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Hydroxylation of Deoxyguanosine at 5′ Site of GG and GGG Sequences in Double-stranded DNA Induced by Carbamoyl Radicals

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Free radicals generated by chemicals can cause sequence-specific DNA damage and play important roles in mutagenesis and carcinogenesis. Carbamoyl group ($CONH_2$) and its derived groups ($CONR_2$) occur as natural products and synthetic chemical compounds. We have investigated the DNA damage by carbamoyl radicals $^{\cdot}(CONH_2)$, one of carbon-centered radicals. Electron spin resonance (ESR) spectroscopic study has demonstrated that carbamoyl radicals were generated from formamide by treatment with H_2O_2 plus Cu(II), and from azodicarbonamide by treatment with Cu(II). We have investigated sequence specificity of DNA damage induced by carbamoyl radicals using ^{32}P-labeled DNA fragments obtained from the human c-Ha-*ras*-1 and *p*53 genes. Treatment of double-stranded DNA with carbamoyl radicals induced an alteration of guanine residues, and subsequent treatment with piperidine or Fpg protein led to chain cleavages at 5′-G of GG and GGG sequences. Carbamoyl radicals enhanced Cu(II)/H_2O_2-mediated formation of 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG) in double-stranded DNA more efficiently than that in single-stranded DNA. These results shows that carbamoyl radicals specifically induce hydroxylation of deoxyguanosine at 5′ site of GG and GGG sequences in double-stranded DNA.

Keywords: Formamide; DNA damage; Hydrogen peroxide; Copper

Abbreviations: DTPA, diethylenetriamine-*N*,*N*,*N*′,*N*″,*N*‴-pentaacetic acid; HPLC, high-performance liquid chromatography; HPLC–ECD, HPLC equipped with an electrochemical detector; 8-oxodG, 8-oxo-7,8-dihydro-2′-deoxyguanosine; POBN, α-(1-oxy-4-pyridyl)-N-*tert*-butyl-nitrone; Fpg, *E-coli* formamidopyrimidine-DNA glycosylase; MO, molecular orbital; HOMO, highest occupied molecular orbital; SCE, saturated calomel electrode

INTRODUCTION

Oxidative stress is capable of causing damage to various cellular constituents, leading to many diseases. Particularly, DNA damage mediated by oxidative stress plays critical roles in carcinogenesis.[1–8] In biological systems, although damaged DNA is repaired enzymatically and the cells regain their normal functions, misrepair of DNA damage could result in mutations, leading to carcinogenesis.[9–12] The sequence specificity of DNA damage plays the key role in the mutagenic processing.[13] Therefore, investigation on sequence specificity of DNA damage would provide us biological significance of DNA damage and beneficial findings for cancer prevention.

Redox potentials of reactive species contribute to the determination of sequence-specific damage to double-stranded DNA. Highly reactive radicals, $^{\cdot}OH$ directly abstracts a hydrogen atom from the DNA deoxyribose-phosphate backbone, resulting in DNA cleavage at every nucleotide without marked site specificity.[14–16] $^{\cdot}OH$ also causes addition and hydrogen abstraction of the DNA bases leading to generation of a variety of oxidative products. Less reactive radicals rather than $^{\cdot}OH$, such as SO_4^{-}[17] and benzoyloxyl radicals[18] cause sequence-specific DNA oxidation depending on their redox potentials. Various organic compounds, endogenous molecules and foods ingredients, can produce their radicals

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under certain conditions.[19–21] Therefore, an investigation of the reactivity of their radicals to cause DNA damage is important to predict their participation in mutagenesis and carcinogenesis.

Carbamoyl group ($CONH_2$) and its derived groups ($CONR_2$) occur as natural products and synthetic chemical compounds, which may produce carbamoyl radicals $^{\cdot}(CONH_2)$, carbon-centered radicals, *in vivo*. In this study, carbamoyl radicals generated from formamide by treatment with H_2O_2 plus Cu(II), and azodicarbonamide by treatment with Cu(II) were detected using an electron spin resonance (ESR) spectrometer. We investigated sequence-specificity of DNA damage caused by carbamoyl radicals derived from formamide and azodicarbonamide, using 5′-end-labeled DNA fragments obtained from the human *p*53 tumor suppressor gene and the c-Ha-*ras*-1 protooncogene. We also analyzed 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG) formation in calf thymus DNA by the carbamoyl radicals from formamide.

MATERIALS AND METHODS

Materials

Restriction enzymes (*Apa* I, *Ava* I, *Eco* RI, *Hind* III, *Pst* I, *Sty* I and *Xba* I) and T4 polynucleotide kinase were purchased from New England Biolabs. Calf intestine phosphatase was from Boehringer Mannheim GmbH. [γ-^{32}P]ATP (222 TBq/mmol) was from New England Nuclear. Diethylenetriamine-*N*,*N*,*N*′,*N*″,*N*″-pentaacetic acid (DTPA) was purchased from Dojin Chemicals Co. (Kumamoto, Japan). Acrylamide, bisacrylamide, piperidine were from Wako Chemicals Co. (Osaka, Japan). Ethanol, copper dichloride dihydrate and D-mannitol were from Nacalai Tesque Inc. (Kyoto, Japan). Superoxide dismutase (SOD, 3000 units/mg from bovine erythrocytes), methional and catalase (45000 units/mg from bovine liver) were from Sigma Chemical Co. Nuclease P1 was from Yamasa Shoyu Co. (Chiba, Japan). α-(1-Oxy-4-pyridyl)-N-*tert*-butyl-nitrone (POBN) was from Aldrich Co. E. coli formamidopyrimidine-DNA glycosylase (Fpg) was from Trevigen.

