Articles

Site-Specific Oxidation at GG and GGG Sequences in Double-Stranded DNA by Benzoyl Peroxide as a Tumor Promoter†

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ABSTRACT: Benzoyl peroxide (BzPO), a free-radical generator, has tumor-promoting activity. As a method for approaching the mechanism of tumor promoter function, the ability of oxidative DNA damage by BzPO was investigated by using ^{32}P-labeled DNA fragments obtained from the human *p53* tumor suppressor gene and c-Ha-*ras*-1 protooncogene. BzPO induced piperidine-labile sites at the 5′-site guanine of GG and GGG sequences of double-stranded DNA in the presence of Cu(I), whereas the damage occurred at single guanine residues of single-stranded DNA. Both methional and dimethyl sulfoxide (DMSO) inhibited DNA damage induced by BzPO and Cu(I), but typical hydroxyl radical (•OH) scavengers, superoxide dismutase (SOD) and catalase, did not inhibit it. On the other hand, H_2O_2 induced piperidine-labile sites at cytosine and thymine residues of double-stranded DNA in the presence of Cu(I). Phenylhydrazine, which is known to produce phenyl radicals, induced Cu(I)-dependent damage at thymine residues but not at guanine residues. These results suggest that the BzPO-derived reactive species causing DNA damage is different from •OH and phenyl radicals generated from benzoyloxyl radicals. BzPO/Cu(I) induced 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG) formation in double-stranded DNA more effectively than that in single-stranded DNA. Furthermore, we observed that BzPO increased the amount of 8-oxodG in human cultured cells. Consequently, it is concluded that benzoyloxyl radicals generated by the reaction of BzPO with Cu(I) may oxidize the 5′-guanine of GG and GGG sequences in double-stranded DNA to lead to 8-oxodG formation and piperidine-labile guanine lesions, and the damage seems to be relevant to the tumor-promoting activity of BzPO.

Benzoyl peroxide (BzPO)[1] is widely used in variety of applications, including as an initiator in free-radical-induced polymerization of monomers to form plastics, as a food additive, and as a component of acne treatments. BzPO acts as a tumor promoter and progressor, though it is not an initiator or complete carcinogen in mouse skin (*1*, *2*). BzPO enhances the rate of malignant change of benign papillomas in the skin (*3*, *4*). Several reports have supported generation of reactive oxygen species and the subsequent oxidative DNA damage is related to the tumor-promoting activity (*5*, *6*).

The sequence specificity of DNA damage may play a major role in the mutagenic processing (*7*). Among the nucleic acid bases, guanine has the lowest oxidation potential. We have found that the exposure of DNA to UVA in the presence of endogeneous photosensitizers can induce hydroxylation specifically at C-8 of the 5′ site of GG in double-stranded DNA (*8*, *9*). A similar photoinduced GG specificity has been observed in several systems (*10*−*12*). The sequence-specificity for guanine photodamage by type I photooxidation could be explained in terms of the lower ionization potentials of stacked guanine base pairs on the basis of theoretical calculation (*13*, *14*). It is, therefore, very interesting to know whether the such sequence-specific DNA damage observed in type I photooxidation also occur in typical free radical-mediated DNA oxidation.

In this study, we investigated the sequence-specific DNA damage by BzPO in the presence of metal ion, using ^{32}P-5′-end-labeled DNA fragments obtained from the human *p53* tumor suppressor gene and c-Ha-*ras*-1 protooncogene. We also analyzed 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG) formation in isolated and cellular DNA treated with BzPO.

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[1] Abbreviations: BzPO, benzoyl peroxide; •OH, hydroxyl free radical; 8-oxodG, 8-oxo-7,8-dihydro-2′-deoxyguanosine (and also known as 8-hydroxy-2′-deoxyguanosine); HPLC-ECD, electrochemical detector coupled to high performance liquid chromatography. DTPA, diethylenetriaminepentaacetic acid; SOD, superoxide dismutase.

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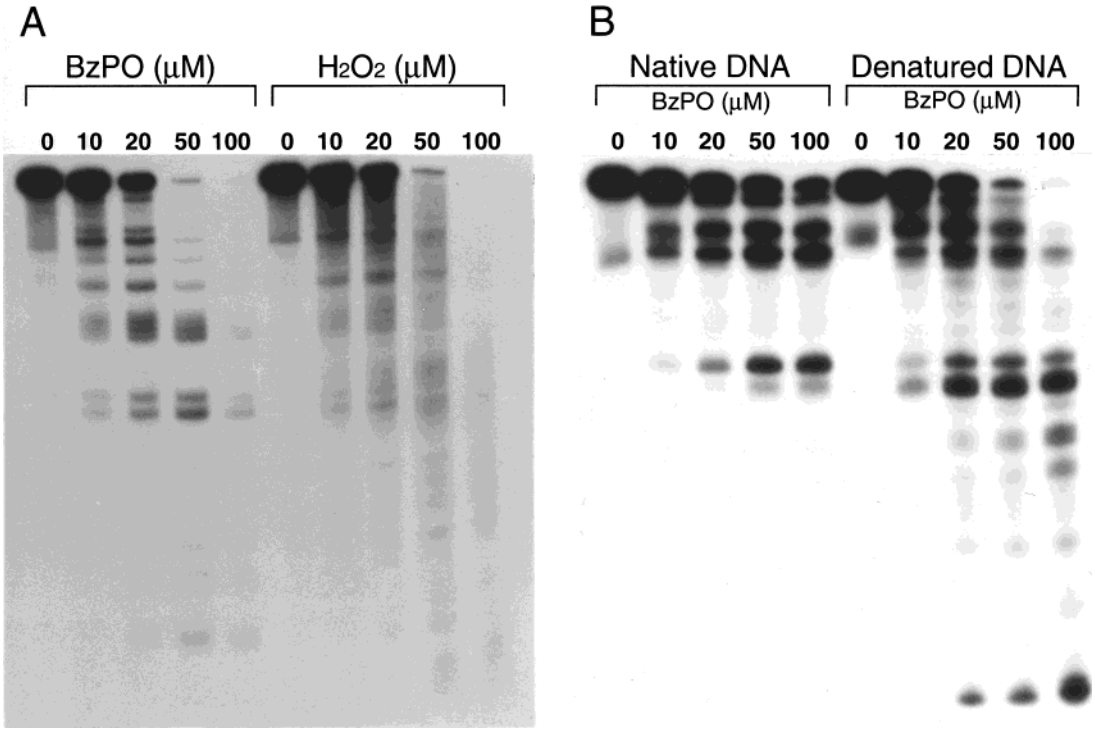

