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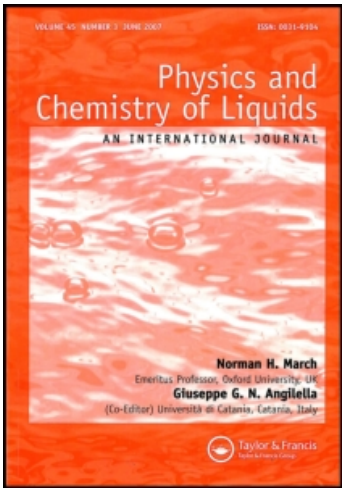

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Thermodynamics of the binding of the three water-soluble porphyrins with DNA

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Interactions of three water soluble porphyrins, tetra (*p*-trimethyl) ammonium phenyl porphyrin iodide (TAPP) as a cationic porphyrin, tetra sodium meso-tetrakis (*p*-sulphonato phenyl) porphyrin (TSPP) as an anionic porphyrin, and manganese tetrakis (*p*-sulphonato phenyl) porphyinato acetate (MnTSPP) as a metal porphyrin, with calf-thymus DNA were comprehensively studied at 1 mM phosphate buffer, pH 7.0, and at various temperatures, using UV–Vis absorption spectroscopy. The binding constant and stoichiometry were determined by analysis of optical absorption spectra of porphyrin at various DNA concentrations using SQUAD software. The results show that the best fitting corresponds to 1 : 1 complex model between base pair of DNA and porphyrin. All the thermodynamic parameters were calculated by van't Hoff equation at various temperatures. The results show that the process is essentially entropy driven. The MnTSSP has the highest affinity respect to TSPP and TAPP that can represent the formation of an axial bond between the phosphate group of nucleotide and Mn in the central core of MnTSSP. However, the higher affinity of TAPP as a cationic porphyrin respect to TSPP as an anionic can be related to the role of electrostatic interactions.

Keywords: DNA; Porphyrin; Thermodynamics of binding; Optical absorption; SQUAD

1. Introduction

Introduction of DNA-cleaving functional groups into an antisepsis oligonucleotide has been shown to generate site-directed damage on the target nucleic acids through chemical or photochemical reactions [1–3]. A variety of chemical molecules have been covalently linked to oligonucleotides which were hybridized to cellular DNA to affect processes such as cellular uptake [4], nuclease resistance [5], and binding affinity [3]. One of the most important groups of those reactive molecules is porphyrins and their

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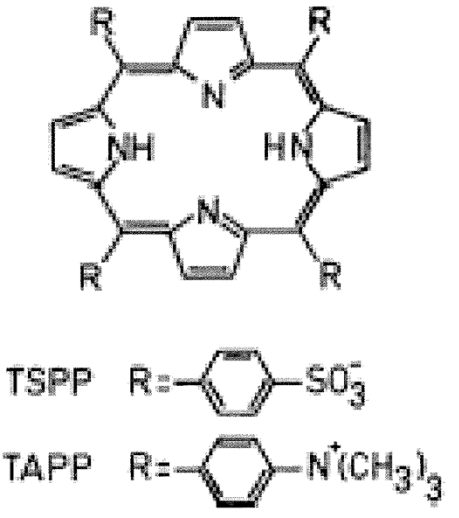

Scheme 1. The chemical structures of tetra (*p*-trimethyle) ammonium phenyl porphyrin iodide (TAPP) and tetra sodium meso-tatrakis (*p*-sulphonato phenyle) porphyrin (TSPP).

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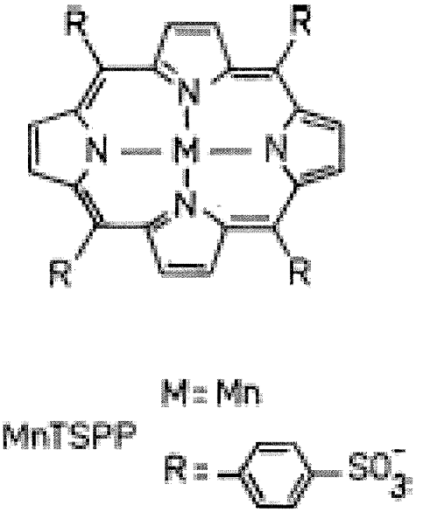

Scheme 2. The chemical structure of manganese tetrakis (*p*-sulphonato phenyle) porphinato acetate (MnTSPP).

metal derivatives [6–8]. These compounds are widely used as probes for nucleic acid structure and dynamics, and have all possible medical applications. The binding strength of porphyrins to DNA is one of the important parameters on its efficacy. The thermodynamic parameters of binding can also help to obtain insights into the molecular nature of the interactions.

