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# Recognition and Incision of Cr(III) Ligand-Conjugated DNA Adducts by the Nucleotide Excision Repair Proteins UvrABC: Importance of the Cr(III)–Purine Moiety in the Enzymatic Reaction

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## Abstract

Hexavalent chromium [Cr(VI)] is an ubiquitous environmental contaminant and a well-known etiological agent of human lung cancer. Inside human cells, Cr(VI) is reduced to Cr(III), which can conjugate with amino acids, ascorbic acids, and glutathiones in the cytoplasm. Conjugated and unconjugated Cr(III) can enter the nucleus to form adducts with DNA and electrostatically interact with the phosphate group of DNA. It has been found that in both human and *Escherichia coli* systems, Cr(III) ligand-conjugated DNA ternary adducts are efficiently repaired by the nucleotide excision repair (NER) pathway. In contrast, DNA adducts formed by unconjugated Cr(III) with DNA are repaired significantly less efficiently by the NER system. These results raise the possibility that the NER system repairs Cr(III) ligand-conjugated DNA adducts and biadducts such as Cr(III)–guanine–phosphate adducts but not Cr(III)–phosphate adducts. To test this hypothesis, we determined the cutting efficiency and the mode of cutting of DNA modified with tannin-conjugated Cr(III) by the *E. coli* NER enzymes UvrABC. Tannin compounds, gallic acid (GA), and ethyl gallate (EGA) can reduce Cr(VI) to Cr(III) to form Cr(III)–$GA_2$ and Cr(III)–$EGA_2$, respectively, which can interact with a single guanine or adenine base but not with the DNA phosphate backbone. We found that UvrABC is able to incise Cr(III)–$GA_2$- and Cr(III)–$EGA_2$-modified plasmid DNA, and the amount of incision increased as a function of tannin concentration used for modifications. In contrast, UvrABC nuclease does not incise GA- and EGA-modified plasmid DNA. Mapping the sequence specificity of Cr(III)–$GA_2$– and Cr(III)–$EGA_2$–DNA formation in the human *p53* gene sequence by UvrABC nuclease cutting, we found that the sequence specificity for both adducts is the same but is much more selective than Cr(III)–guanine–DNA adducts. Together, these results suggest that NER proteins from *E. coli* recognize the purine–Cr(III) adduct but not the Cr(III)–backbone phosphate complex.

## Introduction

Chromium (Cr) compounds are ubiquitous environmental contaminants, and chromates are known human and animal carcinogens (1). The major valences of Cr that exist naturally are VI and III. While Cr(III) compounds are not generally active in cellular assays because of the inability of Cr(III)–aqua complexes to cross cell membranes (2), Cr(VI), under physiological conditions, forms a chromate anion and can be taken in by cellular anion transport mechanisms (3,4). Inside cells, Cr(VI) is reduced to Cr(III) by intracellular reducing agents, such as amino acids, ascorbic acids, and glutathiones (5). Both ligand-conjugated and free Cr(III) can form DNA adducts, mainly with guanine residues, and to a much less extent with adenine residues; Cr(III) can also react with the phosphate group of the DNA backbone, presumably through

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electrostatic interactions (6,7). For the sake of simplicity, we call the former as DNA adducts the latter as complex.

Previously, we found that *Escherichia coli*[1] nucleotide excision repair (NER) enzymes, UvrA, UvrB, and UvrC, working in concert (termed UvrABC), are able to incise both DNA modified with histidine (His)-conjugated Cr(III) and free form Cr(III) with the same sequence specificity but incise DNA modified with the former much efficiently than the latter (8). On the basis of these results, we hypothesized that NER enzymes recognize ternary Cr(III)–DNA adducts such as Cr(III) ligand–guanine–DNA adducts and biadducts such as Cr(III)–guanine–phosphate adducts but not Cr(III)–phosphate complexes (8). Consistent with our hypothesis is the recent finding from Zhitkovich's laboratory that recovery of transfected Cr(III)–cysteine-modified plasmids is much lower than that of transfected Cr(III)-modified plasmids in NER-deficient human XPA cells, a difference that is not observed in NER-proficient cells (9).

It has been well-established that NER is the major mechanism for repair of bulky DNA damage in both human and *E. coli* cells (10,11). In *E. coli* cells, only three proteins (UvrA, UvrB, and UvrC) are needed to recognize and incise bulky DNA damage, while in human cells, six factors with 14 different proteins are needed to perform the same initial incision step (12). Yet, both NER systems have very similar, if not identical, substrate specificity (12). To further test our hypothesis that ternary Cr(III)–ligand–DNA adducts and biadducts, such as Cr(III)–guanine–phosphate adducts, are the NER substrates, we determined the UvrABC incision toward DNA modified with Cr(III)–$(gallate)_2$ [Cr(III)–$GA_2$] and Cr(III)–$(ethyl\ gallate)_2$ [Cr(III)–$EGA_2$] in comparison with DNA modified with gallate and EGA. Previously, we found that Cr(III)–$GA_2$ and Cr(III)–$EGA_2$ bind DNA between the Cr(III) and the DNA bases guanine and adenine but not backbone phosphates (13). We found that the Cr(III)–guanine adduct is important but neither Cr(III)–phosphate complex nor tannin ligands are essential for UvrABC activity. These results suggest that recognition of the conformational changes induced by the Cr(III)–guanine adduct and/or the adduct itself by NER factors is essential for repair of Cr–DNA adducts.

