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Aggregation behavior of tetrakis (2, 3, 5, 6 -tetrafluoro-N, N2, Nº-trimethyl ammonium phenyl) porphyinato acetate manganese (III) and its interaction with calf thymus DNA: A thermodynamic approach

Nasrin Sohrabi*, Nahid Rasouli, Mohammad Hadi Ghafaee
Department of Chemistry, Payame Noor University,PO Box 19395-3697, Tehran, (IRAN)
E-mail: sohrabnas@ pnu.ac.ir; n.rasouli@pnu.ac.ir

ABSTRACT

The association behavior of cationic metalloporphyrin, tetrakis (2, 3, 5, 6 tetrafluoro-N, N2 , Nº-trimethyl ammonium phenyl) porphyinato acetate manganese(III), [Mn(III)(TF_4TMAPP)] was investigated in aqueous solutions at 25 °C and various ionic strengths using optical absorption and resonance light scattering (RLS) spectroscopies. The [Mn(III)(TF_4TMAPP)] does not have any affinity for aggregation due to increasing salt concentration and exists as monomers even in high ionic strengths. Interaction of [Mn(III)(TF_4TMAPP)] with ct-DNA has also been studied by optical absorption, resonance light scattering spectroscopies and thermal denaturation experiments. The appearance of hypochromicity and a bathochromicity shift in UV-vis spectra, decreasing of thermal melting point of ct-DNA and no change in RLS spectra due to interaction with ct-DNA, represent the outside groove binding mode without any aggregate formation. The binding constants were obtained by analysis of the optical absorption spectra at various ct-DNA concentrations using SQUAD software. The thermodynamic parameters were calculated by van't Hoff equation.

KEYWORDS

Calf thymus DNA;
Porphyrin;
SQUAD;
Thermodynamic parameters;
Optical absorption.

INTRODUCTION

Cationic porphyrins and their interaction with DNA has been a subject of intense investigations on molecular recognition since their first synthesis[1] and their prior discovery by Fiel et al.[2]. It has been reported from the viewpoint of their role in biological systems that cationic porphyrins act as an inhibitor of human telomerase[3, 4], a receptor for peptides[5], a DNA cleaver[6–9] and a specific probe of DNA structure[10]. Three major binding modes have been proposed for the binding of cationic porphyrins to DNA: intercalation, outside groove binding and outside binding with self-stacking, in which porphyrins are stacked along the DNA helix[11–14]. The binding of cationic porphyrins to DNA is presumably stabilized by electrostatic interactions between the positively charged substituent on the porphyrin periphery and the negatively charged phosphate oxygen atom of DNA. In the case of intercalation, favorable aromatic π-π stacking inter-

