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Thermodynamic and spectroscopic study on the binding of cationic Zn(II) and Co(II) tetrapyridinoporphyrazines to calf thymus DNA: the role of the central metal in binding parameters

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Binding of water-soluble *N*,*N*′,*N*″,*N*‴-tetramethyltetra-3,4-pyridinoporphyrazinatozinc(II) {[Zn(3,4-tmtppa)]$^{4+}$} and *N*,*N*′,*N*″,*N*‴-tetramethyltetra-3,4-pyridinoporphyrazinatocobalt(II) {[Co(3,4-tmtppa)]$^{4+}$} with calf thymus DNA has been investigated in 1 mM phosphate buffer and 5 mM NaCl at low ionic strength by UV-vis, fluorescence, and circular dichroism (CD) spectroscopic techniques, and also by the viscometric method. The appearance of a dramatic hypochromicity without any shift in the Q-band maximum of Zn(tmtppa) in the presence of DNA and a positive ellipticity in the visible CD of this porphyrazine complexed with DNA are definitive evidence of outside binding of this complex. It can be assumed that [Zn(3,4-tmtppa)]$^{4+}$ in water is axially ligated by one H_2O molecule. Therefore, this complex is inhibited from intercalation and binds externally. The interaction of [Co(3,4-tmppa)]$^{4+}$ with DNA causes a decrease of the absorbance in the Q-band region of porphyrazine and a large red-shift. The visible CD of Co(tmtppa) complexed with DNA manifests a pattern of bisignate CD spectra, which possibly lead us to the coexistence of intercalation and outside binding modes. The binding constants were determined from the changes in the Q-band maximum of the porphyrazine spectra using SQUAD software. The values of K were $(8.9 \pm 0.3) \times 10^4$ and $(2.3 \pm 0.0) \times 10^5$ M^{-1} for [Co(3,4-tmtppa)]$^{4+}$ and [Zn(3,4-tmtppa)]$^{4+}$, respectively, at 27 °C. The higher affinity of the zinc complex towards DNA with respect to the cobalt complex was attributed to the coordination interaction as the major contributing factor, which enables superior interaction of the zinc complex with the DNA duplex. The thermodynamic parameters (ΔG°, ΔH°, ΔS°) were calculated from the van't Hoff equation at various temperatures. The enthalpy and entropy changes were determined to be: $+39.6 \pm 3.0$ kJ mol^{-1} and $+226.7 \pm 10.5$ J mol^{-1} K^{-1} for [Co(3,4-tmtppa)]$^{4+}$ and $+44.0 \pm 3.3$ kJ mol^{-1} and $+249.6 \pm 11.3$ J mol^{-1} K^{-1} for [Zn(3,4-tmtppa)]$^{4+}$. The positive and large values of the entropy and enthalpy suggest that both hydrophobic and electrostatic interactions may play an important role in the stabilization of the complex. The influence of the ionic strength was investigated. It was concluded that the apparent binding constants decrease with increasing [Na^+] as predicted. Titration of DNA with Zn(tmtppa) produces moderate decreases in the solution-reduced viscosity (SRV), indicative of outside binding. The increase in the viscosity of DNA in the presence of the cobalt complex is related to the lengthening of the DNA helix due to the intercalation. The quenching of the DNA–ethidium bromide complex by the above-mentioned porphyrazines was investigated. The values of the quenching constants (K_{SV}) and the rate constants of the quenching (k_q) were determined by the Stern–Volmer equation.

Introduction

DNA plays a fundamental role in the storage and expression of genetic information in a cell. Various ligands interact with DNA and regulate its function, and some of them are used as antitumor or antibiotic agents.[1] Among them, cationic porphyrins containing four pyrrole rings represent an expanding class of compounds, which have applications in biology, medicine and catalysis. In addition to their chemical and photophysical properties, porphyrins have been studied from the viewpoint of their role as DNA cleavers.[2] In addition to their applications in cancer chemotherapy, porphyrin species also appear to exhibit antiviral activity. A patent application and a note[3–5] reveal that even in the absence of light, certain porphyrins can inhibit replication of HIV, the virus which is responsible for acquired human immune deficiency syndrome (AIDS).

Recently, the attention on photosensitizers has been focused on porphyrins and their analogs, because their presence in natural systems makes them ideal candidates for use in biological singlet oxygen generation and DNA cleavage. Porphyrins and their derivatives have the ability to absorb several wavelengths in the UV-vis range. The long-lived triplet states of many porphyrins and their derivatives allow for high quantum yields.[6,7]

Studies on the interaction of cationic porphyrins and their derivatives with DNA have been pursued in recent years. Three major binding modes have been proposed for cationic porphyrin binding to DNA: intercalation and two types of outside binding. The first outside binding involves both placement of porphyrin in the grooves and electronic interaction with the phosphate backbone, while the second type is the stacking of the porphyrin along the DNA helix. The spectroscopic

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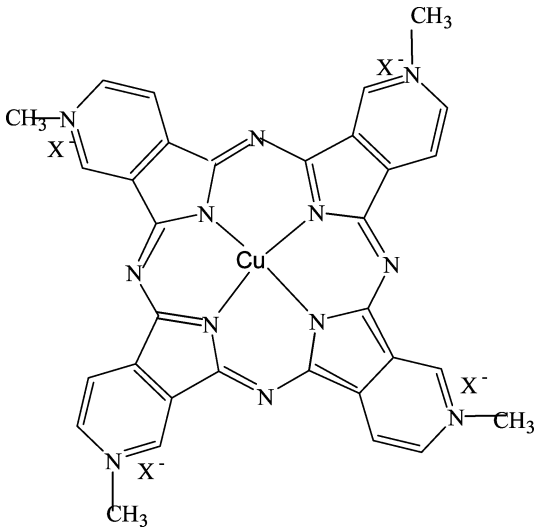
signatures of intercalation include a bathochromic shift of ≥ 8 nm in the Soret region and a considerable hypochromicity, as well as a negative induced CD signal. In contrast, external binding produces a small hypochromism, or even hyperchromism, and a comparatively small bathochromic shift of 4 or 5 nm. Moreover, groove binding generally induces a positive induced CD signal.[8–16] The most extensively studied DNA-binding, water-soluble porphyrins are *meso*-tetrakis(*N*-methylpyridinium-4-yl) porphyrin (H_2TMpyP) and its metal derivatives. Depending on the metal ion, they display different binding modes. Porphyrins without axial ligands (free base, Cu^{2+} and Ni^{2+} derivatives) can readily intercalate between GC base pairs.[17] It has been reported that, for obvious steric reasons, metalloporphyrins with one or more axial ligands (such as Zn^{2+} and Fe^{2+} derivatives) exhibit external binding, mostly at the AT regions.[18]

