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ORIGINAL PAPER

Biophysical, spectroscopic vis-à-vis biochemical investigation on DNA–metalloprotein interaction: a model study involving cobalt(II)-glutathione complex

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Abstract The interaction of cobalt(II)-glutathione (CoGSH) with deoxyribonucleic acid (DNA) has been studied by UV–vis, fluorescence, circular dichroism (CD), thin-film infrared (IR), and viscometric techniques. From the UV-spectroscopic method, binding constant (K_b) was determined and was found to be 2.3×10^6 M^{-1}. In fluorimetric analysis, the quenching of fluorescence intensity of DNA bound to ethidium bromide (EB) was investigated. The Stern–Volmer quenching constant (K_{sv}) was also estimated from this study and was found to be 2.8×10^6 M^{-1}at 37 °C. The solution CD spectra of DNA and DNA–CoGSH indicate that in each case, DNA exists in the 'B' conformation and suggested an intercalative binding mode. Thin-film IR data also reveal that DNA attains the 'B' family of conformations after interaction with CoGSH complex. The increase in DNA viscosity in the presence of CoGSH complexes is attributed to the lengthening of DNA helix due to intercalation.

Keywords Calf thymus DNA · Glutathione · Electronic absorption spectra · Circular dichroism (CD) · Intercalation · Fluorescence quenching · Viscometric studies

Introduction

The genetic information used to build a variety of protein molecules from the constituent amino acids is stored in the nucleic acid molecule, the deoxyribonucleic acid (DNA). The copying of the DNA code in polyribonucleic acid form as messenger ribonucleic acid (mRNA), and the replication of DNA requires the involvement of divalent metal ions [1, 2]. There are reports [3, 4] that the biological functioning of DNA also involves participation of metal ions. Co^{2+} is a biologically essential transition metal ion and is an integral part of vitamin B_{12}. It has also been found to remain present in the active sites of many other enzymes, like a group of aminopeptidases, whereby it coordinates with the biological donor sites available in the protein molecules. Various cobalt complexes are reported to have their utility as drugs [5], in hyperbilirubinemia [6], and in viral diseases [7].

Peptides are the backbones of proteins; thus, they play a crucial role in the biological system. Interaction of calf thymus DNA (CT-DNA) and lysine-rich histone fraction reveals that DNA undergoes various conformational changes [8]. The tripeptide γ-glutamyl-cysteinyl-glycine (GSH) is a major low molecular weight thiol compound in plants and animals [9]. It acts as a biological detoxifier and peroxide scavenger [10]. The hematopoietic physiology is also regulated by this molecule and plays a very vital role in gene expression [11]. The above-mentioned biological aspects prompted this study of the binding of cobalt(II)-glutathione (CoGSH) complex with DNA.

Results and discussion

UV-spectral study

Electronic absorption spectroscopy is usually utilized to determine the interaction ratio and binding of complexes with DNA. A complex bound to DNA through intercalation induces a change in absorbance (hypochromism) and red

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shift in wavelength of absorption of the complex due to the stacking interaction between the aromatic chromophore and the DNA base pairs [12–14]. The electronic spectra of the complex in the presence and absence of DNA was monitored at a wavelength of $\lambda = 399$ and 467 nm, respectively. Upon addition of incremental amounts of DNA, a considerable drop in absorptivity was observed, and a sharp isosbestic point was observed at wavelength $\lambda = 433$ nm. This suggests that CoGSH is bound to CT-DNA mainly due to π–π stacking interaction. There was a red shift (3 nm) in the absorption wavelength of the complex on the addition of DNA (Fig. 1).

From the plot of [DNA]/($\varepsilon_a - \varepsilon_b$) versus [DNA] (Fig. 2), the binding constant (K_b) of the complex with DNA was calculated to be 2.3×10^6 M^{-1}. This binding constant is comparable with other classical intercalators [15].

