

Study on interaction of cationic porphyrazine with synthetic polynucleotides

Hamid Dezhmpanah* and Soghra Fyzolahjani

Laboratory of Physical Chemistry, Faculty of Science, Department of Chemistry, University of Guilan, Rasht, Iran

Abstract. Interactions of cationic tetrakis (N, N', N'', N'''- tetramethyltetra-3, 4-pyridinoporphyrinatozinc (II) (Zn (tmtppa)) with synthetic polynucleotides, poly (G-C) and poly (A-T), and calf thymus DNA have been characterized in 7.5 mM phosphate buffer of pH 7.2 by UV-Vis absorption and fluorescence spectroscopy. The appearance of hypochromicity more than 30% in UV-Vis spectra of porphyrazine due to interaction of both poly (G-C) and poly (A-T) indicates interaction similar to that of porphyrazine with DNA.

The binding constants were determined from the changes in the Q-band maximum of the porphyrazine spectra at various poly (G-C) and DNA concentrations. The values of K were $2.5 \times 10^6 \text{ M}^{-1}$, $2.5 \times 10^6 \text{ M}^{-1}$ and $2.5 \times 10^5 \text{ M}^{-1}$ for poly (G-C), poly (A-T) and DNA, respectively, at 25°C. The thermodynamic parameters (ΔG° , ΔH° , ΔS°) were calculated using the van't Hoff equation at various temperatures. The enthalpy and entropy changes were determined to be $41.14 \text{ kJ mol}^{-1}$ and $260.50 \text{ J mol}^{-1} \cdot \text{K}^{-1}$ for poly (G-C) and $53.59 \text{ kJ mol}^{-1}$ and $285.46 \text{ J mol}^{-1} \cdot \text{K}^{-1}$ for DNA at 25°C. 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 formation. The binding of polynucleotides to porphyrazine quenches fluorescence emission of ethidium bromide (EB), and the quenching process obeys linear Stern-Volmer relationship. The results revealed groove-binding mode of porphyrazine for both AT- and GC-rich polynucleotides of DNA.

Keywords: Zinc porphyrazine, calf thymus DNA, synthetic polynucleotide, groove binding

1. Introduction

The interaction of small molecules with DNA continues to be an extremely important area of research, from both fundamental and practical points of view, as the molecular recognition of DNA is of fundamental importance to life. DNA is an important drug target, in particular in the treatment of cancer, where many compounds that bind covalently and/or non-covalently to DNA, or damage DNA, are used. Interaction of porphyrins and metalloporphyrins with DNA has become a topic of considerable interest due to their medical applications. The ability of porphyrin to selectively

cleave nucleic acid has made the porphyrins to be widely used as a structural probe of DNA [1–5].

In molecular biology, G-quadruplexes (also known as G-tetrads or G_4 -DNA) are nucleic acid sequences that are rich in guanine and are capable of forming a four-stranded structure. Four guanine bases can associate through Hoogsteen hydrogen bonding to form a square planar structure called a guanine tetrad, and two or more guanine tetrads can stack on top of each other to form a G-quadruplex. The quadruplex structure is further stabilized by the presence of a cation, especially potassium, which sits in a central channel between each pair of tetrads.

The G-quadruplex structure plays a very important role in controlling telomerase activity. Therefore, drugs to stabilize this conformation are thought to be effective telomerase inhibitors. Using G-quadruplex as a target to investigate the interaction between quadruplex

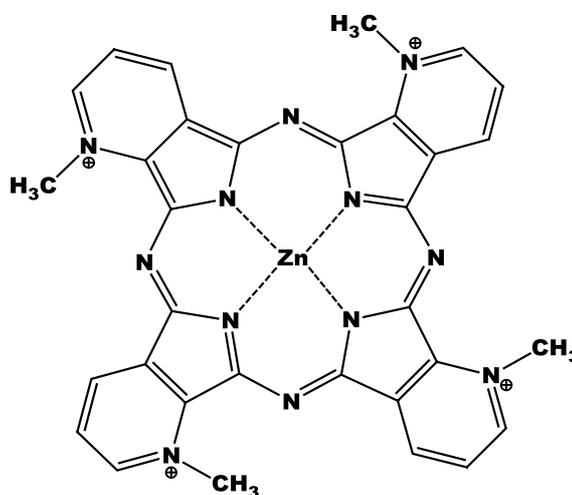
*Corresponding author: Hamid Dezhmpanah, Laboratory of Physical Chemistry, Faculty of Science, Department of Chemistry, University of Guilan, P.O. Box 41335-1914, Rasht 0098, Iran. Tel./Fax: +98 131 3233262; E-mails: h.dpanah@guilan.ac.ir, h_dpanah@yahoo.com.

