*Free Radical Research*, April 2007; 41(4): 461–468

informa
healthcare

# Photosensitized DNA damage induced by NADH: Site specificity and mechanism

KIMIKO ITO[1], YUSUKE HIRAKU[2], & SHOSUKE KAWANISHI[2,3]

[1]*Department of Life Science, Tsu City College, Tsu, Mie 514-0112, Japan,* [2]*Department of Environmental and Molecular Medicine, Mie University Graduate School of Medicine, Tsu, Mie 514-8507, Japan, and* [3]*Faculty of Health Science, Suzuka University of Medical Science, 1001-1 Kishioka, Suzuka, Mie 510-0293, Japan*

Accepted by Professor J. Cadet

*(Received 4 September 2006; in revised form 27 November 2006)*



**Abstract**

Increasing evidence reveals the carcinogenicity of UVA radiation. We demonstrated that UVA-irradiated NADH induced damage to $^{32}$P-labeled DNA fragments obtained from the *p53* gene in the presence of Cu(II). Formamidopyrimidine glycosylase (Fpg)-sensitive lesions were formed at guanine residues, whereas piperidine-labile lesions occurred frequently at thymine residues. Formation of 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG), upon UVA exposure in the presence of Cu(II), increased depending on NADH concentration. Catalase and bathocuproine, a Cu(I)-specific chelator, inhibited the DNA damage, suggesting the involvement of reactive species derived from $H_2O_2$ and Cu(I). UVA-irradiated riboflavin induced DNA cleavage through electron transfer at 5′ guanine of the 5′-GG-3′ sequence with both Fpg and piperidine treatments; Fpg induced less cleavage at the guanine residues than piperidine. These results imply that NADH may participate as an endogenous photosensitizer in UVA carcinogenesis via $H_2O_2$ generation, producing metal-mediated mutagenic lesions such as 8-oxodG.

**Keywords:** *NADH, DNA damage, 8-oxo-7,8-dihydro-2′-deoxyguanosine, UVA, hydrogen peroxide*

## Introduction

Ample evidence is now presented that, in addition to UVB radiation, UVA radiation of sunlight plays an important role in skin carcinogenesis [1–5]. Although the carcinogenic potential of UVA is much lower than that of UVB on the basis of incident energy, UVA constitutes most of the solar UV at the surface of the earth and penetrates deeply into the human skin epidermis reaching the basal cell layers, which are likely to contain the target cells of tumor initiation [6]. It is generally accepted that UV-induced skin cancers result from the accumulation of DNA photolesions in crucial genes such as oncogenes and/or tumor suppressor genes [7]. In UVB wavelengths, pyrimidine photoproducts generated by the direct absorption of UV light by DNA are shown to be relevant to mutagenesis and carcinogenesis [7]. In contrast, it is speculated that UVA can produce mutagenic DNA lesions indirectly through photosensitization reactions mediated by intracellular chromophores, since UVA is hardly absorbed by DNA.

There have been considerable studies demonstrating the importance of oxidative stress in UV-induced carcinogenesis [5,8,9]. It is assumed that photosensitized choromophores in the epidermis react with oxygen to produce reactive oxygen species such as singlet oxygen ($^1O_2$) or superoxide anion radical ($O_2^-$) or directly with DNA via electron transfer. In this regard, we have demonstrated that the exposure of double-stranded DNA to UVA in the presence of various endogenous photosensitizers, such as riboflavin, folic acid, pterin derivatives and menadione, produces promutagenic 8-oxo-7,8-dihydro-2′-deoxyguanosine

Correspondence: S. Kawanishi, Faculty of Health Science, Suzuka University of Medical Science, 1001-1 Kishioka, Suzuka, Mie 510-0293, Japan. Tel: 81 59 381 2388 *204. Fax: 81 59 381 2386. E-mail: kawanisi@suzuka-u.ac.jp

ISSN 1071-5762 print/ISSN 1029-2470 online 
DOI: 10.1080/10715760601145240

(8-oxodG) predominantly at guanine located 5′ to guanine via electron transfer reaction [10–13]. It was also shown that $^1O_2$, formed by hematoporphyrin-mediated photosensitization, induces modifications at most guanine residues [14]. In addition, certain drugs may act as exogenous photocarcinogens. We have demonstrated that nalidixic acid and fluoroquinolone antibacterials, such as lomefloxacin, which have been widely used for the treatment of infectious diseases, mediate UVA-induced DNA damage [15,16].

NADH is an important coenzyme in biological systems, functioning as carriers of hydrogen atoms and electrons in oxidation–reduction reactions. Its concentration in a certain tissue was estimated to be as high as 100–200 μM [17]. It has been reported that hydrogen peroxide ($H_2O_2$) can be generated by photoirradiation of NADH [18,19]. In addition, we have previously demonstrated that NADH could be one of the endogenous reductants inducing metal-dependent DNA damage [20]. Thus, we investigated in this study the DNA damage induced by UVA in the presence of NADH, using $^{32}$P-labeled DNA fragments obtained from the human *p53* tumor suppressor gene. We also analyzed the formation of 8-oxodG by photoirradiated NADH in calf thymus DNA.

## Materials and methods

### *Materials*

[γ-$^{32}$P]-ATP (222 TBq/mmol) was obtained from New England Nuclear (Boston, MA). NADH, superoxide dismutase (SOD, 3000 units/mg from bovine erythrocytes), catalase (45,000 units/mg from bovine liver), and calf thymus DNA were from Sigma Chemical Co. (St Louis, MO). Diethylenetriamine-*N,N,N′,N″,N″*-pentaacetic acid (DTPA) was from Dojin Chemicals Co. (Kumamoto, Japan). Restriction enzymes (*Apa* I and *Hind* III) and $T_4$ polynucleotide kinase were purchased from New England Biolabs (Bervery, MA). Restriction enzyme (*Eco* RI) and calf intestine phosphatase were from Boehringer Mannheim GmbH (Mannheim, Germany). Nuclease $P_1$ was from Yamasa Shoyu Co. (Chiba, Japan). *Escherichia coli* formamidopyrimidine-DNA glycosylase (Fpg) was from Trevigen Co. (Gaithersburg, MD).