This article reports a comprehensive thermodynamic study on interaction of three water-soluble porphyrins, tetra (*p*-trimethyl) ammonium phenyl porphyrin iodide (TAPP) (scheme 1) as a cationic porphyrin, tetra sodium meso-tetrakis (*p*-sulphonato phenyl) porphyrin (TSPP) (scheme 1) as an anionic porphyrin, and manganese tetrakis (*p*-sulphonato phenyl) porphinato acetate (MnTSPP) (scheme 2)

as a metal porphyrin, with DNA at various temperatures. The interaction process has been followed using UV–Vis optical absorption spectroscopy. The spectral data was analyzed in order to obtain the mode of binding and binding constant using SQUAD software.

2. Experimental

2.1. *Materials and methods*

DNA from calf-thymus was obtained from Sigma-Chemical Co. TSPP and TAPP were prepared by methods described previously [9,10]. TSPP was metallated according to the literature method [11]. These complexes were characterized by UV–Vis spectroscopy and elemental analysis. The spectral characteristics of the isolated materials were compared to the literature values and found to be in excellent agreement. All of the chemicals, which have been used for these syntheses, were of analytical grade and purchased from Sigma Chemical Co. All solutions were prepared using double-distilled water. Porphyrin stock solution was made by dissolving the solid porphyrin in buffer solution. Phosphate buffer, 1 mM, pH 7.0, was used as buffer. Porphyrin stock and working solutions were stored at room temperature in the dark to avoid undesired photochemical reactions. UV–Vis measurements were performed on a Cary 100 spectrophotometer using 1 cm quartz cuvettes. To prepare the DNA stock solution, about 2 mg of DNA was dissolved in 1 mL of the phosphate buffer at 4°C for 48 h with occasional stirring to ensure the formation of a homogenous solution. The DNA concentrations were determined using molar extinction coefficients of $\varepsilon_{257\,nm} = 6700\,M^{-1}\,cm^{-1}$. In all experiments, the porphyrins and DNA solutions were freshly prepared before spectral analysis and were protected from direct sun light until they were inserted into the cell compartments.

To observe the salt effect on the porphyrin absorption, the titrations were made by the addition of aliquots of NaCl solution into cuvettes containing the porphyrin solution of appropriate concentration. The obtained spectra were corrected with respect to dilution effect. The titration of porphyrin solution as a function of DNA concentration was performed at pH 7.0, 1 mM phosphate buffer, and at 20, 25, 30, 35, 40 and 45°C.

3. Results and discussion

3.1. *Solution properties of TSPP, MnTSPP, and TAPP*

In order to identify the solution properties of these porphyrins UV–Vis spectroscopy was employed. Optical absorption spectrum of MnTSPP shows four distinct peaks at 563, 466, 400, and 379 nm. The Soret band appears at 466 nm. The molar absorption of the Soret band is $1.82 \times 10^5\,M^{-1}\,cm^{-1}$. The Soret band maximum obeys Beer's law over an extended concentration range between. 1.0×10^{-5} and 2.0×10^{-4} M, in phosphate buffer 1 mM, pH 7.0. After the upper limit of this concentration range, a negative deviation has been observed that corresponds to the self-association of this porphyrin due to the increasing concentration. In spite of MnTSPP, the spectrum

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of TSPP just shows a distinct Soret band at 413 nm, the molar absorption of this band is $1.80 \times 10^5\,M^{-1}\,cm^{-1}$. The Soret band maximum obeys Beer's law in a concentration range between 1.0×10^{-5} and 1.0×10^{-4} M in 1 mM phosphate buffer, pH 7.0. The observed positive deviation from linearity that represents the self-aggregation occurred after this range. The spectral feature of TAPP at the same condition shows a Soret band at 412 nm, with a shoulder. The molar absorption of Soret band was $3.02 \times 10^5\,M^{-1}\,cm^{-1}$. The Soret band obeys Beer's law in the concentration range from 1.50×10^{-5} to 1.10×10^{-4} M. A negative deviation was observed after this range. The results in this part represent the higher tendency of TAPP for concentration self-aggregation with respect to others. However, the lesser tendency of MnTSPP may be related to Mn. It seems that metallation of TSPP with Mn inhibited the intermolecular interactions which are responsible for self-aggregation.