and small molecule is one of the important ways to find anti-tumor drugs [6, 7]. Most of these ligands have the common feature of extended planar aromatic electron-deficient chromophore with cationic substituents. This structural feature facilitates these ligands to intercalate into G-quadruplexes easily and improves the stability of the compounds.

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} [8]. Positively charged phthalocyanines possess promising photosensitizing properties for the photodynamic therapy of cancer, showing a higher photodynamic activity *in vitro* than the commonly used haematoporphyrin [9]. 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 [10].

Cationic tetraazaporphyrins, or porphyrazines, represent an alternative and highly developed class of cationic porphyrinic compounds. Macrocycles hasten the binding on the porphyrazine core, including phthalocyanines, but the replacement of the meso-methylene carbons of porphyrins with nitrogen in porphyrazines creates profound differences [11]. For example, porphyrazines absorb more strongly at longer wavelengths, a critical feature in biological and medical applications [11, 12]. 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 [13] with some of them existing largely as monomers in aqueous solutions [14–16].

The interaction between water-soluble cationic porphyrazine and DNA has been well studied [17, 18], but to our knowledge the binding mode of the complex formed between porphyrazine and synthetic polynucleotide such as poly (A-T) and poly (G-C) has not been reported so far. In this paper, we report the binding characteristics of porphyrazine tetrakis (N, N', N'', N'''-tetramethyltetra-3, 4-pyridinoporphyrazinatozinc (II) (Zn (tmtppa)) (Scheme 1) with polynucleotides such as poly (G-C) and poly (A-T) and DNA on the basis of the binding mode, the binding affinity and thermody-



Scheme 1. Chemical structure of Zn(tmtppa).

amic parameters of the interaction by spectroscopic method.

1.1. Chemicals and preparations

Metal porphyrazine [Zn (tmtppa)] was synthesized according to the previous method [19].

The stock solution of porphyrazine complex (1 mg ml^{-1}) was prepared in 7.5 mM phosphate buffer, pH 7.20, 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 were determined spectrophotometrically by measuring the molar extinction coefficient. Polynucleotides such as poly (G-C) and poly (A-T) and calf thymus DNA were purchased from Sigma Chemical Co. and were used without further purification.

To prepare the DNA stock solution, 2 mg of DNA was dissolved in 1 mL of phosphate buffer the day before the experiment and stored at 4°C. The stock solutions of poly (G-C), poly (A-T) were prepared in phosphate buffer and stored at 4°C. The concentration of poly (G-C), poly (A-T) and DNA was determined from their optical absorption using their molar absorption coefficients. The extinction coefficients of $\epsilon_{254\text{nm}} = 1.68 \times 10^4 \text{ cm}^{-1} \cdot \text{M}^{-1}$, $\epsilon_{262\text{nm}} = 1.32 \times 10^4 \text{ cm}^{-1} \cdot \text{M}^{-1}$ and $\epsilon_{259\text{nm}} = 1.32 \times 10^4 \text{ cm}^{-1} \cdot \text{M}^{-1}$ were used to determine the concentration of base pairs of poly (G-C), poly (A-T) [20] and DNA [21], respectively. The pH values were controlled using a Metrohm-744 pH meter. The