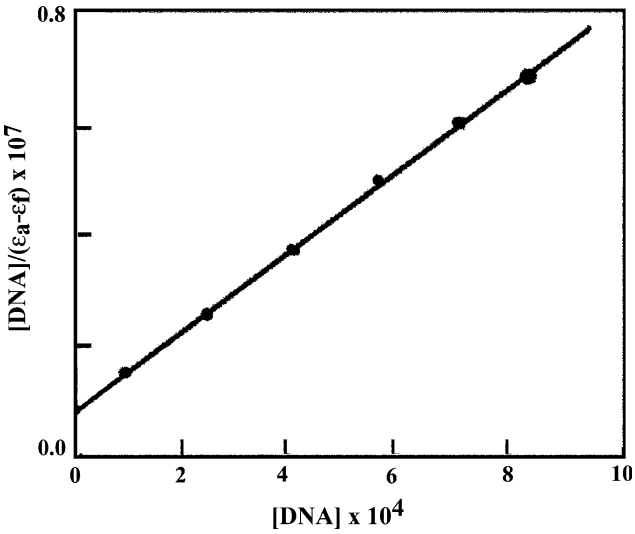

Fig. 2 Plot of [DNA]/($\varepsilon_a - \varepsilon_b$) $\times 10^7$ M/M^{-1} cm^{-1} versus [DNA] $\times 10^4$ M

Fluorimetric study

Fluorimetric competitive binding experiment, using ethidium bromide (EB) as a probe, was performed to establish the binding mode of small molecules to the target molecule, the double-helical DNA. EB emits intense fluorescence in the presence of DNA due to its strong intercalation between the adjacent DNA base pairs. This enhanced fluorescence of EB can be quenched by the addition of a third molecule [16] and that can be used to monitor the mode of binding, thereby indicating the ability of a compound to prevent intercalation of EB to DNA. This kind of quenching of fluorescence by a third molecule has also been found in the case of DNA binding by a number of macrocyclic copper(II) complexes [17, 18].The emission spectra of EB bound to DNA in the absence and in the presence of the complex are given in Fig. 3. The addition of the complex to DNA pretreated with EB causes an obvious reduction in emission intensity, indicating that the complex competes with EB in binding to DNA, which leads to a fast quenching in the fluorescence intensity of the EB-DNA complex system. There occurred a gradual decrease in fluorescence intensity of the EB-DNA system with the concomitant increase in concentration of the CoGSH complex. The significant decrease in intensity lends strong support for the intercalation of complex into the helix of DNA.

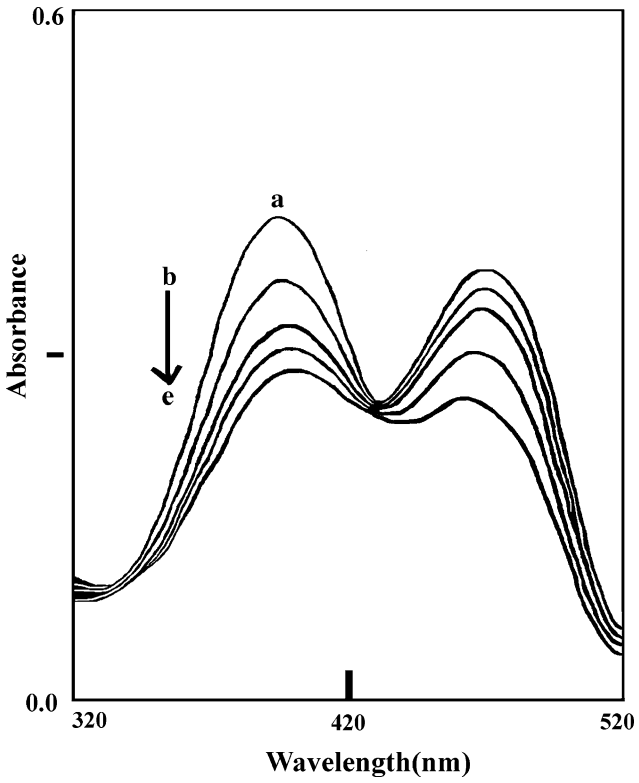

Fig. 1 Absorption spectra of cobalt(II)-glutathione (CoGSH) in the absence (**a**) and presence of increasing amount of DNA (**b–e**), [CoGSH] = 5×10^{-5} M. [DNA]/[CoGSH] = 1.2; 2.0; 2.8; 3.6

Quenching of fluorescence intensity under the influence of the CoGSH complex was further analyzed by the Stern–Volmer equation [19], as indicated below.

$$I_0/\mathrm{I} = 1 + K_{SV}[Q],$$

where I_0 and I are the fluorescence intensities in the absence and presence of quencher (complex), respectively, K_{SV} is the Stern–Volmer quenching constant, which is experimentally obtained from the slope of plot of I_0/I versus [Q]. The shape of the Stern–Volmer plots can be used to characterize the quenching pattern. A plot of I_0/I versus [complex]/[DNA] produces a linear curve (Fig. 4). These changes may suggest that only one kind of