FIGURE 1: Autoradiogram of ^{32}P-labeled DNA fragments incubated with BzPO and H_2O_2 in the presence of Cu(I). (A) The reaction mixture contained the ^{32}P-5′-end-labeled 337-bp fragment (*Pst*I 2345−*Ava*I *2681), 10 μM per base of sonicated calf thymus DNA, 15 μM CuCl, and BzPO or H_2O_2 in 200 μL of 10 mM phosphate buffer (pH 7.8) containing 2.5 μM DTPA. After the incubation at 37 °C for 90 min, followed by piperidine treatment, the treated DNA fragments were electrophoresed on an 8% polyacrylamide/8 M urea gel (12 × 16 cm), and the autoradiogram was obtained by exposing X-ray film to the gel. (B) The reaction mixture contained the ^{32}P-5′-end-labeled 348-bp fragment (*Sty*I 13160−*Eco*RI *13507), 10 μM per base of sonicated calf thymus DNA, BzPO, and 15 μM CuCl in 200 μL of 10 mM phosphate buffer (pH 7.8) containing 2.5 μM DTPA. For DNA denaturation, DNA was treated at 90 °C for 5 min and quickly chilled before incubation at 37 °C for 60 min. The DNA fragments were treated with 1 M piperidine for 20 min at 90 °C and then electrophoresed on an 8% polyacrylamide/8 M urea gel. The autoradiogram was obtained by exposing X-ray film to the gel.

MATERIALS AND METHODS

Materials. Restriction enzymes (*Sma*I, *Eco*RI, *Apa*I, and *Sty*I) were purchased from Boehringer Mannheim GmbH. Restriction enzymes (*Hin*dIII, *Ava*I, and *Pst*I) and T4 polynucleotide kinase were purchased from New England Biolabs. [γ-^{32}P]ATP (222 TBq/mmol) was from New England Nuclear (Boston, MA). Diethylenetriamine-*N*,*N*,*N*′,*N*′′,*N*′′-pentaacetic acid (DTPA) and bathocuproinedisulfonic acid were from Dojin Chemicals Co. (Kumamoto, Japan). BzPO, acrylamide, dimethyl sulfoxide (DMSO), bisacrylamide, and piperidine were from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Ethanol, CuCl, $CuCl_2$, H_2O_2, D-mannitol, sodium formate, and phenylhydrazine were from Nacalai Tesque, Inc. (Kyoto, Japan). Catalase (45 000 units/mg from bovine liver), SOD (3000 units/mg from bovine liver), and methional were from Sigma Chemical Co. (St. Louis, MO).

Preparation of ^{32}P-5′-End-Labeled DNA Fragments. DNA fragments were obtained from the human *p53* tumor suppressor gene (*15*). The 5′-end-labeled 650-bp fragment (*Hin*dIII *13972−*Eco*RI *14621) and 460-bp fragment (*Hin*dIII *13038−*Eco*RI *13507) were obtained by dephosphorylation with calf intestine phosphatase and rephosphorylation with [γ-^{32}P]ATP and T4 polynucleotide kinase. (The asterisk indicates ^{32}P-labeling.) The 650-bp fragment was further digested with *Apa*I to obtain a singly labeled 211-bp fragment (*Hin*dIII *13972−*Apa*I 14182), and the 460-bp fragment was further digested with *Sty*I to obtain a singly labeled 118-bp fragment (*Hin*dIII *13038−*Sty*I 13155), as described previously (*16*). DNA fragment was obtained from the human c-Ha-*ras*-1 protooncogene (*17*). DNA fragment was prepared from plasmid pbcNI, which carries a 6.6-kb *Bam*HI chromosomal DNA segment containing the c-Ha-*ras*-1 gene, and a singly labeled 337-bp fragment (*Pst*I 2345−*Ava*I *2681) was obtained according to the method described previously (*18*, *19*). Nucleotide numbering starts with the *Bam*HI site (*17*).

Detection of Damage to Isolated DNA Induced by BzPO in the Presence of Cu(I). The standard reaction mixture in a microtube (1.5 mL Eppendorf) contained various concentrations of BzPO, CuCl, ^{32}P-labeled DNA fragment, and sonicated calf thymus DNA in 200 μL of 10 mM sodium phosphate buffer (pH 7.8) containing 2.5 μM DTPA. 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 previously described (*18*, *19*).