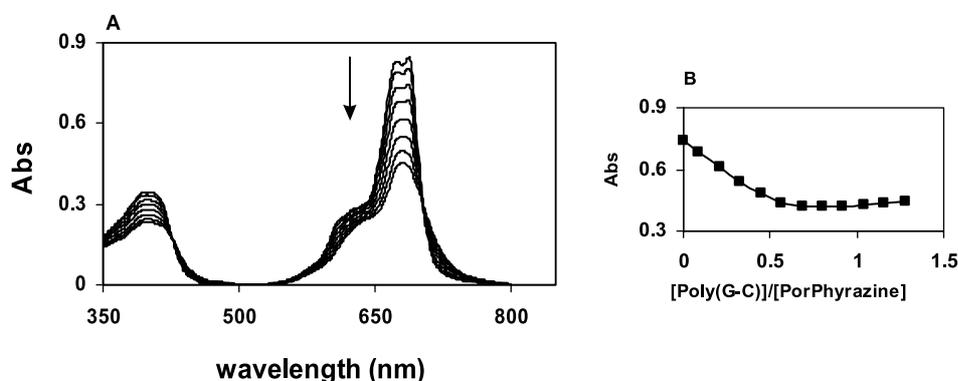


Fig. 1. (A): Absorption spectra of Zn (tmtppa) ($14.5 \mu\text{M}$) in the presence of poly (G-C): 0, 0.16, 0.32, 0.48, 0.63, 0.78, 0.94, 1.09 μM . The arrow indicates the absorbance changes with increasing [poly (G-C)]. (B) The variation of absorbance at the Q-band (688 nm) versus mole ratio of poly (G-C) to Zn (tmtppa).

temperature of the solutions was kept within the range of $\pm 0.1^\circ\text{C}$.

1.2. Optical absorption

The absorption spectra were recorded on a Cary 500 scan UV-vis-NIR spectrophotometer. The porphyrazine solutions were prepared in the concentration range of 4.0 to $40.0 \mu\text{M}$ for optical absorption measurements in the Q-band region. The UV-vis titration experiments were performed by addition of the DNA and polynucleotides stock solutions each into 1 mL cuvettes containing the porphyrazine solution of appropriate concentration. The concentration range of DNA and polynucleotides was 10^{-6} M to 10^{-4} M. The titration experiments were performed at various temperatures with a precision of $\pm 0.1^\circ\text{C}$.

1.3. Fluorescence spectroscopy

Emission spectra of ethidium bromide (EB) bound to DNA in the absence and presence of the Zn(tmtppa) were recorded on a Shimadzu model RF-5000 spectrofluorimeter. In a typical experiment, titration of a mixed DNA with EB in separate solution with porphyrazine in phosphate buffer were performed by stepwise addition of porphyrazine solution in the same buffer directly into the cuvette AQ for clarity. The solutions were excited at 525 nm and the emitted light intensity was measured in the range of 520–800 nm. Both UV-vis and fluorescence spectra were also corrected for dilution. The temperature was kept constant with an accuracy of $\pm 1^\circ\text{C}$ during titration experiments.

2. Results and discussion

2.1. Solution properties of Zn (tmtppa)

In order to identify the solution properties of Zn(tmtppa), we employed UV-vis spectroscopy. The optical absorption spectrum of Zn(tmtppa) shows a Q-band and a Soret band feature, which is a characteristic of the base porphyrazine. The molar absorptivity coefficient of these bands was calculated as $9.92 \times 10^4 \text{ M}^{-1} \cdot \text{cm}^{-1}$ for Q-band ($\lambda = 688 \text{ nm}$) and $4.25 \times 10^4 \text{ M}^{-1} \cdot \text{cm}^{-1}$ for Soret ($\lambda = 401 \text{ nm}$), respectively. The Q-band maximum of Zn(tmtppa) obeys Beer's law over an extended concentration range between 4.0×10^{-6} and 4.0×10^{-5} M in water. From this observation we can conclude that Zn(tmtppa) does not show concentration dependent aggregation.

We have conducted the titration of porphyrazine solution at a fixed concentration of complex (1.52×10^{-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, our porphyrazine exists mainly in the monomer form. Figures 1–3 show a typical titration spectra of Zn(tmtppa) upon increasing addition of poly (G-C), poly (A-T) and DNA, respectively, at 25°C . In both cases, high hypochromism ($H \geq 30\%$) in Q-band was observed, which represents the existence of non-covalent interaction and external groove binding between poly (G-C), poly (A-T), DNA and porphyrazine complex [22, 23].

