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Thermodynamic of Interaction between Some Water-Soluble Porphyrins and DNA by Titration Microcalorimetry

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ABSTRACT

In the present work, the interaction of three water soluble porphyrins, tetra (p-trimethyle) ammonium phenyl porphyrin iodide (TAPP) as a cationic porphyrin, tetra sodium meso-tetrakis (p-sulphonato phenyle) porphyrin (TSPP) as an anionic porphyrin and manganese tetrakis (p-sulphonato phenyl) porphinato acetate (MnTSPP) as a metal porphyrin, with DNA have been studied by isothermal titration microcalorimetry at 1 mM phosphate buffer, pH 7.0 and 25 °C. The values of binding constant, entropy, enthalpy and Gibbs free energy changes for binding of the first MnTSPP, and first and second TSPP and TAPP molecules were estimated from microcalorimetric data analysis. The results represent that the process is both entropy and enthalpy driven and DNA induces self-aggregation of the porphyrins. The results indicate that both columbic and hydrophobic interactions act as self-aggregation driving forces for the formation of aggregates around DNA.

Keywords: Porphyrin; DNA; Isothermal Titration Microcalorimetry; Aggregation

INTRODUCTION

Interaction of porphyrins and their derivatives with proteins and DNA is one of the considerable interests since they are widely used as probes for structure and dynamics of nucleic acid and have possible medical applications [1-3]. It is known that the photochemical and photo physical properties of porphyrins and phtalocyanines are modified when they bind to proteins and RNA. Therefore, binding studies of these photosensitizes with proteins and RNA is of interest [4, 5]. It would be expected that during the interaction of porphyrins with RNA, its interaction with DNA are also involved. The interactions of cationic porphyrins with nucleic acids have received considerable at tension [6, 7]. Several of these strongest DNA binders, with association constant 10^5 M^{-1} to $10^6 M^{-1}$ [8,9], have clinical potential as anticancer agents in photodynamic therapy [10,11], probably as a consequences of their ability to selectivity accumulate on the surface of tumor cells, become internalized, bind to genomic DNA, and then induce DNA strand cleavage

A prerequisite for a deeper insight into the molecular basis of DNA-porphyrin interaction is a thorough characterization of the energetic governing complex formation. This can be done on the level of Gibbs energy, ΔG , enthalpy, ΔH , and entropy, ΔS , changes [12]. In biochemical binding studies it is usually advantageous to determine the change in enthalpy values by direct microcalorimetric techniques. In many instances it is also possible to determine K values for binding reactions by calorimetric titration experiments [13-21].

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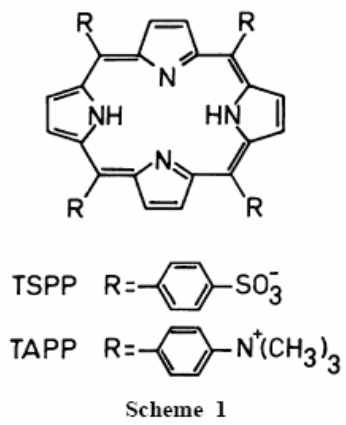
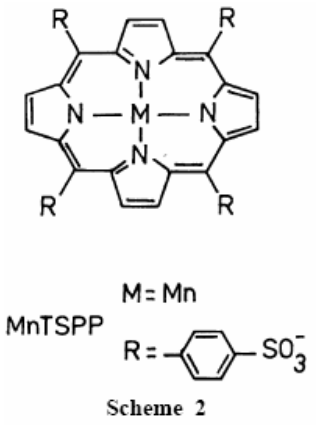
Values for entropy changes, ΔS in solution systems are normally obtained from the difference between Gibbs energy and enthalpy changes, $\Delta G = \Delta H - T\Delta S$.

