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Studies on DNA Binding to Metal Complexes of Sal_2trien

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Abstract—The complexes [Fe(Sal_2trien)]NO_3 and Cu(Sal_2trien) have been synthesized and their interaction with calf thymus DNA has been investigated for the first time using UV spectra, fluorescence spectra, thermal denaturation, and viscosity measurements. The experimental results show conformably that the mode of binding of the complex [Fe(Sal_2trien)]NO_3 to DNA is nonclassical electrostatic action, but the mode of binding of the complex Cu(Sal_2trien) to DNA is classical intercalation.

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Numerous control mechanisms in the living cell are based on the recognition and interaction between biologically active molecules and biomacromolecules [1, 2]. The design of synthetic ligands that read the information in the DNA duplex has been a central goal at the interface of chemistry and biology [3]. Syntheses of DNA binding molecules, such as triplex-forming oligonucleotide [4-7], peptide nucleic acid [8, 9], oligosaccharide [10], and oligopeptide [11, 12] have been exploited. These small molecules are stabilized in binding to DNA through a series of weak interactions, such as the π-stacking interactions associated with intercalation of a planar aromatic group between the base pairs, hydrogen-bonding, and van der Waals interactions of functionalities bound along the groove of the DNA helix [13], and the electrostatic interaction of the cation with phosphate group of DNA. Studies directed toward the design of site- and conformation-specific reagents provide rationales for new drug design as well as a means to develop sensitive chemical probes for nucleic acid structure. Considerable attention has been given to polyamide complexes with metals due to their high affinity and specificity interactions to specific DNA regions [14-18]. Since these polyamides can permeate living cell membranes, they have the potential to control specific gene expression [19, 20]. Furthermore, many useful complexes can be created by polyamides coordinating with other various molecules. The complex [Mg_2(diethylenetriamine)Cl(OH)]$Cl_2 \cdot 2H_2O$ was found to have high cleavage activity toward DNA [21]. Polyamine complex with glucoses synthesized by Liu et al. has great potential in curing congenital genetic and acquired immunity disease because it can transfer nucleic acid medicines safely, innocuously, and highly efficiently [22, 23].

Up to now, study on the spin equilibrium [24], subchronic toxicity [25], and ion flotation [26] of triethylenetetramine–metal complexes have been reported, but there are few reports about the interaction between triethylenetetramine metal complexes and DNA. In this paper the complexes [Fe(Sal_2trien)]NO_3 (Sal = salicylaldehyde, trien = triethylenetetramine) and Cu(Sal_2trien) have been synthesized and their interactions with calf thymus DNA have been investigated for the first time using UV spectra, fluorescence spectra, thermal denaturation, and viscosity measurements.

We have focused our work on complexes of triethylenetetramine–metal complexes, which have been rarely studied concerning their interaction with DNA, but they possess interesting DNA binding properties. The results should be valuable in understanding the mode of the interaction of the complexes with DNA as well as laying a foundation for the rational design of novel powerful agents for probing and targeting nucleic acids.

Abbreviations: CT DNA) calf thymus DNA; DMSO) dimethylsulfoxide; EB) ethidium bromide; Sal) salicylaldehyde; TMS) tetramethyl silicon; trien) triethylenetetramine.

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MATERIALS AND METHODS

Calf thymus DNA (CT DNA) was obtained from Sigma (USA). Ligand was synthesized according to a procedure described elsewhere [24]. Its purity was checked by elemental, FTIR, and NMR analyses. All other chemicals were of analytical reagent grade and used without further purification.

Carbon, nitrogen, and hydrogen analyses were determined using a Perkin-Elmer (USA) 240C elemental analyzer. ^{1}H-NMR and ^{13}C-NMR spectra were measured on a Bruker (Germany) DRX-300 spectrometer in dimethylsulfoxide (DMSO)-d_6 solution, with tetramethyl silicon (TMS) as the internal standard. IR spectra were recorded on a Shimadzu (Japan) FT-IR-8300 instrument using KBr discs in the region 400-4000 cm. Absorbance spectra recorded on a Hewlett-Packard (USA) HP-8453 Chemstation UV-Vis spectrophotometer were employed to check DNA purity ($A_{260}/A_{280} > 1.80$) and concentration (ε = 6600 liter/mol per cm at 260 nm). Fluorescence measurements were made with a Perkin-Elmer Ls-50B spectrophotometer equipped with quartz curettes of 1 cm path length at room temperature. The excitation and emission slit widths were 10 nm.