The presence of isosbestic points in their UV-Vis titration spectra confirms the homogeneous binding

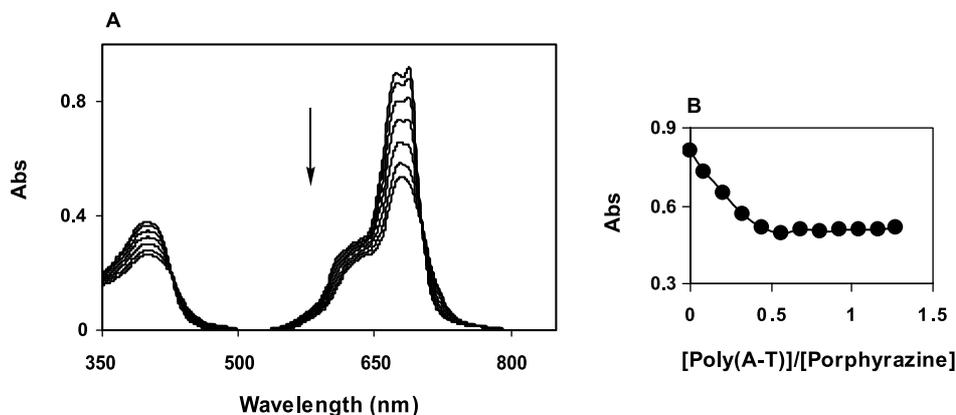


Fig. 2. (A): Absorption spectra of Zn (tmtppa) ($14.5 \mu\text{M}$) in the presence of poly (A-T): 0, 0.16, 0.32, 0.48, 0.63, 0.78, 0.94, 1.09 μM . The arrow indicates the absorbance changes with increasing [poly (A-T)]. (B) The variation of absorbance at the Q-band (688 nm) versus mole ratio of poly (A-T) to Zn (tmtppa).

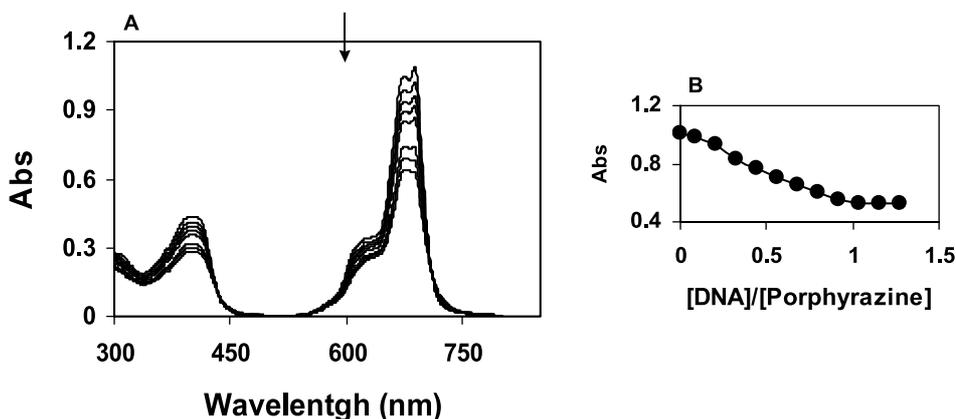


Fig. 3. (A): Absorption spectra of Zn (tmtppa) ($15.4 \mu\text{M}$) in the presence of DNA: 0, 1.82, 3.60, 5.35, 7.07, 10.40, 12.00 and 13.60 μM . The arrow indicates the absorbance changes with increasing [ct-DNA]. (B) The variation of absorbance at the Q-band (688 nm) versus mole ratio of DNA to Zn (tmtppa).

mode. A considerable hypochromicity without any redshift in the Q band of porphyrazine is caused by its interaction with the polynucleotide. These changes in the absorption spectra were similar to those reported for DNA surface. It can be deduced that Zn(tmtppa) in water is axially ligated by one H_2O molecule, as in five-coordinate Zn(tmtppa), which is known as a typical external groove binder [24]. In other words, this complex is inhibited from intercalation and externally binds AQ for clarity. The large hypochromicity suggests that the porphyrazine π electrons are perturbed considerably upon binding to polynucleotide and DNA. Porphyrazine metal complexes with ligands axially bound to the central metal ion (Zn(II) metalloporphyrazine) cannot find a place between base pairs and thus undergo groove binding.

