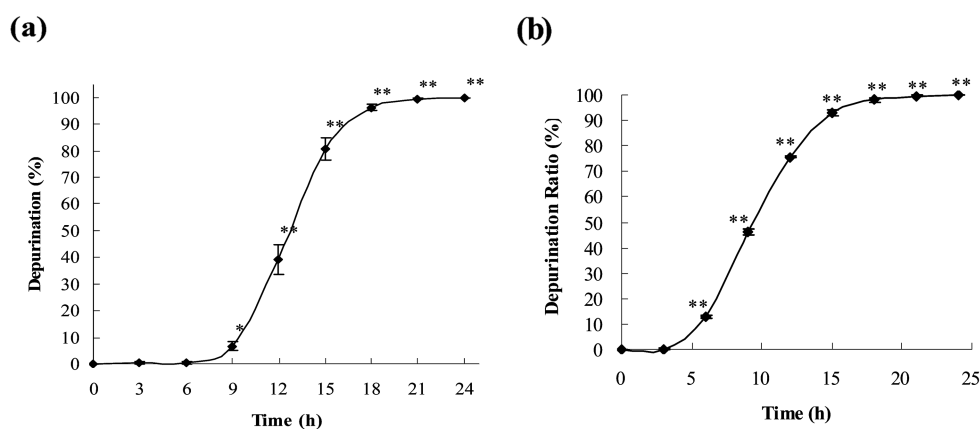
**(a)**

**(b)**



**Fig. 4.** Time response curves of (a) dA and (b) dG by 2-BP. Time response reactions were performed with the 512 equivalents of 2-BP at a time interval of 3 h until the time at which 100% depurination was occurred, which was analyzed by HPLC and LC/MS/MS with the condition mentioned in the materials and methods.
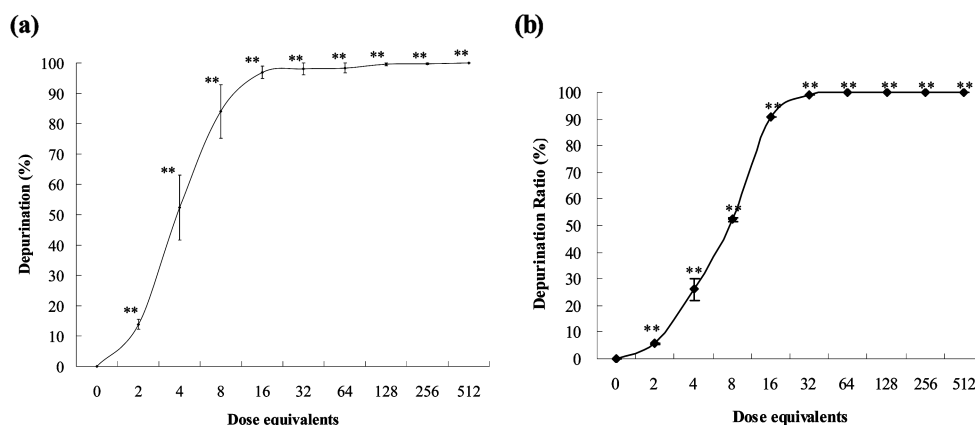
**Fig. 5.** Dose response curves of (a) dA and (b) dG by 2-BP. Dose response reactions were performed with the 0, 2, 4, 8, 16, 32, 64, 128, 256 and 512 equivalents of 2-BP for 24 h, which was analyzed by HPLC and LC/MS/MS with the condition mentioned in the materials and methods.

depurination rate between dA and dG, depurination rate of dG was faster than that of dA.

**Analysis of dose response depurination of dA and dG induced by 2-BP**

Fig. 5 shows dose response curves of depurination rate of dA or dG induced by 2-BP according to dose. Fig. 5(a) indicated dose response curve of deadenylation after incubation of dA and different dose equivalent of 2-BP at the physiological condition for 24 h. Deadenylation begin to occur at 2 dose equivalent of 2-BP, and drastically increase until 16 dose equivalent of 2-BP in dose dependent manner. Complete deadenylation was occurred at 32 dose equivalent of 2-BP. Fig. 5(b) indicated dose response curve of deguanylation after incubation of dG and different dose equivalent of 2-BP at the physiological condition for 24 h. Deguanylation begin to occur at 2 dose equivalent of 2-BP, and drastically increase until 16 dose equivalent of 2-BP in a dose dependent manner. Complete deguanylation was occurred at 32 dose equivalent of 2-BP.

## DISCUSSION

Depurination ratio (%) in nucleosides were calculated on the basis of the decreased amount of nucleosides in percentage by comparing the integration value of the nucleosides in HPLC using the formula mentioned in materials and methods. The depurinated products of nucleosides (dA and dG) are adenine and guanine, respectively. Since the solubility of guanine is relatively low at physiological condition, the guanine formed after depurination precipitates in pH 7.4 buffer solution, which decreases the accuracy of depurination ratio, if we apply the

increasing amount of depurinated product (adenine or guanine) for the determination of depurination ratio. Therefore, we applied the decreasing amounts of nucleosides for the determination of depurination ratio. In Fig. 2 and 3, chromatogram 1 shows the peak of authentic adenine or guanine as references, chromatogram 2 shows that of each nucleosides, dA or dG, along with internal standard's peak, chromatogram 3 and 4 show the peaks of products formed after incubation of dA or dG with 1-BP or 2-BP for 48 h, respectively. It is evident that the peaks of dA or dG are completely disappeared and the peaks of adenine or guanine have appeared after incubation with 2-BP for 48 h (chromatogram 4 in Fig. 2 and 3). However, almost no change of chromatogram was observed after incubation with 1-BP for 48 h (chromatogram 3 in Fig. 2 and 3). These results indicated that 100% depurination was occurred in dA and dG by 2-BP, but practically no depurination was occurred by 1-BP (Table I).

Time and dose response reaction with dA and dG by 2-BP indicated that depurination was increased in time and dose dependent manner (Fig. 4 and 5). In time response reaction, depurination was increased in time dependent manner, and complete depurination was observed after 21 h in both dA and dG by 2-BP (Fig. 4). Compared to depurination rate between dA and dG according to time, depurination rate of dG was observed to be faster than that of dA.

In dose response reaction, depurination was increased in dose dependent manner, and complete depurination was observed with both 32 dose equivalents for 24 h incubation in dA and dG (Fig. 5). In conclusion, we observed depurination of dA and dG induced by 2-BP as a probable mechanism of toxicity. Since the mechanism of depurination is unknown at the

present time, the study for elucidation of mechanism of depurination is in progress.

## ACKNOWLEDGMENTS

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