## Materials and Methods

### Materials

$K_2CrO_4$ and $CrCl_3\ 6H_2O$ was purchased from Sigma (St. Louis, MO). $CrCl_3$ solutions were freshly prepared before each experiment. Gallic acid (GA) and EGA were obtained from Fluka Chemie (Buchs, Switzerland). [$\gamma^{32}$P]ATP (3000 Ci/mmol) was purchased from MEN (Boston, MA). T4 polynucleotide kinase and Taq DNA polymerase were obtained from Promega (Madison, MI). Primers were synthesized by the Midland Certified Reagent Co. (Midland, TX). The plasmid DNA used was double-stranded pGEM-zf11(+)-APRT (adenine phosphoribosyltransferase) [pGEM-APRT] constructed by inserting a 3.9 Kb *Bam*HI fragment containing the Chinese hamster ovary APRT gene into the pGEM-zf11(+) vector purchased from Promega (14).

### Synthesis of Cr(III)–$(Gallate)_2$, [Cr(III)–$GA_2$], and Cr(III)–$(EGA)_2$

The mixtures containing potassium chromate (15 mM) and either GA (15 mM) or EGA (15 mM) in a total volume of 5 mL were incubated at 20 °C for 24 h, and the tannin complexes were purified by a Sephadex G-25 column (13). Elemental analysis for the tannin complexes and fast atom bombardment mass spectrometry (FABMS) and Fourier transform infrared (FTIR) analyses indicate that the Cr(III) cation is six-coordinated with an octahedral geometry

[1]Abbreviations: A, adenine; APRT, adenine phosphoribosyltransferase; C, cytosine; *E. coli*, *Escherichia coli*; EGA, ethyl gallate; FABMS, fast atom bombardment mass spectrometry; FTIR, Fourier transform infrared; G, guanine; GA, gallic acid; NER, nucleotide excision repair; T, thymine; TAE, tris-acetate and EDTA buffer; TBE, tris-borate and EDTA buffer; SDS, sodium dodecyl sulfate; BPB, bromophenol blue.

and is bidentately bound to two GA or EGA residues and two water molecules occupying the two axial positions as shown in Figure 1. Average yields of the Cr(III)–$GA_2$ and Cr(III)–$EGA_2$ preparations obtained from several experiments were determined to be 55% based on the chromium measurement by atomic absorption spectroscopy.

### DNA Fragment Isolation and 5′-End Labeling

Methods for preparing single 5′-end-$^{32}$P-labeled exons 5, 7, and 8 of the human *p53* gene DNA fragments were the same as previously described (15).

### Modifications of the Plasmids and the DNA Fragments with Cr(III)–$GA_2$, Cr(III)–$EGA_2$, and Cr (III)

Plasmid DNA (pGEM-APRT) or 5′-end-$^{32}$P-labeled exons 5, 7, and 8 were modified with Cr (III)–tannin complexes or Cr(III) by mixing the plasmid (2 $\mu$g) or the 5′-end-$^{32}$P-labeled exons ($2 \times 10^5$ cpm, ~20 ng) with different concentrations of the Cr(III)–$GA_2$, Cr(III)–$EGA_2$, or $CrCl_3$ (0–5.0 $\mu$M) in 150 $\mu$L of 0.01 M Mes (pH 6.3) solution. The mixtures were incubated for 30 min to 2 h at 37 °C, and the Cr(III)–tannin-modified plasmid and DNA fragments were purified with ethanol precipitation followed by phenol and ether extractions. The unreacted Cr (III) was further removed using a filter device (YM-30 Amicon), and the filter was washed twice with 500 $\mu$L of $H_2O$. The Cr(III)-modified DNA on the filter was recovered by washing with 10 $\mu$L of $H_2O$.

### Purification of UvrA, UvrB, and UvrC Proteins

The UvrA, UvrB, and UvrC proteins were isolated from the *E. coli* K12 strain CSR603, which carried plasmids *pUNC* 45 (UvrA), *pUN211* (UvrB), or *pDR3274* (UvrC) (15). The bacterial strain and plasmids were kindly provided by Dr. A. Sancar (University of North Carolina, Chapel Hill, NC). The purification procedures were the same as previously described (16,17).

### UvrABC Incision Assay for Plasmid and DNA Fragments

For plasmid DNA used as substrates, UvrABC reactions were carried out in a total volume of 50 $\mu$L containing 50 mM Tris-HCl, pH 7.5, 10 mM $MgCl_2$, 1 mM ATP, 0.1 mM DTT, 100 mM KCl, 15 nM UvrA, UvrB, and UvrC, and substrate plasmids (100 ng). The reaction mixtures were incubated at 37 °C for 60 min. It should be noted that UvrABC remains active after this reaction condition. The reaction mixtures were then added to 10 $\mu$L of 40% (w/v) sucrose containing 1% sodium dodecyl sulfate (SDS) and 0.25% bromophenol blue (BPB) and heated at 65 °C for 5 min. Aliquots of the mixtures were electrophoresed in a 1% agarose gel in tris-acetate and EDTA (TAE) buffer (40 mM Tris-acetate, pH 8.0, 1 mM EDTA) at 2.7 V/cm for 3 h. The gel was stained with 0.5 $\mu$g/mL ethidium bromide to visualize supercoiled and nicked plasmids. The relative amount of each of the two forms of plasmid DNA was determined by ChemiImager (Alpha Innotech). When $^{32}$P-labeled DNA fragments were used as substrates, UvrABC reaction conditions were the same except a higher concentration of UvrA, UvrB, and UvrC (120 nM each) was used, and at the end of reaction, DNA fragments were purified by phenol/ether extractions and ethanol precipitations. The precipitated DNA fragments were dissolved in a denaturing dye mixture containing 95% formamide, 0.25% BPB, and 0.25% xylene cyanol and heated at 90 °C for 5min. The resultant DNA were electrophoresed at 40 V/cm in an 8% (w/v) polyacrylamide sequencing gel containing 45% (w/v) urea in tris-borate and EDTA (TBE) buffer (90 mM Tris-borate, pH 8.0, 2 mM EDTA) in parallel with Maxam and Gilbert sequencing reaction products (18). After electrophoresis, the gel was dried (BioRad gel dryer) and exposed to Kodak X-Dmat RP film at −70 °C.