Binding constants for the interaction of cationic porphyrazine with poly (G-C) and DNA were determined by analysis of absorption spectrophotometric titrations data. The changes in absorbance of the Q-band upon addition of poly (G-C) and DNA were monitored at the maximum of the Q-band. The apparent binding constant K_{app} of cationic porphyrazine to DNA was calculated using Eq. 1

$$\frac{[DNA]_{total}}{(\varepsilon_{app} - \varepsilon_f)} = \frac{[DNA]_{total}}{(\varepsilon_b - \varepsilon_f)} + \frac{1}{K_{app}(\varepsilon_b - \varepsilon_f)} \quad (1)$$

Here ε_{app} , ε_f and ε_b correspond to $A_{observed}/[\text{porphyrazine}]$, the extinction coefficient for the free porphyrazine and the extinction coefficient for the porphyrazine in the fully bound form, respec-

tively. In the plot of $[DNA]_{total}/(|\varepsilon_{app} - \varepsilon_f|)$ versus $[DNA]_{total}$ or $[poly(G-C)]_{total}/(|\varepsilon_{app} - \varepsilon_f|)$ versus $[poly(G-C)]_{total}$, K_{app} is given by the ratio of the slope to the intercept [25–27]. The plots of porphyrzine-poly (G-C) and porphyrzine-DNA binding are shown in Figs. 4 and 5. The apparent binding constant of Zn(tmtppa) was estimated and used for calculation of Gibbs free energy change of reaction at various temperatures. Thermodynamic parameters of porphyrzine-DNA interaction were calculated from the binding constants determined at various temperatures in the range of 20–45°C.

2.2. Thermodynamics of porphyrzine binding process

By measuring the temperature dependence of the binding constant, thermodynamic studies of the interaction between porphyrzine complexes and poly (G-C) and DNA have been carried out. Gibbs free energy was determined from the binding constant according to the following relationship:

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

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 estimated from the Gibbs free energy change and the molar enthalpy was determined as:

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

The obtained binding constants and thermodynamic parameters with their uncertainties for interaction with Zn(tmtppa) 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 use the equilibrium concentrations instead of the activities. Therefore, the value of K may vary with composition. The standard free energy changes (ΔG^o) for porphyrzine-poly (G-C)

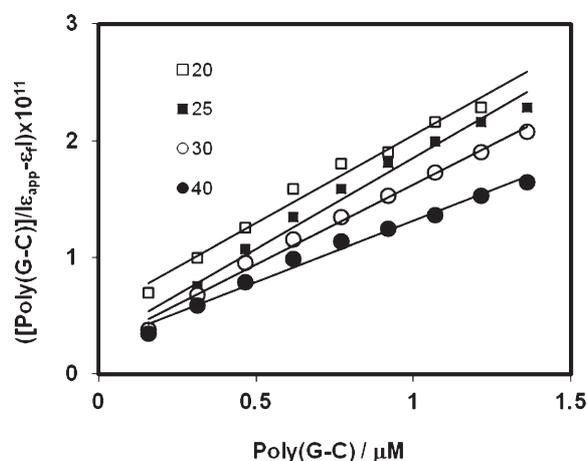


Fig. 4. Typical plots of $[poly(G-C)]_{total}/(|\varepsilon_{app} - \varepsilon_f|)$ versus $[poly(G-C)]_{total}$ for the determination of the equilibrium binding constant (K_{app}) based on Eqn. (1) of the $poly(G-C)$ binding of Zn (tmtppa) at various temperatures.

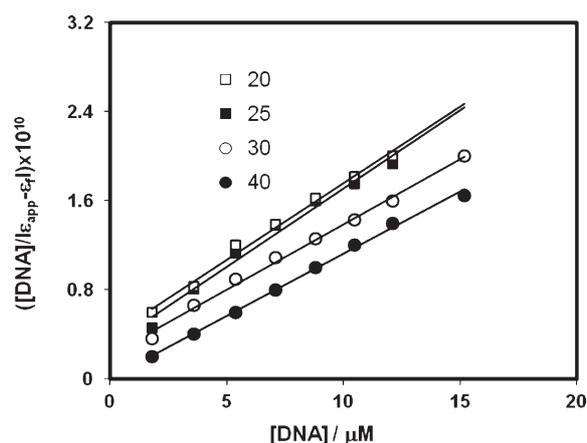


Fig. 5. Typical plots of $[DNA]_{total}/(|\varepsilon_{app} - \varepsilon_f|)$ versus $[DNA]_{total}$ for the determination of the equilibrium binding constant (K_{app}) based on Eq. (1) of the DNA binding of Zn (tmtppa) at various temperatures.

and porphyrzine-DNA interactions are large and negative due to their strong association. It has also been indicated that the binding of porphyrzine to DNA is an endothermic process. The ΔH^o 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, ΔH^o is positive. The positive values of ΔH^o in the porphyrzine-poly (G-C) and porphyrzine-DNA interactions indicate a contribution of the positive entropy changes (ΔS^o), resulting in large $T\Delta S^o$ and

