Articles

Nonenzymatic Reduction of Nitro Derivative of a Heterocyclic Amine IQ by NADH and Cu(II) Leads to Oxidative DNA Damage

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ABSTRACT: Nitro derivative (nitro-IQ) of a carcinogenic heterocyclic amine 2-amino-3-methylimidazo-[4,5-*f*]quinoline (IQ) is known to be a potent mutagen as well as IQ, and nitro-IQ is believed to be activated enzymatically by nitroreductase. We investigated nonenzymatic reduction of nitro-IQ by an endogenous reductant NADH and the ability of inducing DNA damage by nitro-IQ. Nitro-IQ caused DNA damage including 8-oxo-7,8-dihydro-2′-deoxyguanosine in the presence of NADH and Cu(II). Catalase and bathocuproine, a Cu(I)-specific chelator, inhibited the DNA damage, suggesting the involvement of H_2O_2 and Cu(I). Nitro-IQ induced DNA cleavage frequently at thymine and cytosine residues in the presence of NADH and Cu(II). UV−vis spectroscopic study showed that no spectral change of Nitro-IQ and NADH was observed in the absence of Cu(II), while rapid spectral change was observed in the presence of Cu-(II), suggesting that Cu(II) mediated redox reaction of nitro-IQ and NADH. These results suggest that nitro-IQ can be reduced nonenzymatically by NADH in the presence of Cu(II), and the redox reaction resulted in oxidative DNA damage due to the copper−oxygen complex, derived from the reaction of Cu(I) with H_2O_2. We conclude that nonenzymatic reduction of nitro-IQ and resulting in oxidative DNA damage can play a role in carcinogenesis of IQ.

2-Amino-3-methylimidazo[4,5-*f*]quinoline (IQ),[1] a representative member of mutagenic and carcinogenic heterocyclic amines, is isolated from cooked meet (*1*−*3*) and also cigarette smoke condensate (*4*). 2-Nitro-3-methylimidazo[4,5-*f*]quinoline (nitro-IQ) is an analogue of IQ with a nitro group in place of the amino group and detected in urine as one of IQ metabolites (*5*). Oxidative activation of IQ to nitro-IQ has been observed in chemical and enzymatic systems and also by photoirradiation. Chemically, IQ is converted to nitro-IQ by treatment with nitrite (*6*). Exposure to sunlight causes oxidation of IQ to form nitro-IQ (*7*), and thus nitro-IQ may be present by photoactivation in airborne particles from cigarette smoke and frying meat. Therefore, it seems likely that humans are exposed to nitro-IQ. Nitro-IQ exhibits mutagenicity in bacterial systems (*8*−*10*) and genotoxic activities (*11*, *12*), as well as IQ.

Regarding the mechanisms of DNA damage by carcinogenic IQ, DNA adduct formation has been considered to be a major causal factor (*1*, *13*, *14*). Recently, it is reported that several antioxidants (*15*−*17*) significantly inhibited IQ-hepatocarcinogenesis in rats, suggesting that reactive oxygen species may participate in heterocyclic amine-induced tumor development. Kato et al. (*18*) reported that 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG) level in rat liver was increased dose dependently along with the concentration of 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline (MeIQx) in the diet. Relevantly, Hayatsu and his colleagues (*19*, *20*) showed that N-hydroxy derivative of 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-2) treatment produced intracellular reactive oxygen species that can damage DNA in mouse cells in culture. Therefore, DNA adduct formation is a prerequisite, but itself may not be sufficient for their carcinogenic action. There remains a possibility that oxidative DNA damage plays an important role in carcinogenesis induced by IQ.

There has been a long-standing interest in the chemical reactions of NADH in enzyme-free systems. Several studies indicate that NADH may react nonenzymatically with some xenobiotics and mediate their reduction (*21*). A benzene metabolite 1,4-benzoquinone is nonenzymatically reduced by NADH to semiquinone radical (*22*), and diabetogenic alloxan is also reduced by NADH to alloxan radical (*23*). A number of nitroaromatic compounds can be enzymatically reduced by cellular reductases to the corresponding nitro anion radicals (*24*). It is also suggested that nitro-IQ is reduced by nitroreductase to N-hydroxy IQ, and then this derivative is further activated to N-acetoxy derivative (*9*). It

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[1] Abbreviations: IQ, 2-amino-3-methylimidazo[4,5-*f*]quinoline; nitro-IQ, 2-nitro-3-methylimidazo[4,5-f]quinoline; MeIQx, 2-amino-3,8-dimethylimidazo[4,5-*f*]quinoxaline; Trp-P-2, 3-amino-1-methyl-5*H*-pyrido[4,3-*b*]indole; NADH, *β*-nicotinamide adenine dinucleotide (reduced form); NAD^+, oxidized form of NADH; $O_2^{\bullet-}$, superoxide; SOD, superoxide dismutase; DTPA, diethylenetriamine-*N*,*N*,*N*′,*N*″,*N*″-pentaacetic acid; 8-oxodG, 8-oxo-7,8-dihydro-2′-deoxyguanosine (also known as 8-hydroxy-2′-deoxyguanosine); HPLC-ECD, an electrochemical detector coupled to a high-pressure liquid chromatograph.

