

Calf-Thymus DNA Interaction with Cr(III)-Gallate and Cr(III)-Ethyl Gallate Studied by FTIR Spectroscopy and Capillary Electrophoresis

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Cr(VI) salts are well known to be carcinogen, and are reduced by various cellular components to form cross-linked Cr(III) products. Organic compounds, such as gallic acid (GA) and ethyl gallate (EGA), reduce Cr(VI) to Cr(III) to form Cr(III)-gallate [Cr(III)-GA] and Cr(III)-ethyl gallate [Cr(III)-EGA] as final products. These Cr(III)-tannin complexes are DNA binders. The interaction of calf-thymus DNA with Cr(III)-GA and Cr(III)-EGA in aqueous solutions at physiological pH were studied at Cr(III)/DNA (phosphate) molar ratios (r) of 1:160, 1:80, 1:40, 1:20, 1:10, 1:4, and 1:2 using FTIR spectroscopy and capillary electrophoresis. An analysis by FTIR showed that at low concentrations (r = 1/80 and 1/40), Cr(III)-GA and Cr(III)–EGA mainly bind to the guanine N-7 atom of the G–C base pairs with minor perturbations of the A–T base pairs. At r > 1/20, a partial helix opening occurred with major perturbations of the G–C and A–T base pairs. At r >1/10, aggregation of the Cr(III)-tannin–DNA complexes occurred. However, no DNA conformational changes were observed, and the DNA maintained the B-family structure. The binding constants of the Cr(III)-GA–DNA and Cr(III)-EGA–DNA were estimated to be $3.8 \times 10^4\ M^{-1}$ and $6.2 \times 10^4\ M^{-1}$, respectively, by Scatchard plots following capillary electrophoresis. These results suggest that the Cr(III)-tannin complexes are external DNA binders, and do not form a chelate with the DNA.#

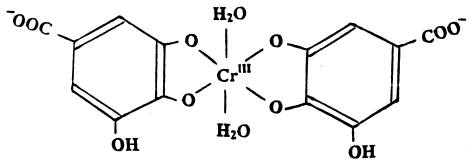
Cr(VI) salts in the environment are considered to be biological hazards because of their mutagenesity[1] and carcinogenesity.[2] Since it is highly reactive with reductive materials, a part of the Cr(VI) salts is converted to Cr(III) by inorganic electron donors, such as Fe^{2+}, S^{2-} or by bioprocess involving organic compounds.[3] Following this conversion, Cr(III) is expected to precipitate as oxides and hydroxides, or to form soluble complexes with a number of organic ligands. During the course of our study on removing the Cr(VI) salts by tannin from withered leaves, we found that the tannin reduced Cr(VI) into Cr(III) through Cr(V) to form water-soluble Cr(III)-tannin complexes.[4,5] When Cr(VI) salts are incorporated into the cell, they are reduced by intracellular reductive agents, ascorbic acid and glutathione[6] into Cr(V), Cr(IV), and Cr(III) as final products. During the conversion of the Cr(VI) into Cr(III), DNA is considered to bind chromium, forming Cr(III)–DNA adducts.[7] DNA damage resulting from Cr(VI) exposure has been reported.[1,8] In biological materials, most of all the cellular chromium is present as Cr(III)–organic compounds9 that form a variety of complexes with nucleic acids and proteins.[10] Several studies were attempted to determine the carcinogenic and mutagenic activities of Cr(III) compounds.[11,12] It has been shown that $CrCl_3$ salt increases mutagenesis when $CrCl_3$-treated phage DNA is transfected into *Escherichia coli*.[13–15] A study of the effect of Cr(III) on DNA replication with a single-strand DNA template and micromolar concentrations of chromium revealed that Cr(III) binds DNA in a dose-dependent manner and prevents DNA replication.[16] However, if the unbound chromium is removed from the system by gel filtration, the rate of replication by polymerase I on Cr(III)-bound template increases and the replication fidelity decreases. When the concentration of Cr(III) increases further, DNA–DNA cross-links occur to inhibit the polymerase activity. It has been also shown that Cr(III) causes a mutational spectrum in shuttle vectors replicated in human cells.[17] Recently, we have reported the complexation of Cr(III) with calf-thymus DNA in which Cr(III) binds primarily with guanine N-7 and the backbone phosphate group to form a chelate.[18] However, it remained unclear how Cr(III)–organic complexes bind DNA in vitro and in vivo. In this context, Cr(III)-amino acid complexes were found to be less reactive than Cr(III) towards DNA.[19]

In the present paper, we report on the characterization of Cr(III)-gallate and Cr(III)-ethyl gallate complexes (Chart 1, A and B) synthesized from potassium chromate and either gallic acid or ethyl gallate. Using FTIR spectroscopy and capillary electrophoresis, the binding mode of the Cr(III)-tannin complexes with calf-thymus DNA was studied. Furthermore, the difference between the nature of the Cr(III)-tannin–DNA complexation and that of Cr(III)–DNA is also discussed. To our knowledge, this is the first spectroscopic study regarding DNA interactions with the Cr(III)-tannin complexes, and should help to elucidate the reactivity of these complexes towards DNA.

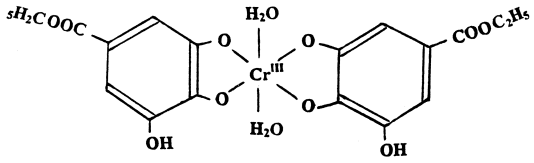
Experimental

Materials. Highly polymerized type-I calf-thymus DNA so-

Abbreviations: GA, gallate; EGA, ethyl gallate; A, adenine; G, guanine; C, cytosine; T, thymine; FTIR, Fourier transform infrared



Cr(III)-gallate (A)



Cr(III)-ethyl gallate (B)

Chart 1.

dium salt (7% Na content) was purchased from Sigma Chemical Co., and was deproteinated by the addition of $CHCl_3$ and isopentyl alcohol in a NaCl solution. Gallic acid and ethyl gallate were purchased from Sigma Chemical Co. and Fluka Co., respectively. All other chemicals used in the present study were of reagent grade.

