Oxygen Radical-Mediated DNA Damage by Redox-Active Cr(III) Complexes

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ABSTRACT: The mechanism of DNA damage induced by Cr(III) complexes is currently unknown even though it is considered to be the ultimate biologically active oxidation state of chromium. In this study, we have employed the *Salmonella* reversion assay to identify mutagenic Cr(III) complexes. Cyclic voltammetry was used to differentiate the redox kinetics between mutagenic and selected nonmutagenic Cr(III) species. Plasmid relaxation of supercoiled DNA was employed to show in vitro interactions with plasmid DNA and correlate the interactions with the electrochemical behavior and biological activity. The results of this study demonstrate that the mutagenic Cr(III) complexes identified in the *Salmonella* reversion assay display characteristics of reversibility and positive shifts of the Cr(III)/Cr(II) redox couple consistent with the ability of these Cr(III) complexes to serve as cyclical electron donors in a Fenton-like reaction. These same mutagenic complexes display an ability to relax supercoiled DNA in vitro, presumably by the induction of single-strand breaks. Nonmutagenic complexes were selected to test different ligands to determine how the ligand directs the activity of Cr(III) complexes. All nonmutagenic complexes tested thus far have shown classical irreversibility, more negative reduction potentials, and an inability to relax supercoiled plasmid DNA. These results suggest that the mechanism by which chromium complexes potentiate mutagenesis involves an oxygen radical as an active intermediate. These data also demonstrate the effect of associated ligands with regard to the ability of a metal to generate an active redox center.

Chromium(VI) compounds were identified as some of the earliest chemicals with the ability to induce cancer in humans (Langard, 1990; Machle & Gregorius, 1948). The consequence of chromium(VI) exposure was first recognized in the chrome-plating (Royle, 1975), pigment (Langard & Norseth, 1975), and tanning industries (Acheson et al., 1970) by a significant increase in lung cancers. This initial recognition led to the most extensive epidemiological investigations of any inorganic compound to date. While the epidemiological evidence has clearly shown that chromium(VI) is carcinogenic, the mechanism by which chromium compounds induce carcinogenesis has yet to be adequately elucidated.

Chromium-induced mutagenicity and carcinogenicity have primarily focused on the +6 oxidation state of chromium because of its biological activity in a variety of assay systems. This activity has been associated with the ability of Cr(VI) compounds to cause DNA single-strand breaks and form DNA–protein and DNA–DNA cross-links (Tsapakos et al., 1983; Sugiyama et al., 1986a,b). Cr(III), formed from the reduction of chromates intracellularly, is considered to be the ultimate active oxidation state of chromium (Leonard & Lauwerys, 1980). Cr(III) salts, however, have shown little or no activity in these assay systems which would warrant such a judgment (Bianchi et al., 1983; Langerwerf et al., 1985; DeFlora & Wetterhahn, 1989). This apparent contradiction is attributed to the inability of Cr(III) salts to traverse the plasma membrane while Cr(VI) passes readily through the plasma membrane by anion carriers and is rapidly reduced intracellularly to an active Cr(III) complex (Connett & Wetterhahn, 1983).

In contrast to Cr(III) salts, certain hexacoordinate Cr(III) amine complexes have demonstrated biological activity in *Salmonella* reversion assays (Warren et al., 1981; Beyersmann, 1984). The mutagenicity of these hexacoordinate Cr(III) complexes is limited to those with two or three aromatic bidentate amines in an octahedral conformation (Warren et al., 1981a). One of these complexes, $[Cr(bpy)_2Cl_2]Cl$,[1] has demonstrated an absolute dependence on oxygen for the induction of mutagenicity in a *Salmonella* reversion assay (Sugden et al., 1990). This oxygen dependence could reflect a requirement for DNA damage induced by reactive oxygen. Alternatively, Cr(III) salts may exert their activity by increasing the processivity of DNA polymerase in vitro with a concomitant decrease in polymerase fidelity (Snow, 1991).

Any compound that can serve as a cyclical electron donor has the potential to generate oxygen radicals under the appropriate conditions. Some examples of these are the inorganic ions of Fe (Starke & Farber, 1985) and Cu (Marshall et al., 1981), certain organic herbicides such as methyl viologen (Moody & Hassan, 1982), and thiols such as cysteine (Rowley, 1982). The two equations which describe this behavior are known as the Haber–Weiss (1) and the Fenton reaction (2).

The Haber–Weiss equation describes the production of superoxide anion, hydrogen peroxide, and hydroxyl radical by consecutive monovalent reductions of molecular oxygen.

$$O_2 \rightarrow O_2^{\bullet -} \rightarrow HOOH \rightarrow HO^{\bullet} + H_2O \rightarrow H_2O \quad (1)$$

The Fenton equation involves the use of a metal ion as an electron donor to reduce hydrogen peroxide to the hydroxyl radical and water.

$$Me^n + e^- \rightarrow Me^{(n-1)} + HOOH \rightarrow Me^n + HO^{\bullet} + H_2O \quad (2)$$

Both the superoxide anion and the hydroxyl radical are considered to have DNA-damaging capability. The hydroxyl radical however is thought to be the only species with the proper reactivity and electrophilicity to be capable of causing strand breakage in DNA (Pryor, 1988).