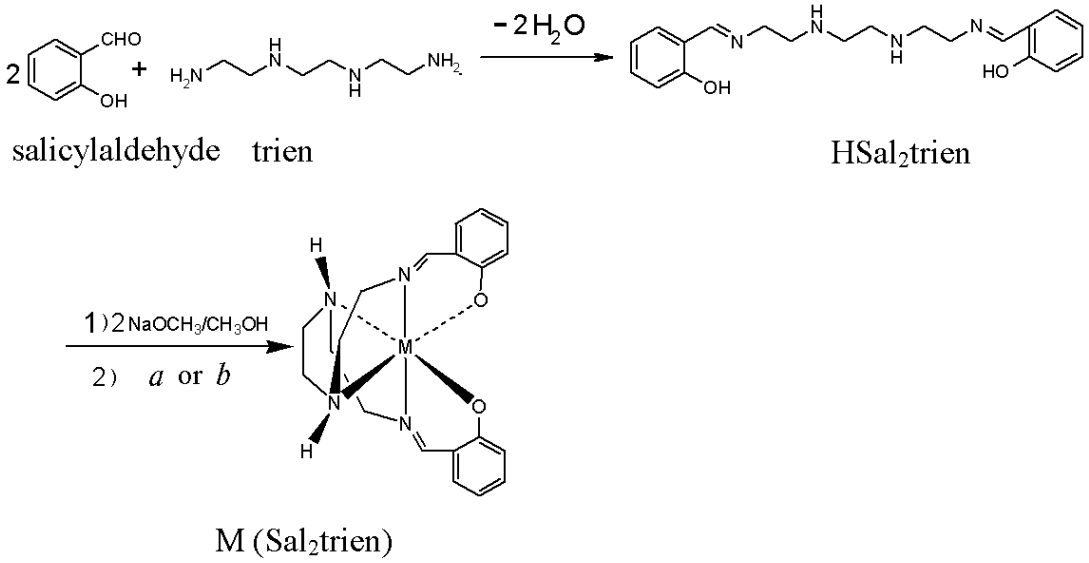
Viscosity was measured using a Ubbelodhe viscosimeter maintained at 25.0 ± 0.1°C. Flow time was measured with a digital stopwatch; mean values of replicated measurements were used to evaluate the viscosity η of the samples. The data are reported as $(\eta/\eta_0)^{1/3}$ vs. the [ML]/[DNA] (ML = [Fe(Sal$_2$trien)]NO_3 or Cu(Sal$_2$trien)) ratio, where η_0 is the viscosity of the DNA solution alone. The DNA melting experiments were carried out by controlling the temperature of the sample cell with a Shimadzu circulation bath, monitoring the absorbance at 260 nm.

The complexes were prepared according to the literature methods [24]. The synthetic route of the complexes is shown on the Scheme below.

The complexes were characterized as follows: [Fe(Sal$_2$trien)]NO_3, black solid. Analytical calculations for $C_{20}H_{24}FeN_5O_5$: C, 51.08; N, 14.89; H, 5.14. Found: C, 51.06; N, 14.86; H, 5.10. ^{1}H-NMR (300 MHz, DMSO-d_6): δ 8.2 (s, 2H), 7.4 (d, 2H), 7.1 (m, 2H), 6.8 (m, 4H), 3.68 (t, 4H), 2.93 (t, 4H), 2.68 (s, 4H), 2.0 (s, 2H); ^{13}C-NMR (DMSO-d_6): δ 163.8, 157.8, 132.5, 130.6, 124.6, 121.5, 116.0, 28.3, 23.2, 21.5; IR (KBr) ν: 3436.9, 3174.6, 2932.1, 1627.8, 1598.4, 1538.6, 1469.7, 1444.6, 1383.3, 1340.0, 1298.0, 1200.6, 1150.9, 1128.3, 1058.8, 1028.0, 896.8, 798.0, 766.2, 756.5, 738.2, 619.6.

