ISSN 0006-2979, Biochemistry (Moscow), 2007, Vol. 72, No. 6, pp. 652-657. © Pleiades Publishing, Ltd., 2007.
Published in Russian in Biokhimiya, 2007, Vol. 72, No. 6, pp. 799-804.

Thermodynamic Study of Interaction of TSPP, CoTsPc, and FeTsPc with Calf Thymus DNA

M. Monajjemi[1]*, H. Aghaie[1], and F. Naderi[1,2]

[1]*Science and Research Campus, Islamic Azad University, P.O. Box 14515-775, Tehran, Iran; fax: (98)21-44804172; E-mail: m_monajjemi@yahoo.com*
[2]*Shahriyar-Shahr e Ghods Branch, Islamic Azad University, Tehran, Iran*

Received November 8, 2006
Revision received December 29, 2006

Abstract—The interaction of two water soluble phthalocyanines, cobalt(II) 4,4′,4″,4‴-tetrasulfo-phthalocyanine (CoTsPc) and iron(II) 4,4′,4″,4‴-tetrasulfo-phthalocyanine (FeTsPc), and one water soluble porphyrin, tetra sodium meso-tetrakis(*p*-sulfophenyl)porphyrin (TSPP), with calf thymus DNA has been studied by UV-Vis spectroscopy at five different temperatures (20, 25, 30, 35, and 40°C). The optical absorption spectra of these materials were analyzed to obtain binding constants and stoichiometries using SQUAD software. The results show that the best fitting corresponds to a 1 : 1 complex model between a base pair of DNA and these materials. All of the studied porphyrin and phthalocyanines showed strong electrolyte effect, and increasing NaCl concentration induced self-aggregation of these materials.

DOI: 10.1134/S0006297907060089

Key words: DNA, porphyrin, phthalocyanine, SQUAD

DNA plays an increasingly important role in bioorganic chemistry, biotechnology, and material science. It has been experimentally shown that DNA double helices can transfer electronic charges through long distances when the charge carrier is a hole.

The DNA double helix is highly negatively charged and it interacts strongly with oppositely charged species [1-3]. Ligand binding to macromolecules plays a key role in biology and medicine as a steering mechanism in biological processes. More specifically, the binding of drugs to proteins and DNA has been of great interest in recent years leading to a large body of structural studies using both experimental and theoretical methods. Owing to the central role of DNA in replication and transcription, DNA has been a major target for antibiotic, anticancer, and antiviral drugs [4].

The effects of nucleic acid binding drugs are known for various diseases such as cancer, malaria, AIDS, and other viral, bacterial, and fungal infections [5]. The majority of DNA drugs are aromatic compounds of low molecular weight often carrying positive charges [6]. The different modes of drug binding to DNA include intercalation between adjacent base pairs and intrusion into the minor groove and into the major groove. Intercalation and minor-groove binding are the predominant DNA-binding modes of small ligands [5-7]. Sequence specificity of drug–DNA binding is limited owing to the restricted size of drugs but is generally higher for minor-groove binders than for intercalators [5, 6]. Phthalocyanines and porphyrins attract large attention because of their role in the human body, ability to accumulate in many kinds of cancer cells, as well as their magnetic and optical properties. These features make them useful in cancer medicine and photodynamic therapy [8-10].

Physicochemical properties of phthalocyanines and porphyrins can be easily adjusted by modification of the electronic distribution on the aromatic ring through peripheral substitutions, which plays an important role in many biological systems and in photochemistry.

The existence of porphyrin complexes is crucial for transport of oxygen (hemoglobin), solar energy transfer (chlorophyll), and electron transfer (cytochrome oxidase) [11-13]. Many phthalocyanines are known to dimerize and further agglomerate in aqueous solutions. A series of water soluble porphyrins can be derived from porphyrin precursors insoluble in water by introducing ionic groups such as COO^-, SO_3^-, $=NH^+$-CH_3, and