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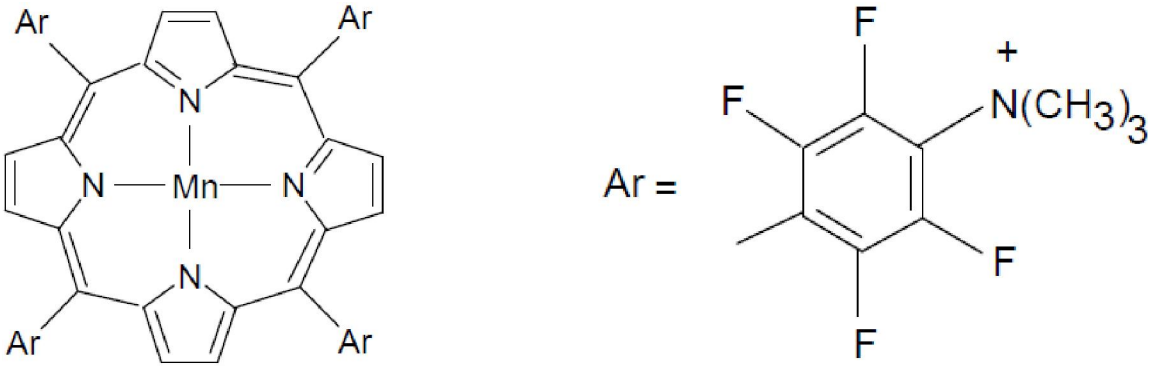
actions between the porphyrin macrocycle and the base pair of nucleic acid are also involved[14]. The porphyrin core is planar but shielded by peripheral substituents. This makes cationic porphyrins different from the conventional intercalators such as ethidium bromide, proflavin and daunomycin in which the planar fused aromatic ring can slip between adjacent base pairs of DNA without any major distortion from the idealized of metalloporphyrin-oligonucleotide complexes have shown that intercalation or groove binding of cationic porphyrin causes a distortion of B-DNA significantly[15, 16]. Besides the metal-free porphyrin, a number of intensive studies on interactions between metalloporphyrin and DNA have been reported. It has also been shown that the binding mode of porphyrin to nucleic acid duplexes, e.g. intercalative or outside binding mode, can be easily tuned by varying the metal center[11, 15, 16, 17]. Except for the experimental conditions such as pH, ionic strength and molar ratio of porphyrin to DNA base pair[11–13, 18], the nature of porphyrins plays an important role in their binding to DNA. The binding strength of porphyrin to DNA is one of the important parameters of its efficiency. The thermodynamic parameters of binding can also help us to obtain more insights into the molecular nature of interactions. Hence, determination of thermodynamic parameters governing ct-DNA-metallo porphyrin complex formation makes for deeper insight into the molecular basis of ct-DNA-metalloporphyrin interactions. In the present report, a comprehensive study on aggregation behavior of tetrakis (2, 3, 5, 6 tetrafluoro-N, N′ , N″-trimethyl ammonium phenyl) porphyinato acetate manganese (III), [Mn (III) (TF_4TMAPP)] (Scheme 1) and its interaction with ct-DNA has been done. Possessing four positive peripheral charges along with electron withdrawing groups such as fluorine make the porphyrin ring more positive, thereby enhancing the intrinsic affinity for cat-DNA. The binding constants were determined by analyzing optical absorption spectra of [Mn(III)(TF_4TMAPP)] at various ct-DNA concentration using SQUAD software. The aggregation behavior has been investigated using RLS spectroscopy as a powerful and simple technique. The mode, strength and nature of binding were determined on the basis of these integrative collected data.

EXPERIMENTAL

Materials and methods

Calf thymus DNA was purchased from Sigma. The [Mn(III)(TF_4TMAPP)] was prepared and purified according to literature methods[19, 20]. This metalloporphyrin was characterized by IR and UV-Vis spectroscopies and elemental analysis. All experiments were run in 5 mM phosphate buffer, pH 7.0 at 25 °C. The buffer consisted of (2.5 mM NaH_2PO_4 and 5 mM Na_2HPO_4) dissolved in Milli-Q water. To prepare the ct-DNA stock solution, 2 mg of ct-DNA was dissolved in 1 mL of phosphate buffer the day before the experiment and stored at 4°C. The experiments were conducted at 25±0.1°C. The concentration of ct-DNA was determined from its optical absorption using its molar absorption coefficients, ε_{259}nm =1.32×10^4 $cm^{-1}.M^{-1}$(i.e. reported in molar base pair)[21, 22]. The pH values were controlled using a Metrohm 744 pH meter. All other reagents were



Scheme 1 : The structure of tetrakis (2, 3, 5, 6 tetrafluoro-N, N2 , Nº-trimethyl ammonium phenyl) porphyinato acetate manganese (III)

TABLE 1 : UV/Vis spectral characteristics of [Mn(III)(TF_4TMAPP)] in5mM phosphate buffer,pH 7.0

Band	λ_{max}(nm)	Molar absorptivity $(M.cm)^{-1}$
Q_1-band	367	$10^4 \times 1.56$
Q_2-band	552	$10^3 \times 3.47$
B-band	456	$10^4 \times 3.47$

