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8-Oxoguanine induces intramolecular DNA damage but free 8-oxoguanine protects intermolecular DNA from oxidative stress

Ja-Eun Kim, Seongwon Choi, Ju-Ah Yoo, Myung-Hee Chung*

Department of Pharmacology, Seoul National University College of Medicine, 28 Yongon-dong, Chongno-gu, Seoul 110-799, South Korea

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Abstract 7,8-Dihydro-8-oxoguanine (8-oxoguanine; 8-oxo-G), one of the major oxidative DNA adducts, is highly susceptible to further oxidation by radicals. We confirmed the higher reactivity of 8-oxo-G toward reactive oxygen (singlet oxygen and hydroxyl radical) or nitrogen (peroxynitrite) species as compared to unmodified base. In this study, we raised the question about the effect of this high reactivity toward radicals on intramolecular and intermolecular DNA damage. We found that the amount of intact nucleoside in oligodeoxynucleotide containing 8-oxo-G decreased more by various radicals at higher levels of 8-oxo-G incorporation, and that the oligodeoxynucleotide damage and plasmid cleavage by hydroxyl radical were inhibited in the presence of 7,8-dihydro-8-oxo-2′-deoxyguanosine (8-oxo-dG). We conclude that 8-oxo-G within DNA induces intramolecular DNA base damage, but that free 8-oxo-G protects intermolecular DNA from oxidative stress. These results suggest that 8-oxo-G within DNA must be rapidly released to protect DNA from overall oxidative damage.

Key words: Oxidative stress; 8-Oxoguanine; Electron transfer

1. Introduction

Guanine is attacked preferentially upon oxidative DNA damage because it has the lowest oxidation potential of the four bases (dG 1.29 V, dA 1.42 V, dC 1.6 V, dT 1.7 V [1]). This results from the fact that guanine has an electron-rich purine structure, which enables it to react with radicals easily [2,3]. In addition, guanine also acts as a 'hot spot' for electron migration [4–6]. A number of studies have shown that the 5′-G of GG or GGG sequences is significantly easier to be oxidized than isolated guanines [7–12], which is why electron transfer from electron-rich guanine along DNA contributes to the further oxidative damage.

The oxidation of guanine generally results in the formation of 7,8-dihydro-8-oxoguanine (8-oxoguanine; 8-oxo-G) [13], which is produced abundantly in vivo and used as a biomarker of oxidative damage [14,15]. 8-Oxo-G is more reactive toward radicals than unmodified base because of its low oxidation potential [16–20], and represents a 'hot spot' and 'electron sink' for oxidative DNA damage [21]. Therefore, it has been suggested that radical reactions in DNA during oxidation are localized to electron-rich moieties such as 8-oxo-G, and that electron transfer along DNA is initiated by the oxidation of the 8-oxo-G.

In this study, we question the effect of the electron-rich property of 8-oxo-G on DNA damage. We first confirm the susceptibility of 8-oxo-G to radical-induced oxidation using singlet oxygen, ionizing radiation, hydroxyl radical and peroxynitrite. We raise the question as to whether the electron-rich property of 8-oxo-G leads to DNA damage, and to what extent this damage depends on the number of 8-oxo-G residues in DNA under various oxidation conditions. On the other hand, we also question whether the electron-rich property of 8-oxo-G protects the intermolecular DNA from oxidative damage by an electron transfer mechanism. In order to address these questions, we investigate the effect of 8-oxo-G within DNA on other intramolecules by treating oligodeoxynucleotides containing 8-oxo-G with various radical species, and examine the intermolecular effect of free 8-oxo-G by treating oligodeoxynucleotide and plasmid DNA with hydroxyl radical in the presence of 7,8-dihydro-8-oxo-2′-deoxyguanosine (8-oxo-dG).

2. Materials and methods

2.1. Materials

Nucleosides dG, dA, dT, dC, 8-oxo-dG and nuclease P1 were obtained from Sigma (MO, USA). Deuterium oxide and alkaline phosphatase were purchased from Aldrich (WI, USA) and from Roche Molecular Biochemicals (Mannheim, Germany), respectively. The plasmid isolation kit was supplied by Bio-Rad (CA, USA). All chemicals used in the study were of analytical quality.

2.2. Oligodeoxynucleotides

The sequences of oligodeoxynucleotides used are shown in Table 1. Oligodeoxynucleotides containing 8-oxo-G purified by high-performance liquid chromatography (HPLC) were purchased from Midland Certified Reagent (TX, USA) and the other oligodeoxynucleotides were synthesized by Bioneer (Korea).

2.3. Treatment of deoxynucleosides using various radical-producing systems

The formation of 8-oxo-G from dG and the high susceptibility of 8-oxo-G toward radicals were confirmed by using various radical-producing systems including the mallet reaction, high-dose ionizing radiation, Fenton reagent and peroxynitrite treatment as follows.

