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Mechanism of guanine-specific DNA damage by oxidative stress and its role in carcinogenesis and aging

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Abstract

Reactive species generated by chemicals and UV radiation can cause sequence-specific DNA damage and play important roles in mutagenesis, carcinogenesis and aging. We have investigated sequence specificity of oxidative stress-mediated DNA damage by using ^{32}P-labeled DNA fragments obtained from the human c-Ha-*ras*-1 and *p53* genes. Free hydroxyl radical causes DNA damage with no marked site specificity. Reactive nitrogen species, sulfate radicals, nitrogen-centered radicals, benzoyloxyl radical and alkoxyl radical show different sequence specificity. Benzoyloxyl radical specifically causes damage to the 5′-G in GG sequence. UVA radiation also causes DNA damage at this site through electron transfer in the presence of certain photosensitizers. The 5′-G in GG sequence is easily oxidized because a large part of the highest occupied molecular orbital is distributed on this site. On the basis of these findings, the sequence specificity of DNA damage is presumably determined by (a) redox potential of reactive species; (b) ionization potential of DNA bases; and (c) site-specific binding of metal ion to DNA. Here we discuss the mechanisms of sequence-specific DNA damage in relation to carcinogenesis and aging.

Abbreviations: ROS, reactive oxygen species; UV, ultraviolet; O_2^-, superoxide radical anion; $^{\bullet}OH$, hydroxyl radical; H_2O_2, hydrogen peroxide; $^1O_2$, singlet oxygen; NO, nitric oxide; $SO_4^{\bullet-}$, sulfate radical; NHE, normal hydrogen electrode; HOMO, the highest occupied molecular orbital; EDTA, ethylenediaminetetraacetate; SOD, superoxide dismutase; 8-oxodG, 8-oxo-7,8-dihydro-2′-deoxyguanosine; FAPy-G, 2,6-diamino-5-formamido-4-hydroxypyrimidine; GSH, glutathione; NADH, reduced form of nicotineamide adenine dinucleotide; NADPH, reduced form of nicotineamide adenine dinucleotide phosphate; $ONOO^-$, peroxynitrite; SIN-1, 3-morpholinosydnonimine-*N*-ethylcarbamide; 8-nitroG, 8-nitroguanine; ESR, electron spin resonance; BzPO, benzoyl peroxide; BHT, butylated hydroxytoluene; BHT-OOH, 2,6-di-*tert*-butyl-4-hydroperoxyl-4-methyl-2,5-cyclohexadienone; NA, nalidixic acid

Keywords: DNA damage; Sequence specificity; Oxygen radical; Reactive species; UV

1. Introduction

Oxidative stress includes reactive oxygen species (ROS)-generating chemicals, ultraviolet (UV) radiation and ionizing radiation. Oxidative stress is capable of causing damage to various cellular constituents, such as DNA, proteins and lipids, leading to carcinogenesis, aging and many other diseases. Particularly, DNA damage mediated by oxidative stress plays critical roles in carcinogenesis and aging [1–5]. In biological systems, damaged DNA is repaired enzymatically and the cells regain their normal functions. However, misrepair of DNA damage could result in mutations such as base substitution and deletion, leading to carcinogenesis [6–9]. The sequence specificity of DNA damage plays the key role in the mutagenic

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processing. Recently, it has been reported that the sites of DNA damage affect the mutation frequency [10]. Therefore, investigation on sequence specificity of DNA damage would provide us biological significance of DNA damage and beneficial findings for cancer prevention. Here we discuss the mechanisms and sequence specificity of DNA damage caused by various reactive species generated from chemical compounds and UVA-activated photosensitizers.

2. Site-specific DNA damage by reactive species

Much endogenous DNA damage arises from intermediates of oxygen reduction [11]. ROS include oxygen free radicals, such as superoxide radical anion (O_2^{-}) and hydroxyl radical ($^{\bullet}OH$), and non-radical oxidants, such as hydrogen peroxide (H_2O_2) and singlet oxygen ($^1O_2$). In addition to ROS, a number of free radicals are capable of causing DNA damage: nitric oxide (NO), lipid hydroperoxides (LOOH), alkoxyl radical ($RO^{\bullet}$), peroxyl radical ($^{\bullet}OOH$), nitrogen-centered radical, sulfate radical ($SO_4^{\bullet -}$) and metal–oxygen complexes. These reactive species have different redox potentials [12,13], and redox potentials of these species play important roles in sequence-specific DNA damage.