Previous studies on the organization and distribution of porphyrins showed that these molecules non-covalently bound to DNA, which considerably affect sensitizing ability of porphyrins via induced aggregation, decreasing of quantum yields, ϕ_T, and consequently, decreasing the quantum yield of singlet oxygen formation [22]. Since there are no reports on the microcalorimetry and thermodynamic study on the binding of porphyrins to DNA, we were interested to investigate the interaction of three water soluble porphyrins (scheme 1 and 2), tetra (p-trimethyle) ammonium phenyl porphyrin iodide (TAPP) as a cationic porphyrin, tetra sodium meso-tetrakis (p-sulphonato phenyle) porphyrin (TSPP) as an anionic porphyrin and manganese tetrakis (p-sulphonato phenyl) porphinato acetate (MnTSPP) as a metal porphyrin, with DNA by isothermal titration microcalorimetry. The thermodynamic parameters of binding have been estimated from heat values of a stepwise calorimetric titration experiment by a modified simple graphical method which has been previously used for data analysis of simple systems [23]. The estimated thermodynamic parameters have been interpreted on the basis of molecular forces.



Scheme 1



Scheme 2

EXPERIMENTAL SECTION

Materials

DNA from Calf–Thymus was obtained from Sigma – Chemical Co. TSPP and TAPP were prepared by the methods described previously [24, 25]. TSPP was metallated according to the literature method [23]. 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 solutions were made by dissolving the solid porphyrin in buffer solution. Phosphate buffer solution of 1 mM concentration, pH=7.0, was used. 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 scanning spectrophotometer using 1cm quartz cuvettes, with thermostat cell compartment that control the temperature around the cell with in $\pm 0.1^\circ c$.

Methods

All solutions were prepared using double distilled water. Dissolving the solid porphyrin in buffer solution made porphyrin stock solution. Phosphate buffer, 1mM, pH 7.0 was used as buffer porphyrin stock and working solution were stored at room temperature in dark to avoid undesired photochemical reactions. To prepare the DNA stock solution about 2mg of DNA was dissolved in 1ml of the phosphate buffer at $4^\circ c$ for 48 hour, with occasional stirring to ensure the formation of a homogenous solution. The DNA concentrations were determined using molar extinction coefficients of $\varepsilon_{258nm} = 6700 M^{-1}cm^{-1}$. In all experiments, the porphyrins and DNA solutions were freshly prepared before spectral analysis and were protected from direct sun lights they were inserted into the cell compartments.

Enthalpy measurements were performed at 25.0±0.002°C using a four-channel commercial micro-calorimetric system, Thermal Activity Monitor 2277, Thermometric, Sweden. The microcalorimeter was interfaced with an IBM PS/2 model PIII computer, "Thermometric Digit am 3" was the software program used. A 1000 µl injection syringe was employed throughout. The enthalpy of interaction between porphyrin and DNA was measured by transferring the porphyrin solution to a 5 ml titration cell. The volume of DNA solution in the measuring cell was 2.5 ml. The concentration of porphyrin inside the syringe was 1 mM and the concentration of DNA in the titration cell was 0.1 % (W/V). The volume of porphyrin solution injected in each step was 20 µl

for TSPP and TAPP and 40 µl for MnTSPP. The enthalpy of dilution of the porphrin due to the injection was corrected by measuring as described above except excluding DNA. The heat of dilution of DNA is negligible. The micro-calorimeter was frequently calibrated electrically during the course of the study.

RESULTS AND DISCUSSION

The data obtained from isothermal titration microcalorimetry of DNA interaction with porphyrins are shown in Figs. 1. Fig. 1a shows the total evolutes heat versus total concentration of porphyrin for TSPP and TAPP and Fig. 1b represents the corresponding results for MnTSPP. Previous study shows that DNA promotes aggregation of the bound monomeric porphyrin [21]. Hence, for analyzing the enthalpy curves for obtaining thermodynamic parameters of binding, we have to consider the following multiple equilibrium between DNA (D) and porphyrin (P):

$$D + P \Leftrightarrow DP$$

$$DP + P \Leftrightarrow DP_2$$

$$\vdots$$

$$DP_{i-1} + P \Leftrightarrow DP_i$$

$$\vdots$$

A stepwise macroscopic association binding constant (equation 1), K_i, and enthalpy change ΔH can be related to any distinguished equilibrium step.