The mallet reaction was used to generate singlet oxygen from hydrogen peroxide and hypochlorite [22]. Solutions (500 μl) of dG, dA, dT, dC or 8-oxo-dG (0.01 mM of each) in 10 mM Tris–HCl buffer, pH 7.4, were incubated with 24 mM hydrogen peroxide and 20 mM

*Corresponding author. Fax: (82)-2-745 7996.
E-mail address: mhchung@snu.ac.kr (M.-H. Chung).

Abbreviations: 8-oxo-G, 7,8-dihydro-8-oxoguanine, 8-oxoguanine, 8-oxo-dG, 7,8-dihydro-8-oxo-2′-deoxyguanosine; OGG1, 8-oxoguanine glycosylase 1

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Table 1
The sequences and numbers of bases in the oligodeoxynucleotides used

Name	Sequence	G	8-oxo-G	A	T	C
NG_{30}	5′-ATCCATATCCATACACTAACTCTTATCATC-3′	–	–	10	10	10
FB_{40}	5′-ATCGCATGAGTCGCATACGACTAGACTGCTGTAGTCAGTC-3′	10	–	10	10	10
NA_{40}	5′-GTCGCGTGGGTCGCGTGCGGCTGGGCTGCTGTGGTCGGTC-3′	20	–	–	0	10
NT_{40}	5′-AGCGCAGGAGGCGCAGACGACGAGACGGCGGGAGGCAGGC-3′	20	–	10	–	10
NC_{40}	5′-ATGGGATGAGTGGGATAGGAGTAGAGTGGTGTAGTGAGTG-3′	20	–	10	10	–
$O5_{35}$	5′-ATCCATXATCXCATACACTAXACTCTXTATCAXTC-3′	–	5	10	10	10
$O5G5_{40}$	5′-ATCGCATXAGTCXCATACGACTAXACTGCTXTAGTCAXTC-3′	5	5	10	10	10
$O10_{40}$	5′-ATCXCATXAXTCXCATACXACTAXACTXCTXTAXTCAXTC-3′	–	10	10	10	10

X; 8-oxo-G (8-oxoguanine).

sodium hypochlorite at room temperature with vigorous stirring. The reaction was also performed in 90% D_2O instead of H_2O to enhance the lifetime of singlet oxygen.

Solutions (500 μl) containing 0.01 mM of each deoxynucleoside in 10 mM Tris–HCl buffer, pH 7.4, were irradiated at room temperature using a ^{137}Cs γ-source (Gammacell 2000, Atomic Energy of Canada Ltd., Quebec, Canada) [23].

Solutions (500 μl) containing 0.01 mM of each deoxynucleoside, 0.1 mM cupric sulfate, 0.1 mM ascorbic acid, 5 mM hydrogen peroxide and 10 mM Tris–HCl, pH 7.4, were incubated at 37°C. After preincubating deoxynucleoside with cupric sulfate for 30 min at room temperature, the reaction was started by adding ascorbic acid and hydrogen peroxide, and terminated by adding EDTA at a final concentration of 2 mM [24].

Solutions (500 μl) containing 0.01 mM of each deoxynucleoside, 0.1 mM sodium peroxynitrite and 10 mM Tris–HCl, pH 7.4, were incubated at room temperature. Sodium peroxynitrite was synthesized by oxidizing nitrous acid with hydrogen peroxide [25,26]. In brief, an aqueous solution (100 ml) containing 10 mmol of sodium nitrite and 10 mmol of hydrogen peroxide was cooled to in an ice bath. Then, 10 ml of cold 1 M hydrochloric acid was added under vigorous stirring. The peroxy-nitrous acid formed was quenched immediately by adding of 15 ml of cold 1 M sodium hydroxide and the solution turned yellow. It was found that the yield of peroxynitrite increased if all the solutions were mixed as fast as possible. The excess hydrogen peroxide was destroyed by granular manganese dioxide under stirring for 5 min, and subsequently filtered to remove the solid fraction. The solution was frozen at −20°C for as long as a week. Peroxynitrite tends to form a yellow top layer due to freeze fractionation, which was collected and used for further studies. The concentration of the sodium peroxynitrite solution was determined by measuring UV absorption at 302 nm ($\varepsilon_{302} = 1670\ M^{-1}\ cm^{-1}$ in 1 M sodium hydroxide).

After each treatment, the formation of 8-oxo-dG from dG and the remaining amount of each deoxynucleoside was measured by using a HPLC equipped with an electrochemical or an UV detector as described previously [18].

