# Binding of new Fe(II)-containing porphyrins to DNA. Effect of ionic strength S. A.A. Sulyman<sup>1,2</sup>, A. A. Ghazaryan<sup>1</sup>, Y. B. Dalyan<sup>1\*</sup>

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### Abstract

We employed Circular Dichroism (CD) and UV/Vis-spectrophotometric methods to study the interaction of two Fe-containg cationic porphyrins: meso-tetra (4N-butylpyridyl) porphyrin [FeTButPyP4] and meso-tetra (4N-oxyethylpyridyl) porphyrin [FeTOEtPyP4] with DNA at various ionic strengths. The temperature-dependent absorption titration data were used to determine the binding constants and stoichiometry for each ligand-DNA complexes. It was shown that the decrease in ionic strength of solution leads to decrease in the number of binding sites of porphyrins to DNA and increase in the efficiency of their binding. The CD spectra analysis shows that the binding mode of both porphyrins to DNA depends on the ionic strength of buffer. It changes from outside random binding at lower ionic strength ( $\mu = 0.02M$ ) to semi-intercalation at higher strength ( $\mu = 0.2M$ ). The magnitude and sign of enthalpy and entropy, calculated using Van't Hoff equation, confirm these conclusions.

Keywords: DNA, Fe-porphyrins, optical absorption, circular dichroism, melting, thermodynamics of binding

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## Introduction

The interactions of water-soluble cationic porphyrins and their metal-containing derivatives with DNA have been an interesting subject for years due to their ability to accumulate in malignant cells [1], as well as their application to photodynamic therapy [2] and virus inhibition [3]. Three binding modes have been proposed for cationic porphyrin binding to DNA: intercalation and two types of outside binding. The first type is the outside binding involving both placement of porphyrin in the minor groove and electronic interaction with the phosphate backbone, and the second type is the stacking of the porphyrin along the DNA helix [4]. These structures of the porphyrin–DNA complexes have been extensively characterized using a variety of physical techniques [5,6].

Binding mode could be modulated by the nature of the metal ion and the size and location of the substitute groups on the periphery of the porphyrin. Generally, the free base porphyrins and planar metalloporphyrins (such as Ni(II) and Cu(II)-containing) tend to intercalate between DNA base pairs (preferably to GC sites). For the metalloporphyrins which have axial ligands (such as Co(II), Mn(II), and Fe(II)-containing metalloporphyrins) or those with bulky substituent on the periphery of the structure intercalation is blocked and outside binding occurs. Recent studies showed that intercalation versus outside binding may also be influenced by the charge on the porphyrin core [6] and the ionic strength of the medium,

which affects self-association of the porphyrin [7,8]. The side of the porphyrin ring fits into the minor groove of DNA or locates in the major groove by electrostatic interaction between the negatively charged phosphate group of DNA and the positively charged pyridylic ring of porphyrin.

It has been principally accepted that thermodynamic parameters of any process relate to its molecular basis. Hence, determination of thermodynamic parameters governing DNA– Porphyrin complex formation makes deeper insight into molecular basis of DNA–Porphyrin interactions. On the basis of this importance, in the present study the UV/visible and circular dichroism (CD) spectroscopy was applied to investigate the interactions of FeTOEPyP4, FeTButPyP4 with DNA duplex at different ionic strength ( $\mu = 0.02$  and  $\mu = 0.2$ ). Change of ionic strength results in the change of screening of porphyrins charge and in the change of conformation DNA double helix. Both of these factors can influence on the mechanism of binding of porphyrins to DNA. Our goal was to determine the influence of ionic strength on the mechanism of binding of these porphyrins to DNA.

### Experimental

The ultrapure calf thymus DNA (protein < 0.1%, RNA < 0.1%, MW >25 MDa: GC=42%) was isolated at laboratory headed by prof. D.Yu. Lando at the Institute of Bioorganic Chemistry (Minsk, Belarus).

The studied meso-tetra (4N-oxyethylpyridyl) porphyrin [FeTOEtPyP4] and meso-tetra (4N-butylpyridyl) prophyrin [FeTButPyP4] were synthesized, purified, and kindly donated by Dr. Robert Ghazaryan (Faculty of Pharmaceutical Chemistry, Yerevan State Medical University, Armenia). The structure of this porphyrin is shown in Fig. 1.

All experiments were carried out in phosphate buffers 1BPSE (ionic strength  $\mu = 0.2$ ) and 0.1BPSE ( $\mu = 0.02$ ), pH = 7.3, where 1BPSE = 6 mM Na<sub>2</sub>HPO<sub>4</sub> + 2 mM NaH<sub>2</sub>PO<sub>4</sub>+185 mM NaCl+ 1 mM EDTA.