## Results

### UvrABC Incision of Cr(III)–$GA_2$- and Cr(III)–$EGA_2$-Modified Plasmid DNA

To determine whether the UvrABC nuclease recognizes Cr(III)–$GA_2$–DNA and Cr(III)–$EGA_2$–DNA adducts and the quantitative relationship between DNA adduct density in the plasmid and the number of UvrABC incisions, the supercoiled form of the pGEM-APRT plasmid (1.5 $\mu$g) was modified with various concentrations of the Cr(III)–tannin complexes (0–5 $\mu$M) in MES buffer. The modified supercoiled plasmid DNAs (200 ng) were incubated with 10-fold molar excess UvrABC nuclease. It is well-established that UvrABC incision of DNA adducts in plasmid DNA produces single-stranded DNA breaks. Consequently, the supercoiled DNA becomes an open circle, and these two forms of DNA can be separated by gel electrophoresis. Results in Figure 2a,b show that although nonspecific UvrABC cutting in unmodified plasmid DNA is observed, the extent of UvrABC incision in Cr(III)–tannin-modified plasmid is higher than nonspecific cutting and, more importantly, is proportional to the concentrations of Cr(III)–tannin used for modifications. The number of UvrABC incisions on the plasmid was calculated based on the Poisson distribution equation, $P(0) = e^{-n}$, where $n$ is UvrABC incision number and $P(0)$ represents the fraction of uncut plasmid DNA. Results in Figure 3a show that the number of UvrABC incisions increases proportionally to the Cr(III)–tannin concentration and reaches peaks of 0.5 and 0.4 at 1 and 2 $\mu$M Cr(III)–$GA_2$ and Cr(III)–$EGA_2$, respectively. The number of UvrABC incisions decreases in plasmid DNA modified with higher concentrations of Cr(III)–$GA_2$ and Cr(III)–$EGA_2$. The reason for this decrease of UvrABC incision is unclear; similar phenomena have been observed in DNA modified with benzo(*a*)pyrene diol epoxide, mitomycin C, and anthramycin (19–21). Presumably, more than one of these DNA lesions within a certain distance in the DNA helix may impact DNA conformation to be unrecognizable by UvrABC. The number of UvrABC incisions in Cr(III)–plasmid plateaus at 1.4 incision per plasmid (Figure 3b). The reason for the low incision number in the Cr(III)–DNA and Cr(III)–tannin–DNA adducts is unclear. It has been found that the recognition and incision of bulky DNA by UvrABC nuclease in supercoiled plasmid DNA are different from linear dependence on the structure of the DNA adducts; while UvrABC nuclease incises with equal efficiency for benzo(*a*)pyrene diol epoxide (BPDE)–DNA adducts in supercoiled DNA and linear DNA, it incises psoralen DNA adducts in supercoiled DNA poorly as compared to the same adducts in linear DNA (21, 22). Nonetheless, an incision assay using supercoiled plasmid DNA as a substrate is a sensitive and specific method for determining whether the DNA damage is a substrate for NER. It has been found that tannins are able to bind proteins, and this binding may reduce the probability of proper sequential Uvr–DNA damage complex formation and consequently reduce UvrABC cutting (23, 24).

It is well-established that tannin is able to bind to DNA (25,26). To investigate whether the GA and EGA moieties in the Cr(III)–tannin–DNA adducts are important for UvrABC recognition and incision, we determined whether UvrABC incised GA- or EGA-modified supercoiled plasmid DNA. Supercoiled form pGEM-APRT (1.5 $\mu$g) DNAs were modified with various concentrations of GA or EGA. A prior FTIR study demonstrated that hydroxyl groups in the polyphenol moiety in some flavonoids bind the DNA backbone phosphate at low concentrations through hydrogen bonding and also bind DNA bases—including guanine, adenine, and thymine—at higher concentrations through noncovalent binding (25). The GA- and EGA-modified DNA was incubated with UvrABC nuclease. The results in Figure 2c,d show that UvrABC does not cut GA- or EGA-modified DNA, indicating that the UvrABC nuclease does not recognize the GA–DNA and EGA–DNA structures.

### Sequence Specificity of Cr(III)–$GA_2$–DNA and Cr(III)–$EGA_2$–DNA Adduct Formation in the *p53* Gene

One possible reason for the low efficiency of UvrABC incision on Cr(III)–tannin-modified plasmids as compared to Cr(III)-modified plasmids is that the formation of Cr(III)–tannin–DNA adduct may be more sequence selective than the formation of the Cr(III)–DNA adduct. To test this possibility, we determined UvrABC incision sites in Cr(III)–tannin- and Cr(III)-modified exons 5, 7, and 8 of the *p53* gene fragments. Results in Figures 4a–c show that there are indeed fewer UvrABC incision bands in Cr(III)–tannin-modified DNA fragments than in Cr(III)-modified DNA fragments; while 33 UvrABC incision sites are observed in Cr(III)–DNA adducts, only 18 UvrABC incision sites were detected in the Cr(III)–tannin–DNA adducts. Furthermore, the results also show that Cr(III)–GA and Cr(III)–EGA have the same sequence specificity of DNA binding.