Cu(Sal$_2$trien), green solid. Analytical calculations for $C_{20}H_{24}CuN_4O_2$: C, 57.75; H, 5.82; N, 13.47. Found: C, 57.71; H, 5.77; N, 13.46. ^{1}H-NMR (300 MHz, DMSO-d_6): δ 8.1 (s, 2H), 7.5 (d, 2H), 7.1 (m, 2H), 6.6 (m, 4H), 3.68 (t, 4H), 2.96 (t, 4H), 2.63 (s, 4H), 2.0 (s, 2H); ^{13}C-NMR (DMSO-d_6): δ 163.8, 157.8, 132.6, 130.6, 124.8, 121.6, 116.0, 28.5, 23.4, 21.8; IR (KBr) ν: 3563, 2300 (wide peak), 1634, 1599, 1542, 1447, 1398, 1349, 1312, 1248, 1197, 1152, 1127, 1093, 901, 862, 767 cm^{-1}.

Buffered aqueous solutions of DNA (0.6 mM) were prepared with water purified on a Millipore (France) apparatus. Phosphate (10 mM) buffer (pH 7.0) containing 1 mM EDTA was used. No added salt or support electrolyte was used. These solutions were dialyzed for three days with continuous agitation on a shaker bath at 30°C. Free complex concentrations were determined from the



Synthetic route of the complexes: *a* = $Fe(NO_3)_3 \cdot 9H_2O$, *b* = $CuCl_2 \cdot 2H_2O$, M = Fe(III) or Cu(II)

Scheme

dialyzate by absorbance measurements at 279 nm (ε = 17,164 $M^{-1}\cdot cm^{-1}$).

Aqueous solutions of the complexes [Fe(Sal_2trien)]NO_3 and Cu(Sal_2trien) were prepared by dissolving in water at a concentration of $1.58\cdot10^{-4}$ and $1.0\cdot10^{-4}$ M, respectively. The absorption titration was performed by keeping the concentration of CT DNA ($1.5\cdot10^{-4}$ M) constant with varying the complex concentration (for [Fe(Sal_2trien)]NO_3, (0-6)$\cdot10^{-6}$ M; for Cu(Sal_2trien), (0-7)$\cdot10^{-6}$ M). The absorption was recorded after each addition of the complex. The fluorescence spectra (λ_{ex} = 520 nm) were also recorded at room temperature. All solutions were allowed to equilibrate thermally for about 30 min before measurements.

RESULTS AND DISCUSSION

DNA binding studies are important for the rational design and construction of new and more efficient drugs targeted to DNA [27]. A variety of small molecules interact reversibly with double stranded DNA, primarily through three modes: (i) electrostatic interactions with the negatively charged nucleic sugar-phosphate structure, which are along the external DNA double helix and do not possess selectivity; (ii) binding interactions with two grooves of DNA double helix; and (iii) intercalation between the stacked base pairs of native DNA. Heterocyclic dyes, such as ethidium, anthracyclines, phenothiazines, and acridine derivatives interact through intercalation with the planar, aromatic group stacked between base pairs [28-30]. So as to explore the binding mode of the complexes with DNA, the experiments as follow have been carried out.

Effect of the complexes on the fluorescence spectra of DNA–ethidium bromide (EB) complex. To investigate the mode of binding of the Fe(III) and Cu(II) complexes to DNA, competitive binding experiments were carried out. The fluorescent emission of EB (2 μM) bound to DNA (20 μM) in the absence and the presence of the complexes are shown in Fig. 1. Ethidium bromide is a conjugate planar molecule. Its fluorescence intensity is very weak, but it is greatly increased when EB is specifically intercalated into the base pairs of double-stranded DNA. When EB is free from DNA, the fluorescence of DNA–EB complex is clearly quenched. Therefore, EB can be used as a probe for DNA structure detection [31]. [Fe(Sal_2trien)]NO_3 and Cu(Sal_2trien) do not show appreciable fluorescence in the spectral region studied, either free of or bound to DNA, and do not quench the fluorescence of EB in the absence of DNA under the conditions of our experiments. The emission band at 590 nm of the DNA–EB system increased in intensity with increasing the concentration of Fe(III) complex, but decreased with increasing the Cu(II) complex concentration (Fig. 1). Since intercalated EB is the only fluorescent species, the fluorescence increase indicates that the Fe(III) complex can make a contraction in the helix axis of DNA by electrostatic binding to the phosphate group of the DNA backbone and the observed fluorescence decrease indicates that the Cu(II) complex can replace EB inside the DNA cavities. Such a characteristic change is often observed in the intercalative DNA interaction [32].