$$K_i = \frac{[DP_i]}{[DP_{i-1}][D]} \qquad (1)$$

The sum of heat evolution following the nth titration step (Q_n) can be expressed as:

$$Q_n = Q_n^{(1)} + Q_n^{(2)} + \cdots + Q_n^{(i)} + \cdots \qquad (2)$$

Where $Q_n^{(i)}$ is the sum of heat evolutions following the nth titration step, due to the formation of DP_i complex from DP_{i-1}.

The value of $Q_n^{(i)}$ relates on the amount of DP_i complex formed, which can be expressed as:

$$Q_n^{(i)} = \Delta H_i \cdot V_n \cdot [DP_i]_n \qquad (3)$$

Where V_n and $[DP_i]_n$ are the volume of the reaction solution and concentration of DP_i in the nth titration step, respectively.

In special case that:

$$K_1 >> K_2 >> K_3 \cdots K_{i-1} >> K_i \cdots$$

it can be assume that DP_i complex is not formed until the complete formation of DP_{i-1}. This situation permits us to divide the titration steps into distinct categories. In the first category, only DP is formed and Q_n should be equal to $Q_n^{(1)}$. In the second set, all of the free DNA converts to DP complex and further addition of porphyrin results in formation of DP_2 complex. So Q_n for the second set should be equal to the total evolved heat for complete conversion of DNA into DP, $Q_n(1)$ plus the evolved heat from partial conversion of DP to DP_2, The similar relations can be written for the third, forth and so on, sets of injections, as follows:

$Q_n = Q_n^{(1)}$	first set of injection	$0 \le n \le n_1$
$Q_n = Q_n(1) + Q_n^{(2)}$	second set of injection	$n_1 < n \le n_2 + n_2$
$Q_n = Q_n(1) + Q_n(2) + Q_n^{(3)}$	third set of injection	$n_1 + n_2 < n \le n_3 + n_2 + n_3$
$\vdots$		
$Q_n = Q_n(1) + Q_n(2) + \cdots + Q_n(i-1) + Q_n^{(i)}$	ith set of injection	$n_1 + n_2 + ... + n_{i\text{-}1} < n \le n_i + n_2 + ... + n_i$

$Q_n(i)$ is the total heat evolution for complete formation of DP_{i-1} complex to DP_i and n_1, n_2 ,…, n_i are the numbers of injections in the first, second, … and ith sets, respectively. In the first injection set that only DP complex is formed, K_1 should be equal to:

$$K_1 = \frac{[DP]_n}{([D_0]_n - [DP]_n)([P_0]_n - [DP]_n)} \qquad (4)$$

where $[D_0]_n$, $[P_0]_n$ and $[DP]_n$ are the total concentration of DNA $([D]+[DP])$, the sum of concentration of bound and unbound ligand and the concentration of the complex following the nth addition of ligand, respectively. With respect to equation (3), $Q_n^{(1)}(n < n_1)$ should be equal to:

$$Q_n^{(1)} = \Delta H_1 \cdot V_n \cdot [DP]_n \quad \text{where} \quad n < n_1 \qquad (5)$$

Combination of equations (4) and (5) leads to:

$$\frac{1}{K_1} = \frac{[D_0]_n \cdot [P_0]_n \cdot \Delta H_1 \cdot V_n}{Q_n} - [D_0]_n - [P_0]_n + \frac{Q_n^{(1)}}{\Delta H_1 \cdot V_n} \qquad (6)$$

Equation (6) contains two unknowns, K_1 and ΔH_1. A series of reasonable values for ΔH_1 are inserted into equation (7) and the corresponding values for $\frac{1}{K_1}$ are calculated and the graph of $\frac{1}{K_1}$

versus ΔH_1 is constructed. If this is done for all titration steps by considering the assumption about the formation of a 1:1 complex with respect to $K_1 >> K_2$, in ideal situation, all the curves intersect at one point. This point represents the true value for $\frac{1}{K_1}$ and ΔH_1. Due to measurement errors, the intersection will in practice cover a limited area. The best pair of values within this area can then be obtained by minimizing the value of $\sum (Q_n^{exp.} - Q_n^{calc.})^2$. This method has been previously used for a simple system in which only a 1:1 complex is formed [20].