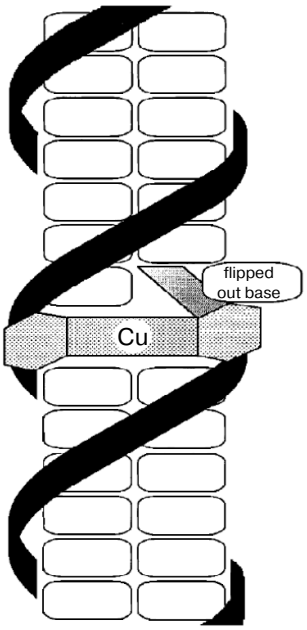
Abbreviations: CoTsPc) cobalt(II) 4,4′,4″,4‴-tetrasulfo-phthalocyanine; FeTsPc) iron(II) 4,4′,4″,4‴-tetrasulfo-phthalocyanine; TSPP) tetra sodium meso-tetrakis(*p*-sulfophenyl)porphyrin.

* To whom correspondence should be addressed.

$-N(CH_3)_3^+$. These so-called peripheral charge groups change chemical, spectral, and redox properties of the compounds and their metal complexes [12, 13].

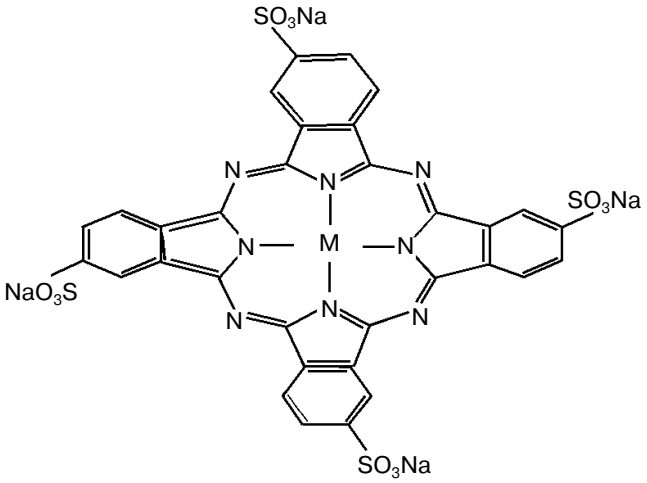
The DNA binding mechanism is very dependent upon not only the sequence of the DNA strands but also the structure perturbation of the phthalocyanine and porphyrin molecules [14-16].

Metal free and four-coordinate cationic porphyrins interact with DNA preferentially in G–C regions by true or hemi-intercalation.



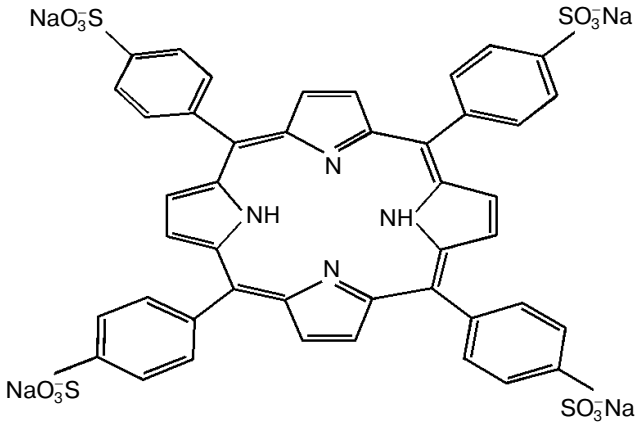
A porphyrin hemi-intercalated in a fragment of DNA

Scheme 1



Structural formulas of cobalt (II) 4,4′,4″,4‴-tetrasulfo-phthalocyanine (CoTsPc) and iron (II) 4,4′,4″,4‴-tetrasulfo-phthalocyanine (FeTsPc)

Scheme 2



Structural formula of tetra sodium meso-tetrakis(*p*-sulfophenyl)porphyrin (TSPP)

Scheme 3

In A–T regions, phthalocyanine binding appears to be external and coulombic, possibly involving the minor groove of DNA [17, 18]. Scheme 1 shows a schematic diagram of a porphyrin hemi-intercalated in a fragment of DNA [19]. This paper reports a thermodynamic study on interaction of cobalt(II) 4,4′,4″,4‴-tetrasulfophthalocyanine (CoTsPc), iron(II) 4,4′,4″,4‴-tetrasulfophthalocyanine (FeTsPc) (Scheme 2), and tetra sodium meso-tetrakis(*p*-sulfophenyl)porphyrin (TSPP) (Scheme 3) with DNA.