Phthalocyanines differ from porphyrins by having nitrogen atoms link the individual pyrrol units. They have been extensively studied as DNA binders. A long triplet lifetime and a relatively high triplet quantum yield, which are useful qualities for a photosensitizer, characterize metallophthalocyanines containing diamagnetic metal ions such as Al^{3+} and Zn^{2+}.[19] Positively charged phthalocyanines possess promising photosensitizing properties for the photodynamic therapy of cancer, showing a higher photodynamic activity *in vitro* than the commonly used haemato porphyrin.[20] It has been observed that the uptake and the killing of cells are higher for positively charged sensitizers than for neutral or negatively charged ones.[21]

Tetrapyridinoporphyrazines are phthalocyanine aza analogs in which four pyridine moieties formally substitute four benzene moieties in the macrocycle. These compounds are synthesized from dicyanopyridine or pyridinedicarboxylic acid. The pyridine nature of these compounds allows easy quaternization in order to modulate the solubility of the tetracationic derivatives. Thus water-soluble compounds can be obtained,[22] which some of them existing largely as monomers in aqueous solutions.[23,24]

Even though there has been considerable research into the application of porphyrins and phthalocyanines for their binding to nucleic acids,[25–27] the tetramethylmethalloporphyrazines have received little attention. The present work was undertaken to determine the equilibrium binding processes of *N*,*N*′,*N*″,*N*‴-tetramethyltetra-3,4-pyridinoporphyrazinatozinc(II) {[Zn(3,4-tmtppa))]$^{4+}$} and *N*,*N*′,*N*″,*N*‴-tetramethyltetra-3,4-pyridinoporphyrazinatocobalt(II) {[Co(3,4-tmtppa)]$^{4+}$}shown in Scheme 1



Scheme 1 Structures of the metalloporphyrazine complexes [M(3,4-tmtppa)]$^{+}$; M = Zn, Co; X = CH_3SO_4.

to calf thymus DNA (ctDNA). In this way binding constants, thermodynamic parameters, binding modes, the values of the Stern–Volmer constant (K_{SV}) and the rate constants for the quenching (k_q) were determined. It is clear that a complete understanding of the porphyrazine–DNA binding modes and factors that influence them helps us to design new anticancer, antiviral and antibacterial drugs.

Experimental

[Zn(3,4-tmtppa)]$^{4+}$ and [Co(3,4-tmtppa)]$^{4+}$ were synthesized and purified by the method described previously.[23,28] The stock solution of porphyrazine complexes (1 mg ml^{-1}) were prepared in 1 mM phosphate buffer, 5 mM NaCl, pH 7.0 and stored in the dark at 5–10 °C. Dilutions of complex stock solutions in the appropriate buffer were prepared immediately before use and their concentrations determined spectrophotometrically by measuring the molar extinction coefficient ε. The molar extinction coefficients for porphyrazines were determined as follows. The absorbances were measured for a 1 mM phosphate buffer, pH 7, 5 mM NaCl, to which porphyrazines had been added over a concentration range of 1.56×10^{-6}–2.28×10^{-5} M and 2.1×10^{-6}–4.2×10^{-5} M for the cobalt and zinc complexes, respectively. Linear plots were obtained with correlation coefficients of 0.996 and 0.990 for Zn(tmtppa) and Co(tmtppa). The value of ε for the zinc complex at 686 nm under our experimental conditions was $(9.99 \pm 0.16) \times 10^4$ M^{-1} cm^{-1} at 27 °C; the corresponding value for the cobalt complex at this temperature was $(9.94 \pm 0.28) \times 10^4$ M^{-1} cm^{-1} at its Q band (656 nm).

Calf thymus DNA was obtained from Sigma Chemical Co. and was used without further purification. Stock solutions of DNA (1 mg ml^{-1}) were prepared in the same phosphate buffer and their concentrations in base pairs determined spectrophotometrically with the extinction coefficient of DNA = 1.32×10^4 M^{-1} cm^{-1} at 260 nm.[29]

All other chemical reagents were analytical grade and purchased from Sigma Chemical Co., Merck and Aldrich companies. All experiments were carried out in doubly distilled water. Porphyrazine–DNA solutions were formed with slow mixing of reagents at appropriate concentrations. Beer's law was followed during the titration.

Absorbance measurements were taken on a Jasco UV-vis double beam spectrophotometer, model V-530, using a 1 cm optical path quartz cell with a Lauda Ecolin RE 104 thermostat, which controlled the temperature of the cell compartment with precision of ±0.1 °C.