It is well-established that the UvrABC nuclease incises bulky damage 6–8 nucleotides 5′ to and 3–4 nucleotides 3′ to a DNA adduct (10,11,27). If the mode of nuclease incision of Cr(III)–$GA_2$–DNA and Cr(III)–$EGA_2$–DNA adducts follows the mode of incision for other bulky DNA adducts (i.e., seven nucleotides 5′ to a DNA adduct), then the Cr(III)–$GA_2$ and $EGA_2$–DNA adducts formed in exon 5 should be located at guanine and adenine residues in codons 143 (GTG), 150 (ACA), 154 (GGC), 156 (CGC), 158 (CGC), and 160 (ATG)G; in exon 7, the adducts should be at the GG sequence in codon 263 (GGA); in exon 8, the adducts should be located in codons 285 (GAG), 291 (AAG), 294 (GAG), 302 (GGG), and 311 (GGA). The positions of the Cr(III)–DNA adducts formed in exon 5 are guanine and adenine residues in codons 137 (CTG)G, 143 (GTG), 146 (TGG), 150 (ACA), 154 (GGC), 156 (CGC), 158 (CGC), and 160 (ATG)G; in exon 7, the adducts should be located in codons 246 (ATG), 248 (CGG), 249 (AGG), 258 (GAA), 261 (AGG), and 263 (GGA); and in exon 8, the adducts should be located in codons 291 (AAG), 293 (GGG), 294 (GAG), 302 (GGG), 307 (GGT), and 311 (GGA). While 24 out of 33 Cr(III)–DNA adducts occur at contiguous –GG–sequences, only 10 out of 18 Cr(III)–tannin–DNA adducts occur at these sequences. These results are consistent with the hypothesis that UvrABC recognizes ternary Cr(III)–ligand-conjugated DNA adducts and biadducts such as Cr(III)–guanine–phosphate– and Cr(III)–$(guanine)_2$–DNA adducts rather than Cr(III)–phosphate–DNA adducts because if UvrABC recognized Cr(III)–phosphate–DNA adducts, then we would expect a UvrABC incision band to occur at every base. It should be noted that although in general Cr(III)–DNA binding is less selective, Cr(III)–tannin binds at codon 285 while Cr(III) does not.

## Discussion

It is well-established that carcinogenic Cr(VI) compounds can induce multiple types of DNA lesions such as Cr(III)–ligand–guanine– and Cr(III)–guanine–phosphate–DNA adducts and Cr(III)–phosphate complex in human cells; however, the repair mechanisms for these different kinds of lesions have not yet been elucidated (28). The recent findings that NER-deficient XPA cells are much more sensitive to Cr(VI)-induced lethality and that XPA cells have a significantly lower recovery for Cr(III)–cysteine-modified plasmid than its wild-type counterpart cells strongly suggest that NER is the major mechanism for repair of ternary Cr(III)–ligand–DNA adducts (9). Intriguingly, there is very little, if any, difference between the recovery of Cr(III)-modified plasmid DNA transfected into XPA cells and NER wild-type cells (9). Because it is well-established that the Cr(III)–phosphate complex is the major lesion formed in in vitro Cr(III)–DNA modifications, these results raise the possibility that the NER system may recognize Cr(III)–ligand–DNA adducts and Cr(III)–guanine–phosphate adducts and does not recognize Cr(III)–phosphate complexes. Consistent with this possibility is our recent finding that the UvrABC nuclease, the NER enzyme complex isolated from *E. coli* cells, is able to catalyze the incision of Cr(III)–His-modified DNA adducts much more efficiently

than Cr(III)-modified DNA in a cell-free system (8). Furthermore, we have found that UvrABC incision shows great sequence specificity for Cr(III)-modified DNA and Cr(III)–His-modified DNA and that the sequence specificities for both types of modified DNA are identical (8). Together, these results lead us to hypothesize that the NER system recognizes the guanine adduct, whether it is a Cr(III)–ligand–DNA or a Cr(III)–phosphate–DNA adduct, but not Cr (III)–phosphate complexes (8).

To further test this hypothesis, we determined which moiety in Cr(III)–compounds–DNA adducts is important for UvrABC nuclease activity. Among many Cr(III) compounds, Cr(III)–$GA_2$ and Cr(III)–$EGA_2$ have been found to react with guanine and adenine residues in DNA to form Cr(III)–$GA_2$–DNA and Cr(III)–$EGA_2$–DNA adducts but do not react with backbone phosphates (13). Our results show that the UvrABC nuclease does indeed incise Cr(III)–$GA_2$ and Cr(III)–$EGA_2$–DNA adducts. We also found that the UvrABC nuclease does not incise GA- and EGA-modified plasmid DNA. These results collectively support the hypothesis that the Cr(III)–guanine and Cr(III)–adenine adducts and possibly the DNA conformational changes induced by these adducts, not the Cr(III)–phosphate or the tannin–guanine complex, are the crucial structures for UvrABC recognition and incision.