Oxidation potentials of DNA bases also contribute to the determination of sequence specificity of DNA damage. Guanine is most easily oxidized among the four DNA bases, because the oxidation potential of guanine is lower [1.29 V versus normal hydrogen electrode (NHE)] than the other three DNA bases, adenine (1.42 V), cytosine (1.6 V) and thymine (1.7 V) [14,15]. The distribution of electrons involved in chemical reaction on DNA bases is an important factor to determine susceptibility to oxidative attacks. The highest occupied molecular orbital (HOMO), which accommodates electrons with the highest energy, confers the reactivity of DNA bases. A large part of electrons of HOMO is located on the 5′-G of GG sequence in B-form double-stranded DNA [16,17], and thus, this guanine is likely to be oxidized. Therefore, sequence specificity of DNA damage appears to be determined by redox potentials of reactive species and HOMO distribution on DNA bases. In addition, steric effects on the interaction of reactive species with DNA may contribute to determination of the sequence specificity of DNA damage. Details of sequence specificity of DNA damage by reactive species are discussed below.

2.1. Free hydroxyl radical

$^{\bullet}OH$ is a highly reactive species and can be generated through a variety of mechanisms. Ionizing radiation causes decomposition of H_2O, resulting in formation of $^{\bullet}OH$ and hydrogen atoms. $^{\bullet}OH$ is also generated by photolytic decomposition of alkylhydroperoxides [18]. It is well known that $^{\bullet}OH$ is generated through Fe(II)-mediated decomposition of H_2O_2 (the Fenton reaction). $^{\bullet}OH$ is responsible for DNA damage caused by the interaction of Fe(II)-ethylenediaminetetraacetate (EDTA) [19] or Fe(III)-nitrilotriacetate [26] with H_2O_2. It has been suggested that the Fenton reaction is involved in H_2O_2-induced DNA damage in vivo [20–25]. Chromate(VI) also induced DNA damage by generating $^{\bullet}OH$ through the interaction with H_2O_2 [27]. H_2O_2 is generated through spontaneous and/or superoxide dismutase (SOD)-catalyzed dismutation of O_2^{-}. O_2^{-} is produced by one-electron reduction of molecular oxygen (O_2) through the reaction with free radicals and the enzymatic reaction catalyzed by xanthine oxidase. $^{\bullet}OH$ directly abstracts a hydrogen atom from the DNA deoxyribose-phosphate backbone, resulting in DNA cleavage at every nucleotide without a marked site specificity [19,26,27]. $^{\bullet}OH$ also causes addition to the DNA bases leading to generation of a variety of oxidative products. The interaction of $^{\bullet}OH$ with guanine leads to the generation of 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG) and 2,6-diamino-5-formamido-4-hydroxypyrimidine (FAPy-G) [14]. Adenine reacts with $^{\bullet}OH$ in a similar manner to guanine, although oxidative adenine lesions are less prevalent in DNA damage [14]. Our previous studies demonstrated that endogenous reductants, such as ascorbate, glutathione (GSH) and reduced form of nicotineamide adenine dinucleotide (NADH), caused DNA damage at every nucleotide in the presence of Fe(III) or Fe(III)–EDTA complex with a little dominance of guanines (Fig. 1) [28]. NADH-induced 8-oxodG formation in the presence of Fe(III)–EDTA and H_2O_2 by generating $^{\bullet}OH$ [28]. The DNA damage was inhibited by typical $^{\bullet}OH$ scavengers and catalase [28,29], suggesting that these reductants cause DNA damage via the Fenton reaction. $^{\bullet}OH$ can also be generated through a