Synthesis of Cr(III)-Gallate and Cr(III)-Ethyl Gallate Complexes. After mixtures containing potassium chromate (15 mM) and either gallic acid (15 mM) or ethy gallate (15 mM) were incubated for 24 h at 20 °C, 5 mL portions of the mixtures were placed on a column of Sephadex G-25 (1 × 85 cm) equilibrated in water. Fifteen fractions (5 mL) were collected, and the concentrations of Cr(VI), tannin and total Cr in each fraction were determined by atomic absorption spectroscopy and complexiometric methods.[20] Then, fraction numbers 7 to 9 were pooled, and lyophylized. These fractions were used for further analysis. The purified Cr(III)-tannin complexes prepared by a Sephadex G-25 column were analyzed for carbon, hydrogen, chromium and potssium contents by a CH analyzer and atomic-absorption spectroscopy. An elemental analysis of the Cr(III)-gallate showed the following: C, 25.48; H, 3.60; Cr,7.81; K,10.6%. Calcd for $C_{14}H_{28}CrK_2O_{21}$: C, 25.38; H, 4.2; O, 50.76; Cr, 7.85; K, 11.78%. The Cr(III)-ethyl gallate showed that found: C, 36.23; H, 4.02; Cr 8.23%. Calcd for $C_{18}H_{32}CrO_{18}$: C, 36.70; H, 5.40; O, 48.98; Cr, 8.84%. FABMS on the Cr(III)-GA and Cr(III)-EGA gave molecular ions of *m*/*z* 424 and 480, respectively. Taken together with the FTIR data, the Cr(III) cation is most likely characterized to be six-coordinated with an octahedral geometry and bidentately bound to two gallic acid or ethyl gallate residues, and two water molecules occupying the two axial positions.

Preparation of Stock Solutions. Sodium-DNA was dissolved to 1% w/w (25 mM DNA (phosphate)) in 50 mM NaCl at 5 °C for 24 h with occasional stirring to ensure the formation of a homogenenous solution. Solutions of Cr(III)-GA and Cr(III)-EGA were prepared in distilled water, and the pH of the solutions was adjusted to 7.2 using a NaOH solution. Then, the Cr(III)-tannin solutions were added to the DNA solution to attain the desired Cr(III) /DNA (P) molar ratios of 1:80, 1:40, 1:20, 1:10, 1:4, and 1:2 at a final DNA(P) concentration of 12.5 mM. The infrared spectra of Cr(III)-GA–DNA and Cr(III)-EGA–DNA complexes were recorded after incubation of the mixtures for 2 h.

FTIR Spectra. Infrared spectra were recorded on a Bomem DA3-0.02 FTIR spectrometer equipped with a nitrogen-cooled HgCdTe detector and a KBr beam splitter. The solution spectra were taken using AgBr windows with a resolution of 2 to 4 cm^{-1} and 100 scans. Each set of infrared spectra were taken (three times) on three identical samples with the same DNA and metal-ion concentrations. Water subtraction was carried out with a 0.1 M (= mol dm^{-3}) NaCl solution used as a reference at neutral pH.[21] A good subtraction was achieved as shown by the flat baseline at around 2200 cm^{-1}, where the water combination mode is located. This method is a rough estimate, but removes the water content in a satisfactory way. The difference spectra [(DNA solution + Cr(III)-tannin complex solution)] − (DNA solution)] were produced using a band at 968 cm^{-1} as an internal reference. This band, due to deoxyribose C–C stretching vibration, exhibits no spectra changes (shifting and intensity variations) on Cr(III)-tannin–DNA complexation, and it was cancelled upon spectral subtraction. The spectra were smoothed buy a Savitzy–Golay procedure.[21] The intensity ratios of several DNA in-plane vibrations related to A–T and G–C base pairs and the PO_2 stretchings were measured (with respect to the reference band of 968 cm^{-1}) as a function of the chromium concentrations with an error of ±3%. These intensity ratios were used to determine the bindings of Cr(III)-tannin complexes to the DNA bases and the backbone phosphate groups.

Capillary Electrophoresis. A P/ACE System MDQ (Beckman) with a PDA (photodiode array) detector was used in this study. An uncoated fused-silica capillary of 75 μm i.d. and 57 cm length was used. The capillary was initially conditioned by washing with 1 M sodium hydroxide for 30 min, followed by a 15-min wash with 0.1 M sodium hydroxide. It was then extensively rinsed with deionized water and a running buffer before use. Samples were injected using a voltage injection at 10 kV for 5 s. Electrophoresis was carried out at a voltage of 25 kV for 10 min using normal polarity. All runs were carried out at 25 °C in a run buffer of 7.5 mM Tris-HCl, pH 7.4 containing 15 mM NaCl. The electropherograms were monitored at 260 nm. Stock solutions of Cr(III)-GA and Cr(III)-EGA (6.25 mM) were prepared in deionized water. The capillary inlet and outlet vials were replenished after each run. The Cr(III)-tannin complexes-binding experiments were performed in a sample buffer containing 1.5 mM Tris-HCl, pH 7.4 and 3 mM NaCl, using constant concentration of calf-thymus DNA and various concentrations of Cr(III)-tannin complexes. Calf-thymus DNA was dissolved in 3 mM Tris-HCl (pH 7.4), and 6 mM NaCl, at a DNA phosphate (P) concentration of 2.5 mM. The stock solutions of Cr(III)-tannin complexes were added to the DNA solutions to attain the desired Cr(III)/DNA(P) molar ratios of 1:160, 1:80, 1:40, 1:20, 1:10, and 1:4. Each sample was allowed to equilibrate for 30 min before injection.

