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Induction of 8-hydroxydeoxyguanosine in DNA by chromium(III) plus hydrogen peroxide and its prevention by scavengers

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The capability of Cr(III) to induce DNA lesions generated by oxidative damage was investigated in this study by examining the formation of 8-hydroxydeoxyguanosine (8-OHdG) in calf thymus DNA by $CrCl_3$ and/or H_2O_2 in 10 mM phosphate buffer. In the presence of 0.5 mM H_2O_2, the formation of 8-OHdG markedly increased with increasing $CrCl_3$ concentration. In contrast, H_2O_2 or $CrCl_3$ alone did not cause any increase in 8-OHdG level above background. The amount of 8-OHdG induced by $CrCl_3$ plus H_2O_2 was time dependent; its generation increased linearly over an incubation period of 90 min. The formation of 8-OHdG was unfavorable in an acidic solution (pH < 6); the highest level of 8-OHdG was observed at pH 7–8. Scavengers of reactive oxygen species markedly inhibited the formation of 8-OHdG by $CrCl_3$ plus H_2O_2; the inhibition effect was sodium azide > D-mannitol > Tris–HCl at an equal concentration. The induction of 8-OHdG by $CrCl_3$ plus H_2O_2 remained unchanged in D_2O. Moreover, an addition of catalase (2.2 U/ml) to the reaction mixture completely inhibited the formation of 8-OHdG by $CrCl_3$/H_2O_2, whereas only 22% of that formation was inhibited by superoxide dismutase (11 U/ml). A large amount of bovine serum albumin (1.1 mg/ml) could reduce the formation of 8-OHdG by $CrCl_3$ plus H_2O_2, thereby implying that Cr(III)-mediated DNA–protein crosslinks are unfavorable for 8-OHdG formation. Furthermore, ascorbate could prevent the formation of 8-OHdG by $CrCl_3$ plus H_2O_2; the extent of prevention increased with increasing ascorbate concentration (10 μM–3 mM). Thus, ascorbate acts as a free radical scavenger in the $CrCl_3$/H_2O_2 system. The above findings suggest that Cr(III)/H_2O_2 could generate oxidative damage to DNA, possibly through a Fenton-like reaction, i.e. $Cr(III) + H_2O_2 \rightarrow Cr(IV) + {}^{\cdot}OH + OH^-$. This study also indicates that Cr(III), previously considered as the ultimate kinetically stable species of Cr(VI) metabolites, is capable of inducing carcinogenic lesions through interaction with a cellular oxygen species.

Introduction

Epidemiological evidence has clearly confirmed that chromium exposure significantly increases risks of cancer in the respiratory tract (1). Chromium can also induce tumors in experimental animals and cause genotoxicity, i.e. chromosome aberrations, sister chromatid exchanges, cell transformation, gene mutations and cell death, in mammalian cell cultures (2–6). Chromium exists in many oxidation states, of which hexavalent Cr is the most effective form for inducing cytotoxicity and carcinogenicity (reviewed in refs 7 and 8). Cr(VI) forms an oxyanion (e.g. CrO_4^{2-}) that mimics physiologically important oxyanions (e.g. SO_4^{2-}) by rapidly entering cells through the anion transport systems, whereas the stable cation form, e.g. Cr(III) salt, is incapable of entering into cells through membrane transporters (9). Nevertheless, Cr(VI) does not directly interact with DNA or nuclei *in vitro* (10–12). An important model accounting for the toxic effects of Cr(VI) is the intracellular reduction of Cr (13,14). Once in the cytoplasm of the cells, Cr(VI) is reduced through short-lived Cr intermediates to the ultimate kinetically stable trivalent species; additionally, several reactive oxygen species (ROS*) are generated (13,14). Furthermore, Cr(III), Cr intermediates and ROS can interact directly with DNA and proteins, forming DNA single-strand breaks, DNA–protein crosslinks, Cr–DNA adducts and base modifications in mammalian cells (13–17).