Figs 2a, 2b and 2c represent the variation of $\frac{1}{K_1}$ versus ΔH_1 for TSPP, TAPP and MnTSPP, respectively. All of the curves in these Figs. intersect in a limited area which confirms our analysis. The values of K_1 and ΔH_1 can be estimated from these Figs.

The value of $Q(1)$ should be equal to:

$$Q(1) = \Delta H_1 \cdot [D_0] \cdot V_0 \qquad (8)$$

Where $[D_0]$ and V_0 are initial concentration and volume of DNA solution in the cell, respectively. By estimating $Q(1)$, the value of $Q_n^{(2)}$ for the second set of titration have been estimated. A similar analysis can be done for second set of titration steps by considering the definition of K_2. Figs. 3a, 3b and 3c show the variation of $\frac{1}{K_2}$ versus ΔH_2 for TSPP, TAPP and MnTSPP, respectively. All of the curves in Figs. 3a and 3b reasonably intersect in a limited region; however, Fig. 3c does not show this situation. It represents that the assumption of $K_2 >> K_3$ is not valid for MnTSPP but acceptable for TSPP and TAPP. The values of ΔH_2 and K_2 for TSPP and TAPP were estimated from these figures. The value of $Q(2)$ should be equal to:

$$Q(2) = \Delta H_2 \cdot [D_0] \cdot V_0 \qquad (9)$$

Hence, the values of $Q_n^{(3)}$ have been calculated for third set of injection. The corresponding curve for this set does not intersect in a limited region and represents the fail of our assumption. The curve for TAPP at this set was shown in Fig. 4. The calculated thermodynamic parameters for binding of TSPP, TAPP and MnTSPP were listed in Table 1.