Detection of Carbamoyl Radicals Derived from Formamide and Azodicarbonamide

ESR spectra were recorded to detect carbamoyl radicals derived from formamide and azodicarbonamide. The spectra were measured at 25°C using a JES-TE100 (JEOL, Tokyo, Japan) spectrometer with 100 kHz field modulation. The spectra were recorded with a microwave power of 4 mW and a modulation amplitude of 50 μT.

Preparation of ^{32}P-5′-end-labeled DNA Fragments

DNA fragments were obtained from the human *p*53 tumor suppressor gene[22] and the c-Ha-*ras*-1 protooncogene.[23] The DNA fragment of the *p*53 tumor suppressor gene was prepared from pUC18 plasmid, ligated fragments containing exons of *p*53 gene. A singly ^{32}P-5′-end-labeled 118-bp fragment (*Hind* III* 13038-*Sty* I 13155) and a 211-bp fragment (*Hind* III* 13972-*Apa* I14182) were obtained according to the method described previously.[24] DNA fragments were also prepared from plasmid pbcNI, which carries a 6.6-kb BamHI chromosomal DNA segment containing the human c-Ha-*ras*-1 protooncogene. A singly labeled 341-base pair fragment (*Xba* I 1906-*Ava* I* 2246), a 337-base pair fragment (*Pst* I 2345-*Ava* I* 2681), a 261-bp fragment (*Ava* I* 1645-*Xba* I 1905) and a 98-bp fragment (*Ava* I* 2247-*Pst* I 2344) were obtained according to the method described previously.[25,26] Nucleotide numbering starts with the *Bam* HI site.[23] The asterisk indicates ^{32}P-labeling.

Detection of DNA Damage by Formamide and Azodicarbonamide

The standard reaction mixture (in a microtube; 1.5 ml; Eppendorf) contained ^{32}P-5′-end-labeled DNA fragment (< 1 μM), calf thymus DNA, 20 μM Cu(II) and formamide plus H_2O_2 or azodicarbonamide in 200 μl of 10 mM sodium phosphate buffer (pH 7.8) containing 5 μM DTPA. DTPA was added to remove trace metals which could be contaminated in sodium phosphate buffer. After incubation at 37°C for the indicated duration, the DNA fragments were heated at 90°C in 1 M piperidine for 20 min where indicated and treated as described previously.[27] After incubation, instead of piperidine treatment, the DNA fragments were treated with 6 units of Fpg protein in 10 μl of the reaction buffer [10 mM HEPES-KOH (pH 7.4), 100 mM KCI, 10 mM EDTA, and 0.1 mg/ml BSA] at 37°C for 120 min. Subsequently, the Fpg-treated DNA fragments were precipitated, and treated as described previously.[27] The preferred cleavage sites were determined by direct comparison of the positions of the oligonucleotides with those produced by the chemical reactions of the Maxam-Gilbert procedure[28] using a DNA-sequencing system (LKB 2010 Macrophor). A laser densitometer (LKB 2222 UltroScan XL) was used for the measurement of the relative amounts of oligonucleotides from treated DNA fragments.

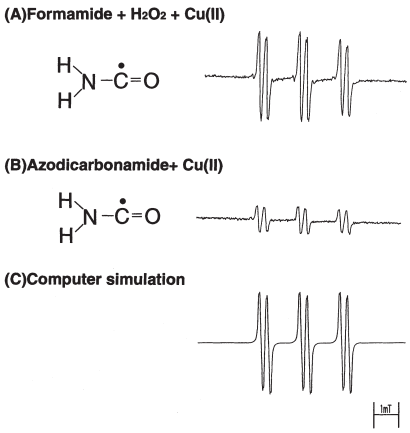

FIGURE 1 ESR spectra of the carbamoyl radicals adduct of POBN produced by formamide plus H_2O_2 or azodicarbonamide in the presence of Cu(II). The reaction mixture contained 20% (v/v) formamide plus 10 mM H_2O_2 (A) or 4.5 mM azodicarbonamide (B), 400 mM POBN and 20 μM $CuCl_2$ in 100 μl of 10 mM Tris–HCl buffer (pH 7.8) containing 5 μM DTPA, were incubated for 10 min (A) or 60 min (B) at 37°C. The ESR spectra were measured immediately after incubation. Computer simulation with the coupling constants of $a^{N(POBN)} = 1.499$ mT, $a^{H(POBN)} = 0.277$ mT; $a^{N(carbamoyl)} = 0.022$ mT and $a^{H(carbamoyl)} = 0.055$ mT, using Isotropic EPR Simulation Ver. 2.2A (Labotec Co., Ltd.) (C).