We also found the puzzling results that the average number of UvrABC incisions on a Cr(III)–tannin-modified plasmid plateaus at 0.4–0.5, while, in contrast, the average numbers of UvrABC incision on a $Cr(III)Cl_3$-modified plasmid DNA plateau at 1.4. The low UvrABC incision rate on Cr(III)–tannin-modified plasmids is not due to insufficient formation of the Cr (III)–tannin–DNA adducts since more than 10 Cr(III)–tannin–DNA adducts per plasmid DNA molecule were detected using radioactively labeled Cr(III)–tannin complexes to modify plasmid DNA (data not shown). One possibility is that the tannin moiety in the Cr(III)–tannin–guanine DNA adduct may reduce the proper sequential Uvr protein–DNA damage complex formation that leads to UvrABC incision. It is known that tannin can form complexes with proteins through hydrophobic forces (29). If UvrC or UvrB binds to the Cr(III)–tannin-guanine–DNA adduct first and the hydrophobic interactions of tannin with these two proteins prevent their dissociation from damaged DNA sites, then UvrA would not be able to bind at the damaged DNA sites and UvrABC incision would therefore not occur. Consistent with this explanation is the result shown in Figure 3 that UvrABC incises the Cr(III)–$GA_2$–modified plasmid more efficiently than the Cr(III)–$EGA_2$–modified plasmid; the latter plasmid is much more hydrophobic than the former due to an ethyl group.

Our results show that Cr(III)–tannin complexes bond almost exclusively to guanine residues and have a higher sequence selectivity than Cr(III) alone. The higher sequence selectivity of the Cr(III)–tannin complexes can be attributed to the lower reactivity of Cr(III) in Cr(III)–tannin complexes. During the process of bonding of Cr(III)–$EGA_2$ to DNA, the centered Cr (III) cation in the complexes is attacked by the guanine N7 atom, which substitutes one of two water molecules coordinated in a trans position to the Cr(III)–tannin complexes; in contrast, the Cr(III) in the Cr(III)–$GA_2$ indirectly binds the guanine N7 and O6 through water molecule (Figure 5). Coordination of the two water molecules in a cis position in the Cr(III)–tannin complexes is not plausible since the 90° bond angle of $H_2O$–Cr(III)–$H_2O$ will allow it to bind both the guanine and the nearest backbone phosphate, and our FTIR results did not show such evidence (13). Hence, we propose that Cr(III)–$GA_2$ and Cr(III)–$EGA_2$ bind DNA through bonding between the guanine and the Cr(III) cations and consequently form mono-functional DNA adducts.

In summary, we conclude that the Cr(III)–purine–base modifications, but not Cr(III)–phosphate modifications, are essential for UvrABC nuclease activity and that the hydrophobicity of tannin ligands affects UvrABC activity.

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## References

1. IARC. IARC Monographs on the Evalutaion of Carcinogeneic Risk to Humans, Chromium, Nickel and Welding. Vol. 48. IARC; Lyon, France: 1990. p. 49-508.
2. Snow ET. Metal carcinogenesis: Mechanistic implications. Pharmacol Ther 1992;53:31–65. [PubMed: 1641401]
3. Sehlmeyer U, Hechtenberg S, Klyszcz H, Beyersmann D. Accumulation of chromium in Chinese hamster V79-cells and nuclei. Arch Toxicol 1990;64:506–508. [PubMed: 2275608]
4. Alexander J, Aaseth J. Uptake of chromate in human blood cells and isolated rat liver cells: The role of anion carrier. Analyst 1995;120:931–933. [PubMed: 7741257]
5. Connett PH, Wetterhahn KE. In vitro reaction of the carcinogen chromate with cellular thiols and carboxylic acid. J Am Chem Soc 1985;107:4282–4288.
6. Costa M. Analysis of DNA-protein complexes induced by chemical carcinogens. J Cell Biochem 1990;45:127–135. [PubMed: 1702790]
7. Zhitkovich A, Voitkun V, Costa M. Glutathione and free amino acids form stable complexes with DNA following exposure of intact mammalian cells to chromate. Carcinognesis 1995;16:907–913.
8. Arakawa H, Wu F, Costa M, Rom W, Tang Ms. Sequence specificity of Cr(III)-DNA adduct formation in p53 gene: NGG sequences are preferential adduct-forming sites. Carcinogenesis 2006;27:639–645. [PubMed: 16251206]
9. Reynolds M, Peterson E, Quievryn G, Zhitkovich A. Human nucleotide excision repair efficiently removes chromium-DNA phosphate adducts and protects cells against chromate toxicity. J Biol Chem 2004;279:30419–30424. [PubMed: 15087443]
10. Sancar A. DNA excision repair. Annu Rev Biochem 1996;65:43–81. [PubMed: 8811174]
11. Sancar A, Tang Ms. Nucleotide excision repair. Photochem Photobiol 1993;57:905–921. [PubMed: 8393197]
12. Friedberg, EC.; Walker, GC.; Siede, W.; Wood, RD.; Schultz, RA.; Ellenberger, T., editors. DNA Repair and Mutagenesis. ASM Press; Washington, DC: 2006. p. 227-343.
13. Arakawa H, Watanabe N, Tajmir-riahi HA. Calf-thymus DNA interaction with Cr(III)-gallate and Cr (III)-ethyl gallate studied by FTIR spectroscopy and capillary electrophoresis. Bull Chem Soc Jpn 2001;74:1075–1082.
14. Feng Z, Hu W, Komissarova E, Pao A, Hung MC, Adair GM, Tang Ms. Transcription coupled DNA repair is genomic context-dependent. J Biol Chem 2002;277:12777–12783. [PubMed: 11821423]
15. Feng Z, Hu W, Rom WN, Beland FA, Tang Ms. N-hydroxy-4-aminobiphenyl-DNA binding in human P53 gene: Sequence preference and the effect of C5 cytosine methylation. Biochemistry 2002;41:6414–6421. [PubMed: 12009904]
16. Thomas DC, Levy M, Sancar A. Amplification and purification of UvrA, UvrB, and UvrC proteins of Escherichia coli. J Biol Chem 1985;260:9875–9883. [PubMed: 2991268]
17. Sancar A, Rupp WD. A novel repair enzyme: UVRABC excision nuclease of Escherichia coli cuts a DNA strand on both sides of the damaged region. Cell 1993;32:249–260.
18. Maxam AM, Gilbert W. Sequencing end-labeled DNA with base-specific chemical cleavages. Methods Enzymol 1980;65:499–560. [PubMed: 6246368]
19. Walter RB, Pierce J, Case R, Tang Ms. Recognition of the DNA helix stabilizing anthramycin-N2-guanine adduct by UVRABC nuclease. J Mol Biol 1988;203:939–947. [PubMed: 3210245]
20. Kohn H, Li VS, Tang Ms. Recognition of mitomycin C-DNA monoadducts by UVRABC nuclease. J Am Chem Soc 1992;114:5501–5509.
21. Tang, Ms; Pierce, JR.; Doisy, RP.; Nazimiec, ME.; MacLeod, MC. Differences and similarities in the repair of two benzo(a)pyrene diol epoxide isomers induced DNA adducts by uvrA, uvrB and uvrC gene products. Biochemistry 1992;31:8429–8436. [PubMed: 1390626]