3.2. *Effect of salts*

The effect of NaCl on the absorption spectrum of MnTSPP, TSPP, and TAPP is shown in figure 1(a), (b), and (c), respectively. As the concentration of NaCl increases, the absorbance at all of the spectral regions of the studied porphyrins significantly decreases. The decrease in the absorbance for MnTSPP spectra is accompanied with red shift and disappearance of Q-bands which represent the formation of well-defined aggregates. These results also represent the strong electrolyte effect on aggregation state of MnTSPP. The bath chromic shift of spectra with the broadening of spectral bandwidth which have been observed for TSPP and TAPP can be related to formation of ill-defined aggregate in the presence of salt. However, the tendency of TSPP for formation of aggregate is more than TAPP.

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3.3. *Interaction of porphyrins with DNA: optical absorption study*

With respect to the previous discussion, it can be concluded that in homogenous aqueous solution at low ionic strength, these studied porphyrins exist mainly as monomers. So, the titration of porphyrin solution was conducted at fixed concentration and varying [DNA] at pH 7.0 and 1 mM phosphate buffer as a low ionic strength medium. Figure 2(a), (b), and (c), shows a representative titration spectrum of MnTSPP, TSPP, and TAPP, respectively, upon increasing the concentration of DNA at 25°C.

In all of the spectral regions, the intensity of Soret band decreases as DNA concentration increases. The absorption data were analyzed in order to calculate the binding parameters using SQUAD program. This program is designed to calculate the best values for the stability constants of the proposed equilibrium model by employing a nonlinear least square approach.

The results represent the formation of 1 : 1 complex model between studied porphyrins and DNA at all temperatures with sum of squares of reduced error between 10^{-3} and 10^{-4}. Hence, a simple equilibrium between free porphyin and DNA pair base exists. The estimated equilibrium constants for the formation of base pair, porphyrin complex at various temperatures are listed in tables 1–3.

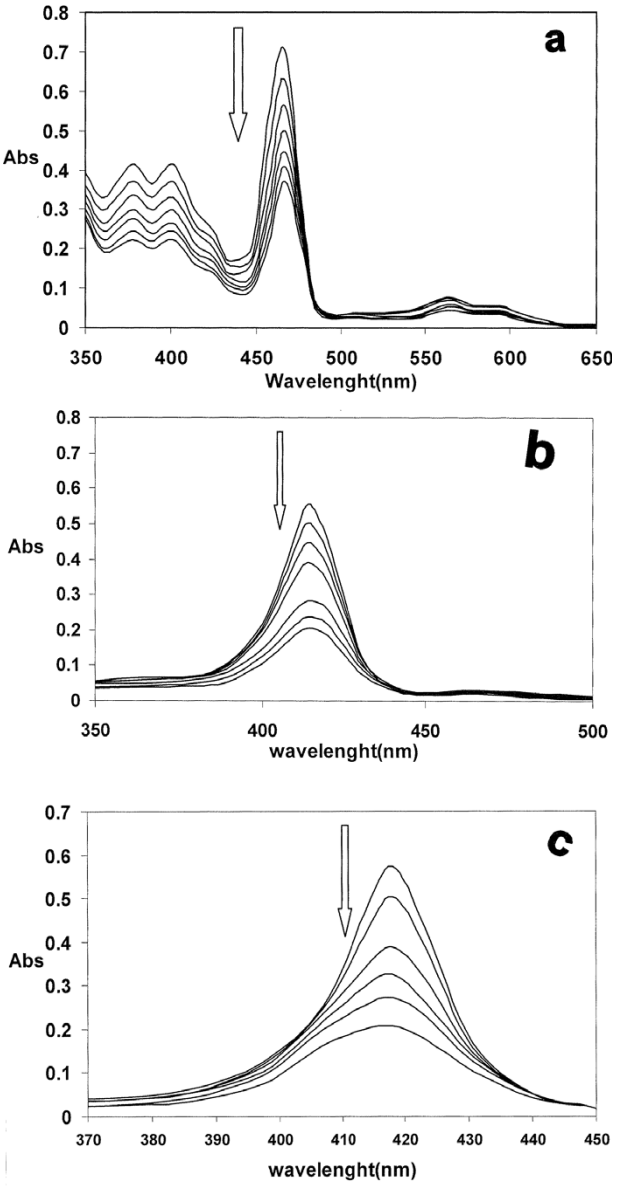

Figure 1. Corrected absorption spectra of MnTSPP (a), TSPP (b), and TAPP (c) upon titration with NaCl in 1 mM phosphate buffer, pH 7.0 at 25°C. The concentration of NaCl is increased in the direction of arrows.

3.4. *Thermodynamics of DNA–porphyrin interaction*

The energetics of DNA–porphyrin equilibrium can be conveniently characterized by three thermodynamic parameters, standard Gibbs free energy, ΔG°, can be calculated from the equilibrium constant, K, of the reaction using the familiar relationship, $\Delta G^{\circ} = -RT\ln K$, in which R and T refers to the gas constant, and the absolute temperature, respectively.