Analysis of 8-oxodG Formation in Calf Thymus DNA by Formamide in the Presence of H_2O_2 and Cu(II)

DNA fragments (100 μM/base) from calf thymus were incubated with formamide. H_2O_2 and Cu(II) for 60 min at 37°C. For DNA denaturation, the fragments were heated at 90°C for 5 min and then chilled quickly before incubation. After ethanol preciptation, DNA was digested to the nucleosides with nuclease P1 and calf intestine phosphatase and analyzed by HPLC–ECD, as previously described.[29]

RESULTS

Generation of Carbamoyl Radicals from Formamide and Azodicarbonamide

An ESR spectrum of a spin adduct of formamide-derived radicals was observed when POBN was added to a mixture solution of formamide, H_2O_2 and Cu(II) (Fig. 1A). The hyperfine splitting constants of the radicals were $a^{N(POBN)} = 1.499$ mT, $a^{H(POBN)} = 0.277$ mT, $a^{N(carbamoyl)} = 0.022$ mT, and $a^{H(carbamoyl)} = 0.055$ mT. The radicals with the same hyperfine splitting constants were observed when azodicarbonamide was treated with Cu(II) (Fig. 1B). Computer simulation pattern provided a satisfactory fit of signals obtained in this study (Fig. 1C). Addition of catalase, methional or bathocuproine to a mixture solution of formamide, H_2O_2, and Cu(II) decreased the yield of the radical adducts of POBN, whereas ethanol did not (data not shown). Methional can scavenge not only free ·OH but also other radicals like cryptohydroxyl radicals.[30] The inhibitory effects of scavenger and bathocuproine on the yield of formamide-derived radicals suggests that H_2O_2 and Cu(I) are involved in the generation of carbamoyl radicals from formamide.

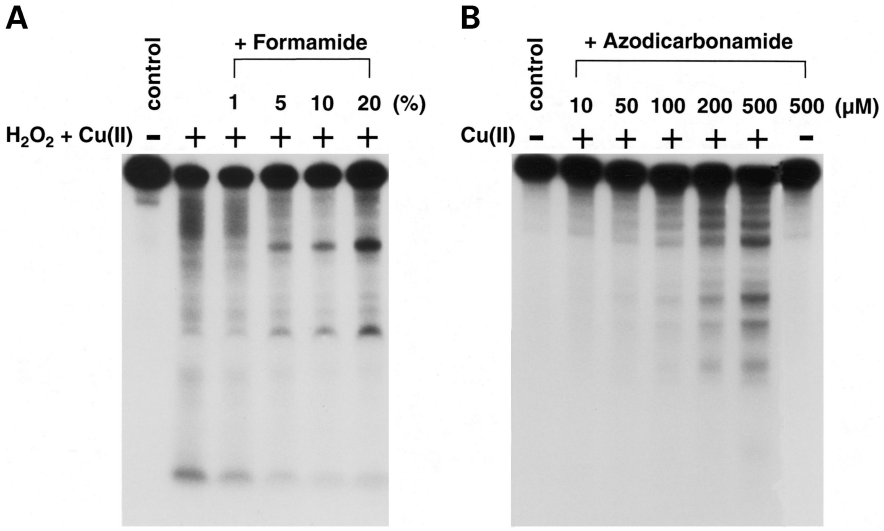

FIGURE 2 Autoradiogram of ^{32}P-DNA fragments incubated with formamide plus H_2O_2 or azodicarbonamide in the presence of Cu(II). (A) The reaction mixture contained ^{32}P-5′-end-labeled 98-bp DNA fragments, 10 μM/base of sonicated calf thymus DNA, indicated concentrations of formamide, 200 μM H_2O_2 and 20 μM $CuCl_2$ in 200 μl of 10 mM sodium phosphate buffer (pH 7.8) containing 5 μM DTPA. (B) The reaction mixture contained ^{32}P-5′-end-labeled 261-bp DNA fragments, 10 μM/base of sonicated calf thymus DNA, indicated concentrations of azodicarbonamide and 20 μM $CuCl_2$ in 200 μl of 10 mM sodium phosphate buffer (pH 7.8) containing 5 μM DTPA. After incubation at 37°C for 60 min, the DNA fragments were treated with 1 M piperidine for 20 min at 90°C, then electrophoresed on an 8% polyacrylamide/8 M urea gel. The autoradiogram was obtained by exposing the gel to an X-ray film.