22. Munn MM, Rupp WD. Interaction of the UvrABC endonuclease with DNA containing a psoralen monoadduct or crosslink. J Biol Chem 1991;266:24748–24756. [PubMed: 1761569]

23. Peleg H, Bodine KK, Nobel AC. The influence of acid on astringency of alum and phenolic compounds. Chem Senses 1998;23:371–378. [PubMed: 9669051]

24. Pripp AH, Vreeker R, van Dyhoven J. Binding of olive oil phenolics to food proteins. J Sci Food Agric 2005;85:354–362.

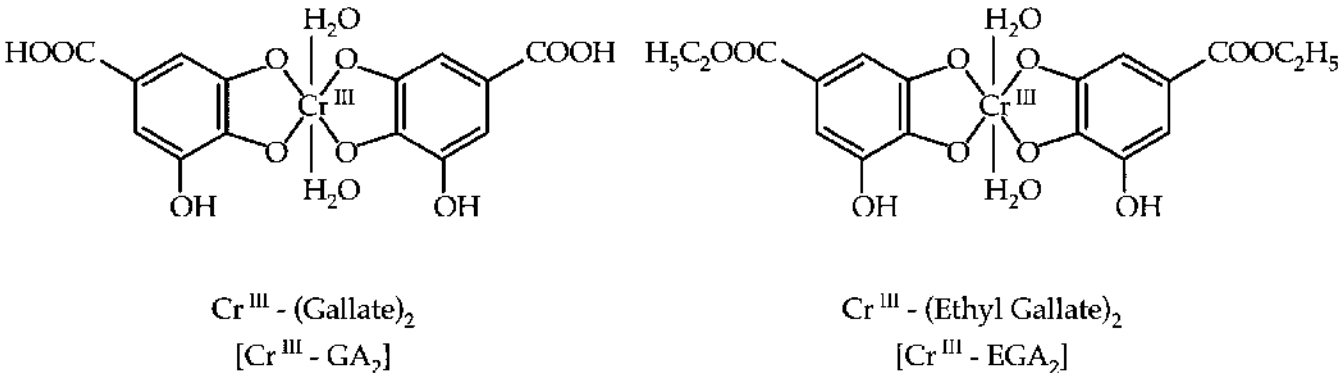
25. Kanakis CD, Tarantilis PA, Polissiou MG, Diamantoglou S, Tajmir-riahi HA. DNA interaction with naturally occurring antioxidant flavonoids quecetin, kaempherol and delphinidin. J Biomol Struct Dyn 2005;22:719–724. [PubMed: 15842176]

26. Labieniec M, Gabryelak T. Interactions of tannic acid and its derivatives (ellagic and gallic acid) with calf thymus DNA and bovine serum albumin using spectroscopic method. J Photochem Photobiol B 2006;82:72–78. [PubMed: 16263304]

27. Tang, Ms. Mapping and quantification of bulky chemicals-induced DNA damage using UvrABC nucleases. In: Pfeifer, G., editor. Technologies for Detection of DNA Damage and Mutation. Vol. Chapter 11. Plenum Press; New York: 1996. p. 139-152.

28. Zhitkovich A. Importance of chromium-DNA adducts in mutagenicity and toxicity of chromium(VI). Chem Res Toxicol 2005;18:3–11. [PubMed: 15651842]

29. Oh HI, Hoff JE, Armstrong GS, Haff LA. Hyrophobic interaction in tannin-protein complexes. J Agric Chem Food 1980;28:394–398.