Scatchard plots. To get a better insight into the nature of complex–DNA binding, the binding of EB to DNA was investigated in the absence and presence of the competing metal complexes by fluorescence spectra, which can be expressed by Scatchard equation [31]:

$$r/C_f = K(n - r),$$

where r is the ratio of bound EB to total DNA concentration, C_f is the concentration of free EB, n is the num-

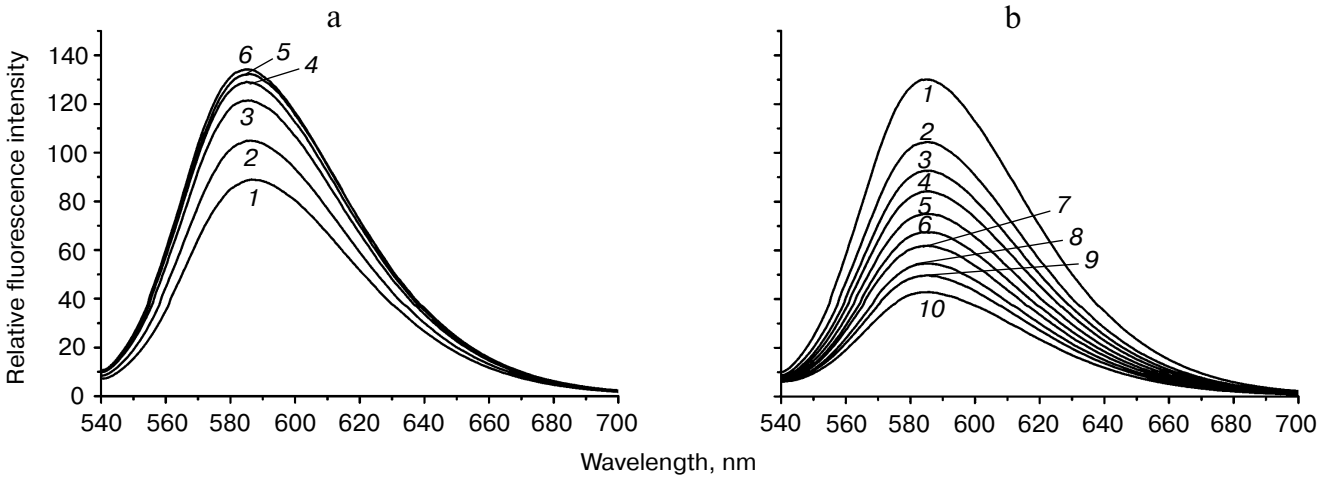


Fig. 1. Fluorescence emission spectra (excited at 520 nm) of EB–DNA in the absence (*1*) and presence of increasing concentrations of the Fe(III) (*2-6*) (a) and Cu(II) (*2-10*) (b) complex (5 μl per scan was added).

ber of binding sites per nucleic acid, and K is the intrinsic binding constant for EB. Fluorescence Scatchard plots for the binding of EB to CT DNA ($1.5 \cdot 10^{-6}$ M) in the absence and presence of the Fe(III) or Cu(II) complexes are given in Fig. 2. In Fig. 2a, the K has nearly no change, which indicates that the Fe(III) complex binds to DNA by a noncompetitive inhibition [27], namely, the interaction between the Fe(III) complex and DNA is through the electrostatic mode. In Fig. 2b, the K change suggests that the Cu(II) complex binds to DNA by a competitive inhibition [33]. This is due to the insertion of the planar benzene ring into DNA, blocking potential intercalation sites of EB and competing for the intercalative binding sites with EB.

Effect of the complexes on UV spectra of DNA. "Hyperchromic" effect and "hypochromic" effect are the spectra features of DNA concerning its double-helical structure [34]. The spectral change process reflects the corresponding changes in DNA in its conformation and structures after the drug bound to DNA. Hypochromism results from the contraction of DNA in the helix axis, as well as from the change in conformation on DNA; in contrast, hyperchromism derives from damage to the DNA double-helix structure [34, 35]. As shown in Fig. 3, the absorption spectra of DNA decrease with increasing the Fe(III) complex concentration. This is a typical "hypochromic" effect. Therefore, the result indicates the interaction between Fe(III) complex and DNA is through

