Regular Article

PHYSICAL CHEMISTRY RESEARCH
Published by the
Iranian Chemical Society
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***Phys. Chem. Res.**, Vol. 4, No. 2, 161-172, June 2016.*
DOI: 10.22036/pcr.2016.12985

Spectroscopic Studies on the Interaction of Co(II) Tetrapyridinoporphyrazine with Synthetic Polynucleotides and DNA

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(Received 25 August 2015, Accepted 25 January 2016)

Interactions of cationic tetrakis (N,N´,N´´,N´´´-tetramethyltetra-3,4-pyridinoporphyrazinatocobalt(II) (Co(tmtppa)) with synthetic polynucleotides, poly(A-T), poly(G-C) and calf thymus DNA have been characterized in 5 mM phosphate buffer, pH 7.2, by optical absorption and fluorescence spectroscopy. The appearance of hypochromicity effect and the red shift in UV-Vis spectrum of porphyrazine was due to the interaction of both poly(A-T) and poly(G-C) which is similar to interaction of porphyrazine with DNA. The binding constants (K) were determined from the changes in the optical absorption spectra at various poly(G-C), poly(A-T) and DNA concentrations. According to the results, the values of K were $2.50 \times 10^6\ M^{-1}$, $2.25 \times 10^6\ M^{-1}$ and $2.25 \times 10^5\ M^{-1}$ for poly(A-T), poly(G-C) and DNA, respectively, at 25 ºC. The thermodynamic parameters were calculated by the van't Hoff equation. The positive values of the entropy and enthalpy suggest that coulombic interactions may play an important role in stabilization of the complex formed. The influence of the ionic strength was also investigated. It was concluded that the apparent binding constants decrease with increasing salt concentration. The fluorescence quenching of the DNA-ethidium bromide complex by the porphyrazine was also investigated. The values of the quenching constant (K_{SV}) was determined by the Stern-Volmer equation. The results revealed groove binding mode of porphyrazine for both A-T and G-C rich region of polynucleotides of DNA.

Keywords: Cobalt(II) Porphyrazine, Calf thymus DNA, Synthetic polynucleotide, Groove binding, Fluorescence quenching

INTRODUCTION

Storage and expression of genetic information in a cell are performed by DNA molecules. Several ligands such as antitumor or antibiotic agents interact with DNA and regulate its function agents [1]. Cationic porphyrins including four pyrrole rings exhibit an expanding class of compounds, which have been applied in biology, medicine and catalysis [2-4]. Cationic porphyrins have been evaluated as DNA binding cleavage, reagents and photosensitizers for photodynamic therapy [5-7], nuclease-resistant delivery agents for anti-sense oligonucleotides and tracers for nucleic acid structure [8].

The major modes of non-covalent interactions with DNA are intercalation and groove binding. While, the intercalation mode does not usually depend on the DNA sequence context, the groove mode of binding is commonly specific to adenine-thymine (AT)-rich sequences [9]. Based on the nearest neighbor hypothesis, the highest possible Porphyrin-base pair ratio for intercalation must be 1:2.

The elongated and ribbon-like structure of groove binding make it possible that one ligand molecule positioned alongside the DNA helix occupies at least 3-5 base pairs [10]. It is, therefore, possible that AT-specificity of the groove-binding molecule needs the presence of at least 3-5 AT base pairs in the sequence.

Porphyrazines (tetraazaporphyrins) belong to a class of macrocycle structures similar to phthalocyanines and porphyrins. It has been found that replacement of the meso methylene carbons of porphyrins with nitrogen in porphyrazines can create profound differences. Porphyrazines are mainly prepared from dicyanopyridine or

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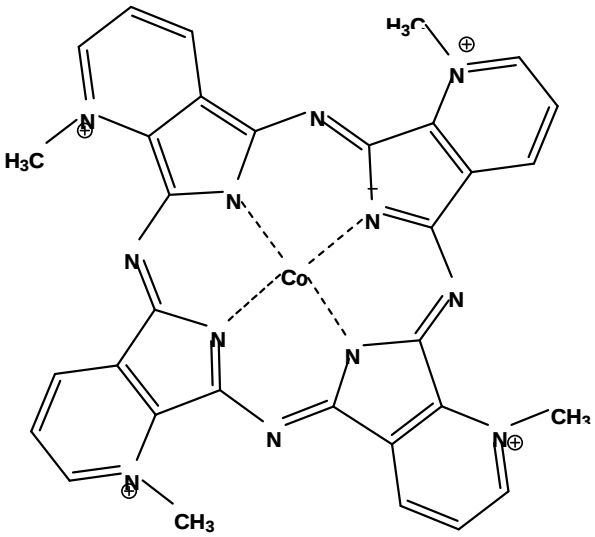
pyridinedicarboxylic acid. The methylation of the nitrogen atom of pyridine ring using suitable agents is the common way for quaternization of these compounds aiming to prepare water soluble porphyrazine [11]. Some of these compounds mainly exist as monomers in aqueous solutions [12,13].