Cr(III) compounds are more effective than Cr(VI) in genotoxicity assays in cell-free systems (7,8). Replication of Cr(III)-treated single-stranded phage DNA *in vitro* can enhance mutations in *Escherichia coli*, possibly because Cr–DNA adducts enhance the processivity of polymerases (18,19). Additionally, Cr(III)-mediated interstrand DNA–DNA crosslinks could result in polymerase arrest in a synthetic DNA template (6,20). Furthermore, hexacoordinate Cr(III) aromatic bidentate amine complexes can induce mutations in the Ames test (21). The mutagenicity of those Cr(III) complexes may result from DNA damages induced by the Cr(III)/Cr(II) redox couple (21). However, the role of ROS in Cr(III) mutagenesis remains unclear.

ROS are known to induce damage to DNA, forming DNA strand breaks and modified bases (22,23). One of the oxidative DNA lesions, 8-hydroxydeoxyguanosine (8-OHdG), causes misincorporation during replication and subsequently leads to G → T transversions (24–26). 8-OHdG has been reported to be an important biomarker relevant to carcinogenesis (27,28). Also, electron spin resonance studies have demonstrated that Cr(III) is capable of generating hydroxyl radicals from H_2O_2 in a DNA-free system (29). In this study, the capability of the specific kind of ROS generated by Cr(III) plus H_2O_2 to induce DNA damage was investigated by examining the formation of 8-OHdG in calf thymus DNA with $CrCl_3$ and/or H_2O_2 using HPLC with UV and electrochemical detection. Several ROS scavengers inhibit the formation of 8-OHdG by Cr(III) plus H_2O_2. Those results demonstrate that $CrCl_3$ in the presence of H_2O_2 can induce 8-OHdG formation in DNA, possibly through the generation of hydroxyl radical. Ascorbate functions as a free radical scavenger to inhibit the formation of 8-OHdG by Cr(III) plus H_2O_2. Furthermore, ROS generated by reoxidation of Cr(III) may be a critical factor in Cr(VI)-induced genotoxicity and carcinogenicity.

*Abbreviations: ROS, reactive oxygen species; 8-OHdG, 8-hydroxydeoxyguanosine; BSA, bovine serum albumin; DTPA, diethylenetriamine pentaacetic acid; SOD, superoxide dismutase; dG, deoxyguanosine.

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Materials and methods

Materials

Calf thymus DNA (activated type XV), ascorbate, diethylenetriamine pentaacetic acid (DTPA), deoxyguanosine, bovine serum albumin (BSA) and D_2O were purchased from Sigma Chemical Co. (St Louis, MO). $CrCl_3 \cdot 6H_2O$ (99.995% purity) was obtained from Aldrich Chemical Co (Milwaukee, WI). Perhydrol (30% H_2O_2), EDTA (disodium salt dihydrate), potassium phosphate and D-mannitol were obtained from Merck (Darmstadt, Germany). Tris, catalase, superoxide dismutase (SOD), DNase I, nuclease P1 and calf intestinal alkaline phosphatase were obtained from Boehringer Mannheim GmbH (Germany). Sodium azide was purchased from Ishizu Pharmaceutical (Osaka, Japan). Standard 8-OHdG was obtained from Chemsyn Science Lab (Lenexa, KS). Chelex 100 chelating resin was purchased from BioRad Lab (Richmond, CA). DNA was dissolved in MilliQ-purified H_2O at 1 mg/ml, aliquot, and stored at –20°C. $CrCl_3 \cdot 6H_2O$ was freshly prepared in H_2O. 10-X potassium phosphate buffer (100 mM) of various pH values were prepared from 1 M K_2HPO_4 and 1 M KH_2PO_4 as described by Sambrook *et al.* (30).

