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Effects of bromide upon reaction of nucleosides with hydrogen peroxide induced by ultraviolet light

Toshinori Suzuki *, Naofumi Moriwaki, Kazuko Kurokawa †, Michiyo Inukai

School of Pharmacy, Shujitsu University, Okayama 703-8516, Japan

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ABSTRACT

When ultraviolet light was irradiated on a neutral solution of deoxynucleosides and hydrogen peroxide, concentrations of all the deoxynucleosides decreased greatly. Addition of bromide in the system suppressed the reactions of 2′-deoxycytidine, 2′-deoxythymidine, and 2′-deoxyadenosine, but not that of 2′-deoxyguanosine. Addition of hydroxyl radical scavengers suppressed the reaction. The effect of deuterium oxide, an enhancer of singlet oxygen, was small. It is reported that hydroxyl radical, generating from hydrogen peroxide by ultraviolet irradiation, can react with bromide forming bromine radical, and that bromine radical reacts with bromide forming dibromide radical anion. Our result of dose dependency of bromide suggests that dibromide radical anion is the reaction species to react only with 2′-deoxyguanosine.

Ultraviolet (UV) light is a major cause of human skin cancer.[1] DNA can react with UV light directly to form products including pyrimidine dimers and hydroxylated monomers.[2] In addition, UV light reacts with hydrogen peroxide (H_2O_2) generating hydroxyl radical ($HO^{\cdot}$), a highly reactive oxygen species.[3–5] $HO^{\cdot}$ reacts with DNA at its various sites causing various types of DNA damage such as strand breaks, release of bases, and hydroxylation of bases.[6] Bromine is one of the most abundant and ubiquitous trace elements. Seawater contains 65–67 mg/kg bromide (Br^-), about 1/300 the concentration of chloride (Cl^-).[7,8] The first identified use of Br^- by enzymes in humans appears to be a role in defense mechanisms against parasites mediated by the preferential oxidation of Br^- by eosinophil peroxidase.[9] An eosinophil peroxidase/H_2O_2/Br^- system in the presence of a plasma concentration of Cl^- can react with nucleosides to form brominated nucleosides.[10–12] It has also been reported that a myeloperoxidase/H_2O_2/Cl^- system in the presence of a plasma concentration of Br^- generates 5-bromo-2′-deoxycytidine from 2′-deoxycytidine (dCyd).[13] Recently, we indicated that ONOOH and Br^- react efficiently with dCyd in the presence of ammonium ion or amines, forming 5-hydroxy-2′-deoxycytidine and 5-bromo-2′-deoxycytidine.[14] However, little is known about the effect of Br^- on the reaction of nucleosides with $HO^{\cdot}$. In the present study, we examined the effects of Br^- on the reaction of deoxynucleosides and H_2O_2 irradiating UV right.

To obtain information about the effects of halides on the reaction of deoxynucleosides with $HO^{\cdot}$, a solution mixture of dCyd, 2′-deoxyguanosine (dGuo), 2′-deoxythymidine (dThd), and 2′-deoxyadenosine (dAdo) (100 μM each) containing 10 mM H_2O_2 and 10 mM NaF, NaCl, NaBr, or NaI in 100 mM potassium phosphate buffer (pH 7.4) was irradiated with UV light originating from a 200 W high pressure mercury lamp at 37 °C for 60 s. The intensity of the UV light was 173 mW/cm^2 for 254 nm. The nucleoside concentrations were determined by reversed phase high performance liquid chromatography (RP-HPLC) analysis detected at 260 nm. Table 1 shows the nucleosides concentrations in the UV irradiated solutions. Addition of NaF and NaCl showed no effect on any of the nucleoside concentrations after UV irradiation. NaBr inhibited the reactions of dCyd, dThd, and dAdo but not that of dGuo. NaI repressed the reaction of all the nucleosides. We focused on the effects of NaBr because of its selective inhibition of the reactions. To know whether reactive species are generated directly from Br^- by UV light, the nucleoside mixture was irradiated with UV light without H_2O_2. Figure 1A shows the time course when UV light irradiated the nucleosides without Br^-. The concentration of dCyd slightly decreased as the reaction time increased, while those of other nucleosides were not changed. A similar result was obtained for the reaction with 10 mM Br^- as shown in Figure 1B. In both reactions, when the reaction mixture was allowed to stand at 5 °C for 1 day, the concentration of dCyd recovered to the initial concentration (data not shown). It has been reported that a reversible photohydration takes place on C5–C6 double bond of dCyd forming diastereomers of 6-hydroxy-5,6-dihydro-2′-deoxycytidine.[2,15] The results indicate that direct reaction of the nucleosides with the UV light was slight, and that no reactive species for nucleosides is generated from Br^- with UV light.