2.4. Treatment of oligodeoxynucleotides using various radical-producing systems

The oxidizing effect of 8-oxo-G present within DNA on other base components in the same DNA molecule was determined by detecting the remaining amounts of intact deoxynucleosides in oligodeoxynucleotides, after exposure to radical-producing systems. Solutions (200 μl) containing 1 μM of each oligodeoxynucleotide in 10 mM Tris–HCl, pH 7.4, were treated using the mallet reaction for 120 min, ionizing radiation at 400 Gy, Fenton reagent for 20 min, or peroxynitrite for 60 min at room temperature as described above. After treatment, oligodeoxynucleotides were precipitated using 3 M sodium acetate and 100% ethanol, washed with 70% ethanol and dried in air. Precipitated oligodeoxynucleotides were solubilized in 10 mM Tris–HCl, pH 7.4, for deoxynucleoside analysis. The oligodeoxynucleotides were then hydrolyzed to the deoxynucleoside level using nuclease P1 and alkaline phosphatase, as previously described [27]. The amount of each dT and dA was measured using a HPLC equipped with an UV detector.

2.5. Treatment of oligodeoxynucleotides with Fenton reagent in the presence of 8-oxo-G

The inhibitory effect of free 8-oxo-G on oxidative damage of oligodeoxynucleotides was investigated by detecting the remaining amount of dT by Fenton reagent in the presence 8-oxo-dG. Solutions (200 μl) containing 1 μM of each oligodeoxynucleotide, 0.1 mM cupric sulfate, 0.1 mM ascorbic acid, 5 mM hydrogen peroxide and 10 mM Tris–HCl, pH 7.4,, were incubated at room temperature for 20 min in the absence or presence of 0.1 mM of 8-oxo-dG. After digestion of oligodeoxynucleotides to deoxynucleoside, the remaining amount of dT was measured as described above.

2.6. Treatment of thymidine or plasmid DNA with Fenton reagent in the presence of 8-oxo-G or hydroxyl radical scavengers

The effect of 8-oxo-G, not present within DNA, on intermolecules during oxidation was determined by detecting the degree of free dT degradation or cleavage of plasmid DNA by Fenton reagent in the presence of 8-oxo-dG. Solutions (500 μl) containing 0.01 mM of free dT, 0.1 mM cupric sulfate, 0.1 mM ascorbic acid, 5 mM hydrogen peroxide and 10 mM Tris–HCl, pH 7.4, were incubated at 37°C for 120 min as described above. Nucleosides, i.e. 8-Oxo-dG, dG, dA, dT and dC, and hydroxyl radical scavengers, i.e. sodium azide, sodium formate, mannitol, dimethylsulfoxide (DMSO) and *tert*-butyl alcohol, were added to a final concentration of 0.2 mM. After treatment, the remaining amount of dT was measured.

Supercoiled plasmid DNA was prepared from *Escherichia coli* strain containing a 3900 bp plasmid by the alkaline lysis method using the Quantum Prep plasmid maxiprep kit according to the manufacturer's instructions (Bio-Rad, CA, USA). Reaction mixtures (100 μl) containing 10 μg ml^{-1} plasmid DNA, 40 μM cupric sulfate, 40 μM hydrogen peroxide, 40 μM ascorbic acid and 10 mM Tris–HCl, pH 7.4, were incubated at room temperature for 10 min. Nucleosides or hydroxyl radical scavengers were added to a final concentration of 4 mM. The reaction was terminated by adding EDTA at a final concentration of 0.8 mM containing 50% (W/V) sucrose and 0.1% (W/V) bromophenol blue. The supercoiled, open circular and linear forms of the plasmid DNA were analyzed by agarose gel electrophoresis (0.8% agarose; running buffer, 45 mM Tris–HCl/45 mM boric acid/1 mM EDTA) [28]. The gel was stained with ethidium bromide, viewed, and photographed on an UV transilluminator. The amount of supercoiled DNA was estimated from the photograph using a microcomputer imaging device (MCID, Imaging Research INC., Canada), and expressed as a percentage of the total plasmid.

3. Results

3.1. Degradation of 8-oxo-dG in various radical-producing systems

The purpose of this study was to determine the effect of the high reactivity of 8-oxo-G toward radicals on DNA both inter- and intramolecularly. Initially, we investigated the formation of 8-oxo-dG from dG in various radical-producing systems including the mallet reaction, high-dose ionizing radiation, Fenton reagent and peroxynitrite treatment. Next, we examined the reactivity of 8-oxo-G toward various radical species. The reaction was performed at the deoxynucleoside level by detecting the amount of intact deoxynucleoside remaining after treatment with the various radical species.