CD spectra were recorded on a Jasco J-715 spectropolarimeter (Japan). In the visible region, results were expressed as Δε (in M^{-1} cm^{-1}). The spectra were obtained from ten and two times scans of the visible region. The instrument was calibrated with (+)-10-camphor sulfonate, assuming $[\theta]_{291} = 7820$ deg cm^2 dmol^{-1}, and with Jasco standard non-hygroscopic ammonia (+)-10-camphor sulfonate, assuming $[\theta]_{290.5} = 7910$ deg cm^2 dmol^{-1}. Noise in the data was smoothed using Jasco J-715 software including the fast Fourier-transform noise reduction routine, which allows enhancement of most noisy spectra without distorting their peak shape.[30]

Fluorescence titration experiments were performed at 27 °C, using a Perkin–Elmer LS-2B spectrophotometer. The emission spectra were recorded through a fluorimeter optical cut-off filter using a continuous interference filter fixed at 604 nm as the emission wavelength. We used a xenon lamp as light source. The excitation wavelength was 490 nm.

The viscosity measurements were conducted using an Ostwald capillary viscometer. The temperature of the measurement was maintained at 27 ± 0.1 °C using a temperature-controlled circulating water bath. Several time readings were obtained at each titration point.

Results and discussion

Absorption spectroscopy

The results of a titration experiment involving [Co(3,4-tmtppa)]$^{4+}$ and [Zn(3,4-tmtppa)]$^{4+}$ with ctDNA are shown in Figs. 1 and 2. The DNA concentration varied from zero to 8.35×10^{-5} M and the concentration of porphyrazines was 1.00×10^{-5} M for the zinc complex and 1.08×10^{-5} M for the cobalt complex. The absorption spectrum of [Zn(3,4-tmtppa)]$^{4+}$ displays a Q band at 686 nm. A considerable hypochromicity without any red-shift in the Q band of porphyrazine is caused by its interaction with the DNA surface. It can be deduced that [Zn(3,4-tmtppa)]$^{4+}$ in water is axially ligated by one H_2O molecule, as in five-coordinate Zn(TMpyP), which is known as a typical external groove binder.[31] In other words, this complex is inhibited from intercalation and externally binds. The large hypochromicity suggests that the porphyrazine π electrons are perturbed considerably upon binding to DNA. The interaction of [Co(3,4-tmppa)]$^{4+}$ with DNA causes a decrease in absorbance in the Q-band region of porphyrazine and a substantial red-shift from 656 nm to 670 nm. This shift possibly points to noncovalent interaction of the porphyrazine–DNA complex, which reduces the exposure of [Co(3,4-tmtppa)]$^{4+}$ to water due to the intercalation binding mode.

The absorption spectra changes of zinc and cobalt porphyrazines in the presence of DNA reflect the extent of porphyrazine–DNA complexation and make possible the determination of the binding constant, K, using the SQUAD program. This program has been developed to enable the evaluation of the best set of binding constants of the proposed equilibrium model by employing a nonlinear least-squares approach.[32,33] The input data consist of the absorbance values and the total DNA and porphyrazine concentrations. The Gauss–Newton nonlinear least-squares algorithm is used for minimizing the residual sum of squares, U, which is calculated from eqn. (1):.

$$U = \sum_{i=1}^{I} \sum_{k=1}^{NW} \left(A_{i,k}^{cal} - A_{i,k}^{obs} \right)^2 \qquad (1)$$

where $A_{i,k}^{obs}$ is the absorbance value of the i^{th} solution at k^{th} wavelength, give a total of I solutions and a grand total of NW wavelengths (in our experiments $I = 15$ and $NW = 50$). The output data are the logarithm of the macroscopic binding constant (K_{ij}) for formation of D_iP_j, where D is DNA and P is porphyrazine corresponds to the following equilibrium:

$$iD + jP \leftrightarrow D_iP_j \qquad (2)$$

The values of U and the percent error represent the uncertainty in $\log K_{ij}$ calculated by the program. The absorption data were analyzed by assuming 1 : 1 or 2 : 1 and/or simultaneous 1 : 1 and 2 : 1 molar ratios of porphyrazine to DNA. Fitting of the experimental data (15 points), to the proposed stoichiometric models was evaluated by the sum of squares of the calculated points by the model. The results show that the best fitting corresponds to the 1 : 1 complex model at studied temperatures with the residual sum of squares, U, ranging between 10^{-4}–10^{-3}.

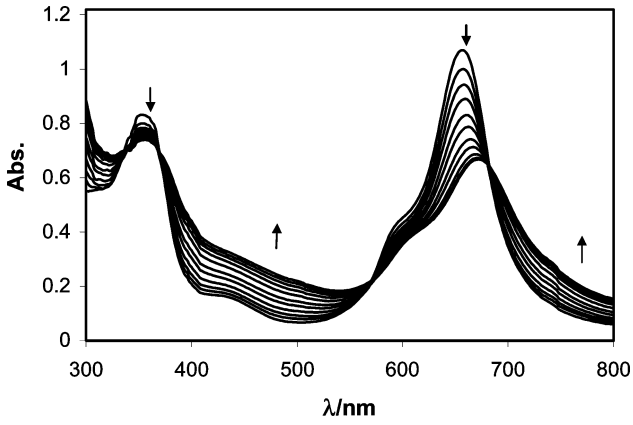


Fig. 1 Electronic absorption spectra for the titration of 11 μM [Co(3,4-tmtppa)]$^{4+}$ with DNA at pH 7.0 in 5 mM NaCl and 1 mM phosphate buffer. DNA concentrations were 0–83.5 μM.

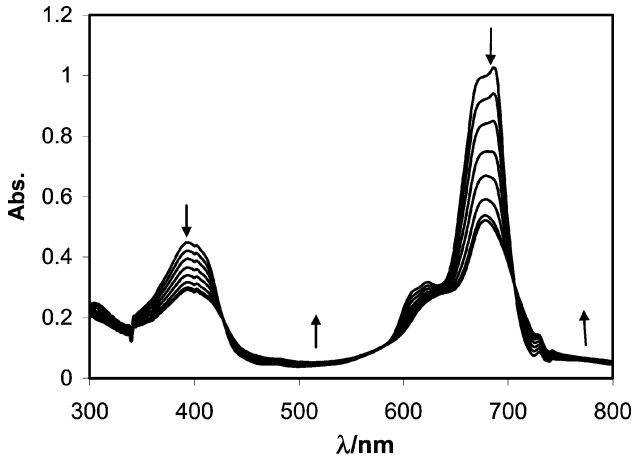


Fig. 2 Electronic absorption spectra for the titration of 10 μM [Zn(3,4-tmtppa)]$^{4+}$ with DNA at pH 7.0 in 5 mM NaCl and 1 mM phosphate buffer. DNA concentrations were 0–83.5 μM.

