Nucleic Acids Research, 1999, Vol. 27, No. 10 **2219–2226**

Oxidative damage of DNA by chromium(V) complexes: relative importance of base versus sugar oxidation

Rathindra N. Bose*, Shadi Moghaddas, Paula A. Mazzer, Lea P. Dudones, Lamis Joudah and Diane Stroup[1]

Department of Chemistry, Kent State University, Kent, OH 44242, USA and [1]Department of Biochemistry, Northeast Ohio Universities College of Medicine, Rootstown, OH 44272, USA

Received December 5, 1998; Revised March 17, 1999; Accepted March 26, 1999

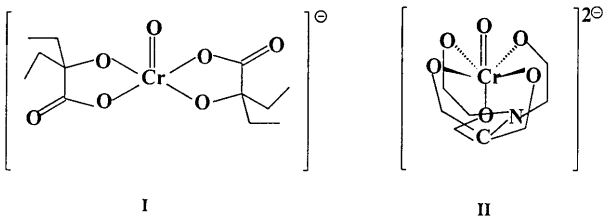
ABSTRACT

Chromium(V)-mediated oxidative damage of deoxyribonucleic acids was investigated at neutral pH in aqueous solution by utilizing bis(2-ethyl-2-hydroxybutanato)oxochromate(V) (I) and bis(hydroxyethyl)amino-tris(hydroxymethyl)methane)oxochromate(V) (II). Single-stranded and double-stranded (ds) calf thymus and human placenta DNA, as well as two oligomers, 5′-GATCTAGTAGGAGGACAAATAGTGTTTG-3′ and 5′-GATCCAAGCAAACACTATTTGTCCTCCTACTA-3′, were reacted with the chromium(V) complexes. Most products were separated and characterized by chromatographic and spectroscopic methods. Polyacrylamide gel electrophoresis experiments reveal more damage at G sites in comparison to other bases. Three primary oxidation products, 5-methylene-2-furanone (5-MF), furfural and 8-oxo-2′-deoxyguanosine, were characterized. A minor product, which appears to be thymine propenal, was also observed. The dsDNA produces more furfural than furanone. The formation of these two products resulted from hydrogen abstraction or hydride transfer from C1′ and C5′ positions of the ribose to the oxo-chromium(V) center. Since no enhancements of these products (except propenal) were observed in the presence of oxygen, mechanisms pertaining to the participation of activated oxygen species may be ruled out. The oxidation of the G base is most likely associated with an oxygen atom transfer from the oxo-metallates to the double bond between C8 and N7 of the purine ring. The formation of the propenal may be associated with an oxygen-activated species, since a marginal enhancement of this product was observed in the presence of oxygen. The formation of furfural in higher abundance over 5-MF for dsDNA was attributed to the ease of hydrogen abstraction (or hydride transfer) from the C5′ compared to C1′ position of the ribose within a Cr(V)–DNA intermediate in which the metal center is bound to the phosphate diester moiety.

INTRODUCTION

Chromium(V) and -(IV) complexes are believed to be the ultimate carcinogens in chromium(VI)-induced carcinogenesis (1–5). These two atypical oxidation states are formed by reductions with cellular reducing agents (6–14). Recently, we have shown that single-stranded (ss) DNA is oxidized by two chromium(V) complexes (15), bis(2-ethyl-2-hydroxy- butanato)-oxochromate(V) (**I**) and bis(hydroxyethyl)amino-tris(hydroxymethyl)methane)oxochromate(V) [Cr(V)-BT; BT = bis(hydroxyethyl)amino tris(hydroxymethyl)methane] (**II**) (Scheme 1). These two complexes were used as a model to gain mechanistic insight into the DNA oxidations. In particular, we have shown that one of the major pathways of oxidation is through the coordination of the phosphate diester moiety followed by hydrogen abstraction (or hydride transfer) from the C1′ position of the ribose (15). Polyacrylamide gel electrophoresis experiments with a 32-oligomer revealed that >60% of cleavage took place at the G bases, even though the 32mer contained only 29% of the G base. The amount of released guanine estimated from the HPLC experiments appeared to be somewhat lower (<50%) than that predicted from the gel electrophoresis based on the cleavage at G sites. Our experiments were not designed to measure the guanine oxidation products. We have now carried out new experiments to assess other oxidation products. An understanding of various pathways of oxidation is important since we have yet to evaluate factors that control the reactivity and selectivity (sites of DNA oxidation) of this redox process. For example, structural variations of chromium(V) complexes and the nature of the coordinated ligands might dictate their selectivity and reactivity. It is, therefore, conceivable that product distributions resulting from oxidations at different sites may be varied for different chromium(V) compounds. Furthermore, chromium(V)-mediated oxidation products from double-stranded (ds) DNA should be compared with those obtained from ss analogs for understanding mechanistic variations with nucleic acid structures. Finally, a variety of sequences of oligonucleotides need to be examined for establishing base or sequence specificity toward the DNA cleavage. The present study is primarily aimed toward gaining insight into the extent of base oxidation compared to that of the sugar moiety, to recognize base specificity by selecting different sequences and to compare the sugar oxidation products from dsDNA with that from the ss counterpart.

*To whom correspondence should be addressed. Tel: +1 330 672 2032; Fax: +1 330 672 3816; Email: rbose@platinum.kent.edu



I II

Scheme 1. Structures of [Cr(V)(HEBA)$_2^-$; **I**] and [Cr(V)-BT; **II**] complexes.

MATERIALS AND METHODS

Reagents

Chromium(V) complexes, bis(2-ethyl-2-hydroxy-butanato)oxochromate(V) [Cr(V)(HEBA)$_2^-$; **I**] and bis(hydroxyethyl)amino-tris(hydroxymethyl)methane)oxochromate(V) [Cr(V)-BT; **II**] were synthesized following the literature methods (16–18). *Caution: Chromium(V) complexes are carcinogens and mutagens and should be handled with care*. ss and ds calf thymus and human placenta DNA were obtained from Sigma. The oligonucleotides GATCTAG-TAGGAGGACAAATAGTGTTTGCTTTG-OH (oligo-I) and GA-TCCAAAGCAAACACTATTTGTCCTCCTACTA-OH (oligo-II) were synthesized by Gibco/BRL (Gaithersburg, MD). The radioactive isotope [α-^{32}P]dCTP (3000 Ci/mol) was purchased from Dupont/NEM (Boston, MA). The modified guanine base, 8-oxo-deoxyguanosine (8-oxo-dG), was purchased from ESA (Boston, MA). Nucleosides, deoxymononucleotides, nuclease P1 and alkaline phosphatase were purchased from Sigma. Organic solvents used for HPLC separations were of highest purity. Dimethyl sulfate was obtained from Acros (Geel, Belgium). Hydrazine, piperidine (Sigma) and formic acid (Fisher Scientific) were used without further purification.