As shown in Fig. 1, 8-oxo-dG was formed from dG with concomitant decrease of the dG time and dose dependently in

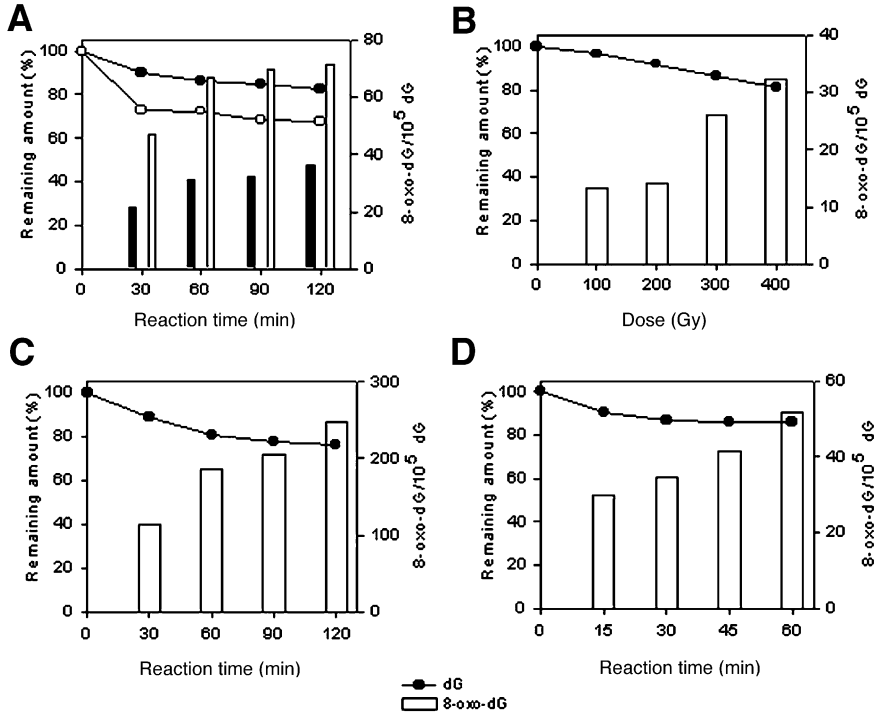

Fig. 1. The formation of 8-oxo-dG from dG in various radical-producing systems. 0.01 mM of dG was treated with (A) 24 mM hydrogen peroxide and 20 mM sodium hypochlorite at room temperature (open symbols, in D_2O; closed symbols, in H_2O), (B) ^{137}Cs γ-ionizing radiation, (C) 0.1 mM cupric sulfate, 0.1 mM ascorbic acid and 5 mM hydrogen peroxide at 37°C, and (D) 0.1 mM sodium peroxynitrite at room temperature. The amounts of 8-oxo-dG were measured by HPLC-UV or ECD and then expressed as the number of 8-oxo-dG per 10^5 dG.

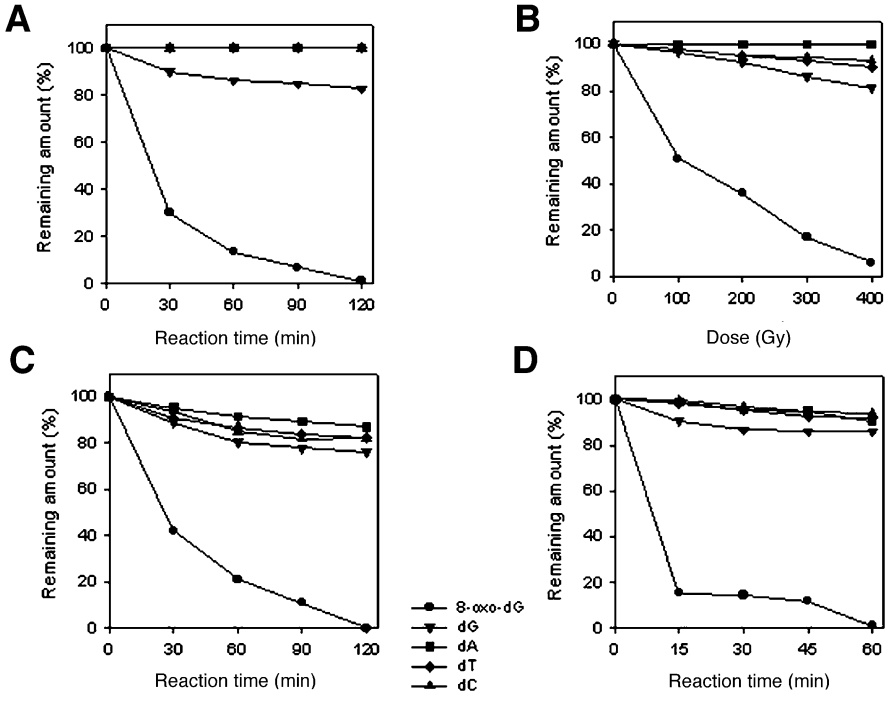

Fig. 2. The degradation of 8-oxo-dG and normal deoxynucleosides by various radical-producing systems. 0.01 mM of each deoxynucleoside was treated with (A) 24 mM hydrogen peroxide and 20 mM sodium hypochlorite at room temperature, (B) ^{137}Cs γ-ionizing radiation, (C) 0.1 mM cupric sulfate, 0.1 mM ascorbic acid and 5 mM hydrogen peroxide at 37°C, and (D) 0.1 mM sodium peroxynitrite at room temperature. The remaining amount of each deoxynucleoside was measured by HPLC-UV.