Treatment of DNA with Cr(III) and/or H_2O_2

Calf thymus DNA (100 μg) was treated with $CrCl_3$ (1 μM–1 mM) in the presence or absence of H_2O_2 (0.5 mM) in 10 mM potassium phosphate buffer, pH 7.5, at a final volume of 450 μl. The reaction was carried out at 37°C for 1 h and terminated by the addition of sodium acetate (0.3 M, final concentration) and 2 vol of cold ethanol. Next, the DNA sample was precipitated, washed once with 70% ethanol, dried and dissolved in 200 μl, 10 mM Tris–HCl (pH 7.4). The reaction was also performed at various pH values or incubation for various time courses at 37°C in the same condition. In experiments investigating the effect of ROS scavengers, D-mannitol, sodium azide or Tris was added to the Cr(III)–DNA mixture before the addition of H_2O_2. The reaction was also performed in D_2O (replacing H_2O) to study the involvement of singlet oxygen (31). For those experiments, DNA in phosphate buffer was vacuum dried and dissolved in D_2O. Meanwhile, Cr(III) and H_2O_2 prepared in D_2O were added to DNA solution and the reaction mixture was incubated at 37°C for 1 h. Moreover, the effects of catalase, SOD or BSA on the 8-OHdG formation by Cr(III) plus H_2O_2 were determined by treatment of DNA with Cr(III) plus H_2O_2 and incubation for 30 min; catalase, SOD, or BSA were then added to the reactions and incubation continued for another 60 min. In experiments to determine the effect of ascorbate, all of the materials were freshly prepared in Chelex 100-treated water, and 1 μM DTPA was included in the reaction mixture. Thus, trace transitional metals, i.e. iron and copper, were removed, thereby eliminating the effect of ascorbate autooxidation (32). Higher concentrations of DTPA (5 μM) would reduce the induction of 8-OHdG by Cr(III) plus H_2O_2 possibly due to it chelating some of the Cr(III).

Determination of deoxyguanosine (dG) and 8-OHdG

DNA sample was digested to deoxynucleotides at 37°C with 40 units of DNase I for 30 min and followed by 2.5 units of nuclease P1 for 60 min. The solution containing deoxynucleotides was incubated with 2.5 units of calf intestinal alkaline phosphatase at 37°C for 30 min. Proteins in the reaction mixture were removed by precipitation with 5 ml acetone. The supernatant was dried, dissolved in 0.4 ml H_2O and passed through a 0.22 μM nylon filter before analyzing the amounts of dG and 8-OHdG.

The amounts of dG and 8-OHdG in the DNA were analyzed as previously described by Floyd *et al.* (33) using HPLC with UV absorbance detection (Beckman diode array detector 168) and electrochemical detection (BAS amperometric electrochemical detector; West Lafayette, ID) respectively. Nucleoside mixture (50–100 μl) was injected into a reverse-phase C18 Cosmosil column (5 μm, 4.6×250 mm) and the mobile phase was 50 mM phosphate buffer (pH 5.5) containing 8% methanol. Under this condition, dG and 8-OHdG were eluted at ~11.2 and ~16.3 min respectively. The amount of dG was calculated from the standard peak area detected by the absorbance at 254 nm with a UV detector in line with (before) the electrochemical detector. Next, the amount of 8-OHdG in sample was quantitated by injecting known amounts of pure 8-OHdG as standard based on the peak area detected with the electrode set at 0.8 V and 20 nA. The 8-OHdG level is expressed as 8-OHdG molecules/10^5 dG.