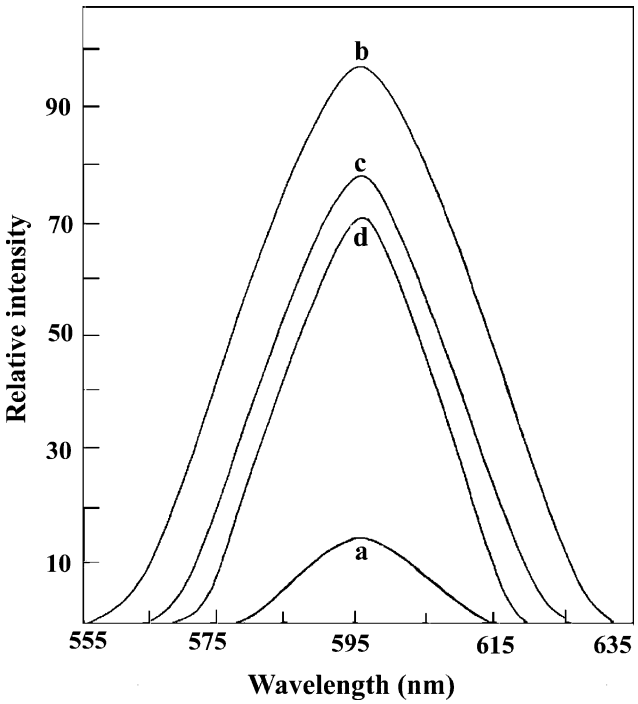

Fig. 3 Effect of addition of the cobalt(II)-glutathione (CoGSH) complex to the emission intensity of the 60 μM calf thymus (CT)-DNA-bound ethidium bromide (EB) (12.5 μM): EB only (**a**) DNA + EB (**b**), DNA + EB + 5×10^{-5} M CoGSH complex (**c**), and DNA + EB + 5×10^{-4} M CoGSH complex (**d**)

quenching process, i.e., dynamic quenching, is involved. The Stern–Volmer quenching constant (K_{SV}) was found to be 2.8×10^6 M^{-1} at 37 °C. The result of this experiment is also in agreement with that of the electronic spectral study.

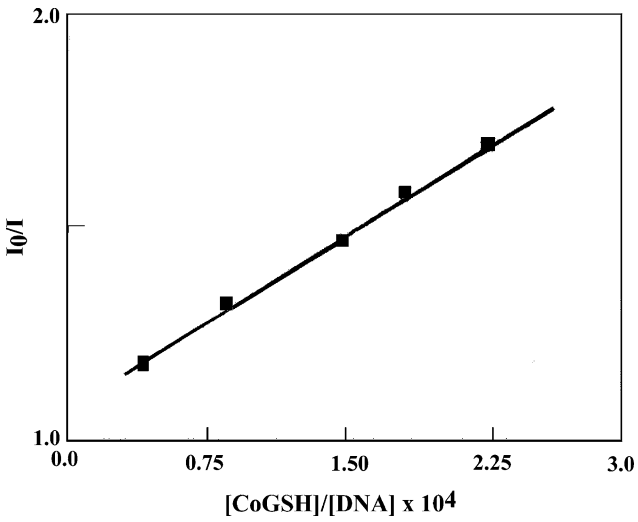

Fig. 4 Stern–Volmer plot for the quenching of fluorescence ethidium bromide (EB) DNA complex caused by the cobalt(II)-glutathione (CoGSH) complex

Circular dichroism study

The results of circular dichroism (CD) studies are shown in Fig. 5. Pure DNA produced a characteristic spectrum with a positive band around 275 nm and a negative band around 245 nm. The solution CD spectra of DNA–CoGSH and that of pure DNA was recorded after 3 h of incubation. It showed that the positive band of pure DNA at 275 nm as well as the negative band at 245 nm retained their positions, with slight decrease in intensity of the negative band and a marginal increase in intensity of the positive band. There appeared another sharp positive band at 220 nm. This type of spectra favours the formation of a more compact arrangement of the DNA backbone.