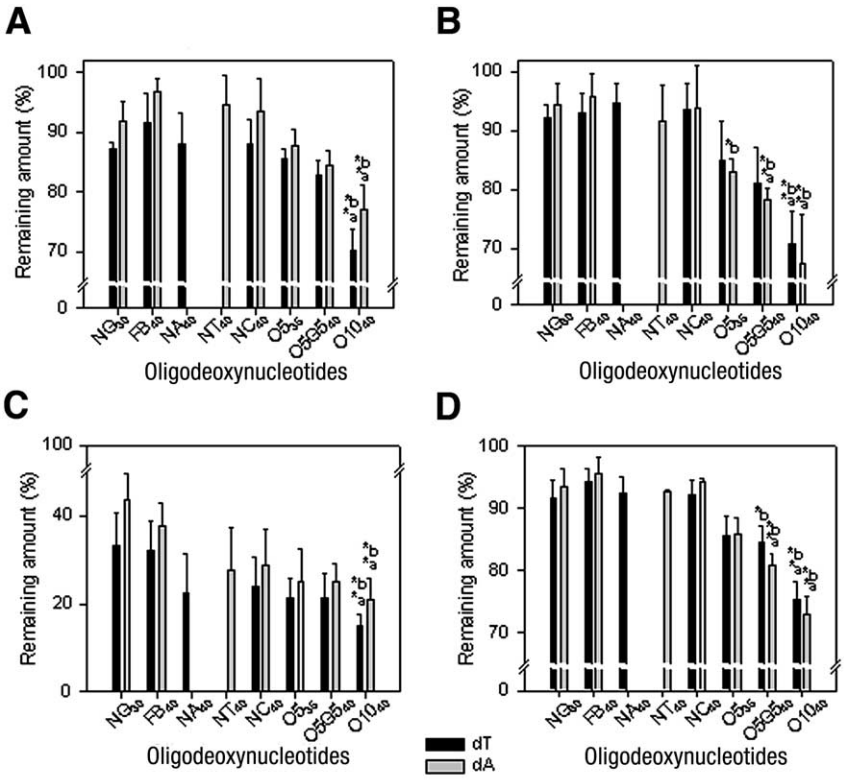

Fig. 3. The degradation of dT and dA in oligodeoxynucleotides containing 8-oxo-G by various radical-producing systems. One μM of each oligodeoxynucleotide was treated with (A) 24 mM hydrogen peroxide and 20 mM sodium hypochlorite at room temperature for 120 min, (B) ^{137}Cs γ-ionizing radiation at 400 Gy, (C) 0.1 mM cupric sulfate, 0.1 mM ascorbic acid and 5 mM hydrogen peroxide at room temperature for 20 min, and (D) 0.1 mM sodium peroxynitrite at room temperature for 60 min. After digestion of oligodeoxynucleotide to deoxynucleoside, the remaining amounts of dT and dA were detected by HPLC-UV. Data are expressed as means ± S.E.M. $^{*a}P < 0.05$, $^{*b}P < 0.05$; significantly different from oligo NG_{30} and oligo FB_{40}, respectively.

all radical-producing systems. Deuterium oxide was used to confirm that singlet oxygen was formed in the reaction between hypochlorite and hydrogen peroxide. Deuterium oxide is known to extend the half-life of singlet oxygen. As shown in Fig. 1A, the degradation rate of dG in D_2O was faster than that of dG in H_2O. Furthermore, the formation of 8-oxo-dG increased about two-fold when D_2O was replaced with H_2O.

However, 8-oxo-dG was found to be unstable under various oxidation conditions. When the four normal deoxynucleosides and 8-oxo-dG were treated in all radical-producing systems studied, 8-oxo-dG was found to have the greatest reactivity (Fig. 2). Of the four normal deoxynucleosides, dG was reduced the fastest. These findings indicate that guanine is the most reactive as compared to other bases. It is easily converted into 8-oxo-G, one of modified guanine adducts, and in turn 8-oxo-G is more reactive than the parent guanine.

Treatment with superoxide produced from the gamma-radiolysis in formate solution or with hydrogen peroxide did not lead to the degradation of deoxynucleosides (data not shown).