### *Preparation of $^{32}$P-5′-end-labeled DNA fragments*

The DNA fragment of the human *p53* tumor suppressor gene was prepared from pUC18 plasmid, ligated fragments containing exons of the *p53* gene [21]. The 5′-end-labeled 650-base pair (*Hind* III⋆ 13972-*EcoR* I⋆ 14621) fragment was obtained as described previously [22]. The fragment was further digested with *Apa* I to obtain the singly $^{32}$P-5′-end-labeled double-stranded 443-base pair fragment (*Apa* I 14179-*EcoR* I⋆14621). The asterisk indicates $^{32}$P-labeling.

DNA fragments were also prepared from plasmid pbcNI which carries a 6.6-kilobase *Bam* HI chromosomal DNA segment containing human c-Ha-*ras*-1 protooncogene [23]. A singly labeled 337-base pair fragment (*Pst* I 2345–*Ava* I⋆ 2681) was obtained as described previously [23]. The asterisk indicates[32] Nucleotide numbering and starts with *Bam* HI site [24].

### *Detection of DNA damage induced by NADH plus UVA light*

The standard reaction mixture in a microtube (1.5-ml Eppendorf) contained $^{32}$P-labeled DNA fragment, 2 μM/base sonicated calf thymus DNA, 200 μM NADH and 20 μM $CuCl_2$ in 100 μl of 10 mM sodium phosphate buffer (pH 7.8). Denatured DNA fragments were prepared by heating double-stranded DNA fragments at 90°C for 5 min and quick chilling. The reaction mixtures were exposed to UVA light using 10-W UV lamps (365-nm, UVP Inc., CA) placed at a distance of 20 cm. The reaction mixture was protected from direct sunlight and the temperature was maintained at 0°C. In a certain experiment, $CuCl_2$ was added to the mixture after UVA irradiation. After irradiation, the DNA fragments were treated with 1 M piperidine at 90°C for 20 min or two units of Fpg protein in the reaction buffer (10 mM HEPES-KOH (pH 7.4), 100 mM KCl, 10 mM EDTA and 0.1 mg/ml bovine serum albumin) for 2 h at 37°C. The DNA fragments were subjected to electrophoresis on an 8 M urea/8% polyacrylamide gel. The autoradiogram was obtained by exposing an X-ray film to the gel. 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 [25] using a DNA sequencing system (LKB2010 Macrophor). A laser densitometer (LKB 2222 UltroScan XL) was used for the measurement of the relative amounts of oligonucleotides from treated DNA fragments.

### *Measurement of 8-oxodG in calf thymus DNA exposed to UVA light in the presence of NADH*

Calf thymus DNA (100 μM/base) was exposed to 10 J/cm$^2$ UVA light (365 nm, 10 W) with various concentrations of NADH and 20 μM $CuCl_2$ in 200 μl of 4 mM sodium phosphate buffer (pH 7.8). After ethanol precipitation, DNA was digested to the nucleosides with nuclease $P_1$ and calf intestine phosphatase, and analyzed with an HPLC-ECD as described previously [26].

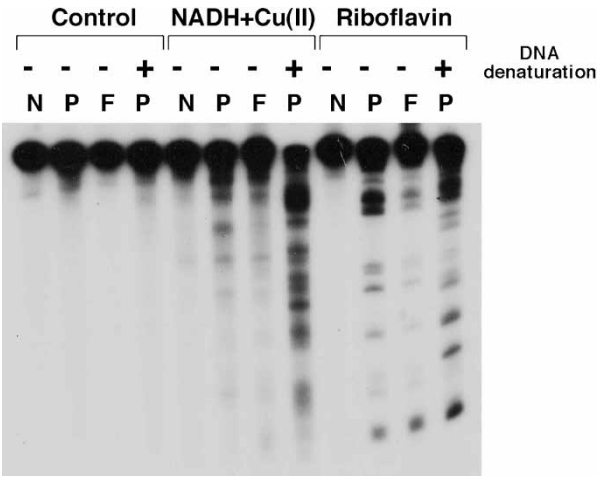


Figure 1. Autoradiogram of DNA fragments exposed to UVA light in the presence of NADH + Cu(II) or riboflavin. The reaction mixtures contained the $^{32}$P-labeled 443-base pair DNA fragment, 2 μM/base of sonicated calf thymus DNA, 200 μM NADH plus 20 μM $CuCl_2$, or 25 μM riboflavin in 100 μl of 10 mM sodium phosphate buffer (pH 7.8) containing 5 μM DTPA. The reaction mixtures were exposed to 10 J/cm$^2$ UVA light (365-nm). The DNA fragments were then treated with piperidine or Fpg, and analyzed by the method described in the Materials and methods. N, no treatment; P, piperidine treatment; F, treatment with Fpg protein.