Data Analysis. The binding constants for the Cr(III)-tannin–DNA complexes and Cr(III)–DNA were determined by capillary electrophoresis using the Scatchard analysis.[22–24] The average number (R_f) of Cr(III) cations bound per one binding site of the DNA was determined from the change of the peak height due to the presence of the Cr(III)-cation by the equation

$$R_f = (h - h_0)/(h_s - h_0), \quad (1)$$

where h is the change in the peak height measured for any added Cr(III) cation concentrations, while h_0 and h_s correspond to the peak height of the free DNA and Cr(III)-cation saturated DNA, re-

spectively. Using the equation for the binding constant,

$$K_b = [\text{Cr(III)-cation–DNA}]/[\text{DNA}][\text{Cr(III)-cation}], \quad (2)$$

the experimental Cr(III)-cation binding constant (K_b) was then computed by fitting the experimental values of the R_f and Cr(III)-cation concentrations to the equation

$$R_f = K_b[\text{Cr(III)-cation}]/(1 + K_b[\text{Cr(III)-cation}]). \quad (3)$$

The last equation gives a convenient form for Scatchard analysis:

$$R_f/[\text{Cr(III)-cation}] = K_b - K_b R_f. \quad (4)$$

In recent years, capillary electrophoresis has been widely used to determine the binding constants of DNA–protein and metal–protein complexes.[25–28]

Results

Interaction of the Cr(III)-Tannin Complexes with DNA Studied by FTIR Spectroscopy.

In order to examine the interactions of calf-thymus DNA with the Cr(III)-tannin complexes, the FTIR spectra of DNA adducts formed from a constant concentration of free DNA and various concentrations of either Cr(III)-GA or Cr(III)-EGA were recorded (Figs. 1 and 2). The spectra showed an increase in the intensity of the bands derived from the DNA-adducts bases (1700–1500 cm^{-1}), compared with those of free DNA. To study on the rate of increase in the intensity, the intensity ratios of the bands in the several prominent DNA in-plane vibrations at 1717 cm^{-1} (G, T), 1663 cm^{-1} (T, G, A, C), 1609 cm^{-1} (A, C), 1492 cm^{-1} (C), and 1222 cm^{-1} (PO_2 asymmetric stretch) with respect to the band at 968 cm^{-1}, which is not perturbed during the complexation, were calculated.[21,29–35]

At a low concentration of Cr(III)-GA (r = 1/80), the intensity ratios of the bands at 1717 cm^{-1} and 1663 cm^{-1} increased by 30% and 5% relative to free DNA with no significant spectral shifting, whereas a minor decrease in the intensity ratios was observed in the bands at 1609 cm^{-1} and 1222 cm^{-1} (Fig. 3). The spectra of Cr(III)-EGA–DNA showed that the intensity ratios for the bands at 1717 cm^{-1} and 1663 cm^{-1} increased by 70% and 30%, associated with a reduction in the intensity

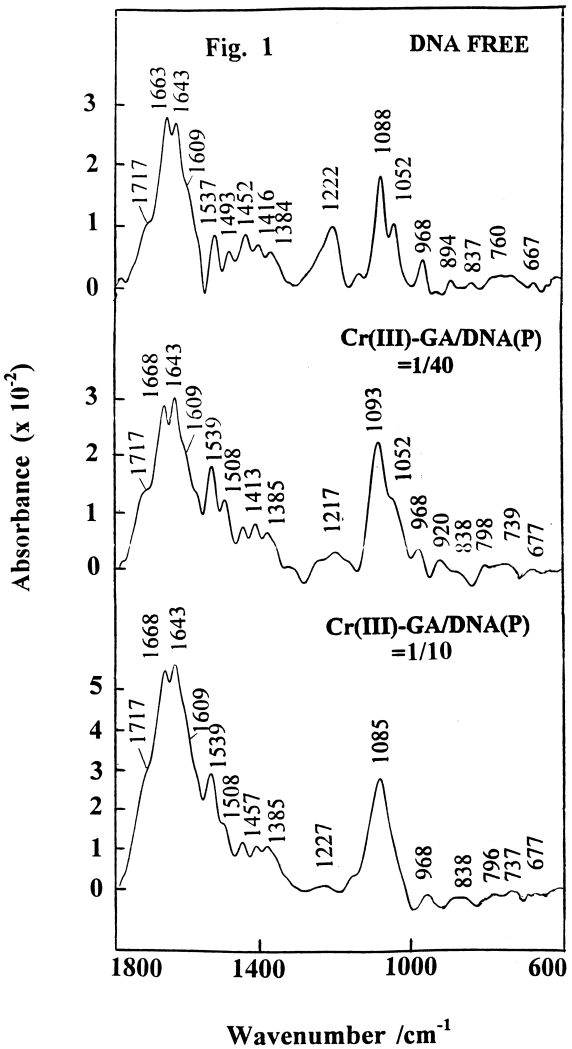


Fig. 1. FTIR spectra of calf-thymus DNA and Cr(III)-GA–DNA complexes formed in Cr(III)-GA/DNA(P) ratios of 1/40 and 1/10 in the region of 1800–600 cm^{-1}.