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 (*20*) by using a DNA sequencing system (LKB 2010 Macrophor, Pharmacia Biotech, Uppsala, Sweden). A laser densitometer (LKB 2222 UltroScan XL, Pharmacia Biotech, Uppsala, Sweden) was used for the measurement of the relative amounts of oligonucleotides from treated DNA fragments.

Analysis of 8-OxodG Formation in Calf Thymus DNA by Reaction of BzPO in the Presence of Cu(I). Calf thymus

DNA fragments (100 μM per base) were incubated with BzPO and CuCl for the indicated duration at 37 °C. For the experiment with denatured DNA, calf thymus DNA was treated at 90 °C for 5 min and quickly chilled before incubation. After ethanol precipitation, DNA was enzymatically digested to the nucleosides and analyzed by HPLC-ECD, as described previously (*21*).

Measurement of 8-OxodG in Cultured Cells. Human leukemia HL-60 cells and their H_2O_2-resistant clone HP100 cells were grown in RPMI 1640 supplemented with 6% FCS at 37 °C under 5% CO_2 in a humidified atmosphere. Catalase activity of HP100 cells was 18 times higher than that of HL-60 cells (*22*, *23*). Cells (10^6 cells/mL) were incubated with BzPO for 2.5 h at 37 °C and immediately washed three times with PBS, and the DNA was extracted by using a DNA extractor WB kit (Wako Pure Chemical Industries, Ltd.). The DNA was dissolved in H_2O and treated with 8 units of nuclease P1 and then with 1.2 units of bacterial alkaline phosphatase. The content of 8-oxodG was determined by the method described previously (*21*, *23*).

RESULTS

Damage to ^{32}P-Labeled DNA Fragments Induced by BzPO and H_2O_2 in the Presence of Cu(I) or Cu(II). Figure 1A shows that DNA damage increased with increasing concentrations of BzPO and H_2O_2 in the presence of Cu(I). Neither BzPO nor H_2O_2 alone caused DNA damage. When Cu(II) was added instead of Cu(I), H_2O_2 caused DNA damage, whereas BzPO did not. Although BzPO/Cu(I) caused no DNA cleavage without piperidine treatment, piperidine treatment led to chain cleavage (data not shown). This result indicated that BzPO plus Cu(I) caused base modification without breakage of the deoxyribose−phosphate backbone. The damage to denatured single-stranded DNA was greater than that to native double-stranded DNA (Figure 1B).

Effects of Scavengers and Bathocuproine on DNA Damage Induced by BzPO in the Presence of Cu(I). Figure 2 shows the effects of scavengers and bathocuproine on Cu(I)-mediated DNA damage by BzPO. The DNA damage was not inhibited by ethanol (data not shown) and mannitol. SOD and catalase also showed no inhibitory effects on the DNA damage. Methional and DMSO, both of which are sulfur compounds, inhibited the DNA damage completely, and sodium formate did to some extent. The addition of bathocuproine inhibited it.

Site Specificity of Cu(I)-Mediated DNA Damage Induced by BzPO. To investigate site specificity of Cu(I)-mediated DNA cleavage, ^{32}P-5′-end-labeled DNA fragments incubated with BzPO in the presence of Cu(I), followed by piperidine treatment, were electrophoresed and the autoradiograms were scanned with a laser densitometer. Figure 3 shows the site-specific DNA cleavage induced by BzPO in the presence of Cu(I). BzPO and Cu(I) caused preferential DNA damage at the 5′-site guanine of GGG and GG sequences in double-stranded DNA (Figure 3A). When denatured single-stranded DNA was used, DNA cleavage occurred frequently at single guanine residues (Figure 3B). Figure 4 shows the comparison of site specificity of DNA damage by BzPO (Figure 4A) and H_2O_2 (Figure 4B) in the presence of Cu(I) in double-stranded DNA. BzPO/Cu(I) induced cleavage at the guanine residue of a known hot spot in codon 248 (CGG) of the *p53* tumor suppressor gene (*24*). When H_2O_2 was used instead of BzPO, Cu(I)-mediated DNA cleavage occurred at cytosine and thymine residues. Figure 5 shows the comparison of site-specific DNA cleavages induced by BzPO and phenylhydrazine. BzPO induced DNA cleavage more frequently at consecutive guanine residues in the presence of Cu(I) (Figure 5A). Phenylhydrazine induced DNA cleavage frequently at thymine in the presence of Cu(II) (data not shown). Our previous study revealed that phenyl radicals and H_2O_2 were generated in the reaction mixture of phenylhydrazine and Cu(II) (*25*). Figure 5B shows the site specificity of DNA damage by phenylhydrazine and Cu(II) observed when an excess dose of catalase was added to remove H_2O_2. This result suggests that phenyl radicals cause DNA damage frequently at thymine residues and excludes the possibility that phenyl radicals are responsible for BzPO/Cu(I)-induced polyguanine-specific damage.