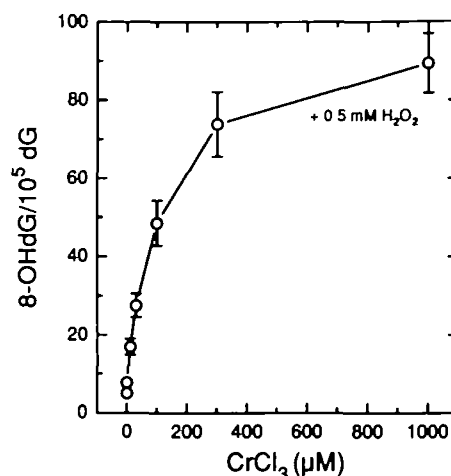

Fig. 1. The formation of 8-OHdG in DNA by $CrCl_3/H_2O_2$. Calf thymus DNA was reacted with 1–1000 μM $CrCl_3$ plus 0.5 mM H_2O_2 in 10 mM potassium phosphate buffer, pH 7.5, at 37°C for 1 h. The reaction was terminated by ethanol precipitation and the DNA sample was digested to the nucleoside level for the 8-OHdG analysis as described in Materials and methods. The amount of 8-OHdG in DNA was determined according to the average of 4–16 experiments. The bars denote the standard error.

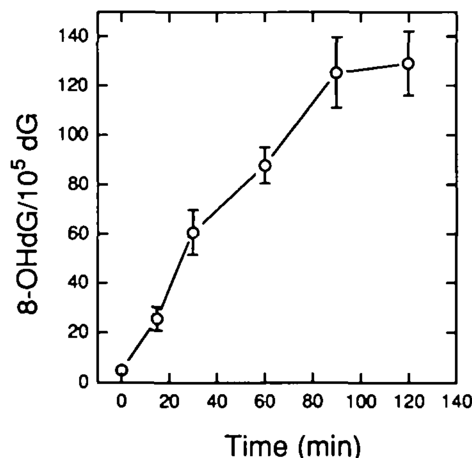

Fig. 2. Time-dependent formation of 8-OHdG in DNA by $CrCl_3/H_2O_2$. Calf thymus DNA was reacted with 1 mM $CrCl_3$ plus 0.5 mM H_2O_2 for 0–120 min under the same conditions as described in the legend to Figure 1. The amount of 8-OHdG in DNA was determined according to the average of 3–16 experiments. The bars denote the standard error.

Results

Figure 1 shows 8-OHdG formation in DNA obtained with $CrCl_3$ (1–1000 μM) plus H_2O_2 (0.5 mM) in 10 mM potassium phosphate buffer (pH 7.5) at 37°C for 1 h. The amount of 8-OHdG in DNA markedly increased when $CrCl_3$ concentration was increased from 1 μM (7.8/10^5 dG) to 1 mM (90/10^5 dG). Control experiments with $CrCl_3$ (1 mM) or H_2O_2 (0.5 mM) alone did not enhance 8-OHdG formation in DNA above the background level. Calf thymus DNA (type XV) used here has a background 8-OHdG level of 5/10^5 dG, which is low compared to those observed in most commercial sources of DNA (5–50/10^5 dG). These results have indicated that trace metals did not affect the induction of 8-OHdG by $CrCl_3/H_2O_2$ in our experimental conditions.

The time-course-dependent formation of 8-OHdG by $CrCl_3/H_2O_2$ is shown in Figure 2. In the presence of 1 mM $CrCl_3$ and 0.5 mM H_2O_2, the formation of 8-OHdG in DNA increased linearly with an increase in the incubation time; the level increased ~25-fold of the background in 2 h. Again, control experiments with $CrCl_3$ or H_2O_2 alone were similar to those in the background level.

Figure 3 shows that the formation of 8-OHdG by $CrCl_3/H_2O_2$ is dependent on the pH values of the reaction buffer. At pH values below pH 6, 1 mM $CrCl_3$ plus 0.5 mM H_2O_2 did not generate any significant amount of 8-OHdG above the

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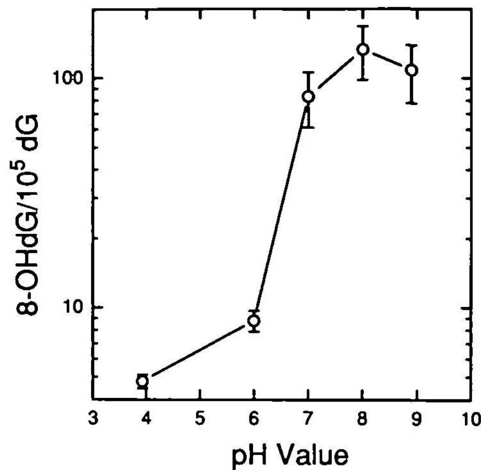