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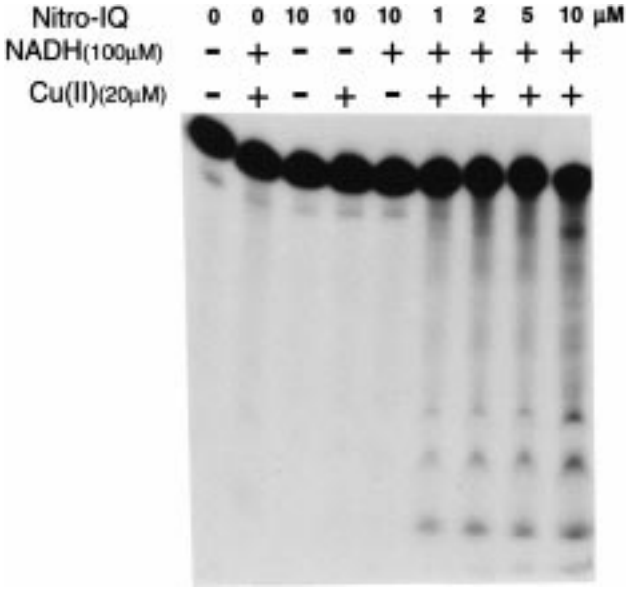

FIGURE 1: Autoradiogram of ^{32}P-labeled DNA fragments incubated with nitro-IQ in the presence of NADH and Cu(II). The reaction mixture contained ^{32}P-5′-end-labeled 98 bp DNA fragments, 5 μM/base of sonicated calf thymus DNA, indicated concentrations of nitro-IQ, 100 μM NADH, and 20 μM $CuCl_2$ in 200 μL of 10 mM sodium phosphate buffer (pH 7.8) containing 2.5 μM DTPA. The mixture was incubated at 37 °C for 1 h, followed by piperidine treatment, as described in the Materials and Methods. The DNA fragments were electrophoresed on an 8% polyacrylamide/8 M urea gel and the autoradiogram was obtained by exposing X-ray film to the gel.

is very interesting to clarify whether the reduction of nitro-IQ occurs nonenzymatically or not.

To obtain the answers for the questions described above, we investigated nonenzymatic reduction of nitro-IQ and the ability of inducing DNA damage by the experiments with UV−vis spectroscopy and ^{32}P-labeled DNA fragments obtained from the human *p53* tumor suppressor gene and the c-Ha-*ras*-1 protooncogene. We also analyzed 8-oxodG formation in calf thymus DNA by HPLC-ECD, as an indicator of oxidative DNA damage.

MATERIALS AND METHODS

Materials. Restriction enzymes (*Sma*I, *Eco*RI, and *Apa*I) and calf intestine phosphatase were purchased from Boehringer Mannheim (Germany). Restriction enzymes (*Hin*dIII, *Avr*I, and *Xba*I) and T_4 polynucleotide kinase were purchased from New England Biolabs. [γ-^{32}P]ATP (222 TBq/mmol) was from New England Nuclear. 2-Nitro-3-methylimidazo-[4,5-f]quinoline (nitro-IQ) was kindly supplied by Dr. T. Matsushima (Japan Bioassay Research Center). β-Nicotinamide adenine dinucleotide disodium salt (reduced form) (NADH) was purchased from Kohjin, Co. (Tokyo, Japan). Diethylenetriamine-*N*,*N*,*N*′,*N*′′,*N*′′-pentaacetic acid (DTPA) and bathocuproinedisulfonic acid were from Dojin Chemicals Co. (Kumamoto, Japan). Superoxide dismutase (SOD, 3000 units/mg from bovine erythrocytes), methional and catalase (45 000 units/mg from bovine liver) was from Sigma Chemical Co. Ethanol, $CuCl_2$, D-mannitol, and sodium formate were from Nakalai Tesque Inc. (Kyoto, Japan). Nuclease P_1 was from Yamasa Shoyu Co. (Chiba, Japan).

Preparation of ^{32}P-5′-End-Labeled DNA Fragments. DNA fragments obtained from the human *p53* tumor suppressor gene (*25*) containing exons 7 and 8 were prepared, as described previously (*26*). The 5′-end-labeled 650 bp fragment (*Hin*dIII* 13972−*Eco*RI* 14621) was obtained by dephosphorylation with calf intestine phosphatase and rephosphorylation with [γ-^{32}P]ATP and T_4 polynucleotide kinase (*, ^{32}P-labeled). The 650 bp fragment was further digested with *Apa*I to obtain a singly labeled 443 bp fragment (*Apa*I 14179−*Eco*RI* 14621) and a 211 bp fragment (*Hin*dIII* 13972−*Apa*I 14182). DNA fragment was also obtained from the human c-Ha-*ras*-1 protooncogene (*27*). 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 98 bp fragment (*Avr*I* 2247−*Pst*I 2344) and a 337 bp fragment (*Pst*I 2345−*Avr*I* 2681) were obtained according to the method described previously (*28*). Nucleotide numbering starts with the *Bam*HI site (*27*).