The melting curves of porphyrin/DNA complexes were recorded by using a Lambda-40 spectrophotometer. For all experiments the heating rate was 1°C/min while absorbance at 260 nm was recorded. Absorbance spectra were recorded by means of a Lambda 800 (Perkin Elmer) UV-Vis spectrophotometer thermostated with PTP-6 at 18, 25, 35, and 45 °C. Circular dichroism spectra were recorded by using a Roussel Jouan-II dichrograph.



**Fig. 1**. Chemical structure of Fe(II)-porphyrins.

### **Results and Discussions**

Absorption spectra. The interaction of FeTOEPyP4 and FeTButPyP4 with DNA at ionic strength 0.02M and 0.2M was monitored at Soret region of absorption spectra (near 420 nm). The solutions of constant concentration of porphyrins  $(10^{-6} \text{ M})$  were titrated with a stock solution of DNA. The Soret region of absorption spectra (380-500 nm) of porphyrins at increasing DNA concentrations is shown in Fig. 2.



**Fig. 2.** Soret region of absorption spectra of porphyrins in the presence of DNA at 25 °C at different ionic strengths. The concentration of stock solution of DNA is  $C_{DNA} = 10^{-4} \text{ M bp}$ .

The analysis of Fig.2 reveals that the increasing DNA concentration leads to hypocromic effect and red shift of Soret peak of FeTOEtPyP4 and FeTButPyP4. In the case of lower ionic strength there is a slight red shift ( $\sim$ 3-6 nm) and large hypochromicity (20-21%) of the Soret absorption band for both porphyrins. In the case of higher ionic strength the effects of the red shift are more evident ( $\sim$  6-8 nm) and moderate hypochromicity could be observed (14-15%) for the same complexes. One set of isosbestic points was observed in each case, and the binding was found to proceed apparently in a single step. This allowed us to use the non-cooperative neighbor exclusion model proposed by McGhee and von Hippel [9] to analyze the titration data.

*Melting.* For investigation of interaction of Fe-porphyrins with DNA the thermal melting experiments of complexes were performed. The binding with drugs changes a stability of DNA. The measurement of the melting temperature,  $T_m$ , of DNA in the presence of porphyrin in solutions reflects the strength of the interaction between porphyrin and DNA.

To study the changes of  $T_m$  upon addition of FeTOEtPyP4 and FeTButPyP4 to the solution of DNA  $T_m$  was measured in the range of relative concentrations 0.001 < r < 0.1. It appeared that at high ionic strength ( $\mu = 0.2$ ) the melting process of DNA/porphyrin complexes finishes above 98°C which made it impossible to record the entire melting transition. Consequently, it is not possible to accurately determine melting curve parameters under these conditions. However, it can be concluded that the addition of porphyrins strongly stabilizes the structure of DNA.

Our experiments at relatively lower ionic strength ( $\mu = 0.02$ ) allow acquisition of the entire melting curve and thereby accurate determination of melting curve parameters (Table1).

$r = C_{por}/C_{DNA}$	FeTButPyP4			FeTOEtPyP4		
	$T_{m,o}C$	ΔT,°C	Δh,%	T <sub>m</sub> , <sup>o</sup> C	ΔT,°C	Δh,%
0	71.99	9.31	36.1	71.99	9.31	36.1
0.001	72.28	8.49	37.09	71.84	9.39	37.15
0.005	72.18	8.04	36.72	72.23	9.46	38.03
0.01	71.97	8.76	36.67	72.53	9.79	37.30
0.05	74.90	12.55	37.90	77.13	12.61	38.12
0.1	78.31	15.54	38.98	aggregate		

**Table 1.** The melting temperature (T<sub>m</sub>), melting interval ( $\Delta$ T) and hypochromic effect ( $\Delta$ h) at  $\lambda$ =260nm of DNA complexes with different concentrations of Fe-porphyrins. Buffer–0.1BPSE ( $\mu$ =0.02 M), C<sub>DNA</sub> = 3.5×10<sup>-5</sup> M of base pairs.