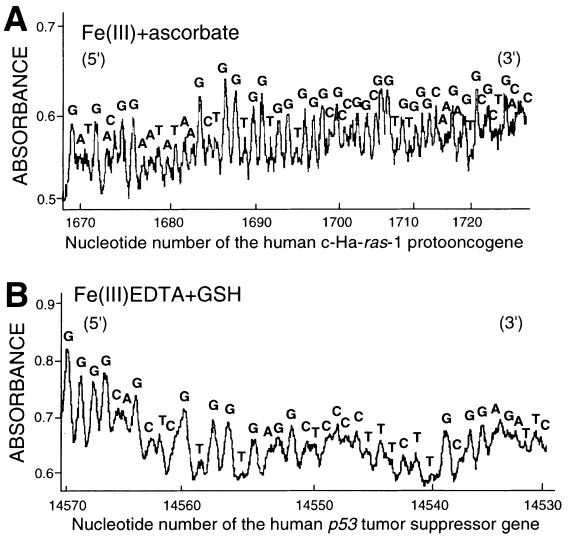

Fig. 1. Site specificity of DNA damage induced by endogenous reductants in the presence of Fe(III). The reaction mixture containing the ^{32}P-5′-end-labeled 261-base pair fragment (*Ava*I* 1645–*Xba*I 1905) obtained from the c-Ha-*ras*-1 gene, 1 μM sonicated calf thymus DNA, 50 μM $FeCl_3$ and 50 μM ascorbate (A) or containing the ^{32}P-5′-end-labeled 109-base pair fragment (PU8* 14404-*Hpa*II 14512) obtained from the *p53* gene, 10 μM sonicated calf thymus DNA, 20 μM Fe(III)EDTA, 20 μM H_2O_2 and 20 μM GSH (B) in 10 mM sodium phosphate buffer at pH 7.9 was incubated for 60 min at 37°C. Then, the DNA fragments were treated with 1 M piperidine for 20 min at 90°C, and electrophoresed on an 8% polyacrylamide/8 M urea gel using a DNA-sequencing system, The autoradiogram was obtained by exposing an 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. The horizontal axis shows the nucleotide number.

non-Fenton reaction: hydrazine and its derivatives caused DNA damage at every nucleotide through the Mn(II)-dependent generation of $^{\bullet}$OH, generated from O_2^- in an H_2O_2-independent manner [30].

2.2. Copper–hydroperoxo complex

We have demonstrated that H_2O_2 plus Cu(I) caused DNA damage at thymine residues adjacent to guanine residues, particularly at the 5′-G<u>T</u>C-3′ sequence [31,32]. Endogenous reductants, such as ascorbate, GSH, NADH and reduced form of nicotineamide adenine dinucleotide phosphate (NADPH), generated H_2O_2 in the presence of Cu(II), and showed a similar pattern of DNA damage [28,29]. Recently, piperidine-labile oxidative products of thymine, 5-formyluracil [33] and formylamine [34], have been detected, and these products may be responsible for DNA damage at thymines. H_2O_2 plus Cu(II) cause DNA damage particularly at thymines in phosphate buffer, whereas the 5′-G of GG sequence was specifically damaged in bicarbonate buffer [28]. In addition, NADH plus Cu(II) generated much larger amount of 8-oxodG in bicarbonate buffer than in phosphate buffer (unpublished data). Typical $^{\bullet}$OH scavengers had little or no inhibitory effect on Cu(II)-mediated DNA damage, suggesting that the primary reactive species in DNA damage may not be $^{\bullet}$OH but a copper–oxygen complex with similar reactivity to $^{\bullet}$OH [27,35]. Several studies have suggested that reactive intermediates such as Cu(I)-hydroperoxo complex [Cu(I)OOH] are formed during the reaction of H_2O_2 with Cu(I) [35–37].

However, the possibility that $^{\bullet}$OH is involved in copper-mediated oxidative DNA damage cannot be neglected. Cu(II) preferentially binds to the charged phosphate group and the N-7 atom of guanine [38]. It can be speculated that Cu(II) bound to DNA at specific sites is reduced to Cu(I) to react with H_2O_2, resulting in the formation of the DNA–Cu(I)OOH complex. This complex may be a bound hydroxyl radical to release $^{\bullet}$OH, which immediately attacks DNA constituents adjacent to copper-binding sites before being scavenged by $^{\bullet}$OH scavengers [39]. Therefore, site specificity of Cu(II)-mediated DNA damage may be determined by binding sites of Cu(II) in DNA.

2.3. Peroxynitrite

Recently, DNA damage caused by reactive nitrogen species has been drawing interest. DNA damage mediated by NO has been discussed in relation to the association between chronic inflammation and carcinogenesis. In inflamed tissues, NO and O_2^- are produced by activated phagocytes. These reactive species interact with each other, resulting in the formation of a highly reactive species, peroxynitrite ($ONOO^-$), at almost diffusion-controlled rate. We demonstrated that $ONOO^-$ and 3-morpholinosydnonimine-*N*-ethylcarbamide (SIN-1), which generates NO and O_2^- simultaneously and continuously, caused DNA cleavage at every nucleotide with a little dominance of guanines

[40]. $ONOO^-$ alone and the combination of an NO-generating agent and an O_2^--generating system [41] showed similar site specificity. $ONOO^-$ and SIN-1 induced a significant increase in the formation of 8-oxodG [40], which is an oxidative product of guanine. The DNA damage and 8-oxodG formation were inhibited by typical $^{\bullet}OH$ scavengers (ethanol and sodium formate), but not inhibited by catalase. Transition metal ions [Cu(II) and Fe(III)] had no effect on DNA damage, suggesting that the $ONOO^-$ formation is independent of metal ions. Recently, it has been suggested that NO^- may play an important role in inflammation in the presence of H_2O_2 and transition metal ions [42].