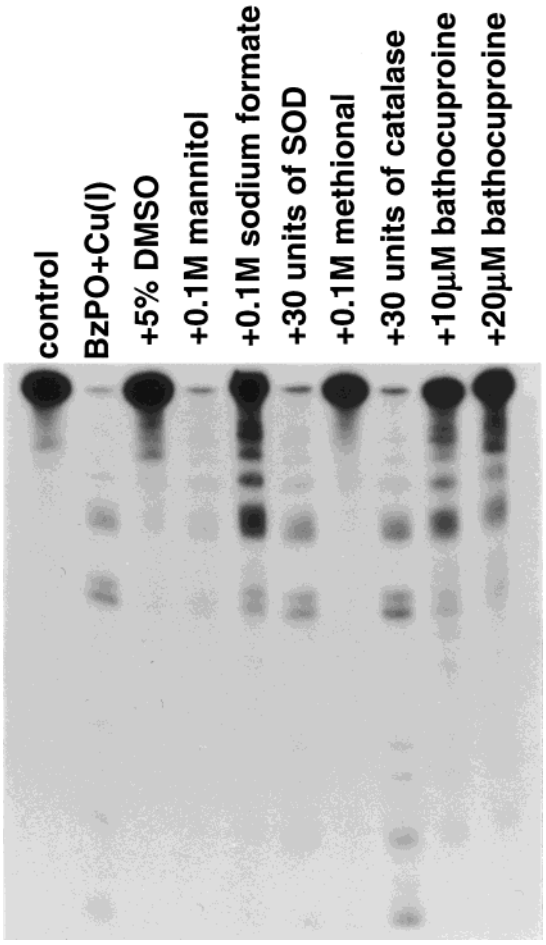

FIGURE 2: Effects of scavengers on DNA cleavage induced by BzPO in the presence of Cu(I). The reaction mixture contained the ^{32}P-5′-end-labeled 337-base pair fragment (*Pst*I 2345−*Ava*I *2681), 10 μM per base of sonicated calf thymus DNA, 50 μM BzPO, 15 μM CuCl, scavenger, and 2.5 μM DTPA in 200 μL of 10 mM sodium phosphate buffer at pH 7.8. Scavenger or bathocuproine was added where indicated. After the incubation at 37 °C for 90 min, followed by the piperidine treatment, the DNA fragments were analyzed by the method described in the Figure 1 caption.

Formation of 8-OxodG in Calf Thymus DNA by BzPO in the Presence of Cu(I). Using HPLC-ECD, we measured 8-oxodG content in calf thymus DNA treated with BzPO in the presence of Cu(I). The amount of 8-oxodG increased with time (Figure 6). BzPO/Cu(I) very efficiently induced 8-oxodG formation in native double-stranded DNA, whereas

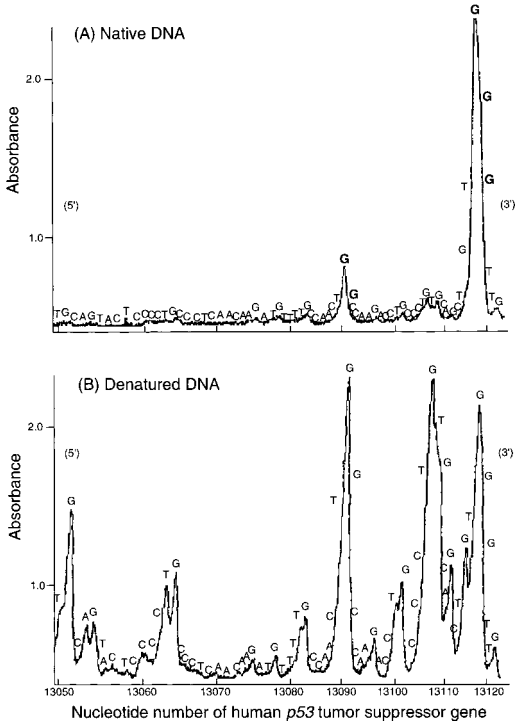

FIGURE 3: Site specificity of DNA cleavage by BzPO in the presence of Cu(I). The reaction mixture contained the ^{32}P-5′-end-labeled 118-bp fragment (*Hin*dIII *13038−*Sty*I 13155), 10 μM per base of sonicated calf thymus DNA, 50 μM BzPO, and 15 μM CuCl in 200 μL of 10 mM sodium phosphate buffer at pH 7.8 containing 2.5 μM DTPA. For DNA denaturation, DNA was treated at 90 °C for 5 min and quickly chilled before incubation. After incubation at 37 °C for 90 min, followed by piperidine treatment, the DNA fragments were electrophoresed on an 8% polyacrylamide/8 M urea gel using a DNA sequencing system, and the autoradiogram was obtained by exposing X-ray film to the gel. The relative amounts of oligonucleotides produced were measured by a laser densitometer (LKB 2222 UltroScan XL). The piperidine-labile sites of the treated DNA were determined by direct comparison with the same DNA fragment after undergoing DNA sequence reaction according to the Maxam−Gilbert procedure (*20*). The horizontal axis shows the nucleotide number of the human *p53* tumor suppressor gene (*15*).

BzPO/Cu(I) induced a little formation of 8-oxodG in denatured single-stranded DNA. 8-OxodG formation was not observed in the absence of Cu(I) or BzPO (data not shown).