TABLE 2 : UV/Vis spectral characteristics of [Mn(III)(TF_4TMAPP)] solution (2×10^{-5} M) up on increasing the NaCl

[NaCl] M	Abs(max)	λ_{max}(B-band)(nm)	$W_{1/2}$(nm)
0	0.61285	456	17
0.45	0.60088	456	17
1	0.59011	457	17
1.67	0.56923	457	18
2.14	0.54746	457	18
2.5	0.52926	458	19

analytical reagent grade and used without further purification. Double distilled water was used throughout the experiments.

Optical absorption

The absorption spectra were recorded on a Cary 500 scan UV-Vis-NIR spectrophotometer. The [Mn(III)(TF_4TMAPP)] solutions were prepared in the concentration range of 8 to 20 μM for optical absorption measurements in the Q band region. The UV/Vis titration experiments were made by the addition of the ct-DNA stock solution into a 1 mL cuvette containing the porphyrin solution of appropriate concentration. The concentration range of ct-DNA was 10^{-6} M to 10^{-4} M. The titration experiments were performed at various temperatures with a precision of ± 0.1 °C.

Resonance light scattering

The light scattering measurements were done on a Shimadzu model RF-5000 spectrofluorimeter. The scattered light intensity was monitored using the right angle in the synchronous scanning regime of the excitation and emission monochromators in the region of 300 to 600 nm. The experimental light-scattering spectra were corrected taking into account the solution optical absorption and instrument sensitivity dependence on the wavelength as described elsewhere[23, 24]. All experimental data are the averaged values of at least five independent experiments.

Thermal denaturation of ct-DNA

The thermal denaturation of ct-DNA was monitored on a Cary 500scan UV-Vis-NIR spectrophotometer equipped with a temperature controller and thermocouple monitor using a temperature rise of 1 deg/min, at λ = 259 nm.

RESULTS AND DISCUSSION

Solution properties of [Mn(III)(TF_4TMAPP)]

In order to identify the solution properties of [Mn(III)(TF_4TMAPP)], we employed UV-Vis and RLS spectroscopies. The optical absorption spectrum of [Mn(III)(TF_4TMAPP)] shows Q_1 and Q_2 bands and a Soret band feature (B-band), which is a characteristic of the base porphyrin[25]. The molar absorptivity coefficient of these bands was calculated at $1.58 \times 10^4\ M^{-1}.cm^{-1}$ for Q_1 band (λ = 367 nm), $3.47 \times 10^3\ M^{-1}.cm^{-1}$, for Q_2 band (λ = 352 nm) and $3.47 \times 10^4\ M^{-1}.cm^{-1}$ for Soret band (λ = 456 nm), respectively. TABLE 1 summarizes the molar absorptivity of these bands. The B band maximum of [Mn(III)(TF_4TMAPP)] obeys Beer's law over an extended concentration range between 3.78×10^{-6} to 2.52×10^{-5} M in water. From this observation, we can conclude that [Mn (III) (TF_4TMAPP)] does not show a concentration-dependent aggregation.

Effects of inorganic salts

The effect of NaCl on the absorption spectrum of [Mn(III)(TF_4TMAPP)] (2×10^{-5} M) in water is shown in Figure 1 and the data concerning these spectral changes are presented in TABLE 2.