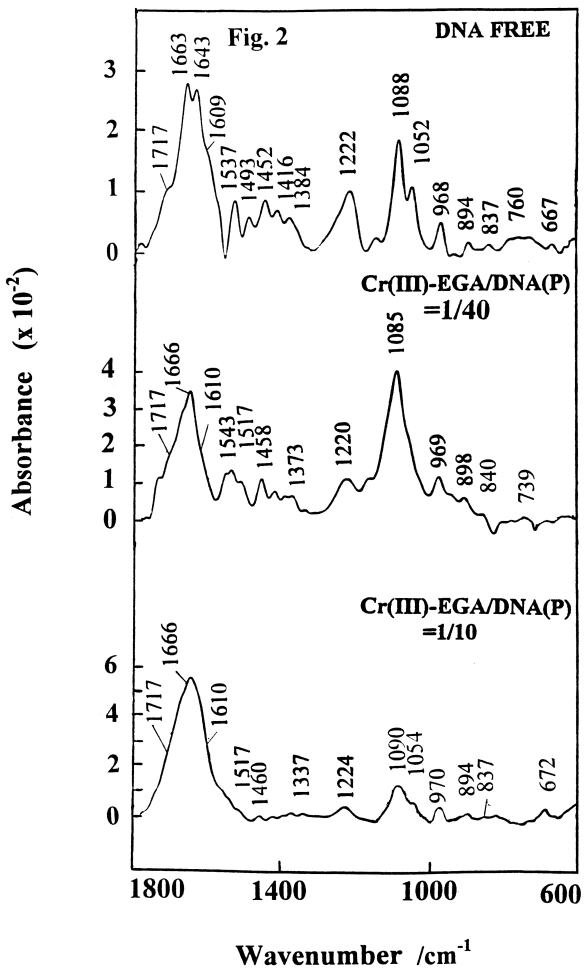


Fig. 2. FTIR spectra of calf-thymus DNA and Cr(III)-EGA–DNA complexes formed in Cr(III)-EGA/DNA(P) ratios of 1/40 and 1/10 in the region of 1800–600 cm^{-1}.

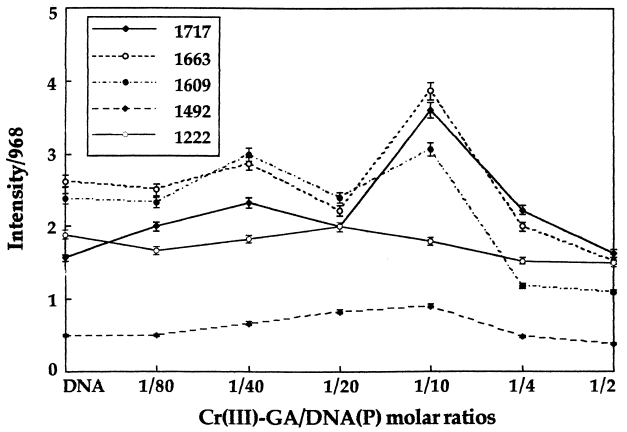


Fig. 3. Intensity ratio variations for several DNA in-plane vibrations at 1717 (G, T), 1663 (T, G, A, C), 1609 (A, C), 1494 (C), and 1222 cm^{-1} (PO_2 asymmetric stretch) in Cr(III)-GA/DNA(P) ratios of 1:80, 1:40, 1:20, 1:10, 1:4, and 1:2. Each intensity ratio was determined from values of ratios of the intensity of each DNA band and that of band at 968 cm^{-1}.

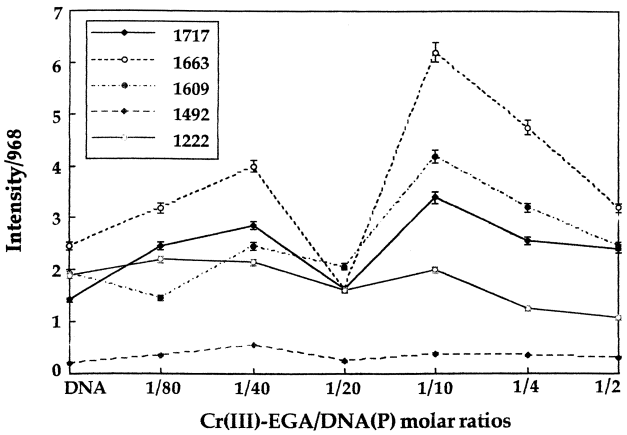


Fig. 4. Intensity ratio variations for several DNA in-plane vibrations at 1717(G, T), 1663 (T, G, A, C), 1609 (A, C), 1494 (C), and 1222 cm^{-1} (PO_2 asymmetric stretch) in Cr(III)-EGA/DNA(P) ratios of 1:80, 1:40, 1:20, 1:10, 1:4, and 1:2. Each intensity ratio was determined from values of ratios of the intensity of each DNA band and that of band at 968 cm^{-1}.

ratio of the band at 1609 cm^{-1} (Fig. 4). The band at 1222 cm^{-1} exhibited a slight increase in the intensity ratio. The band at 1492 cm^{-1} exhibited no major spectral changes upon Cr(III)-tannin–DNA complexation (Figs. 3 and 4). It has been suggested that the infrared spectral changes in the region of 1700–1500 cm^{-1} can be attributed to the formation of cation–DNA complexes at the guanine N-7 atom, but not at other guanine sites, such as C6-O, N-1 and C2–NH_2 groups which are involved with the Watson–Crick hydrogen bondings.[21,29] Also, Cr(III) has been reported to preferentially bind to guanine-containing DNA.[36] Thus, the present FTIR spectra indicate that Cr(III)-GA and Cr(III)-EGA most likely bind to guanine of the G–C base pairs. Similar infrared spectral changes were observed for the Cr(III)–DNA complexes, where direct coordination of Cr(III) to guanine N-7 occurred.[18] It is important to note that besides a major intensity variation of the guanine band at 1717 cm^{-1} in the spectrum of Cr(III)–DNA, this band was shifted towards a lower frequency at 1709 cm^{-1}. However, the Cr(III)-GA–DNA complexes showed no such spectral shifting. Consequently, the observed spectral change in the present study indicates that Cr(III)-GA indirectly binds to guanine N-7 sites. The extent of the increase in intensity ratio for the band at 1717 cm^{-1} in Cr(III)-EGA is larger than that of Cr(III)-GA, suggesting that the binding ability of the former complex is stronger than that of the latter complex. Moreover, minor interactions of the Cr(III)-tannin complexes with thymine can not be ruled out at this stage.