In addition to 8-oxodG, other products are generated by $ONOO^-$-mediated guanine modification. 8-Nitroguanine (8-nitroG) [43] and 4,5-dihydro-5-hydroxy-4-(nitrosooxy)-2′-deoxyguanosine [44], are formed through $ONOO^-$ formation, followed by depurination. In bicarbonate buffer, $ONOO^-$ reacts with CO_2 or HCO_3^-, resulting in formation of $ONO_2CO_2^-$ [45], which probably mediates the formation of 8-nitroG [46]. We have recently found that SIN-1 caused DNA damage at the 5′-G of GG and GGG sequences in bicarbonate buffer. These findings suggest that NO and O_2^- form 8-nitroG at this guanine in bicarbonate buffer, whereas 8-oxodG is formed at every guanine without marked sequence specificity in phosphate buffer.

2.4. Sulfate radical

Sulfate radical ($SO_4^{\bullet -}$) is a powerful one-electron oxidant with a redox potential estimated to be 2.5–3.1 V versus NHE [13] and capable of causing DNA damage. This radical is produced by metal-catalyzed autoxidation of sulfite (SO_3^{2-}), which is used as a preservative in foods and formed by hydration of an air pollutant, sulfur dioxide in the lung. Our study demonstrated that sulfite caused DNA damage in the presence of Co(II), although sulfite itself was incapable of causing DNA damage [47]. DNA damage by sulfite plus Co(II) was caused specifically at the 5′-G of GG sequence, DNA damage was inhibited by primary and secondary alcohols, which readily react with $SO_4^{\bullet -}$. Electron spin reasonance (ESR)-spin trapping method using 5,5-dimethyl-1-pyrroline-*N*-oxide has confirmed that $SO_4^{\bullet -}$ is generated through autoxidation catalyzed by Co(II) [47,48]. These findings suggest that $SO_4^{\bullet -}$ is the primary species in Co(II)-mediated DNA damage by sulfate. It has been reported that sulfite causes guanine-specific DNA damage through its autoxidation to $SO_4^{\bullet -}$, catalyzed by a certain Ni(II)-peptide [49]. $SO_4^{\bullet -}$ bound to a nickel compound reacts with guanines. A certain square planar macrocyclic Ni(II) complex causes oxidative damage to guanine and uridine residues in RNA by generating $SO_4^{\bullet -}$ [50].

2.5. Benzoyloxyl radical

Recently, we have reported that in the presence of Cu(I), benzoyl peroxide (BzPO) caused DNA damage specifically at 5′-G of GG and GGG sequences in double-stranded DNA (Fig. 2) [51]. The amount of 8-oxodG was larger in double-stranded DNA than in single-stranded DNA, suggesting that the DNA double-helical structure plays an important role in DNA damage. Typical $^{\bullet}OH$ scavengers, SOD and catalase showed no inhibitory effects on DNA damage. No significant difference in the amounts of 8-oxodG was observed in HL60 and $HP100^{-1}$ cells [51], which are approximately 340-fold more resistant to H_2O_2 than the parent HL60 cells [52]. This finding confirms that H_2O_2 does not participate in BzPO-caused DNA damage. An ESR spin-trapping technique using α-phenyl-*N*-*tert*-butylnitrone demonstrated that the benzoyloxyl radical was formed through copper-mediated decomposition of BzPO [53]. These results suggest that the benzoyloxyl radical probably abstracts an electron from the 5′-G of GG and GGG sequences, resulting in formation of 8-oxodG.

2.6. Alkoxyl radicals

We have previously demonstrated that a metabolite of a food additive butylated hydroxytoluene (BHT), 2,6-di-*tert*-butyl-4-hydroperoxyl-4- methyl-2,5-cyclohexadienone (BHT-OOH), specifically caused DNA damage at guanines in the presence of Cu(II) [54]. Bathocuproine inhibited DNA damage, whereas catalase had no inhibitory effect. It has been reported that BHT-OOH is converted into phenoxyl and alkoxyl radicals [55]. BHT-OOH showed similar sequence specificity to hydroperoxide of linoleic acid, which may produce the corresponding alkoxyl radical [56].