Formation of 8-OxodG in Human Cultured Cells by BzPO. To investigate cellular induction of oxidative DNA damage, we measured the content of 8-oxodG, a relevant indicator of oxidative base damage, in HL-60 and HP100 cells treated with BzPO. Formation of 8-oxodG in DNA extracted from the treated HL-60 cells was increased in a dose-dependent manner (Figure 7). HP100 cells were used to assess whether H_2O_2 participates in BzPO-induced oxidative DNA lesion. BzPO increased the amount of 8-oxodG in HP100 cells. The content of 8-oxodG of DNA in HL-60 and HP100 cells treated with 0.2 mM BzPO was significantly increased in comparison with nontreated cells (Figure 7). However, there were no significant differences between HL-60 cells and HP100 cells treated with 0.2 mM BzPO. It was reported that HP100 cells were approximately 340-fold more resistant to H_2O_2 than the parent cells, HL-60 (*22*). These results suggest that BzPO can cause oxidative DNA damage in human cultured cells and that H_2O_2 does not participate in BzPO-induced oxidative DNA lesion.

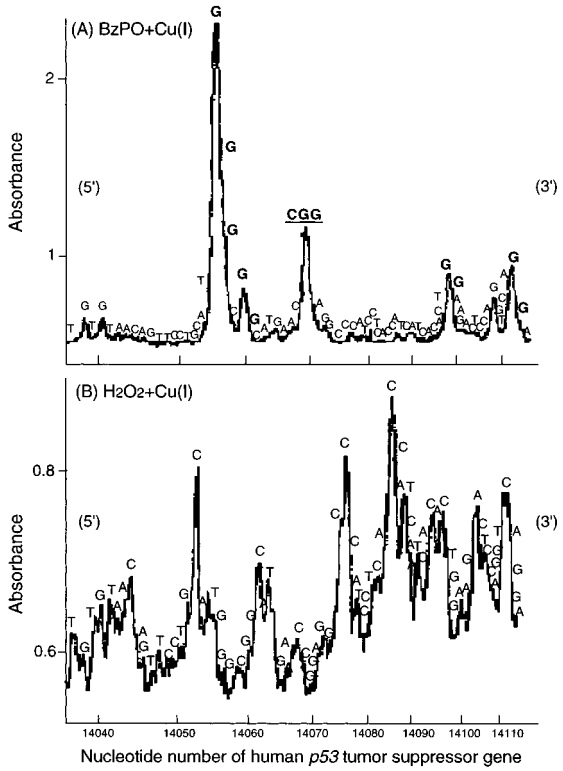

FIGURE 4: Site specificity of DNA cleavage by BzPO and H_2O_2 in the presence of Cu(I). The reaction mixture contained the ^{32}P-5′-end-labeled 211-bp fragment (*Hin*dIII *13972−*Apa*I 14182), 20 μM per base of sonicated calf thymus DNA, 15 μM CuCl, and 50 μM BzPO (A) or 50 μM H_2O_2 (B) in 200 μL of 10 mM sodium phosphate buffer at pH 7.8 containing 2.5 μM DTPA. After incubation at 37 °C for 60 min, followed by the piperidine treatment, the DNA fragments were analyzed by the method described in the caption to Figure 3.

DISCUSSION

The present study has demonstrated that BzPO has an ability to cause polyguanine-specific oxidation to native double-stranded DNA and guanine-specific oxidation to denatured single-stranded DNA in the presence of Cu(I). Although Hazlewood and Davies (*26*) reported that BzPO/Cu(I) caused both strand breaks and high yields of altered bases via the formation of base adducts, BzPO/Cu(I) induced only base alteration under the conditions used. Catalase and SOD showed no inhibitory effect on BzPO/Cu(I)-induced base alteration, suggesting no involvement of O_2^- and H_2O_2. Furthermore, we demonstrated that the content of 8-oxodG in HL-60 and HP100 cells was increased by the BzPO treatment. Although the catalase activity of HP100 cells was reported to be higher than that of HL-60 cells (*22*), there were no significant differences between HL-60 cells and HP100 cells treated with the same dose of BzPO. These results suggest that the BzPO-derived reactive species that is responsible for DNA damage is different from H_2O_2.

To speculate what kinds of reactive species cause oxidative DNA damage, experiments using various scavengers were performed. Typical $^{\bullet}$OH scavengers, ethanol and mannitol, did not offer DNA protection from BzPO/Cu(I). Other $^{\bullet}$OH scavengers such as DMSO and methional, which are reported to be able to scavenge species with reactivity weaker than $^{\bullet}$OH (*27*, *28*), inhibited the DNA damage. In addition, it is worth noting that BzPO/Cu(I) causes the polyguanine-specific base modification in double-stranded DNA. It is

