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One-Electron Reduction of Chromium(VI) by α-Lipoic Acid and Related Hydroxyl Radical Generation, dG Hydroxylation and Nuclear Transcription Factor-κB Activation

Fei Chen,* Jianping Ye,† Xiaoying Zhang,† Yon Rojanasakul,‡ and Xianglin Shi§,[1]

*Department of Pathology, Milton S. Hershey Medical Center, Pennsylvania State University, Hershey, Pennsylvania 17033; †Oncology Center, John Hopkins University, Baltimore, Maryland 21231; ‡Department of Basic Pharmaceutic Sciences, West Virginia University, Morgantown, West Virginia 26506; and §Pathology and Physiology Research Branch, Health Effects Laboratory Division, National Institute for Occupational Safety and Health, Morgantown, West Virginia 26505

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Reaction of chromium(VI) with α-lipoic acid (reduced form, also called 1,2-dithiolane-3-pentanoic acid) generated Cr(V) and hydroxyl radical (˙OH) as measured by electron spin resonance and ESR spin trapping. 5,5-Dimethyl-1-pyrroline was used as a spin trapping agent. Catalase inhibited the ˙OH generation and enhanced the Cr(V) formation. Superoxide dismutase had an opposite effect. H_2O_2 enhanced the ˙OH generation and decreased the Cr(V) formation in a dose-dependent manner. Metal chelators, EDTA, diethylenetriaminepentaacetic acid, deferoxamine, and 1,10-phenanthroline inhibited ˙OH radical generation in the order of EDTA > 1,10-phenanthroline > DTPA > deferoxamine. Oxygen consumption measurements indicated that molecular oxygen was used to generate ˙OH radical in the mixture of Cr(VI) and α-lipoic acid. H_2O_2 and superoxide radical (O_2^-) were involved as reactive intermediates. The ˙OH radical was generated via Cr(V)-mediated Fenton-like reaction (Cr(V) + H_2O_2 → Cr(VI) + OH^- + ˙OH). HPLC measurements show that the ˙OH radical generated by this reaction is capable of generating 8-hydroxyl-2′-deoxyguanosine from 2-deoxyguanosine. Incubation of Cr(VI) with cultured Jurkat cells resulted in an activation of DNA binding activity of the nuclear factor (NF)-κB. Addition of α-lipoic acid enhanced the NF-κB activation, while the ˙OH radical scavenger, sodium formate, inhibited it, showing that α-lipoic acid enhanced Cr(VI)-induced NF-κB activation via free radical reactions. The results indicate that while α-lipoic acid is considered to be an antioxidant, it may be a cellular one-electron Cr(VI) reductant and could be involved in the mechanism of Cr(VI)-induced carcinogenesis.

Although chromium is necessary for some physiological functions, chromium(VI) compounds are generally considered as human carcinogens (1). They have mutagenic effects on bacteria (2) and induce transformation of mammalian cells (3). They caused respiratory cancers in humans and animals (4–9). While the detailed biochemical mechanism of Cr(VI)-induced carcinogenesis is unclear, the carcinogenic effect of Cr(VI) is generally attributed to cellular uptake because Cr(VI) and not Cr(III) actively enters cells by the sulfite transport system (10). Once inside the cell, Cr(VI) is reduced to its lower oxidation states, such as Cr(IV) and Cr(V). These reactive intermediates could directly cause DNA damage. They also generate hydroxyl radical (˙OH) from H_2O_2 via Fenton-like reactions as follows (11–13):

$$Cr(IV) + H_2O_2 \rightarrow Cr(V) + OH^- + {}^{\cdot}OH \quad [a]$$

$$Cr(V) + H_2O_2 \rightarrow Cr(VI) + OH^- + {}^{\cdot}OH \quad [b]$$

The ˙OH radicals generated can cause cellular damage, such as hydroxylation of 2′-deoxyguanosine (dG)[2] and

[1] To whom correspondence should be addressed. Fax: (304) 285-6126.

[2] Abbreviations used: dG, deoxyguanosine; NF-κB, nuclear transcription factor-κB; 8-OHdG, 8-hydroxyl-2′-deoxyguanosine; DTPA, diethylenetriaminepentaacetic acid; PMA, phorbol 12-myristate 13-acetate; DPPH, 1,1-diphenyl-2-picrylhydrazyl; DMPO, 5,5-dimethyl-1-pyrroline *N*-oxide; PMSF, phenylmethylsulfonyl fluoride; DTT, di-

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DNA strand breakage. Very recently, it has been reported that Cr(VI) is able to induce activation of nuclear transcription factor (NF)-κB via free radical reactions, which was suggested as an important mechanism of Cr(VI)-induced carcinogenesis (14). It is generally believed that most of the genotoxic effects of Cr(VI) are associated with reactive chromium species, such as Cr(IV) and Cr(V), and oxygen free radicals generated by these intermediates (6, 7, 15–20). Pretreatment with certain antioxidants are reported to alter the genotoxic effects of Cr(VI) (21, 22). Among a variety of antioxidants, the role of glutathione and ascorbate in the mechanism of Cr(VI)-induced carcinogenesis is most intensively studied (10, 17, 23–27).

In this communication, we report our recent studies on the reduction of Cr(VI) by α-lipoic acid (reduced form, also called 1,2-dithiolane-3-pentanoic acid) and related $^{\bullet}$OH generation, dG hydroxylation, and NF-κB activation. α-Lipoic acid was chosen because it is considered as a biological antioxidant via its reaction with reactive oxygen species (28–30). It also acts as an antioxidant via its weak metal-chelating activity (29). Second, α-lipoic acid has –SH thiyl group in its structure. Most of these thiyl containing molecules, such as glutathione, cysteine, penicillamine, and acetyl-cysteine, function as Cr(VI) reductants (10, 31). Finally, α-lipoic acid has been used for the treatment of heavy metal poisoning (29, 30). Our results show that reduction of Cr(VI) by α-lipoic acid in fact generates Cr(V) intermediate and $^{\bullet}$OH radicals. These radicals are able to cause dG hydroxylation and NF-κB activation. The reduction of Cr(VI) by α-lipoic acid may represent an important Cr(VI) reduction pathway and could be involved in the mechanism of Cr(VI) carcinogenesis.