As the Cr(III)-GA concetration increased (r = 1/40), the intensity ratios of the bands at 1717 cm^{-1}, 1663 cm^{-1}, and 1609 cm^{-1} increased by 50%, 10%, and 20% relative to the free DNA (Fig. 3). The band at 1222 cm^{-1} exhibited no major intensity change relative to the band at r = 1/80. In the spectra of Cr(III)-EGA–DNA, the intensity ratios for the bands at 1717 cm^{-1}, 1663 cm^{-1}, and 1609 cm^{-1} increased; the rate of the increase was estimated to be 85%, 35%, and 25%, respectively (Fig. 4). These increases in the intensities of the vibrations were further characterized by the positive peaks at 1720–1615 cm^{-1}1 in the difference spectrum of Cr(III)-GA–DNA and at 1708–1610 cm^{-1} and 1226 cm^{-1} in that of Cr(III)-EGA–DNA (Figs. 5 and 6, r = 1/40). These data indicate that binding of the Cr(III)-tannin complexes to guanine continues, while binding of the complexes to the A–T base pairs and the backbone phosphate can not be ruled out here. Since the difference spectra of Cr(III)-EGA–DNA at 1/40 showed a positive peak at 1708 cm^{-1}, the Cr(III)-EGA probably caused direct coordination with guanine. It should also be noted that the intensity ratio of the band at 1663 cm^{-1} is affected by the vibration of guanine and adenine besides thymine. Furthermore, this band is affected by a composite water band at 1643 cm^{-1}. Consequently, the interactions of the Cr(III)-tannin complexes with the A–T base pairs probably occurs through adenine N-7 as well as thymine O-2 atom.

At r = 1/20, some reduction in the intensity ratios of the bands at 1717 cm^{-1}, 1663 cm^{-1}, and 1609 cm^{-1} were observed (Figs. 3 and 4, r = 1/20). The observed loss of intensity ratios were probably due to helix stabilization induced by the interactions of Cr(III)-GA and Cr(III)-EGA with the DNA. As the Cr(III)-tannin complexes concentrations increased to r = 1/10, major increases of intensity ratios in the DNA vibrations occurred at 1717 cm^{-1}, 1663 cm^{-1}, and 1609 cm^{-1} upon Cr(III)-GA–DNA and Cr(III)-EGA–DNA complexations (Figs. 3 and 4 r = 1/10). Strong positive peaks were also observed at 1720–1615 cm^{-1} and 1720–1610 cm^{-1} in the difference spectra of Cr(III)-GA–DNA and Cr(III)-EGA–DNA, respectively (Figs. 5 and 6, r = 1/10). The gains in the intensity ratios are attributed to helix destabilization by associating with a large amount of the Cr(III)-tannin complexes. A similar increase in the intensity ratios of several DNA vibrations was also observed when DNA was incubated with high concentrations of chlorophyll[37] or diethylstilbestrol.[38] Moreover, a positive peak was observed at 1720 cm^{-1} in the difference spectra of Cr(III)-EGA–DNA, indicating that indirect binding of Cr(III)-EGA to

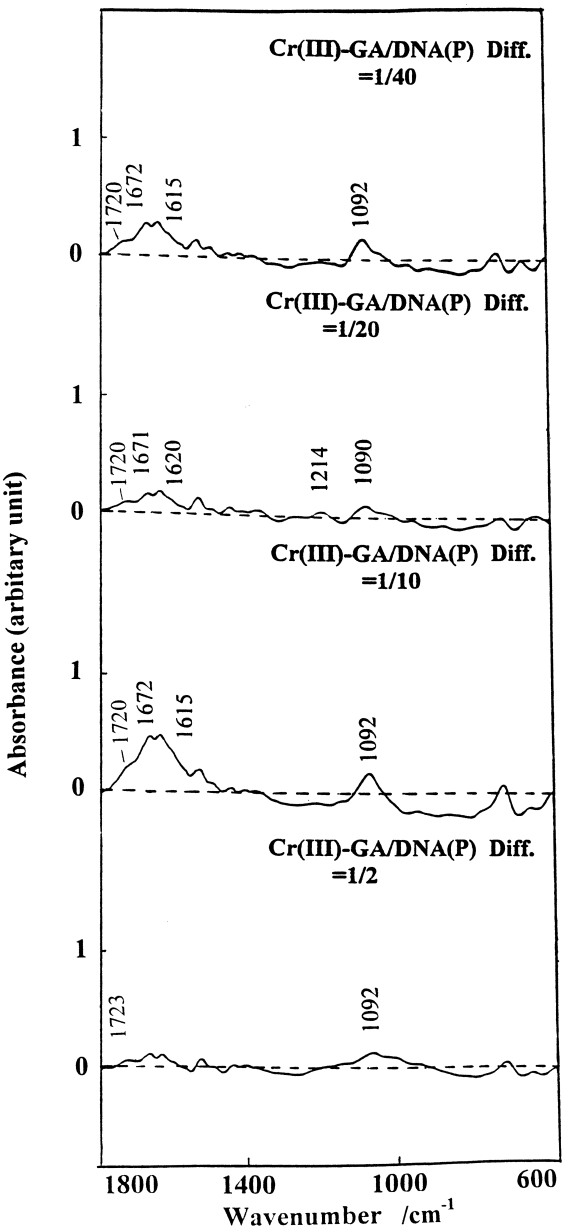


Fig. 5. FTIR difference spectra of Cr(III)-GA–DNA complex. The spectra were obtained by subtraction of calf-thymus DNA spectrum from spectra of Cr(III)-GA–DNA complex formed in Cr(III)-EGA/DNA(P) ratios of 1:40, 1:20, 1:10, and 1:2.