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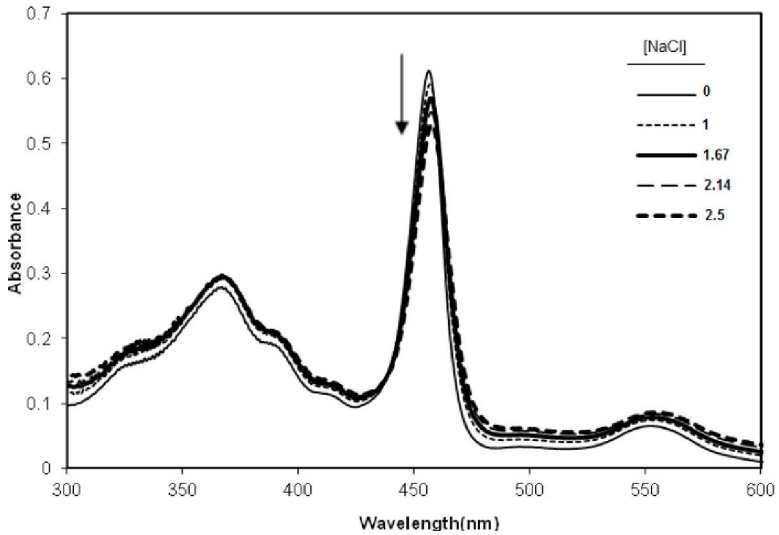

Figure 1 : Effect of ionic strength on spectra of 0.2 µM [Mn(III)(TF$_4$TMAPP)] in 5 mM phosphate buffer, pH 7.0 and at 25ºC

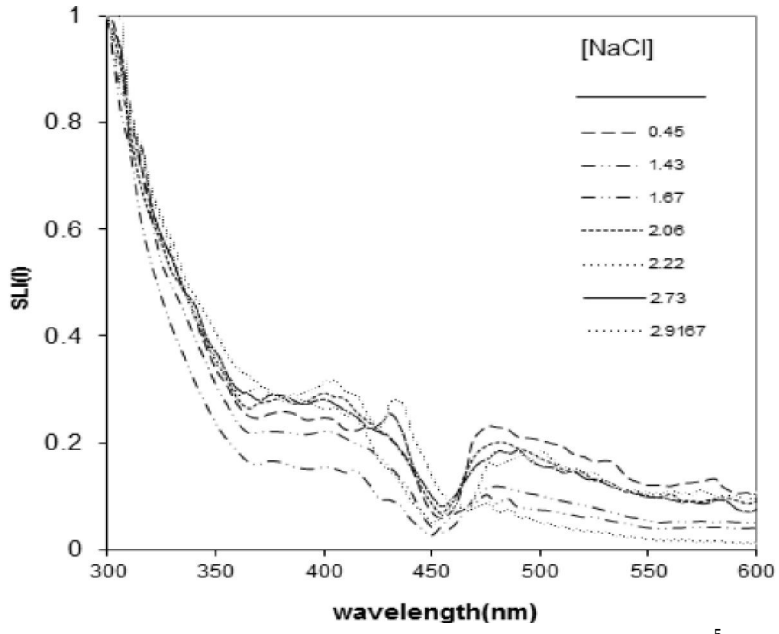

Figure 2 : Spectra of relative scattered light of [Mn(III)(TF$_4$TMAPP)] solution (2×10^{-5}M) upon addition of NaCl in 5 mM phosphate buffer,pH 7.0 at 25ºC

As can be seen in Figure 1, the bandwidth at half height, $W_{1/2}$, and the wavelength of maximum absorption, λ_{max}, of the B band do not show considerable changes by the increase of NaCl. Further, by increasing the concentration of NaCl, no new band appears even at high concentrations of the salt. This means that [Mn(III)(TF$_4$TMAPP)] does not form well-defined aggregates (i.e. H or J type) even at high concentrations of the salt. The lack of NaCl-induced self-association of [Mn(III)(TF$_4$TMAPP)] was also supported by resonance light scattering experiments.