MATERIALS AND METHODS

Materials. Potassium dichromate ($K_2Cr_2O_7$), sodium ascorbate, sodium formate, EDTA, deferoxamine, diethylenetriaminepentaacetic acid (DTPA), α-lipoic acid (reduced form), 1,10-phenanthroline, H_2O_2, phorbol 12-myristate 13-acetate (PMA), and 1,1-diphenyl-2-picrylhydrazyl (DPPH) were purchased from Sigma (St. Louis, MO). The spin trap, 5,5-dimethyl-1-pyrroline *N*-oxide (DMPO), was purchased from Aldrich (Milwaukee, WI). DMPO was purified by charcoal decolorization. This method consisted of successively treating the DMPO with activated charcoal until all free radical impurities disappeared as verified by electron spin resonance spectroscopy. Chelex 100 chelating resin was purchased from Bio-Rad Laboratories (Richmond, CA). The phosphate buffer (pH 7.4) was treated with Chelex 100 to remove transition metal ion contaminants.

ESR measurements. Short-lived free radical intermediates were detected by ESR spin trapping (32, 33). All ESR measurements were made using an E-4 ESR spectrometer and a flat cell assembly. The *g* values and hyperfine splittings were measured (to 0.1 G) directly from magnetic field separation using DPPH as a reference standard. Reactants were mixed in test tubes in a total final volume of 450 μl. The reaction mixture was then transferred to a flat cell for ESR measurement. The concentrations given in the figure legends are final concentrations. All the experiments were carried out at room temperature.

Oxygen consumption measurements. Oxygen consumption experiments were performed using a Clark oxygen electrode. The sample contained 10 mM α-lipoic acid and 2 mM $K_2Cr_2O_7$ in a phosphate buffer (pH 7.4).

HPLC measurements. A BAS 200A HPLC system was used for HPLC measurements using a biophase ODS C^{18} column (4.6 × 250 mm). The solvent used for elution was a mixture of 50 mM KH_2PO_4/7% methanol at a flow rate of 1.5 ml/min. The 8-OHdG was monitored at 254 nm and by an electrochemical detector set at 700 mV and 5–50 nA full scale. Under these conditions, the retention time for dG was 8.1 min and that for 8-OHdG was 11.3 min. The identification of the 8-OHdG peaks in experimental sample was determined by spiking the sample with synthesized reference 8-OHdG. The HPLC system was calibrated with standard solution of dG and 8-OHdG. The detection limit of the HPLC analytical procedure was 0.28 pmol 8-OHdG/μg dG.

Oligonucleotide. Oligonucleotides were synthesized by the phosphoramidite method on a DNA/RNA synthesizer (Applied Biosystems, Model 392, Foster City, CA). A NF-κB binding sequence (5′-GAAATTCCAAAGAGTCATCAGA-3′) from the promoter region of the human IL-2 gene was used to synthesize a NF-κB binding oligonucleotide. The synthesized single-stranded oligonucleotide were deprotected at 50°C for overnight, dried in a speed vacuum, and then dissolved in the TE buffer. Complimentary strands were denatured at 80°C for 5 min and annealed at room temperature. The double-stranded probe was labeled with [^{32}P]dCTP (Amersham, Arlington Heights, IL) using Klenow fragment (BRL, Gaithersburg, MD).

Cell lines. Jurkat cells ($CD4^+$ human lymphoblast cell line) were cultured in RPMI 1640 supplemented with 10% fetal calf serum, 2 mM glutamine, and 100 U/ml penicillin–streptomycin (complete medium). Cells (2×10^6/ml) were used for variety of treatments in this study.

Nuclear extraction. The nuclear extracts were prepared according to the method described earlier (34). Briefly, 2×10^7 cells were treated with 500 μl lysis buffer (50 mM KCl, 0.5% NP-40, 25 mM Hepes, pH 7.8, 1 mM PMSF, 10 μg/ml leupeptin, 20 μg/ml aprotinin, 100 μM DTT) on ice for 4 min. After 1-min centrifugation at 14,000 rpm, the supernatant was saved as a cytoplasmic extract. The nuclei were washed once with the same volume buffer without NP-40 and then were put into a 300-μl volume of extraction buffer (500 mM KCl, 10% glycerol with the same concentrations of Hepes, PMSF, leupeptin, aprotinin, and DTT as the lysis buffer) and pipetted several times. After centrifugation at 14,000 rpm for 5 min, the supernatant was harvested as the nuclear protein extract and stored at −70°C. The protein concentration was determined by BCA (Pierce, Rockford, IL).

Electrophoretic mobility shift assay (EMSA). The DNA–protein binding reaction was conducted in a 24-μl reaction mixture including 1 μg poly(dI·dC) (Sigma), 3 μg nuclear protein extract, and 3 μg BSA, 4×10^4 cpm of ^{32}P-labeled oligonucleotide probe and 12 μl of 2× Y buffer (34). This mixture was incubated on ice for 10 min in the absence of the radiolabeled probe and then incubated for 20 min at room temperature in the presence of radiolabeled probe. After the incubation, the DNA–protein complexes were resolved on a 5% acrylamide gel (National Diagnostics, Atlanta, GA) that had been prerun at 110 V for 1 h with 0.5× TBE buffer. The loaded gel was run at 210 V for 90 min, dried, and then placed on Kodak X-OMAT film (Eastman Kodak, Rochester, NY) for autoradiography. The film was developed after overnight exposure at −70°C.

thiothreitol; EMSA, electrophoretic mobility shift assay; SOD, superoxide dismutase.