Fig. 3. Effects of pH value on the formation of 8-OHdG in DNA by $CrCl_3/H_2O_2$. Calf thymus DNA was reacted with 1 mM $CrCl_3$ plus 0.5 mM H_2O_2 at various pH values for 1 h at the same condition as described in the legend to Figure 1. The amount of 8-OHdG in DNA was determined according to the average of 4–16 experiments. The bars denote the standard error.

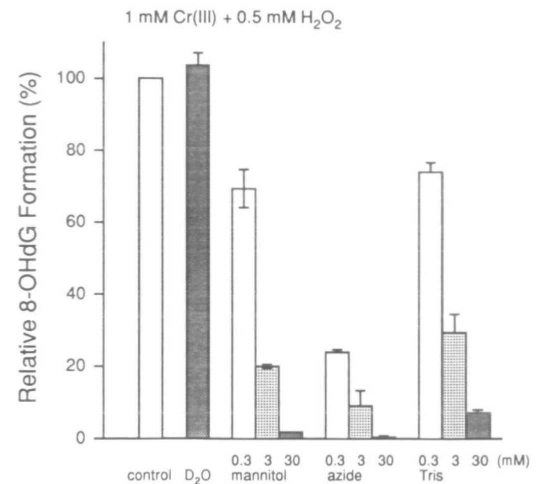

Fig. 4. Effects of ROS scavengers on the formation of 8-OHdG in DNA by $CrCl_3/H_2O_2$. Various concentrations (0.3–30 mM) of sodium azide, Tris-HCl, or D-mannitol were added to the DNA solutions before the addition of 1 mM Cr(III) and 0.5 mM H_2O_2. The reactions were performed at the same condition as described in the legend to Figure 1. The reaction was also performed in D_2O as described in Materials and methods. The relative 8-OHdG formation was determined by dividing the amount of 8-OHdG obtained in each experiment containing ROS scavengers or D_2O by those obtained in $Cr(III)/H_2O_2$. Data were derived from the average of two or three experiments and the bars denote the standard error.

background level, whereas those contents markedly increased at pH values 7–8.9. The formation of 8-OHdG at pH 8.9 was lower than that observed at pH 8, which could be due to a higher degree of strand breakage, resulting in a small fragments (data not shown), and thereby causing more difficulty in isolating DNA for analysis.

The participation of ROS in the formation of 8-OHdG by $CrCl_3/H_2O_2$ was next determined by examining the effect of ROS scavengers, i.e. sodium azide, D-mannitol and Tris–HCl. The average amount of 8-OHdG generated was $90/10^5$ dG when DNA was incubated with 1 mM $CrCl_3$ plus 0.5 mM H_2O_2 at 37°C for 1 h. Additions of sodium azide, D-mannitol or Tris-HCl to those reaction mixtures caused a marked decrease in the generation of 8-OHdG in DNA (Figure 4). At equal concentrations of these ROS scavengers, the inhibitory effect was sodium azide > D-mannitol > Tris–HCl. At 0.3 mM, sodium azide reduced 76% of 8-OHdG formation by $CrCl_3/H_2O_2$; at 30 mM, it almost completely inhibited this reaction. These results suggest that hydroxyl radical, singlet oxygen and/or superoxide radicals may participate in the formation of 8-OHdG by $CrCl_3$ plus H_2O_2.