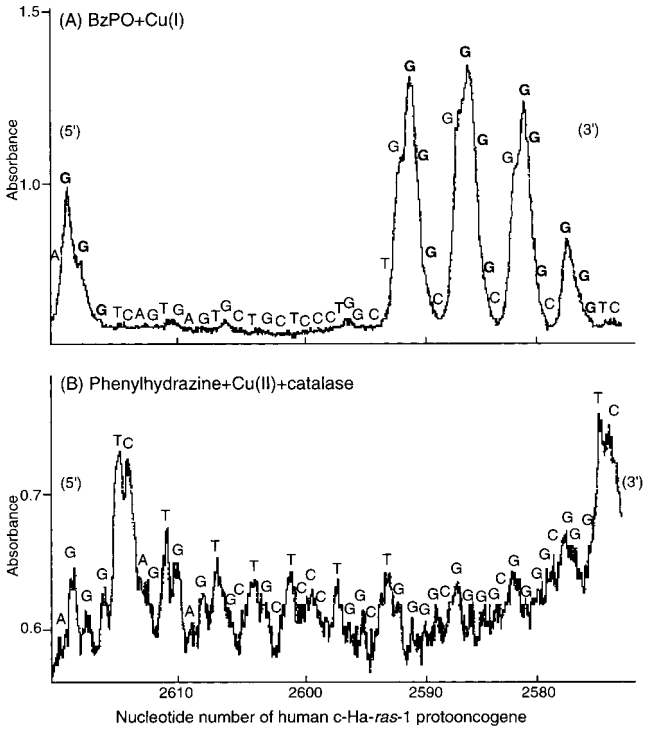

FIGURE 5: Comparison with site specificity of DNA cleavage by BzPO plus Cu(I) and by phenylhydrazine plus Cu(II) in the presence of catalase. The reaction mixture contained the ^{32}P-5′-end-labeled 337-base pair fragment (*Pst*I 2345−*Ava*I *2681), 20 μM per base of sonicated calf thymus DNA, and 50 μM BzPO plus 15 μM CuCl (A) or 1 mM phenylhydrazine and 20 μM $CuCl_2$ plus 1000 units of catalase (B) in 200 μL of 10 mM sodium phosphate buffer at pH 7.8 containing 2.5 μM DTPA. After incubation at 37 °C for 60 min, followed by the piperidine treatment, the DNA fragments were analyzed by the method described in the caption to Figure 3.

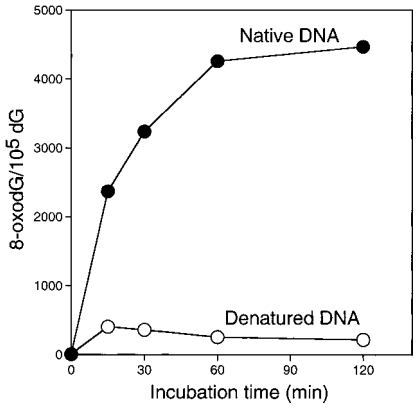

FIGURE 6: Formation of 8-oxodG in calf thymus DNA by BzPO in the presence of Cu(I). Calf thymus DNA fragments (100 μM per base) were incubated with 20 μM BzPO plus 15 μM CuCl at 37 °C for the indicated duration. For DNA denaturation, DNA was treated at 90 °C for 5 min and quickly chilled before incubation. After ethanol precipitation, DNA was digested to the nucleosides with nuclease P1 and calf intestine phosphatase and analyzed by HPLC-ECD.

generally considered that •OH causes DNA cleavage at any nucleotide with little site specificity (*29*−*32*). These results support the idea that a reactive species other than •OH is responsible for the damage. On the other hand, it was reported that exposure to BzPO/Cu(I) caused up to 20-fold increases in the levels of adenine-derived modified bases, up to 4-fold increases in guanine- and cytosine-derived modified bases, and only a <2-fold increase in thymine-

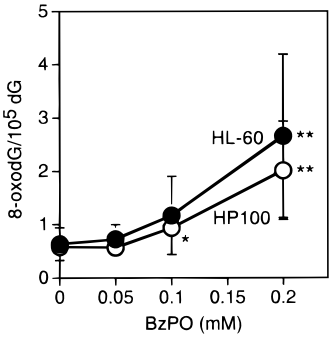

FIGURE 7: Contents of 8-oxodG in DNA of HL-60 and HP100 cells treated with BzPO. The HL-60 (●) and HP100 (○) cells (1.0 × 10^6 cells/mL) were incubated with various concentrations of BzPO for 2.5 h and the treated DNA was extracted immediately. The extracted DNA was subjected to enzyme digestion and analyzed by HPLC-ECD as described under Materials and Methods. Results are expressed as means ± SD of values obtained from six independent experiments. Asterisks indicate significant differences compared with control by *t*-test (*, $P < 0.01$; **, $P < 0.05$).

derived modified bases (*33*). DMSO inhibited BzPO/Cu(I)-induced base modification by 10−50%. Mannitol was a less effective inhibitor than DMSO (*33*). These weak effects could be explained by the formation of •OH close to the site of a DNA base. The •OH might react with a DNA base immediately so that scavengers cannot reach that position on time. Therefore, Akman et al. (*33*) have speculated that •OH or a similar reactive intermediate is responsible for the damage.

BzPO plus Cu(I) induced specific DNA cleavage at 5′-site guanine of GGG and GG sequences. On the other hand, in the presence of Cu(I), H_2O_2 induced piperidine-labile sites at cytosine and thymine residues but rarely at guanine and adenine residues. Akman et al. (*34*) have observed that BzPO/Cu(I) induced mutational clusters at d(pGGG)-d(pCCC) and suggested that the mutagenesis may be induced by site-specific DNA damage by interacting of BzPO with Cu(I) bound to G-C base pairs. However, the difference in the site specificity of DNA damage between BzPO/Cu(I) and H_2O_2/Cu(I) can be explained by a difference of generated reactive species rather than preferential binding of Cu(I) to DNA.