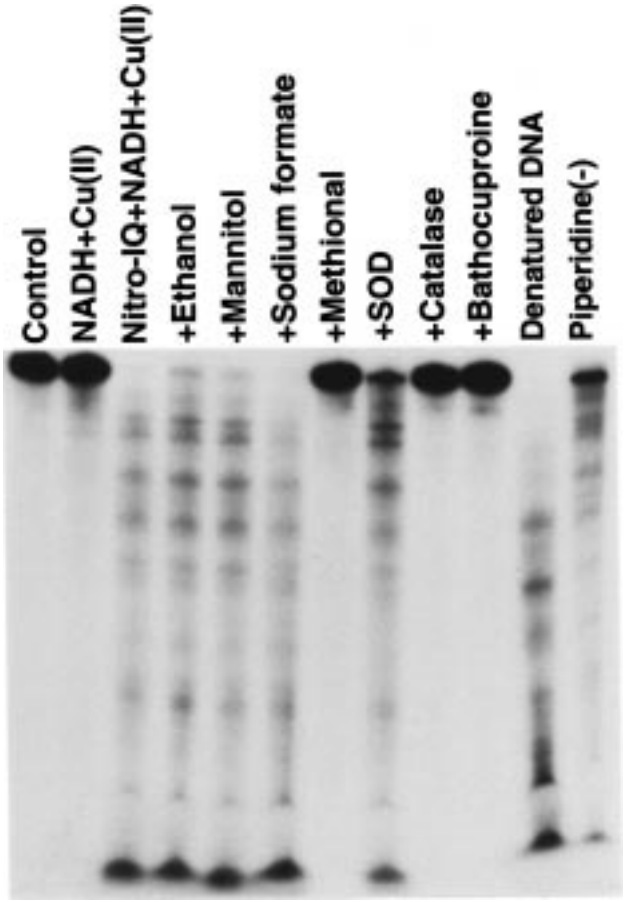

FIGURE 2: Effects of scavengers and bathocuproine on DNA damage induced by nitro-IQ in the presence of NADH and Cu(II). The reaction mixture contained ^{32}P-5′-end-labeled 443 bp DNA fragments, 5 μM/base of sonicated calf thymus DNA, 25 μM nitro-IQ, 200 μM NADH, and 20 μM $CuCl_2$ in 200 μL of 10 mM sodium phosphate buffer (pH 7.8) containing 2.5 μM DTPA. The mixture was incubated at 37 °C for 1 h, followed by piperidine treatment where indicated. The DNA fragments were analyzed as described in the legend to Figure 1. The concentration of scavengers and bathocuproine was as follows: 5% (v/v) ethanol; 0.1 M mannitol; 0.1 M sodium formate; 0.1 M methional; 30 units of SOD; 30 units of catalase; 50 μM bathocuproine. DNA fragments were denatured before the incubation where indicated.

Detection of DNA Damage by Nitro-IQ in the Presence of NADH and Cu(II). The standard reaction mixture (in a