The involvement of the ROS species was further characterized by performing the reaction in D_2O, in which singlet oxygen can exist longer than in H_2O (31). As shown in Figure 4, the formation of 8-OHdG by $CrCl_3$ plus H_2O_2 was not enhanced in D_2O solution. In addition, the effect of catalase and SOD was examined. DNA was treated with $CrCl_3$ plus H_2O_2 and the reaction proceeded for 30 min, followed by the addition of enzyme or protein and the reaction continued for another 1 h. Meanwhile, $CrCl_3$ plus H_2O_2 treatments for 30 and 90 min were performed simultaneously. Figure 5 shows that 2.2 U/ml of catalase completely inhibited the formation of 8-OHdG by $CrCl_3$ plus H_2O_2, and that heat-denatured catalase (2.2 U/ml) did not influence those formations. This result confirmed that H_2O_2 is required for the formation of 8-OHdG. However, 11 U/ml of heat-denatured catalase reduced ~30% of 8-OHdG formation by $CrCl_3/H_2O_2$, possibly due to the non-specific scavenger activity of proteins. SOD (11 U/ml = 2.2 μg/ml) reduced 22% of 8-OHdG formation by $CrCl_3$ plus H_2O_2, and heat-inactivated SOD left the 8-OHdG formation unchanged (Figure 5). This finding indicated that SOD might scavenge some of superoxide ion and partially inhibit 8-OHdG formation.

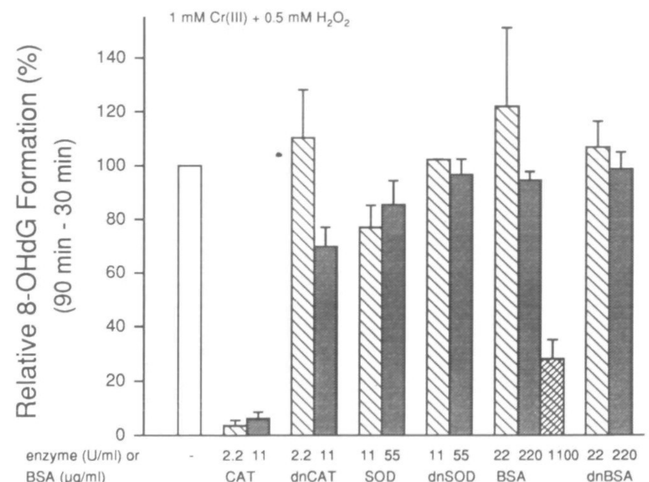

Fig. 5. Effects of catalase, SOD and BSA on the formation of 8-OHdG in DNA by $CrCl_3/H_2O_2$. DNA was reacted with 1 mM Cr(III) plus 0.5 mM H_2O_2 as described in the legend to Figure 1 and incubated for 30 min. Next, various amount of catalase, SOD or BSA were added to the reactions and incubation continued for another 60 min. The increased amounts of 8-OHdG formation between 90 min and 30 min incubation in Cr(III) plus H_2O_2 treatment of DNA were defined as 100%. The relative amounts of 8-OHdG formation were calculated by dividing those increased amounts obtained in experiments containing enzymes or proteins by those increased amounts obtained in experiments without enzymes or proteins. CAT represents catalase, and dnCAT, dnSOD, and dnBSA represents heat-denatured catalase, SOD, and BSA respectively. The data were derived from the average of two or three experiments. The bars denote the standard error.

The effects of DNA–protein crosslinks mediated by $CrCl_3$ may interfere with the formation of 8-OHdG (17). BSA was added to the DNA solution that had been treated with $CrCl_3$

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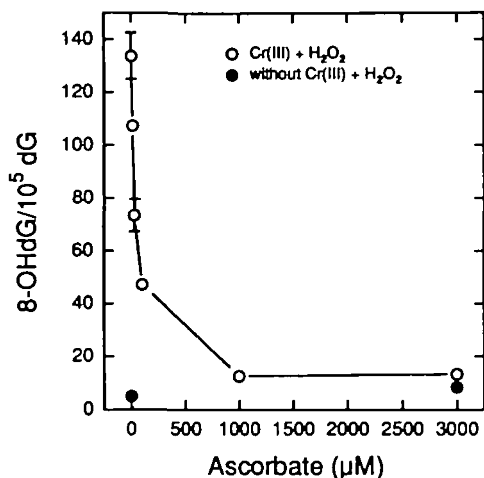