There is a wide range of research work on the interaction of porphyrins and phthalocyanines for their binding to nucleic acids [14-16]. However, tetramethylmethalloporphyrazines have received less attention and, to the best of our knowledge, their binding with poly polynucleotides has not been reported so far.

The present work has been undertaken to determine the equilibrium binding processes of porphyrazine tetrakis (N,N´,N´´,N´´´-tetramethyltetra-3,4-pyridinoporphyrazinatocobalt(II) ((Co(tmtppa)) shown in Scheme 1 to poly(A-T), poly(G-C) and DNA. To do so, binding constants, binding modes, the values of the Stern-Volmer constant (K_{SV}) and the rate constants for the quenching (k_q) are determined by the optical absorption and fluorescence spectroscopy. It is clear that a complete understanding of the porphyrazine-DNA binding modes and factors affecting them helps us to design new anticancer, antiviral and antibacterial drugs.



Scheme 1. Chemical structure of Co(tmtppa)

Chemicals and Preparations

Metal porphyrazine [Co(tmtppa)] was synthesized according to a method described by Marti in 2000 [17]. A solution of Co(tmtppa) complex (1 mg ml^{-1}) in 5 mM phosphate buffer, pH 7.20, was first prepared as stock, stored in the dark at 5-10 ºC and used to make required dilution just before experiment. The concentrations of diluted samples were then determined by measuring their molar extinction coefficient on spectrophotometer. Ethidium bromide, poly(A-T), poly(G-C) and Calf thymus DNA were purchased from Sigma™ and used without further purification.

The DNA stock solution was freshly prepared by dissolving 2 mg of DNA in 1 ml of phosphate buffer stored at 4 ºC. The stock solutions of poly(A-T), poly(G-C) were also made in phosphate buffer and stored at 4 ºC. The concentration of poly(A-T), poly(G-C) and DNA were determined from their optical absorption using their molar absorption coefficients. The extinction coefficients of $\varepsilon_{254\,nm}$ = 1.68×10^4 cm^{-1} M^{-1}, ε_{262nm} = 1.32×10^4 cm^{-1} M^{-1} and ε_{259nm} = 1.32×10^4 cm^{-1} M^{-1} were used to determine the concentration in base pairs of poly(A-T), poly(G-C) [18] and DNA [19], respectively. The pH values were controlled on a Metrohm-744 pH-meter. The temperature of solution was kept constant within a range of ±0.1 ºC.

UV-Vis Spectroscopy

The absorption spectra were recorded by a Cary 500 scan UV-Vis-NIR spectrophotometer. The Co(tmtppa) solutions were prepared in a concentration range of 4.0-40.0 μM for optical absorption measurements in the Q-band region (for the beer's law experiment). The UV-Vis titration experiments were made by addition of the polynucleotides and DNA stock solutions into a 1 ml cuvette containing the Co(tmtppa) solution of appropriate concentration (9.7 μM). The concentration range of polynucleotides and DNA were 0, 1.82, 3.60, 5.35, 7.07, 10.40, 12.00 and 13.60 μM. The titration experiments were performed at various temperatures with a precision of ±0.1 ºC.

Fluorescence Spectroscopy

Emission spectra of ethidium bromide (EB) bound to DNA in the absence and presence of the Co(tmtppa) were recorded on a Shimadzu model RF-5000 spectrofluorimeter.

A 1.5 ml sample of an ethidium bromide solution with fixed concentration (70 μM) and 330 ml of DNA solution (209 μM) were placed in a cell. The mixture of DNA-EB was titrated by 5 ml Co(tmtppa) solution (8.5 μM) at 27 ºC in phosphate buffer. The solutions were excited at 490 nm and the emitted light intensity was measured in the range of 500-800 nm. Both UV-Vis and fluorescence spectra were also corrected for dilution. The temperature was kept constant at ±1 °C during titration experiments.

RESULTS AND DISCUSSION

Solution Properties of Co(tmtppa)

As mentioned before, the properties of Co(tmtppa) solution were identified using UV-Vis spectroscopy. The optical absorption spectrum of Co(tmtppa) showed the characteristic of the Co(tmtppa) base, a Q- and a B- band. The molar absorptivity coefficient of these bands were calculated by Beer's line 9.92×10^4 M^{-1} cm^{-1} for Q-band (λ = 656 nm) and 4.25×10^4 M^{-1} cm^{-1} for B-band (λ = 360 nm), respectively. The Q-band maximum of Co(tmtppa) obeyed Beer's law over an extended concentration range between 1.50×10^{-5}-1.25×10^{-4} M in water. This observation shows that Co(tmtppa) does not aggregate in a concentration dependent manner.