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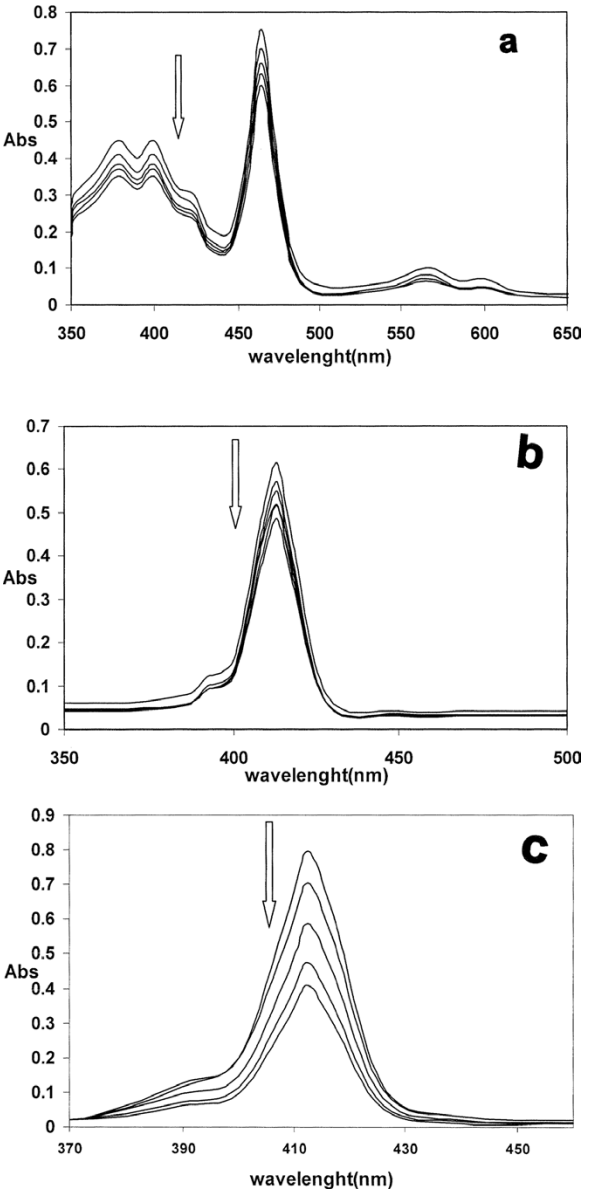

Figure 2. Corrected absorption spectra of MnTSPP (a), TSPP (b), and TAPP (c) upon titration with DNA in 1 mM phosphate buffer, pH 7.0 at 25°C. The concentration of DNA has been increased in the direction of arrow.

The van't Hoff equation

$$\frac{d\ln K}{d(1/T)} = \frac{-\Delta H^{\circ}}{R} \tag{1}$$

gives a linear plot of ln$K$ *versus* $1/T$, if the heat capacity change for the reaction is essentially zero. The ΔH° can be calculated from the slope of the straight line, $-\Delta H^{\circ}/R$

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Table 1. Thermodynamic parameters for binding of MnTSPP to DNA in 1 mM phosphate buffer, pH 7.0, and at various temperatures.

T (°C)	$K \times 10^{-4} \pm \Delta K$	$\Delta G^\circ \pm \Delta\Delta G^\circ$ (K J mol^{-1})	$\Delta H^\circ \pm \Delta\Delta H^\circ$ (K J mol^{-1})	$\Delta S^\circ \pm \Delta S^\circ$ (J K^{-1} mol^{-1})
20	9.333 ± 1.023	-27.897 ± 0.056	45.519 ± 0.055	250.438 ± 0.191
25	12.302 ± 1.031	-29.057 ± 0.072	45.519 ± 0.055	250.129 ± 0.242
30	14.135 ± 1.033	-30.071 ± 0.083	45.519 ± 0.055	249.349 ± 0.274
35	21.878 ± 1.026	-31.507 ± 0.063	45.519 ± 0.055	249.963 ± 0.205
40	28.841 ± 1.028	-32.666 ± 0.077	45.519 ± 0.055	249.673 ± 0.227
45	40.738 ± 1.035	-34.175 ± 0.093	45.519 ± 0.055	250.492 ± 0.249

Table 2. Thermodynamic parameters for binding of TSPP to DNA in 1 mM phosphate buffer, pH 7.0, and at various temperatures.