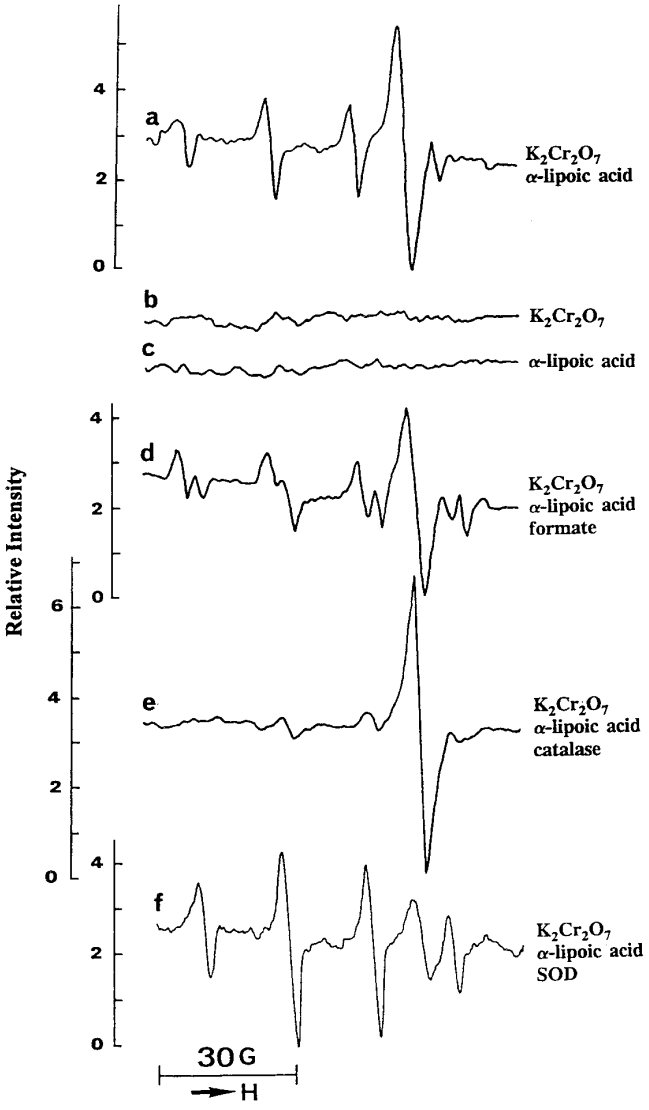

FIG. 1. (a) ESR spectrum recorded from phosphate buffer (pH 7.4) containing 1 mM $K_2Cr_2O_7$, 2 mM α-lipoic acid, and 80 mM DMPO. The spectrum was recorded after 2 min of mixing the components. (b) Same as a but without α-lipoic acid. (c) Same as a but without Cr(VI). (d) Same as a but with 50 mM sodium format added. (e) Same as a but with 20,000 units/ml catalase added. (f) Same as a but with 500 units/ml SOD added. The spectrometer settings were as follows: receiver gain, 8.0×10^3; modulation amplitude, 1.0 G; field, 3470 ± 100 G.

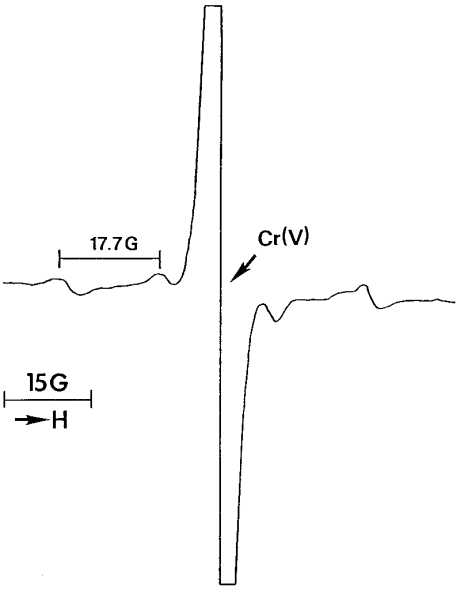

FIG. 2. ESR spectrum recorded from phosphate buffer (pH 7.4) containing 2 mM $K_2Cr_2O_7$, and 10 mM α-lipoic acid. The spectrum was recorded after 2 min of mixing the components. The spectrometer settings were as follows: receiver gain, 1.0×10^4; modulation amplitude, 1.0 G; field, 3470 ± 100 G.

RESULTS

·OH Radical Generation

Figure 1a shows the ESR spectrum obtained from a mixture of 1 mM $K_2Cr_2O_7$ and 2 mM α-lipoic acid in a phosphate buffer (pH 7.4). The spectrum is a composite of the spin adduct signal (1:2:2:1 quartet) and that of Cr(V), with a broad peak at $g = 1.992$ (Fig. 1a). To verify the assignment of Cr(V), ESR spectrum was recorded at a higher gain from the mixture containing 2 mM $K_2Cr_2O_7$ and 10 mM α-lipoic acid in phosphate buffer (pH 7.4). The spectrum was shown in Fig. 2, which exhibited four satellite peaks due to ^{53}Cr (9.55% abundance, $I = 3/2$) hyperfine splittings. The observed ^{53}Cr hyperfine splittings of 17.7 G are also typical of Cr(V) species (13), thus confirming the assignment of Cr(V) intermediate. The intensity of Cr(V) signal decreased as a function of time (Fig. 3a), indicating that

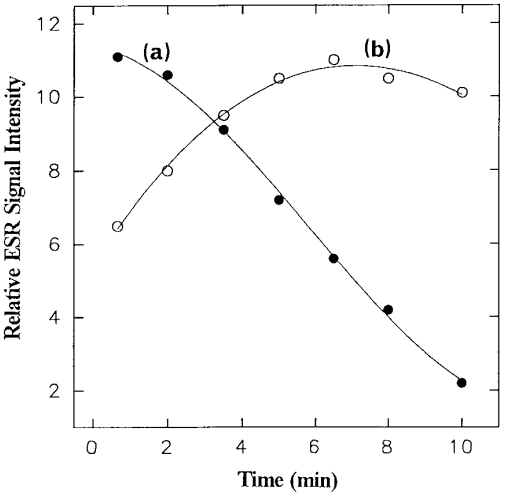

FIG. 3. Time courses of (a) Cr(V) and (b) DMPO/·OH. The experimental conditions were the same as those in Fig. 1a.