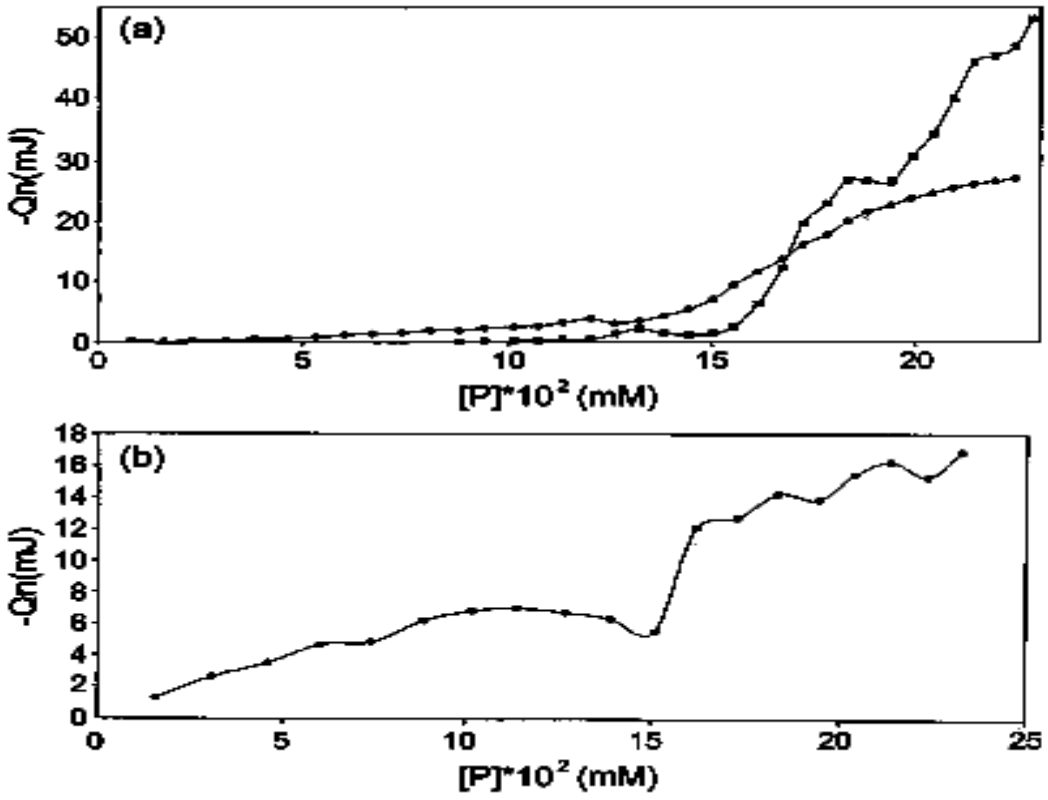


Fig.1. (a) Experimental heat quantities (Q_n) plotted versus total concentration of porphyrin ([P]). (■) **TAPP**, (●) **TSPP**. (b) Experimental heat quantities (Q_n) plotted versus total concentration of **MnTSPP** ([**P**]).