Preparation of ^{32}P-labeled oligonucleotides

Synthetic ss oligomers were labeled at the 3′-end by adding [α-^{32}P]dCTP (3000 Ci/mol) with terminal deoxynucleotidyl transferase (Promega, Madison, WI) and gel purified by excising the labeled oligomer from a 15% polyacrylamide gel. Sizes of chromium cleavage products were determined by comparison to a sequencing ladder generated by chemical cleavage of the same probe by the method of Maxam and Gilbert (19).

Preparation of single- and double-stranded DNA

Solutions of calf thymus or human placenta ssDNA were prepared by heating at 75°C for 5 min followed by immediate cooling on an ice bath. The concentration was determined by using the molar extinction coefficient, 6.60×10^3 M^{-1} cm^{-1} at 260 nm. The concentration of dsDNA was also determined spectrophotometrically by utilizing a conversion factor of 1 absorbance unit to 50 μg/ml equivalent at 260 nm.

High performance liquid chromatographic separation

Chromatographic separations were done either on a Waters system equipped with a diode array detector or Varian gradient system equipped with a UV-Vis detector. Data acquisitions and retrieval on the Waters system were done by Millennium software. Separations were accomplished on a reversed-phase C-18 column (Waters, Novapak) either by an isocratic or a gradient elution technique. Usually, detections were made at 260 nm. In a typical isocratic separation, 50 mM ammonium formate or phosphate buffers (pH 4.0 or 7.0, respectively) in 90% water: 10% acetonitrile (v/v) were used as mobile phases. For gradient separation, the mobile phase consisted of 50 mM ammonium formate (pH 4.0 or 7.0; solvent A) and 100% acetonitrile or methanol (solvent B). A linear gradient from 0 to 100% B in 20 min was set up for one-step gradient. In the two-step gradient, 0–15% B was initially used for the first 15 min followed by 15–100% of the same for an additional 25 min.

Mass spectrometric measurements

Mass spectra were collected on a modified VG Autospec tandem mass spectrometer with an EBE geometry. The samples were ionized by an electron impact ionization source (70 eV).

Cleavage of synthetic oligonucleotides by chromium(V) complexes

The chromium(V) cleavage reactions were initiated by incubating Cr(O)(HEBA)$_2$ (**I**) complex (1.0 mM) with the oligonucleotides (10 000 c.p.m. of the end-labeled probes) with or without BT (30 mM) at pH 7.0 for 2 h. The final volume of the reaction mixture was adjusted to 100 μl. In some cases, EDTA (50 mM, pH 8.0) was added at the end of the reaction and incubated for an additional 30 min at room temperature. The samples were precipitated by adding 25 μl of DMS stop solution (1.5 M sodium acetate, 1.0 M β-mercaptoethanol and 100 μg yeast tRNA/ml) and 300 μl ethanol. The precipitate was collected by centrifugation. The ethanol precipitation was repeated and the cleavage was initiated by heating at 90°C in the presence of 10% piperidine for 30 min. To reduce volatile components, the samples were dried and resuspended in water twice. The pellet was dissolved in 15 μl formaldehyde loading solution (95% formamide, 20 mM EDTA, 0.05% bromophenol blue and 0.05% xylene cyanol FF). After heating, the samples were separated on an 8% (29:1 cross-linking) acrylamide sequencing gel containing 6 M urea. The bands were visualized by exposing X-ray film and were quantified with IP Lab Gel software (Signal Analytics Corp., Vienna, VA) in conjunction with Molecular Dynamics PhosphorImager 445Si.

Reactions of Cr(V) complexes with single- and double-stranded DNA

Typically, reactions between DNAs (5–10 mM) and chromium(V) complexes (1–2 mM) were carried out in BT (25–30 mM) buffer at pH 7.0 for 2–13 h at 37°C. At the end of these reaction times, EDTA (50 mM, pH 8.0) was added and the mixtures were incubated for an additional 25 min to release coordinated bases and phosphate moieties (20). The oxidation products were analyzed by two methods: acid precipitation and enzymatic hydrolysis, followed by HPLC separation. For the acid precipitation, the EDTA-treated Cr(V)–DNA reaction mixtures were treated with perchloric acid (1.0 M) for 5 min. The unreacted DNA was separated by centrifugation. The solution was immediately adjusted to pH 7.0 by Na_2CO_3 and subjected to HPLC separation. Control experiments without Cr(V) were performed in an identical manner to assess any damage without the metal center.

In another set of experiments, the Cr(V)–DNA reaction mixtures were enzymatically hydrolyzed to nucleosides by