It was concluded from CD studies that DNA when bound with CoGSH remained in the 'B' family of conformation with a more compact $(B)_n$ type of structure, as was also suggested by Shin and Eichhorn [20] in a different situation.

Viscometric study

Viscometric studies provide the most unambiguous results to corroborate DNA binding pattern in solution. The intercalative binding leads to lengthening of the DNA helix, which in turn increases the viscosity of DNA. On the other

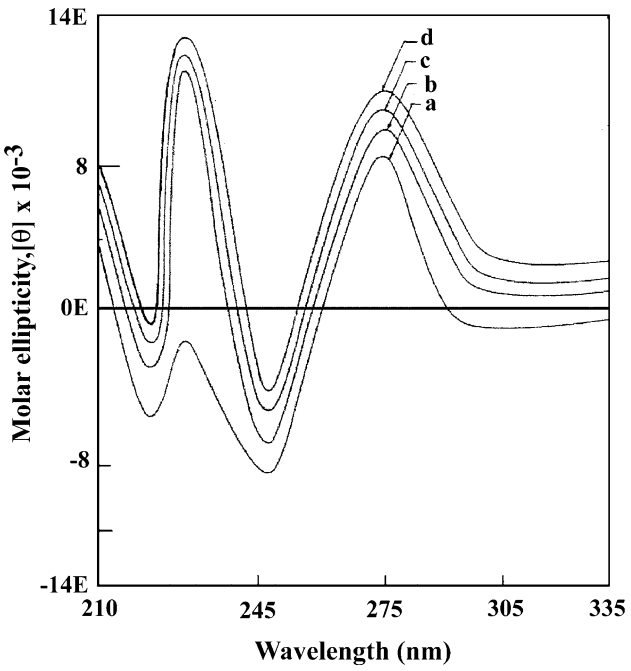

Fig. 5 Circular dichroism (CD) spectrum (deg cm^2 $dmol^{-1}$) of DNA solution in the absence and presence of cobalt(II)-glutathione (CoGSH) complex: 60 μM DNA (**a**), DNA + 5×10^{-5} M CoGSH (**b**), DNA + 1.2×10^{-4} M CoGSH (**c**), DNA + 5×10^{-4} M CoGSH (**d**)

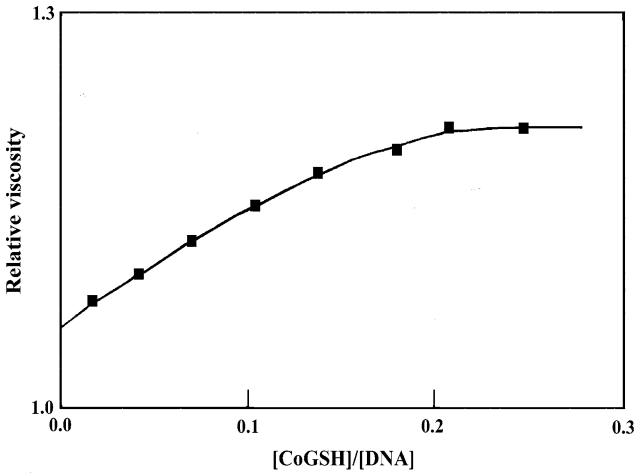

Fig. 6 Effect of increasing the amount of cobalt(II)-glutathione (CoGSH) on the relative viscosity of calf thymus (CT)-DNA at 30 °C

hand, nonintercalative binding could lead to shortening of the DNA helix, thereby reducing the viscosity of DNA, or it may even be static if no appreciable effect on the length of the CT-DNA helix is imparted by the binding molecule [21, 22]. Results of viscometric studies are presented in Fig. 6. It was observed that a steady increase in the viscosity occurred with the addition of the CoGSH complex. The addition of the complex to the CT-DNA solution led to an increase in viscosity of the CT-DNA, thereby clearly demonstrating the intercalative binding of CT-DNA by the CoGSH complex.

Thin film IR study

Thin-film IR spectra of DNA were taken for assessing DNA conformation. There are certain diagnostic IR vibrations that indicate specific DNA conformation. The bands at 835 cm^{-1} (weak) appeared in pure DNA as well as in DNA and CoGSH complex at 84% and 96% relative humidities (RH), which strongly suggest that DNA and its CoGSH complex both remained in the 'B' family of conformation (Fig. 7) [23]. However, pure DNA at 66% RH showed bands at 835 (weak), 1,175 (shoulder), and 860 (shoulder) cm^{-1}, indicating either a coexistence of both 'A' and 'B' forms [24] or a change in the sugar phosphate geometry within the 'B' framework. That the latter proposition is reasonably correct is evident from CD spectrum as well. However, in DNA–CoGSH at 66% RH, the last two bands were not observed, which suggests that DNA in the presence of the above complex remains exclusively in the physiologically stable 'B' family.