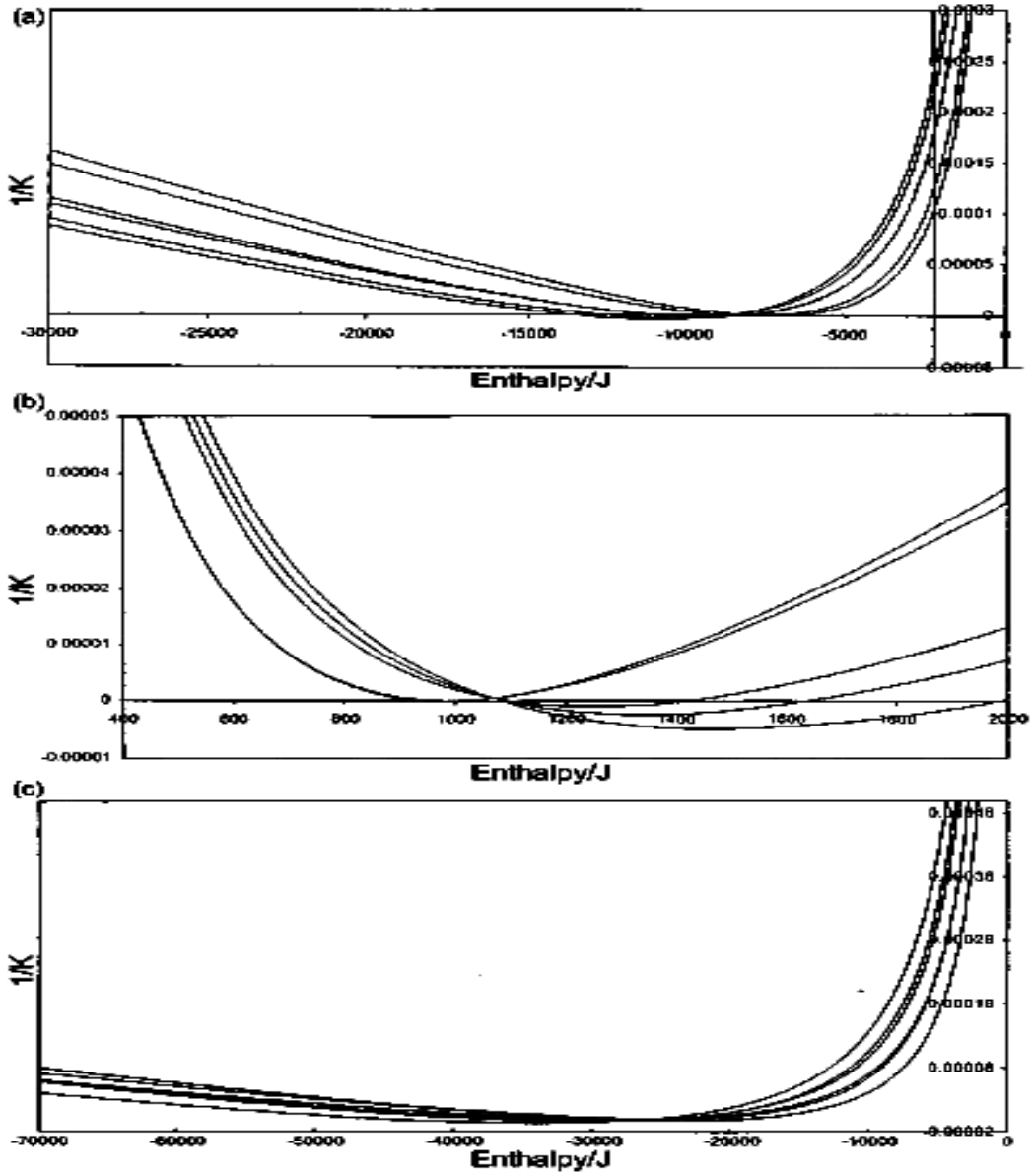


Fig.2. (a) Evaluation of K_1 and ΔH_1 for interaction of **TSPP** with **DNA**. The total concentration of TSPP lies between 0 to 0.086 mM. The injections of 1 to 12 are correspond to this set. Some of the curves have been deleted for better presentation of figure. (b) Evaluation of K_1 and ΔH_1 for interaction of **TAPP** with **DNA** from. The total concentration of TAPP lies between 0 to 0.097 mM. The injections of 1 to 13 are correspond to this set. Some of the curves have been deleted for better presentation of figure. (c) Evaluation of K_1 and ΔH_1 for interaction of **MnTSPP** with **DNA**. The total concentration of MnTSPP lies between 0 to 0.198 mM. The injections of 1 to 16 are correspond to this set. Some of the curves have been deleted for better presentation of figure.