Swauger et al. (*35*) confirmed that the labile O−O bond of BzPO is readily cleaved to give initially benzoyloxyl radicals and then phenyl radicals as a result of decarboxylation. However, phenyl radicals did induce strand cleavage at thymine residues but not at guanine residues (Figure 5B). As shown in Figure 8, a possible mechanism of oxidative DNA damage by BzPO and Cu(I) is proposed on the basis of our results and previous literature (*35*). Cu(I) catalyzes the decomposition of BzPO into benzoyloxyl radicals and benzoate anion. It is conceivable that benzoyloxyl radicals, which are precursor of phenyl radicals, would be capable of causing polyguanine-specific DNA damage. Oxidation of organic compounds in combination with BzPO and Cu(I)−Cu(II) extensively studied by Kharasch et al. (*36*) in the early 1970s has long been known as a synthetically useful reaction. The benzoyloxyl radicals are a kind of carboxyl radicals, which may well be able to undergo electron transfer with electron-rich 5′-site guanine at consecutive G sequences (*37*). It is known that the energy level of the highest occupied molecular orbital (HOMO) of 5′-site guanine of GG se-

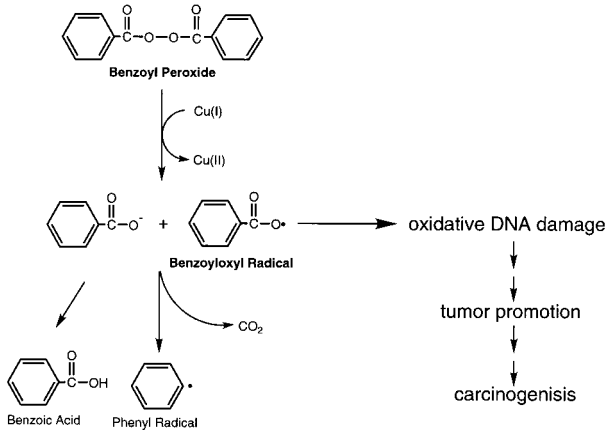

FIGURE 8: Possible mechanism of DNA damage induced by BzPO in the presence of Cu(I).

quences is high among these of other nucleobases, and accordingly, 5′-site guanine of GG sequences is oxidized most easily (*13*). Recently, Saito et al. (*14*) reported that the effectiveness of GG doublets and GGG triplets acted as a trap in long-range oxidative damage to DNA through the double-stranded DNA caused by one-electron oxidations. The predominant guanine alteration in BzPO/Cu(I)-induced DNA damage can be explained by assuming that benzoyloxyl radicals have less oxidizing power than •OH. The experiment with ^{32}P-labeled DNA showed that BzPO/Cu(I) caused piperidine-labile lesions specifically at the 5′ guanine of GG and GGG sequences of double-stranded DNA. BzPO/Cu(I) also induced 8-oxodG formation in double-stranded DNA to a greater extent than that in single-stranded DNA. These results suggest that 8-oxodG formation and the more piperidine-labile guanine lesions should occur at the polyguanine sequences of double-stranded DNA. Hole migration has been thought to be more efficient through duplex DNA but to be inefficient with single stranded DNA (*38*). From the calculated ionization potentials in duplex DNA, it has been speculated that 5′-guanines of GGG and GG are the major site of oxidation (*38*). Thus, it may be considered that benzoyloxyl radicals would oxidize the consecutive guanine residues in double-stranded DNA to yield cation radicals at the 5′ guanine. The formed guanine cation radical could react with a water molecule to form the C-8 OH adduct radical, followed by oxidation, leading to the formation of 8-oxodG in double-stranded DNA to a greater extent than in single-stranded DNA.

These DNA damage may occur in vivo, since copper ions are an essential component of chromatin and are known to accumulate preferentially in the heterochromatic regions (*39*, *40*). It has been reported that 8-oxodG formation can lead to G to T transversion via DNA misreplication, resulting in a mutation (*41*−*43*). The present study showed that BzPO/Cu(I) induced cleavage at the guanine residue of the CGG sequence of codon 248, a known hot spot (*24*) of the *p53* tumor suppressor gene. The genetic damage might lead to inactivation of *p53* tumor suppressor gene, which is consistent with the step of tumor promotion in a multistage process of carcinogenesis. As Greenblatt et al. (*44*) has reviewed, mutations at codon 248 were most frequently observed in various types of human cancers. Among seven mutational hot spots of the *p53* gene including codon 248, four hot spots have GG sequence. Polyguanine-specific DNA damage by BzPO/Cu(I) may have some relation with the *p53* mutation that leads to tumor promotion and progression. Thus, oxidative DNA damage by BzPO/Cu(I) seems to be relevant to the tumor promoter activity of BzPO. Although it is reported that BzPO does not present a carcinogenic risk to humans (*45*), this work requires further studies on safety and risk assessment of BzPO.