The titration of Co(tmtppa) solution was conducted at a fixed concentration of complex (9.70×10^{-5} M) and varying concentrations of DNA and polynucleotides in 5 mM phosphate buffer pH 7.2 with sufficient binding capacity. Regarding the results of the previous section, Co(tmtppa) exists mainly as monomer form. Figures 1-3 show a typical titration spectra of Co(tmtppa) upon increasing poly(A-T), poly(G-C) and DNA addition, respectively, at 25 °C. In both cases, a bathochromic shift of $\Delta\lambda \leq 8$ nm and moderate hypochromism ($H \leq 20\%$) in Q-band were observed, which represents the existence of Coulombic interaction and groove binding, between poly(A-T), poly(G-C), DNA and Co(tmtppa) complex [19,20].

The appearance of isosbestic points in the spectra clearly indicates the existence of a simple equilibrium between free Co(tmtppa) and 1:1 Co(tmtppa)-DNA complex:

$$\text{Co(tmtppa)} + \text{DNA} \rightarrow \text{Co(tmtppa)} - \text{DNA} \quad (1)$$

This situation was observed in all the temperatures studied. The positions of Q-band and isosbestic points did not show significant difference at various temperatures. The Q-band position of free Co(tmtppa) was at 656 nm and Co(tmtppa)-DNA or Co(tmtppa)-polynucleotide at >656 nm (Figs. 1- 3). The above equilibrium returned to the left by the addition of NaCl:

$$\text{Co(tmtppa)} - \text{DNA} + \text{Na}^+ \rightarrow \text{Co(tmtppa)} + \text{DNA} - \text{Na}^+ \quad (2)$$

This result corresponds to titration absorption spectra of Co(tmtppa)-DNA upon addition of NaCl in phosphate buffer, pH 7.2, at 25 °C (Fig. 4). The molar ratio of [DNA]/[Co(tmtppa)] was 2.0 in the experiment. Molecular dynamic (MD) simulations, solution NMR and crystallographic results confirmed that the mono valent cations Na^+, K^+, Cs^+, Rb^+ prefer direct binding (inner sphere) at the A-T step in A-track in minor groove of DNA [21-24]. So, it can be concluded that Co(tmtppa) binds to the minor groove of DNA, and addition of Na^+ diminishes this binding leading to the separation of Co(tmtppa) from double strand DNA.

Binding constants for the interaction of cationic Co(tmtppa) with poly(A-T), poly(G-C) and DNA were determined by the analysis of absorption spectrophotometric titrations data. The changes in absorbance of the Q band upon addition of poly(A-T), poly(G-C) and DNA were monitored at maximum of the Q band. The apparent binding constant, K_{app} of cationic Co(tmtppa) to DNA was calculated using Eq. (3),

$$\frac{[\text{DNA}]_{\text{total}}}{(|\varepsilon_{\text{app}} - \varepsilon_{\text{f}}|)} = \frac{[\text{DNA}]_{\text{total}}}{(|\varepsilon_{\text{b}} - \varepsilon_{\text{f}}|)} + \frac{1}{K_{\text{app}}(|\varepsilon_{\text{b}} - \varepsilon_{\text{f}}|)} \quad (3)$$

where ε_{app}, $\varepsilon_{f \text{ and }} \varepsilon_b$ correspond to $A_{obsorved}$/[Co(tmtppa)], the extinction coefficient for the free Co(tmtppa) and the extinction coefficient for the Co(tmtppa) in the fully bound form, respectively. In the plot of $[DNA]_{total}/(|\varepsilon_{app} - \varepsilon_f|)$ *vs.* $[DNA]_{total}$, $[poly(A-T)]_{total}/(|\varepsilon_{app} - \varepsilon_f|)$ *vs.* $[poly(A-T)]_{total}$, and $[poly(G-C)]_{total}/(|\varepsilon_{app} - \varepsilon_f|)$ *vs.* $[poly(G-C)]_{total}$ K_{app} is given by the ratio of the slope to the intercept [25-27]. These plots of Co(tmtppa)-poly(A-T), Co(tmtppa)-poly(G-C) and Co(tmtppa)-DNA binding are shown in Figs. 5-7,