MATERIALS AND METHODS

DNA from calf thymus was purchased from Sigma (USA). Highly purified preparations of phthalocyanines and porphyrin were provided by the Shahid Beheshti Uinversity, Tehran. All solutions were prepared using double-distilled water. Phosphate buffer (1 mM, pH 7.4) was used. All of the working solutions were made by dissolving the solid materials in buffer solution. The phthalocyanines and porphyrin solutions were freshly prepared before spectral analysis. DNA solutions were prepared in a cold room (4°C) for 2 days with stirring in to ensure the formation of a homogeneous solution. The titration of phthalocyanine and porphyrin solutions as a function of DNA concentration was performed at 20, 25, 30, 35, and 40°C. UV-Vis spectrophotometric scanning was performed in the spectral range 200-800 nm using a spectrophotometer with 1-cm quartz cuvettes and thermostatted cell compartment that controls the temperature around the cell within ±0.1°C.

The binding constant and stoichiometry were determined by the analysis of optical absorption of phthalocyanine and porphyrin at various DNA concentrations using SQUAD software.

RESULTS AND DISCUSSION

DNA. DNA solutions after preparation were stored in a refrigerator at 4-5°C. Determination the calf thymus DNA concentration was based upon the reported ε_{258} value of 6700 $M^{-1}\cdot cm^{-1}$ and the Beer–Lambert equation:

$$A = \varepsilon \cdot b \cdot C, \quad (1)$$

where A is the absorbance, ε is the molar extinction coefficient, b is the length of cell, and C is the molar concentration.

Phthalocyanines and porphyrin. Electronic absorption spectra of phthalocyanines and porphyrin are sensitive to processes such as metallation, protonation (pH), substitution, or dimerization, which make phthalocyanines and porphyrins useful sensors of their environmental surroundings.

Figure 1 shows that the Soret band maximum obeys Beer's law over the concentration range $0.2\cdot10^{-5}$-$5.0\cdot10^{-5}$ M. Figure 2 shows absorption spectra of CoTsPc, FeTsPc, and TSPP. The spectrum of FeTsPc shows a band at 635 nm. The band of CoTsPc consists of two components (631.5 and 670 nm). The spectrum of TSPP shows a Soret band at 423 nm. All porphyrin and phthalocyanine solutions were titrated with 5 M NaCl in phosphate buffer, pH 7.4 at 25°C. The hypochromicity of 30-50% can be related to formation of agglomerate in the presence of salt. The UV-Vis spectra are shown in Figs. 3-5.

Interaction with DNA. All porphyrin and phthalocyanine solutions were titrated with a stock solution of calf thymus DNA. It can be assumed that the concentration of phthalocyanines and porphyrin in the solution remained

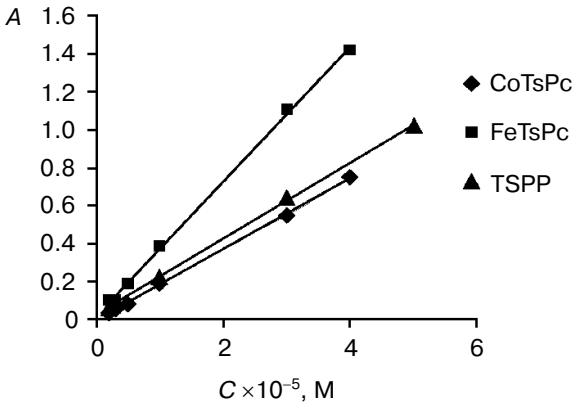


Fig. 1. Absorbance as a function of concentration at 25°C for CoTsPc, FeTsPc, and TSPP over concentration range between $0.2\cdot10^{-5}$ and $5.0\cdot10^{-5}$ M in their Soret bands.