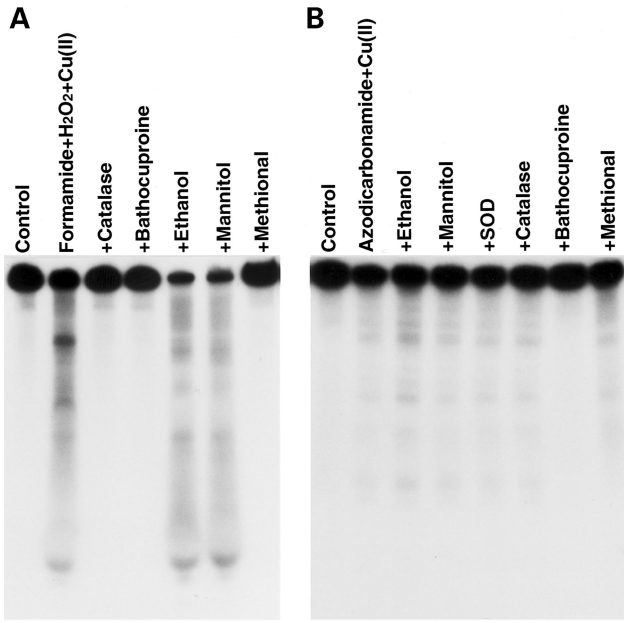

FIGURE 3 Effects of scavengers and metal chelator on Cu(II)-dependent DNA damage induced by formamide plus H_2O_2 or azodicarbonamide. (A) The reaction mixture contained ^{32}P-5′-end-labeled 98-bp DNA fragments, 10 μM/base of sonicated calf thymus DNA, 10% (v/v) formamide, 200 μM H_2O_2 and 20 μM $CuCl_2$ in 200 μl of 10 mM sodium phosphate buffer (pH 7.8) containing 5 μM DTPA. (B) The reaction mixture contained ^{32}P-5′-end-labeled 261-bp DNA fragments, 10 μM/base of sonicated calf thymus DNA, 200 μM azodicarbonamide and 20 μM $CuCl_2$ in 200 μl of 10 mM sodium phosphate buffer (pH 7.8) containing 5 μM DTPA. The mixture was incubated at 37°C for 60 min, and treated by the method described in Fig. 2. The concentration of scavengers and metal chelator was as follows: 30 units of catalase; 30 units of SOD; 0.1 M methional; 5% (v/v) ethanol; 0.1 M mannitol; 50 μM bathocuproine.

Cu(II)-mediated Damage of ^{32}P-labeled DNA Fragments by Formamide plus H_2O_2 and Azodicarbonamide

Figure 2A shows an autoradiogram of DNA fragments treated with formamide in the presence of H_2O_2 and Cu(II). H_2O_2 and Cu(II) in the absence of formamide could damage DNA.[27] When formamide was added, the DNA cleavage patterns were changed. The site specificity of DNA damage by formamide was quite different from that induced by H_2O_2 and Cu(II) (described in latter). The patterns of DNA cleavage changed depending on concentrations of formamide, although totally DNA damage did not increased. This result suggests that formamide inhibits DNA damage induced by H_2O_2 and Cu(II), however, induces site specific DNA damage through radicals generated by the reaction of formamide with H_2O_2 and Cu(II). On the other hand, azodicarbonamide induced DNA damage in the presence of Cu(II) (Fig. 2B). The intensities of DNA damage increased depending on concentrations of azodicarbonamide.

Effects of Scavengers and Bathocuproine on DNA Damage Induced by Formamide and Azodicarbonamide

Figure 3 shows the effects of scavengers and bathocuproine, a Cu(I)-specific chelator, on DNA damage induced by formamide in the presence of H_2O_2 plus Cu(II), and by azodicarbonamide in the presence of Cu(II). Typical $^{\cdot}$OH scavengers, ethanol and mannitol did not inhibit DNA damage induced by formamide in the presence of H_2O_2 and Cu(II) (Fig. 3A). Methional, catalase, and bathocuproine inhibited DNA damage induced by formamide in the presence of H_2O_2 and Cu(II). DNA damage induced by azodicarbonamide in the presence of Cu(II) was inhibited by bathocuproine (Fig. 3B), whereas typical OH scavengers, methional, catalase, and SOD did not inhibit the DNA damage. No inhibitory effect of methional on this DNA damage suggests that azodicarbonamide-derived radicals are neither free $^{\cdot}$OH nor cryptohydroxyl radicals.

Site Specificity of Cu(II)-mediated DNA Cleavage by Formamide plus H_2O_2 and Azodicarbonamide

An autoradiogram was obtained and scanned with a laser densitometer to measure relative intensity of DNA cleavage in the human *p*53 tumor suppressor gene and the c-Ha-*ras*-1 protooncogene as shown in Figs. 4 and 5. Figure 4 shows a comparison of site specificity of Cu(II)-mediated DNA cleavage by H_2O_2 and that by formamide plus H_2O_2. H_2O_2 and Cu(II) induced cleavage at cytosine, thymine and guanine residues (Fig. 4A). Formamide in the presence of H_2O_2 and Cu(II) (Figs. 4B and 5A) induced piperidine-labile site at 5′-G in GG and GGG sequences in both double-stranded DNA fragments from the human *p*53 tumor suppressor gene and the c-Ha-*ras*-1 protooncogene. When azodicarbonamide was used instead of formamide and H_2O_2, similar results were observed (Fig. 5B). When denatured single-stranded DNA was used, preferential damage occurred at guanine by treatment with H_2O_2 plus Cu(II) in the presence or absence of formamide (Figs 4C and D).