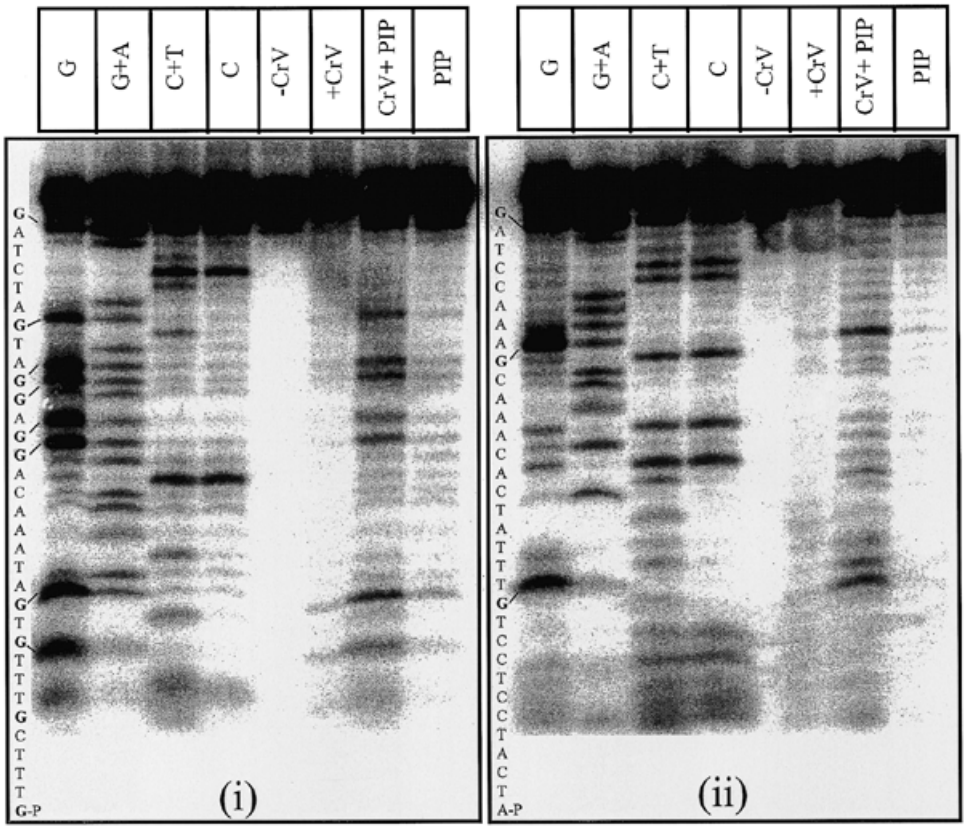


Figure 1. Electropherograms of polyacrylamide gels of two oligomers, 5′- GATCTAGTAGGAGGACAAATAGTGTTTGCTTTG-3′ and 5′-GATCCAAGCAAAC-ACTATTTGTCCTCCTACTA-3′, and the products of the reactions with Cr(V) complexes (**I** and **II**). Four lanes corresponding to Maxam–Gilbert sequence are indicated. Lanes marked with –CrV and PIP represent control experiments in the absence of Cr(V) and in the presence of piperidine, respectively. Lanes marked with +CrV and CrV+PIP represent the reactions of the oligonucleotides with Cr(V) complexes and subsequent piperidine treatment at the end of Cr(V)–DNA reactions.

nuclease P_1 (10–15 U/ml) for 30 min followed by alkaline phosphatase (20 U/ml) for an additional 30 min (21). The digested mixtures were then subjected to gradient HPLC separations utilizing mobile phases and gradients outlined earlier. Control experiments without the chromium(V) complexes were carried out in an identical manner.

The chromium(V) reactions with DNA were also carried out under nitrogen. In these experiments, individual reactants were purged with oxygen-free nitrogen for at least 15 min. Nitrogen gas was passed through Cr(II) towers to remove any oxygen present in the gas.

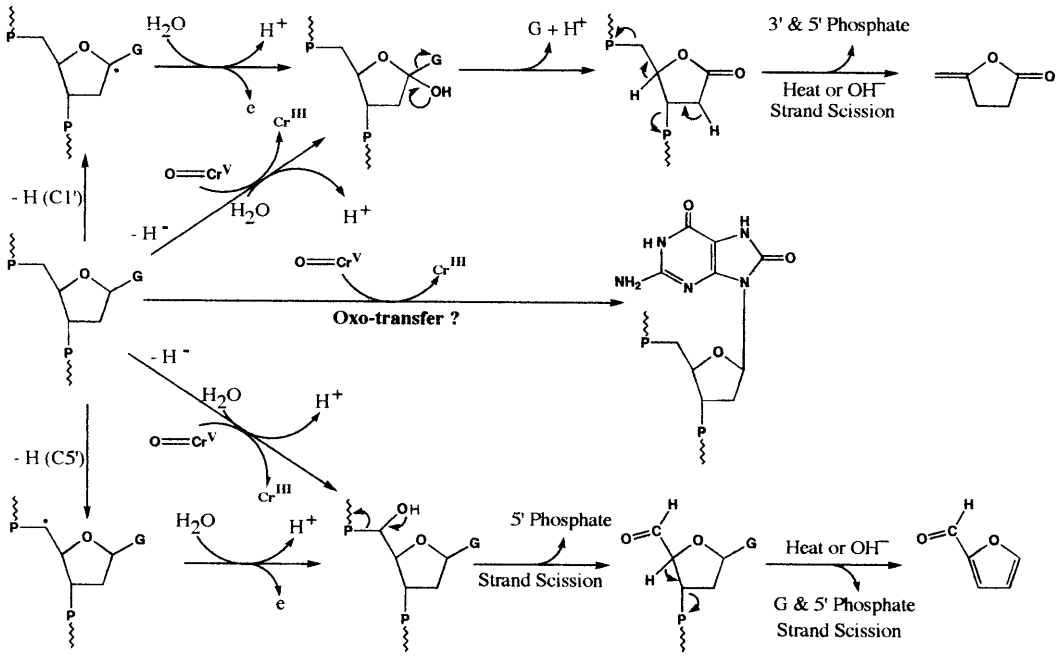
RESULTS

Figure 1 shows cleavage of two oligonucleotides, 5′-GATCTAG-TAGGAGGACAAATAGTGTTTG-3′ and 5′-GATCCAAGCA-AACACTATTTGTCCTCCTACTA-3′, by the chromium(V) complexes, **I** and **II** on polyacrylamide gels. In the absence of piperidine, the cleavage was minimal. These small cleavages were observed predominantly at G sites. The cleavages were intensified upon treatment with piperidine. Other sites of cleavage became apparent, although G sites were more prominent. For the piperidine-treated cleavage process, quantitative determinations based on the density measurements revealed that 62% of cleavage took place at G sites for the first mentioned oligonucleotide, even though the G base represents only 32% of the sequence in the 28 bases of the 33mer that have been observed in the gel. In contrast, the A base represented 36% of the sequence, yet only 21.5% of cleavage was observed at A sites. The remaining cleavages took place at C (4%) and at T (12%) bases. Note that C and T represent 8 and 24% of the bases in the 28mer. Similar cleavage patterns were observed for the other oligomer. These results confirm our earlier study (15) that the cleavage is predominantly restricted at G sites. Table 1 summarizes the cleavage of three oligonucleotides of varied sequences.

Table 1. Percent of observed cleavage[a] at individual bases in three randomly selected oligonucleotides.