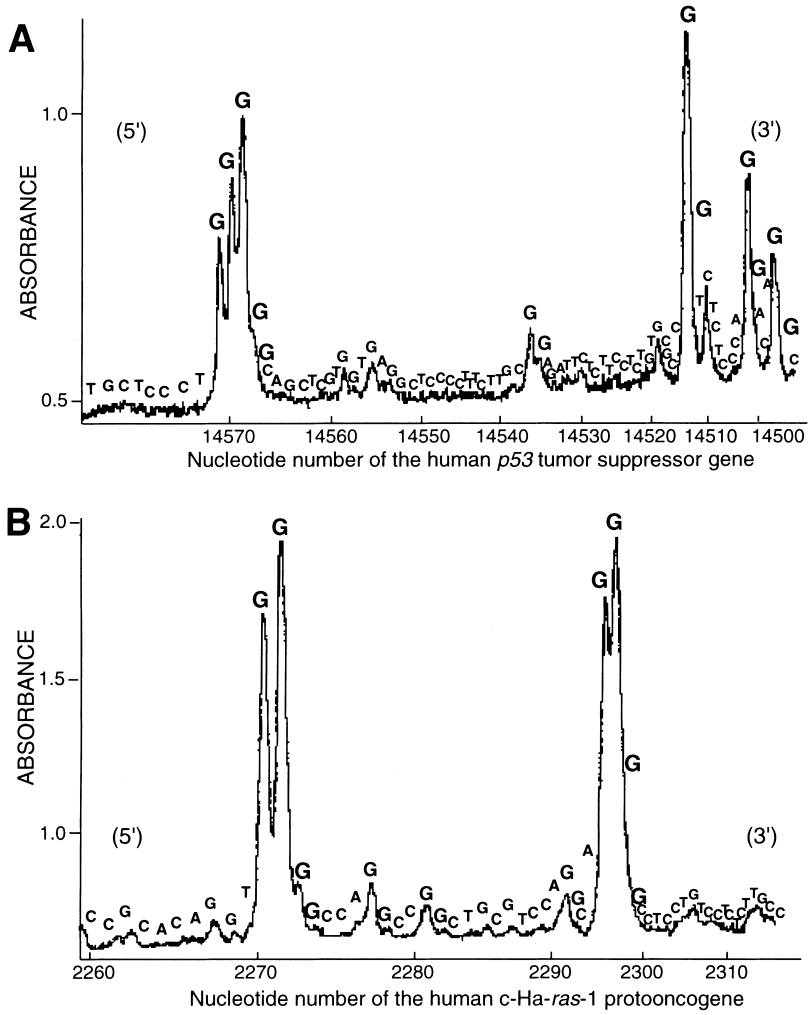

Fig. 2. Site specificity of DNA damage induced by BzPO plus Cu(I). The reaction mixture contained the ^{32}P-5′-end-labeled 443-base pair fragment (*Apa*I 14179–*Eco*RI* 14621) obtained from the *p53* gene (A) or 98-base pair fragment (*Ava*I * 2247–*Pst*I 2344) obtained from the c-Ha-*ras*-1 gene (B), 20 μM/base of sonicated calf thymus DNA, 50 μM BzPO, and 15 μM CuCl in 10 mM sodium phosphate buffer at pH 7.8 containing 2.5 μM DTPA. After incubation at 37°C for 60 min, the DNA was treated and electrophoresed and the autoradiogram was analyzed by the methods in the legend to Fig. 1. The horizontal axis shows the nucleotide number.

Therefore, DNA damage induced by BHT-OOH may be attributed to the alkoxyl radical generated through metal-catalyzed reaction.

3. Site-specific DNA damage by UVA radiation in the presence of photosensitizers

Solar UV light containing UVA (320–380 nm, 95%) and UVB (280–320 nm, 5%) is the major source of human exposure to UV radiation. Solar radiation is a well-known carcinogen. It was believed that UVB component is primarily responsible for induction of skin cancer [57]. UVB has been shown to cause direct photoactivation of the DNA molecule to form cyclobutane pyrimidine dimers and pyrimidine (6-4)- pyrimidone photoadducts [58], leading to mutagenesis and carcinogenesis [58]. However, recently studies have demonstrated that UVA radiation induces skin tumors in animals as well as UVB [59]. It has been reported

that the mutagenic specificity of mutational spectrum of solar light in cells is not determined entirely by the UVB component and that UVA greatly contributes to solar light-induced mutation [60]. UVA-induced DNA damage presumably involves indirect mechanisms by which UV-absorbing substances (photosensitizers) are activated to produce reactive species causing DNA damage, because only little UVA can be absorbed by the DNA molecule. Therefore, solar carcinogenesis would involve not only UVB-induced DNA photoadduct formation but also UVA-induced oxidative DNA damage mediated by activated photosensitizers. Repeated exposure of human skin to solar UV radiation leads to not only skin carcinogenesis but also photoaging through DNA damage. UV-induced photoaging results in the accumulation of massive amounts of abnormal elastic material in the dermis of skin.

In this section, we focused on UVA light-induced carcinogenesis and summarize the mechanisms of sequence-specific DNA damage mediated by photosensitization. UVA-induced DNA photodamage through excited photosensitizers involves Type I mechanism (electron transfer, 5′-G of GG doublets) and major (singlet oxygen, guanine) and minor (H_2O_2 plus metal ions) Type II mechanisms [61] (Fig. 3).