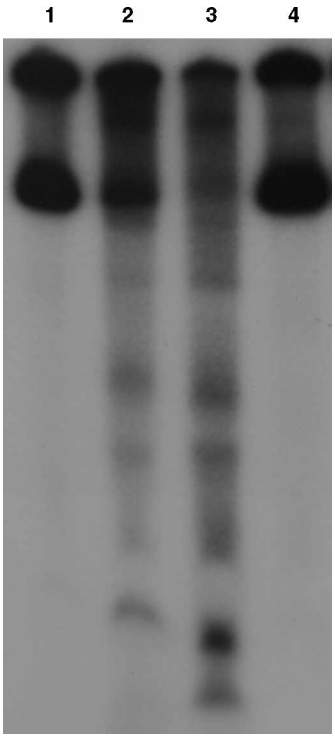


Figure 2. NADH-induced DNA photodamage by addition of Cu(II) after irradiation. The $^{32}$P-labeled 337-base pair DNA fragments were exposed to 10 J/cm$^2$ UVA light (365-nm) in the presence of 200 μM NADH with (lane 2) or without (lane 3) 20 μM $CuCl_2$ in 100 μl of 10 mM phosphate buffer (pH 7.8) containing 2 μM/base of sonicated calf thymus DNA and 5 μM DTPA. In lane 3, 20 μM $CuCl_2$ was added to the reaction mixture after the irradiation, followed by incubation for 20 min at 37°C. Then DNA fragments were treated with piperidine and analyzed by the method described in the Materials and methods. Lane 1, DNA fragments were treated with only 10 J/cm$^2$ UVA light; lane 4, DNA fragments were kept in the dark in the presence of 200 μM NADH for the same duration as required for 10 J/cm$^2$ irradiation, and incubated with 20 μM $CuCl_2$ for 20 min at 37°C.

## Results

### *DNA damage induced by UVA irradiation of NADH*

Figure 1 shows the autoradiogram of $^{32}$P-end-labeled DNA fragments exposed to UVA light in the presence of NADH or riboflavin. UVA irradiation of NADH induced degradation of double-stranded DNA in the presence of Cu(II). DNA damage was observed upon treatment with piperidine or Fpg protein, whereas no damage was observed without these treatments, suggesting that the DNA damage is due to base modification with little or no strand breakage. When the double-stranded DNA was denatured to the single-stranded DNA by heating at 90°C, the DNA damage was significantly enhanced. No oligonucleotide was produced without Cu(II) or without UVA radiation (data not shown). In agreement with our previous findings [10], UVA irradiation of double-stranded DNA in the presence of riboflavin, followed by treatment with piperidine or Fpg protein, induced DNA cleavage. Interestingly, as shown in Figure 2, DNA damage was induced when NADH was irradiated by UVA in the absence of Cu(II) and then incubated with Cu(II). The cleavage pattern (lane 3) was similar to that induced by UVA irradiation of NADH in the presence of Cu(II) (lane 2), or $H_2O_2$ plus Cu(I) (data not shown). Non-irradiated NADH plus Cu(II) did not induce DNA damage under the condition used (lane 4). These results suggest $H_2O_2$ formation via $O_2^-$ generated by one-electron transfer from photoexcited NADH to oxygen (minor type II).

### *Effects of scavengers on the photosensitized DNA damage in the presence of NADH and Cu(II)*

As shown in Figure 3, catalase and bathocuproine, a Cu(I)-specific chelator, inhibited the DNA damage induced by UVA irradiation of NADH in the presence of Cu(II), whereas typical free hydroxyl radical (·OH) scavengers (ethanol, mannitol and sodium formate) and SOD showed no inhibitory effects.

### *Site specificity of DNA photodamage induced by NADH or riboflavin*

Figure 4A shows that exposure of double-stranded DNA to UVA in the presence of NADH and Cu(II), followed by piperidine treatment, resulted in the cleavage frequently at thymine residue, located 3′ to guanine or adenine. As shown in Figure 4B, Fpg treatment induced the cleavage mainly at guanine residue of the 5′-ACG-3′ sequence complementary to codon 273. In addition, guanine residues at other sequences and some cytosine residues were cleaved

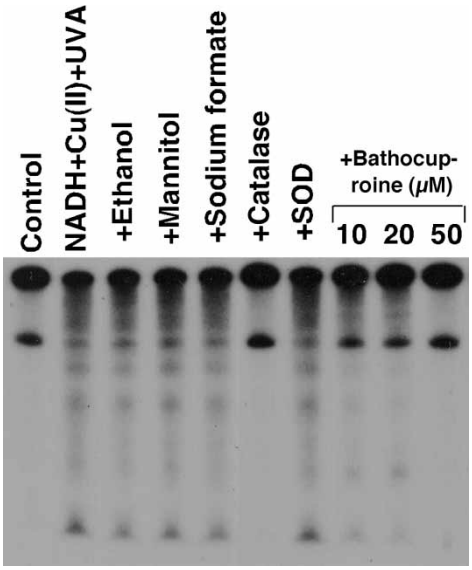


Figure 3. Effects of scavengers and bathocuproine on DNA photodamage induced by NADH in the presence of Cu(II). The reaction mixture contained the $^{32}$P-labeled 337-base pair DNA fragment, 2 μM/base of sonicated calf thymus DNA, 200 μM NADH, 20 μM Cu(II) and 5 μM DTPA in 100 μl of 10 mM sodium phosphate buffer (pH 7.8). DNA fragments were denatured as described in the Materials and methods. The reaction mixtures were exposed to 10 J/cm$^2$ UVA light (365-nm). Then, the DNA fragments were treated with piperidine and analyzed by the method described in the Materials and methods. Where indicated, 1 M ethanol, 0.1 M mannitol, 0.1 M sodium formate, 30 units of catalase, 30 units of SOD or indicated concentration of bathocuproine was included.

to a lesser extent. For comparison, Figure 5 shows that photoirradiated riboflavin induced DNA cleavage specifically at the guanine residues located 5′ to guanine in double-stranded DNA after treatment with Fpg as well as piperidine. In addition, it is noteworthy that Fpg induced less cleavage at the guanine residues than piperidine.