The higher values of K for the zinc complex with respect to the cobalt complex can be attributed to the higher affinity of the former for DNA binding. It possibly results that the major contributing factor in the binding of the zinc complex to DNA is the coordination interaction, while for the binding of the cobalt complex it is the electrostatic and π-π interactions that are most important.

It has been reported that methyl green, a non-intercalating dye that can bind to DNA electrostatically, is displaced from DNA by 0.15 M NaCl.[34] Therefore, additional support to confirm the contribution of coordination in the Zn(tmtppa)–DNA interaction can be obtained from an NaCl titration of the Zn(tmtppa)–DNA complex. A 2.5 ml aliquot of a zinc complex solution with fixed concentration (1.0×10^{-5} M) and 220 μL of a DNA solution (7.2×10^{-4} M) were placed in a cell. The mixture of DNA–zinc complex was titrated by 2 M NaCl solutions (20 μL). There was not any change in the Zn(tmtppa)–DNA absorbance during titration with NaCl (Fig. 3). This means that NaCl is unable to displace Zn(tmtppa) from the DNA duplex and possibly shows that the zinc complex is coordinated to the DNA base pairs. In other words, the zinc center of porphyrazine is presumably coordinated by the carbonyl group of thymine and/or the ring nitrogen of the

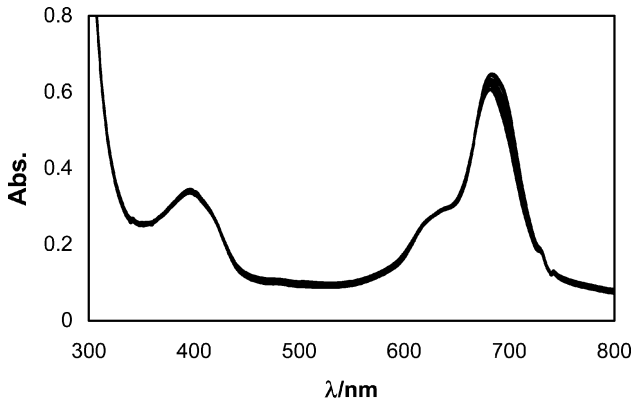


Fig. 3 Electronic absorption spectra for the titration of [Zn(3,4-tmtppa)]$^{4+}$–DNA (10 μM : 61 μM) complex with NaCl. NaCl concentrations were 0.02, 0.03, 0.05, 0.06, 0.08, 0.09, 0.11, 0.12 M.

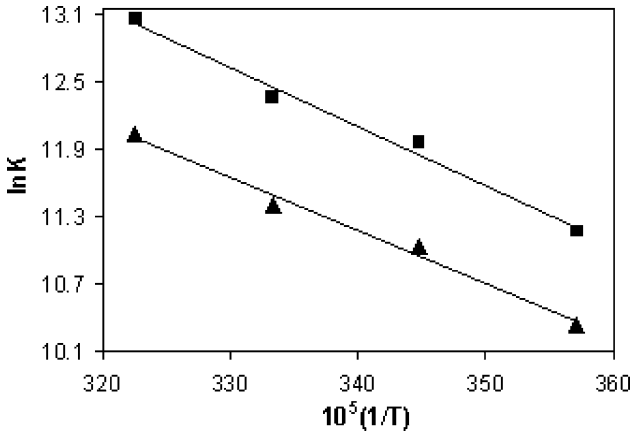


Fig. 4 The van't Hoff plots of DNA binding of (■) [Zn(3,4-tmtppa)]$^{4+}$ and (▲) [Co(3,4-tmtppa)]$^{4+}$.

base pairs. Such a coordination interaction has been reported in the literature.[35]

Thermodynamics of the porphyrazine–DNA complex interactions

By measuring the temperature dependence of the binding constant, thermodynamic studies of the interaction between porphyrazine complexes and DNA have been carried out. The Gibbs free energy was determined from the binding constant according to the following relationship:

$$\Delta G^\circ = -RT\ln K \qquad (3)$$

where R and T are the gas constant and the absolute temperature, respectively. The binding enthalpy was calculated from a plot of the temperature dependence of the binding constant according to the van't Hoff relationship:

$$\partial \ln K = -(\Delta H^o/R)\partial\left(\frac{1}{T}\right) \qquad (4)$$

The van't Hoff plots of porphyrazine–DNA binding are shown in Fig. 4. The molar entropy was estimated from the Gibbs free energy change and the molar enthalpy as:

$$\Delta S^\circ = (\Delta H^\circ - \Delta G^\circ)/T \qquad (5)$$

The obtained binding constants and thermodynamic parameters are presented in Tables 1 and 2.

Since for our system, like most other reactions involving biological macromolecules, activity coefficients are not known, the usual procedure is to assume a value of unity and to use the equilibrium concentrations instead of the activities. Therefore, the value of K may vary with composition. Since DNA–ligand equilibrium often depend on many factors such as pH, buffering compounds, salts, *etc.* and their influence on K is seldom known, it is appropriate to adapt the terminology of apparent equilibrium constants, $K^{o\prime}$, Gibbs free energy change, $\Delta G^{o\prime}$, enthalpy, $\Delta H^{o\prime}$, and entropy, $\Delta S^{o\prime}$.