Figure 2, illustrates the RLS profile of [Mn(III)(TF$_4$TMAPP)] in neat water and at different NaCl concentrations. By increasing the concentration of NaCl, the resonance light scattering signal at decreased quickly, which shows that the prominent feature can be assigned to no extended aggregates of electronically coupled metalloporphyrin. So, it can be concluded that [Mn(III)(TF$_4$TMAPP)] does not have any afûnity for aggregation by increasing of the salt concentration and exists as monomers in homogeneous aqueous solutions even at high ionic strength (more than 1

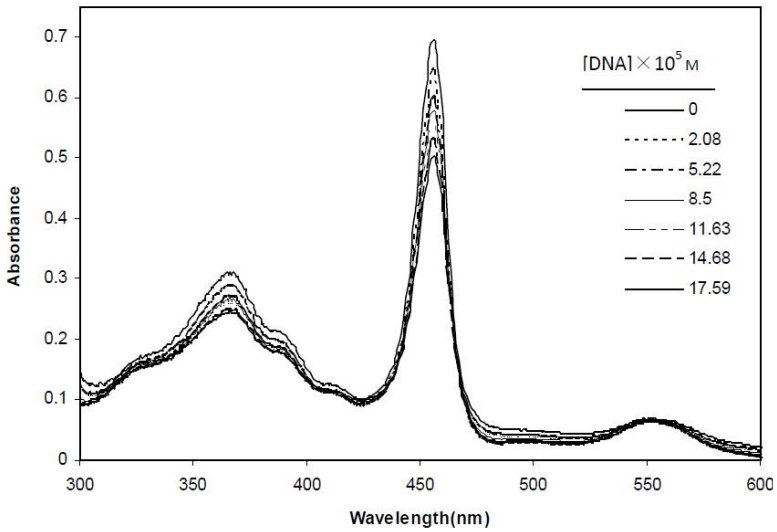

Figure 3 : The absorption spectral change of [Mn(III)(TF TMAPP)] (2×10^{-5}M) titrated with stock solution of ct-DNA (4×10^{-4} M) in 5 mM phosphate buffer, pH 7.0 and at 25°C

TABLE 3 : Thermodynamic parameters and affinity constants for binding of [Mn(III)(TF_4TMAPP)] to ct-DNA in 5 mM phosphate buffer, pH 7.0 at various temperatures

T(K)	logK_1(M^{-1})	ΔG_1^o(KJ/mol)	ΔH_1^o(KJ/mol)	ΔS_1^o(J.mol^{-1}.K^{-1})
298	4.26±0.27	-24.30±1.54	12.36±1.07	123.02±8.76
308	4.34±0.25	-25.59±1.47	12.36±1.07	123.20±8.25
328	4.46±0.18	-28.00±1.13	12.36±1.07	123.05±6.71
338	4.52±0.28	-29.25±1.81	12.36±1.07	123.09±8.52

M of NaCl). On the other hand, the addition of NaCl does not have any effect on the monomer-aggregate equilibrium of metalloporphyrin.

Binding of [Mn(III)(TF_4TMAPP)] to ct-DNA

Optical absorption. We have conducted the titration of metalloporphyrin solution, ([Mn(III)(TF_4TMAPP)]) at a fixed concentration of (2×10^{-5}M) and varying concentrations of ct-DNA in 5 mM phosphate buffer of pH 7.0 with a sufficient binding capacity. Regarding the results of the previous section, our metalloporphyrin exists mainly as monomers. Figure 3 shows a typical titration spectra of [Mn(III)(TF_4TMAPP)] upon addition of ct-DNA at 25 °C. A little bathochromicity shift and weak hypochromism in the soret band were observed, which represents the existence of non-covalent interaction and external groove binding between ct-DNA and the ([Mn(III)(TF_4TMAPP)])[26, 27].

The binding constant at any specified temperature was determined by the concentration dependence of UV-Vis absorption data using SQUAD program. This program has been developed for evaluation of the best set of binding constants of the proposed equilibrium model by employing a nonlinear least-squares approach. The input data consists of (a) the absorbance values, and (b) the total ct-DNA and metalloporphyrin concentrations. The absorption data were analyzed by assuming 1:1 or 2:1 and/or simultaneous 1:1and 2:1 molar ratios of metalloporphyrin to ct-DNA. Fitting of the experimental data (15 point), to the proposed stoichiometric models was evaluated by the sum of squares of the calculated points by the model. The results showed that the best fitting corresponds to the 2:1 complex model. These results are also confirmed by the existence of two simultaneous isosbestic points in the titration stage. The calculated binding constants are given in TABLE 3 and 4. As can be seen in this TABLE, the binding constant increases with increasing of temperature. This phenomena is due to increasing the complex stability at higher temperature and shows a higher binding constant value. The *K* value indicates a high binding affinity of [Mn(III)(TF_4TMAPP)] for ct-DNA.