*Formation of 8-oxodG induced by UVA plus NADH*

Figure 6 shows that UVA irradiation of NADH in the presence of Cu(II) increased 8-oxodG content in both double-stranded and single-stranded DNA with the NADH concentrations. In single-stranded DNA, the formation of 8-oxodG by photoexcited NADH was enhanced compared to double-stranded DNA, Without UVA radiation, NADH plus Cu(II) did not cause 8-oxodG formation under the conditions employed (data not shown). Riboflavin also induced 8-oxodG formation upon UVA irradiation, and in contrast to NADH, the amount of 8-oxodG in double-stranded DNA was larger than that in single-stranded DNA.

## Discussion

The present study has demonstrated that UVA irradiation of NADH induces DNA damage, mainly base modification, in the presence of Cu(II). DNA cleavage sites observed with piperidine treatment were frequently at thymine, located 3′ to guanine or adenine. With Fpg treatment, the cleavage occurred mainly at guanine residues, especially at the 5′-ACG-3′ sequence complementary to codon273 of the *p53* gene, and cytosine residues to some extent. In contrast, in accordance with our previous report [10], photoexcited riboflavin has been shown to induce DNA cleavage specifically at 5′ guanine of 5′-GG-3′ with Fpg treatment as well as piperidine treatment. It is generally accepted that the photosensitized oxidation reactions are classified in types I and II processes. The type I process involves direct interaction of an excited sensitizer with a substrate via electron transfer. In type II process, an excited sensitizer can react with oxygen, generating mainly $^1O_2$ by energy transfer (major type II), or giving in some cases $O_2^-$ by electron transfer from the excited sensitizer to oxygen (minor type II). We have previously demonstrated that photoexcited riboflavin induces DNA damage through type I reaction [10]. In addition, studies with various endogenous and exogenous photosensitizers, such as pterins, folic acid, nalidixic acid, and xanthone analogues, have shown that the type I photosensitized oxidation induces piperidine-labile damage in double-stranded DNA predominantly at 5′ guanine of 5′-GG-3′ sequence [10–13,15,16]. Since the site specificity of photosensitized DNA damage by NADH is quite different from that by riboflavin, it is suggested that type I reaction is not involved in NADH-induced DNA photodamage.

DNA damage induced by UVA in the presence of NADH and Cu(II) was almost completely inhibited by catalase and bathocuproine, a Cu(I)-specific chelator, but not by SOD and typical free hydroxyl radical ·OH scavengers (ethanol, mannitol and sodium formate). These results suggest that reactive species derived from $H_2O_2$ and Cu(I) are primarily responsible for the damage, but ·OH does not play an important role. We have previously demonstrated that a high concentration of NADH induces DNA damage without UVA irradiation in the presence of Cu(II) and suggested that optimal molar ratios of DNA/metal ion yield copper with a high redox potential which oxidizes unirradiated NADH to ·NAD being further oxidized to $NAD^+$ with generation of $O_2^-$, and that $H_2O_2$ reacts with Cu(I) to give reactive oxygen species causing DNA damage [20]. In the present condition, however, unirradiated NADH at a lower concentration did not induce DNA damage in the presence of Cu(II). In addition, DNA photodamage occurred when NADH was irradiated in the absence of Cu(II) and then incubated with Cu(II). It is speculated, therefore, that although the same reactive species are involved in DNA damage in these studies, they are generated through different mechanisms. Accordingly, a possible reaction mechanism is speculated as follows.