The standard free energy changes ($\Delta G^{o\prime}$) for porphyrazine–DNA interactions are large and negative due to their strong

Table 1 Thermodynamic parameters and binding constants for the binding of [Co(3,4-tmtppa)]$^{4+}$ to ctDNA in 1 mM phosphate buffer, pH = 7.0, 5 mM NaCl

T/K	log K/M^{-1}	ΔG°/kJ mol^{-1}	ΔH°/kJ mol^{-1}	ΔS°/J mol^{-1} K^{-1}
280	4.5 ± 0.1	−24.1 ± 0.3	+39.6 ± 3	+227.3 ± 11.8
290	4.8 ± 0.0	−27.1 ± 0.1	+39.6 ± 3	+229.9 ± 10.5
300	5.0 ± 0.0	−28.4 ± 0.2	+39.6 ± 3	+226.7 ± 10.5
310	5.2 ± 0.0	−31.0 ± 0.1	+39.6 ± 3	+227.8 ± 9.8

Table 2 Thermodynamic parameters and binding constants for the binding of [Zn(3,4-tmtppa)]$^{4+}$ to ctDNA in 1 mM phosphate buffer, pH = 7.0, 5 mM NaCl

T/K	log K/M^{-1}	ΔG°/kJ mol^{-1}	ΔH°/kJ mol^{-1}	ΔS°/J mol^{-1} K^{-1}
280	4.8 ± 0.0	−26.0 ± 0.1	+44.1 ± 3.3	+250.2 ± 12.1
290	5.2 ± 0.0	−28.8 ± 0.1	+44.1 ± 3.3	+251.1 ± 11.7
300	5.4 ± 0.0	−30.8 ± 0.1	+44.1 ± 3.3	+249.6 ± 11.3
310	5.7 ± 0.0	−33.7 ± 0.2	+44.1 ± 3.3	+250.7 ± 11.3

association. It has also been indicated that the binding of porphyrazine to DNA is an endothermic process. The $\Delta H^{0\prime}$ value and its sign are dependent on two factors: the solvation effect and the complex heat of formation. Although the heat of bond formation is negative because of bond formation, $\Delta H^{o\prime}$ is positive. The positive values of $\Delta H^{o\prime}$ in the porphyrazine–DNA interactions indicate a contribution of the positive entropy changes ($\Delta S^{o\prime}$), resulting in large $T\Delta S^{o\prime}$ and more negative $\Delta G^{o\prime}$, which favors the binding process. As summarized in Tables 1 and 2, it seems that the major contributing factor in the stabilization of the porphyrazine–DNA complex is entropic in origin. It can be concluded that the positive entropy changes are the driving forces in the Coulombic interactions between the positively charged porphyrazines and negatively charged DNA.[35] Therefore, the positive entropy changes of the porphyrazine–DNA interaction probably come from the electrostatic interaction between the positively charged pyridine rings and negatively charged phosphate oxygens. Upon this interaction, water molecules bound to porphyrazine and DNA are released, which leads to positive entropy changes in the overall thermodynamics of the interactions.

The interaction of zinc porphyrazine is more endothermic than the cobalt complex. This can be attributed to the coordination of the zinc center to the DNA base pairs. Thus, the binding of Zn(tmtppa) to DNA consumes more energy than Co(tmtppa). This can lead to greater changes in DNA local charge density, which undergoes release of the condensed counter-ions from the interacting surface.[36] On the other hand, in the Zn(tmtppa)–DNA interaction, the greater release of water molecules or counter-ions compared with Co(tmtppa) results in more positive entropy and enthalpy values for the former.

It has also been reported that cationic porphyrins can usually intercalate into G/C sites and outside bind at A/T sites. The more flexible A/T site is able to bend, flex or kink around molecules bound externally; it can increase the strength of interactions between the complex, thereby stabilizing external complexes. Such a process is disfavored for the less flexible and more stable G/C duplex. Outside binding of the polycationic porphyrin molecules at A/T sites relative to G/C sites results in an increase in the Coulombic force of attraction.[37]

The G/C base pairs are less hydrated than the A/T ones (G/C and A/T base pairs have 18.5 and 20 water molecules bound per base pair, respectively). Therefore, upon binding of porphyrazine, the A/T site will apparently release more water molecules. Consequently, outside binding of zinc porphyrazines at A/T sites probably results in more positive enthalpy and entropy than intercalation into the G/C sites.[38]

Ionic strength dependence

An important question regarding to the binding of porphyrins to nucleic acids is that of the influence of ionic strength. Literature reports of their binding constants show a dependence on counter-ion concentration (for example [Na^+]).[15,39]

The work reported in this section was undertaken to determine the ionic strength, μ, (in terms of Na^+ concentrations) dependence of the binding of [Co(3,4-tmtppa)]$^{4+}$ and

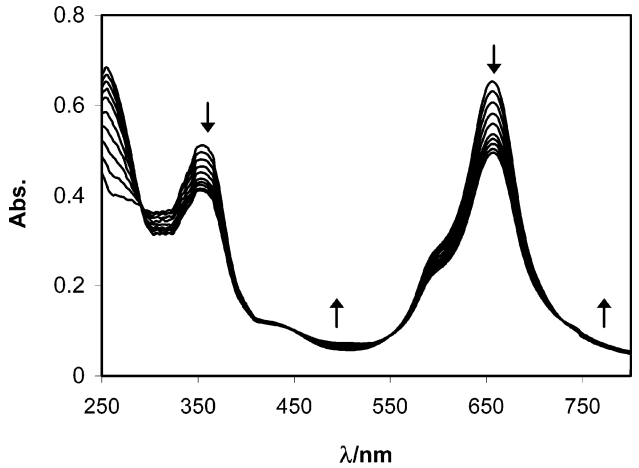


Fig. 5 Electronic absorption spectra for the titration of 11 μM $[Co(3,4\text{-tmtppa})]^{4+}$ with DNA in pH 7.0 in 30 mM NaCl and 1 mM phosphate buffer. DNA concentrations were 0–83.5 μM.