3.2. Damage of intramolecular oligodeoxynucleotides containing 8-oxo-G

Having confirmed the high reactivity of 8-oxo-G toward various radical species, we investigated its effects on other base components within DNA under oxidation conditions. The deoxynucleoside compositions of the oxidized oligodeoxynucleotides containing 8-oxo-G were analyzed after treatment with singlet oxygen, high-dose ionizing radiation, Fenton reagent or peroxynitrite. After digestion of the oxidized oligodeoxynucleotides and their control strands to the deoxynucleoside level, the amounts of intact dT and dA were detected. The results showed that the remaining amounts of dT and dA in the oligodeoxynucleotide (oligo $O10_{40}$) containing 10 8-oxo-G were significantly lower than that of an oligodeoxynucleotide containing no guanine and 10 guanines (oligo

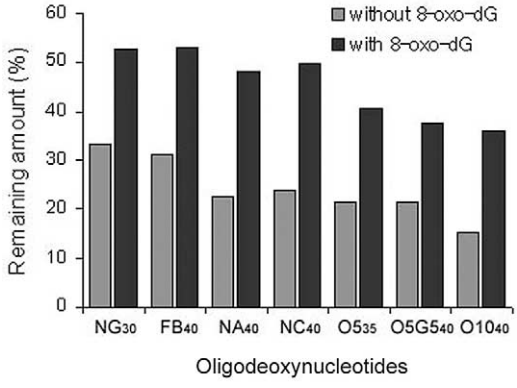

Fig. 4. The inhibition of oligodeoxynucleotide damage by free 8-oxo-dG. One μM of each oligodeoxynucleotide was treated with 0.1 mM cupric sulfate, 0.1 mM ascorbic acid and 5 mM hydrogen peroxide at room temperature for 20 min in the absence or presence of 0.1 mM of 8-oxo-dG. After digestion of oligodeoxynucleotide to deoxynucleoside, the remaining amount of dT was detected by HPLC-UV.