Site Specificity of Cu(II)-mediated DNA Cleavage by Formamide plus H_2O_2 Treated with Fpg Protein

Figure 6 shows the comparison of site specificity of Cu(II)-mediated DNA cleavage by formamide plus H_2O_2 treated with piperidine and Fpg protein. Formamide in the presence of H_2O_2 and Cu(II) caused preferential DNA damage at 5′G in GG and GGG sequences in double-stranded DNA fragments, by both Fpg protein treatment (Fig. 6A) and piperidine treatment (Fig. 6B). When azadicarbonamide was used instead of formamide and H_2O_2,

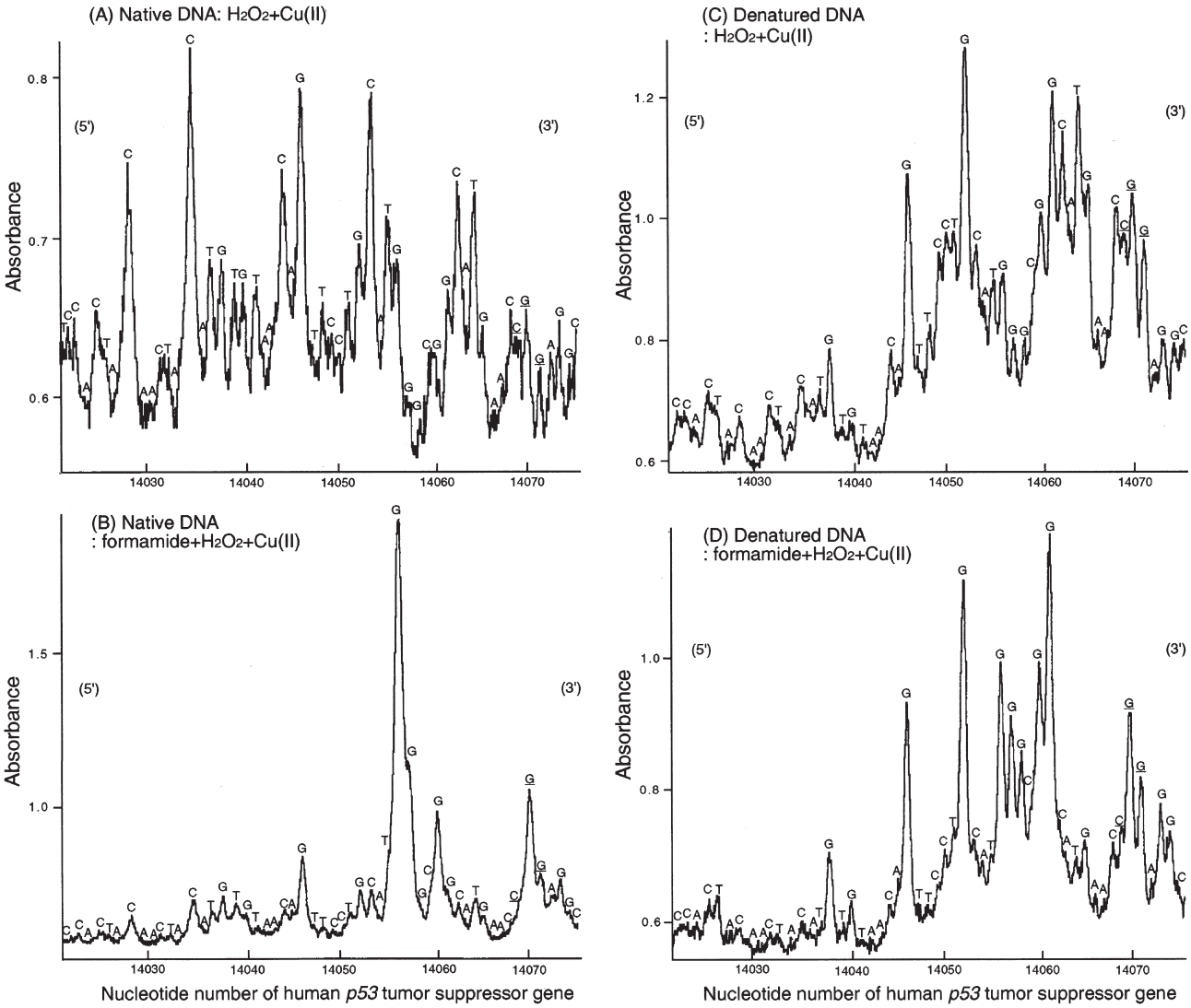

FIGURE 4 Comparison of cleavage sites in double-stranded DNA and in single-stranded DNA induced by H_2O_2 or formamide plus H_2O_2 in the presence of Cu(II). The reaction mixture containing the ^{32}P-5′-end-labeled 211-bp DNA fragment, 10 μM/base of calf thymus DNA, 20 μM $CuCl_2$ and 50 μM H_2O_2 (A) and (C), 10% (v/v) formamide plus 100 μM H_2O_2 (B) and (D), in 10 mM phosphate buffer (pH 7.8) containing 5 μM DTPA was incubated at 37°C for 60 min (native DNA) or 30 min (denatured DNA). For the experiment with denatured single-stranded DNA, 5′-end-labeled DNA fragment was treated at 90°C for 5 min and quickly chilled before the addition of the agents. After piperidine treatment, DNA cleavage sites were determined as described in Fig. 2. The relative amounts of oligonucleotide were measured by scanning the autoradiogram with a laser densitometer (LKB 2222 UltroScan XL, Pharmacia Biotech). The horizontal axis shows the nucleotide number of the *p*53 tumor suppressor gene.[22]

similar results were observed (data not shown). Fpg protein is known to recognize 8-oxodG as well as Fapy residues.[31] Without Fpg protein treatment, formamide plus H_2O_2 and azodicarbonamide did not cause oligonucleotides formation (data not shown). These results suggest that carbamoyl radicals do not induce the breakage of deoxyribose-phosphate backbone, but induce the base alteration, and produce 8-oxodG in double-stranded DNA fragments.