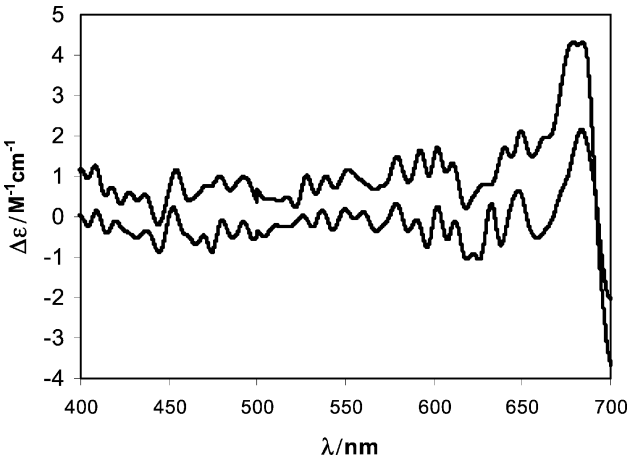


Fig. 7 Induced circular dichroism spectra in the Q-band region of $[Zn(3,4\text{-tmtppa})]^{4+}$ upon titration with DNA. The spectra were recorded at pH 7.0 in 5 mM NaCl and 1 mM phosphate buffer. The porphyrazine concentration was 4.5 μM. DNA concentrations were 16 and 30 μM.

$[Zn(3,4\text{-tmtppa})]^{4+}$ to DNA using UV-visible absorption. We observed that the binding affinity of DNA to porphyrazine was reduced when the ionic strength was increased in the range from 5 to 30 mM. Typical titration data involving $[Co(3,4\text{-tmtppa})]^{4+}$ and ctDNA at $\mu = 30$ mM is shown in Fig. 5.

We determined the dependence of K on $[Na^+]$. Plots of log$K$ *vs.* −log $[Na^+]$ for $[Co(3,4\text{-tmtppa})]^{4+}$ and $[Zn(3,4\text{-tmtppa})]^{4+}$ are shown in Fig. 6. The equations of the lines that best fit these points are:

$$\log K_{Co} = 3.97 - 0.44 \cdot \log[Na^+] \quad (6)$$

$$\log K_{Zn} = 3.82 - 0.69 \cdot \log[Na^+] \quad (7)$$

It is also seen that at higher ionic strengths, there is no longer any red-shift in the Q bands of cobalt–porphyrazine complexes in the presence of DNA. It is concluded that increasing the ionic strength changes the intercalation mode to outside binding. It has been assumed that increasing the ionic strength increase the neutralization of the negative charges of the DNA phosphate groups and therefore, prevents cationic porphyrazine complexes from coming closer to DNA, this favoring the outside binding mode.

It is reasonable to say that in DNA–Co(tmtppa) binding, Coulombic interactions favor a short distance approach between the porphyrazine and DNA, resulting in partial intercalation. In terms of the thermodynamic parameters, the increase in the ionic strength can decrease the negative charges on DNA. Therefore, the electrostatic interaction and the positive entropy changes ($\Delta S^{o\prime}$) originating from it will be decreased. Thus, a decline in the standard free energy ($\Delta G^{o\prime}$) results in a decrease in the binding constant.

Induced circular dichroism measurements

CD studies have been extensively used to study the binding of small molecules, including porphyrins, to DNA in the visible range; the porphyrins alone do not show any ellipticity. However, an induced CD (ICD) spectrum is obtained if there is binding between the porphyrin and DNA. The shapes of the curves obtained give information about the nature of the binding.[40–42]

The binding of our porphyrazines to DNA was also monitored through circular dichroism measurements. None of these porphyrazines showed any ellipticity in the visible or UV range. In a typical experiment, 2.5 ml of porphyrazine solution (4.5×10^{-6} M) was transferred to a quartz cell and titrated with the stock solution of DNA [1.8×10^{-4} M; 250 and 500 μl were added to Zn(tmtppa) and 250 μl was added to Co(tmtppa)]. CD spectra related to porphyrazine were recorded in the visible region. Interaction of the porphyrazines with ctDNA resulted in induced CD spectra in the visible range that are due to the interaction between the transition moments of the achiral porphyrazine and the chirally arranged DNA base transitions. $[Zn(3,4\text{-tmtppa})]^{4+}$ upon binding to calf thymus DNA showed a strong positive peak centered at about 680 nm (Fig. 7), whereas $[Co(3,4\text{-tmtppa})]^{4+}$ showed a weak

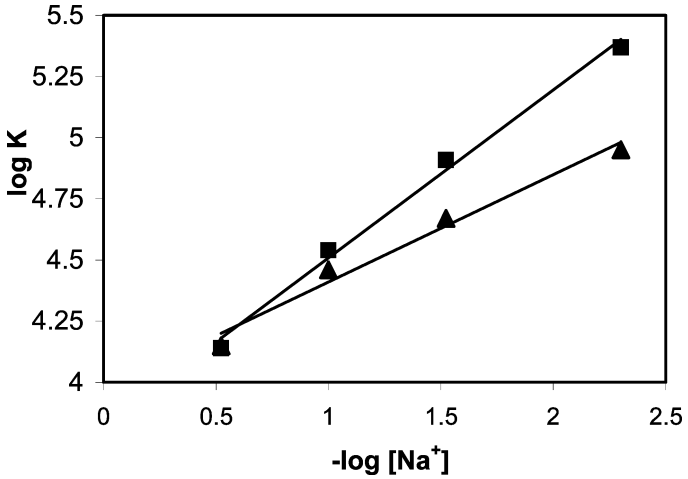


Fig. 6 Plots of log$K$ *vs.* −log$[Na^+]$ for DNA binding of (■) $[Zn(3,4\text{-tmtppa})]^{4+}$ and (▲) $[Co(3,4\text{-tmtppa})]^{4+}$.