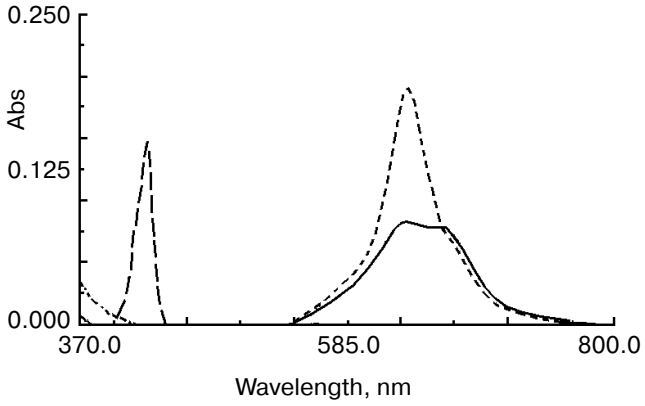


Fig. 2. Absorption spectra of CoTsPc (solid line), FeTsPc (dotted line), and TSPP (dashed line) at concentration of $5\cdot10^{-6}$ M.

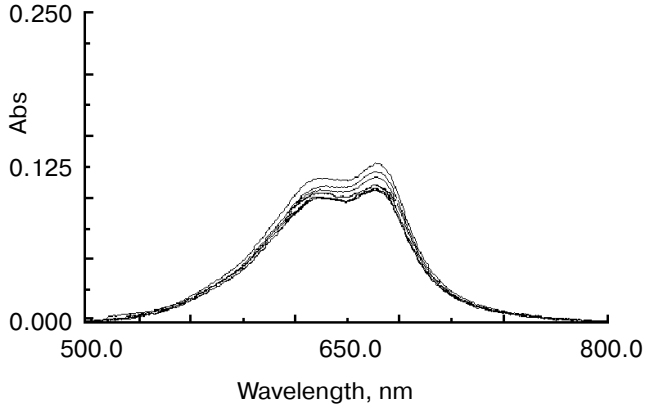


Fig. 3. Absorption spectra of CoTsPc upon titration with NaCl in phosphate buffer, pH 7.4, at 25°C (the absorption maximum decreases with increasing NaCl concentration).

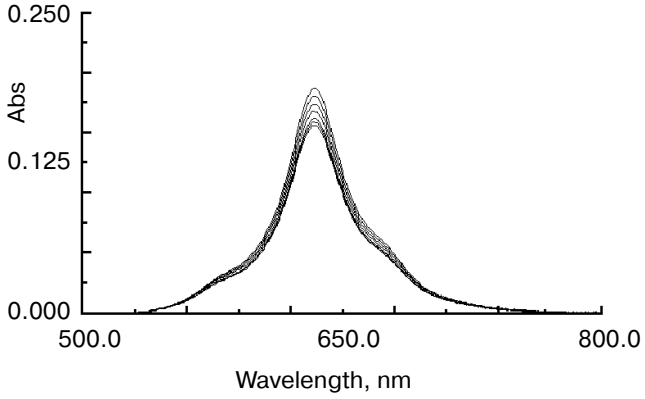


Fig. 4. Absorption spectra of FeTsPc upon titration with NaCl in phosphate buffer, pH 7.4, at 25°C (the absorption maximum decreases with increasing NaCl concentration).

the same because the total solution volume change was less than 2%. In CoTsPc, Soret bands were shifted with hypochromicity of 15-30%; in FeTsPc, the Soret band was shifted with hypochromicity of 15-20%. In TSPP it was about 20-40%. The values for hypochromicity suggest that the interaction was via both the intercalative type of binding and external binding [19]. The UV-Vis spectra from representative experiments are shown in Figs. 6-8.

To analyze the spectral data of porphyrin and phthalocyanines at various concentrations of DNA in titration experiments, 50 wavelengths showing suitable absorbance variations upon addition of DNA were selected from spectra of porphyrin and phthalocyanines. The values of absorbance of these selected wavelengths at various DNA concentrations were analyzed to calculate equilibrium formation constants using SQUAD software. This program is designed to calculate the best values for the stability constants by employing a nonlinear least square approach.

Fiel et al. were the first to suggest that porphyrins and phthalocyanines intercalate in DNA; they also bind via outside non-intercalative modes [20]. The mode of binding is controlled by porphyrin and phthalocyanine shape and charge. Ligands at the fifth and sixth coordination sites of the metal sterically block intercalation. So metal-free and four-coordinate porphyrins and phthalocyanines intercalate, while five- and six-coordinate porphyrins bind via non-intercalative modes [21].