Formation of 8-oxodG in Calf Thymus DNA by Formamide in the Presence of H_2O_2 and Cu(II)

We measured 8-oxodG content in calf thymus DNA treated with formamide in the presence of H_2O_2 and Cu(II) using an HPLC–ECD (Fig. 7). The addition of formamide increased the amount of 8-oxodG formation induced by H_2O_2 and Cu(II) in native double-stranded DNA, whereas formamide decreased the amount of 8-oxodG formation in denatured single-stranded DNA.

DISCUSSION

In the present study, the formamide-derived radicals were detected with a trapping agent POBN in the presence of H_2O_2 and catalytic amount of Cu(II). On treatment with H_2O_2 plus catalytic amounts of Fe(II), formamide produces carbamoyl radicals.[32] Since catalytic activity of copper was found to be more

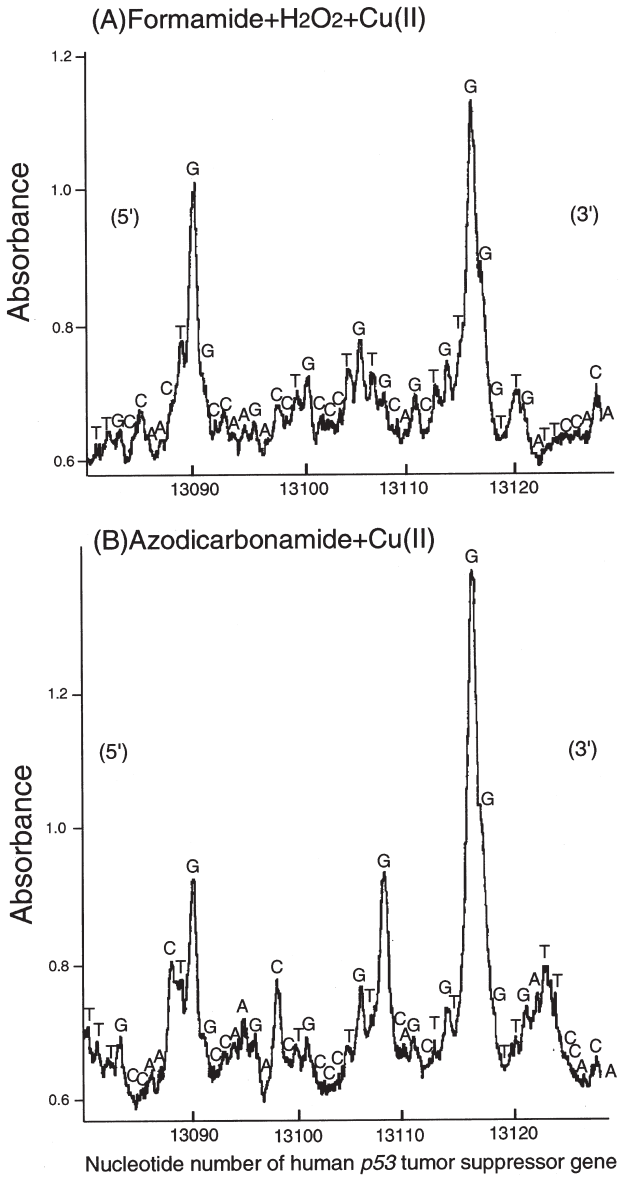

FIGURE 5 Site specificity of Cu(II)-dependent DNA cleavage induced by formamide plus H_2O_2 or by azodicarbonamide. The reaction mixture containing the 32P-5′-end-labeled 118-bp DNA fragment, 10 μM/base of calf thymus DNA, 20 μM $CuCl_2$ and 10% (v/v) formamide plus H_2O_2 (A) of 500 μM azodicarbonamide (B), in 10 mM phosphate buffer (pH 7.8) containing 5 μM DTPA was incubated for 60 min at 37°C. After piperidine treatment, DNA cleavage sites were determined as described in Fig. 4. The horizontal axis shows the nucleotide number of *p*53 tumor suppressor gene.[22]

than iron toward activation of H_2O_2,[33] we used copper instead of iron. On the basis of the present study and the previous report,[33] we proposed a possible mechanism of the formation of carbamoyl radicals ($CONH_2$) by H_2O_2 and Cu(II) as follows:

$$H_2O_2 + 2Cu(II) \rightarrow O_2 + 2H^+ + 2Cu(I) \quad (1)$$

$$H_2O_2 + Cu(I) \rightarrow Cu(I)OOH + H^+ \quad (2)$$

$$HCONH_2 + Cu(I)OOH + H^+ \rightarrow {}^{\cdot}CONH_2 + H_2O + Cu(II) + OH^- \quad (3)$$