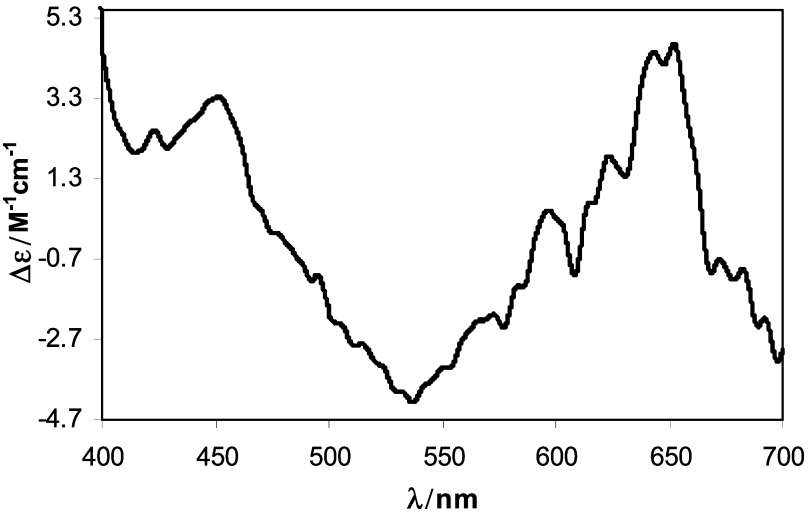


Fig. 8 Induced circular dichroism spectra in the Q-band region of $[Co(3,4\text{-tmtppa})]^{4+}$ upon titration with DNA. The spectra were recorded at pH 7.0 in 5 mM NaCl and 1 mM phosphate buffer. The porphyrazine concentration was 4.5 μM. DNA concentrations were 16 μM.

negative peak centered at about 660 nm and a positive one at 668 nm (Fig. 8).

The nature of the ICD spectra of the zinc complex indicates an outside mode of binding. Analysis of the ICD spectra of the cobalt complex can be accounted for by the coexistence of intercalative (full or partial) and outside binding modes, in which one is associated with a negative CD and another positive. This feature is different from excitonic CD spectra, which originate from aggregation or stacking.[43]

Fluorescence results

We studied the fluorescence pattern of the interaction of the two porphyrazines to DNA in the presence of ethidium bromide, a well-known DNA intercalator. Nucleic acid-bound ethidium bromide generally exhibits marked changes in fluorescence properties as compared to its spectral characteristics.[44]

A 2.5 ml sample of an ethidium bromide solution with fixed concentration (7.1×10^{-5} M) and 330 μL of DNA solution (2.09×10^{-4}M) were placed in a cell. The mixture of DNA–ethidium bromide was titrated by 5 μL porphyrazine solutions (8.93×10^{-6}M). Excitation and emission wavelengths were 490 and 604 nm, respectively. A considerable drop in fluorescence intensity was seen in the presence of cobalt porphyrazine but zinc porphyrazine quenching of ethidium–DNA was much less than that of the cobalt complex.

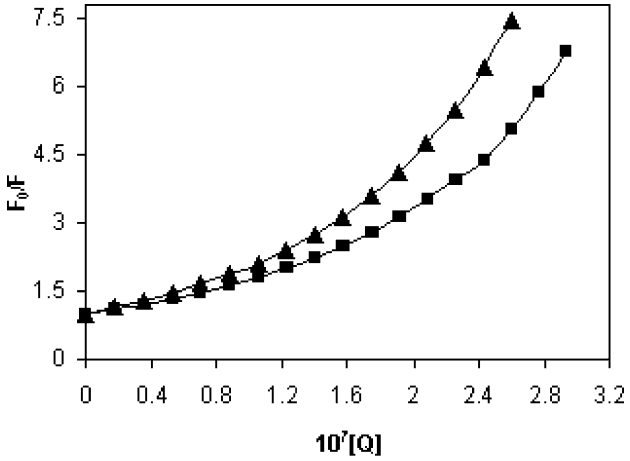


Fig. 9 Stern–Volmer plots for ethidium–DNA quenching by the porphyrazine complexes: (■) $[Zn(3,4\text{-tmtppa})]^{4+}$ and (▲) $[Co(3,4\text{-tmtppa})]^{4+}$. The porphyrazine concentration range was 0.02–0.24 μM; [ethidium bromide] = 71 μM; DNA concentration was 17.7 μM. $\lambda_{ex} = 290$ nm, $\lambda_{em} = 604$ nm.

The quenching of ethidium–DNA followed the Stern–Volmer relationship of the form:

$$\frac{F_o}{F} = 1 + K_{SV}[Q] = 1 + k_q\tau_o[Q] \qquad (8)$$

where F_o and F are the fluorescence intensities of the excited DNA–ethidium in the absence and presence of porphyrazines, $[Q]$ is the quencher concentration and K_{SV}, the Stern–Volmer constant, is the product between the rate constant for quenching (k_q) and the lifetime of the luminescence in the absence of quencher (τ_o). The plots of F_o/F *vs.* porphyrazine concentration $[Q]$ evolve quadratically and are affected by $[Q]$ (Fig. 9). The nonlinearity of this relationship can account for the possible the existence of either dynamic or static quenching mechanisms. From the quasi-linear portions of these plots (at low $[Q]$), values for K_{SV} were determined (given in Table 3). The quenching rate constants (k_q) were calculated by measuring K_{SV} and τ_o. The excited lifetime of ethidium bromide in the presence of DNA is lengthened to 23 ns.[45] The values of k_q were estimated using eqn. (8) and are also listed in Table 3. Two mechanisms have been proposed to account for the quenching: (a) the displacement of ethidium bromide from DNA and (b) electron transfer from the excited ethidium to an acceptor.[46] The UV-Vis, CD results and the considerable cobalt porphyrazine quenching of ethidium bromide fluorescence in the presence of DNA is in agreement with the former hypothesis, which suggests porphyrazine-induced displacement of the ethidium bromide ion from DNA. Concerning the small porphyrazine quenching of the DNA–ethidium emission, UV-Vis and CD results, we can conclude that the zinc complex cannot displace ethidium bromide ion from DNA. Therefore, the second mechanism can account for ethidium bromide quenching by the zinc complex.