Oligonucleotide	%A	%C	%G	%T
Oligo-I[b]	22 (36)	4 (12)	62 (32)	12 (24)
Oligo-II[c]	25 (36)	21 (29)	30 (11)	23 (25)
Oligo-III[d]	12 (20)	11 (20)	60 (40)	17 (20)

[a]Percent cleavage and percent composition are based on the part of the sequences observed on the polyacrylamide gels. Percent numbers may not add to 100 due to rounding.
[b]5′-GATCTAGTAGGAGGACAAATAGTGTTTGCTTTG-3′.
[c]5′-GATCCAAGCAAACACTATTTGTCCTCCTACTA-3′.
[d]5′-GATCTATGGACTTAGTTCAAGGCCGGGTAATGCTA-3′.
The numerical values in parentheses represent percent compositions of the bases in the oligonucleotides.



Scheme 2. Sugar and base oxidation products formed by chromium(V) oxidation of DNA.

In order to understand the chemical nature of the cleavage processes, the products were characterized by HPLC and mass spectrometry as described below. The chromatographic characterization was done based on matching retention times with authentic samples (often spiked with the reaction mixture) and UV spectra recorded by the diode array detector. Due to the higher concentration requirements, ss human placenta and calf thymus DNA were used rather than the oligonucleotides described above. Figure 2 shows a typical chromatogram of the reaction mixture which was subjected to exonuclease digestion at the end of the reaction. Four intense peaks resulted from the enzymatic hydrolysis of unreacted DNA, corresponding to the four nucleosides dA, dC, dG and dT. The two overlapped peaks (not shown) were identified as Cr(VI) and Cr(III) products with retention times of <2 min. The remaining peaks are DNA oxidation products and released bases. Peaks for the four bases appear right after the elution of Cr(III) and Cr(VI), except that the peak for adenine base appeared to be broad. A peak for furfural is clearly seen in the chromatogram while the peak for 5-methylene-2-furanone (5-MF) coelutes with dG. This assignment can be supported by the observation that the peak for the dG is more intense than dA although the latter base has the highest molar absorptivity at 260 nm. Furthermore, when the undamaged DNA was separated by acid precipitation and the acid soluble products were subjected to HPLC separation (without enzymatic cleavage), two distinct peaks for furfural and 5-MF were observed. The inset of Figure 2 shows the separation of furfural and 5-MF from the acid precipitation method by utilizing a different mobile phase. The third product was identified to be 8-oxo-guanosine. Two bands at 293 and 245 nm were observed for this product as reported by Kasai and Nishimura (22) which also matched with the standard sample. Another peak (F) with a matching retention time corresponding to trans-thymine propenal was also observed. We have yet to identify the product for peak E which exhibits an absorption band at 259 nm.

In the earlier report, we have shown that the reaction of Cr(V) and ssDNA predominantly afforded 5-MF which was separated from the unreacted DNA by acid precipitation of the nucleic acid. The peak for the above mentioned ketone in the HPLC chromatogram was quite broad. The assignment was supported by mass spectroscopic data for which a molecular ion at m/z = 96 and the fragmentation patterns were quite similar to those observed for an authentic sample. However, in the present work, the broad HPLC peak is now resolved into two components using a gradient separation technique and different mobile phases. Indeed, the major component is 5-MF, while the minor component is furfural, another sugar oxidized product which we had overlooked due to the overlapping nature of the chromatographic peaks. In fact, the mass spectra of the two sugar oxidation products, 5-MF and furfural, show the parent ion at m/z at 96 and almost identical fragmentation patterns. The apparent difference between the mass spectra of these two carbonyl compounds is that a new peak at m/z = 79 observed for furfural was absent for 5-MF. Sugar oxidation products from reactions between dsDNA and Cr(V) complexes were also examined after acid precipitation. Although both furfural and 5-MF were detected, the latter is no longer the dominant product in the dsDNA.

The two products, 5-MF and furfural, are formed by the oxidation of ribose initiated through C1′ and C5′ positions. These oxidation processes are accompanied by the release of bases. Therefore, the quantities of released bases determined by HPLC can be related to the extent of DNA damage through the ribose oxidations. Table 2 shows the relative percentages of the released bases during the oxidation of ssDNA. These data indicate that more G is released compared to other three bases, implying that the ribose attached to G is susceptible to oxidation. We should add

that the distribution of the various oxidation products depends on pH and the concentration of chromium employed. At higher chromium concentrations, the formation of 5-MF appears to be minimal. If the reaction mixture was left in the dark for several days at room temperature in the presence of EDTA, a substantial increase in 5-MF production was observed. This increase may be related to the slow conversion of an intermediate lactone to 5-MF without undergoing substantial decomposition, as opposed to heating the sample at 90°C which accelerated the decomposition or polymerization.

Table 2. HPLC determination of relative percentage of released bases from highly polymerized ssDNA estimated by acid precipitation

	%A	%C	%G	%T
ct-ssDNA[a,b]	15 (25)	20 (24)	50 (25)	15 (25)
hp-ssDNA[b]	10 (25)	27 (25)	38 (25)	25 (25)

[a]Almost equal percent composition of four bases was observed from HLPC chromatograms resulted from the complete digestion of the free DNA by nuclease P_1 and alkaline phosphatase. The numerical values in parentheses represent compositions of the bases in DNA[a].
[b]See ref. 15.

The formation of 2-deoxy-8-oxo-guanosine is indicative of the oxidation of the oxo-purine base. This oxidized nucleoside was detected by enzymatic cleavage with nucleases. A comparison of the extent of G and 2-oxo-dG formation may be useful to understand the relative ease of oxidation of the ribose (attached to G) and G base itself. Unfortunately, it is difficult to quantify G and modified G from the same chromatographic experiments. The difficulty of estimating both G and 8-oxo-dG in the same experiment is due to the fact that enzyme digestion is carried out at neutral pH and the solubility of guanine is extremely low so that only a portion of the released G is seen in the chromatogram. In fact, when bases were estimated through acid precipitation, the amount of G was found to be substantially higher than that observed at neutral pH. However, to a first approximation, the HPLC data on hand do support an additional ~10–15% damage at G due to the formation of 8-oxo-dG.