Table 1
Calculated thermodynamic parameters for binding of Zn(tmtppa) to Poly (G-C) in 5 mM phosphate buffer, pH 7.2, at 25°C

T, K	$(K \pm \Delta K) \times 10^6 (M^{-1})$	$\Delta G^\circ, \text{kJ.mol}^{-1}$	$\Delta H^\circ, \text{kJ.mol}^{-1}$	$\Delta S^\circ, \text{J. mol}^{-1} \cdot \text{K}^{-1}$
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 2
Calculated thermodynamic parameters for binding of Zn(tmtppa) to DNA in 5 mM phosphate buffer, pH 7.2, at 25°C

T, K	$(K \pm \Delta K) \times 10^5 (M^{-1})$	$\Delta G^\circ, \text{kJ.mol}^{-1}$	$\Delta H^\circ, \text{kJ.mol}^{-1}$	$\Delta S^\circ, \text{J. mol}^{-1} \cdot \text{K}^{-1}$
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

more negative ΔG° , which favors the binding process. As summarized in Tables 1 and 2, it seems that the major contributing factor in the stabilization of the porphyrine–poly (G-C) and porphyrine–DNA complexes 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 porphyrines and negatively charged DNA [28]. Therefore, the positive entropy changes of the porphyrine–poly (G-C) and porphyrine–DNA interactions probably come from the electrostatic interaction between the positively charged pyridine rings and negatively charged phosphate oxygens. Upon this interaction, water molecules bound to porphyrine, poly (G-C) and DNA are released, which leads to positive entropy changes in the overall thermodynamics of the interactions. The interaction of DNA is more endothermic than that of poly (G-C). 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 the binding to poly (G-C). This can lead to greater changes in DNA local charge density, by the release of condensed counterions from the interacting surface [29]. On the contrary, the greater release of water molecules or counterions in the Zn(tmtppa) - DNA interaction, compared with poly (G-C), results in more positive entropy and enthalpy values. It has also been reported that cationic porphyrins can usually intercalate into G-C sites and bind outside 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 interac-

tions 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 [30]. 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 porphyrine, the A-T site will apparently release more water molecules. Consequently, outside binding of zinc porphyrine at A-T sites probably results in more positive enthalpy and entropy than intercalation into the G-C sites [30].

2.3. Fluorescence spectroscopic studies

No luminescence was observed for the porphyrine complex at room temperature in aqueous solution or in the presence of calf thymus DNA. So the binding of Zn(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. It was previously reported that the fluorescent light could be quenched by the addition of a second molecule [31, 32]. The quenching extent of fluorescence of EB bound to DNA is used to determine the extent of binding between the second molecule and DNA. The addition of the complex to DNA pretreated

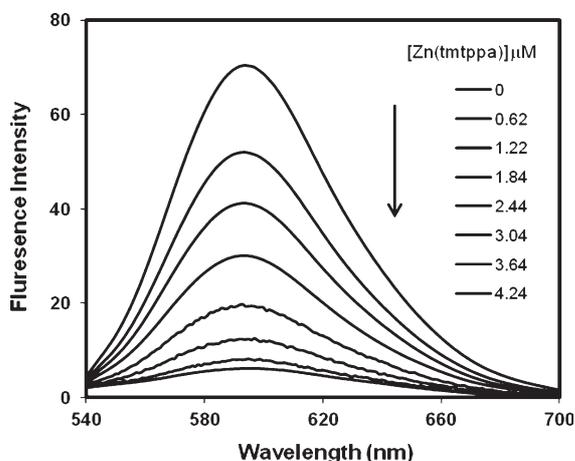


Fig. 6. Emission spectra of EB (5.00 μM) bound to DNA (12.00 μM) in the absence and the presence of different Zn(tmtppa) concentration in 5.5 mM phosphate buffer, pH 7.2, $\lambda_{\text{ex}} = 510 \text{ nm}$

with EB causes appreciable reduction in the emission intensity, indicating the replacement or electron transfer of the EB fluorophore by the complex, resulting in a decrease in the binding constant of the ethidium to the DNA.