T (°C)	$K \times 10^{-4} \pm \Delta K$	$\Delta G^\circ \pm \Delta\Delta G^\circ$ (K J mol^{-1})	$\Delta H^\circ \pm \Delta\Delta H^\circ$ (K J mol^{-1})	$\Delta S^\circ \pm \Delta S^\circ$ (J K^{-1} mol^{-1})
20	1.023 ± 1.030	-22.508 ± 0.032	105.551 ± 0.017	436.836 ± 0.109
25	1.622 ± 1.028	-24.035 ± 0.030	105.551 ± 0.017	434.634 ± 0.101
30	3.631 ± 1.026	-26.469 ± 0.028	105.551 ± 0.017	435.494 ± 0.093
35	7.081 ± 1.023	-28.619 ± 0.026	105.551 ± 0.017	435.405 ± 0.083
40	14.125 ± 1.033	-30.878 ± 0.083	105.551 ± 0.017	435.667 ± 0.265
45	28.841 ± 1.035	-33.259 ± 0.074	105.551 ± 0.017	436.304 ± 0.193

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Table 3. Thermodynamic parameters for binding of TAPP to DNA in 1 mM phosphate buffer, pH 7.0, and at various temperatures.

T (°C)	$K \times 10^{-4} \pm \Delta K$	$\Delta G^\circ \pm \Delta\Delta G^\circ$ (K J mol^{-1})	$\Delta H^\circ \pm \Delta\Delta H^\circ$ (K Jmol^{-1})	$\Delta S^\circ \pm \Delta S^\circ$ (J K^{-1} mol^{-1})
20	5.754 ± 1.026	-26.717 ± 0.061	57.777 ± 0.117	288.228 ± 0.208
25	7.080 ± 1.023	-27.691 ± 0.057	57.777 ± 0.117	286.661 ± 0.191
30	10.715 ± 1.030	-29.196 ± 0.076	57.777 ± 0.117	286.898 ± 0.254
35	16.982 ± 1.028	-30.858 ± 0.072	57.777 ± 0.117	287.636 ± 0.117
40	24.547 ± 1.023	-32.318 ± 0.060	57.777 ± 0.117	287.706 ± 0.138
45	33.884 ± 1.030	-33.688 ± 0.079	57.777 ± 0.117	287.490 ± 0.254

and the standard entropy from its intercept, $\Delta S^\circ/R$ or

$$\Delta S^\circ = \frac{\Delta H^\circ - \Delta G^\circ}{T} \tag{2}$$

The van't Hoff plots for binding of these porphyrins to DNA in the phosphate buffer are shown in figure 3. All of the thermodynamic parameters with their uncertainies for interaction with MnTSPP, TSPP, and TAPP were calculated, and reported in tables 1–3, respectively.

The negative slopes of the lines in van't Hoff plots (figure 3) represent the endothermicity of the reaction. The high correlation coefficient of the lines indicate the lesser value of heat capacity change of reaction. With respect to the values of ΔG°, the binding affinity of TAPP is more than TSPP at all studied temperatures. This can be related to the role of electrostatic interactions in the formation of complex. The strong attractive electrostatic interaction between TAPP as a cationic porphyrin with DNA chains with negative charge density, in comparison to TSPP as an anionic porphyrin,

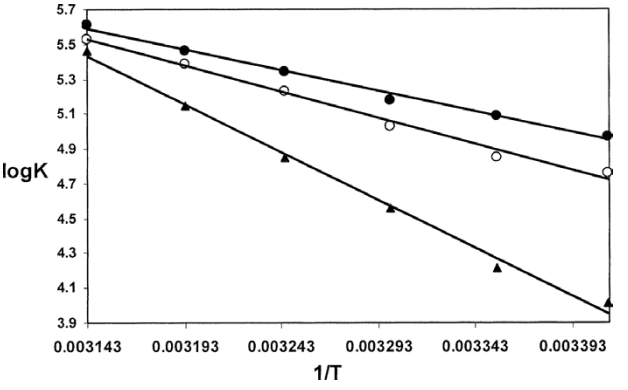

Figure 3. The van't Hoff plot for binding of MnTSPP (●), TSPP (▲), and TAPP (○) to DNA in 1 mM phosphate buffer, pH 7.0.

is responsible for this observation. However, MnTSPP has the highest affinity to DNA with respect to other porphyrins. This can be related to the special role of Mn in formation of complex with DNA. Probably, the axial ligation of Mn with phosphate group increases its affinity to DNA. The comparison between enthalpy and entropy values represents the essential role of entropy in reaction driven. In conclusion, the formation process is essentially entropy driven, and the metal in the central core of porphyrin has an important role in enhancing the interaction with DNA.

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Acknowledgments

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