REFERENCES

1. Slaga, T. J., Klein-Szanto, A. J. P., Triplett, L., and Yotti, L. P. (1981) *Science 213*, 1023−1025.
2. Reiners, J. J., Nesnow, S., and Slaga, T. J. (1984) *Carcinogenesis 5*, 301−307.
3. O'Connell, J. F., Klein-Stanzo, A. J. P., DiGiovanni, D. M., Fries, J. W., and Slaga, T. J. (1986) *Cancer Res. 46*, 2863−2865.
4. Athar, M., Lloyd, J. R., Bickers, D. R., and Mukhter, H. (1989) *Carcinogenesis 10*, 1841−1845.
5. Giri, U., Iqbal, M., and Athar, M. (1996) *Carcinogenesis 17*, 2023−2028.
6. Kensler, T., Guyton, K., Egner, P., McCarthy, T., Lesko, S., and Akman, S. (1995) *Prog. Clin. Biol. Res. 391*, 103−116.
7. Hanrahan, C. J., Bacolod, M. D., Vyas, R. R., Liu, T., Geacintov, N. E., Loechler, E. L., and Basu, A. K. (1997) *Chem. Res. Toxicol. 10*, 369−377.
8. Ito, K., and Kawanishi, S. (1997) *Biochemistry 36*, 1774−1781.
9. Ito, K., and Kawanishi, S. (1997) *Biol. Chem. 378*, 1307−1312.
10. Saito, I., Takayama, M., Sugiyama, H., Nakatani, K., Tsuchida, A., and Yamamoto, M. (1995) *J. Am. Chem. Soc. 117*, 6406−6407.
11. Ly, D., Kan, Y., Armitage, B., and Schuster, G. B. (1996) *J. Am. Chem. Soc. 118*, 8747.
12. Hall, D. B., Holmlin, R. E., and Barton, J. K. (1996) *Nature 382*, 731−735.
13. Sugiyama, H., and Saito, I. (1996) *J. Am. Chem. Soc. 118*, 7063−7068.
14. Saito, I., Nakamura, T., Nakatani, K., Yoshioka, Y., Yamaguchi, K. and Sugiyama, H. (1998) *J. Am. Chem. Soc. 120*, 12686−12687.
15. Chumakov, P. (1990) EMBL Data Library, Accession Number X54156.
16. Yamashita, N., Murata, M., Inoue, S., Hiraku, U., Yoshinaga, T., and Kawanishi, S. (1998) *Mutat. Res. 397*, 191−201.
17. Capon, J. D., Chen, Y. E., Levinson, D. A., Seeburg, H. P., and Goeddel, V. D. (1983) *Nature 302*, 33−37.
18. Kawanishi, S., and Yamamoto, K. (1991) *Biochemistry 30*, 3069−3075.
19. Yamamoto, K., and Kawanishi, S. (1991) *J. Biol. Chem. 266*, 1509−1515.
20. Maxam, A. M., and Gilbert, W. (1980) *Methods Enzymol. 65*, 499−560.
21. Ito, K., Inoue, S., Yamamoto, K., and Kawanishi, S. (1993) *J. Biol. Chem. 268*, 13221−13227.
22. Kasugai, I. and Yamada, M. (1992) *Leuk. Res. 16*, 173−179.
23. Tada-Oikawa, S., Oikawa, S., and Kawanishi, S. (1998) *Biochem. Biophys. Res. Commun. 247*, 693−696.
24. Denissenko, M. F., Pao, A., Tang, M., and Pfeifer, G. P. (1996) *Science 274*, 430−432.
25. Yamamoto, K., and Kawanishi, S. (1992) *Chem. Res. Toxicol. 5*, 440−446.
26. Hazlewood, C., and Davies, M. J. (1996) *Arch. Biochem. Biophys. 332*, 79−91.
27. Youngman, R. J., and Elstner, E. F. (1981) *FEBS Lett. 129*, 265−268.
28. Pryor, W. A., and Tang, R. H. (1978) *Biochem. Biophys. Res. Commun. 81*, 498−503.
29. Kawanishi, S., Inoue, S., Sano, S. (1986) *J. Biol. Chem. 261*, 5952−5958.
30. Inoue, S., and Kawanishi, S. (1987) *Cancer Res. 47*, 6522−6527.

31. Celander, D. W., and Cech, T. R. (1990) *Biochemistry 29*, 1355−1361.
32. Oikawa, S., and Kawanishi, S. (1998) *Biochim. Biophys. Acta 1399*, 19−30.
33. Akman, S. A., Kensler, T. W., Doroshow, J. H., and Dizdaroglu, M. (1993) *Carcinogenesis 14*, 1971−1974.
34. Akman, S. A., Doroshow, J. H., and Kensler, T. W. (1992) *Carcinogenesis 13*, 1783−1787.
35. Swauger, J. E., Dolan, P. M., Zweier, J. L., Kuppusamy, P., and Kensler, T. W. (1991) *Chem. Res. Toxicol. 4*, 223−228.
36. Hiatt, R. (1971) in *Organic peroxides* (Swern, D., Ed.) Vol. II, pp 799−929, Wiley, New York.
37. Oikawa, S., Nishino, K., Oikawa, S., Inoue, S., Mizutani, T., and Kawanishi, S. (1998) *Biochem. Pharmacol. 56*, 361−370.
38. Burrows, C. J., and Muller, J. G. (1998) *Chem. Rev. 98*, 1109−1151.
39. Dijkwel, P. A., and Wenink, P. W. (1986) *J. Cell Sci. 84*, 53−67.
40. Burkitt, M. J. (1994) *Methods Enzymol. 234*, 66−79.
41. Floyd, R. A. (1990) *Carcinogenesis 11*, 1447−1450.
42. Shibutani, S., Takeshita, M., and Grollman, A. P. (1991) *Nature 349*, 431−434.
43. Cheng, K. C., Cahill, D. S., Kasai, H., Nishimura, S., and Loeb, L. A. (1992) *J. Biol. Chem. 267*, 166−172.
44. Greenblatt, M. S., Bennett, W. P., Hollstein, M., and Harris, C. C. (1994) *Cancer Res. 54*, 4855−4878.
45. Kraus, A. L., Munro, I. C., Orr, J. C., Binder, R. L., LeBoeuf, R. A., and Williams, G. M. (1995) *Regul. Toxicol. Pharmacol. 21*, 87−107.

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