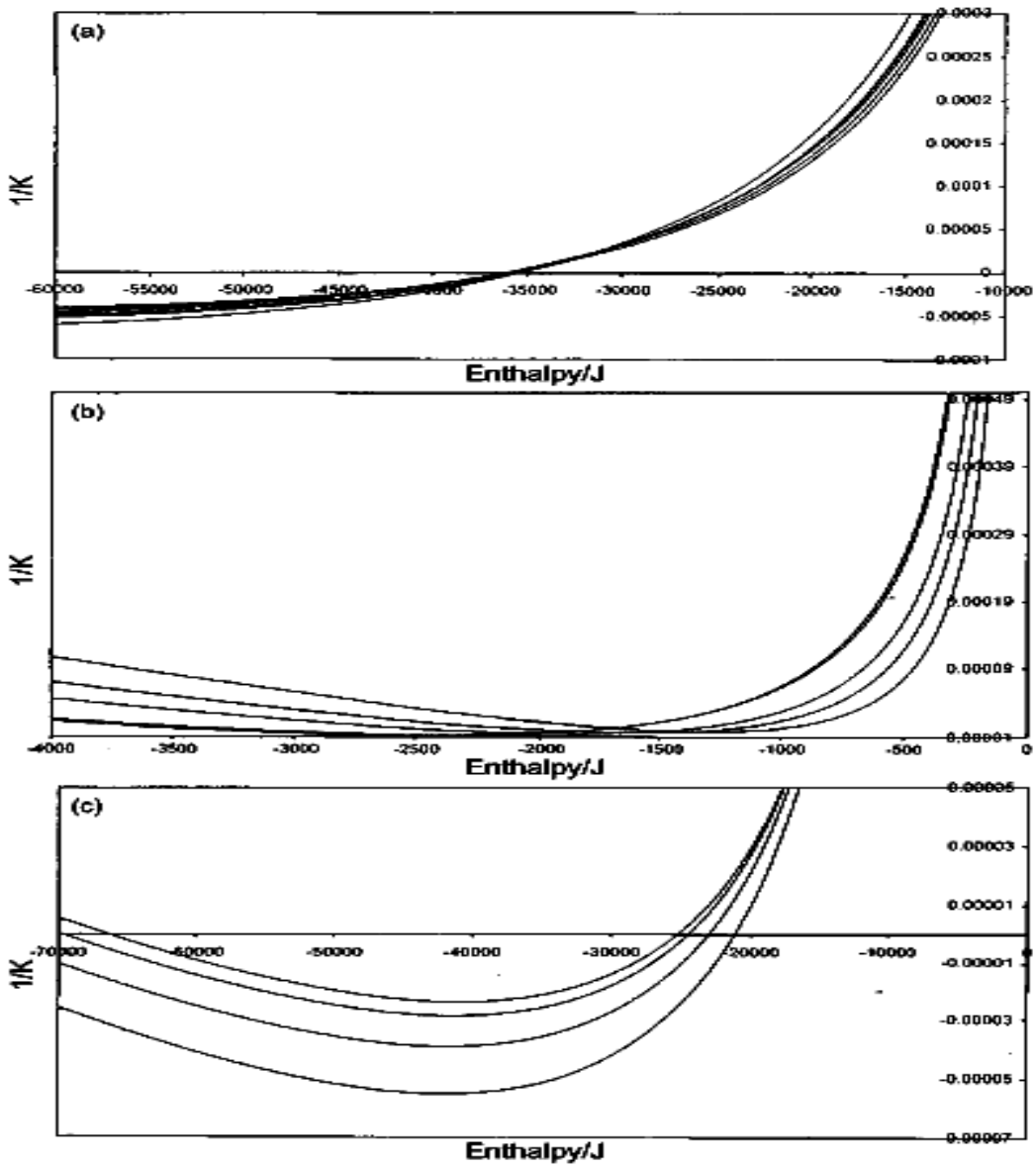


Fig.3. (a) Evaluation of K_2 and ΔH_2 for interaction of **TSPP** with **DNA**. The total concentration of TSPP lies between 0.081 to 0.155 mM. The injections of 12 to 22 are correspond to this set. Some of the curves have been deleted for better presentation of figure. (b) Evaluation of K_2 and ΔH_2 for interaction of **TAPP** with **DNA**. The total concentration of TAPP lies between 0.087 to 0.127 mM. The injections of 13 to 20 are correspond to this set. Some of the curves have been deleted for better presentation of figure. (c) Evaluation of K_2 and ΔH_2 for interaction of **MnTSPP** with **DNA**. The curves in this figure do not intersect in one point and represents the fail of our assumption. The total concentration of MnTSPP is more than 0.215 mM. The injections of 16 to 40 are correspond to this set. Some of the curves have been deleted for better presentation of figure.