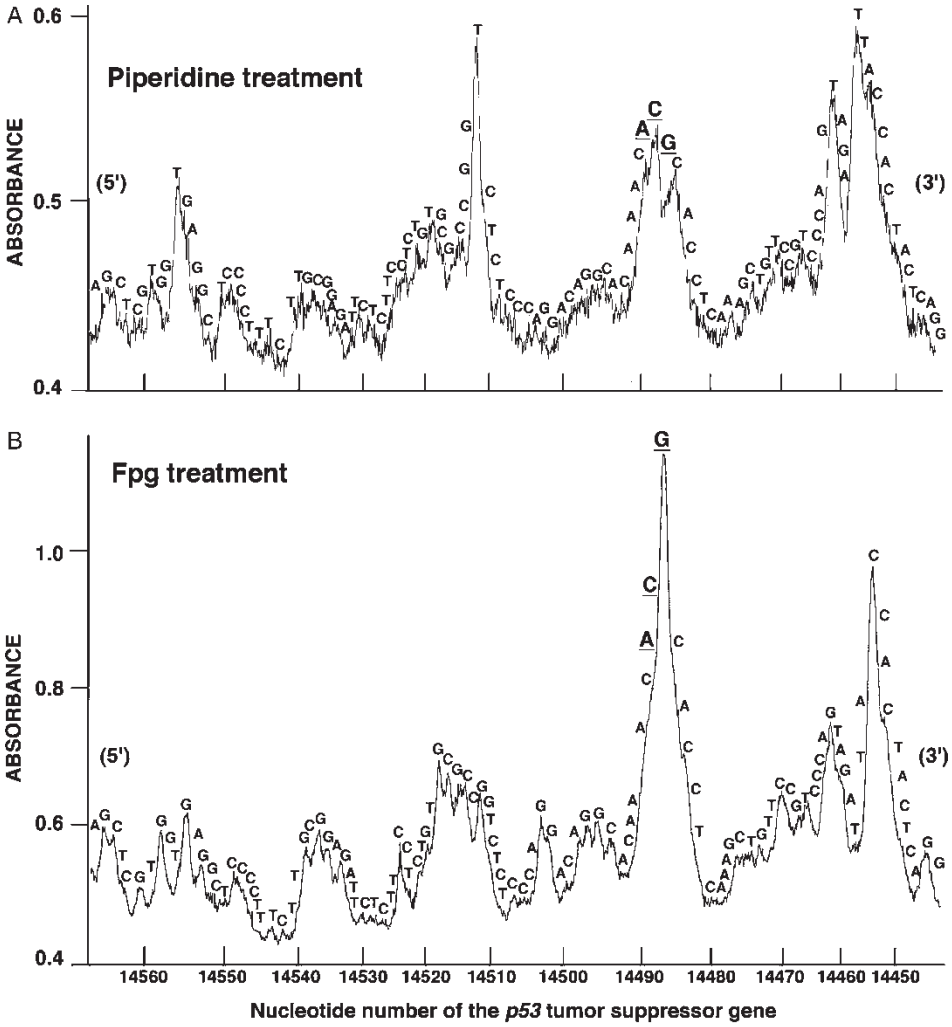


Figure 4. Site specificity of DNA photodamage induced by NADH in the presence of Cu(II). The reaction mixture contained the $^{32}$P-labeled 443-base pair DNA fragment, 2 μM/base sonicated calf thymus DNA, 200 μM NADH, 20 μM Cu(II) and 5 μM DTPA in 100 μl of 10 mM sodium phosphate buffer (pH 7.8). The reaction mixtures were exposed to 10 J/cm$^2$ UVA light (365-nm). Subsequently, the DNA fragments were treated with piperidine (A) or Fpg (B). The DNA was analyzed and the relative amounts of oligonucleotides were measured by the methods described in the Materials and methods. Underscoring shows the complementary sequence to codon 273. *Horizontal axis*, nucleotide numbers of the *p53* tumor suppressor gene.

Irradiation of NADH with UVA in the presence of oxygen causes one-electron oxidation of NADH to produce $O_2^-$ and $NADH^{+\cdot}$, and deprotonation of the latter gives ·NAD which reacts with oxygen at an almost diffusion-controlled rate ($1.9 \times 10^9\,M^{-1}\,s^{-1}$), generating $NAD^+$ and $O_2^-$ [27]. Involvement of the initial one-electron transfer in the photooxidation of NADH has been well investigated [27,28]. The resulting $O_2^-$ is autodismutated to $H_2O_2$, and reduces Cu(II) to Cu(I). Finally, $H_2O_2$ reacts with Cu(I) to form reactive species such as Cu(I)—OOH, which is capable of inducing DNA damage [29]. These reaction schemes are supported by the earlier findings that NADH induces $H_2O_2$ formation during photoirradiation in the presence of oxygen [18,19]. Although it was considered that photoexcited NADH might react with DNA-bound Cu(II), we found that DNA damage was induced by the addition of Cu(II) to the reaction mixture after irradiation of NADH without Cu(II) and the cleavage pattern remained the same as that induced by irradiation of NADH in the presence of Cu(II). These findings suggest that photoexcited NADH reacts with oxygen to generate reactive oxygen species, which participate in Cu(II)-mediated DNA damage. Thus, this is the first study showing the site specificity of photosensitized DNA damage via minor type II mechanism.

Fpg catalyzes the excision of piperidine-resistant 8-oxoG [30], as well as piperidine-labile 8-oxoG-derived lesions such as oxazolone, oxaluric acid, spiroiminodihydantoin, guanidinohydantoin and iminoallantoin [31–35]. We have found that Fpg treatment of DNA exposed to UVA in the presence of NADH and Cu(II) resulted in the cleavage mainly at guanine residues. On the other hand, piperidine treatment induced the cleavage frequently at thymine residues and the cleavage at guanine was greatly reduced compared with Fpg treatment. Therefore, it is assumed that

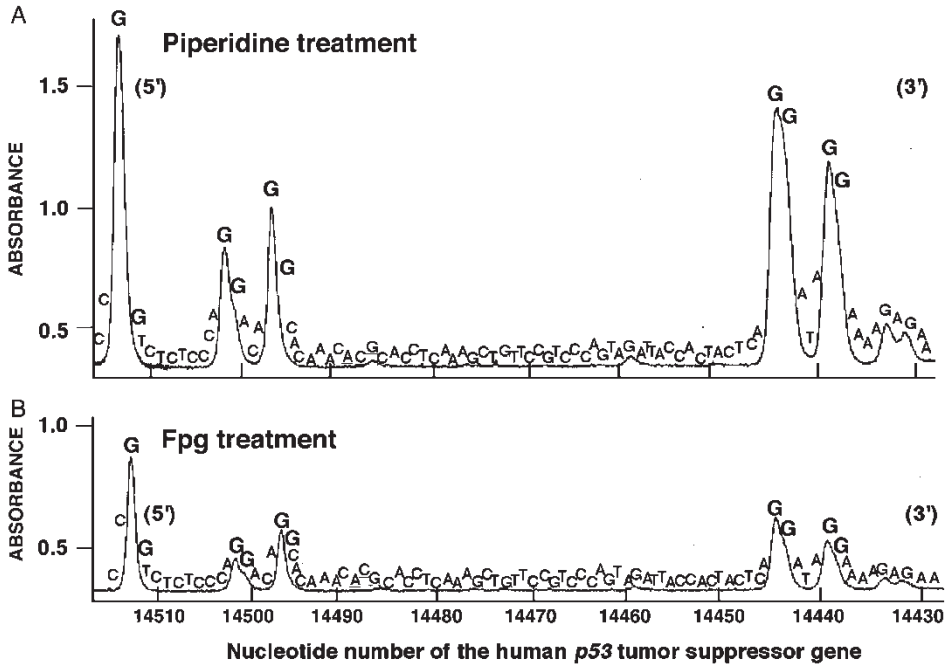


Figure 5. Site specificity of DNA photodamage induced by riboflavin. The reaction mixture contained the $^{32}$P-labeled 443-base pair DNA fragment, 2 μM/base sonicated calf thymus DNA, 25 μM riboflavin and 5 μM DTPA in 100 μl of 10 mM sodium phosphate buffer (pH 7.8). The reaction mixtures were exposed to 10 J/cm$^2$ UVA light (365-nm). Subsequently, the DNA fragments were treated with piperidine (A) or Fpg (B). The DNA was analyzed and the relative amounts of oligonucleotides were measured by the methods described in the Materials and methods. Underscoring shows the complementary sequence to codon 273. *Horizontal axis*, nucleotide numbers of the *p53* tumor suppressor gene.