Fig. 6. Effects of ascorbate on the formation of 8-OHdG in DNA by $CrCl_3$/H_2O_2. All of the materials were freshly prepared in Chelex 100-treated water. DNA was treated with 1 mM Cr(III) plus 0.5 mM H_2O_2 in phosphate buffer (10 mM, pH 7.5) containing 1 μM DTPA and various concentrations of ascorbate (0–3 mM). The reaction was incubated at 37°C for 1 h. The amount of 8-OHdG in DNA was determined from the average of two experiments. The bars denote the standard error.

plus H_2O_2 for 30 min to determine the effect of protein on the 8-OHdG formation. The relative 8-OHdG formation was unaltered by 22–220 μg/ml of BSA, whereas 1.1 mg/ml BSA reduced 72% of the 8-OHdG adducts generated by $CrCl_3$ plus H_2O_2 (Figure 5). This observation indicated that a high level of Cr(III)-mediated DNA–protein crosslinks could inhibit the formation of 8-OHdG. The catalase and SOD concentrations used here were much lower than that of BSA, thereby implying that DNA–protein crosslinks play an insignificant role in the formation of 8-OHdG by Cr(III)/H_2O_2 in the presence of catalase or SOD. Denatured BSA at a concentration of 1.1 mg/ml became aggregated and its effect was not analyzed here.

Figure 6 shows that the formation of 8-OHdG by $CrCl_3$/H_2O_2 was markedly prevented by ascorbate. The degree of prevention of 8-OHdG induction increased when the concentration of ascorbate was increased (10–3000 μM). Ascorbate at 100 μM reduced ~66% of the amount of 8-OHdG formation by 1 mM $CrCl_3$ plus 0.5 mM H_2O_2; at millimolar level, it almost completely inhibited those 8-OHdG formations. Ascorbate alone, or ascorbate plus Cr(III), or ascorbate plus H_2O_2 did not significantly enhance the formation of 8-OHdG above background level. The final pH value (pH 7–7.5) of the reaction mixture was not changed by the addition of ascorbate. Therefore, the reduced number of adducts by ascorbate is due to its antioxidant capability.

Discussion

This study has demonstrated that Cr(III) plus H_2O_2 is capable of inducing 8-OHdG in DNA. The level of 8-OHdG generated increases when the Cr(III) concentrations as well as the incubation times are increased. Sodium azide, D-mannitol and Tris–HCl prevented the formation of 8-OHdG by Cr(III) plus H_2O_2, indicating that ROS is involved. Also, catalase completely inhibits those formations, revealing that H_2O_2 is necessary for DNA damages. H_2O_2 is a normal metabolite in the cell whose steady-state concentrations range from 10^{-8} to 10^{-9} M (34). The concentration of H_2O_2 may markedly increase upon irradiation, during metabolism of carcinogens, and at sites of inflammation (35–37). Although the concentration of H_2O_2 used here for *in vitro* assay is much higher than the physiological concentration, exposure of cells to chromate may increase the amount of H_2O_2 during its metabolism. Our results have suggested that Cr(III), having been shown to be the stable and abundant form of Cr(VI) metabolites in cells, may cause DNA damages through the oxidative pathway.