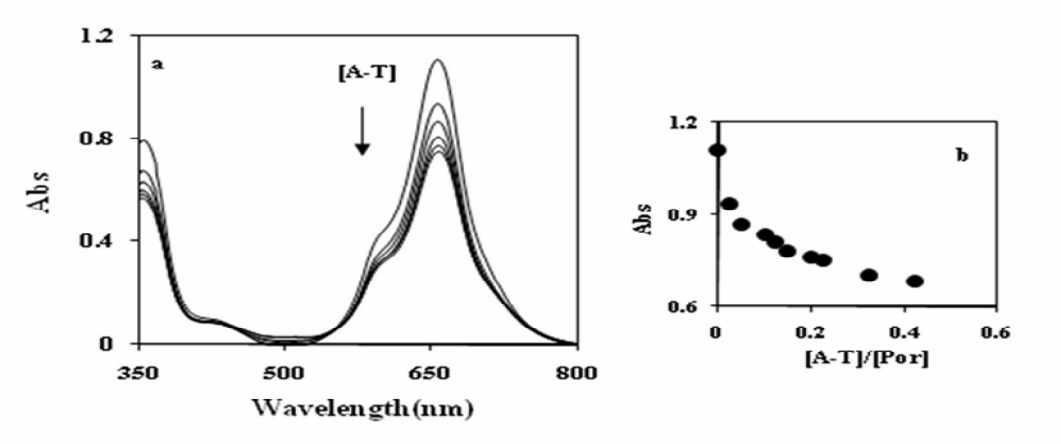


Fig. 1. (a) Absorption spectra of Co(tmtppa). (9.70 μM) in the presence of poly(A-T):0, 1.82, 3.60, 5.35, 7.07, 10.40, 12.00 and 13.60 μM. The arrow indicates the absorbance changes with increasing [poly(A-T)]. (b) Variation of absorbance at the Q-band (656 nm) *vs*. mole ratio of poly(A-T) to Co(tmtppa).

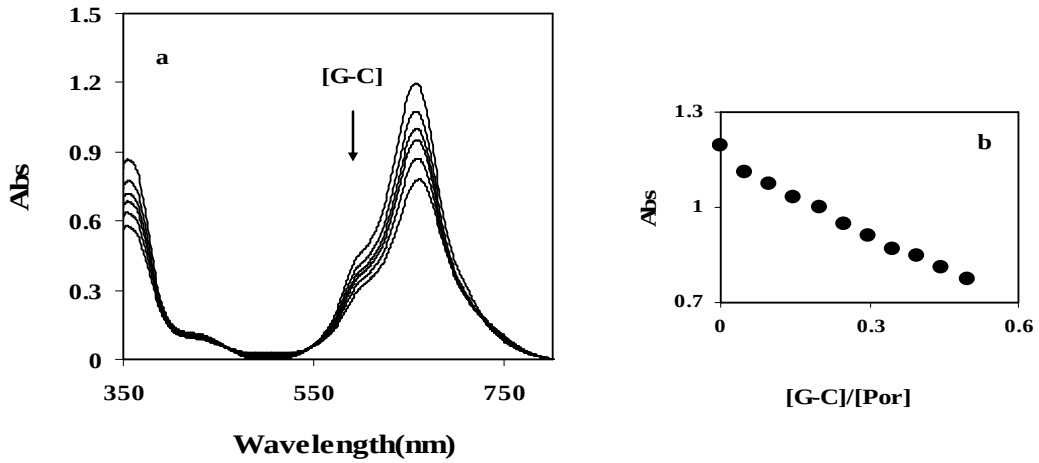


Fig. 2. (a) Absorption spectra of Co(tmtppa) (9.70 μM) in the presence of poly(G-C): 0, 1.82, 3.60, 5.35, 7.07, 10.40, 12.00 and 13.60 μM. The arrow indicates the absorbance changes with increasing [poly(G-C)]. (b) Variation of absorbance at the Q-band (656 nm) *vs*. mole ratio of poly(G-C) to Co(tmtppa).

respectively. The values for apparent binding constants of Co(tmtppa)-DNA, Co(tmtppa)-poly(A-T), Co(tmtppa)-poly(G-C) and Co(tmtppa)-DNA in our experimental conditions were calculated to be 1.25×10^6 M^{-1}, 2.50×10^6 M^{-1} and 4.50×10^5 M^{-1}, respectively at 25 °C. Comparison of our results has revealed that the affinity of Co(tmtppa) to poly(G-C) is greater than that to poly(A-T). Thus Co(tmtppa) is selective for G-C riches sites.