In an attempt to understand the nature of the various species, e.g. Cr(V) or Cr(IV) that are responsible for the formation of a variety of oxidation products, we have analyzed chromium products. For example, at neutral pH, ~80% chromium(V) disproportionates to Cr(III) and Cr(VI), while at lower pH (≤4), this reaction is <50%. On the other hand, at lower pH we did not detect the phosphato-chromium(V) intermediate by epr spectroscopy as shown in our earlier work. It appears then, that at higher pH, the oxidation, at least in part, is dominated by the phosphato intermediate. The involvement of Cr(IV) through the disproportionation reaction must be minimal. This is based on the fact that Wetterhahn and co-workers (23) have observed an insignificant amount of oxidation by the Cr(IV) intermediate.

DISCUSSION

Furfural, 5-MF and 8-oxo-dG are formed due to ribose and guanine oxidations of DNA. The first two mentioned products originate from the oxidation of ribose initiated at C1′ and C5′ positions. However, the formation of neither the oxo-species nor the 5-MF necessarily breaks the DNA backbone as indicated in

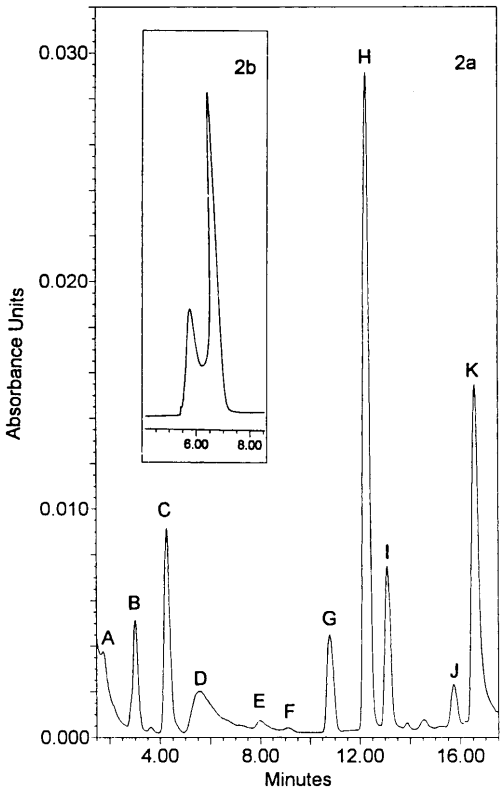


Figure 2. HPLC chromatogram of Cr(V)–DNA reaction mixture consisting of 1.0 mM of the complex I and 2.0 mM hp-ssDNA which was subjected to exonuclease cleavages at the end of the reaction. Components were separated on a C-18 column by using a gradient mobile phase consisting of ammonium formate and acetonitrile described in Materials and Methods. Peaks are detected at 260 nm and identified as follows: A, cytosine; B, guanine; C, thymine plus 2′-deoxycytidine; D, adenine; E, an unidentified product; F, thymine propenal; G, furfural; H, 2′-deoxyguanosine plus 5-MF; I, thymidine; J, 8-hydroxy-2′-deoxyguanosine; K, 2′-deoxyadenosine. (Inset) Portion of an HPLC chromatogram thus resulted after removing unreacted DNA by acid precipitation without utilizing nucleases. The two peaks are identified as furfural and 5-MF (the more intense peak). Note that the mobile phase, which is different from that in the main figure, is phosphate buffer (50 mM) and acetonitrile with a linear gradient from phosphate to 100% acetonitrile in 20 min.

Scheme 2. The formation of furfural, on the other hand, gives rise to strand scission as shown in Scheme 2. It is commonly known that the glycoside bond is substantially weakened due to the formation of a ribono-lactone, an intermediate leading to 5-MF, and the oxidized G (24–33). Treatment with piperidine generates the base labile scission due to the weakening of the glycoside bond which we have observed in our polyacrylamide gels. Besides 8-oxo-dG, two other oxidized bases, 2,2-diamiooxazolone and 2-aminoimidazolone, have been identified in the radiation damage of DNA (34). We did not observe the latter two products in our reaction. There also appears to be some controversy as to whether 8-oxo-dG would give rise to base labile scission of DNA (24). In any event, in order to understand the relative contributions from base versus sugar oxidations, independent measurements of the sugar and base oxidation products are necessary. Unfortunately, 8-oxo-dG is difficult to detect in the piperidine-treated milieu due to the limited lifetime of the oxo-species under the conditions of the treatment (24,35). Likewise, 5-MF polymerizes easily under similar conditions. The detection of this ketone was accomplished

by removing unreacted DNA through acid precipitation as discussed in the earlier paper. In order to measure the oxo-base, we have followed the procedure adopted by Barton and co-workers (21) in which the DNA oxidation products were subjected to exonuclease treatments which readily release the bases without completely decomposing the base oxidation products.

The amount of 8-oxo-dG recovered by the process described above may still be under-estimated for several reasons. First, it has been shown that the oxo-base undergoes decomposition with a half-life of ≤30 min at 90°C. Although the enzymatic cleavage requires much milder conditions, some oxo-base would certainly suffer decomposition during the digestion process (24,35). In the absence of kinetic data, activation energy of decomposition in particular, it is difficult to estimate how much oxidized base is missed. Second, the exonucleases may not recognize chromium-bound base and, therefore, skip the scission at the base coordinated sites. For example, when platinum(II) is bound to G- and A-bases of DNA, these enzymes skip the platinated sites during the cleavage process (36,37). We do not know, however, whether chromium would parallel the platinum–DNA chemistry toward the enzymatic cleavage process. Although addition of EDTA does release the base, this sequestering agent significantly inhibits the enzyme activities toward the cleavage process. Finally, when oxidations at G-base and at the C1′ position of the ribose of the same nucleoside take place, 8-oxo-guanine will be released. Such oxidations are observed in the DNA damage initiated by γ-radiation (38). In fact, an unidentified peak, E, in the HPLC chromatogram might correspond to this product.