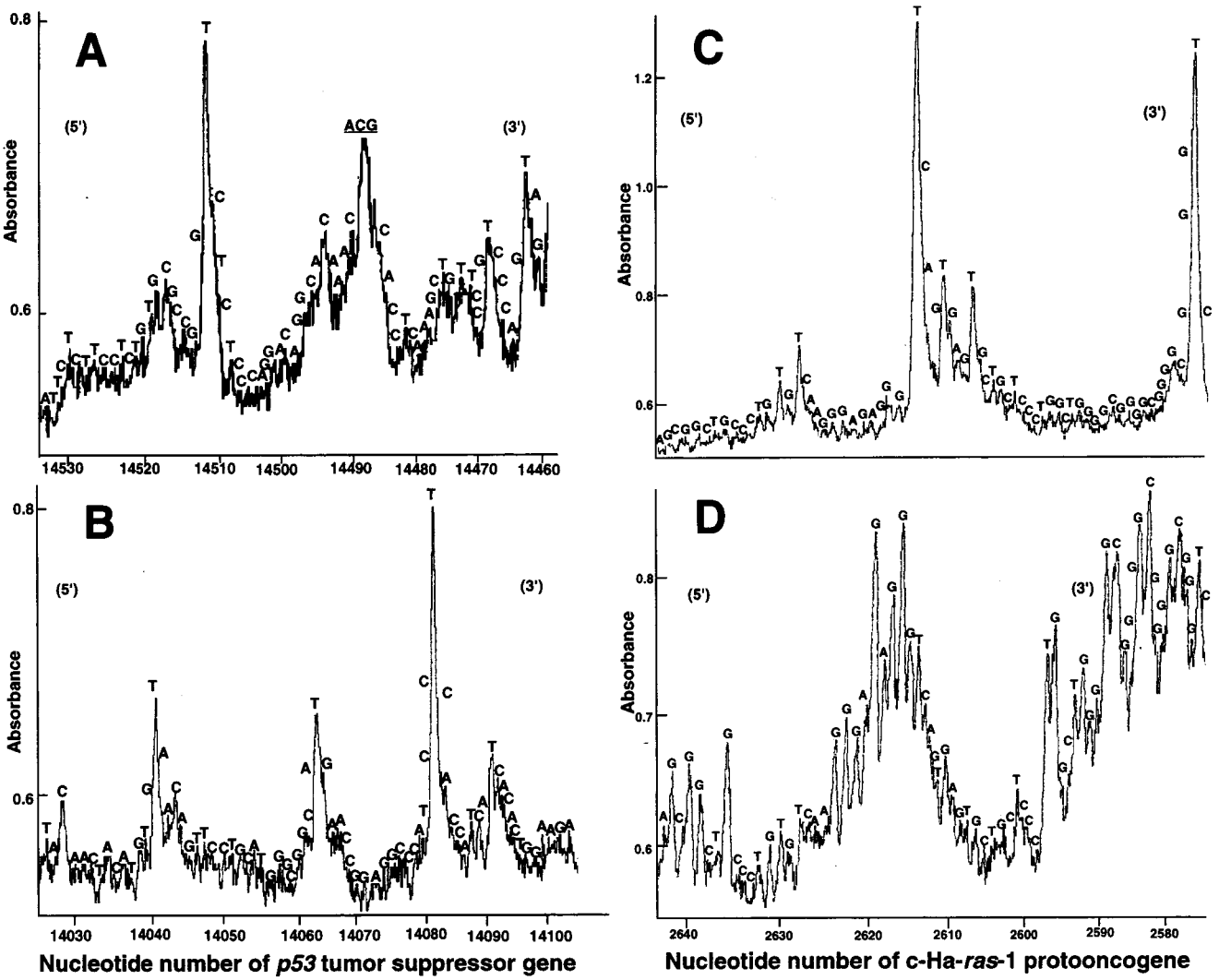

FIGURE 3: Site specificity of DNA cleavage induced by nitro-IQ in the presence of NADH and Cu(II). The reaction mixture contained ^{32}P-5′-end-labeled DNA fragment, 10 μM/base of sonicated calf thymus DNA and 10 μM nitro-IQ, 200 μM NADH and 20 μM $CuCl_2$ in 200 μL of 10 mM sodium phosphate buffer (pH 7.8) containing 2.5 μM DTPA. Native (A, B, C) and denatured (D) DNA was used. DNA fragments were denatured before the incubation where indicated. Reaction mixtures were incubated at 37 °C for 1 h. After the 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 oligonucleotide were measured by scanning the autoradiogram with a laser densitometer (LKB 2222 UltroScan XL). (A) 443 bp fragment (*Apa*I 14179−*Eco*RI*14621), (B) 211 bp fragment (*Hin*dIII* 13972−*Apa*I 14182) of the *p53* tumor suppressor gene, and (C, D) 337 bp fragment (*Pst*I 2345−*Ava*I* 2681) of the c-Ha-*ras*-1 protooncogene. The horizontal axis shows the nucleotide number of the human *p53* tumor suppressor gene (*25*) and underscoring shows complementary sequence to codon 273 (nucleotide number 11486−11488). The horizontal axis shows the nucleotide number of the human c-Ha-*ras*-1 protooncogene starting with *Bam*HI site. (*27*).

microtube; 1.5 mL; Eppendorf) contained nitro-IQ, NADH, and $CuCl_2$, ^{32}P-5′-end-labeled DNA fragments, and sonicated calf thymus DNA (5 μM/base) in 200 μL of 10 mM sodium phosphate buffer (pH 7.8) containing 2.5 μM DTPA. After incubation at 37 °C for 1 h, the DNA fragments were heated at 90 °C in 1 M piperidine for 20 min where indicated and treated as described previously (*29*).

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 (*30*) using a DNA-sequencing system (LKB 2010 Macrophor). The relative amounts of oligonucleotides from the treated DNA fragments were measured with a laser densitometer (LKB 2222 UltroScan XL).

Analysis of 8-oxodG Formation in Calf Thymus DNA by Nitro-IQ in the Presence of NADH and Cu(II). Native or denatured DNA fragments (100 μM/base) from calf thymus were incubated with nitro-IQ, NADH, and $CuCl_2$ for 1 h at 37 °C. For DNA denaturation, the fragments were heated at 90 °C for 5 min and then chilled quickly before incubation. After ethanol precipitation, DNA was digested to the nucleosides with nuclease P_1 and calf intestine phosphatase and analyzed by HPLC-ECD, as previously described (*31*).

UV−Vis Spectra Measurement of Nitro-IQ. Spectral changes for oxidation of NADH and reduction of nitro-IQ were measured with a UV−vis spectrometer (UV-2500PC, Shimadzu, Kyoto, Japan) in the presence and absence of Cu(II). The reaction mixture contained nitro-IQ, NADH, and $CuCl_2$ in 10 mM phosphate buffer (pH 7.8). The spectra of the mixture were measured repeatedly at 37 °C for the indicated duration.

RESULTS

Damage of ^{32}P-Labeled DNA Fragments by Nitro-IQ in the Presence of NADH and Cu(II). Figure 1 shows an autoradiogram of DNA fragments treated with nitro-IQ in the presence of NADH and Cu(II). When Cu(II) or NADH was omitted, nitro-IQ did not induce DNA damage. In the presence of both NADH and Cu(II), nitro-IQ caused DNA damage and the intensity increased with concentration of nitro-IQ and incubation time (data not shown).

Effects of Scavengers and Bathocuproine on DNA Damage Induced by Nitro-IQ in the Presence of NADH and Cu(II). Figure 2 shows the effects of scavengers and bathocuproine, a Cu(I)-specific chelator, on DNA damage induced by nitro-IQ in the presence of NADH and Cu(II). Inhibition of DNA damage by catalase and bathocuproine suggests the involvement of hydrogen peroxide (H_2O_2) and Cu(I). Methional inhibited the DNA damage, although other typical free hydroxyl radical (•OH) scavengers, ethanol, mannitol and sodium formate, did not. SOD showed little inhibitory effect on DNA damage. When denatured DNA was used, the intensity of DNA damage increased. Without piperidine treatment, nitro-IQ-induced direct breakage of the deoxyribose phosphate backbone was observed in the presence of NADH and Cu(II). Piperidine treatment enhanced DNA cleavage by nitro-IQ, suggesting the involvement of base modification.