3.1. Type I mechanism

Type I mechanism involves electron transfer from DNA bases, particularly guanine, to an excited photosensitizer, resulting in formation of the guanine cation radical. Guanine has the lowest oxidation potential among the four DNA bases [14,15]. Particularly, the 5′-G in GG sequence is considered to have the lowest oxidation potential, because this guanine has the lowest ionization potential among the guanine-containing dinucleotides [16]. Therefore, this guanine is most likely to be oxidized through

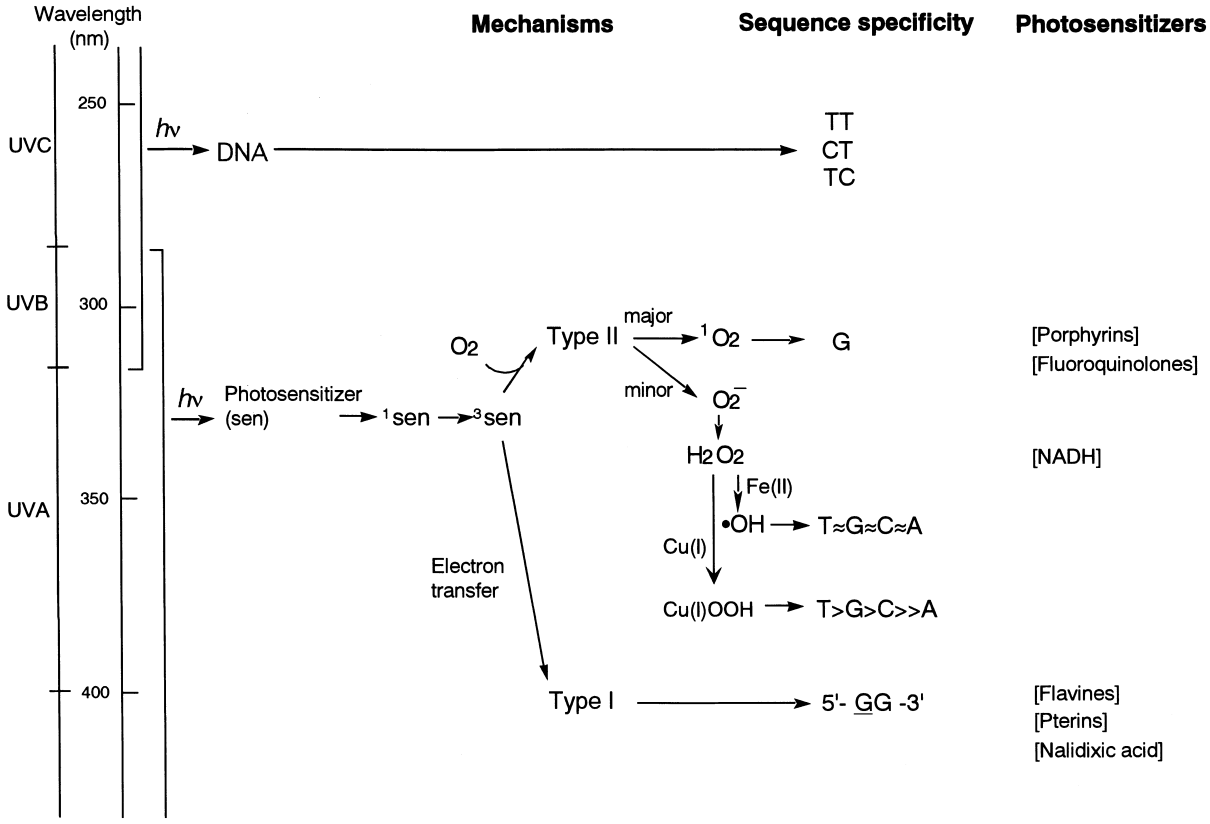

Fig. 3. Mechanisms of UVA-induced DNA damage mediated by photosensitizers and its sequence specificity.

electron transfer. The guanine cation radical can be formed through long-range electron transfer (up to 200 Å) initiated by guanine oxidation at a remote site [73,74], and GG doublets and GGG triplets act as the traps of hole (the cation radical) [17]. Electron transfer induces 8-oxodG formation through the formation of the guanine cation radical and subsequent hydration and oxidation [62]. In addition to 8-oxodG, other products such as 2-aminoimidazolone and 2,2-diaminooxazolone may be produced through the formation of guanine cation radicals [14].