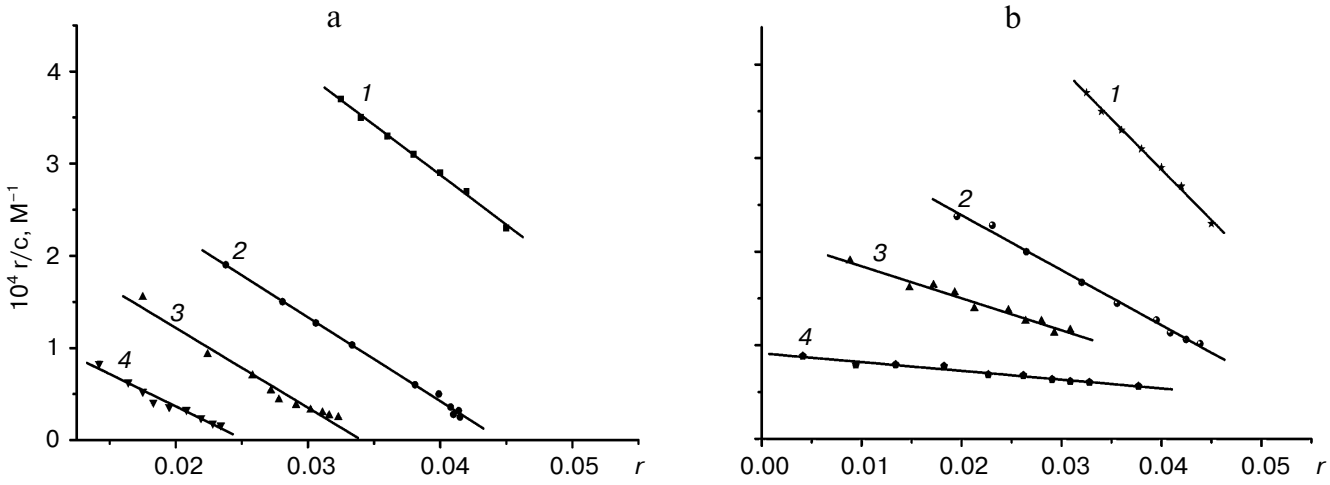


Fig. 2. Fluorescence Scatchard plots of the binding of EB to CT DNA in the absence (*1*) and the presence (*2-4*) of increasing Fe(III) (a) and Cu(II) (b) complex concentrations. For graphs *2* to *4*, [Fe(III) or Cu(II) complex]/[DNA] ratios are 0.25, 0.5, 1.0, respectively. $C_{DNA} = 1.5 \cdot 10^{-6}$ M.

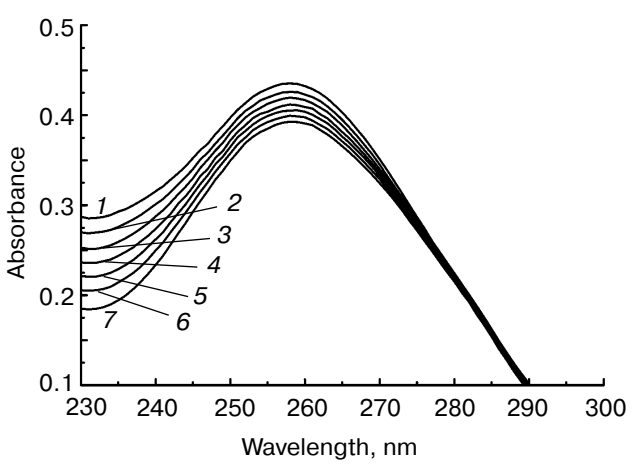


Fig. 3. Absorption spectra of calf thymus DNA ($1.5 \cdot 10^{-4}$ M) in Tris-HCl buffer upon the addition of [Fe(Sal_2trien)]NO_3. For spectra *1-7*, [Fe(Sal_2trien)]NO_3 concentrations are 0, 1, 2, 3, 4, 5, 6 multiplied by 10^{-6} (M), respectively.