the Cr(V) intermediate was unstable and reactive. The computer simulation analysis of the 1:2:1:1 quartet (Fig. 1a) exhibited hyperfine splittings of $a_N = a_H = 15.0$ G, where $a_N = a_H$ represent hyperfine splittings of the nitroxyl nitrogen and hydrogen, respectively. This DMPO spin adduct was assigned to DMPO/$^{\bullet}$OH based on the hyperfine splittings of this adduct reported in the literature (32). The time course of DMPO/$^{\bullet}$OH formation and its decay was shown in Fig. 3b. Omission of any one component from the reaction mixture eliminated the ESR signal (Figs. 1b and 1c). Addition of $^{\bullet}$OH radical scavenger, formate, attenuated the DMPO/$^{\bullet}$OH signal with the concomitant generation of a new DMPO spin adduct signal with hyperfine splittings of $a_N = 15.8$ and $a_H = 18.8$ G (Fig. 1d). These splittings were typical of those of DMPO/$COO^{\bullet -}$ (35), providing an additional support for the assignment of DMPO/$^{\bullet}$OH in Fig. 1a. Addition of catalase, which removed H_2O_2, sharply reduced the DMPO/$^{\bullet}$OH signal and enhanced the Cr(V) signal (Fig. 1e). In contrast, addition of SOD, which removed superoxide radical ($O_2^{\bullet -}$) and generated H_2O_2, enhanced the DMPO/$^{\bullet}$OH signal and decreased the intensity of Cr(V) signal (Fig. 1f).

Figure 4a shows the effect of H_2O_2 on the $^{\bullet}$OH and Cr(V) generation from a reaction mixture containing 1 mM $K_2Cr_2O_7$ and 2 mM α-lipoic acid in a phosphate buffer (pH 7.4). In comparison with Fig. 1a, H_2O_2 significantly enhanced the intensity of DMPO/$^{\bullet}$OH signal. At 1 mM H_2O_2 Cr(V) signal is nondetectable (Fig. 4a). When 0.06 mM H_2O_2 was added, DMPO/$^{\bullet}$OH signal was less intensive than that in Fig. 4a and Cr(V) peak became observable (Fig. 4b). On decreasing the H_2O_2 concentration to 0.03 mM, the Cr(V) signal increased while the DMPO/$^{\bullet}$OH signal decreased but Cr(V) signal was still weaker and DMPO/$^{\bullet}$OH signal stronger than those in Fig. 1a.

Figure 5 shows the effect of chelators on the $^{\bullet}$OH generation in the reaction of α-lipoic acid with Cr(VI) with or without H_2O_2. EDTA essentially eliminated the $^{\bullet}$OH generation and also inhibited the Cr(V) formation (Figs. 5a and 5b). Similar results were obtained for DTPA (Figs. 5c and 5d), deferoxamine (Figs. 5e and 5f), and 1,10-phenanthroline (Figs. 5g and 5h). The inhibitory effect was in the order of EDTA > 1,10-phenanthroline > DTPA > deferoxamine.

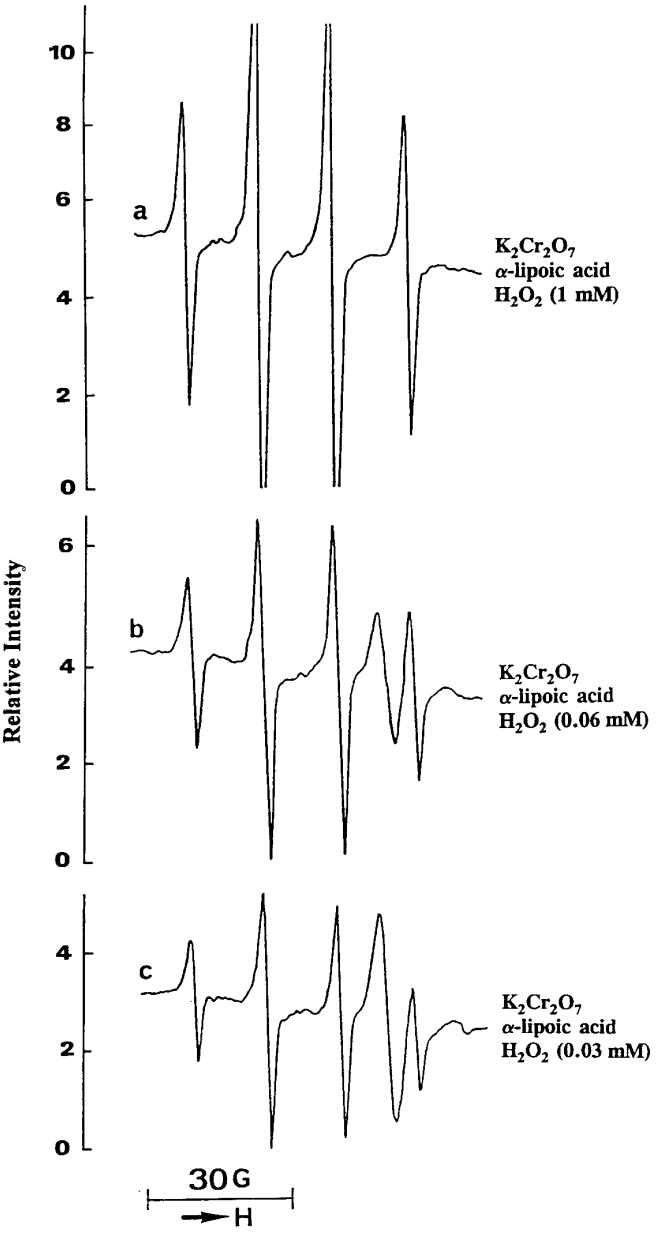

FIG. 4. (a) ESR spectrum recorded from phosphate buffer (pH 7.4) containing 1 mM $K_2Cr_2O_7$, 2 mM α-lipoic acid, 1 mM H_2O_2, and 80 mM DMPO. The spectrum was recorded after 2 min of mixing the components. (b) Same as a but using 0.06 mM H_2O_2. (c) Same as a but using 0.03 mM H_2O_2. The spectrometer settings were the same as in Fig. 1.