In order to understand the mechanism of DNA damage by Cr(III) complexes and the role of oxygen radicals, it is

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[1] Abbreviations; bpy, 2,2′-bipyridyl; phen, 1,10-phenanthroline; en, ethylenediamine. FAB-MS, fast atom bombardment mass spectrometry; TBE, 89 mM boric acid, 89 mM Tris base, and 2.5 mM EDTA, pH 8.0.

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necessary to ascertain the physical characteristics that mutagenic complexes possess versus structurally similar nonmutagenic complexes and how these characteristics might influence radical generation. In this investigation, the structure/activity relationships of a variety of related Cr(III) complexes are examined. Mutagenicity, as determined by the *Salmonella* reversion assay, shows that the mutagenic complexes have similar ligand types yet varying mutagenic activity. The redox kinetics of the mutagenic chromium complexes all demonstrate a degree of reversibility of the redox couples while the nonmutagenic complexes demonstrate both irreversibility and anodic shifts to more negative reduction potentials. In addition, plasmid relaxation studies with the mutagenic Cr(III) complexes exhibit conformational changes in supercoiled DNA that are consistent with an oxygen radical-mediated DNA cleavage mechanism of damage. The nonmutagenic Cr(III) complexes are unable to induce DNA conformational changes in the same assay.

MATERIALS AND METHODS

Synthesis and Purification of Cr(III) Compounds. $[Cr(bpy)_2Cl_2]Cl$ and $[Cr(phen)_2Cl_2]Cl$ were prepared by dissolving 2,2′-bipyridyl or 1,10-phenanthroline in 95% ethanol containing anhydrous chromic chloride according to previous work (Burstall & Nyholm, 1952). Purification by liquid chromatography on Sephadex LH-20 support with an aqueous eluent yields two major bands from each synthesis. The leading red band in each case is the aquated species of the above compounds corresponding to $[Cr(bpy)_2(H_2O)_2]Cl_3$ and $[Cr(phen)_2(H_2O)_2]Cl_3$. The second band in each case was either a brown compound corresponding to $[Cr(bpy)_2Cl_2]Cl$ or an olive green band of $[Cr(phen)_2Cl_2]Cl$. Structural assignments were verified by visible and ultraviolet absorption spectrophotometry and closely matched those previously reported (Garner & House, 1970). FAB-MS on the $[Cr(bpy)_2Cl_2]Cl$ and $[Cr(phen)_2Cl_2]Cl$ species gave molecular ions of m/z 434 and 482, respectively, for both the dichloro and diaquo species.

$[Cr(bpy)_3](ClO_4)_3$ was prepared using a modification of previous work (Burstall & Nyholm, 1952). This modification was the direct synthesis of the chromous bromide intermediate from a 1:10 w/v mixture of chromium metal and hydrobromic acid under argon. After oxidation and recrystallization a single yellow band was observed with liquid chromatography on Sephadex LH-20 support and aqueous eluent. The structure was confirmed by ultraviolet and visible absorption spectrophotometry which closely matched that reported previously for this complex (Garner & House, 1970). FAB-MS showed a molecular ion at m/z of 520.

$[Cr(en)_3]Cl_3$ was prepared by dissolving chromic chloride in absolute methanol with a catalytic amount of zinc dust (*hazardous procedure; synthesis should be performed in explosion-proof hood, wear gloves*). This solution was allowed to reflux for 15 min prior to the dropwise addition of anhydrous ethylenediamine. The vigorous exothermic reaction formed a yellow precipitate which was recrystallized and used without further purification. The visible absorption spectrum was identical with previously reported data for this complex (Garner & House, 1970).

$[Cr(en)_2Cl_2]Cl$ was prepared from $[Cr(en)_3]Cl_3$ by recrystallizing the $[Cr(en)_3]Cl_3$ from a 1% aqueous solution of ammonium chloride and subsequent heating at 210 °C as described previously (Rollison & Bailar, 1946). The red-violet compound was recrystallized and used without further purification. Visible absorption spectra closely matched that reported previously for this complex (Garner & House, 1970).

$[Cr(NH_3)_4Cl_2]Cl$ was prepared from the chloroaquotetraamminechromium(III) chloride species by heating in an oven at 120 °C as described previously (Kyuno et al., 1967). This compound was used without further purification. The visible absorption spectra closely matched that previously reported for this complex (Garner & House, 1970).

$K_3Cr(CN)_6$ was obtained from stock on hand. The ultraviolet absorption spectra of this complex, log ϵ_m in parentheses, showed a maximum at 264 nm (3.15).

Mutagenicity Assay. For this assay, *Salmonella typhimurium* strains TA102 and TA2638 were chosen for their sensitivity to oxidative mutagens. These strains were constructed by Levin et al. (1982) and were gifts from Dr. B. N. Ames. Both TA102 and TA2638 have an A·T base pair at the site of the mutation in *hisG428*. The *hisG428* allele in TA102 resides on the plasmid *pAQ1* and is in its normal genomic location in TA2638. One hundred microliters of an overnight culture of the *Salmonella* strain was challenged with varying concentrations of the chemical in 0.5 mL of M9 buffer and allowed to preincubate for 30 min at room temperature prior to plating in standard plate overlays (Maron & Ames, 1983). The plates were counted for His^+ revertant colonies after incubation at 37 °C for 72 h.