The mechanism of formation of 8-oxo-dG by the oxochromate needs to be addressed. Usually, three mechanisms have been postulated for the formation of the oxidized base. An attack by a hydroxyl radical at the C8 position of dG followed by subsequent tautomerization leads to the formation of the product (39–41). In fact, a hydroxyl radical is shown to be involved in the formation of the oxo-base through a Fenton type reaction with Cr(V) and Cr(IV) with hydrogen peroxide (42). The second mechanism involves a [4 + 2] cycloaddition reaction of singlet oxygen to the C4–C5 and N7–C8 double bonds of guanine followed by a rearrangement from endo-peroxide to C8-hydroperoxide (43–46). The third mechanism is based on the photosensitized oxidation through guanine radical generation (47–49). As stated in Materials and Methods, the oxo-base was detected in the reaction mixture that was purged with highly purified nitrogen. Therefore, the amount of oxygen in the reaction mixture that would assist in the formation of reactive oxygenated species is minimal. Furthermore, the amount of the oxo-base, 5-MF and furfural in the nitrogen purged reaction mixture were no different from that obtained without purging. Therefore, an active oxygenated species including hydroxyl radical may not be involved in the production of oxo-guanosine. Among other possibilities, an oxo atom transfer from the chromium(V) center to the purine double bond appears to be attractive. This possibility has been implicated by Thorp and co-workers (50) in the oxo-ruthenium-mediated DNA oxidation. The fact that an oxo atom transfer from oxochromium(V) and -(IV) complexes to olefinic double bonds to form epoxides is well established (51,52). Moreover, compound **I** indeed reacts with olefins to form diols, perhaps through the oxygen atom transfer reaction (53). Therefore, an oxo transfer reaction initially producing an epoxide, followed by further rearrangements to yield the oxo-base is likely.

The HPLC chromatograms also exhibit furfural. This aldehyde is more abundant in reactions with dsDNA compared to those with ss analog. The mechanistic implications for this differential distribution must not be overlooked. For example, in the earlier paper (15), we have documented that Cr(V) initially binds the phosphate diester moiety, followed by hydrogen abstraction (or hydride transfer) from the C1′ position of the ribose. However, for a dsDNA, the hydrogen at this location is not readily accessible by the chromium(V) species due to its restricted conformation, whereas the hydrogen atom on the C5′ position of the dsDNA is quite close to the Cr(V)–phosphate bond. It is, therefore, conceivable that hydrogen abstraction or hydride transfer is predominantly initiated from this site when the penta-valent species is coordinated to the phosphate moiety of the dsDNA. Hydride transfer reactions for a number of organic oxidations by oxochromate(V) species have been reported (54,55). This selectivity appears to parallel that observed for the hydroxyl radical initiated DNA degradation in that the hydrogen abstraction from the C5′ position is much preferred over the C1′ (56). The pathways differ in that the hydroxyl radical-mediated pathway encounters substantial damage through the C4′ position (56) which we did not observe.

In addition, HPLC chromatograms also reveal the existence of two minor products. Retention times for one (peak F) closely matches that of thymine propenal. The formation of base propenals usually proceeds through the hydrogen abstraction from C4′ position of the ribose followed by an attack by O_2, and has been addressed by several authors utilizing a variety of oxidizing agents (28,57–59). In fact, a small but detectable increase in intensity for this product was observed in the presence of oxygen. We have yet to identify the second peak (E) in the chromatogram (Fig. 2). Since the intensities of these two peaks (including the propenal) were weak, we were unable to record meaningful mass spectra of these components after collecting the fractions from the HPLC column.

Reasons for higher selectivity toward the G base need to be addressed. This particular base is the most easily oxidized based on the ionization potential data (60,61). However, we have observed more sugar oxidation than that of the base itself. There appears to be a good correlation between the two two-electron oxidants, oxo-ruthenium(IV) [$Ru(tpy)(bpy)O^{2+}$] (25) and the oxo-chromium(V) complexes, in oxidizing DNA, although there are subtle differences. In the former case, oxidation through the C5′ position was not observed. Furthermore, G-oxidation appears to be more than what we have observed with the oxochromate(V) centers. In the ruthenium case, an outer sphere complex is easily formed due to the electrostatic attraction between the dipositively charged complex and the negatively charged DNA. Therefore, the ease of oxidation by the ruthenium center can be understood by analyzing the potential data of the redox partners. We have shown earlier that the Cr(V)-mediated oxidation proceeds, at least in large part, through a Cr(V)-phosphato intermediate. Although we have reported the reduction potentials of oxo-Cr(V/IV) and oxo-Cr(IV/III) redox couples (62), we do not know the reduction potential of the phosphato intermediate. It is interesting to note that Hecht and co-workers have indicated that the C1′ position of the attached to G is more reactive than others (63).

The variety of pathways of DNA damage by chromium(V) complexes described above needs to be appraised in the context of chromium(VI)-mediated carcinogenesis. Long-lived chromium(V) intermediates generated by biological reducing agents including

glutathione and ascorbic acid are considered to be putative DNA damaging agents (6–14). Although exact coordination environments of these intermediates have yet to be established, these complexes are oxo species and the reducing ligands are believed to be coordinated to the chromium(V) center. In fact, epr data did support the coordination of ascorbate ligand through the alcoholic group to oxo-chromium(V) (11,64). In this context, the coordinated carboxylate and alcoholic functionalities that we have used in our studies are quite relevant. However, there are conflicting reports regarding the nature of the chromium(V) species involved in the DNA oxidation. For example, Kortenkamp and co-workers (64–66) have observed the formation of apurinic/apyrimidinic sites (AP sites) and strand breakage in PM2 DNA during the ascorbate and glutathione reductions of chromate. These authors suggested that AP sites and strand breakage in these reactions resulted from a reactive intermediate, most likely a peroxidic species coordinated to the chromium, since the addition of catalase prevented the DNA damage. Molecular oxygen may react with hypervalent chromium centers to generate such peroxo complexes. Since we did not observe any accelerated reaction or formation of new products or more of the same products (except base propenals) in the presence of oxygen with our model chromium complexes, involvement of peroxo complexes in forming major products in our system can be ruled out. This does not mean that other chromium(V) and -(IV) complexes would not react with molecular oxygen and generate reactive peroxo species. In fact, well characterized peroxo-chromium(IV) complexes have been reported (67–69). However, Lay and Lavina (70) have demonstrated the absence of direct activation of oxygen by chromium(VI), -(V) and -(IV) complexes in the presence of oxygen and cellular reducing agents. However, our data, specifically the release of bases during the oxidation process, certainly support the formation of apurinic and apyrimidinic sites. Furthermore, we also observed the strand breakage.