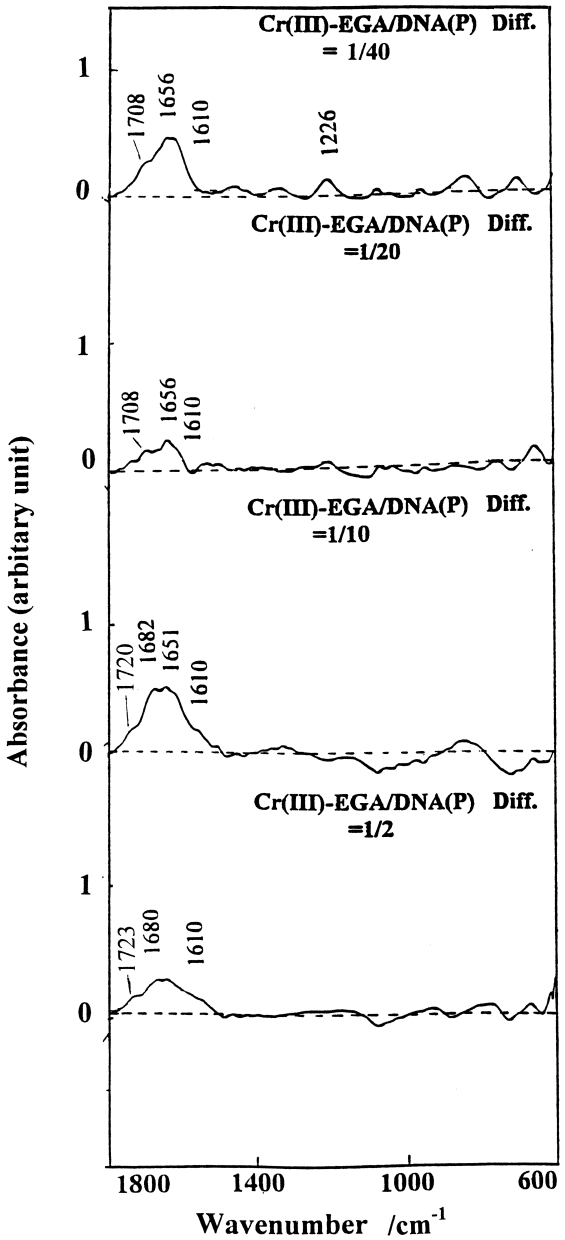


Fig. 6. FTIR difference spectra of Cr(III)-EGA–DNA complex. The spectra were obtained by subtraction of calf-thymus DNA spectrum from spectra of Cr(III)-EGA–DNA complex formed in Cr(III)-EGA/DNA(P) ratios of 1:40, 1:20, 1:10, and 1:2.

guanine bases occurred at this concentration.

At $r = 1/2$, a major loss of the intensity ratios in the bands at 1717 cm^{-1}, 1663 cm^{-1}, and 1609 cm^{-1} was observed (Figs. 3 and 4, $r = 1/2$). Reduction in the intensity ratios of these vibrations were also observed in the difference spectra (Figs. 5 and 6, $r = 1/2$). This is attributed to biopolymer aggregation in the presence of a high concentration the Cr(III)-tannin complexes. The aggregation of DNA by different metal cations is well known, and spectral modifications in the DNA vibrational frequencies by this phenomenon have been reported.[39–41]

The conformational changes in the calf-thymus DNA did not occur during the interaction with the Cr(III)-tannin complexes. It is known that in a B- to A-transition the B-maker band at 837 cm^{-1} (phosphodiester mode) shifts to about 810 cm^{-1}, 860 cm^{-1}, and 885 cm^{-1} and the band at 1717 cm^{-1} appears at 1700 cm^{-1}, while the band at 1222 cm^{-1} shifts toward a higher frequency at 1240 cm^{-1} (Fig. 1).[33,42] In a B- to Z-conformational changes, the sugar-phosphate band at 837 cm^{-1} appears at 800–780 cm^{-1}, and the band at 1717 cm^{-1} displaces to 1690 cm^{-1}, while the band at 1222 cm^{-1} shifts to 1216 cm^{-1}.[33,42] Since such spectral changes did not occur for the infrared marker bands here, DNA remained in the B-conformation in the presence of the Cr(III)-tannin complexes.

Interaction of the Cr(III)-Tannin Complexes with DNA

Studied by Capillary Electrophoresis. The binding properties of the Cr(III)-tannin complexes with calf-thymus DNA were further studied by capillary electrophoresis. Mixtures containing various concentrations of the Cr(III)-tannin complexes and a constant concentration of calf-thymus DNA were subjected to capillary electrophoresis using an uncoated silica capillary (75 μm, 57 cm) at 25 kV. The electropherogram was monitored at 260 nm in a run buffer of Tris-HCl pH 7.4 (7.5 mM) containing 15 mM of NaCl at 25 °C. As shown in Fig. 7, free DNA (Panel A-1 and B-1), Cr(III)-GA–DNA (Panel A-2-4), and Cr(III)-EGA–DNA (Panel B-2-4) were observed as a single peak at migration times of 3.3–3.5 min. The peak height of the Cr(III)-tannin–DNA gradually increased as the molar ratios of Cr(III)-tannin/DNA increased. The maximal peak height was achieved at molar ratios of 1/4 and 1/10 for Cr(III)-GA–DNA and Cr(III)-EGA–DNA, respectively. Then, Scatchard plots were performed using the data of R_f (the number of Cr(III)-tannin complexes bound per one-site of the DNA) and R_f/concetration of Cr(III)-tannin. Figure 8 shows the Scatchard plots for Cr(III)-GA–DNA (Panel A) and Cr(III)-EGA–DNA (Panel B). Both slopes showed a monophasic character, and the binding constants for the Cr(III)-GA–DNA and Cr(III)-EGA–DNA complexes towards DNA were estimated to be $3.8 \times 10^4\ M^{-1}$ and $6.2 \times 10^4\ M^{-1}$, respectively. These results are in agreement with the idea that the primary binding site of the Cr(III)-tannin complexes is the guanine residues. Also, the smaller binding constant of the Cr(III)-GA–DNA than the Cr(III)-EGA–DNA accounts for the fact that the former complex indirectly bound to the DNA, but the latter complex showed a somewhat direct coordination nature at lower concentrations. A Scatchard plot of Cr(III)–DNA complex following the capillary electrophoresis of mixtures containing $Cr(NO_3)_3$ and calf-thymus DNA showed a biphasic slope (Fig. 8 Panel C). This result indicates the presence of low-affinity (ionic-type) and high-affinity (covalent-type) binding sites. The binding constants for the low- and high-affinity binding sites were estimated to be $3.6 \times 10^4\ M^{-1}$ and $2.3 \times 10^5\ M^{-1}$, respectively. These are consistent with those of the infrared spectroscopic results for Cr(III)–DNA complex,[18] which showed two main binding sites; one is the backbone phosphate and the other guanine N-7 atom. The larger stability constant of the Cr(III)–DNA complex than the Cr(III)-tannin–DNA complexes is attributed to the direct binding to the two sites, which form stable chelate bondings in the former complex.