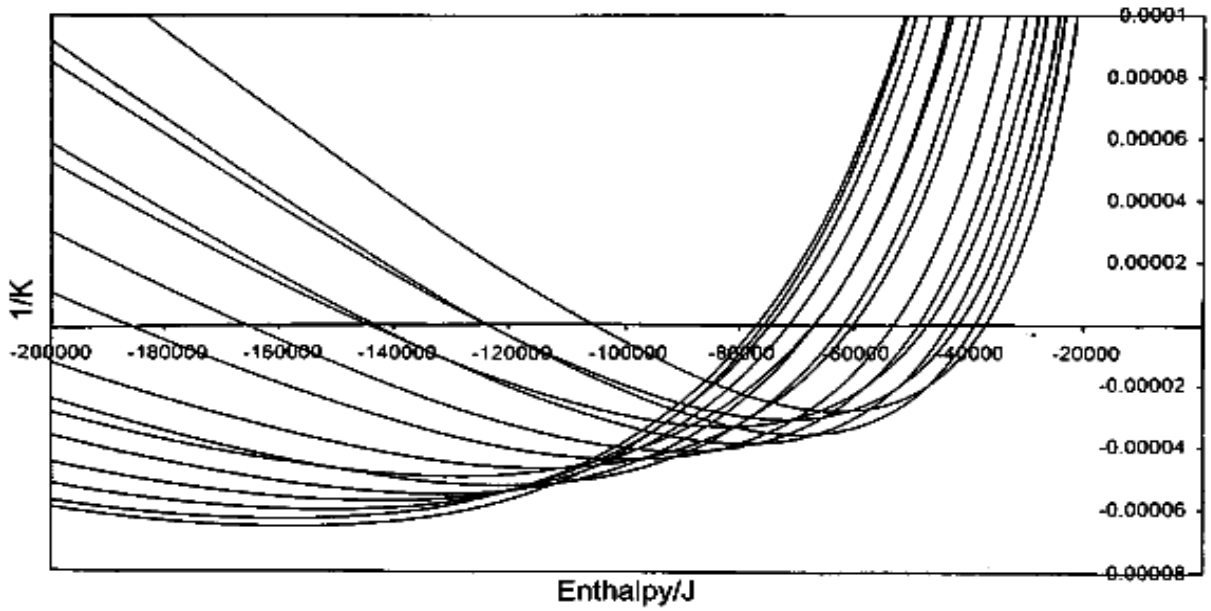


Fig.4. Evaluation of K_3 and ΔH_3 for interaction of **TAPP** with **DNA**. The curves in this figure do not intersect in one point and represents the fail of our assumption. The total concentration of TAPP is more than 0.134 mM. The injections of 19 to 40 are correspond to this set. Some of the curves have been deleted for better presentation of figure.

Table 1. Thermodynamic parameters for interaction of **Porphyrins** to **DNA**, in 1 mM phosphate buffer, pH 7.0 and 25 ºC

Porphyrin Number of injection set	Number of injection in ith set $n_{i-1} - n_{i+1}$	$\frac{[\text{Porphyrin}]}{[\text{DNA}]}$ (molar ratio)	Concentration range of porphyrin (mM)	ΔH_i(kJ/mol)	$K_i \times 10^{-5}$	ΔG_i(kJ/mol)	ΔS_i(J/mol.K)
TSPP 1	1-12	1.11	0.0-0.086	-8.11 ± 0.570	2.706 ± 0.188	-31.191 ± 0.172	$+77.41 \pm 2.42$
TSPP 2	12-22	2.35	0.081-0.155	-31.564 ± 1.455	0.622 ± 0.025	-27.521 ± 0.210	-16.83 ± 5.34
TAPP 1	1-13	1.37	0.0-0.087	$+1.160 \pm 0.045$	33.580 ± 1.780	-37.390 ± 0.128	$+127.97 \pm 4.31$
TAPP 2	13-20	1.87	0.087-0.127	-1.790 ± 0.158	2.822 ± 0.242	-31.284 ± 0.192	$+97.98 \pm 1.22$
MnTSPP1	1-16	3.20	0.0-0.198	-32.240 ± 1.522	1.212 ± 0.061	-29.281 ± 0.131	-13.5 ± 5.83

CONCLUSIONS

Our results represent that DNA induces the self-aggregation of porphyrins. These observations have been previously confirmed by spectroscopic techniques [19]. Estimated thermodynamic parameters represent the binding processes of TSPP and MnTSPP are considerable exothermic but for TAPP, it is endothermic for first ligand and inconsiderable exothermic for second ligand. With respect to positive charge of DNA and negative charge of TSPP and MnTSPP, it can be concluded that columbic attraction has considerable role in the process of binding. However, the considerable positive value of entropy changes, especially for binding of TAPP and binding of first TSPP, represent the role of hydrophobic environment in the process. Hence, DNA induces self aggregation of porphyrins and both columbic and hydrophobic interactions act as self-aggregation driving forces for the formation of aggregates.

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