We have demonstrated that 365 nm UVA radiation in the presence of endogenous sensitizers, riboflavin (Vitamin B_2) [63], pterin and its derivatives [64], causes damage to double-stranded DNA fragment specifically at the 5′-G of GG sequences whereas little or no damage was found at single guanines. In bacteria, riboflavin photosensitization increased the amounts of 8-oxodG and caused $G \cdot C \rightarrow T \cdot A$ transversion, presumably resulted from mispairing of 8-oxodG with adenine [65]. Nalidixic acid (NA) is a quinolone antibacterial used for the treatment of urinary tract infections. NA has been reported to cause skin tumors in experimental animals exposed to UVA [66,67]. In our study, NA plus UVA caused DNA damage at the 5′-G of GG sequences through electron transfer as well as riboflavin and pterin derivatives [68]. This result indicates that NA can act as an exogenous photocarcinogen.

3.2. Type II mechanisms

The major Type II mechanism involves energy transfer from an excited photosensitizer to molecular oxygen to produce $^1O_2$ with a relatively long lifetime. $^1O_2$-mediated DNA damage is specifically caused at every guanine. UVA radiation caused DNA photolesions at guanine residues through the generation of $^1O_2$ in the presence of hematoporphyrin [63,69] and methylene blue [70]. We have examined DNA photodamage induced by fluoroquinolone antibacterials as potential exogenous photocarcinogens. Fluoroquinolones have been used for the treatment of a variety of infectious diseases because of their broad range of antimicrobial activities. Fluoroquinolones are capable of causing tumors in UVA-exposed experimental animals, and showed much more potent photocarcinogenic effects than NA [66,67], suggesting that fluoroquinolones may act as exogenous photocarcinogens. It has been reported that DNA photodamage by fluoroquinolones involves the formation of ROS, particularly $^1O_2$ [71,72]. We have recently found that lomefloxacin, a fluoroquinolone antibacterial, caused DNA photodamage specifically at guanines through the generation of $^1O_2$ (unpublished data).

The minor Type II mechanism involves the formation of O_2^- through electron transfer from an excited photosensitizer to molecular oxygen. H_2O_2 is formed by dismutation of O_2^-. In our study, UVA plus sepiapterin caused DNA photodamage at thymines of 5′-G<u>T</u>C-3′ sequence in the presence of Cu(II) [64]. DNA photodamage mediated by this sensitizer is attributed to the formation of the copper–oxygen complex. In the presence of Fe(II), $^{\bullet}$OH is generated through the Fenton reaction and DNA photodamage is caused at every nucleotide without site specificity.

4. Mechanism of polyguanine-specific DNA damage

The relationship between reactive species and the sequence specificity of DNA damage is summarized in Table 1. Highly reactive species such as $^{\bullet}$OH and $ONOO^-$ cause DNA damage at every nucleotide,

Table 1
Site specificity of DNA damage induced by free radicals

Radical species	Site specificity of DNA damage	Oxidation–reduction potential
$^{\bullet}$OH, $ONOO^-$	G∼T∼C∼A	High
Nitrogen-centered radical	G	
$SO_4^{\bullet -}$ radical	GG > G	
Benzoyloxyl radical	5′-G of polyG	Low

whereas less reactive species cause DNA damage specifically at guanines. Guanine is most easily oxidized among the four DNA bases, because the oxidation potential of guanine is lower than the other DNA bases [14,15]. Recently, molecular orbital calculations have revealed that the GG sequence in B-form DNA has the lowest ionization potential among the guanine-containing dinucleotides [16]. A large part of HOMO is located on the 5′-G of GG and GGG sequences [16], and therefore, this guanine is likely to be oxidized. The site specificity of DNA damage induced by reactive species less reactive than •OH, such as benzoyloxyl radical, could be consistent with HOMO distribution and its energy level.

Binding of metal ion to DNA may contribute to polyguanine-specific DNA damage. Metal ions bind to GG sequence more preferentially than other guanine-containing sequences [38]. Sequence-selective metal ion binding to DNA could be explained by sequence-dependent variations of HOMO distribution and molecular electrostatic potentials of guanines [38]. The model of the structure of copper-modified guanine bases in B-DNA revealed that copper forms a regular octahedral complex, with four water ligands in the equatorial plane and a fifth water along with N-7 of the purine base at the axial positions [75]. In this model, a peroxide bridge appears to be formed between two Cu(II)-complexes at consecutive guanines. HOMO distribution may determine the DNA-binding sites of copper and account for the sequence specificity of DNA damage at consecutive guanines.