Site Specificity of DNA Cleavage by Nitro-IQ in the Presence of NADH and Cu(II). An autoradiogram was obtained and scanned with a laser densitometer to measure relative intensity of DNA cleavage in the human *p53* tumor suppressor gene and the c-Ha-*ras*-1 protooncogene as shown in Figure 3. Nitro-IQ induced piperidine-labile sites relatively at thymine and cytosine residues in the presence of NADH and Cu(II) in the *p53* gene (Figure 3, panels A and B). It is noteworthy that the cytosine residue of the ACG sequence is complementary to codon 273, which is a known hotspot (*32*) of the *p53* gene (Figure 3A). DNA cleavage was observed frequently at thymine and cytosine also in the c-Ha-*ras*-1 protooncogene (Figure 3C). When denatured DNA (Figure 3D) was used, preferential damage occurred more frequently at guanine sites. Without piperidine treatment, nitro-IQ-induced direct breakage of the deoxyribose phosphate backbone showed no clear site specificity (data not shown).

Formation of 8-oxodG in Calf Thymus DNA by Nitro-IQ in the Presence of NADH and Cu(II). Using HPLC-ECD, we measured 8-oxodG content in calf thymus DNA treated with nitro-IQ in the presence of NADH and Cu(II) (Figure 4). The amount of 8-oxodG increased with the concentration of nitro-IQ in the presence of NADH and Cu(II). The formation of 8-oxodG increased by DNA denaturation.

Redox Reaction of Nitro-IQ and NADH in the Presence of Cu(II). Figure 5 shows spectral changes of the mixture of nitro-IQ and NADH in the presence of Cu(II). In the absence of Cu(II), spectra of nitro-IQ and NADH did not change for 60 min, suggesting no reduction of nitro-IQ and no oxidation of NADH (data not shown). When Cu(II) was added to the reaction mixture of nitro-IQ and NADH (Figure 5A, dotted line), the rapid spectral changes were observed for 10 min (Figure 5A, solid lines). Nitro-IQ has the absorbance maximum at 293 and 360 nm (*33*), NADH (reduced form) at 340 nm, and NAD^+ (oxidized form) at 260 nm. Although

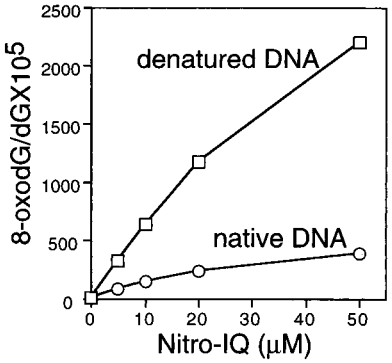

FIGURE 4: Formation of 8-oxodG in calf thymus DNA by nitro-IQ in the presence of NADH and Cu(II). Calf thymus DNA fragments (100 μM/base) were incubated with indicated concentration of nitro-IQ, 200 μM NADH, and 20 μM $CuCl_2$ for 1 h at 37 °C. DNA fragments were denatured before the incubation where indicated. After ethanol precipitation, DNA was enzymatically digested to the nucleosides and analyzed by the HPLC-ECD.

spectra of NADH and nitro-IQ are overlapped, the absorbance at 340 nm due to NADH and that at 293 nm due to nitro-IQ were decreased, and absorbance at 260 nm due to NAD^+ was increased. It is suggested that Cu(II) may mediate the reduction of nitro-IQ by NADH. Figure 6B showed the decrease of absorbance at 340 nm with various concentrations of nitro-IQ and NADH in the presence of Cu(II) for 10 min. The higher the concentration of nitro-IQ reacted, the more the amount of NADH decreased. These results indicate that the amount of reduction of nitro-IQ increases depending on the concentrations of both nitro-IQ and NADH.

DISCUSSION

The present study has demonstrated that nitro-IQ is reduced nonenzymatically by an endogenous reductant NADH, and it has an ability to cause oxidative DNA damage including 8-oxodG in the presence of Cu(II). We confirmed that nitro-IQ was not further reduced to nitroso-IQ by NADH, using HPLC-photodiode array. In addition, we detected the carbon-centered radical which is assigned to NAD• (*21*) in the reaction mixture of nitro-IQ and NADH in the absence of Cu(II) by electron spin resonance spectroscopy with a trapping agent α-(4-pyridy-1-oxide)-*N*-*tert*-butylnitrone (data not shown). UV−vis spectroscopic study showed that addition of Cu(II) led to the rapid spectral changes of NADH (reduced form) to NAD^+ (oxidized form), suggesting requirement of Cu(II) mediation. Oxygen consumption by nitro-IQ also required both NADH and Cu(II) (data not shown). Possible mechanism of nonenzymatic reduction of nitro-IQ and resulting in oxidative DNA damage, as shown in Figure 6, can be envisioned as accounting for most of the observations. Nitro-IQ is reduced to nitro radical anion by NADH in the presence of Cu(II). For undergoing the redox reaction of nitro-IQ and NADH, the presence of Cu(II) was required. Although we could not confirm whether Cu(II) or nitro-IQ initially accepts one electron from NADH, our data strongly suggested that the oxidation of NADH to NAD^+ via $NAD^•$ takes place with two electron transfers in the presence of nitro-IQ and Cu(II). The nitro radical anion reduces O_2 with production of $O_2^{•-}$ and the consequent regeneration of the parent nitro-IQ. Subsequently, the generation of H_2O_2 by $O_2^{•-}$ dismutation and reduction of Cu(II) to Cu(I) concomitantly occur. Inhibitory effects of