According to the classical Stern-Volmer equation [31]:

$$\frac{I_o}{I} = 1 + K_{SV}r, \quad (5)$$

where I_o and I are the fluorescence intensities in the absence and the presence of complex, respectively. 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 fluorescence quenching curve of EB bound to DNA by the Zn(tmtppa) complex is shown in Fig. 6. The quenching plots illustrate that the quenching of EB bound to DNA by porphyrine is in good agreement with the linear Stern-Volmer equation, which also indicates that the porphyrine binds to DNA. In the plot of I_o/I versus [porphyrine]/[DNA], K_{SV} is given by the ratio of the slope to intercept (Fig. 7). The nonlinearity of this relationship can account for the possible existence of either dynamic or static quenching mechanisms.

From the quasi-linear portions of this plot (at low $[r]$), values for K_{SV} were determined ($K_{SV} = 7.8 \times 10^6 \text{ M}^{-1}$).

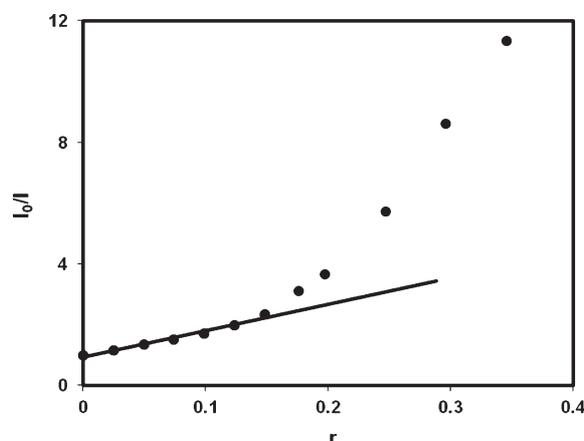


Fig. 7. The Stern-Volmer plot for quenching process of EB by Zn(tmtppa).

3. Conclusion

Zn(tmtppa) does not show concentration dependent aggregation over an extended concentration to 10^{-5} M in water. Addition of NaCl shows no significant electrolyte effect; no new band appears even at high concentration of the salt. This result means that Zn(tmtppa) does not form well-defined aggregates (i.e. H or J type) even at high concentrations of the salt. These observations may be due to the high hydrophobic and large size of peripheral pyridine groups of Zn(tmtppa). It seems that the required symmetric conditions for formation of well-defined aggregates are not available in this porphyrine. Our results show low affinity of this porphyrine for the formation of aggregates even at high salt concentration. This can be taken as an advantage for using of this porphyrine in Photodynamic Therapy (PDT).

Zn(tmtppa) binds to external regions in the G-C step of DNA, which is placed in minor grooves, because these are the binding sites of Na^+ ions. It is assumed that the steric constraints presented by the water axial ligand at the metal center of the zinc porphyrine serve to prevent intercalation into DNA. The higher affinity of the Poly (G-C) with respect to the DNA can be attributed to the role of nucleotide axial ligation in the former.

The Poly (G-C)-binding and DNA-binding processes were endothermic for Zn(tmtppa) and have a large positive entropy. These can represent the predominant role of hydrophobic interactions and out-

side groove binding mode. The existence of high hypochromicity without any red-shift in the UV-vis results of the Zn(tmtpa) spectra suggests an outside binding mode. In spite of this, the UV-vis results represent external binding (to minor or major grooves) with no stake formation of porphyrazine on the DNA surface and polynucleotides.