5. Role of sequence-specific DNA damage in carcinogenesis and aging

A variety of reactive species mediate DNA damage and play critical roles in human diseases, particularly cancer. DNA damage causes mutations, which can lead to activation of protooncogenes and inactivation of tumor suppressor genes, resulting in carcinogenesis. As described above, we have investigated the mechanisms of guanine-specific DNA damage mediated by reactive species. Particularly, 8-oxodG is an important oxidative product of guanine, and 8-oxodG formation can cause DNA misreplication that may lead to mutation such as G · C → T · A transversion, and carcinogenesis [76,77]. Oxidized form of 8-oxodG (probably guanidinohydantoin) can lead to G → T and G → C transversions [78]. 2,2-Diaminooxazolone, which is a piperidine-labile oxidative product of guanine, can cause G → C transversion [79]. Site-specific DNA damage induced by ROS can induce tandem CC → TT mutation [80]. Recently, it has been reported that oxidative DNA damage causes microsatellite instability [81].

In humans, telomere shortening is believed to be associated with cell senescence. Telomere plays essential roles in chromosomal structure and function, including stabilization of the chromosome during DNA replication and possible prevention of aberrant chromosomal recombination. Telomere in vertebrates contains highly conserved repeats of a characteristic hexameric sequence (5′-TTAGGG-3′). In human fibroblasts, the telomere length is decreased by 50–200 base pairs (on average, approximately 90 base pairs) per cell division under the normal condition. Recently, Zglinicki et al. reported an increase of the rate of telomere shortening by oxidative stress in human fibroblasts [82]. Telomere is shortened by approximately 500 base pairs per cell division under hyperoxic conditions. Age-dependent telomere shortening is slowed down by enrichment of intracellular Vitamin C via suppression of oxidative stress [83]. However, the mechanism for the increase of telomere shortening rate by oxidative stress remains to be clarified. To clarify the mechanism of the acceleration of telomere shortening, we investigated site-specific DNA damage induced by oxidative stress using ^{32}P 5′ end-labeled DNA fragment containing the telomere sequence (5′-TTAGGG-3′). H_2O_2 plus Cu(II) caused DNA damage, including 8-oxodG formation, specifically at the GGG sequence in the telomere sequence (5′-TTAGGG-3′) [84]. UVA radiation plus riboflavin also induced 8-oxodG formation specifically at the GGG sequence in telomere through electron transfer (unpublished data). Human 8-oxodG-DNA glycosylase introduces a chain break in a double-stranded oligonucleotide specifically at an 8-oxodG residue [85]. Therefore, the formation of 8-oxodG at the GGG triplet in telemore sequence induced by oxidative stress could participate in acceleration of telomere shortening (Fig. 4). Finally, it is concluded that sequence-specific oxidative damage to DNA may

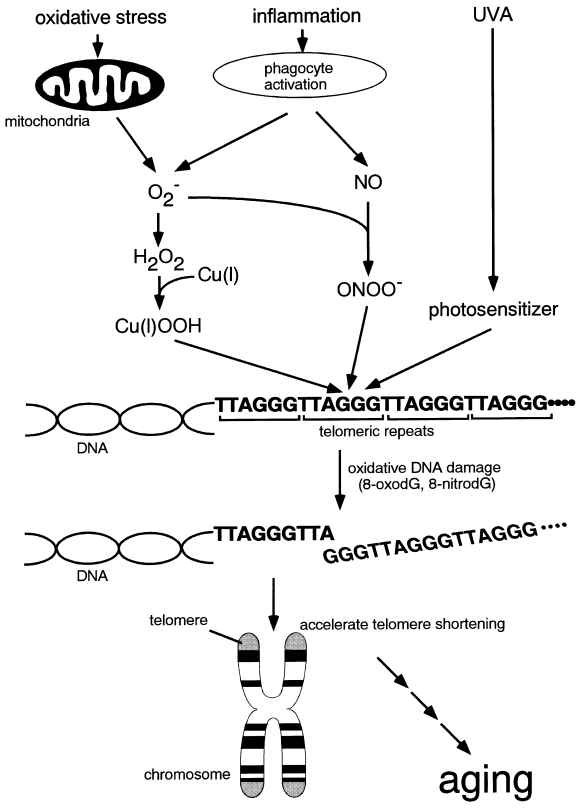

Fig. 4. Proposed mechanism of acceleration of telomere shortening by oxidative stress.

play important roles in not only carcinogenesis but also aging.

6. Conclusions

We have investigated the mechanisms of DNA damage induced by chemicals and UVA radiation. We found that various reactive species mediate DNA damage in sequence-specific manners. On the basis of our findings, the sequence specificity of DNA damage is presumed to be determined by (a) redox potential of reactive species: $^{\bullet}$OH is a highly reactive species, which causes DNA damage at every nucleotide, while other less reactive species cause guanine-specific DNA damage; (b) lowering of ionization potential and 5′-localization of HOMO on GG bases; (c) sequence-specific binding of metal ions to DNA, probably due to HOMO distribution and molecular electrostatic potentials.

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