Cyclic Voltammetry. Cyclic voltammetric data were obtained using a Forth-based voltammetry program interfaced with an Apple IIe computer. Solutions (1.0 mM) of the metal complex in 0.1 M KCl electrolyte were scanned at varying sweep rates using a mercury-coated platinum working and counter flag electrode and a 1.0 M Ag/AgCl reference electrode. The pH of the complexes was adjusted to 7.0 prior to the run using 1.0 M NaOH. No buffers were used due to their tendency to be effective ligands.

Plasmid Relaxation. Supercoiled plasmid was isolated from *Escherichia coli* HB101 containing the *puc-18* plasmid by a previous method (Maniatis et al., 1982). Gel electrophoresis was carried out on 10-μL reaction mixtures containing 2.0 μL of 6.0 mM plasmid DNA, 2.0 μL of 2.0 mM metal, 2.0 μL of 5.0 mM 1:1 ascorbate/H_2O_2 as reductant/oxidant, and 4.0 μL of 10 mM Tris-HCl buffer. The metal–DNA solutions were incubated at 37 °C for 30 min, the reductant was then added, and the solutions were allowed to incubate at ambient temperature for 15 min prior to electrophoresis at 100 V in a 1% agarose gel with 0.5X TBE buffer. DNA bands were resolved by adding 0.5 μL of a 10 mg/mL solution of ethidium bromide per 100 mL of agarose gel prior to pouring.

RESULTS

Synthesis of Chromium Compounds. The first objective was to synthesize a series of chromium complexes to demonstrate which structural themes and physical parameters can confer biological activity. All chromium complexes used in this study are hexacoordinate with a +3 oxidation state and octahedral conformation (Figure 1). Those complexes which possess geometrical isomerism are cis. The structural and physical themes investigated are as follows: (1) the influence of monodentate versus bidentate ligands; (2) the relationship between aromatic and nonaromatic amine ligands; (3) the influence of different spectrochemical series ligands on activity.

Comparison of Biological Activity. The relative reversion frequency of the Cr(III) compounds were determined using Ames strains TA102 and TA2638. Figures 2A and 3A show the relative response for the mutagenic Cr(III) complexes in TA102 and TA2638, respectively. In both *S. typhimurium* strains, the order of reversion frequency is $[Cr(phen)_2Cl_2]^{1+}$ > $[Cr(phen)_2(H_2O_2]^{3+}$ > $[Cr(bpy)_2Cl_2]^{1+}$ > $[Cr(bpy)_2(H_2O)_2]^{3+}$ > $[Cr(bpy)_3]^{3+}$. The revertants/nanomole for these

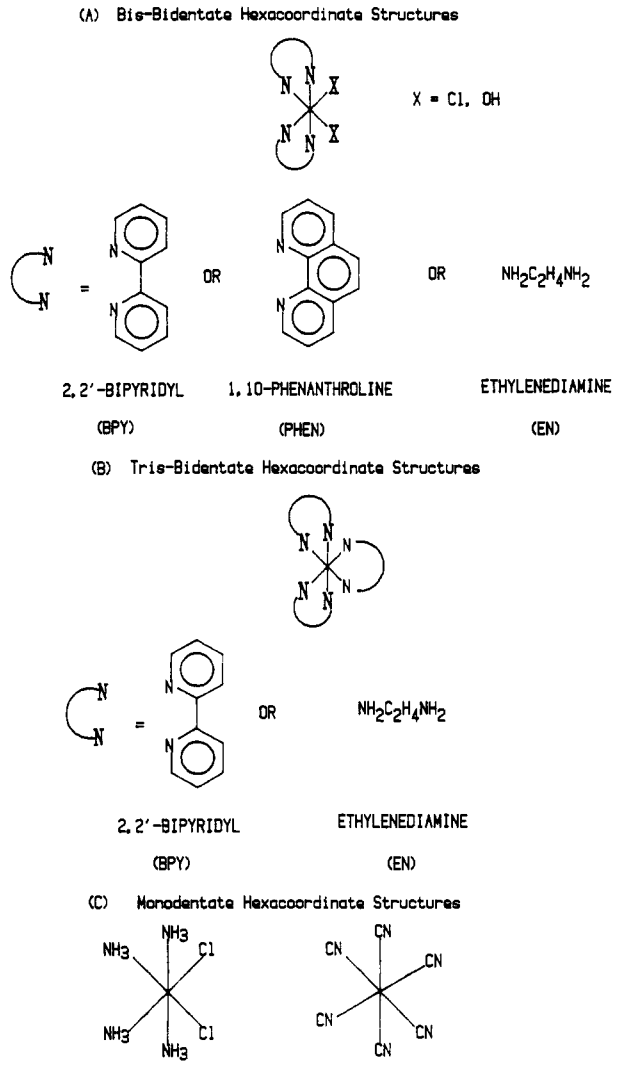

FIGURE 1: Structures of the hexacoordinate Cr(III) complexes used in this study. (A) Schematic representation of the five bis-bidentate species, $[Cr(bpy)_2Cl_2]^{1+}$, $[Cr(phen)_2Cl_2]^{1+}$, $[Cr(bpy)_2(OH)_2]^{1+}$, $[Cr(phen)_2(OH)_2]^{1+}$, and $[Cr(en)_2Cl_2]^{1+}$. (B) Representation of the two tris-bidentate species, $[Cr(bpy)_3]^{3+}$ and $[Cr(en)_3]^{3+}$. (C) Representation of the two monodentate species, $[Cr(NH_3)_4Cl_2]^{1+}$ and $[Cr(CN)_6]^{3+}$.