Thermodynamics of [Mn(III)(TF_4TMAPP)]-ct-DNA binding process. A prerequisite for deeper insight into the molecular basis of metalloporphyrin-DNA

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TABLE 4 : Thermodynamic parameters and affinity constants for binding of [Mn(III)(TF_4TMAPP)] to ct-DNA in 5 mM phosphate buffer, pH 7.0 at various temperatures

T(K)	logK_2(M^{-1})	ΔG_2^o(KJ/mol)	ΔH_2^o(KJ/mol)	ΔS_2^o($J.mol^{-1}.K^{-1}$)
298	2.88±0.08	-16.43±0.45	67.62±1.86	282.04±7.75
308	3.45±0.08	-20.34±0.47	67.62±1.86	285.58±7.56
328	3.93±0.10	-25.74±0.63	67.62±1.86	284.64±7.59
338	4.30±0.02	-27.82±0.13	67.62±1.86	282.37±5.89

interactions is thorough characterization of the energetics governing complex formation. The energetics of metalloporphyrin-ct-DNA equilibrium can be conveniently characterized by thermodynamic parameters such as standard Gibbs free energy change, $\Delta G°$, standard molar enthalpy change, ($\Delta H°$) and standard molar entropy change, $\Delta S°$. The standard Gibbs energy change is usually calculated from the equilibrium constant (K) of the reaction by the following relationship:

$$\Delta G° = -RT \ln K \quad (1)$$

Where R and T are the gas constant and the absolute temperature, respectively. Since the activity coefficients of the reactions are not known, the usual procedure is to assume them to be unity and to use the equilibrium concentrations instead of the activity. Therefore, it would be appropriate to adjust the terminology of apparent equilibrium constant K' , and Gibbs free energy $\Delta G^{o\prime}$. Apparent standard enthalpies per mole of cooperative unit can be obtained from the dependence on temperature of the apparent binding constant $K2$, by van't Hoff equation:

$$\partial \ln K2 = -(\Delta H°2 /R)\partial(1/T) \quad (2)$$

This is the so-called van't Hoff enthalpy. The apparent standard entropy change, $\Delta S^{o\prime}$can be derived from Equation 3:

$$\Delta S°' = (\Delta H°' - \Delta G°')/T \quad (3)$$

The van't Hoff plot for interaction [Mn(III)(TF_4TMAPP)] with ct-DNA is shown in Figure.4a and 4b. The calculated thermodynamic parameters for binding of [Mn(III)(TF_4TMAPP)] to ct-DNA are listed in TABLE 3 and 4.

Resonance light scattering. Figure 5 demonstrated the relative SLI spectra of [Mn(III)(TF_4TMAPP)] solution in the presence of different ct-DNA concentrations. The SLI in the region of 300 to 600 nm at first decreased dramatically with the addition of ct-DNA and then remained approximately unchanged upon further increase of ct-DNA. This result

