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EFFECT OF CHROMIUM(III) ON POLY(dG-dC) CONFORMATION

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The interaction of chromium(III) with poly(dG-dC) inhibits the B to Z transition and results in the condensation of the polymer at high Cr/nucleotide ratios. At low Cr/nucleotide ratios chromium(III) enhanced the ability of ethanol to induce the B to Z transition of poly(dG-dC). The effects of chromium(III) on the conformation of DNA may be related to the carcinogenicity of chromium compounds.

Chromium(VI) compounds have been identified as carcinogens on the basis of epidemiological studies, animal tests and mutagenicity studies in bacteria and mammalian cells (1). Chromium(VI) produced DNA cross-links in rat tissues in vivo (2) and in cultured cells in vitro (3-6). Although chromium(VI) damaged nuclear DNA in whole cells, no reaction of chromium(VI) with isolated DNA occurred in vitro at physiological pH in the absence of a metabolizing system (7,8). The "uptake-reduction" model for the carcinogenicity of chromium(VI) (9,10) proposed that intracellular reduction of chromium(VI) ultimately produces chromium(III) and results in chromium(III) complexes with DNA and other cellular macromolecules. Since chromium(III) is the ultimate form of chromium within the cell, the interaction of chromium(III) with DNA may play a critical role in the carcinogenic action of chromium(VI) compounds.

The ability of alternating purine-pyrimidine sequences of DNA to undergo conformational changes from the right-handed B form to the left-handed Z form has been implicated as a factor in the transcriptional activity of genes and, therefore, the differentiated state of cells (11).

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The action of certain carcinogens, e.g., modification of guanine by N-7 methylation or by reaction at C-8 with N-acetoxy-N-2-acetylaminofluorene, facilitated the B→Z transition of poly(dG-dC) (12-14). However, modification of poly(dG-dC) with the antitumor drug cis-diammine-dichloroplatinum(II), a known cross-linking agent, prevented the B→Z transition (15,16). Cations such as various alkali ions, alkaline earth ions, transition metal ions, lanthanide ions and polyamines were effective in inducing the B→Z transition of poly(dG-dC) and poly(dG-m^5dC) (17-21).

The ability of chromium(III) to affect the B→Z transition in appropriate polynucleotides has not been examined. The possible effects of chromium(III) on the B→Z transition are unclear since chromium(III) produces DNA cross-links *in vitro* (4), but forms cationic complexes in aqueous solutions. Since the carcinogenicity of chromium compounds may be related to the interaction of chromium(III) with DNA sequences capable of undergoing the B→Z transition and affecting gene activation, we have examined the interaction of chromium(III) with poly(dG-dC).

MATERIALS AND METHODS

Chemicals. Poly(dG-dC) was purchased from P-L Biochemicals. The concentration of DNA-P was determined from the absorbance at 255 nm, ε = 8400 $M^{-1}cm^{-1}$. $Cr(NO_3)_3 \cdot 9H_2O$ and $CrCl_3 \cdot 6H_2O$ were purchased from Fisher Scientific. The concentration of chromium was determined from the absorbance at 372 nm, ε = 4830 $M^{-1}cm^{-1}$, in 0.05 M KOH after oxidation of chromium(III) to chromium(VI) with H_2O_2 (22).

Circular Dichroism Studies. Circular dichroism spectra were recorded from 230-340 nm at 25°C using a Cary 60 spectropolarimeter with 6001 CD accessory. Freshly prepared aqueous solutions of chromium(III) were added to solutions of poly(dG-dC) in 5.0 mM Tris-HCl, pH 7.4, containing either 50 mM or 1.0 mM NaCl. "Metal-free" buffers were prepared by treatment with sodium equilibrated AG 50W-X2 cation exchange resin (BioRad Laboratories). In order to insure that poly(dG-dC) had attained its equilibrium conformation for a given solvent composition, samples were heated at 50°C for 10 min and then were allowed to cool to room temperature before recording the CD spectrum (18). The initial nucleotide concentration of poly(dG-dC) ranged from 60-90 μM and the formal ratio of chromium(III) to DNA nucleotide (DNA-P), r, was varied between 0.02 and 4.4.