Molecular Oxygen Consumption

The consumption of dissolved molecular oxygen was monitored to study the role of molecular oxygen in the $^{\bullet}$OH radical generation from a mixture of α-lipoic acid and Cr(VI). As shown in Fig. 6, a mixture of α-lipoic acid and $K_2Cr_2O_7$ in a phosphate buffer (pH 7.4) rapidly consumed molecular oxygen. α-Lipoic acid alone or $K_2Cr_2O_7$ alone did not consume any significant amount of molecular oxygen (data not shown).

dG Hydroxylation

dG hydroxylation by Cr(VI) reaction was measured using HPLC with electrochemical detection. Table I shows a comparison of 8-OHdG formation in the incubation mixtures. A solution containing dG alone, dG and Cr(VI) without α-lipoic acid, or dG and α-lipoic acid without Cr(VI) did not generate any detectable amount of 8-

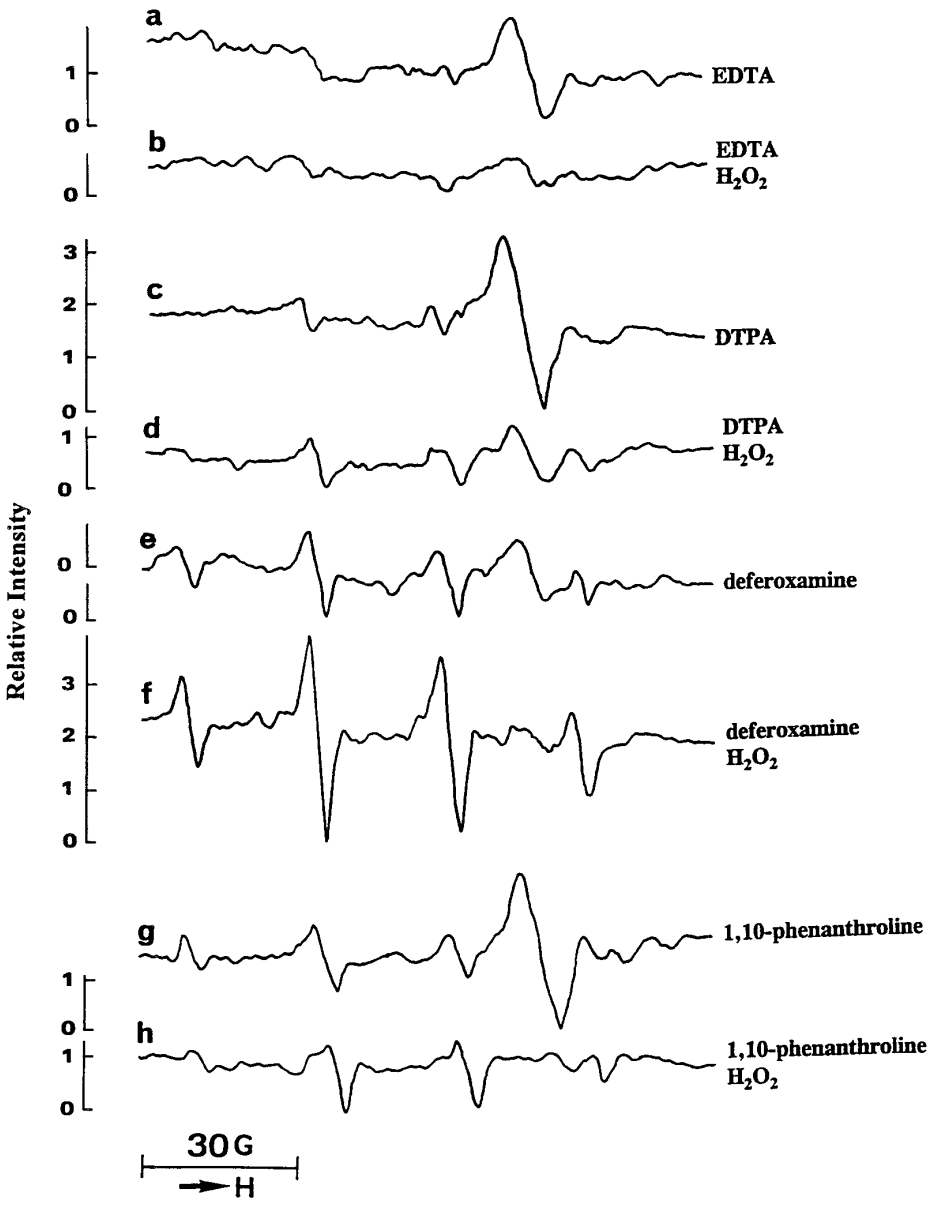

FIG. 5. ESR spectra recorded from phosphate buffer (pH 7.4) containing (a) 1 mM $K_2Cr_2O_7$, 2 mM α-lipoic acid, 1 mM EDTA, and 80 mM DMPO; (b) 1 mM $K_2Cr_2O_7$, 2 mM α-lipoic acid, 0.0325 mM H_2O_2, 1 mM EDTA, and 80 mM DMPO; (c) 1 mM $K_2Cr_2O_7$, 2 mM α-lipoic acid, 1 mM DTPA, and 80 mM DMPO; (d) 1 mM $K_2Cr_2O_7$, 2 mM α-lipoic acid, 0.0325 mM H_2O_2, 1 mM DTPA, and 80 mM DMPO; (e) 1 mM $K_2Cr_2O_7$, 2 mM α-lipoic acid, 1 mM deferoxamine, and 80 mM DMPO; (f) 1 mM $K_2Cr_2O_7$, 2 mM α-lipoic acid, 0.0325 mM H_2O_2, 1 mM deferoxamine, and 80 mM DMPO; (g) 1 mM $K_2Cr_2O_7$, 2 mM α-lipoic acid, 1 mM 1,10-phenanthroline, and 80 mM DMPO; (h) 1 mM $K_2Cr_2O_7$, 2 mM α-lipoic acid, 0.0325 mM H_2O_2, 1 mM 1,10-phenanthroline, and 80 mM DMPO. The spectra were recorded after 2 min of mixing the components. The spectrometer settings were the same as those in Fig. 1.