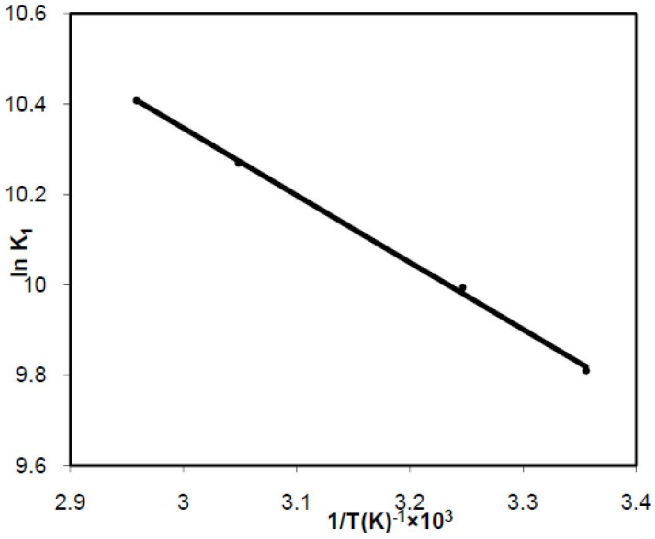

Figure 4a : The van't Hoff plot [Mn(III)(TF_4TMAPP)] binding to ct-DNA

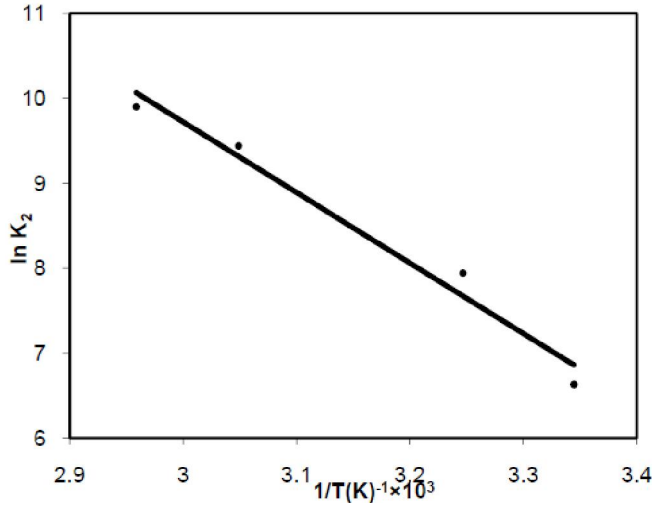

Figure 4b : The van't Hoff plot [Mn(III)(TF_4TMAPP)] binding to ct-DNA

clearly shows that addition of ct-DNA does not have any effect on the monomer-aggregate equilibrium of [Mn(III)(TF_4TMAPP)].

Thermal Denaturation of ct-DNA. The melting curves of both free ct-DNA and [Mn(III)(TF_4TMAPP)]–ct-DNA complex in phosphate buffer were obtained by measuring the hyperchromicity of ct-

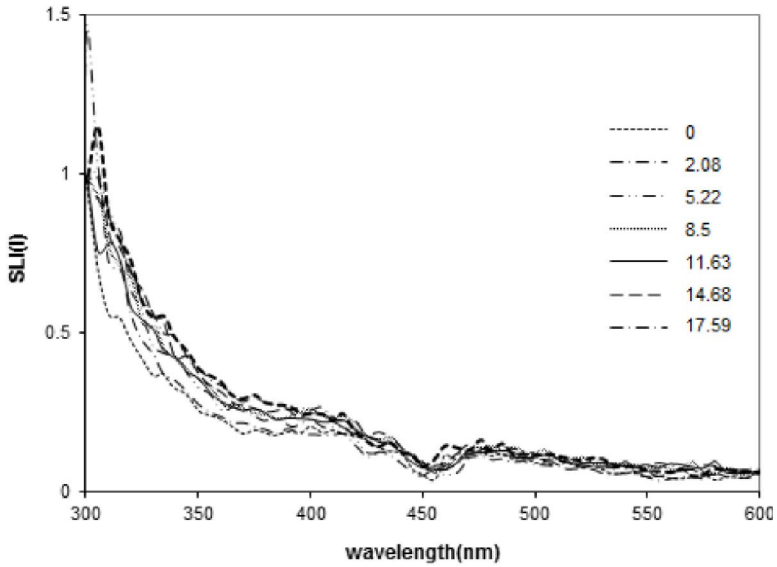

Figure 5 : Spectra of relative scattered light of [Mn(III)(TF$_4$TMAPP)] solution (2×10^{-5} M) in 5 mM phosphate buffer, pH 7.0 and at 25ºC