$Cr^{III}$ - $(Gallate)_2$
$[Cr^{III} - GA_2]$

$Cr^{III}$ - $(Ethyl\ Gallate)_2$
$[Cr^{III} - EGA_2]$

**Figure 1.**
Chemical structures of Cr(III)–$(gallate)_2$ and Cr(III)–$(EGA)_2$.

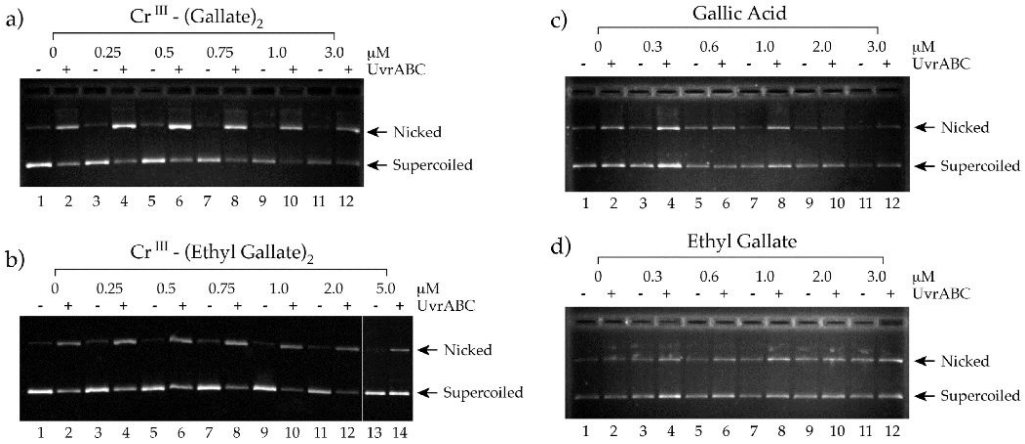



**Figure 2.**

UvrABC incisions of (a) Cr(III)–$(gallate)_2$, (b) Cr(III)–$(EGA)_2$, and (c) GA- and (d) EGA-modified plasmid DNA. Plasmid DNA (pGEM-APRT) was modified with various concentrations of Cr(III)–$(gallate)_2$, Cr(III)–$(EGA)_2$, gallate, and EGA (0–5.0 $\mu$M), and the modified plasmids were treated with UvrABC as described in the Materials and Methods. The resultant DNA was separated by 1% agarose gel electrophoresis in TAE buffer, and the gel was stained with ethidium bromide (0.5 $\mu$g/mL).

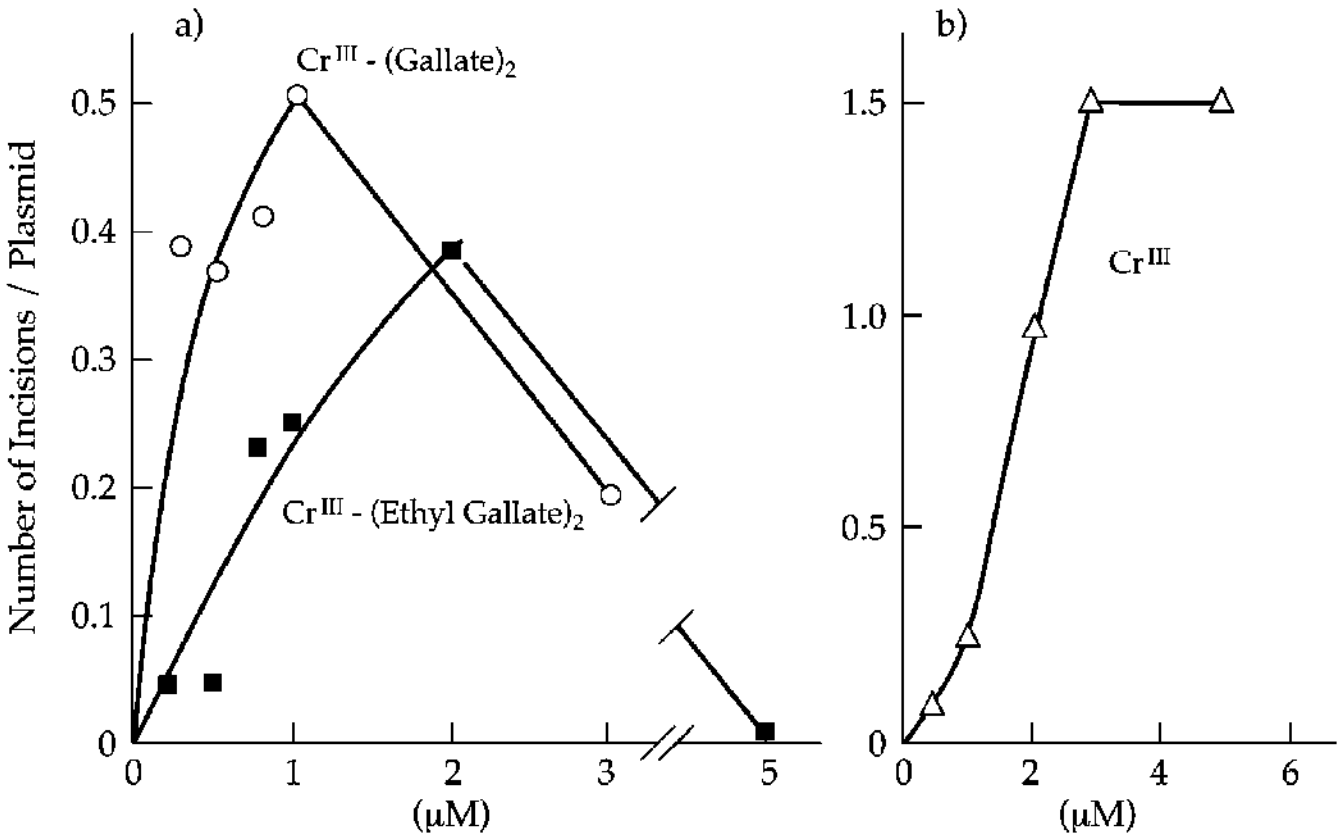



**Figure 3.**

Quantitation of UvrABC incision on Cr(III)–(gallate)$_2$-, Cr(III)–(EGA)$_2$-, and Cr(III)-modified plasmid DNA. The intensities of the supercoiled and nicked forms of the plasmid DNA in Figure 2 were scanned, and the fraction of uncut plasmid DNA, $P(0)$ = supercoiled plasmid/supercoiled + nicked plasmid, was calculated. The number of UvrABC incision sites on a plasmid was calculated based on the Poisson distribution equation, $P(0) = e^{-n}$, where $n$ = number of UvrABC incisions. The data for Cr(III)-modified DNA were cited from our previous paper (8).