OHdG. A mixture of dG, Cr(VI), and α-lipoic acid generated a significant amount of 8-OHdG. H_2O_2 (0.5 mM) caused a threefold increase in the 8-OHdG yield. An increase in H_2O_2 concentration further increased the yield.

NF-κB Activation

Jurkat cells were used to detect Cr(VI)-induced NF-κB activation. The cells were incubated in the presence of 1 μM $K_2Cr_2O_7$ for 3 h and then were harvested for extraction of the nuclear proteins. These nuclear proteins were analyzed by the EMSA for the DNA binding activity of NF-κB. As shown in Fig. 7, lane 1, the untreated Jurkat cells did not exhibit any significant NF-κB activity. After treatment with Cr(VI), the cells showed NF-κB binding activity (Fig. 7, lane 2) and this activity was enhanced upon addition of α-lipoic acid

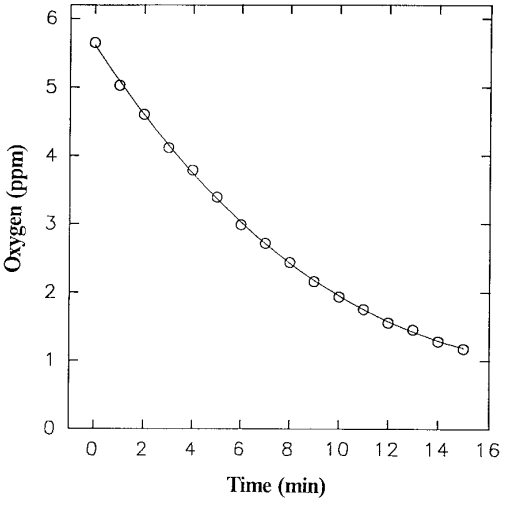

FIG. 6. Dissolved oxygen concentration in parts per million (ppm). The reaction mixture contained 2 mM $K_2Cr_2O_7$, 10 mM α-lipoic acid in a phosphate buffer (pH 7.4).

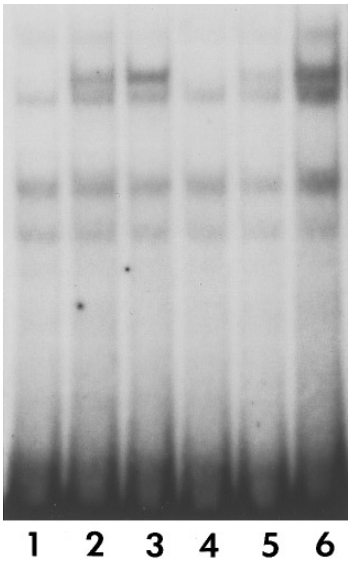

FIG. 7. Induction of DNA binding activity of NF-κB protein by Cr(VI). Jurkat cells were adjusted to a density of 2×10^6/ml and treated for 3 h with different stimulations and then subjected to extraction of the nuclear proteins as stated under Materials and Methods. Cr(VI), α-lipoic, sodium formate, and PMA were applied to the cells, and DNA binding activity of the NF-κB protein was detected with a probe of ^{32}P-labeled double-stranded NF-κB binding oligonucleotide by an EMSA assay. Lane 1, untreated cells; lane 2, cells + 2 μM Cr(VI); lane 3, cells + 2 μM Cr(VI) + 4.5 mM α-lipoic acid; lane 4, cells + 1 mM α-lipoic acid; lane 5, cells + 2 μM Cr(VI) + 5 μM sodium formate; lane 6, cells + 10 ng/ml PMA.

(Fig. 7, lane 3). α-Lipoic acid alone did not exhibit any observable activity (Fig. 7, lane 4). Sodium formate, an $^{\cdot}$OH radical scavenger, blocked Cr(VI)-induced NF-κB activation (Fig. 7, lane 5). Figure 7, lane 6 shows PMA-induced NF-κB activation as a positive control.

DISCUSSION

The present study demonstrates that α-lipoic acid is able to cause one-electron reduction of Cr(VI) leading to the generation of Cr(V) and $^{\cdot}$OH radical. The $^{\cdot}$OH generation involves Fenton-like reaction (Cr(V) + H_2O_2 → Cr(VI) + $^{\cdot}$OH + HO^-). The following experimental observations support this conclusion. (a) Catalase inhibited $^{\cdot}$OH generation and enhanced Cr(V) generation. (b) Addition of H_2O_2 had an opposite effect, i.e., enhancing $^{\cdot}$OH generation and inhibiting Cr(V) generation. During the Cr(VI) reduction by α-lipoic acid, molecular oxygen was reduced to O_2^-, which then generated H_2O_2. This is supported by the following observations: (i) $^{\cdot}$OH radical was generated in a mixture of Cr(VI) and α-lipoic acid without adding H_2O_2. (ii) A mixture of Cr(VI) and α-lipoic acid consumed molecular oxygen. (iii) SOD enhanced $^{\cdot}$OH generation and inhibited Cr(V) formation.

TABLE I

Hydroxylation of dG by Cr(VI) Reaction

Reaction mixture[a]	8-OHdG (pmol/μg dG ± SD)
dG	Nondetectable
dG + Cr(VI)	Nondetectable
dG + α-lipoic acid	Nondetectable
dG + Cr(VI) + α-lipoic acid	5.6 ± 0.18
dG + Cr(VI) + α-lipoic acid + H_2O_2 (0.5 mM)	16.8 ± 0.23
dG + Cr(VI) + α-lipoic acid + H_2O_2 (2 mM)	54.2 ± 0.56

[a] Concentrations in the reaction mixtures were as follows: dG, 0.5 mM; $K_2Cr_2O_7$, 0.4 mM; α-lipoic acid, 0.5 mM. The reaction mixtures were incubated in a phosphate buffer (pH 7.4) at room temperature for 30 min under ambient air.