Several studies have shown that hydroxyl radical and singlet oxygen could induce the formation of 8-OHdG (38,39). Superoxide ion and H_2O_2, although not directly causing DNA damage under physiological conditions, participate in a metal ion catalyzed reaction (the Haber–Weiss reaction) and generate a highly reactive hydroxyl radical and singlet oxygen (22,23,40). Additionally, the induction of 8-OHdG in DNA was found in superoxide ion-generating cells (41,42). Electron spin resonance evidence has shown that hydroxyl radicals are generated in a DNA-free solution containing Cr(III) and H_2O_2 (29). Singlet oxygen is generated in a solution containing Cr(III) and H_2O_2 as determined by electron paramagnetic resonance using 2,2,6,6-tetramethyl-4-piperidone as a trapping agent (results to be published). This is despite the fact that the level of 8-OHdG induced by Cr(III) plus H_2O_2 was not further enhanced when the reaction was performed in D_2O. The fact that the effect of D_2O on 8-OHdG formation was not observed in our experimental conditions may be due to that the level of hydroxyl radical generated is much higher than singlet oxygen and/or that the former is much more reactive to DNA than the latter. Our results indicated that hydroxyl radical is the major species that causes the formation of 8-OHdG by Cr(III) plus H_2O_2.

Because ROS is very reactive and its half-life is very short, the formation of 8-OHdG is possibly due to those radicals generated near DNA. Cr(III) is capable of interacting with nucleic acids and may prefer to coordinate with guanine (10,20). The ROS may be formed near guanine residues in the presence of H_2O_2. In addition, the hexaquo form of Cr(III) is deprotonated at physiological pH and this species subsequently forms polymers containing OH bridges (43). The polymer form of hexaquo Cr(III) may have a stronger coordination with DNA than the monomer form, thereby enhancing ROS and the 8-OHdG formations when H_2O_2 is present.

Ascorbate acts as an anti-oxidant to inhibit 8-OHdG formation by Cr(III) plus H_2O_2. Our preliminary data from electron paramagnetic resonance studies revealed that the level of hydroxyl radical was reduced, whereas ascorbic radical and Cr(V)-complex signals appeared with the addition of ascorbate to phosphate buffer containing Cr(III) and H_2O_2, whereas no Cr(V) signal was observed in phosphate buffer containing $CrCl_3/H_2O_2$ without ascorbate (results to be published). This finding would support the notion that Cr(III) may be oxidized to Cr(IV) at physiological pH. Ascorbate scavenges hydroxyl radicals generated during Cr(III) oxidation; it probably does not function to reduce Cr(IV) to Cr(III). Furthermore, $Cr(H_2O)_6^{3+}$ is a stable species at an acidic solution, whereas the hexavalent state of Cr is the predominant species at pH ranges above 7 (43). This phenomenon has suggested that Cr(III) may not reduce to Cr(II) at physiological pH. Cr(III) may directly interact with H_2O_2 through a Fenton-like reaction: $Cr(III) + H_2O_2 \rightarrow Cr(IV) + {}^{\cdot}OH + OH^-$. In fact, Cr(III) is capable of oxidizing to Cr(VI) that is attributed to the presence of manganese oxide (44). Our results have demonstrated that

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Cr(III) may be reoxidized to Cr(IV) and further contribute to cytotoxicity and genotoxicity.

Ascorbate, although functioning as an anti-oxidant in the Cr(III) plus H_2O_2 system, potentiates ROS, DNA strand breaks and 8-OHdG formation by Cr(VI) plus H_2O_2 (45). Ascorbate has been shown to be the major reductant of Cr(VI) *in vivo* (46). The reaction between Cr(VI) and ascorbate produces Cr(V), Cr(IV), Cr(III) and carbon-base radicals as reactive intermediates (47). Hydrogen peroxide may be produced during reduction of Cr(VI) by ascorbate (48). The ratio of ascorbate to Cr(VI) is critical to the varieties of intermediates generated and could also influence the degree of DNA damage (47,49). For instance, the highest carbon-base radicals, Cr(V), Cr–DNA adducts and DNA strand breaks were observed at the 1:1 reaction ratio; those radicals, intermediates and DNA damage decreased [except Cr(III) and Cr(IV) level] at excess ascorbate (49). Our study has demonstrated that ascorbate could prevent the formation of ROS-mediated base damages generated by Cr(III) plus H_2O_2. Thus, the role of ascorbate in the chromium detoxification or toxification process in cells should be carefully interpreted.

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