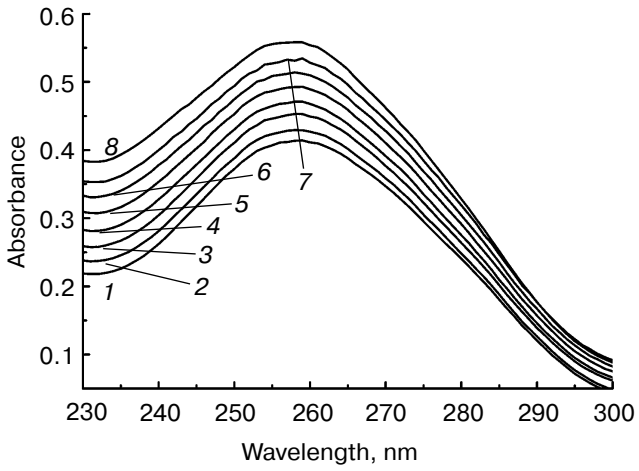


Fig. 4. Absorption spectra of calf thymus DNA ($1.5 \cdot 10^{-4}$ M) in Tris-HCl buffer upon addition of Cu(Sal_2trien). For spectra *1-8*, Cu(Sal_2trien) concentrations are 0, 1, 2, 3, 4, 5, 6, 7 multiplied by 10^{-6} (M), respectively.

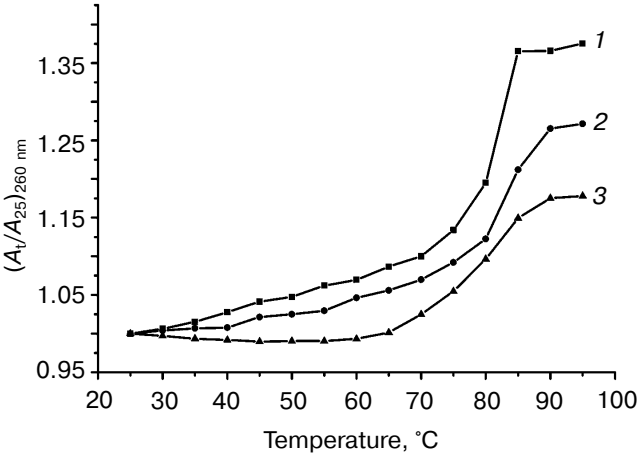


Fig. 5. Plots of $(A_t/A_{25})_{260nm}$ of DNA ($1.24 \cdot 10^{-4}$ M) vs. temperature: *1*) DNA in absence of the complexes; *2*) DNA with a 9 : 1 molar ratio of DNA to $Cu(Sal_2trien)$; *3*) DNA with a 9 : 1 molar ratio of DNA to $[Fe(Sal_2trien)]NO_3$.

electrostatic mode, namely, the Fe(III) complex can not only cause contraction in the helix axis of DNA by electrostatic binding to the phosphate group of DNA backbone, but also results in the change of DNA conformation. In contrast, as shown in Fig. 4, the absorption spectra of DNA increase with increasing Cu(II) complex concentration. This is a typical "hyperchromic" effect, which suggests that the DNA double-helix structure is damaged after the Cu(II) complex bound to DNA through intercalation mode.

Thermal denaturation experiments. Other strong evidence for the binding mode between the complexes and DNA was obtained from DNA melting studies. The intercalation of small molecules into the double helix is known to significantly increase the helix melting temperature, at which the double helix denatures into single-stranded DNA [36, 37]. The extinction coefficient of DNA bases at 260 nm in the double-helical form is much less than that in the single-stranded form [38, 39]; hence, the melting of the helix leads to an increase in the absorption at this wavelength. Thus, the helix-to-coil transition temperature can be determined by monitoring the absorbance of DNA bases at 260 nm as a function of temperature (T_m). However, the T_m will increase lightly (<0.6°C) on the interaction of small molecules with DNA through nonspecific electrostatic interactions with the phosphate backbone of DNA [40]. The DNA melting curves in the absence and in the presence of the complexes are presented in Fig. 5. The T_m of DNA is 80°C in the absence of the complexes and 80.3 and 85°C in the presence of $[Fe(Sal_2trien)]NO_3$ and $Cu(Sal_2trien)$, respectively. Therefore, the interaction of $[Fe(Sal_2trien)]NO_3$ with DNA is through nonspecific electrostatic interactions with the phosphate backbone of DNA, while the interaction between $Cu(Sal_2trien)$ and DNA is intercalation.