Acknowledgments

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

References

- [1] G. Pratviel, J. Bernadou and B. Meunier, *Metal Ions in Biological Systems* **33** (1996), 399.
- [2] Á. Orosz, G. Mező, L. Herényi, J. Habdas, Z. Majer, B. Myśliwa-Kurziel, K. Tóth and G. Csik, *Biophysical Chemistry* **14** (2013), 177–178.
- [3] E. Krestyn, H. Kolarova, R. Bajgar and K. Tomankova, *Toxicology in Vitro* **24**(1) (2010), 286.
- [4] C. Uslan and B.Ş. Sesalan, *Dyes and Pigments* **94**(1) (2012), 135.
- [5] Y. Zhang, H. Kong, Y. Fang, K. Nishinari and G.O. Phillips, *Bioactive Carbohydrates and Dietary Fibre* **1**(1) (2013), 53.
- [6] G. Saretzki, *Cancer Lett* **194** (2003), 209.
- [7] A.J. Kowaltowski and M.S. Baptista, *Photochem Photobiol* **79**(3) (2004), 227.
- [8] B. Paquette and J.E. van Lier, in *phthalocyanines and Related Compounds: Basic Principles and Clinical Applications*, eds. B.W. Henderson and T.J. Dougherty, Marcel Decker, Inc., New York, Basel, Hong Kong, 1992, pp. 145.
- [9] D. Wöhrle, N. Iskandar, G. Grachev, H. Sinn, E. A. Friedrich, W. Maier-Borst, J. Stern and P. Schlag, *Photochem Photobiol* **51** (1990), 351.
- [10] S.R. Wood, J.A. Hotroyd and S.B. Brown, *Photochem Photobiol* **65** (1997), 397.
- [11] M. Gouterman, In *The porphyrins*, 3, Dolphin D. (Ed.) Academic Press: New York, 1978, pp. 1–165.
- [12] I. Rosenthal, E. Ben-Hur, C.C. Leznoff and A.B.P. Lever, *Phthalocyanines: Properties and Applications*, 1, VCH publishers: New York, 1989, p. 393.
- [13] M.J. Fuchter, L. Scott Beall, S.M. Baum, A.G. Montalban, E.G. Sakellariou, N.S. Mani, T. Miller, B.J. Vesper, A.J.P. White, D.J. Williams, A.G.M. Barretta and B.M. Hoffman, *Tetrahedron* **61** (2005), 6115.
- [14] M. Thamae and T. Nyokong, *J Electroanal Chem* **470** (1999), 126.
- [15] M. Thamae and T. Nyokong, *J Porphyrins Phthalocyanines* **5** (2001), 839.
- [16] C. Marti, S. Nonell, M. Nicolau and T. Torres, *Photochem Photobiol* **71** (2000), 53.
- [17] M. Asadi, E. Safaei, B. Ranjbar and L. Hasani, *New J Chem* **2**(8) (2004), 1227.
- [18] H. Dezhampannah, A.K. Bordbar, Z. Salimian and E. Safaei, *J Porphyrins Phthalocyanines* **14** (2010), 354.
- [19] D.E.V. Schmechel and D.M. Crothers, *Biopolymers* **10**, (1971), 465.
- [20] R.F. Pasternack, C.B. Bustamante, P.J. Collings, A. Giannetto and E.J. Gibbs, *J Am Chem Soc* **115** (1993), 5393.
- [21] R.F. Pasternack and E.J. Gibbs, *Met Ions Biol Syst* **33** (1996), 367.
- [22] R.F. Pasternack, E.J. Gibbs and J.J. Villafraca, *Biochemistry* **22** (1983), 2406.
- [23] J.L. Hoard, in *Porphyrins and Metalloporphyrins*, ed. K.M. Smith, Elsevier, Amsterdam, 1979, p. 318.
- [24] A.M. Pyle, J.P. Rehman, R. Meshoyrer, C.V. Kumar, N.J. Turro and J.K. Barton, *J Am Chem Soc* **111** (1989), 3051.
- [25] J. Onuki, A.V. Ribas, M.H.G. Medeiros, K. Araki, H.E. Toma, L.H. Catalani and P.D. Mascio, *Photochem Photobiol* **63** (1996), 272.
- [26] S. Mettath, B.R. Munson and R.K. Pandey, *Bioconj Chem* **10** (1999), 94.
- [27] H. Ogoshi, T. Mizutani, T. Hayashi and Y. Kuroda, In *The Porphyrin Handbook*, eds. K.M. Kadish, K.M. Smith and R. Guilard, Academic Press, Burlington, MA, USA, 1990, vol. 6, ch. 46, p. 279.
- [28] J.B. Chairs, *Biopolymers* **44** (1997), 201.
- [29] R.F. Pasternack and E.J. Gibbs, in *Metal DNA Chemistry (ACS Symposium Series 402)*, ACS, Washington DC, 1989, p. 59.
- [30] D.H. Tjahjono, S. Mima, T. Akutsu, N. Yoshioka and H. Inoue, *J Inorg Biochem* **85** (2001), 219.
- [31] B.C. Baguley and M. LeBret, *Biochemistry* **23** (1984), 937.
- [32] J.R. Lakowicz and G. Webber, *Biochemistry* **12** (1973), 4161.



Hindawi
Submit your manuscripts at
<http://www.hindawi.com>