RESULTS

The effect of chromium(III) nitrate on the CD spectrum of poly(dG-dC) is shown in Figure 1. Chromium(III) had no effect on the normal B-DNA CD

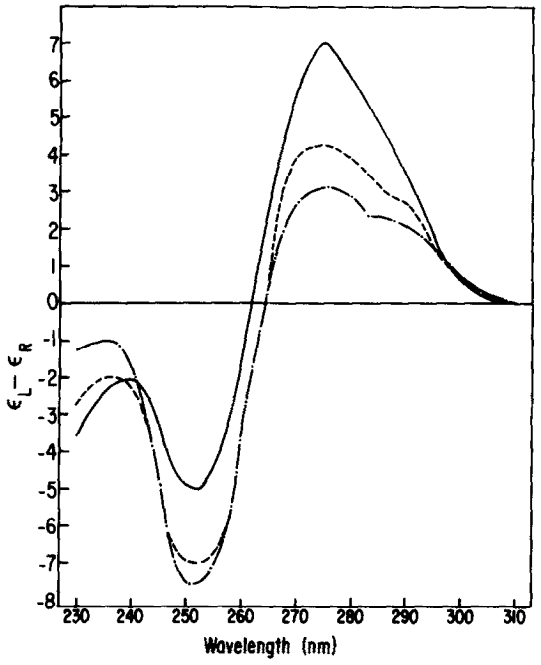

Figure 1. Circular dichroism spectra of poly(dG-dC) in the presence (-----, r=1.57; ———, r=2.27) and absence (-•-•-) of chromium(III) nitrate. Spectra were run at 25°C with [poly(dG-dC)] = 63 μM in 5 mM Tris-HCl, 50 mM NaCl, pH 7.4.

spectrum at r ≤ 1 with NaCl concentrations of either 50 or 550 mM. In contrast, at r ≥ 1 the ellipticity from 260-295 nm increased while that from 230-260 nm became less negative (Figure 1). At r = 4.4 precipitation of the DNA occurred immediately upon adding chromium(III) nitrate. At low NaCl concentration, 1.0 mM, chromium(III) chloride had no effect on the normal B-DNA spectrum at r ≤ 0.4, however, above this ratio a flocculent precipitate formed slowly over a period of 72 hr. The supernatants from polymers precipitated with either chromium(III) chloride of chromium(III) nitrate exhibited a CD signal similar to that shown at high r values in Figure 1, but greatly reduced in intensity.

The effect of chromium(III) chloride on the ethanol-induced B→Z transition of poly(dG-dC) is shown in Figure 2. At low concentrations of ethanol, 20% (v:v), chromium(III) chloride (r = 0.02-0.47) had no effect on the normal B form of the polymer. The midpoint of the B→Z transition upon titration with ethanol decreased from 50% to 41% (v:v) in the presence of