Viscosity study. To further clarify the nature of the interaction between the complexes and DNA, viscosity was measured. DNA is a polyanion. In solution, repulsion among negative charges makes the DNA molecule more extended. When the cations of complex bind by electrostatic interaction to the phosphate group of DNA backbone, the negative charges of DNA are partially neutralized. This leads to the contraction of the DNA helix, shortening of the DNA molecule, and decrease in viscosity of the DNA. In Fig. 6, the specific viscosity of the DNA sample clearly decreases with the addition of the Fe(III) complex. Therefore, it can be confirmed that the binding between the Fe(III) complex and DNA is through electrostatic interaction with the phosphate group of the DNA backbone. In Fig. 7, the specific vis-

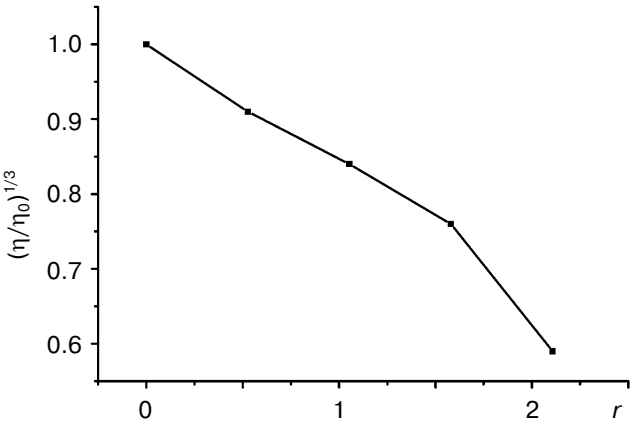


Fig. 6. Effect of increasing amount of $Fe(Sal_2trien)NO_3$ on the relative viscosity of CT DNA at 25.0 ± 0.1°C. [DNA] = 0.15 mM; r = [FeL]/[DNA] ($\times 10^{-2}$).

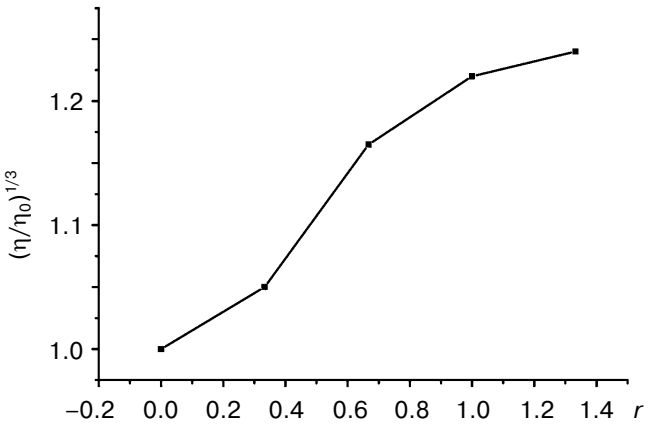


Fig. 7. Effect of increasing amount of $Cu(Sal_2trien)$ on the relative viscosity of CT DNA at 25.0 ± 0.1°C. [DNA] = 0.15 mM; r = [CuL]/[DNA] ($\times 10^{-2}$).

cosity of the DNA sample clearly increases with the addition of the Cu(II) complex. The viscosity studies provide a strong argument for intercalation [41]. The viscosity increase of DNA is ascribed to the intercalative binding mode of the drug because this could cause the effective length of the DNA to increase [42]. In essence, the length of the linear piece of B-form DNA is given by the thickness of the base pairs that are stacked along the helix axis in van der Waals contact with each other. Introducing another aromatic molecule into the stack therefore increases the length. So, we think the viscosity increase of the DNA caused by the addition of the Cu(II) complex can provide further support for the intercalative mode of the Cu(II) complex.

The experimental results described above together suggested that the interaction between the Fe(III) complex and DNA is attributed to the electrostatic effect, while the interaction of the Cu(II) complex with DNA is through intercalation. This can be explained by the points as follows. On one hand, $[Fe(Sal_2trien)]^+$ has a positive charge which makes it apt to act at the phosphate group of DNA electrostatically, while being blocked from insertion of the benzene ring into the base pairs of DNA. On the other hand, $Cu(Sal_2trien)$ is able to intercalate into the base pairs of DNA owing to suitable steric exclusion and no positive charge.

In summary, the experimental results taken together indicate that the mode of binding of the complex $[Fe(Sal_2trien)]NO_3$ to DNA is electrostatic action, whereas the mode of binding of the complex $Cu(Sal_2trien)$ to DNA is intercalation. This is probably because $[Fe(Sal_2trien)]^+$ has one positive charge and $Cu(Sal_2trien)$ has no charge. The results should be valuable in understanding the mode of interaction of the complexes with DNA as well as laying a foundation for the rational design of novel powerful agents for probing and targeting nucleic acids.

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