Thermodynamics of Porphyrazine Binding Process

By measuring the temperature dependence of binding, constant, thermodynamic studies of the interaction of Co(tmtppa) complex with poly(A-T), poly(G-C) and DNA have been carried out at the temperature range of 20-40 °C. The Gibbs free energy was determined from the binding constant according to the following relationship:

$$\Delta G^\circ = -RT\ln K^\circ \quad (2)$$

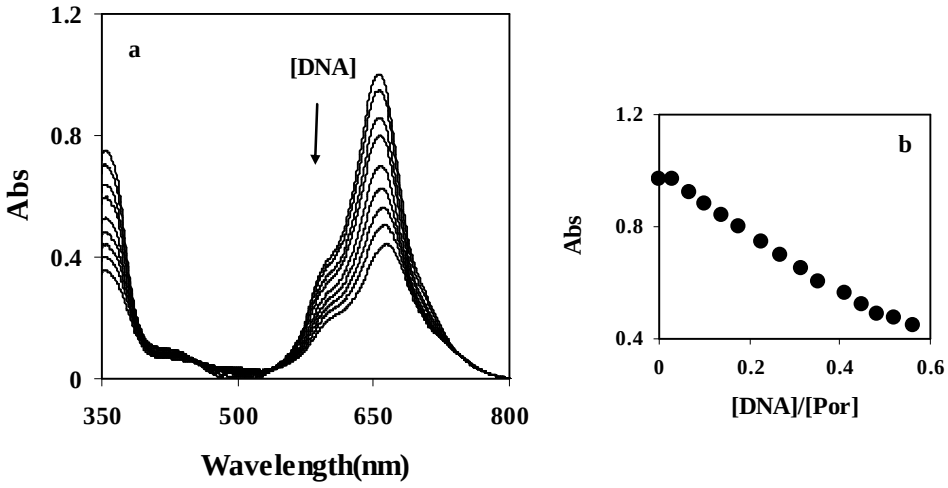


Fig. 3. (a) Absorption spectra of Co(tmtppa). (9.70 μM) in the presence of DNA: 0, 1.82, 3.60, 5.35, 7.07, 10.40, 12.00 and 13.60 μM. The arrow indicates the absorbance changes with increasing [ct-DNA]. (b) Variation of absorbance at the Q-band (656 nm) *vs*. mole ratio of DNA to Co(tmtppa).

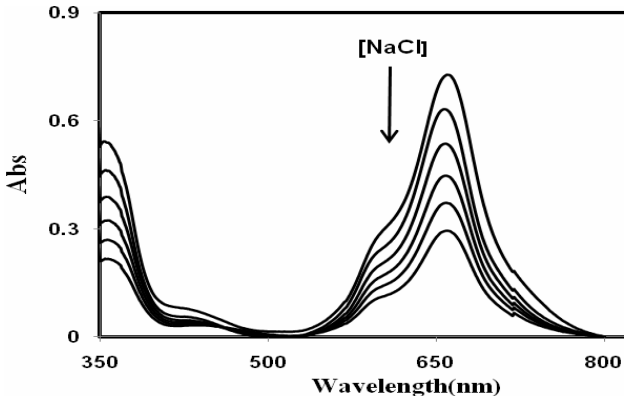


Fig. 4. The titration absorption spectra of Co(tmtppa)-DNA solution (DNA:porphyrazine ratio: 2:1) by NaCl in 5 mM phosphate buffer pH 7.2 at 25 °C. The arrow indicates the absorbance changes with increasing NaCl.

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:

$$\frac{d\ln K^{o}}{d(1/T)} = -\frac{\Delta H^{o}}{R} \quad (3)$$

The molar entropy was calculated from the Gibbs free energy change and the molar enthalpy as:

$$\Delta S^{o} = \frac{\Delta H^{o} - \Delta G^{o}}{T} \quad (4)$$

The obtained binding constants and thermodynamic

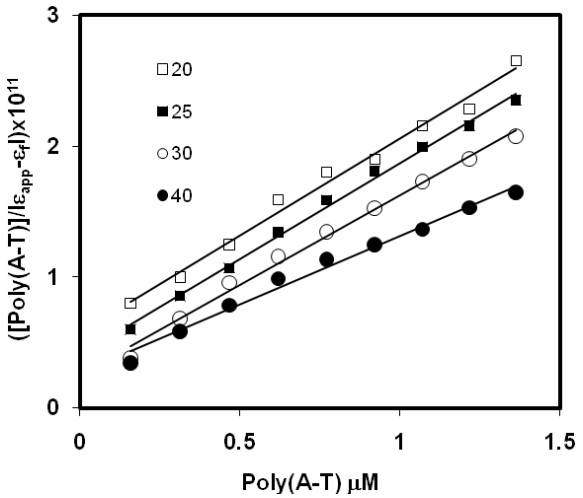


Fig. 5. Typical plots of $[poly(A\text{-}T)]_{total}/(|\varepsilon_{app} - \varepsilon_f|)$ *vs.* $[poly(A\text{-}T)]_{total}$ for determination of the equilibrium binding constant (K_{app}) based on Eq. (1) of poly(A-T) binding of Co(tmtppa) at various temperatures.