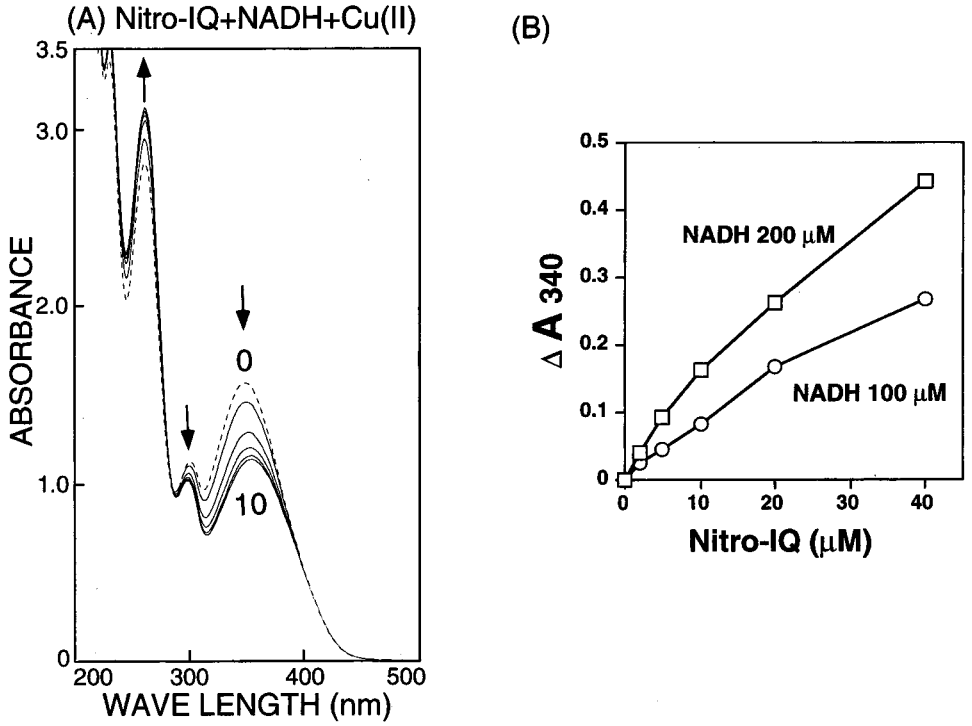

FIGURE 5: Spectral changes of nitro-IQ and NADH in the presence and absence of Cu(II). (A) The reaction mixture contained 40 μM nitro-IQ and 200 μM NADH in 10 mM phosphate buffer (pH 7.8). Spectra were measured with a UV−vis spectroscopy at 37 °C for 60 min (dotted line), and then added 20 μM $CuCl_2$ to the reaction mixture and measured every 2 min for 10 min (solid lines). (B) The deference of absorbance at 340 nm was measured between 0 and 10 min after the addition of Cu(II) in the reaction mixture with indicated concentration of nitro-IQ and NADH

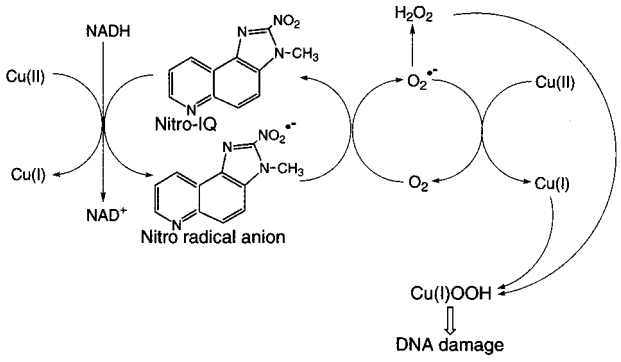

FIGURE 6: Proposed mechanism of nonenzymatic reduction of nitro-IQ and oxidative DNA damage in the presence of NADH and Cu-(II).

catalase and bathocuproine suggested that H_2O_2 and Cu(I) were required for the DNA damage. Typical •OH scavengers did not offer DNA protection from nitro-IQ in the presence of NADH and Cu(II). One possible explanation is that •OH is not involved in DNA damage. Namely, DNA-associated Cu(I) participates in generation of other oxidants including a copper−peroxo intermediate, such as Cu(I)−OOH (*34*), which is formed from reaction of H_2O_2 and Cu(I). This may be supported by the inhibitory effect of methional on the DNA damage. The reactive species is a kind of crypto-OH radical, copper−peroxo intermediate, because it is reported that the radical is reactive with sulfur derivatives such as methional (*35*), but not reactive with traditional •OH scavengers. Relevantly, it was reported that methional can react with not only free •OH but also crypto-OH radicals with formation of ethylene (*35*, *36*). Alternatively, it is possible that copper ions are bound to DNA and damage is induced by a site-specific formation of •OH so that •OH scavengers cannot interfere with this reaction.