8-oxodG represents the main oxidation product of guanine in DNA exposed to UVA in the presence of NADH and Cu(II). This idea is supported by our observation in HPLC-ECD study that 8-oxodG formation was increased in DNA treated with photoirradiated NADH and Cu(II). In contrast, photoirradiated riboflavin induced DNA cleavage specifically at 5′ guanine of 5′-GG-3′ sequence via electron transfer both with Fpg and piperidine treatments, and the cleavages at guanine were greatly and equally enhanced with piperidine treatment. Our HPLC-ECD analysis showed an increase in 8-oxodG formation by exposure to photoirradiated riboflavin. Considering these results, it is likely that in agreement with our previous report, 8-oxodG is formed at the guanine of 5′-GG-3′ sequence; it is however a relatively minor product of guanine oxidation, and other oxidized guanine products arising from 8-oxodG

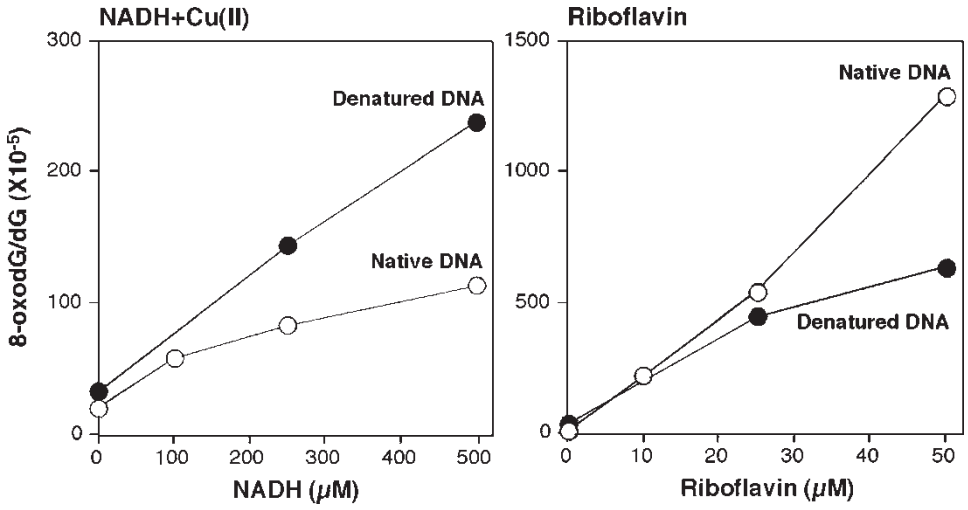


Figure 6. Formation of 8-oxodG in DNA irradiated with UVA in the presence of NADH + Cu(II) or riboflavin. The reaction mixture containing 100 μM/base calf thymus DNA, NADH + 20 μM Cu(II) or riboflavin and 5 μM DTPA in 200 μl of 4 mM sodium phosphate buffer (pH 7.8) was exposed to 10 J/cm$^2$ UVA light (365-nm). Where indicated, the DNA fragments were denatured by heating at 90°C for 5 min, followed by quick chilling on ice. *Open circles*, native (double-stranded) DNA. *Closed circles*, denatured (single-stranded) DNA.

oxidation is involved in the guanine lesions at 5′-GG-3′ sequence, as was discussed previously [12]. Our finding is supported by earlier works suggesting that 8-oxodG formed in the GG sequence is more easily oxidized than guanine in this sequence [36,37], and that piperidine-labile 2-aminoimidazolone is formed at 5′-GG-3′ sequence by photoirradiated riboflavin [38]. Concerning the cleavages at pyrimidine residues, it has been reported that Cu(II)/$H_2O$ system preferentially damages pyrimidine bases in DNA giving rise to various oxidatively generated products [39,40]. Among them, thymine glycol [41], 5-formyluracil [42] and 5-hydroxyluracil [43] are alkali-labile. These products may be generated at thymine residues in our experiments.

It has been shown that irradiation of mammalian cells with UVA and visible light induced $H_2O_2$ production [44,45]. Recently, Sakurai et al. have reported *in vivo* detection of $O_2^-$ in the UVA-exposed skin of mice using a sensitive chemiluminescence method [46]. In addition, Besaratinia et al. have demonstrated in an HPLC-MS/MS analysis that 8-oxodG is generated in the genomic DNA of normal human skin fibroblast exposed to UVA1 [47]. They have also identified the formation of Fpg-sensitive sites along the *p53* gene of the UVA1-irradiated cells dependent on radiation dose. Furthermore, immunohistochemical study by Agar et al. revealed that 8-oxodG accumulation showed basal localization in the epidermis of both squamous cell carcinomas and solar keratosis, and it correlated with the basal epithelial distribution of G:C → T:A transversion [5]. These results strongly suggest that oxidative DNA damage induced by UVA in the presence of photosensitizer plays an important role in skin carcinogenesis. In conclusion, we have shown that UVA irradiation of NADH in the presence of Cu(II) induces DNA modification frequently at thymine and guanine residues by a mechanism involving $O_2^-$ (minor Type II). Although neither $O_2^-$ nor $H_2O_2$ can cause DNA damage by themselves, $H_2O_2$ is long-lived and can diffuse in the cytoplasm to react with metal ions. It is, therefore, considered that NADH may participate as an endogenous photosensitizer in UVA carcinogenesis via $H_2O_2$ generation, producing metal-mediated mutagenic lesions such as 8-oxodG.