The ESR signal with the same hyperfine coupling constants of formamide-derived radicals were detected when azodicarbonamide was treated with catalytic amount of Cu(II), indicating that azodicarbonamide ($H_2NCON = NCONH_2$) also produces carbamoyl radicals by the catalysis of Cu(II) as follows:

$$H_2NCON = NCONH_2 \rightarrow 2\,{}^{\cdot}CONH_2 + N_2 \quad (4)$$

The present study showed that formamide caused sequence-specific damage to DNA in the presence of H_2O_2 plus Cu(II). H_2O_2 plus Cu(II) induced DNA damage especially at T and C residues in the absence of formamide as previously reported.[27] The addition of formamide changed the sequence-specificity of DNA damage by H_2O_2 plus Cu(II) from T and C residues to that 5′-G in GG and GGG sequences in double-stranded DNA. The similar sequence-specificity was observed when azadicarbonamide was used instead of formamide and H_2O_2. To confirm that carbamoyl radicals cause the sequence-specific DNA damage, we have examined the effects of scavengers on the DNA damage and the generation of carbamoyl radicals. Bathocuproine, catalase and methional inhibited the DNA damage induced by formamide in the presence of H_2O_2 and Cu(II), whereas typical ·OH scavengers did not inhibit it. The inhibitory effects of scavengers on the generation of carbamoyl radicals showed similar results. The correlation between the inhibitory effects of scavengers on the DNA damage and those on the carbamoyl radicals production lead us to speculate that carbamoyl radicals cause the sequence-specific DNA damage. Formamide rather than DNA bases should react with the react with the reactive species generated from H_2O_2 plus Cu(II) such as ·OH and/or Cu(I)OOH to produce carbamoyl radicals, because the concentration of formamide was much greater than that of DNA bases under the conditions used. Alternatively, a high concentration of formamide may induce denaturation of double-stranded DNA.[34,35] If so, the Cu(II)-mediated DNA cleavage pattern by H_2O_2 plus formamide in double-stranded DNA should be similar to that in single-stranded DNA. However, Fig. 4B and D showed a difference of formamide-mediated DNA cleavage pattern between single-stranded DNA and double-stranded DNA. Additionally, Fig. 7 showed that 8-oxodG formation by formamide in double-stranded DNA was higher than that of in single-stranded DNA. These results exclude the possibility of altered conformation or partial denaturation of double-stranded DNA by formamide.

On the basis of the reason given below, we have proposed a possible mechanism of sequence-specific DNA damage by carbamoyl radicals: a hydrogen abstraction or an electron transfer from 5′-G of GG