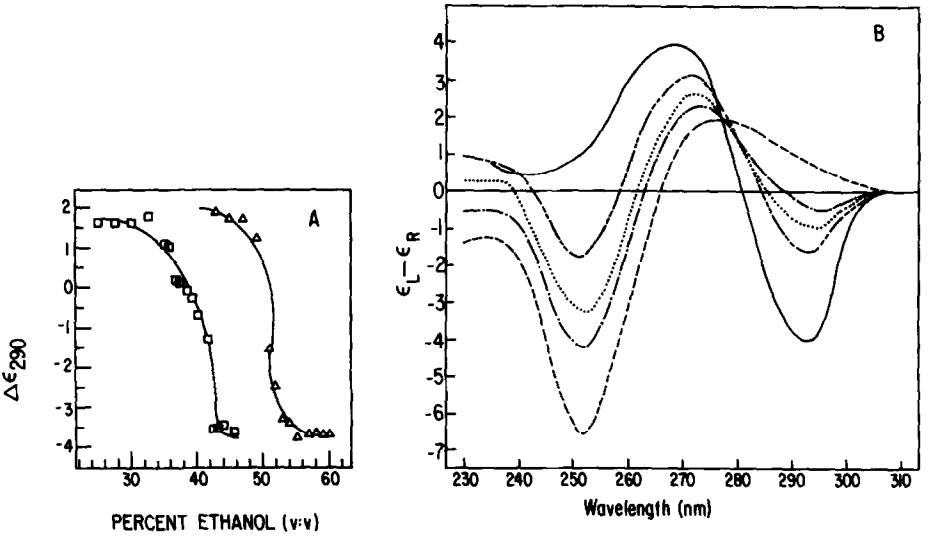

Figure 2. Effect of chromium(III) chloride on the ethanol-induced B→Z transition of poly(dG-dC) at 25°C in 5 mM Tris-HCl, 1 mM NaCl, pH 7.4. (A) Ellipticity of poly(dG-dC) at 290 nm in the presence (□, r=0.08) and absence (△) of chromium(III). (B) Effect of ethanol concentration (-----, 35.2%; -•-•-, 38.5%; •••••, 40.1%; —·—·—, 41.7%; ———, 42.5%) on the CD spectra of poly(dG-dC) in the presence of chromium(III) (r=0.08). Initial concentration of poly(dG-dC) was 80 μM.

chromium(III) at r = 0.08 (Figure 2A). The lack of an isobestic point in the CD spectra (Figure 2B) indicates that the B→Z transition in the presence of chromium(III) is a complex process. The Z form quantitatively reverted to the B form upon dilution of the ethanol indicating that chromium(III) does not interfere with the reversibility of the transition under these conditions. However, at r = 0.2 or 0.25 the ethanol-induced B→Z transition was inhibited and the ellipticity 230-260 nm became less negative as the concentration of ethanol was increased (Figure 3). At these r values, aggregation was apparent at ethanol concentrations of 40-43% (v:v). The conformational changes induced by chromium(III) and ethanol were irreversible since no changes in the CD spectra were observed upon dilution of the ethanol.

Chromium(III) nitrate (r = 0.06-0.10) also lowered the percentage ethanol required to induced the B→Z transition of poly(dG-dC) by 8-12% (v:v). However, at a higher ratio, r = 0.34, chromium(III) prevented the B→Z transition and caused aggregation at high concentrations of ethanol.

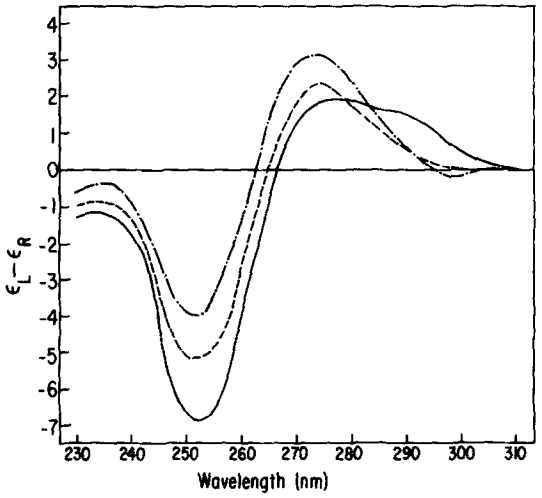

Figure 3. Circular dichroism spectrum of poly(dG-dC) in the presence of ethanol at 25°C in the presence (-•-•-, r=0.25, 41.8% ethanol (v:v); -----, r=0.20, 41.4% ethanol (v:v)) and absence (———, 42.9% ethanol (v:v)) of chromium(III) chloride. Initial concentration of poly(dG-dC) was 90 μM in 5 mM Tris-HCl, 1 mM NaCl, pH 7.4.