* Corresponding author. Tel.: +81 86 271 8346; fax: +81 86 271 8320.
E-mail address: tsuzuki@shujitsu.ac.jp (T. Suzuki).
† Present address: Okayama University, Okayama 700-8558, Japan.

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Table 1
Effects of halides on the reactions of nucleosides and hydrogen peroxide with UV light[a]

Additives	dCyd (μM)	dGuo (μM)	dThd (μM)	dAdo (μM)
None	22.7 ± 1.4	31.4 ± 1.7	23.1 ± 1.7	42.7 ± 0.8
10 mM NaF	25.6 ± 1.5	38.7 ± 0.9	29.8 ± 1.3	48.4 ± 0.7
10 mM NaCl	25.0 ± 0.2	36.5 ± 0.3	28.2 ± 0.2	46.3 ± 0.4
10 mM NaBr	87.8 ± 0.2	40.7 ± 1.6	89.2 ± 0.2	95.3 ± 0.3
10 mM NaI	83.9 ± 0.2	95.9 ± 0.2	97.5 ± 0.1	91.8 ± 0.2

[a] A solution mixture of dCyd, dGuo, dThd, and dAdo (100 μM each) containing 10 mM H_2O_2 and 10 mM sodium halides in 100 mM potassium phosphate buffer (pH 7.4) was irradiated with UV light originating from a 200 W high pressure mercury lamp at 37 °C for 60 s. The intensity of the UV light was 173 mW/cm^2 for 254 nm. The nucleoside concentrations were determined by RP-HPLC analysis detected at 260 nm. Means ± SD (n = 3) are present.

Then UV was irradiated on the nucleoside mixture containing 10 mM H_2O_2. Figure 2A shows the time course of concentration change of the nucleosides when the UV light was irradiated upon the nucleoside mixture containing H_2O_2 without Br^-. All the nucleosides concentrations decreased greatly with increasing irradiation time. As shown in Figure 2B, when the UV light was irradiated upon the nucleoside mixture containing 10 mM H_2O_2 and 10 mM Br^-, the reactions of dCyd, dThd, and dAdo were suppressed efficiently, but dGuo was consumed as well as the reaction without Br^-.

To obtain information on the reactive species in these reactions, the effects of two hydroxyl radical scavengers, thiourea and ethanol, a singlet oxygen scavenger, NaN_3, which also acts as a hydroxyl radical scavenger, and an enhancer of singlet oxygen reaction, D_2O, were investigated. Table 2 shows the effects of the additives on the reaction of the nucleosides mixture containing 10 mM H_2O_2 by UV irradiation. All the reactions of the nucleosides were suppressed efficiently by thiourea and ethanol. NaN_3 also suppressed the reactions although the efficiency for dGuo was relatively low. No effect was observed for D_2O. The results show that HO· is the reactive species in the H_2O_2/UV system. Table 3 shows the effects of the additives on the reaction of the nucleosides mixture containing 10 mM H_2O_2 and 10 mM Br^- by UV irradiation. The reaction of dGuo was suppressed efficiently by thiourea and ethanol. NaN_3 also suppressed the dGuo reaction with a low efficiency. Little effect was observed for D_2O. The results suggest that HO· is included in the H_2O_2/Br^-/UV system and $^1O_2$ is not a major reactive species.

UV light affects the O–O bond of H_2O_2 to give HO·.[3–5]

$$H_2O_2 + h\nu \rightarrow 2HO^{\cdot} \quad (1)$$

HO· can react with Br^- to generate bromine radicals (Br·).[16,17]

$$HO^{\cdot} + Br^- \rightarrow HO^- + Br^{\cdot} \quad (2)$$

The bromine radicals thus formed react with Br^- to form dibromide radical anion ($Br_2^{\cdot -}$):[16,17]