It was observed from thin-film IR studies of DNA–CoGSH at various RH values that DNA under the influence of CoGSH preferred to remain essentially in the 'B' family of conformation, irrespective of RH values. This indicated that there occurred no serious change in the DNA backbone. Hence, from the above results, it became apparent that CoGSH helped DNA to remain in the 'B' family of conformation, with a minor change in the packing pattern.

Conclusions

From our studies, it becomes apparent that DNA is bound by the CoGSH complex. EB displacement fluorimetric, CD, and viscometric studies indicate that the binding mode is intercalative. The conformation of DNA was ascertained by CD and thin-film IR studies. DNA attains 'B' conformation after interaction with CoGSH, which is evident from characteristic CD bands. Thin-film IR studies also support this proposition. The intercalative binding of small molecules to DNA is important, particularly in relation to DNA functioning and drug design. Therefore, this study, which reveals the intercalative mode of DNA binding by metallopeptide, indicates that CoGSH may have far-reaching consequences in relation to DNA functioning.

Experimental

Materials

Analytical-grade solvents used for physicochemical studies were further purified by the literature method [25] before use wherever necessary. CT-DNA was purchased from Sigma Chemical Company, USA. Solutions of CT-DNA in Tris–hydrochloric acid (HCl)/sodium chloride (NaCl) (pH = 7.2) buffer medium gave a ratio of A_{260}/A_{280}, of approximately 1.8–1.9, indicating that the DNA was in sufficiently pure form [26]. DNA concentration per nucleotide was determined by absorption spectroscopy using the molar absorption coefficient (6,600 M^{-1} cm^{-1}) at 260 nm [27]. Stock solutions were stored at 4 °C and used within 4 days. While measuring the absorption spectra, equal amount of DNA was added to both the complex solution and the reference solution to eliminate the absorbance of DNA itself.

Preparation of Cobalt(II)-glutathione complex

CoGSH was isolated by following our earlier methods [28]. The compound is tetra coordinated, with a planar geometry around the cobalt center.

UV–Vis spectral study

UV–Vis spectra were recorded in a Hitachi U-3410 spectrophotometer. The electronic spectra of the complex (λ_{399}