Dalal and co-workers (42,71) have examined the role of hydroxyl radicals generated by Fenton type reaction between Cr(IV) and peroxide in DNA oxidation. These authors have shown that in the presence of peroxide, the oxidation exclusively takes place at the G base yielding 8-oxo-dG. These reactions are quite facile. A small cellular peroxide concentration may be sufficient to generate hydroxyl radical to cause base oxidation leading to DNA damage. Wetterhahn and co-workers (23) reported limited oxidation [<1% of Cr(V) used] of thymine-5′-di- and tri-phosphate by the $Cr(HEBA)_2$ complex. These workers (23) observed oxidation at the C4′ position of the ribose and concluded that the formation of one of the products is oxygen dependent. Since the chromium(V) complex undergoes facile disproportionation reaction through the intermediate Cr(IV) oxidation state, these authors postulated that chromium(IV) species were mainly involved in the oxidation process. Since we have retarded the disproportionation in our reaction by binding with BT, it is most likely that we are observing primary reactions between DNA and oxo-chromium(V) complex. The above argument does not imply that Cr(IV) is excluded from the process since the tetra-valent oxidation state will be encountered in the reduction of Cr(V) to Cr(III).

In conclusion, we have shown that ss- and dsDNA are oxidized by oxo-chromium(V) complexes without the involvement of any activated oxygenated species. For both the DNAs, 8-oxo-guanosine is one of the oxidation products, and this oxo-species may be formed by a direct atom transfer reaction. In addition, hydrogen atom abstraction or hydride transfer from the C5′ position leads to the formation of furfural. In both cases, other minor oxidation products, including thymine propenal, were also formed in both cases. There is, however, some difference between the ss and ds deoxynucleic acids. For the ssDNA, an oxidation at the C1′ position of the ribose is more significant than C5′. For the dsDNA, the C5′ position appears to be equally or more susceptible to oxidation. This difference may be attributed to the accessibility of the hydrogen atoms at the above two sites by the oxo-metalates due to the conformational differences of the nucleic acids.

ACKNOWLEDGEMENTS

We thank Professor Chrys Wesdemiotis of Akron University for allowing us to utilize their mass spectrometry facility and assisting with the experiments. Funding of this research by the National Institutes of Health (CA67297 to R.N.B. and HL01956 to D.S.) is highly appreciated. The instrumentation facility was instituted through a grant to R.N.B. from the Ohio Board of Regents.

REFERENCES

1 Sugiyama,M., Wang,X.W. and Costa,M. (1986) *Cancer Res.*, **14**, 4547–4551.
2 DeFlora,S., Bagnasco,M., Serra,D. and Zanacchi,P. (1990) *Mutat. Res.*, **238**, 99–172.
3 Cohen,M.D., Kargacin,B., Klein,C.B. and Costa,M. (1993) *Crit. Rev. Toxicol.*, **23**, 255–281.
4 Leonard,A. and Norseth,T. (1975) *Br. J. Ind. Med.*, **32**, 62–65.
5 Enterline,P.E. (1974) *J. Occup. Med.*, **16**, 523–526.
6 Bose,R.N., Moghaddas,S. and Gelerinter,E. (1992) *Inorg. Chem.*, **31**, 1987–1994.
7 Shi,X. and Dalal,N.S. (1988) *Biochem. Biophys. Res. Commun.*, **156**, 137–142.
8 Standeven,A.M. and Werrerhahn,K.E. (1991) *Pharmacol. Toxicol.*, **68**, 449–476.
9 Goodgame,D.M.L. and Joy,A.M. (1986) *J. Inorg. Biochem.*, **26**, 219–224.
10 O'Brien,P. and Ozolins,Z. (1989) *Inorg. Chim. Acta.*, **161**, 261–266.
11 Stearns,D.M. and Wetterhahn,K.E. (1983) *Struct. Bonding*, **54**, 93–124.
12 Goodgame,D.M.L. and Joy,A.M. (1987) *Inorg. Chim. Acta.*, **135**, 115–118.
13 Connett,P.H. and Wetterhahn,K.E. (1983) *Struct. Bonding*, **54**, 93–124.
14 Shi,X. and Dalal,N.S. (1989) *Biochem. Biophys. Res. Commun.*, **163**, 627–634.
15 Bose,R.N., Fonkeng,B.S., Moghaddas,S.M. and Stroup,D. (1998) *Nucleic Acids Res.*, **26**, 1588–1596.
16 Krumpolc,M. and Rocek,J. (1979) *J. Am. Chem. Soc.*, **101**, 3206–3209.
17 Fonkeng,B.S., Gelerinter,E. and Bose,R.N. (1995) *J. Chem. Soc. Dalton*, 4129–4130.
18 Fonkeng,B.S., Moghaddas,S. and Bose,R.N. (1998) *J. Inorg. Biochem.*, **72**, 163–171
19 Maxam,A.M. and Gilbert,W. (1980) *Methods Enzymol.*, **65**, 499–569.
20 Easom,K.A. and Bose,R.N. (1988) *Inorg. Chem.*, **27**, 2331–2334.
21 Arkin,M.R., Stemp,E.D., Puiver,S.C. and Barton,J.K. (1997) *Chem. Biol.*, **4**, 389–400.
22 Kasai,H. and Nishimura,S. (1984) *Nucleic Acids Res.*, **12**, 2137–2145.
23 Sugden,K.D. and Wetterhahn,K.E. (1996) *J. Am. Chem. Soc.*, **118**, 10811–10818.
24 Cullis,P.H., Malone,M.E. and Merson-Davis,L.A. (1996) *J. Am. Chem. Soc.*, **118**, 2775–2781.
25 Cheng,C.-C., Goll,J.G., Neyhart,G.A., Welch,T.W., Singh,P. and Thorp,H.H. (1995) *J. Am. Chem. Soc.*, **117**, 2970–2980.
26 Chen,X.Y., Burrows,C.J. and Rokita,S.E. (1992) *J. Am. Chem. Soc.*, **114**, 322–325.
27 Chen,X.Y., Burrows,C.J. and Rokita,S.E. (1991) *J. Am. Chem. Soc.*, **113**, 5884–5886.
28 Pratviel,G., Bernadou,J. and Meunier,B. (1995) *Angew Chem. Int. Edn English*, **34**, 746–769.
29 Pratviel,G., Pitie,M. and Bernadou,J. (1991) *Nucleic Acids Res.*, **19**, 6283–6288.