Viscometric measurements

Viscometric methods, which are sensitive to changes in the length of DNA, are the most critical test of the classical

Table 3 The Stern–Volmer quenching parameters for DNA–ethidium bromide by porphyrazines in 1 mM phosphate buffer, pH = 7.0, 5 mM NaCl

Complex	$10^{-7} \times K_{SV}/M^{-1}$	$\tau_{0\ DNA\text{-}Et}$[a]/ns	$10^{-14} \times k_q/M^{-1}\ s^{-1}$
$[Co(3,4\text{-tmtppa})]^{4+}$	1.4 ± 0.1	23.0	6.2 ± 0.4
$[Zn(3,4\text{-tmtppa})]^{4+}$	0.8 ± 0.1	23.0	3.6 ± 0.2

[a] Ethidium bromide

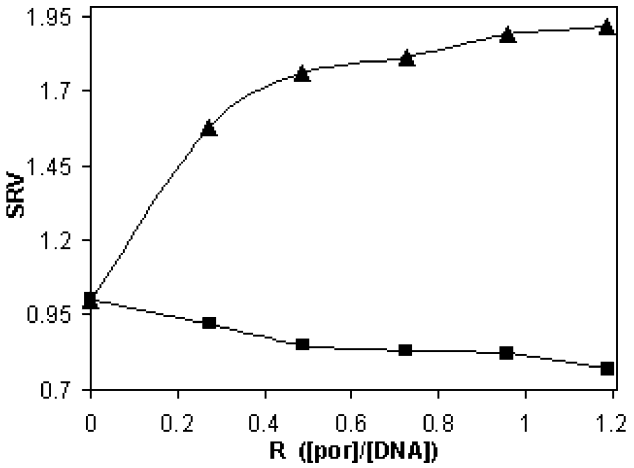


Fig. 10 Plots of SRV *vs.* *R* ([porphyrazine] : [DNA] base pair) in 1 mM phosphate buffer, pH = 7.0, 5 mM NaCl at 27 °C for (■) $[Zn(3,4\text{-tmtppa})]^{4+}$ and (▲) $[Co(3,4\text{-tmtppa})]^{4+}$. *R* = 0, 0.27, 0.49, 0.73, 0.96 and 1.2.

intercalation model.[47] To carry out these studies, typically 5 ml of phosphate buffer was added to the viscometer. Then a small amount of DNA stock solution was added to give a 3.58×10^{-6} M DNA concentration. The flow time of this solution was read. A 50 µl aliquot of porphyrazine solution was added to the DNA. After thermal equilibration (60 min), the flow times of the samples were measured. Each measured point is the average of five readings. The data are presented as the specific-reduced viscosity (SRV) of DNA in the presence and absence of porphyrazine *versus* *R* ([porphyrazine] : [DNA] base pair). It results that the viscosity of the DNA solution is increased considerably with increasing additions of cobalt complex. This can be related to lengthening of the DNA helix due to the intercalation.[38] Such an increase in the relative viscosity was also seen for the ethidium bromide–DNA interaction. This intercalator produces a significant increase in the SRV of DNA (SRV = 2).[48] It is also seen that the zinc complex causes only a slightly decrease in DNA viscosity (Fig. 10). This suggests that the zinc complex fails to unwind DNA. These results are consistent with the conclusions from the visible, ICD and fluorescence spectroscopies.

Conclusions

UV-vis spectroscopy enabled us to determine the porphyrazine–DNA binding constants and thermodynamic parameters. It was deduced that the binding interactions are endothermic and entropy driven. The existence of a high hypochromicity without any red-shift and positive ICD in the visible part of the ($[Zn(3,4\text{-tmtppa})]^{4+}$) spectra suggest an outside binding mode. Analysis of the moderate hypochromicity with a large red-shift and bisignate ICD in the visible part of the $[Co(3,4\text{-tmtppa})]^{4+}$ spectra lead us to conclude for the coexistence of intercalation (full or partial) and outside binding modes. It is assumed that the steric constraints presented by the water axial ligand at the metal center of the zinc porphyrazine serve to prevent intercalation into DNA. The higher affinity of the zinc complex with respect to the cobalt complex can be attributed to the role of nucleotide axial ligation in the former.

The influence of the ionic strength on the binding parameters was investigated, showing that increase in ionic strength causes a decrease of the binding constants. It was also concluded that an increase in ionic strength changed the intercalation to the outside binding mode with the cobalt complex.

The increase of DNA viscosity during its interaction with Co porphyrazine complex is consistent with lengthening of the DNA due to intercalation, while the zinc complex fails to unwind DNA and slightly decreases the viscosity of DNA.

Quenching studies of the ethidium bromide–DNA complex by porphyrazine complexes gave us the Stern–Volmer constants and the rate constants of the quenching. It was also found that cobalt porphyrazine quenching of the DNA–ethidium bromide fluorescence is in agreement with the binding of porphyrazine complexes to DNA while the ethidium bromide is expelled from it. On the other hand, the zinc complex cannot displace ethidium bromide from DNA because of the outside binding mode.

In summary, we believe that these complexes have a good affinity for DNA binding. The diamagnetic zinc complex, as an outside binder, probably acts as a photosensitizer. The cobalt complex can intercalate into the DNA helix. Since unwinding of DNA is generally considered as an important characteristic of intercalating agents, it may unwind DNA and thus damage it.

As a result we have proposed an explanation for the relationship between binding mode, binding affinity and porphyrazine central metal.

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