$$Br^{\cdot} + Br^- \rightarrow Br_2^{\cdot -} \quad (3)$$

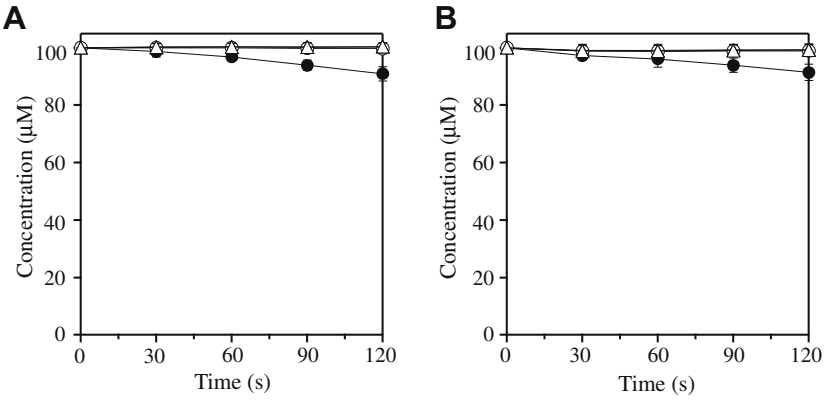

Figure 1. The time course of the concentration changes in dCyd (closed circle), dGuo (open circle), dThd (closed triangle), and dAdo (open triangle) when a solution mixture of dCyd, dGuo, dThd, and dAdo (100 μM each) in 100 mM potassium phosphate buffer (pH 7.4) without (A) and with (B) 10 mM NaBr was irradiated with UV light originating from a 200 W high pressure mercury lamp at 37 °C. The intensity of the UV light was 173 mW/cm^2 for 254 nm. The nucleoside concentrations were determined by RP-HPLC analysis detected at 260 nm. Means ± SD (n = 3) are shown.

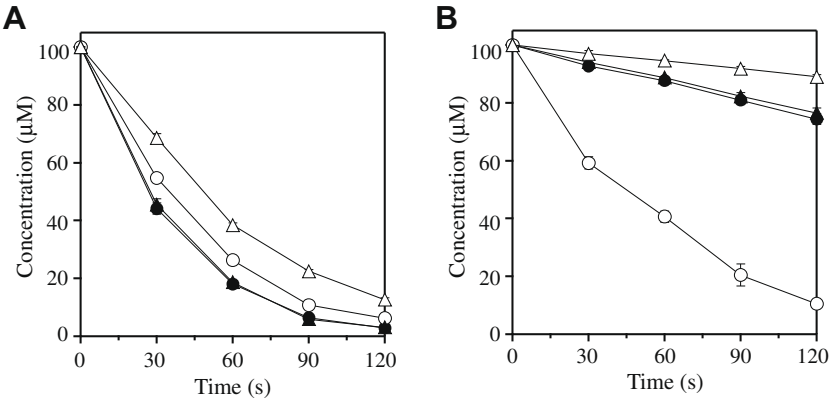

Figure 2. The time course of the concentration changes in dCyd (closed circle), dGuo (open circle), dThd (closed triangle), and dAdo (open triangle) when a solution mixture of dCyd, dGuo, dThd, and dAdo (100 μM each) containing 10 mM H_2O_2 in 100 mM potassium phosphate buffer (pH 7.4) without (A) and with (B) 10 mM NaBr was irradiated with UV light at 37 °C. The nucleoside concentrations were determined by RP-HPLC analysis detected at 260 nm. Means ± SD (n = 3) are shown.

Table 2
Effects of additives on the reactions of nucleosides and hydrogen peroxide with UV light[a]

Additives	dCyd (μM)	dGuo (μM)	dThd (μM)	dAdo (μM)
None	22.7 ± 1.4	31.4 ± 1.7	23.1 ± 1.7	42.7 ± 0.8
10 mM thiourea	72.8 ± 1.7	85.5 ± 0.9	76.1 ± 1.8	85.1 ± 0.8
1% EtOH	91.9 ± 0.6	96.2 ± 0.5	93.9 ± 0.6	96.6 ± 0.4
10 mM NaN_3	67.5 ± 1.7	50.7 ± 0.7	75.9 ± 1.6	84.8 ± 1.1
99.8% D_2O	27.5 ± 0.3	37.4 ± 0.3	30.1 ± 0.3	47.0 ± 0.4

[a] A solution mixture of nucleosides (dCyd, dGuo, dThd, and dAdo, 100 μM each) and 10 mM H_2O_2 containing the additives in 100 mM potassium phosphate buffer (pH 7.4) was irradiated with UV light at 37 °C for 60 s. The nucleoside concentrations were determined by RP-HPLC analysis. Means ± SD (n = 3) are present.

Table 3
Effects of additives on the reactions of nucleosides and hydrogen peroxide containing NaBr with UV light[a]

Additives	dCyd (μM)	dGuo (μM)	dThd (μM)	dAdo (μM)
None	87.8 ± 0.2	40.7 ± 1.6	89.2 ± 0.2	95.3 ± 0.3
10 mM thiourea	90.5 ± 1.4	96.3 ± 0.3	94.7 ± 0.4	96.4 ± 0.6
1% EtOH	93.8 ± 0.4	91.7 ± 0.8	97.3 ± 0.7	98.6 ± 0.3
10 mM NaN_3	84.0 ± 1.1	55.4 ± 4.9	92.9 ± 1.0	95.0 ± 0.6
99.8% D_2O	85.5 ± 0.5	34.0 ± 0.5	87.9 ± 0.7	89.4 ± 0.7

[a] A solution mixture of nucleosides (dCyd, dGuo, dThd, and dAdo, 100 μM each), 10 mM H_2O_2, and 10 mM NaBr containing the additives in 100 mM potassium phosphate buffer (pH 7.4) was irradiated with UV light at 37 °C for 60 s. The nucleoside concentrations were determined by RP-HPLC analysis. Means ± SD (n = 3) are present.