30 Gasmi,G., Pasdeloup,M., Pratviel,G., Pitie,M., Barnadous,J. and Meunier,B. (1991) *Nucleic Acids Res.*, **19**, 2835–2839.
31 Sigman,D.S. (1986) *Acc. Chem. Res.*, **19**, 180–186.
32 Sugiyama,H., Tsutsumi,Y., Fujimoto,K. and Saito,I. (1993) *J. Am. Chem. Soc.*, **115**, 4443–4448.
33 Duff,R.J., de Vroom,E., Geluk,A., Hecht,S.M., van der Marel,G.A. and van Boom,J.H. (1993) *J. Am. Chem. Soc.*, **115**, 3350.
34 Kino,K., Saito,I. and Sugiyama,H. (1998) *J. Am. Chem. Soc.*, **120**, 7373–7374.
35 Chung,M.-H., Kiyosawa,H., Ohtsuka,E., Nishimura,S. and Kasai,H. (1992) *Biochem. Biophys. Res. Commun.*, **88**, 1–7.
36 Eastman,A. (1986) *Biochemistry*, **25**, 3912–3915.
37 Blommaert,F.A., van Dijk-Knijnenburg,H.C.M., Dijt,F.J., den Engelse,L., Baan,R.A., Berends,F. and Fichtinger-Schepman,A.M.J. (1995) *Biochemistry*, **34**, 8474–8480.
38 Doddridge,Z.A., Cullis,P.M., Jones,J.D.D. and Malone,M.E. (1998) *J. Am. Chem. Soc.*, **120**, 10998–10999.
39 Steenken,S. (1989) *Chem. Rev.*, **89**, 503–520.
40 Dizdaroglu,M. (1991) *Free Radical Biol. Med.*, **10**, 225–242.
41 Floyd,R.A., Watson,J.J., Wong,P.K., Altmiller,D.H. and Rickard,R.C. (1986) *Free Radical Res. Commun.*, **1**, 163–172.
42 Luo,H., Lu,Y.D., Shi,X.L., Mao,Y. and Dalal,N.S. (1996) *Annals Clin. Lab. Sci.*, **26**, 185–191.
43 Devasagayam,T.P.A., Steenken,S., Obendorf,M.S.W., Schulz,W.A. and Sies,H. (1991) *Biochemistry*, **30**, 6283–6289.
44 Epe,B. (1991) *Chem.-Biol. Interact.*, **80**, 239–260.
45 Sheu,C. and Foote,C.S. (1993) *J. Am. Chem. Soc.*, **115**, 10446–10447.
46 Sheu,C. and Foote,C.S. (1995) *J. Am. Chem. Soc.*, **117**, 6439–6442.
47 Cadet,J., Berger,M., Decarroz,C., Wagner,J.R., van Lier,J.E., Ginot,Y.M. and Vigny,P. (1986) *Biochimie*, **68**, 813–834.
48 Kasai,H., Yamaizuni,Z., Berger,M. and Cadet,J. (1992) *J. Am. Chem. Soc.*, **114**, 9692–9694.
49 Stemp,E.D.A., Arkin,M.R. and Barton,J.K. (1997) *J. Am. Chem. Soc.*, **119**, 2921–2925.
50 Neyhart,G.A., Cheng,C.-C. and Thorp,H.H. (1995) *J. Am. Chem. Soc.*, **117**, 1463–1471.
51 Garrison,J.M. and Bruice,T.C. (1989) *J. Am. Chem. Soc.*, **111**, 191–198.
52 Samsel,E.G., Srinivasan,K. and Kochi,J.K. (1985) *J. Am. Chem. Soc.*, **107**, 7606–7617.
53 Bose,R.N. and Gould,E.S. (1989) ACS National Meeting, Dallas, TX, Abst. No. 265.
54 Scott,S.L., Bakac,A. and Espenson,J.H. (1992) *J. Am. Chem. Soc.*, **114**, 4205–4213.
55 Al-Ajlouni,A., Bakac,A. and Espenson,J.H. (1994) *Inorg. Chem.*, **33**, 1014.
56 Pogozelski,W.K. and Tullius,T.D. (1998) *Chem. Rev.*, **98** 1089–1107.
57 Hecht,S.M. (1986) *Acc. Chem. Res.*, **19**, 383–391.
58 Natarajan,A. and Hecht,S.M. (1994) In Neidle,S. and Waring,M.J. (eds), *Molecular Aspects of Anticancer Drug-DNA Interactions*. MacMillan Press, London, UK, Vol. 2, pp. 197–242.
59 Ajmera,S., Wu,J.C., Worth,L., Rabow,L.E., Stubbe,J. and Kozarich,J.W. (1986) *Biochemistry*, **25**, 6586–6592.
60 Seidel,C.A.M., Schulz,A. and Sauer,H.M. (1996) *J. Phys. Chem.*, **100**, 5541–5553.
61 Steenken,S. and Javanovic,S.V. (1997) *J. Am. Chem. Soc.*, **119**, 617–618.
62 Bose,R.N., Fonkeng,B.S., Barr-David,G., Farrell,R.P., Judd,R.J., Lay,P.A. and Sangster,D.F. (1996) *J. Am. Chem. Soc.*, **118**, 7139–7144.
63 Kane,S.A., Sasaki,H. and Hecht,S.M. (1995) *J. Am. Chem. Soc.*, **117**, 9107–9118.
64 Stearns,D.M., Kennedy,L.J., Courtney,K.D., Giangrande,P.H., Phieffer,L.S. and Wetterhahn,K.E. (1995) *Biochemistry*, **34**, 910–919.
65 Kortenkamp,A., Casadevall,M. and CaCruz Fresco,P. (1996) *Ann. Clin. Lab. Sci.*, **26**, 160–175.
66 Kortenkamp,A., Casadevall,M., Faux,S.P., Jenner,A., Shayer,R.O.J., Woodbridge,N. and O'Brien,P. (1996) *Arch. Bichem. Biophys.*, **329**, 199–207.
67 House,D.A. and Garner,C.S. (1965) *Nature*, **208**, 776.
68 House,D.A., Hughes,R.G. and Garner,C.S. (1966) *Inorg. Chem.*, **6**, 1077–1082.
69 Dickman,M.H. and Pope,M.T. (1994) *Chem. Rev.*, **94**, 569–584.
70 Lay,P.A. and Levina,A. (1998) *J. Am. Chem. Soc.*, **120**, 6704–6714.
71 Luo,H., Lu,Y., Mao,Y., Shi,X. and Dalal,N.S. (1996) *J. Inorg. Biochem.*, **64**, 25–35.