complexes range from 13.4 to 4.5 in TA102 and from 2.0 to 0.8 in TA2638. The relative reversion frequencies for the Cr(III) complexes are 2–5-fold over background in TA102 and 4–8-fold over background in TA2638.

Figures 2B and 3B show no reversion for the nonmutagenic Cr(III) compounds, $[Cr(CN)_6]^{3-}$, $[Cr(en)_3]^{3+}$, $[Cr(en)_2Cl_2]^{1+}$, and $[Cr(NH_3)_4Cl_2]^{1+}$, in both strains and the same concentration range. The bipyridyl and phenanthroline ligands themselves have shown no activity in either strain used in this study or in a previous study using *S. typhimurium* strains TA92, TA100, and TA98 (Warren et al., 1981b).

Comparison of Reduction Potentials, Reversibility, and Structural Changes between Different Cr(III) Complexes Using Cyclic Voltammetry. Cyclic voltammetry is an electrochemical technique used to study the redox state of a compound. Cyclic voltammetry is used to generate a new redox species on the forward (negative) scan and then probe the redox couple and chemical stability of the complexes on the reverse (positive) scan. Chemical reversibility occurs when the electronic and geometric structure of the reactant and the product are similar. This similarity can be obtained through the solvent or by the nature of the ligand associated with the metal. Figure 4 shows a single-scan cyclic voltammogram

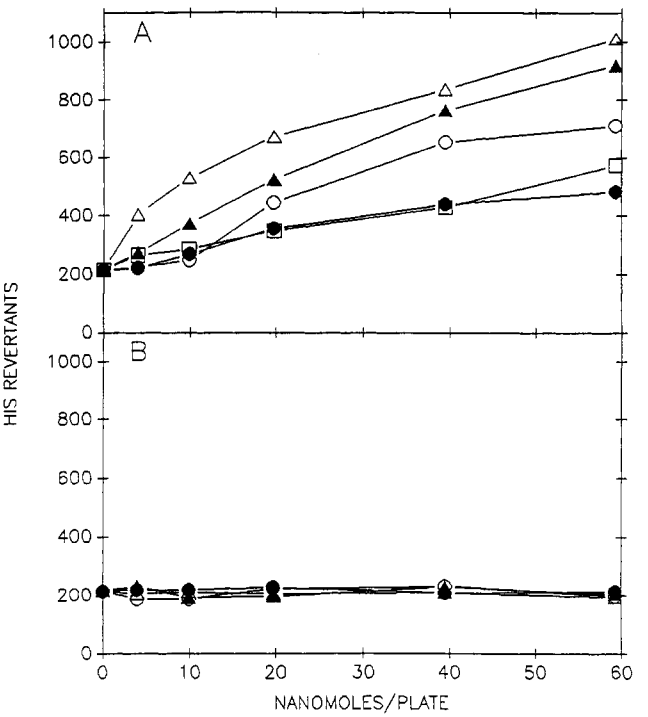

FIGURE 2: *Salmonella* reversion assay with Ames strain TA102. Panel A shows the relative response of the mutagenic Cr(III) complexes. These are in order of decreasing activity: △ = $[Cr(phen)_2Cl_2]^{1+}$; ▲ = $[Cr(phen)_2(OH)_2]^{1+}$; ○ = $[Cr(bpy)_2Cl_2]^{1+}$; □ = $[Cr(bpy)_2(OH)_2]^{3+}$; ● = $[Cr(bpy)_3]^{3+}$. Panel B shows the relative response of the nonmutagenic Cr(III) complexes over the same concentration range. ○ = $K_3Cr(CN)_6$; ● = $[Cr(en)_3]^{3+}$; △ = $[Cr(en)_2Cl_2]^{1+}$; ▲ = $[Cr(NH_3)_4Cl_2]^{1+}$. Each point represents the average of six replicates.