$Br^{\cdot}$ and $Br_2^{\cdot-}$ are candidate reactive species in the H_2O_2/Br^-/UV system. Figure 3 shows the Br^- dose dependence of the nucleoside concentration on the UV irradiation reaction. Addition of Br^- up to 30 μM did not affect the reaction. From 100 μM to 1 mM Br^-, the reaction of all the nucleosides was suppressed. Above 1 mM Br^-, the consumption of dGuo increased, while those of other nucleosides decreased. Thus, a low concentration of Br^- acts as an inhibitory agent for dGuo as well as other nucleosides. This result implies that a low concentration of Br^- is converted by $HO^{\cdot}$ to $Br^{\cdot}$, which causes no reaction for all the nucleosides, and that a high concentration of Br^- causes further reactions of $Br^{\cdot}$ and Br^-, resulting in formation of $Br_2^{\cdot-}$. dGuo is sensitive to oxidants because of its low oxidation potential. Oxidation potentials versus normal hydrogen electrode (NHE) for DNA nucleosides at pH 7 are reported to be 1.29 V for guanosine, 1.42 V for adenosine, 1.6 V for cytidine, and 1.7 V for thymidine.[18–20] The standard oxidation potential of $Br_2^{\cdot-}$ (versus NHE at pH 0) is calculated to be 1.63 V.[21] Oxidation potentials decrease by 59 mV when pH increases by one pH unit.[22] Thus, the oxidation potential of $Br_2^{\cdot-}$ at pH 7 is estimated as 1.22 V, which is comparable to that of dGuo (1.29 V). The ratio per pH unit (−59 mV/pH) can vary depending on the oxidants and reductants involved. In addition, oxidation potentials are affected by temperature. $Br_2^{\cdot-}$ may irreversibly oxidize only dGuo among the nucleosides used in this study, resulting in a decrease of dGuo concentration in the H_2O_2/Br^-/UV system.

The concentration of Br^- in human plasma is low, ranging over levels of 39–84 μM.[23] At the plasma concentration of Br^-, the effect on the reaction of nucleosides with $HO^{\cdot}$ would be small. In the nineteenth century, bromides such as KBr and NaBr were widely used as an antiepileptic drug with good success.[24] Bromides are no longer a mainstay of epilepsy therapy because of their significant toxicity, termed bromism.[25] However, bromides are still used in the treatment of patients with refractory seizures, particularly in pediatrics. The therapeutic serum concentration of Br^- is high and ranges over levels of 10–35 mM.[24] On the other hand, excessive cola consumption causes elevation of serum Br^- concentration

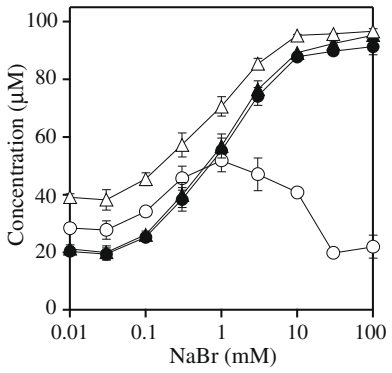

Figure 3. The NaBr dose dependence of the concentration changes in dCyd (closed circle), dGuo (open circle), dThd (closed triangle), and dAdo (open triangle) when a solution mixture of dCyd, dGuo, dThd, and dAdo (100 μM each) containing 10 mM H_2O_2 in 100 mM potassium phosphate buffer (pH 7.4) with 0.01–100 mM NaBr was irradiated with UV light at 37 °C. The nucleoside concentrations were determined by RP-HPLC analysis detected at 260 nm. Means ± SD (n = 3) are shown.

up to 40 mM.[26] Cola contains brominated vegetable oil, which can release Br^- through the biotransformation in humans. Brominated vegetable oil is widely used as an emulsifier in citrus-flavored soft drinks. At such dose levels, Br^- would suppress the nucleoside damages caused by $HO^{\cdot}$ except for dGuo.

In conclusion, we found that the reactions of dCyd, dThd, and dAdo by the H_2O_2/UV system were suppressed by addition of Br^-, while dGuo still reacted under high concentrations of Br^-. Our result of dose dependency of Br^- suggests that $Br_2^{\cdot-}$ is the reactive species. A high concentration of Br^- may change the mutation spectra caused by $HO^{\cdot}$ in humans.

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