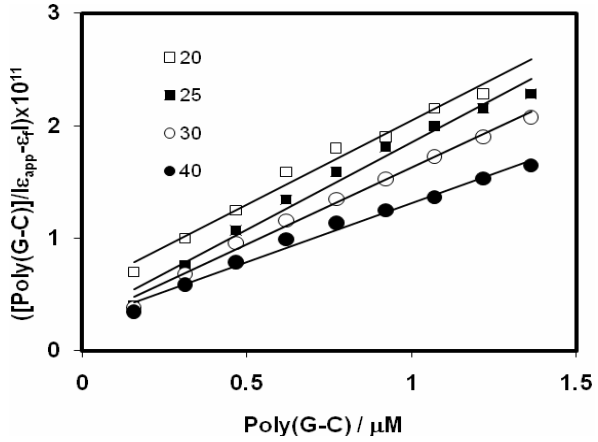


Fig. 6. Typical plots of $[poly(G\text{-}C)]_{total}/(|\varepsilon_{app} - \varepsilon_f|)$ *vs.* $[poly(G\text{-}C)]_{total}$ for determination of the equilibrium binding constant (K_{app}) based on Eq. (1) of poly(G-C) binding of Co(tmtppa) at various temperatures.

parameters of poly(A-T), poly(G-C) and DNA with their uncertainties for interaction with Co(tmtppa) are presented in Tables 1-3.

Since for these systems, in common with all 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 activities. The standard free energy changes ($\Delta G°$) for Co(tmtppa)-poly(A-T), Co(tmtppa)-poly(G-C) and Co(tmtppa)-DNA interactions were large and negative due to their strong association. The positive values of $\Delta H°$ also indicated that the binding of Co(tmtppa) to poly(A-T), poly(G-C) and DNA is an endothermic process. The positive values of $\Delta H°$ in Co(tmtppa)-poly(G-C) and Co(tmtppa)-DNA interactions indicated contribution of the positive entropy changes ($\Delta S°$), resulting in large $T\Delta S°$

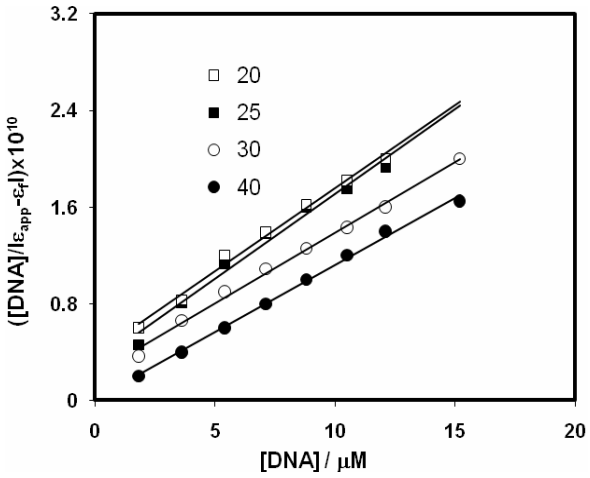


Fig. 7. Typical plots of $[DNA]_{total}/(|\varepsilon_{app} - \varepsilon_f|)$ *vs.* $[DNA]_{total}$ for the determination of equilibrium binding constant (K_{app}) based on Eq. (1) of DNA binding of Co(tmtppa) at various temperatures.

Table 1. Calculated Thermodynamic Parameters for Binding of Co(tmtppa) to Poly(A-T) in 5 mM Phosphate Buffer, pH 7.2, at 25 °C

T (K)	$(K \pm \Delta K) \times 10^6$ (M^{-1})	$\Delta G°$ (kJ mol^{-1})	$\Delta H°$ (kJ mol^{-1})	$\Delta S°$ (J mol^{-1} K^{-1})
293.15	1.59 ± 1.03	-34.07 ± 0.05	38.84 ± 0.11	248.71 ± 0.22
298.15	2.25 ± 1.02	-36.25 ± 0.04	38.84 ± 0.11	251.85 ± 0.25
303.15	2.98 ± 1.04	-37.57 ± 0.06	38.84 ± 0.11	252.05 ± 0.28
313.15	4.45 ± 1.03	-39.85 ± 0.04	38.84 ± 0.11	251.28 ± 0.25

Table 2. Calculated Thermodynamic Parameters for Binding of Co(tmtppa) to Poly(G-C) in 5 mM Phosphate Buffer, pH 7.2, at 25 °C