Discussion

The present study by FTIR showed that Cr(III)-GA indirectly binds to the guanine N-7 atom at a low concentration of the complexes (r = 1/80), and that some perturbations of the A–T base pairs also occurred at higher concentrations (r = 1/40). The Cr(III)-EGA showed a similar binding nature with the Cr(III)-GA. However, this complex seems to have a stronger binding ability than that of Cr(III)-GA, since Cr(III)-EGA showed direct binding to guanine at a low concentration; also, the increase in the intensity in the bands for DNA bases is larger than that of Cr(III)-GA. In fact, the binding constants for Cr(III)-GA–DNA and Cr(III)-EGA–DNA were estimated to be $3.8 \times 10^4\ M^{-1}$ and $6.2 \times 10^4\ M^{-1}$, respectively, by Scathcard plots following capillary electrophoresis. The weaker binding of the Cr(III)-GA to the DNA is probably attributed to a negative charge in the carboxyl group of the gallate ligand, which might repel the negative charge in the sugar phosphate of the DNA. Moreover, the electron-withdrawing inductive effect of

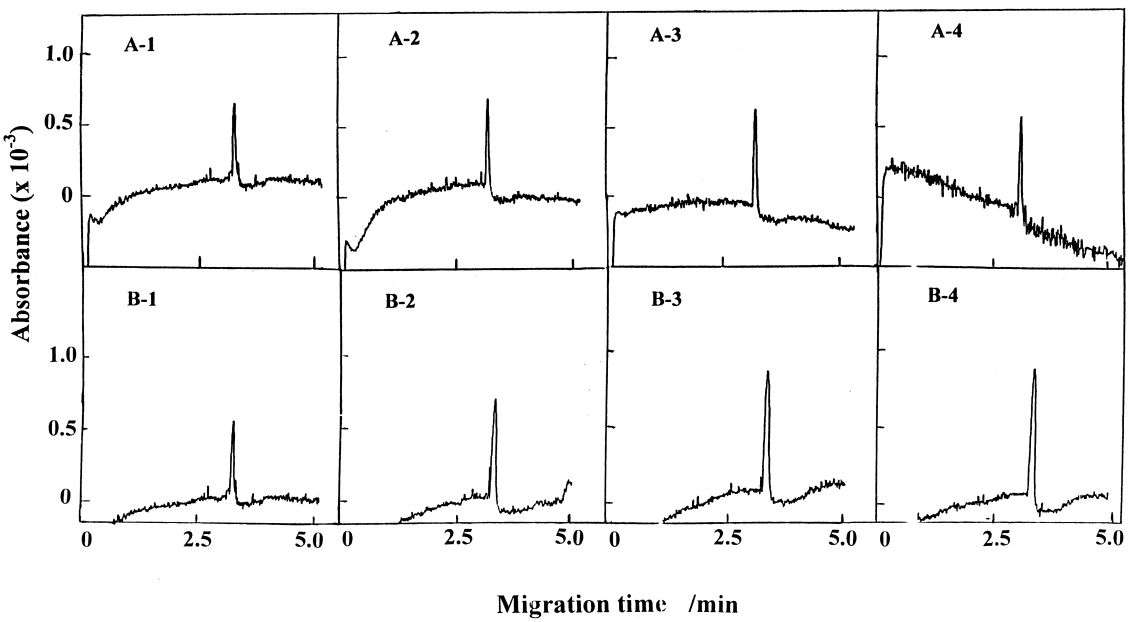


Fig. 7. Panel A shows electropherograms for mixtures with calf-thymus DNA (A-1) and Cr(III)GA–DNA complexes formed in Cr(III)-GA/DNA(P) ratios of 1:80 (A-2), 1:10 (A-3), and 1:4 (A-4) monitored at 260 nm. Panel B shows electropherograms for mixtures with calf-thymus DNA (B-1) and Cr(III)-EGA–DNA complexes formed in Cr(III)-EGA/DNA(P) ratios of 1:160 (B-2), 1:80 (B-3), and 1:10 (B-4). The capillary electrophoresis was performed at 25 kV in a run buffer of 7.5 mM Tris-HCl, pH 7.4 containing of 15 mM NaCl.