Oxidation of NADH appears to proceed by initial rate-limiting one-electron transfer from NADH to the oxidizing agent (*37*). Mason and Holtzman (*38*) first demonstrated that nitro anion radical was formed from nitro compound by a one-electron transfer from reduced flavoenzyme, which donated one electron from NADH. It is noteworthy to find that, instead of enzyme, Cu(II) may act as the agent for mediating redox reaction of NADH and nitro-IQ. In the presence of Cu(II), endogenous reductants, such as NADH, can reduce nonenzymatically nitro derivatives to nitro radical anion with the generation of $O_2^{\bullet-}$, as same as nitroaromatic compounds are reduced by nitroreductase with the generation of $O_2^{\bullet-}$ (*39*). Relevantly, Sato et al. (*40*) detected $O_2^{\bullet-}$ generation from various heterocyclic amines, including IQ with cytochrome P-450 reductase/NADPH. The possibility that metal ion plays an important role in nonenzymatic activation of NADH should be considered. The concentration of NAD(P)H in certain tissue was estimated to be as high as 100−200 μM (*41*). Copper has been found in the nucleus and to be closely associated with chromosomes (*42*). Therefore, nitro-IQ possibly can cause oxidative DNA damage in tissues without nitroreductase.

The DNA-sequencing experiments revealed that nitro-IQ induced piperidine-labile sites frequently at thymine and cytosine in the presence of NADH and Cu(II). Recently, it has been reported that 5-hydroxyuracil (*43*), which is formed by oxidation of cytosine residues, and 5-formyluracil (*44*), which is detected as a major oxidative modification of thymine, are piperidine labile. In addition, formylamine (*45*), a major hydroxyl radical-mediated degradation product of both thymine and cytosine bases, has been shown to be piperidine labile. The site-specific modification of DNA

bases observed here may contain such oxidation products of thymine and cytosine. In detail, the methyl group of thymine is likely to be more accessible to hydrogen abstraction by oxidizing species such as a copper−peroxo intermediate in native DNA. On the other hand, guanine residues are expected to exhibit higher reactivity in single-stranded DNA than native DNA. When denatured DNA was used, preferential damage occurred more frequently at guanine sites. It is known that the 8-oxodG-containing site is not efficiently cleaved by hot piperidine, whereas imidazolone- and oxazolone-containing sites are piperidine labile (*46*, *47*). The formed 8-oxodG is much more easily oxidized than guanine itself. It is reported that 8-oxodG may be further oxidized to the imidazolone and other piperidine-labile derivatives (*48*). Therefore, it is speculated that the preferential damage at guanine residues in DNA, especially denatured form, observed here may contain such piperidine-labile oxidation products of guanine.

There are many reports concerning carcinogenesis of IQ in animals (*1*, *49*−*51*). Genetic alteration in *p53* gene was observed in monkey treated with IQ (*51*). Noteworthily, we showed that nitro-IQ induced the cytosine residue of the ACG sequence complementary to codon 273, a known hotspot (*32*) of the *p53* gene. It is concluded that nonenzymatic reduction of nitro-IQ by NADH results in oxidative DNA damage in the presence of Cu(II), and it may play some roles in carcinogenicity of IQ.