## Acknowledgements

This work was supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan.

## References

[1] IARC Working Group. Solar and ultraviolet radiation. IARC monographs on the evaluation of the carcinogenic risk of chemicals to humans, Vol. 55 Lyon: IARC; 1992.

[2] Setlow RB, Grist E, Thompson K, Woodhead AD. Wavelengths effective in induction of malignant melanoma. Proc Natl Acad Sci USA 1993;90:6666–6670.

[3] Drobetsky EA, Turcotte J, Chateauneuf A. A role for ultraviolet A in solar mutagenesis. Proc Natl Acad Sci USA 1995;92:2350–2354.

[4] Runger TM. Role of UVA in the pathogenesis of melanoma and non-melanoma skin cancer. A short review. Photodermatol Photoimmunol Photomed 1999;15:212–216.

[5] Agar NS, Halliday GM, Barnetson RS, Ananthaswamy HN, Wheeler M, Jones AM. The basal layer in human squamous tumors harbors more UVA than UVB fingerprint mutations: A role for UVA in human skin carcinogenesis. Proc Natl Acad Sci USA 2004;101:4954–4959.

[6] Morris RJ. Keratinocyte stem cells: Targets for cutaneous carcinogens. J Clin Invest 2000;106:3–8.

[7] Ananthaswamy HN, Pierceall WE. Molecular mechanisms of ultraviolet radiation carcinogenesis. Photochem Photobiol 1990;52:1119–1136.

[8] Tyrrell RM, Keyse SM. New trends in photobiology. The interaction of UVA radiation with cultured cells. J Photochem Photobiol B 1990;4:349–361.

[9] Kunisada M, Sakumi K, Tominaga Y, Budiyanto A, Ueda M, Ichihashi M, Nakabeppu Y, Nishigori C. 8-Oxoguanine formation induced by chronic UVB exposure makes Ogg1 knockout mice susceptible to skin carcinogenesis. Cancer Res 2005;65:6006–6010.

[10] Ito K, Inoue S, Yamamoto K, Kawanishi S. 8-Hydroxydeoxyguanosine formation at the 5′ site of 5′-GG-3′ sequences in double-stranded DNA by UV radiation with riboflavin. J Biol Chem 1993;268:13221–13227.

[11] Ito K, Kawanishi S. Photoinduced hydroxylation of deoxyguanosine in DNA by pterins: Sequence specificity and mechanism. Biochemistry 1997;36:1774–1781.

[12] Ito K, Kawanishi S. Site-specific DNA damage induced by UVA radiation in the presence of endogenous photosensitizer. Biol Chem 1997;378:1307–1312.

[13] Hirakawa K, Suzuki H, Oikawa S, Kawanishi S. Sequence-specific DNA damage induced by ultraviolet A-irradiated folic acid via its photolysis product. Arch Biochem Biophys 2003; 410:261–268.

[14] Kawanishi S, Inoue S, Sano S, Aiba H. Photodynamic guanine modification by hematoporphyrin is specific for single-stranded DNA with singlet oxygen as a mediator. J Biol Chem 1986;261:6090–6095.

[15] Hiraku Y, Ito H, Kawanishi S. Site-specific hydroxylation at polyguanosine in double-stranded DNA by UVA radiation with nalidixic acid. Biochem Biophys Res Commun 1998;251: 466–470.

[16] Hiraku Y, Kawanishi S. Distinct mechanisms of guanine-specific DNA photodamage induced by nalidixic acid and fluoroquinolone antibacterials. Arch Biochem Biophys 2000; 382:211–218.

[17] Malaisse WJ, Hutton JC, Kawazu S, Herchuelz A, Valverde I, Sener A. The stimulus-secretion coupling of glucose-induced insulin release. XXXV. The links between metabolic and cationic events. Diabetologia 1979;16:331–341.

[18] Czochralska B, Kawczynski W, Bartosz G, Shugar D. Oxidation of excited-state NADH and NAD dimer in aqueous medium involvement of $O_2^-$ as a mediator in the presence of oxygen. Biochim Biophys Acta 1984;801:403–409.

[19] Cunningham ML, Johnson JS, Giovanazzi SM, Peak MJ. Photosensitized production of superoxide anion by monochromatic (290–405 nm) ultraviolet irradiation of NADH and NADPH coenzymes. Photochem Photobiol 1985;42: 125–128.

[20] Oikawa S, Kawanishi S. Site-specific DNA damage induced by NADH in the presence of copper(II): Role of active oxygen species. Biochemistry 1996;35:4584–4590.