Adding 5 M NaCl to the phthalocyanine and porphyrin solutions reveals a negative relationship between NaCl concentration and the absorbance of the solutions.

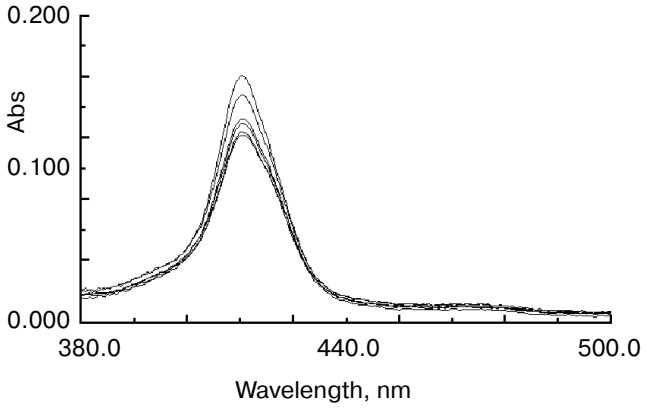


Fig. 5. Absorption spectra of TSPP upon titration with NaCl in phosphate buffer, pH 7.4, at 25°C (the absorption maximum decreases with increasing NaCl concentration).

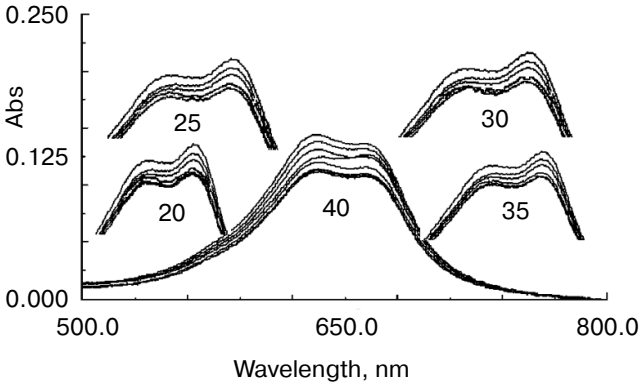


Fig. 6. Absorption spectra of CoTsPc upon titration with DNA in phosphate buffer, pH 7.4, at 20, 25, 30, 35, and 40°C (the absorption maximum decreases with increasing DNA concentration).

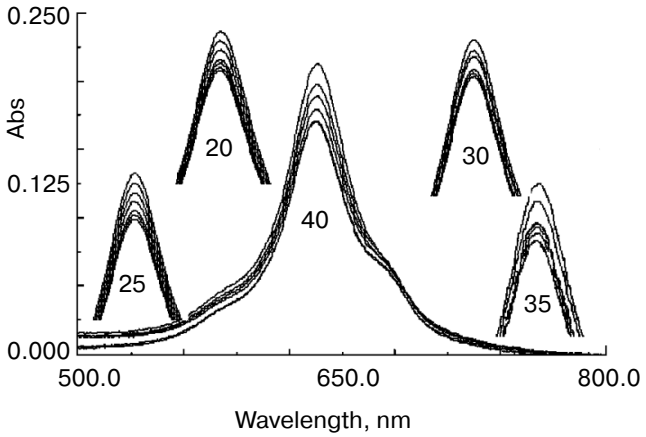


Fig. 7. Absorption spectra of FeTsPc upon titration with DNA in phosphate buffer, pH 7.4, at 20, 25, 30, 35, and 40°C (the absorption maximum decreases with increasing DNA concentration).

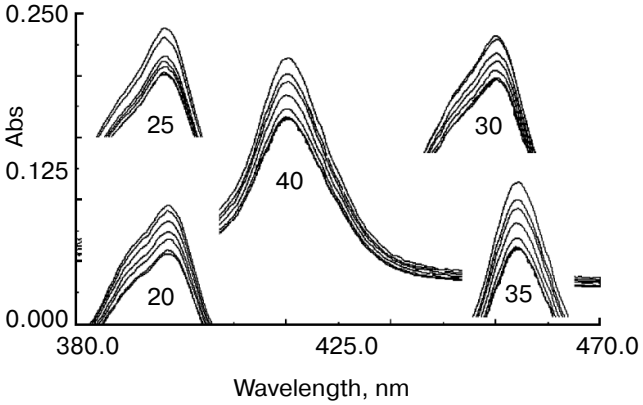


Fig. 8. Absorption spectra of TSPP upon titration with DNA in phosphate buffer, pH 7.4, at 20, 25, 30, 35, and 40°C (the absorption maximum decreases with increasing DNA concentration).