for the known oxygen radical generator methyl viologen (paraquat). Determination of a formal reduction potential is typically made by taking the average of the forward (reduction) and return (oxidation) waves. This approximation, however, is only accurate for reversible systems. A reversible system is defined as a reaction that is fast enough to maintain the concentrations of the reduced and oxidized forms of the analyte in equilibrium at the electrode surface. The separation between the two peak potentials, ΔE_p (where $\Delta E_p = E_{pc} - E_{pa}$), in a reversible electron-transfer process should be close to $58/n$ mV, where n is the number of electrons transferred. However, many systems will display reversibility when the voltage is scanned slowly but at increasing scan rates, and ΔE_p will be greater than $58/n$. Reversibility can then be viewed as a matter of degree depending on a variety of factors that are both compound and environment specific. Determination of the half-wave potential ($E_{1/2}$), which roughly corresponds to the formal electrode potential ($E^{\circ\prime}$), can be made off the voltammogram by taking the potential where the current is half the value of the peak current i_{pc}. This is the half-peak potential, $E_{p/2}$, that is related to the $E_{1/2}$ by the equation:

$$E_{p/2} = E_{1/2} \pm (28.0/n \text{ mV})$$

(sign is positive for a reduction process). Chemically irreversible reactions are those that yield products which cannot electrochemically regenerate the original reactant. The lack of a return peak and the changing cathodic peak potential, E_{pc}, with scan rate preclude an accurate reduction potential calculation using the above method.

In the current study, cyclic voltammetry is used to determine if the accompanying ligand can direct the biological activity of Cr(III) complexes by shifting the reduction potentials and

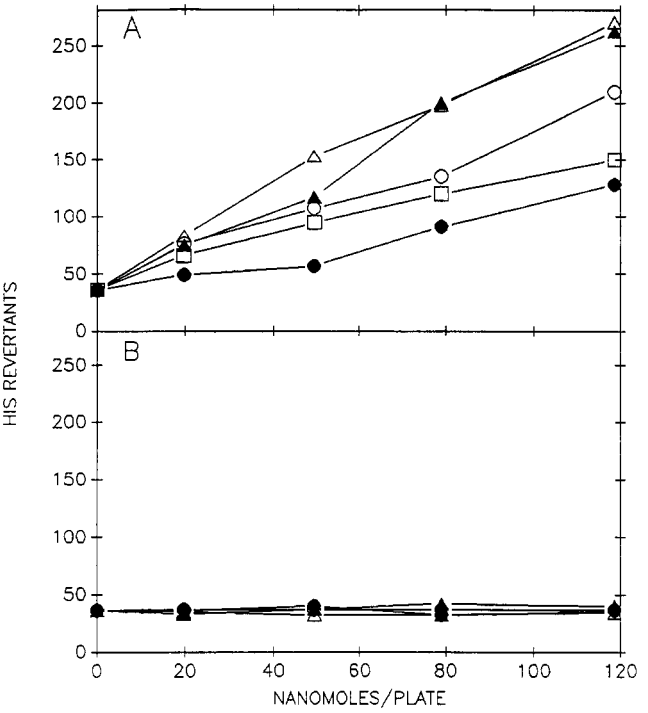

FIGURE 3: *Salmonella* reversion assay with Ames strain TA2638. Panel A shows the relative response of the mutagenic Cr(III) complexes. These are in order of decreasing activity: △ = $[Cr(phen)_2Cl_2]^{1+}$; ▲ = $[Cr(phen)_2(OH)_2]^{1+}$; ○ = $[Cr(bpy)_2Cl_2]^{1+}$; □ = $[Cr(bpy)_2(OH)_2]^{3+}$; ● = $[Cr(bpy)_3]^{3+}$. Panel B shows the relative response of the nonmutagenic Cr(III) complexes over the same concentration range. ○ = $K_3Cr(CN)_6$; ● = $[Cr(en)_3]^{3+}$; △ = $[Cr(en)_2Cl_2]^{1+}$; ▲ = $[Cr(NH_3)_4Cl_2]^{1+}$. Each point represents the average of six replicates.

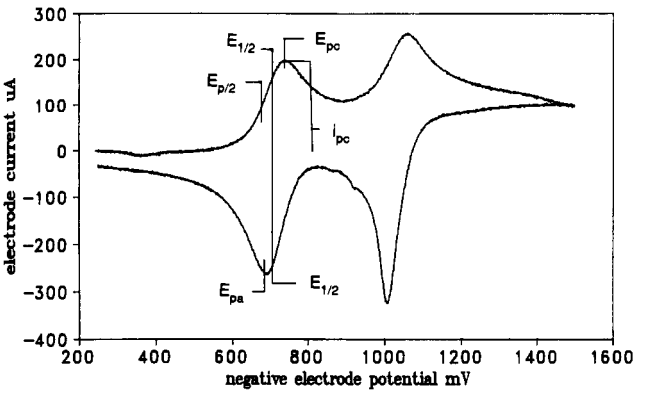

FIGURE 4: Cyclic voltammogram of 1.0 mM methyl viologen in 0.1 M KCl supporting electrolyte. Initial and end potentials were −250 mV with reversing potentials at −1500 and −250 mV versus the 1.0 M Ag/AgCl reference electrode. The sweep resolution was 1.00 mV/data point with a sweep rate of 614 mV/s.

conferring reversibility of the redox couples to allow the compound to serve as a cyclical electron donor.

Figure 5 shows a typical cyclic voltammogram for one of the mutagenic complexes and its aquated analog at varying sweep rates. The electrochemical behavior of both this complex, $[Cr(bpy)_2Cl_2]^{1+}$, and its tris-bidentate analog, $[Cr(bpy)_3]^{3+}$, has been the subject of several studies (Nadler et al., 1985; Soignet & Hargis, 1972, 1973; Baker & Dev Mehta, 1965). The other mutagenic complex, $[Cr(phen)_2Cl_2]^{1+}$, because of the ligand similarity, demonstrates redox behavior much like that of the $[Cr(bpy)_2Cl_2]^{1+}$ complex.