TABLE 5 : DNA melting temperature changes up on increasing the molar ratio of [Mn(III)(TF$_4$TMAPP)] to ct-DNA

[DNA]	[Mn(III)(TF$_4$TMAPP)]			
	0	**0.026**	**0.0523**	**0.1012**
T_m(K)	341.66	336.34	335.74	334.65

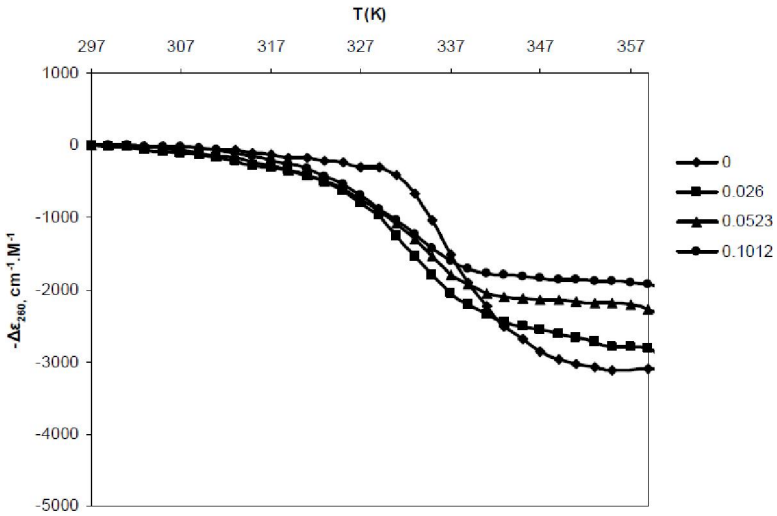

Figure 6 : Melting profiles ($\lambda_{(max)}$ = 260 nm) for the free ct-DNA in the absence of [Mn(III)(TF$_4$TMAPP)] and in different molar ratios of ct-DNA to [Mn(III)(TF$_4$TMAPP)]

DNA absorbance at 259 nm as a function of temperature. The temperature was scanned from 24 to 90° 0C at a speed of 1 °C/min. The melting temperature (T_m)was taken as the midpoint of the hyperchromic transition. The melting temperature (T_m) of ct-DNA is sensitive to its double helix stability and the binding of compounds to ct-DNA alters the T_m values, depending on the strength of interaction[28]. Therefore, it can be used as an indicator of binding properties of metalloporphyrin to ct-DNA and their binding strength. The obtained results of such studies for [Mn(III)(TF$_4$TMAPP)]–ct-DNA complex show decreasing T_m values upon addition of [Mn(III)(TF$_4$TMAPP)] (TABLE 5 and Figure 6). The above results revealed that the studied metalloporphyrin [Mn(III)(TF$_4$TMAPP)], interacts with ct-DNA to stabilize the duplex structure to thermal denaturation. Hence it can be concluded that external bind-

ing has occurred.

CONCLUSION

Studying the aggregation behavior of [Mn (III) (TF_4TMAPP)] indicated that increasing the concentration of complex doesn't affect the tendency to aggregation. The addition of NaCl shows no significance electrolyte effect, no new band appears even in high concentration of salt. This result means that [Mn(III)(TF_4TMAPP)] does not form well defined aggregates even in high concentration of salt. The [Mn(III)(TF_4TMAPP)] binds to external regions of ct-DNA. The DNA-binding process was endothermic for [Mn(III)(TF_4TMAPP)] and has the large positive entropy value. These can be represent the predominate role of hydrophobic interactions and outside binding mode. The decreasing of melting temperature (T_m) of DNA upon addition of metalloporphyrin represents the existence of an outside binding mode

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