REFERENCES

1. Sugimura, T. (1997) *Mutat. Res. 376*, 211−219.
2. Felton, J. S., Knize, M. G., Wood, C., Wuebbles, B. J., Healy, S. K., Stuermer, D. H., Bjeldanes, L. F., Kimble, B. J., and Hatch, F. T. (1984) *Carcinogenesis 5*, 95−102.
3. IARC Working Group (1993) *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans*, vol. 56, pp 165−195, IARC, Lyon, France.
4. Yamashita, M., Wakabayashi, K., Nagao, M., Sato, S., Yamaizumi, Z., Takahashi, M., Kinae, N., Tomita, I., and Sugimura, T. (1986) *Jpn. J. Cancer Res. 77*, 419−422.
5. Fay, L. B., and Turesky, R. J. (1992) *Biol. Mass Spectrom. 21*, 463−469.
6. Sasagawa, C., Muramatsu, M., and Matsushima, T. (1988) *Mutat. Res. 203*, 386.
7. Hirose, M., Wakabayashi, K., Grivas, S., De Flora, S., Arakawa, N., Nagao, M., and Sugimura, T. (1990) *Carcinogenesis 11*, 869−871.
8. Morrison, L. D., Eling, T. E., and Josephy, P. D. (1993) *Mutat. Res. 302*, 45−52.
9. Dirr, A., and Wild, D. (1988) *Mutagenesis 3*, 147−152.
10. Asan, E., Fasshauer, I., Wild, D., and Henschler, D. (1987) *Carcinogenesis 8*, 1589−1593.
11. Graf, U., Wild, D., and Würgler, F. E. (1992) *Mutagenesis 7*, 145−149.
12. Sawada, S., Daimon, H., Asakura, S., Kawaguchi, T., Yamatsu, K., Furihata, C., and Matsushima, T. (1994) *Carcinogenesis 15*, 285−290.
13. Snyderwine, E. G., Davis, C. D., Nouso, K., Roller, P. P., and Schut, H. A. J. (1993) *Carcinogenesis 14*, 1389−1395.
14. Yanagawa, Y., Sawada, M., Deguchi, T., Gonzalez, F. J., and Kamataki, T. (1994) *Cancer Res. 54*, 3422−3427.
15. Hirose, M., Iwata, S., Ito, E., Nihro, Y., Takahashi, S., Mizoguchi, Y., MiKi, T., Satoh, T., Ito, N., and Shirai, T. (1995) *Carcinogenesis 16*, 2227−2232.
16. Hirose, M., Futakuchi, M., Tanaka, H., Orita, S.-I., Ito, T., Miki, T., and Shirai, T. (1998) *Eur. J. Cancer Prevent. 7*, 61−67.
17. Hirose, M., Hasegawa, R., Kimura, J., Akagi, K., Yoshida, Y., Tanaka, H., Miki, T., Satoh, T., Wakabayashi, K., Ito, N., and Shirai, T. (1995) *Carcinogenesis 16*, 3049−3055.
18. Kato, T., Hasegawa, R., Nakae, D., Hirose, M., Yaono, M., Cui, L., Kobayashi, Y., Konishi, Y., Ito, N., and Shirai, T. (1996) *Jpn. J. Cancer Res. 87*, 127−133.
19. Wakata, A., Oka, N., Hiramoto, K., Yoshioka, A., Negishi, K., Wataya, Y., and Hayatsu, H. (1985) *Cancer Res. 45*, 5867−5871.
20. Wataya, Y., Yamane, K., Hiramoto, K., Ohtsuka, Y., Okubata, Y., Negishi, K., and Hayatsu, H. (1988) *Jpn. J. Cancer Res. 79*, 576−579.
21. Oikawa, S., and Kawanishi, S. (1996) *Biochemistry 35*, 4584−4590.
22. Hiraku, Y., and Kawanishi, S. (1996) *Cancer Res. 56*, 5172−5178.
23. Murata, M., Imada, M., Inoue, S., and Kawanishi, S. (1998) *Free Radical Biol. Med. 25*, 586−595.
24. Krainev, A. G., Williams, T. D., and Bigelow, D. J. (1998) *Chem. Res. Toxicol. 11*, 495−502.
25. Chumakov, P. (1990) *EMBL Data Library*, accession number X54156.
26. Yamashita, N., Murata, M., Inoue, S., Hiraku, Y., Yoshinaga, T. and Kawanishi, S. (1998) *Mutat. Res. 39*, 191−201.
27. Capon, D. J., Chen, E. Y., Levinson, A. D., Seeburg, P. H., and Goeddel, D. V. (1983) *Nature 302*, 33−37.
28. Yamamoto, K., and Kawanishi, S. (1989) *J. Biol. Chem. 264*, 15435−15440.
29. Kawanishi, S., Inoue, S., and Kawanishi, M. (1989) *Cancer Res. 49*, 164−168.
30. Maxam, A. M., and Gilbert, W. (1980) *Methods Enzymol. 65*, 499−560.
31. Ito, K., Inoue, S., Yamamoto, K., and Kawanishi, S. (1993) *J. Biol. Chem. 268*, 13221−13227.
32. Levine, A. J, Momand, J., and Finlay, C. A. (1991) *Nature 351*, 453−456.
33. Wolz, E., Wild, D., and Degen, G. H. (1995) *Arch. Toxicol. 69*, 171−179.
34. Yamamoto, K., and Kawanishi, S. (1991) *J. Biol. Chem. 266*, 1509−1515.
35. Pryor, W. A., and Tang, R. H. (1978) *Biochem. Biophys. Res. Commun. 81*, 498−503.
36. Youngman, R. J., and Elstner, E. F. (1981) *FEBS Lett. 129*, 265−268.
37. Carlson, B. W., Miller, L. L., Neta, P., and Grodkowski, J. (1984) *J. Am. Chem. Soc. 106*, 7233−7239.
38. Mason, R. P., and Holtzman, J. L. (1975) *Biochemistry 14*, 1626−1632.
39. Orna, M. V., and Mason, R. P. (1989) *J. Biol. Chem. 264*, 12379−12384.
40. Sato, K., Akaike, T., Kojima, Y., Ando, M., Nagao, M., and Maeda, H. (1992) *Jpn. J. Cancer Res. 83*, 1204−1209.
41. Malaisse, W. J., Hutton, J. C., Kawazu, S., Herchuelz, A., Valverde, I., and Sener, A. (1979) *Diabetologia 16*, 331−341.
42. Burkitt, M. J. (1994) *Methods Enzymol. 234*, 66−79.
43. Fujimoto, J., Tran, L., and Sowers L. C. (1997) *Chem. Res. Toxicol. 10*, 1254−1258.
44. Saito, I., Takayama, M., and Kawanishi, S. (1995) *J. Am. Chem. Soc. 117*, 5590−5591.
45. Bourdat, A.-G., Gasparutto, D., and Cadet, J. (1999) *Nucleic Acids Res. 27*, 1015−1024.
46. Kino, K., Saito, I., and Sugiyama, H. (1998) *J. Am. Chem. Soc. 120*, 7373−7374.
47. Gasparutto, D., Ravanat, J.-L., Gérot, O., and Cadet, J. (1998) *J. Am. Chem. Soc. 120*, 10283−10286.
48. Adam, W., Saha-Möller, C. R., and Schönberger, A. (1996) *J. Am. Chem. Soc. 118*, 9233−9238.
49. Dooley, K. L., Von Tungeln, L. S., Bucci, T., Fu, P. P., and Kadlubar, F. F. (1992) *Cancer Lett. 62*, 205−209.
50. Thorgeisson, U. P., Gomez, D. E., Lindsay, C. K., Sinha, C. C., and Adamson, R. H., (1996) *Liver 16*, 71−83.
51. Fujimoto, Y., Hampton, L. L., Snyderwine, E. G., Nagao, M., Sugimura, T., Adamson R. H., and Thorgeirsson, S. S., (1994) *Jpn. J. Cancer Res. 85*, 506−509.

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