DISCUSSION

Circular dichroism studies of poly(dG-dC) in the presence of chromium(III) are consistent with the ability of cationic chromium(III) complexes to affect the conformation of the polymer through interacting with the DNA sugar-phosphate backbone.

At high ratios of Cr to DNA-P, both hexaquochromium(II) (from chromium(III) nitrate) and trans-dichlorotetraaquochromium(III) (from chromium(III) chloride) caused aggregation of poly(dG-dC) and resulted in CD spectra more "A-like" than the original B form of the DNA polymer. The slower condensation of poly(dG-dC) with trans-dichlorotetraaquochromium(III) (+1 charge) may be due to the slow hydrolysis of the complex to more highly charged species. At pH 7.4, hexaquochromium(III) (+3 charge) slowly undergoes hydrolysis and results in monomeric complexes (+1 and +2 charge) and a trimeric complex (+5 charge) (23). Highly charged cations (+3 and +4), including hexaquochromium(III), hexamminecobalt(III), tris(ethylenediamine)cobalt(III), spermidine and spermine, caused condensation of λ-DNA into toroidal conformations which had the CD spectrum of B-DNA (24,25). It was suggested that these cations noncovalently cross-

link adjacent helices of the DNA forming electrostatic bridges which cause DNA condensation (24). Since the chromium(III) complexes may covalently cross-link poly(dG-dC) as well as ion pair with the phosphate backbone, aggregation of DNA by chromium(III) is expected to be more complicated than that observed for the inert cobalt(III) complexes and polyamines.

Chromium(III) prevented the ethanol-induced B→Z transition of poly(dG-dC), caused irreversible aggregation of the polymer and resulted in CD spectra only slightly more "A-like" than the original B form of the polymer at Cr to DNA-P ratios lower than required to aggregate the polymer in the absence of ethanol. Modification of poly(dG-dC) with aflatoxin B1 (24), cis- or trans-diamminedichloroplatinum(II) (15,16) inhibited the salt- or ethanol-induced B→Z transition of the polymer. It was suggested that cross-linking of DNA by the bifunctional platinum complexes, whereas the bulk and hydrogen bonding interactions of aflatoxin, were responsible for preventing the B→Z transition (15,16,26). It is likely that chromium(III) cross-links poly(dG-dC), thereby preventing the B→Z transition and enhancing irreversible aggregation of the polymer in the presence of ethanol. Ethanol has been shown to induced the B→A transition and cause aggregation in calf thymus DNA (27).

At low ratios of Cr to DNA-P, the presence of chromium(III) lowered the concentration of ethanol needed to induce the B→Z transition in poly(dG-dC) and no aggregation occurred. It has been proposed that agents providing positive charge which shields the repulsions between the negatively charged sugar-phosphate backbones of the two strands facilitate the B→Z transition since the phosphates in opposite strands are closer in Z-DNA than in B-DNA (12,18). Facilitation of the B→Z transition has been observed upon covalent modification of the polymer at the N-7 of guanine by chloroethylenetriamineplatinum(II) (15,16) and dimethyl sulfate (12). Hexamminecobalt(III) induces the B→Z transition in poly(dG-dC) through noncovalent interactions with the polymer (18). It is likely that chromium(III) acts in a manner similar to cations which may bind either

directly or indirectly (through hydrogen bonding) to the bases or phosphates of the polymer, e.g., Mn^{2+}, Co^{2+}, Ni^{2+}, Tb^{3+}, Na^{+}, Mg^{2+}, Ca^{2+} and Ba^{2+} (17-21), to facilitate the B→Z transition.

Since chromium(III) causes condensation of DNA and either facilitates or inhibits the conformational change of B-DNA to Z-DNA depending upon the ratio of Cr to DNA-P, it appears that the biological effects of chromium(III) will depend critically on its concentration in the nucleus. Chromium(III) may interfere with gene expression and gene regulation through its ability to alter the B→Z transition and cause DNA condensation in active chromatin.

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