Increasing NaCl concentration induces self-aggregation of TSPP and phthalocyanines.

The absorbance data were analyzed to calculate the binding parameters using SQUAD program. SQUAD refines stability constants by employing a nonlinear least square approach. The results represent the formation of 1 : 1 complex model between porphyrins, phthalocyanines, and DNA. The obtained binding constants at different temperatures are summarized in the table.

To compare all thermodynamic parameters the change in standard Gibbs free energy (ΔG^0) should be calculated according to Eq. (2):

$$\Delta G^0 = -RT\ln K, \quad (2)$$

where K is the association binding constant, T is absolute temperature, and R is the gas constant. The Van't Hoff equation is:

$$d(\ln K)/d(1/T) = -\Delta H^0/R. \quad (3)$$

The Van't Hoff equation gives a linear plot of $\ln K$ versus $1/T$ if the heat capacity change for the reaction is essentially zero.

Thermodynamic parameters for binding of CoTsPc, FeTsPc, and TSPP to DNA in 1 mM phosphate buffer, pH 7.4, at various temperatures

T, °C	K × 10^{-4}	ΔG^0, kJ/mol	ΔH^0, kJ/mol	ΔS^0, J/mol
		CoTsPc		
20	3.1659	–25.2567	53.6577	269.1946
25	4.5789	–26.6019	53.6577	269.1922
30	7.0326	–28.1298	53.6577	269.7922
35	10.6862	–29.6656	53.6577	270.3988
40	19.0308	–31.6495	53.6577	272.4164
		FeTsPc		
20	4.0623	–25.8643	50.6663	261.0630
25	5.3061	–26.9675	50.6663	260.3852
30	8.3166	–28.5524	50.6663	261.3187
35	10.8923	–29.7146	50.6663	260.8499
40	21.6966	–31.9908	50.6663	263.9538
		TSPP		
20	23.3561	–30.1273	76.7332	364.5251
25	37.4108	–31.8089	76.7332	364.0522
30	62.6936	–33.6466	76.7332	364.0999
35	100.6409	–35.4122	76.7332	363.9315
40	173.6704	–37.4062	76.7332	364.4880

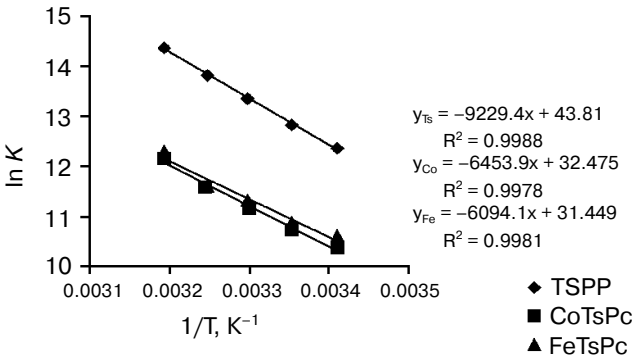


Fig. 9. A linear plot of $\ln K$ versus $1/T$ for binding of these porphyrin and phthalocyanines to DNA in phosphate buffer, pH 7.4.

The standard entropy can be calculated from the following equation:

$$\Delta G^0 = \Delta H^0 - T\Delta S^0. \quad (4)$$

Figure 9 displays a linear dependence of $\ln K$ versus $1/T$ for binding of these porphyrin and phthalocyanines to DNA in the phosphate buffer at pH 7.4. The negative slopes in plots point to the endothermicity of reaction, and the high correlation coefficients of the lines indicate that heat capacity changes are independent from temperature. Characteristic thermodynamic parameters obtained for these reactions are collected in the table.

The authors would like to thank Islamic Azad University and Shahid Beheshti University for their financial and other support.

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