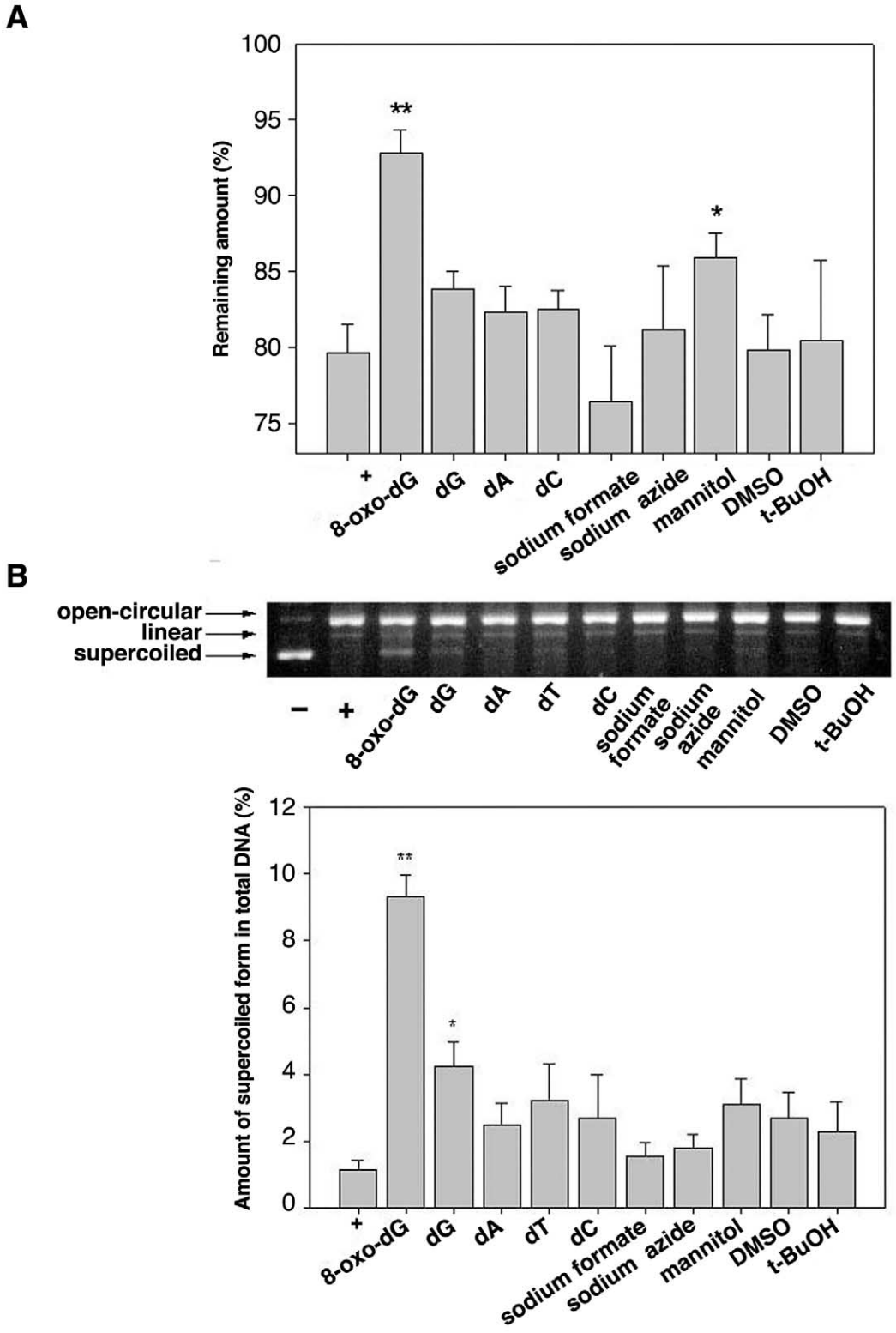

Fig. 5. The protective effects of deoxynucleosides and hydroxyl radical scavengers on (A) dT degradation and (B) plasmid cleavage. (A) 0.01 mM of dT was incubated with 0.1 mM cupric sulfate, 0.1 mM ascorbic acid and 5 mM hydrogen peroxide at 37°C for 120 min in the presence of 0.2 mM of each deoxynucleoside or hydroxyl radical scavenger, i.e. sodium azide, sodium formate, mannitol, DMSO and *tert*-butyl alcohol. The remaining amount of dT was detected by HPLC-UV. (B) Supercoiled DNA was incubated at room temperature for 10 min in the presence of 4 mM of each deoxynucleoside or hydroxyl radical scavenger. The open circular and linear DNAs were separated by agarose gel electrophoresis. The amounts of supercoiled DNA were estimated from the photograph and expressed as a percentage of the total DNA. Data are expressed as means ± S.E.M. $^{**}P < 0.01$, $^{*}P < 0.05$; significantly different from the positive control; −, negative control; +, positive control.

NG_{30} and FB_{40}, respectively) (*[a]P and *[b]$P < 0.05$, Fig. 3). Of the three oligodeoxynucleotides containing 8-oxo-G (oligo $O5_{35}$, $O5G5_{40}$ and $O10_{40}$), the oligodeoxynucleotide containing 10 8-oxo-G (oligo $O10_{40}$) showed the lowest amount of dT and dA after treatment.

3.3. Inhibition of intermolecular damage by 8-oxo-G

The effect of 8-oxo-G on intermolecular damage was determined by treating oligodeoxynucleotide, free dT or plasmid DNA with Fenton reagent in the presence of free 8-oxo-dG. As shown in Fig. 4, the amount of intact dT in the oxidized oligodeoxynucleotides was detected to see the effect of highly reactive 8-oxo-G intermolecularly. The degradation of dT was inhibited by free 8-oxo-dG. Furthermore, the capacity of 8-oxo-dG to inhibit degradation of free dT and cleavage of plasmid DNA by Fenton reagent was compared to that of each of the other deoxynucleosides or hydroxyl radical scavengers. The remaining amount of intact dT increased significantly in the presence of 8-oxo-dG (**$P < 0.01$) as well as mannitol (*$P < 0.05$) (Fig. 5A). As shown in Fig. 5B, the isolated supercoiled plasmid was converted to the open circular form on treatment with Fenton reagent. Under the same condition, 8-oxo-dG was found to most efficiently protect plasmid cleavage (**$P < 0.01$). dG was also found to act as an efficient radical scavenger (*$P < 0.05$). The inhibitory effect of deoxynucleosides on plasmid cleavage decreased in the following order: 8-oxo-dG > dG > dT > dC > dA, which is similar to the order of reactivity to Fenton reagent (Fig. 2C).

4. Discussion

The present study confirms that 8-oxo-G is highly reactive toward various radical species. The susceptibility of 8-oxo-G to oxidation was evidenced by its rapid degradation when treated with singlet oxygen, high-dose ionizing radiation, Fenton reagent, or peroxynitrite (Fig. 2). In this study, we examined whether this high reactivity of 8-oxo-G to oxidation has a harmful or beneficial effect on DNA. Our results show that 8-oxo-G present within DNA induces other base damages, whereas free 8-oxo-G protects DNA from damage under oxidation conditions. The damage-inducing effect of 8-oxo-G on intramolecular DNA under various oxidation conditions was supported by the observation that the remaining amounts of intact nucleosides in oxidized oligodeoxynucleotides decreased depending on the number of 8-oxo-G moieties within the oligodeoxynucleotide (Fig. 3). In contrast to its damage-inducing effect, the protective effect of free 8-oxo-G on intermolecular DNA damage under the oxidation condition was supported by the finding that oligodeoxynucleotide damage and plasmid cleavage were significantly inhibited in the presence of 8-oxo-dG (Figs. 4 and 5). These findings indicate that 8-oxo-G induces base damage of intramolecular DNA due to its facile oxidation, and suggest that once 8-oxo-G is removed from DNA, this free 8-oxo-G protects DNA against further oxidative damage.