The result shows that all metal chelators tested, EDTA, DTPA, deferoxamine, and 1,10-phenanthroline, inhibited $^{\cdot}$OH generation in the reaction between Cr(VI) and α-lipoic acid with or without H_2O_2. The effect is in the order of EDTA > 1,10-phenanthroline > DTPA > deferoxamine. The difference may be due to the different binding capability of these chelatiors. The results obtained from the present study indicate that proper chelation may be used to inhibit Cr(V) and $^{\cdot}$OH formation and to prevent or attenuate Cr(VI)-mediated cellular injury.

The current study shows that $^{\cdot}$OH radicals generated by the reaction of Cr(VI) with α-lipoic acid with or without exogenous H_2O_2 are capable of reacting with dG to form 8-OHdG. This result is significant because $^{\cdot}$OH radicals generated in the reaction of H_2O_2 with

some other metal ions, such as nickel (36), copper, or zinc containing superoxide dismutase (37), exhibit very limited reactivity. For example, ·OH radicals generated by these systems cannot be scavenged by either ethanol or formate. The reason for this nonreactivity is thought to be that ·OH radicals are generated within the domain of certain macromolecules and are not "free" to exhibit significant activity (36, 37). The present study shows that the ·OH radicals generated in the reaction of Cr(VI) with α-lipoic acid with or without H_2O_2 are free and do have the potential to react with ethanol, formate, and dG. This observation further suggests that Cr(VI)-generated ·OH radicals may cause DNA damage via hydroxylation reaction.

In this study, we have demonstrated that α-lipoic acid is able to enhance Cr(VI)-induced DNA binding activity of NF-κB via enhancement of Cr(VI)-mediated ·OH radical generation. Cr(VI) compounds are widely considered as human carcinogens. It is generally recognized that chromosome aberration (38), DNA mutation (39) and DNA–protein cross-linking (40) are associated with carcinogenic effects of Cr(VI). Using a human Jurkat T lymphoma cell line as a model to study Cr(VI)-mediated oncogene regulation, Ye *et al.* have reported that Cr(VI) can activate transcription factor NF-κB (14). Since activation of NF-κB is associated with formation of lymphoma (49), Cr(VI) may induce overexpression of c-*myc* protooncogene via NF-κB activation. Because overexpression of the c-*myc* protooncogene is associated with carcinogenesis (38, 41), Ye *et al.* have hypothesized that NF-κB activation and a subsequent protooncogene activation might serve as a mechanism for induction of neoplastic transformation by Cr(VI) (14). They have shown that Cr(VI) reduction is required for induction of NF-κB activity (14). In the cellular system, certain small molecules such as ascorbate (24, 25, 27) and glutathione (23) and several flavoenzymes such as glutathione reductase (11) can reduce Cr(VI) with Cr(V) as intermediate and generate free radicals. The present study has demonstrated that α-lipoic acid may be another important cellular Cr(VI) reductant, thus contributing to the overall mechanism of Cr(VI)-induced carcinogenesis.

Recently, a great deal of attention has been given to possible antioxidant functions for α-lipoic acid (28–30). It has been reported to scavenge O_2^-, ·OH, and peroxide radicals (28–30, 42, 43). It also enhances other water- or lipid-soluble antioxidants (ascorbate, vitamin E, and GSH) by regenerating them via reduction of their radicals or its oxidized form (GSSG) (44, 45). α-Lipoic acid has also been considered as a good candidate for the treatment of heavy metal poisoning due to its possible chelating effects (28, 29). These metals include arsenite, cadmium, and mercury. α-Lipoic acid has been shown to be a potent inhibitor of NF-κB activation in human T cell due to its antioxidant effect (46). It may be noted that Connett and Wetterhahn (10) proposed that ascorbate and physiological thiol-containing molecules, i.e., GSH and cysteine, would be the most likely candidates for nonenzymatic reduction of Cr(VI). Recently, intensive studies have been carried out on the reduction of Cr(VI) by ascorbate and GSH (17, 24–27, 47). These studies include the generation of chromium intermediates, ascorbate-derived free radicals, and oxygen free radicals. Although ascorbate and GSH are well-known antioxidants, reactive chromium intermediates and free radicals generated by reactions of Cr(VI) with these antioxidants cause DNA damage and may play an important role in the mechanism of Cr(VI) carcinogenicity. Similarly, α-lipoic acid is also an antioxidant. As shown by the present study, this antioxidant is able to reduce Cr(VI) to generate Cr(V). During the reduction process, ·OH radicals are generated via Fenton-like reaction. The ·OH radicals thus generated cause dG hydroxylation and NF-κB activation.

In conclusion, the present study demonstrates that α-lipoic acid reduces Cr(VI) to generate Cr(V). During the reduction process, molecular oxygen was reduced to O_2^- and then to H_2O_2. The Cr(V) reacted with H_2O_2 to generate ·OH radical via Fenton-like reaction. EDTA, 1,10-phenanthroline, DTPA, and deferoxamine inhibited Cr(V) and ·OH generation. The ·OH radicals generated by Cr(VI) reaction were able to cause dG hydroxylation and NF-κB activation. Although α-lipoic acid has been considered an important cellular antioxidant, it is also an one-electron Cr(VI) reductant and could be involved in the mechanism of Cr(VI) carcinogenesis.

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REFERENCES

1. De Flora, S., Bagnasco, M., Serra, D., and Zanacchi, P. (1990) *Mutat. Res.* **238,** 99–172.
2. Petrilli, F. L., and De Flora, S. (1977) *Appl. Environ. Microbiol.* **33,** 805–809.
3. Dipaolo, J. A., and Levis, A. G. (1979) *Mutat. Res.* **67,** 231–238.
4. Alcedo, J. A., and Wetterhahn, K. E. (1990) *Int. Rev. Exp. Pathol.* **31,** 85–108.
5. De Flora, S., and Wetterhahn, K. E. (1989) *Life Chem. Rep.* **7,** 169–244.
6. Cohen, M. D., Kargacin, B., Klein, C. B., and Costa, M. (1993) *Crit. Rev. Toxicol.* **23,** 255–281.
7. Snow, E. (1992) *Pharmacol. Ther.* **53,** 31–65.
8. Langard, A., and Lauwerys, R. R. (1980) *Mutat. Res.* **76,** 227–239.
9. IARC (1990) IARC Monogr. Eval. Carcinog. Risks Hum. 49.