T (K)	$(K \pm \Delta K) \times 10^6$ (M^{-1})	$\Delta G°$ (kJ mol^{-1})	$\Delta H°$ (kJ mol^{-1})	ΔS (J mol^{-1} K^{-1})
293.15	1.66 ± 1.02	-34.88 ± 0.06	41.14 ± 0.12	259.45 ± 0.21
298.15	2.50 ± 1.03	-36.49 ± 0.6	41.14 ± 0.12	260.50 ± 0.25
303.15	3.33 ± 1.03	-37.83 ± 0.05	41.14 ± 0.12	260.62 ± 0.28
313.15	5.00 ± 1.02	-40.13 ± 0.07	41.14 ± 0.12	259.64 ± 0.25

Table 3. Calculated Thermodynamic Parameters for Binding of Co(tmtppa) to DNA in 5 mM Phosphate Buffer, pH 7.2, at 25 °C

T (K)	$(K \pm \Delta K) \times 10^5$ (M^{-1})	ΔG° (kJ mol^{-1})	ΔH° (kJ mol^{-1})	ΔS° (J mol^{-1} K^{-1})
293.15	2.00 ± 1.02	-30.27 ± 0.06	53.59 ± 0.12	286.21 ± 0.21
298.15	2.50 ± 1.03	-36.49 ± 0.6	53.59 ± 0.12	285.46 ± 0.25
303.15	3.30 ± 1.03	-37.83 ± 0.05	53.59 ± 0.12	285.94 ± 0.28
313.15	5.00 ± 1.02	-40.13 ± 0.07	53.59 ± 0.12	286.07 ± 0.25

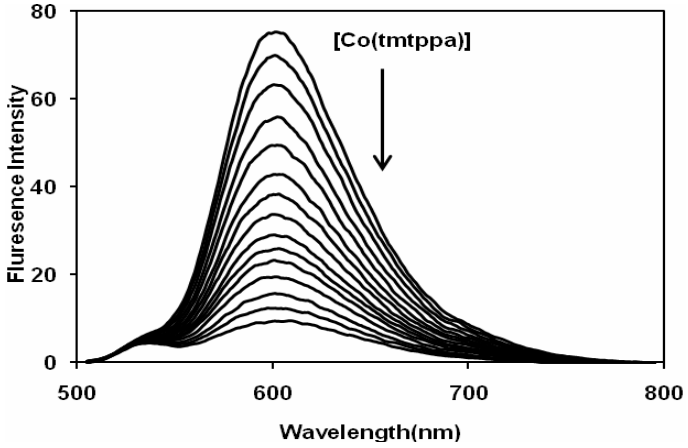


Fig. 8. Emission spectra of EB (5.00 μM) bound to DNA (12.00 μM) in the absence and the presence of different Co(tmtppa) concentration in 5 mM phosphate buffer, pH 7.2, λ_{ex} = 490 nm.

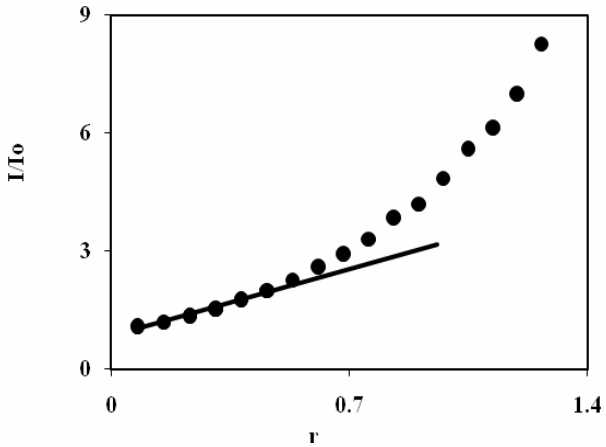


Fig. 9. The Stern-Volmer plot for quenching process of EB by Co(tmtppa).

and more negative ΔG°, which favors the binding process. As summarized in Tables 1-3, it seems that the major contributing factor in the stabilization of the Co(tmtppa)-poly(A-T), Co(tmtppa)-poly(G-C) and Co(tmtppa)-DNA complexes are entropic in origin. It can be concluded that the positive entropy changes are the driving forces in the Coulombic interactions between the positively charged Co(tmtppa) and negatively charged poly(A-T), poly(G-C) and DNA [28]. Therefore, the positive entropy changes of the Co(tmtppa)-poly(A-T), Co(tmtppa)-poly(G-C) and Co(tmtppa)-DNA interaction probably originates from the electrostatic interactions between the positively charged pyridine rings and negatively charged phosphate oxygens. Upon this interaction, water molecules bound to Co(tmtppa), poly(A-T), poly(G-C) and DNA are released, leading to positive entropy changes in the overall thermodynamics of the interactions. In the case of the binding of the Co(tmtppa) to poly(A-T), poly(G-C) and DNA desolvation occurring at the ionic interaction is also significant as shown by the positive enthalpy changes.