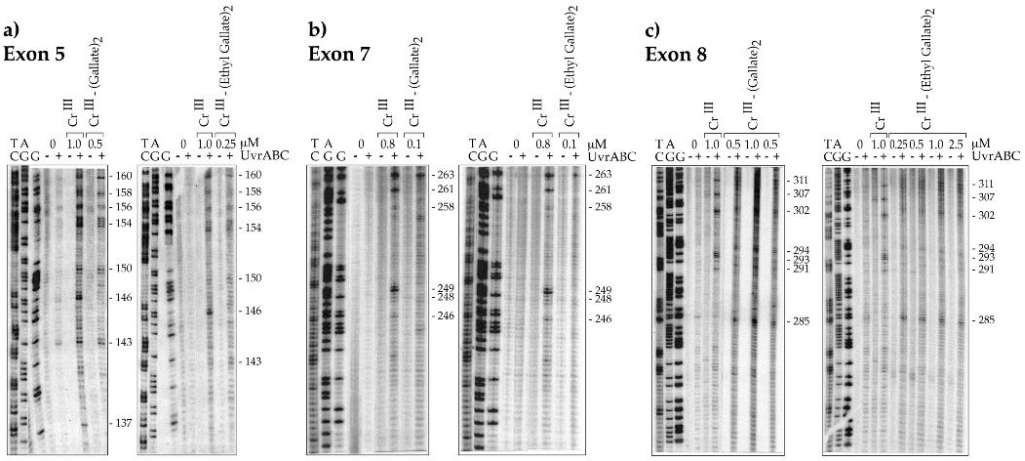



**Figure 4.**

Identification of Cr(III)–(gallate)$_2$ and Cr(III)–(EGA)$_2$ adduct formation sites in exon 5 (A), exon 7 (B), and exon 8 (C) in the *p53* gene sequence by the UvrABC incision method. Single 5′-$^{32}$P-end-labeled exon 5, 7, and 8 DNA fragments of the human *p53* gene were obtained by PCR and purified by polyacrylamide gel electrophoresis. The DNA fragments were modified with Cr(III)–(gallate)$_2$, Cr(III)–(EGA)$_2$, and $CrCl_3$ and treated with or without UvrABC, and the fragments were separated by electrophoresis in an 8% denatured polyacrylamide gel as described in the Materials and Methods. A + G, G, and T + C are Maxam–Gilbert sequencing reaction products. The numbers to the right-hand side of each UvrABC incision correspond to the *p53* gene codon number.

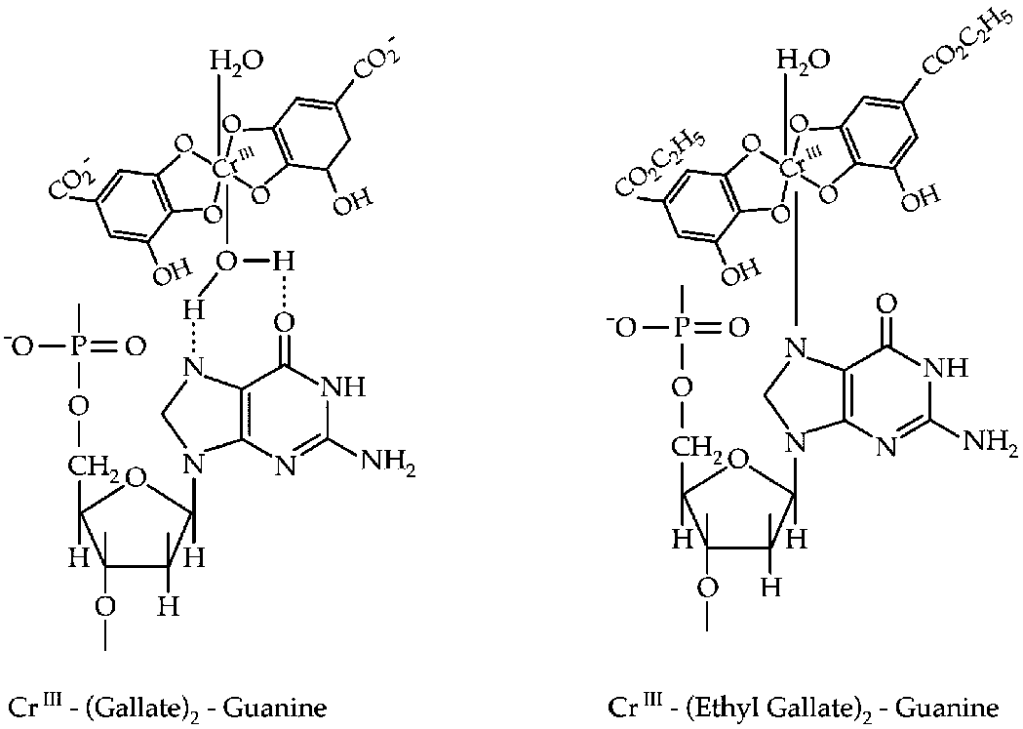



**Figure 5.**
Proposed structures of Cr(III)–(gallate)$_2$–DNA and Cr(III)–(EGA)$_2$–DNA adducts.