The cyclic voltammogram of the $[Cr(bpy)_2Cl_2]^{1+}$ complex shows that reduction proceeds in three steps which become ill-defined at faster sweep rates. The half-wave potentials ($E_{1/2}$) for the three reduction waves are −0.76, −0.97, and

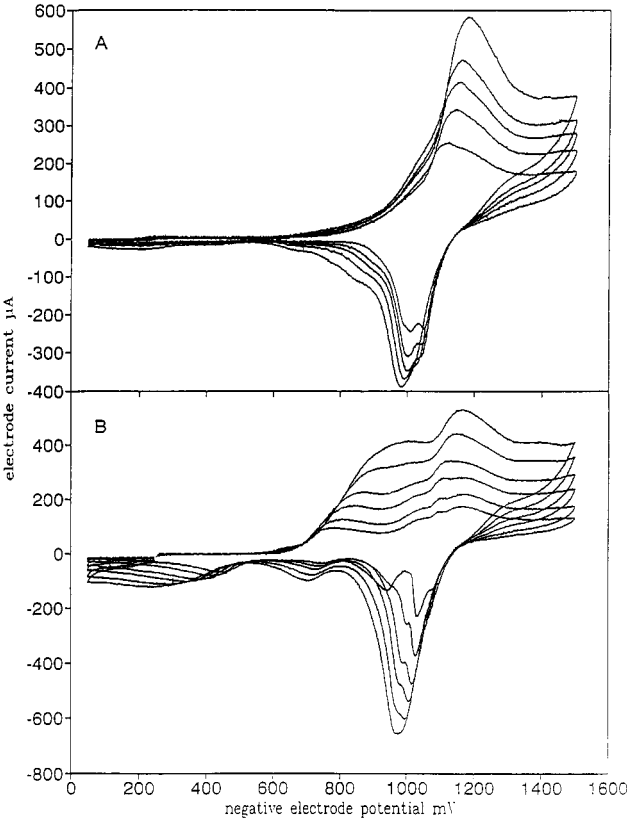

FIGURE 5: Cyclic voltammogram of 1.0 mM $[Cr(bpy)_2Cl_2]^{1+}$ (B) and the dihydroxy analogue $[Cr(bpy)_2(OH)_2]^{1+}$ (A) with 0.1 M KCl supporting electrolyte. Initial and end potentials were −250 mV with reversing potentials at −1500 and −50 mV versus 1.0 M Ag/AgCl reference electrode. Sweep resolution was 1.00 mV/data point with sweep rates by increasing current of 226, 397, 614, 971, and 1436 mV/s. The pH of the solution was adjusted to 7.0 prior to running.

−1.13 V. These $E_{1/2}$ potentials shift to slightly more negative values with increasing scan rate. Comparison of these waves with 1.0 mM cadmium chloride solution shows that each reduction wave roughly corresponds to the transfer of one electron. The oxidation waves are of a complex nature but are consistent with redox cycling. The pattern of the voltammograms, while complicated, are indicative of an EC (electrochemical/chemical) mechanism where the first reduction is followed by a chemical reaction relating to solvolytic ligand exchange of the chloride halogens for water. Further reduction results in the loss of a phenanthroline or bipyridyl ligand that can allow formation of the tris-amine complex on the return sweep. It is likely, however, that the only physiologically significant redox couple is in the range of the Cr(III)/Cr(II) couples. In both the bis-phenanthroline and bis-bipyridyl case, the polarographic behavior suggests the following reactions (am = bpy or phen):

step 1: $[Cr(am)_2Cl_2]^{1+} + e^- \leftrightarrow [Cr(am)_2Cl_2]^0$

$E_{1/2} = -0.73, -0.76$ V

step 2: $[Cr(am)_2Cl_2]^0 + 2H_2O \rightarrow [Cr(am)_2(H_2O)_2]^{2+} + 2Cl^-$

step 3: $[Cr(am)_2(H_2O)_2]^{2+} \leftrightarrow [Cr(am)_2(H_2O)_2]^{3+} + e^-$

$E_{1/2} = -0.96, -1.10$ V

With the tris-bipyridyl complex, the reaction is similar, but the first reduction results in the loss of a bipyridyl ligand accompanied by a catalytic electron-transfer process, again producing the bis-bipyridyl diaquo complex. This is shown