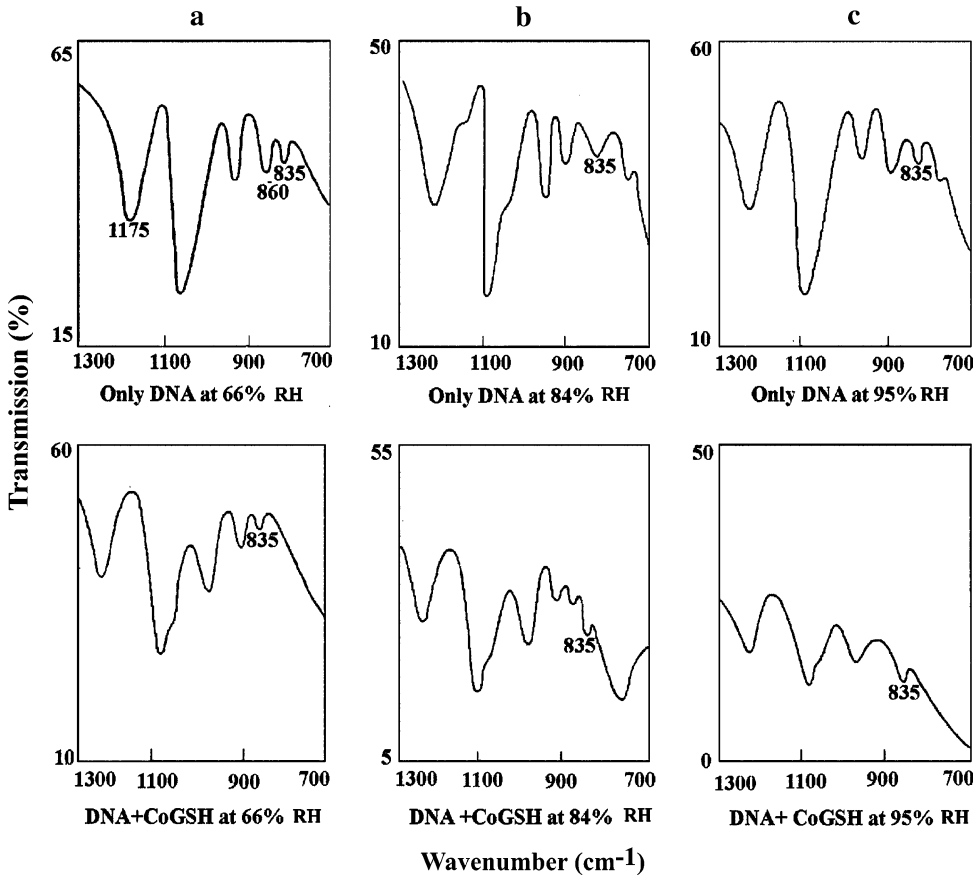

Fig. 7 Thin-film infrared (IR) spectra of 60 μM DNA and different molar ratios of DNA + cobalt(II)-glutathione (CoGSH) mixture: DNA + 5×10^{-5} M CoGSH (**a**), DNA + 1.2×10^{-4} M CoGSH (**b**), DNA + 5×10^{-4} M CoGSH (**c**)

and λ_{467} nm bands) were monitored both in the presence and absence of DNA. The interaction ratio and binding constant for the interaction of complex with DNA was obtained from absorption titration data. A fixed concentration of complex (60 μM) was titrated with increasing amounts of DNA over a range of 60–180 μM. The binding constant was determined using the equation: [29]

$$[\mathrm{DNA}]/(\varepsilon_a - \varepsilon_b) = [\mathrm{DNA}]/(\varepsilon_b - \varepsilon_f) + 1/K_b(\varepsilon_b - \varepsilon_f),$$

where [DNA] is the concentration of DNA in base pairs, the apparent absorption coefficients ε_a, ε_f and ε_b correspond to A_{obsd}/[complex], the extinction coefficient for the free complex, and the extinction coefficient for the complex in the fully bound form, respectively. Plots of [DNA]/($\varepsilon_a - \varepsilon_b$) versus [DNA] gave a slope $1/(\varepsilon_b - \varepsilon_f)$ with intercept $1/K_b$ ($\varepsilon_b - \varepsilon_f$); K_b obtained from the ratio of the slope to the intercept.

Fluorimetric study

Fluorescence emission intensity measurements were carried out using JASCO FP 6500 spectroflourometer. In this binding experiment, 60 μl of CT-DNA (400 μM) solution in Tris–HCl/NaCl buffer (50 mM Tris and 50 mM NaCl, pH 7.2) was added to 2.0 ml of EB (150 μM) in the same buffer medium to get the maximum fluorescence intensity. Aliquots of 1.0 mM stock solution of the complex in H_2O were added to the EB-bound CT-DNA solution and the fluorescence was measured for each test solution after 2 h of incubation at 37 °C. The solutions were excited at 530 nm, whereas the excitation and emission slit was 5 nm and the spectra were recorded in the range 555–635 nm with an emission maximum at 595 nm.

CD study

CD measurements of 60 μM DNA and DNA–CoGSH solution mixture were done in a Jasco-600 spectropolarimeter using a quartz cuvette of path length 1 cm, and spectrum were recorded from 210 to 350 nm.

Viscometric studies

Viscosity of sonicated DNA was measured [30] by fabricated microviscometer immersed in a thermostat at 30 °C. Data are presented as $(\eta/\eta_0)^{1/3}$ versus the ratio of the concentration of metal complex to that of the CT-DNA, where η and η_0 are the viscosities of CT-DNA solutions in the presence and absence of the complex, respectively. Specific viscosity values were calculated from the observed

flow time of CT-DNA-containing solutions (t) and corrected for buffer solution (t_0), $\eta = t - t_0/t_0$.

Thin-film study

Thin film IR spectra of CoGSH–DNA were recorded over IRTran-2 cells. The thin films were prepared by the method described elsewhere [31]. The films formed were equilibrated for 24 h at 66%, 84%, and 95% RH successively, and IR spectra at each relative humidity were recorded.

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