Once 8-oxo-G is formed in DNA by either direct oxidation or by the incorporation of 8-oxo-dGTP, it represents a premutagenic lesion since it promotes the incorporation of dATP residues opposite the lesion during replication and leads to transversion mutation (GC→TA) [29]. In addition to the mutagenic action of 8-oxo-G, its damage-inducing effect on DNA, as observed in the present study, is also a harmful action of 8-oxo-G within DNA. Koizume et al. [30] also found that DNA damage by $KMnO_4$ is initiated the oxidation of 8-oxo-G. However, this lesion is removed in eukaryotic cells by the repair enzyme, 8-oxoguanine glycosylase 1 (OGG1) [31–33]. But if this repair activity is inadequate, it leads to further DNA damage and may trigger cell death. Therefore, OGG1 may play an important role in the prevention both of mutagenesis by mismatch and of further neighboring base damage by 8-oxo-G oxidation.

From a different point of view, 8-oxo-G in DNA, which escapes from the repair system and is not excised, is very labile to oxidation, and thus forms a variety of detrimental lesions, such as cyanuric acid, oxaluric acid or oxazolones by singlet oxygen oxidation [16,34,35] or peroxynitrite oxidation [36–38] and guanidinohydantoin, iminoallantoin, or spiroiminodihydantoin by one-electron oxidation [39,40]. We also identified several degradation products of 8-oxo-G by photooxidation with methylene blue. Oxaluric acid, spiroiminodihydantoin and iminoallantoin were determined by LC-ESI-MS (liquid chromatography electospray ionization mass spectrometry) (unpublished data). These lesions have mutagenic and/or DNA synthesis blocking effects [16,41–43]. Therefore, when 8-oxo-G is abundantly present due to oxidative stress, it results in severe DNA damage and finally mutagenesis. For this reason, the 8-oxo-G formed in DNA should be rapidly removed by OGG1 in order to avoid further mutation.

In this context, we raise the question about the action of 8-oxo-G released from DNA to cytosol. In this study, we used deoxynucleoside 8-oxo-dG instead of base 8-oxo-G for solubility reason. The plasmid cleavage caused by the Fenton reagent was inhibited in the presence of free 8-oxo-dG. The mechanism probably involves that the intermolecular electron transfer between free 8-oxo-dG and plasmid DNA inhibits the intramolecular electron transfer within plasmid DNA. The present study supports the possibility that if the repair activity is adequate, 8-oxo-G excised from DNA by OGG1 may act as a radical scavenger in an oxidizing environment and consequently protect the DNA from further oxidative damage.

8-Oxo-dG in urine or in cellular DNA has been used as a useful biomarker for detecting oxidative DNA damage [14,15]. In the case of urinary 8-oxo-dG, its increased excretion has been equated to the presence of high levels of oxidative stress, since it is considered as evidence of elevated DNA damage. However, it is uncertain whether urinary 8-oxo-dG originates from the repair of oxidized DNA or from the hydrolysis of oxidized guanine nucleotides. 8-Oxo-dG can be released from 8-oxo-dGTP by sequential actions of hMTH (human MutT(8-oxo-GTPase) homolog) and nucleotidase [44]. In addition, more recently, it has been suggested that there are nucleotide excision repair and specific endonuclease which release 8-oxo-dG nucleoside, not 8-oxo-G base [45]. Therefore, though 8-oxo-dG in urine has been believed to represent oxidative stress status, free 8-oxo-dG may imply high repair activity against oxidative damage [46]. Moreover, in the context of protective role of 8-oxo-dG, the released 8-oxo-dG in urine may have a role as an efficient radical scavenger. On the basis of these findings, a free base or nucleoside containing an oxoguanine moiety in the cytosol can be interpreted as a 'good phenomenon'.

In the present study, we showed the high reactivity of 8-oxo-G toward various radical species. This observed reactivity of 8-oxo-G results in further intramolecular DNA dam-

age, and also contributes to the protection of intermolecular DNA damage during oxidative stress Therefore, our results suggest that 8-oxo-G in DNA must be rapidly removed from DNA to avoid further DNA damage and that 8-oxo-G released to cytosol may have a protective effect as a scavenger. 8-Oxo-G can be interpreted as a 'suicide' molecule in that 8-oxo-G is attacked preferentially by oxidative stress and sacrifices itself to protect other molecules.

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