[21] Chumakov P. EMBL Data Library 1990; Accession Number X54156.
[22] Yamashita N, Murata M, Inoue S, Hiraku Y, Yoshinaga T, Kawanishi S. Superoxide formation and DNA damage induced by a fragrant furanone in the presence of copper(II). Mutat Res 1998;397:191–201.
[23] Ito K, Yamamoto K, Kawanishi S. Manganese-mediated oxidative damage of cellular and isolated DNA by isoniazid and related hydrazines: Non-Fenton-type hydroxyl radical formation. Biochemistry 1992;31:11606–11613.
[24] Capon DJ, Chen EY, Levinson AD, Seeburg PH, Goeddel DV. Complete nucleotide sequences of the T24 human bladder carcinoma oncogene and its normal homologue. Nature 1983; 302:33–37.
[25] Maxam AM, Gilbert W. Sequencing end-labeled DNA with base-specific chemical cleavages. Methods Enzymol 1980;65: 499–560.
[26] Kasai H, Crain PF, Kuchino Y, Nishimura S, Ootsuyama A, Tanooka H. Formation of 8-hydroxyguanine moiety in cellular DNA by agents producing oxygen radicals and evidence for its repair. Carcinogenesis 1986;7:1849–1851.
[27] Land EJ, Swallow AJ. One-electron reactions in biochemical systems as studied by pulse radiolysis. IV. Oxidation of dihydronicotinamide-adenine dinucleotide. Biochim Biophys Acta 1971;234:34–42.
[28] Czochralska B, Lindqvist L. Biphotonic one-electron oxidation of NADH on laser excitation at 353 nm. Chem Phys Lett 1983;101:297–299.
[29] Yamamoto K, Kawanishi S. Hydroxyl free radical is not the main active species in site-specific DNA damage induced by copper (II) ion and hydrogen peroxide. J Biol Chem 1989;264: 15435–15440.
[30] Boiteux S, Gajewski E, Laval J, Dizdaroglu M. Substrate specificity of the *Escherichia coli* Fpg protein (formamidopyrimidine-DNA glycosylase): Excision of purine lesions in DNA produced by ionizing radiation or photosensitization. Biochemistry 1992;31:106–110.
[31] Spassky A, Angelov D. Influence of the local helical conformation on the guanine modifications generated from one-electron DNA oxidation. Biochemistry 1997;36: 6571–6576.
[32] Duarte V, Gasparutto D, Jaquinod M, Cadet J. *In vitro* DNA synthesis opposite oxazolone and repair of this DNA damage using modified oligonucleotides. Nucleic Acids Res 2000;28: 1555–1563.
[33] Burrows CJ, Muller JG, Kornyushyna O, Luo W, Duarte V, Leipold MD, David SS. Structure and potential mutagenicity of new hydantoin products from guanosine and 8-oxo-7,8-dihydroguanine oxidation by transition metals. Environ Health Perspect 2002;110(Suppl 5):713–717.
[34] Tretyakova NY, Wishnok JS, Tannenbaum SR. Peroxynitrite-induced secondary oxidative lesions at guanine nucleobases: Chemical stability and recognition by the Fpg DNA repair enzyme. Chem Res Toxicol 2000;13:658–664.
[35] Dherin C, Gasparutto D, O'Connor TR, Cadet J, Boiteux S. Excision by the human methylpurine DNA *N*-glycosylase of cyanuric acid, a stable and mutagenic oxidation product of 8-oxo-7,8-dihydroguanine. Int J Radiat Biol 2004;80:21–27.
[36] Prat F, Houk KN, Foote CS. Effect of guanine stacking on the oxidation of 8-oxoguanine in B-DNA. J Am Chem Soc 1998; 120:845–846.
[37] Ravanat JL, Saint-Pierre C, Cadet J. One-electron oxidation of the guanine moiety of 2′-deoxyguanosine: Influence of 8-oxo-7,8-dihydro-2′-deoxyguanosine. J Am Chem Soc 2003;125: 2030–2031.
[38] Kino K, Saito I, Sugiyama H. Product analysis of GG-specific photooxidation of DNA via electron transfer: 2-Aminoimidazolone as a major guanine oxidation product. J Am Chem Soc 1998;120:7373–7374.
[39] Dizdaroglu M, Rao G, Halliwell B, Gajewski E. Damage to the DNA bases in mammalian chromatin by hydrogen peroxide in the presence of ferric and cupric ions. Arch Biochem Biophys 1991;285:317–324.
[40] Evans MD, Dizdaroglu M, Cooke MS. Oxidative DNA damage and disease: Induction, repair and significance. Mutat Res 2004;567:1–61.
[41] Higurashi M, Ohtsuki T, Inase A, Kusumoto R, Masutani C, Hanaoka F, Iwai S. Identification and characterization of an intermediate in the alkali degradation of (6–4) photoproduct-containing DNA. J Biol Chem 2003;278:51968–51973.
[42] Ono A, Okamoto T, Inada M, Nara H, Matsuda A. Nucleosides and nucleotides. 131. Synthesis and properties of oligonucleotides containing 5-formyl-2′-deoxyuridine. Chem Pharm Bull (Tokyo) 1994;42:2231–2237.
[43] Simon P, Gasparutto D, Gambarelli S, Saint-Pierre C, Favier A, Cadet J. Formation of isodialuric acid lesion within DNA oligomers via one-electron oxidation of 5-hydroxyuracil: Characterization, stability and excision repair. Nucleic Acids Res 2006;34:3660–3669.
[44] Peak JG, Peak MJ. Induction of slowly developing alkali-labile sites in human P3 cell DNA by UVA and blue- and green-light photons: Action spectrum. Photochem Photobiol 1995;61: 484–487.
[45] Hockberger PE, Skimina TA, Centonze VE, Lavin C, Chu S, Dadras S, Reddy JK, White JG. Activation of flavin-containing oxidases underlies light-induced production of $H_2O_2$ in mammalian cells. Proc Natl Acad Sci USA 1999;96: 6255–6260.
[46] Sakurai H, Yasui H, Yamada Y, Nishimura H, Shigemoto M. Detection of reactive oxygen species in the skin of live mice and rats exposed to UVA light: A research review on chemiluminescence and trials for UVA protection. Photochem Photobiol Sci 2005;4:715–720.
[47] Besaratinia A, Synold TW, Chen HH, Chang C, Xi B, Riggs AD, Pfeifer GP. DNA lesions induced by UVA1 and B radiation in human cells: Comparative analyses in the overall genome and in the *p53* tumor suppressor gene. Proc Natl Acad Sci USA 2005;102:10058–10063.