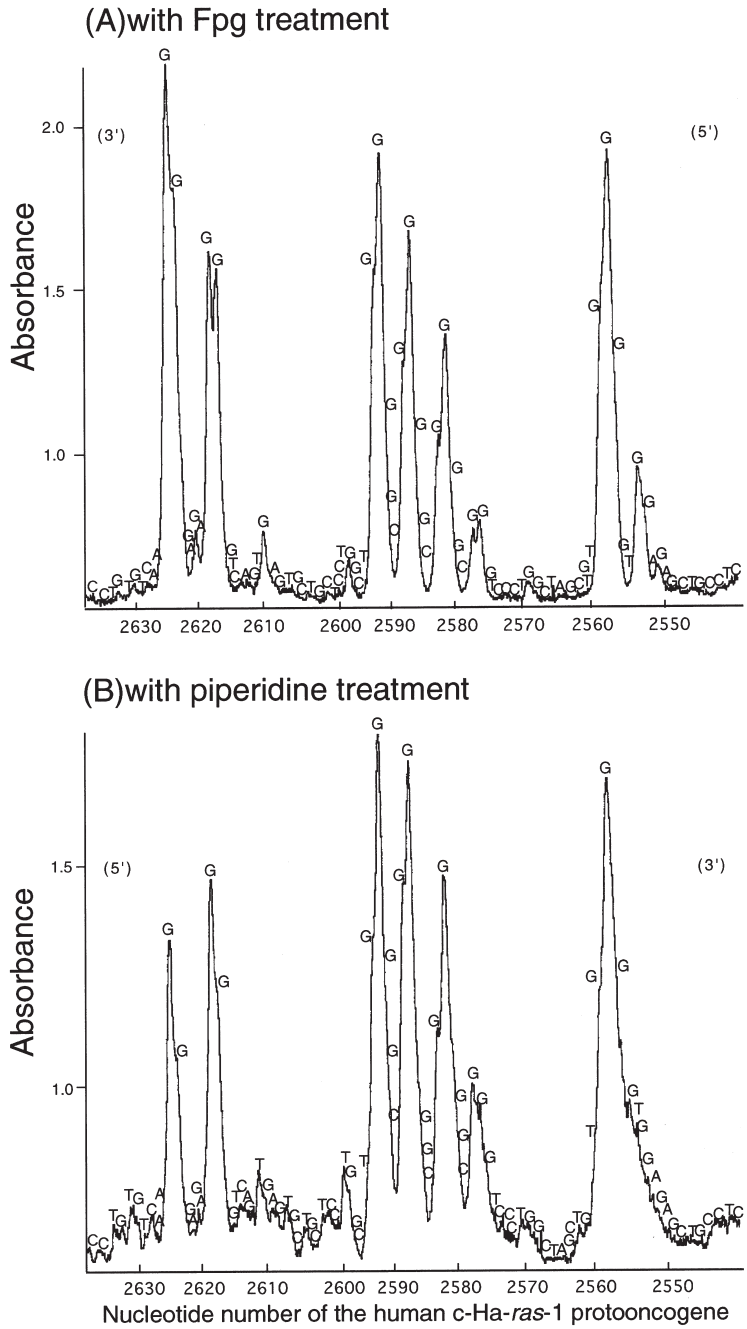

FIGURE 6 Site specificity of Cu(II)-dependent DNA cleavage induced by formamide plus H_2O_2. The reaction mixture contained ^{32}P-5′-end-labeled 337-bp DNA fragments, 1 μM/base of sonicated calf thymus DNA, 10% (v/v) formamide, 100 μM H_2O_2 and 20 μM $CuCl_2$ in 200 μl of 10 mM sodium phosphate buffer (pH 7.8) containing 5 μM DTPA. After incubation at 37°C for 60 min, the DNA fragments were treated with 6 units of Fpg protein at 37°C for 120 min (A) or piperidine treatment (B). DNA cleavage sites were determined as described in Fig. 4. The horizontal axis shows the nucleotide number of the c-Ha-*ras*-1 protooncogene.[23]

and GGG and followed by hydroxylation of the oxidized guanine to produce 8-oxodG. Highly reactive species such as $^{\cdot}OH$ and $ONOO^-$ cause DNA damage at every nucleotide, whereas less reactive species cause DNA damage specifically at guanines.[3] Guanine is most easily oxidized among the four DNA bases, because the oxidation potential of guanine is lower than the other DNA bases.[36,37] Recently, molecular orbital (MO) calculations have revealed that the GG sequence in double-stranded DNA has the lowest ionization potential among the guanine-containing dinucleotides.[38] A large part of the highest occupied molecular orbital (HOMO) is located on the 5′-G of GG and GGG sequences in double-stranded DNA,[38] and therefore, this guanine is likely to be oxidized. The sequence-specificity of DNA damage induced by reactive species less reactive than $^{\cdot}OH$ could be consistent

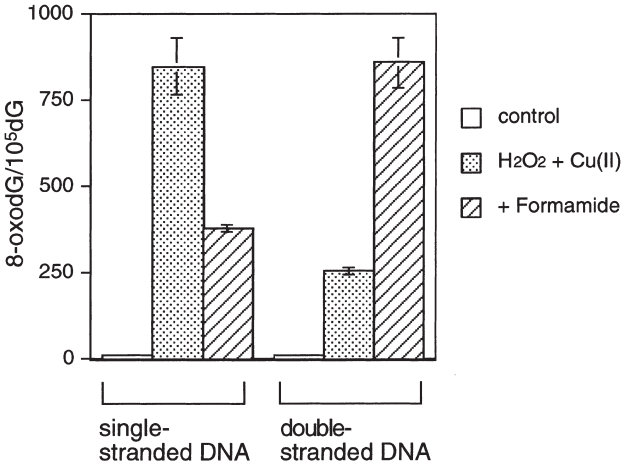

FIGURE 7 8-oxodG formation induced by formamide plus H_2O_2 in the presence of Cu(II). The reaction mixture contained calf thymus DNA fragments (100 μM/base), 20 μM $CuCl_2$, 20% (v/v) formamide and 20 μM H_2O_2 in 400 μl of 4 mM phosphate buffer (pH 7.8) containing 5 μM DTPA. For DNA denaturation, DNA was treated at 90°C for 5 min and quickly chilled before the addition of the agents. After incubation for 60 min at 37°C, DNA fragment was enzymatically digested into nucleosides, and 8-oxodG formation was measured with an HPLC–ECD as described in "Materials and Methods".

with HOMO distribution and its energy level. Redox potentials of various carbon-centered radicals ($E°(CR/^{-}CR)$) are estimated to be about −1 V vs Saturated Calomel Electrode (SCE).[39] They are clearly less reactive oxidant than $^{\cdot}OH$ ($E°(^{\cdot}OH, H^{+}/H_2O) = 2.63$ V vs SCE). Carbamoyl radicals, one of the carbon-centered radicals, are also considered to be less reactive oxidant than $^{\cdot}OH$. Therefore, carbamoyl radicals specifically oxidized the 5′-G of GG and GGG sequences in double-stranded DNA, although there remains the possibility that the radicals derived from carbamoyl radicals cause the DNA damage. The decrease in formation of 8-oxodG in single-stranded DNA can be reasonably explained by the result of MO calculations indicating that the 5′-G of GG and GGG in double-stranded DNA is easily oxidized than G in single-stranded DNA.[38,40]

It is concluded that carbamoyl radicals can cause sequence-specific DNA oxidation. Carbamoyl group ($CONH_2$) and its derived groups ($CONR_2$) occur as natural products and synthetic chemical compounds. We have obtained the experimental data that carcinogenic semicarbazide, which is one of the derivatives of hydrazine, generates carbamoyl radicals in the presence of Cu(II). The generation of this free radicals may relevant to the expression of carcinogenicity of semicarbazide. This study requires further investigation of chemical compounds, especially endogenous molecules and/or ingredients of foods, which produce carbamoyl radicals, from the standpoint of toxicology of organic radicals.

Acknowledgements

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