10. Connett, P. H., and Wetterhahn, K. E. (1983) *Struct. Bond.* **54,** 93–124.
11. Shi, X., and Dalal, N. S. (1989) *Biochem. Biophys. Res. Commun.* **163,** 627–634.
12. Shi, X., and Dalal, N. S. (1990) *Arch. Biochem. Biophys.* **281,** 90–95.
13. Shi, X., and Dalal, N. S. (1990) *Arch. Biochem. Biophys.* **277,** 342–350.
14. Ye, J., Zhang, X., Young, A. A., Mao, Y., and Shi, X. (1995) *Carcinogenesis* **16,** 2401–2405.
15. Kasprzak, K. S. (1991) *Chem. Res. Toxicol.* **4,** 604–615.
16. Klein, C. B., Frenkel, K., and Costa, M. (1991) *Chem. Res. Toxicol.* **4,** 592–604.
17. Standeven, A. M., and Wetterhahn, K. E. (1991) *Chem. Res. Toxicol.* **4,** 616–625.
18. Stohs, S. J., and Bagchi, D. (1995) *Free Radical Biol. Med.* **18,** 321–336.
19. Sugiyama, M. (1994) *Cell. Biol. Toxicol.* **10,** 1–22.
20. Kasprzak, K. S. (1995) *Cancer Invest.* **13,** 411–430.
21. Sugiyama, M., Tsuzuki, K., and Ogura, R. (1991) *J. Biol. Chem.* **266,** 3383–3386.
22. Sugiyama, M. (1992) *Free Radical Biol. Med.* **12,** 397–407.
23. Shi, X., and Dalal, N. S. (1988) *Biochem. Biophys. Res. Commun.* **156,** 137–142.
24. Stearns, D. M., and Wetterhahn, K. E. (1994) *Chem. Res. Toxicol.* **7,** 219–230.
25. Stearns, D. M., Kennedy, L. J., Courtney, K. D., Giangrande, P. H., Phieffer, L. S., and Wetterhahn, K. E. (1995) *Biochemistry* **34,** 910–919.
26. Fresco, P., Shacker, F., and Kortenkamp, A. (1995) *Chem. Res. Toxicol.* **8,** 884–890.
27. Shi, X., Mao, Y., Kanpton, A. D., Ding, M., Rojanasakul, Y., Gannett, P. M., Dalal, N. S., and Liu, K. (1994) *Carcinogenesis* **15,** 2475–2478.
28. Packer, L., Witt, E. H., and Tritschler, H. J. (1995) *Free Radical Biol. Med.* **19,** 227–250.
29. Ou, P., Trischler, H. J., and Wolff, S. P. (1995) *Biochem. Pharmacol.* **50,** 123–126.
30. Matsugo, S., Yan, L., Han, D., Trischler, H. J., and Packer, L. (1995) *Biochem. Biophys. Res. Commun.* **208,** 161–167.
31. Shi, X., Dong, Z., Dalal, N. S., and Gannett, P. M. (1994) *Biochim. Biophys. Acta* **1226,** 65–72.
32. Janzen, E. G., and Blackburn, B. J. (1968) *J. Am. Chem. Soc.* **90,** 5909–5910.
33. Mottley, C., and Mason, R. P. (1989) *Biol. Magn. Reson.* **8,** 489–546.
34. Ye, J., Ghosh, P., Cippitelli, M., Subleski, J., Hardy, K. J., Ortaldo, J. R., and Young, H. A. (1994) *J. Biol. Chem.* **269,** 25728–25734.
35. Buettner, G. R. (1987) *Free Radical Biol. Med.* **3,** 259–303.
36. Inoue, S., and Kawanishi, S. (1989) *Biochem. Biophys. Res. Commun.* **159,** 445–451.
37. Yim, B. B., Chock, P. B., and Stadtman, E. R. (1990) *Proc. Natl. Acad. Sci. USA* **87,** 5006–5010.
38. Tsuda, H., and Kato, K. (1977) *Mutat. Res.* **46,** 87–94.
39. Bianchi, V., Celott, L., Lanfranchi, G., Majone, F., Marin, G., Montaldi, A., Sponza, G., Tamino, G., Venier, P., Zantedeschi, A., and Levis, A. G. (1983) *Mutat. Res.* **117,** 279–300.
40. Wedrychowski, A., Ward, W. S., Schmidt, W. N., and Hnilica, L. S. (1985) *J. Biol. Chem.* **260,** 7150–7155.
41. Ji, L., Arcinas, M., and Boxer, L. M. (1994) *Mol. Cell. Biol.* **14,** 7967–7974.
42. Suzuki, Y. J., Tsuchiya, M., and Packer, L. (1991) *Free Radical Res. Commun.* **15,** 255–263.
43. Scott, B. C., Aruoma, O. I., Evans, P. J., O'Neill, C., van der Vliet, A., Cross, C. E., Tritschler, H., and Halliwell, B. (1994) *Free Radicals Res.* **20,** 119–137.
44. Kagan, V. E., Shvedova, A., Serbinova, E., Khan, S., Swanson, C., Powell, R., and Packer, L. (1992) *Biochem. Pharmacol.* **44,** 1637–1649.
45. Scholich, H., Murphy, M. E., and Sies, H. (1989) *Biochim. Biophys. Acta* **1001,** 256–261.
46. Suzuki, Y. J., Aggarwal, B. B., and Packer, L. (1992) *Biochem. Biophys. Res. Commun.* **189,** 1709–1715.
47. Aiyar, J., Berkovits, H. J., Floyd, R. A., and Wetterhahn, K. E. (1990) *Chem. Res. Toxicol.* **3,** 595–603.