Upon interactions of Co(tmtppa) with poly(A-T), poly(G-C) and DNA, the axial ligands, such as water molecules, are presumably substituted by the carbonyl group of thymine and/or the ring nitrogen of the base pairs and exchange polynucleotides or DNA conformation. Such a coordination interaction has been reported for several metalloporphyrins [27]. In addition, the interaction of Co(tmtppa) with DNA induces a more significant distortion in the DNA structure compared to that of poly(A-T) and poly(G-C). The distortion results in a change in the local charge density, which results in condensed counter-ion release from the interacting surface. So that, more distortion of DNA and the condensed co counter-ion release leads to large positive enthalpy and entropy changes for it [29].

On the other hand, in the Co(tmtppa)-DNA interaction, the greater release of water molecules or counter-ions compared with poly(A-T) and poly(G-C) results in more positive entropy and enthalpy values for the former [29].

Fluorescence Spectroscopic Studies

No luminescence was observed for the porphyrazine complex at room temperature in aqueous solution or in the presence of calf thymus DNA. So, the binding of Co(tmtppa) complex and DNA cannot be directly presented in the emission spectra. In a previous study, ethidium bromide (EB) was shown to emit intense fluorescence light in the presence of DNA, due to its strong intercalation between the adjacent DNA base pair [30]. It has been previously reported that the fluorescent light could be quenched by the addition of a second molecule [31-32]. The quenching extent of fluorescence of the EB binding to DNA was used to determine the extent of binding between the second molecule and DNA. Addition of the complex to DNA pretreated with EB caused appreciable reduction in the emission intensity, indicating the replacement or electron transfer of the EB fluorophore by the complex which resulted in a decrease of the binding constant of ethidium to DNA.

Based on Stern-Volmer equation [31]:

$$\frac{I_o}{I} = 1 + K_{SV} r = 1 + K_q \tau_o r \qquad (5)$$

where I_0 and I are the fluorescence intensities in the absence and the presence of complex respectively, and K_{SV} is a linear Stern-Volmer quenching constant dependent on the ratio of r (the ratio of the total concentration of complex to the concentration of polynucleotide or DNA). The Stern-Volmer constant, is the product of the rate constant for quenching (K_q) and the lifetime of the luminescence in the absence of quencher (τ_0) ($K_{SV} = K_q \tau_0$). The excited lifetime of ethidium bromide in the presence of DNA is lengthened to 23 ns [33].

Figure 8. In the plot of I_0/I *vs.* [porhyrazine]/[DNA], K_{SV} is given by the ratio of the slope to intercept (Fig. 9). The nonlinearity of this relationship can account for the possibility of the existence of either dynamic or static quenching mechanisms [34]. Nevertheless, at [porhyrazine]/[DNA] concentration ratio lower than 0.6 the dependence is linear and quenching mechanism is static. At concentration ratio higher than 0.6 (for nonlinear region Stren-Volmer plot) both dynamic and static quenching mechanisms take place.

The quenching plots illustrate that quenching of EB bound to DNA by Co(tmtppa) is in a good agreement with the linear Stern-Volmer equation (at the low r), also indicating that porphyrazine binds to DNA. In these cases we can calculate the quenching constant. The slope of the

plot yields (for linear region of r) K_{SV} and K_q values (K_{SV} = 2.61×10^6 M^{-1} and $K_q = 1.13 \times 10^{14}$ M^{-1} S^{-1}, respectively, (τ_0 = 23 ns and $K_q = K_{SV}/\tau_0$).

CONCLUSIONS

Co(tmtppa) does not show concentration dependent aggregation over an extended concentration to 10^{-4} M in water. The Poly(A-T)-binding, Poly(G-C)-binding and DNA-binding process were endothermic for Co(tmtppa) with a large positive entropy. These can represent the predominant role of coulombic interactions and groove binding mode. The existence of a moderate hypochromicity and shift of wavelength in the UV-Vis spectra of Co(tmtppa) also suggests a groove binding mode with no stake formation of Co(tmtppa) on the DNA surface and Poly nucleotides.

The enthalpy and entropy changes in binding of Co(tmtppa) to DNA are larger than those in binding of Co(tmtppa) to poly(A-T) and Co(tmtppa) to poly(G-C), suggesting that upon interaction of Co(tmtppa) with DNA more water molecules are released compared to the case of poly(G-C) and poly(G-C).

The fluorescence quenching results showed that the Stern-Volmer equation is linear at low quencher concentration and quenching is static. Consequently, the binding characteristic of Co(tmtppa) to the polynucleotides and DNA duplexes is tuned by varying the metal center.

ACKNOWLEDGMENTS

We gratefully acknowledge the Research Council of University of Guilan for financial supporting this work.

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