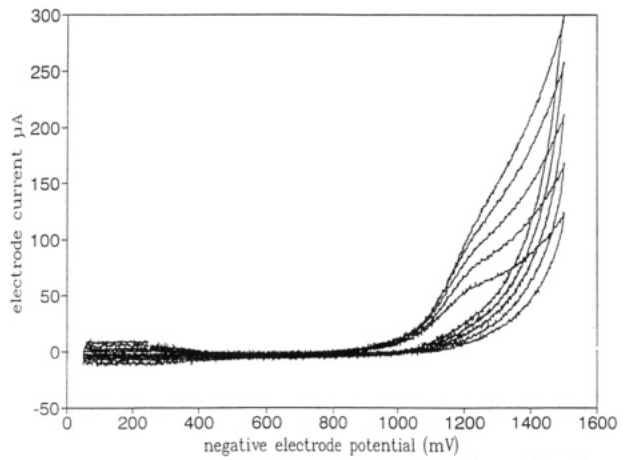

FIGURE 6: Cyclic voltammogram of 1.0 mM $[Cr(en)_2Cl_2]^{1+}$ with 0.1 M KCl supporting electrolyte. Initial and end potentials were –250 mV with reversing potentials at –1500 and –50 mV versus 1.0 M Ag/AgCl reference electrode. Sweep resolution was 1.00 mV/data point with sweep rates by increasing current of 226, 397, 614, 971, and 1436 mV/s. The pH of the solution was adjusted to 7.0 prior to running.

in the following reactions:

step 1: $[Cr(bpy)_3]^{3+} + e^- \leftrightarrow [Cr(bpy)_3]^{2+}$

$E_{1/2} = -0.93$ V

step 2: $[Cr(bpy)_3]^{2+} + 2H_2O \rightarrow [Cr(bpy)_2(H_2O)_2]^{2+} +$ bpy

step 3: $[Cr(bpy)_3]^{3+} + [Cr(bpy)_2(H_2O)_2]^{2+} \rightarrow [Cr(bpy)_3]^{2+} + [Cr(bpy)_2(H_2O)_2]^{3+}$

step 4: $[Cr(bpy)_2(H_2O)_2]^{3+} + e^- \leftrightarrow [Cr(bpy)_2(H_2O)_2]^{2+}$

$E_{1/2} = -1.10$ V

The oxidation in step 2 is reversible in the presence of free bipyridyl ligand and can result in the reformation of the tris complex, but the kinetics favor the bis-bipyridyl diaquo complex. The pK_a's of the diaquo complexes have been measured, giving pK_{a1} of 3.5, 3.4 and pK_{a2} of 6.1, 6.0 for $[Cr(bpy)_2(H_2O)_2]^{3+}$ and $[Cr(phen)_2(H_2O)_2]^{3+}$, respectively. These results are similar to that obtained previously (Inskeep & Bjerrum, 1961). Thus the complex present at intracellular pH is the dihydroxy species of the bipyridyl and phenanthroline complexes. Figure 5A shows a representative complex analyzed using cyclic voltammetry after adjusting the pH of the diaquo species to 7.0. As in the case of the dichloro species, these complexes demonstrate electrochemical behavior consistent with redox cycling.

In Figure 6, the nonmutagenic complex $[Cr(en)_2Cl_2]^{1+}$ shows a classical irreversible redox couple with a first reduction further negative than that of the mutagenic complexes. This type of behavior is similar for the $[Cr(NH_3)_4Cl_2]^{1+}$ complex while the $[Cr(en)_3]^{3+}$ and $K_3Cr(CN)_6$ complexes show no reduction prior to the catalytic generation of hydrogen at the electrode surface. The bipyridyl and phenanthroline ligands themselves show no reduction or oxidation in the voltage range scanned.

DNA Conformational Changes Induced by Cr(III) Complexes. The interactions which occur between DNA and metal complexes can be used to help define the mechanism of damage. Figure 7 shows the conformational changes induced in DNA when Cr(III) complexes and a reductant/oxidant are allowed to react in the presence of DNA. The change in DNA from a supercoiled conformation to a relaxed conformation is seen for a known hydroxyl radical generator, Fe–EDTA, as well

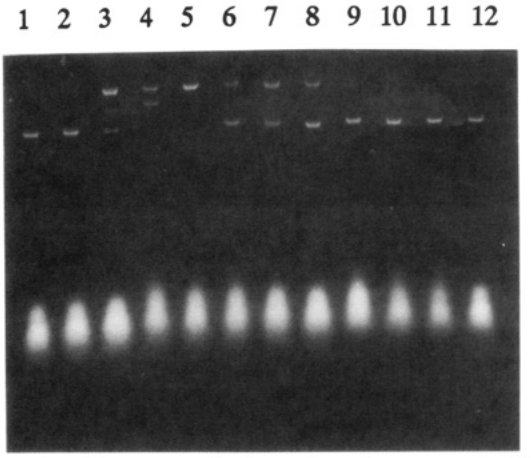

FIGURE 7: 1% agarose gel electrophoresis pattern of ethidium bromide-stained *puc-18* plasmid DNA from *E. coli* HB101. From left to right: lane 1, DNA control; lane 2, DNA + Asc/H_2O_2 control; (lanes 3–12 all contain DNA and ascorbate/H_2O_2 with the corresponding metal complex) lane 3, Fe–EDTA; lane 4, $[Cr(phen)_2Cl_2]^{1+}$; lane 5, $[Cr(phen)_2(OH)_2]^{3+}$; lane 6, $[Cr(bpy)_2Cl_2]^{1+}$; lane 7, $[Cr(bpy)_2(OH)_2]^{3+}$; lane 8, $[Cr(bpy)_3]^{3+}$; lane 9, $[Cr(en)_3]^{3+}$; lane 10, $[Cr(en)_2Cl_2]^{1+}$; lane 11, $[Cr(NH_3)_4Cl_2]^{1+}$; lane 12, $[Cr(CN)_6]^{3-}$. The reaction mixtures were incubated at 37 °C for 30 min prior to the addition of the ascorbate/H_2O_2. The final reaction mixture was allowed to stand for 15 min at ambient temperature prior to electrophoresis.