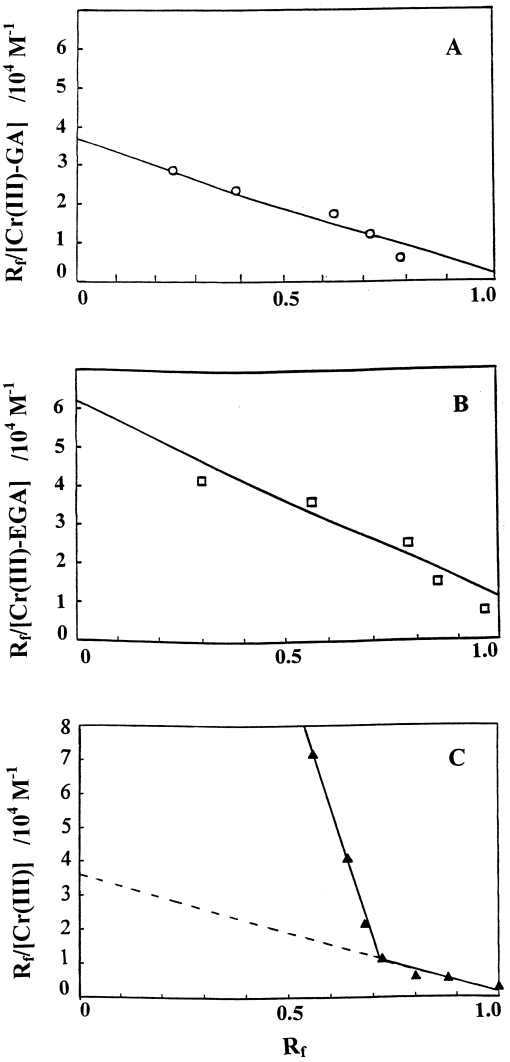


Fig. 8. Scatchard plots for Cr(III)-GA–DNA (A), Cr(III)-EGA–DNA (B), and Cr(III)-DNA (C).

the carboxyl group may strengthen the binding the water ligand to the centered Cr(III). This probably caused indirect binding of the Cr(III)-GA to guanine residues through the water ligand. In contrast, the electron-donating induction effect of the ethyl group in the Cr(III)-EGA may function in a reversed way. As a result, the water ligand might be substituted to form direct coordination-bonding between Cr(III) and the guanine residue. However, at a higher concentration of the Cr(III)-EGA, nonspecific indirect-binding of the complexes to the DNA probably occurred.

In the Cr(III)-tannin complexes, Cr(III) cation bidentately binds two gallic acid or ethyl gallate residues and two water molecules in the fifth and sixth coordination position. This specific structural arrangement prevents chelate and interchelate formations. Furthermore, our FTIR data showed that in the presence of the Cr(III)-tannin complexes the intensity of the band at 1717 cm^{-1}, which corresponds to O6 carbonyl stretching, primarily increased. This implies that the Cr(III)-tannin complexes most likely interact to the guanine N7 moiety. At a higher concentration of the complexes, although the bands for the A–T base pairs were also perturbed, the sugar phosphate band was not significantly perturbed. These results suggest that the Cr(III)-tannin complex primarily binds to a major groove including the guanine residue in the DNA. However, at higher concentrations of the Cr(III)-tannin complexes, the binding may extend to other sites, including A–T base pairs.

At r = 1/20, a reduction in the intensity of the bands for DNA bases was observed. Our study using FTIR did not show any evidence for a conformational change in the calf-thymus DNA. Thus, this phenomenon is not due to a conformational change of the DNA. Rather, a partial structural alteration of the DNA may account for the reduction in the intensity. At r = 1/10, a major increase in the intensity of the DNA vibrations was observed. This probably occurred by helix opening when a large amount of the Cr(III)-tannin complexes bind to the DNA, breaking the hydrogen bondings between the base pairs.

At the highest concentration of the Cr(III)-tannin complexes, a major decrease in the intensity for the DNA vibrations was observed. It is known that the interaction of Cr(III) with calf-thymus DNA caused DNA condensation by chelate formation through guanine N-7 and the proximal backbone phosphate.[18,43] However, the Cr(III)-tannin complexes may not cause condensation because the complexes monodentately bind to guanine N-7 and, as a result, do not form a chelate. It may be speculated that Cr(III)-tannin complexes in one DNA molecule bind to other DNA molecules, and many DNA molecules are gathered by cross-linking through the complexes. Consequently, the binding of Cr(III)-tannin complexes to the DNA causes aggregation rather than condensation.

It has been reported that $CrCl_3 \cdot 6H_2O$ and $Cr(NO_3)_3 \cdot 9H_2O$ initially exist as trans-$[Cr(H_2O)_4Cl_2]^+$ and $[Cr(H_2O)_6]^{3+}$ species at physiological pH and room temperature.[44] These positive-charged species bind to DNA through electrostatic interaction with negative-charged backbone phosphate groups to form an Cr(III)–DNA adducts.[45] This reaction leads to the formation of stable chelate bondings between the phosphate groups and the nearest guanine N-7 in the Cr(III)–DNA complexes, since the chloride and water ligands are good leaving groups and can be readily substituted by guanine residues. A study of the binding of Cr(III)–amino acid complexes to salmon nuclear DNA in vitro showed that the complexes with a large ligand, such as K_2[Cr(III)(L-glutathionate)$_2$]$\cdot 3H_2O$, are more reactive than the adducts with small ligand, like Na[Cr(III)(L-cysteine)$_2$]$\cdot 2H_2O$.[19] It has been speculated that the bulkiness of the glutathionate ligands of the Cr(III)-glutathionate may be responsible for the lability and the ease of displacement of the reaction with the nuclear DNA. Other researchers have reported that the incubation of a plasmid DNA (supercoiled form) with K[Cr(III)(L-cysteine)$_2$]$\cdot 2H_2O$ in the presence of hydrogen peroxide yielded only a slight amount of the open circular form.[46] The amount of the open circular form was almost the same level as that of the control experiment without any Cr(III) compounds. The result implies that the Cr(III)-complex in which the ligand tightly binds to the centered Cr(III) cation is generally inactive towards DNA. Our study using FTIR and capillary electrophoresis showed that Cr(III)-tannin complexes are weakly bound to calf-thymus DNA compared with $CrCl_3$ and $Cr(NO_3)_3$ salts, since stable water and tannin ligands make

it difficult for substitution with the potential sites on the DNA duplex. As a result, the Cr(III)-tannin complexes largely bound to guanine bases indirectly through the water or tannin ligands. Consequently, it is suggested that Cr(III)-tannin complexes are less reactive towards DNA compared with hydrated Cr(III). Taken together, tannin substances such as a gallic acid, might reduce the genotoxicity of chromium by the coordination of Cr(III), following the reduction of Cr(VI) inside cells.

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