as the five mutagenic Cr(III) complexes. In contrast, the nonmutagenic complexes of Cr(III) as well as the bipyridyl and phenanthroline ligands themselves show only background levels of relaxation comparable to the DNA/reductant control. Incubation of all Cr(III) complexes with DNA in the absence of a reductant results in no relaxation of the supercoiled DNA.

DISCUSSION

We have established a ligand-directed dependence on the observed biological activity of closely related hexacoordinate chromium complexes. The identical order of reversion in the two *S. typhimurium* strains qualitatively suggests that the ligand imparts a fundamental structural contribution to the activity of Cr(III) compounds. This structural contribution is apparent in the electrochemical behavior between the mutagenic and the nonmutagenic complexes. In all the complexes tested thus far, the aromatic bidentate amines (bpy, phen) are the only ligands that confer activity. The aliphatic bidentate amine ligand (en), monodentate ammine (NH_3), and the high ligand field strength, monodentate ligand (CN^-) all lack biological activity. This lack of activity can be correlated with their electrochemical behavior which is markedly different from that of the mutagenic complexes. The ability of the bipyridyl and phenanthroline ligands to confer a degree of reversibility and positive shifts of the redox couples could allow these complexes to serve as cyclical electron donors in a Fenton-like reaction for the generation of oxygen radicals. The positive shifts of the Cr(III)/Cr(II) redox couple in the bipyridyl and phenanthroline complexes may shift the reduction potentials into the range of cellular reductants such as NADH, glutathione, and ascorbate. These cellular reductants are readily available in the cell in millimolar concentrations and have reduction potentials ranging from –0.17 to –0.92 versus the standard hydrogen electrode (SHE). The first reduction of the dichloro and tris-bipyridyl complexes results in the formation of the diaquo species which at intracellular pH would form the dihydroxy complex due to the acid dissociation constants. Cyclic voltammetry of the dihydroxy complexes demonstrates a degree of reversibility comparable to their parent compounds. Thus, the dihydroxy

analogs of the mutagenic Cr(III) complexes are likely to be the ultimate form of these complexes which cause DNA damage. This does not, however, preclude the formation of μ-hydroxy-bridged dimers of these complexes although the low concentration and neutral conditions present in the cell make this unlikely. Conversely, the nonmutagenic complexes display classical irreversibility of the redox couple or, like the ligands themselves, fail to show any redox reactions at these potentials. This irreversibility coupled with more negative reduction potentials would serve to prohibit a Fenton-like mechanism of oxygen radical generation.

The mechanism by which Cr(III) complexes may damage DNA is further shown using a plasmid relaxation assay. The mutagenic complexes all demonstrate conformational changes in supercoiled DNA to the relaxed state. To generate the relaxed state, a cleavage of the phosphodiester backbone must take place. This cleavage we believe is due to an oxygen radical intermediate (most likely the hydroxyl radical) abstracting a proton from the C-4 position of the deoxyribose sugar, leading to ring collapse and subsequent cleavage of the phosphodiester backbone. The hydroxyl radical is also capable of inducing mutations by electrophilic addition to DNA bases. The fact that removal of the reductant in this assay leads to no relaxation suggests that intracellular reductants must play a major role in the production of oxygen radicals with Cr(III) complexes.

Similar relationships between ligand type, electrochemical behavior, and activity toward DNA have been demonstrated with Ni(II) complexes (Chen et al., 1991). The work of Chen et al. describes a similar ligand-directed dependence upon the activity toward DNA with electrochemical behavior.

A principle issue not addressed in this paper is the mode of interaction of the Cr(III) complexes with DNA. The high reactivity of the hydroxyl radical would prohibit direct DNA damage unless it was generated at or very near the DNA itself. This spatial centering may occur by several methods such as purely electrostatic attraction or intercalation as seen with similar ligand complexes of ruthenium (Barton et al., 1984; Barton et al., 1986). As well, the interaction of the metal with DNA has the potential to alter the electrochemical behavior of the chromium complex itself. These factors need to be considered before a complete assessment of the ability for a metal complex to form oxygen radicals and damage DNA can be made.

Conclusions. The activity of Cr(III) complexes is dependent on the associated ligands. Bidentate aromatic amine ligands have the ability to alter the electrochemical behavior of chromium to generate a redox-active center. This redox-active center has the potential to generate oxygen radicals in a Fenton-like reaction. The oxygen radicals formed by this cyclical electron donation have shown an ability to relax supercoiled plasmid DNA. It is possible that these mutagenic Cr(III) complexes mimic those compounds formed from the intracellular reduction of Cr(VI). These results also suggest that risk assessment based solely on one or two metal–ligand complexes does not guarantee that all complexes of that metal will be biologically analogous.

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