Obtained data show that interaction of both studied porphyrins with DNA leads to relatively moderate stabilization of duplex structure. Moreover, the increase in porphyrin-to-DNA concentration ratio, r, in the range  $0.001 \le r \le 0.01$  weakly affects  $T_m$  and  $\Delta T$  of the melting curves. The hypochromicity change,  $\Delta h$ , of the complexes is also not perturbed over the latter concentration range ( $\delta(\Delta h) \sim 1-2\%$ ). At greater porphyrin/DNA ratios,  $0.05 \le r \le 0.1$ , both the  $T_m$  and  $\Delta T$  values increase with the increasing concentration of porphyrins. At r > 0.2 aggregation effects are observed which hinder acquisition of the melting curves. The obtained results specify that at low porphyrin/DNA ratios ( $r \le 0.01$ ) the external binding mode is more reasonable.

*Circular Dichroism*. CD spectra of DNA–porphyrin complexes are characterized by two bands: UV-region band, which is a consequence of natural asymmetry of DNA, and a visible region band or induced CD (ICD), which is caused by the asymmetry of porphyrin packing on DNA. Judging by the sign of ICD it is possible to determine the mechanism of binding of porphyrins with DNA. The ICD spectra of FeTButPyP4 and FeTOEtPyP4 with duplex DNA at 25°C were measured. For these experiments we use the five time smaller concentration of DNA than usually. The small initial DNA concentrations  $(1.5 \times 10^{-5} - 2 \times 10^{-5}M)$  were chosen to avoid aggregation, which are observed at high DNA concentrations.

At lower ionic strength no change in Soret region upon addition of porphyrins to DNA duplex occurs. This should mean that no ordered structures are formed in case of  $\mu = 0.02M$ . An example of such binding mechanism is outside random binding.

In the case of higher ionic strength the addition of aliquots of porphyrin to a known amount of DNA solution induces a large negative CD band for both complexes (Fig. 3). This picture is consistent with an intercalation binding mode for these compounds at higher  $r = C_{por}/C_{DNA}$  ratio.



**Fig. 3**. The induced circular dichroism spectra of DNA with (a) FeTOEtPyP4, 0.5 < r < 1.9 (b) FeTButPyP4, 0.5 < r < 2.6 at 25 °C in 1BPSE. The direction of increasing concentration of porphyrin is represented by the arrow.

Usually the increasing of ionic strength results first in the stronger screening of porphyrins charges, and second, to increasing of twisting of DNA helix. Both these factors together promote the intercalation mode of binding. But as known from literature [10], Feporphyrins have two axial ligands which prevent metalloporphyrins to intercalate into DNA duplex. We assume that the observed effect is a result of so-called semi-intercalation binding mode of porphyrins during which one of the periphery rings intercalates into the DNA helix, but the center of porphyrin core stays out near the phosphate groups of DNA.

*Thermodynamics of binding.* Thermodynamics of interaction of both Fe-porphyrins with DNA at different ionic strengths was investigated in order to understand the molecular forces of complex formation between cationic porphyrins and DNA.

The results obtained from titration experiments were used for calculation of binding parameters: the binding constant  $K_b$  and the number of binding sites per base pair (or exclusion parameter) n.

Calculation was made by the McGee and von Hippel model using the equation (1) of Correia and co-workers [11]:

$$C_{f} = -\frac{r}{K_{b}} \left[ \frac{nr-1}{nr-r-1} \right]^{-n} (nr-r-1),$$
(1)

where  $C_f$  is the free porphyrins concentration in solution,  $r = C_b/C_{DNA}$ ,  $C_b$  is the concentration of bound porphyrins,  $C_{DNA}$  is the concentration of DNA base pairs,  $K_b$  is the binding constant and n is the number of binding sites per base pair. Calculated parameters are presented in Table 2.

Porphyrin	μ, (Mol)	$K_b, 10^6 \mathrm{M}^{-1}$	п	$\Delta G$ (kJ mol <sup>-1</sup> )	$\Delta H$ (kJ mol <sup>-1</sup> )	$\Delta S \ (J \text{ mol}^{-1} \text{K}^{-1})$
FeTButPyP4	0.02	$6.6 \pm 0.41$	$1.9\ \pm 0.1$	$-8.06 \pm 0.04$	$7.4 \pm 0.9$	52.1 ± 3.1
	0.2	$1.14 \pm 0.16$	$5.3 \pm 0.1$	-7.9±0.14	$1.4 \pm 0.1$	31.2 ± 5.1
FeTOEtPyP4	0.02	$2.6 \pm 0.24$	$2.8 \pm 0.1$	-7.5±0.03	$4.5 \pm 0.46$	$40.5 \pm 1.41$
	0.2	$1.55 \pm 0.36$	9.7 ± 0.2	$-8.03 \pm 0.13$	$2.5 \pm 0.7$	$35.2 \pm 5.8$

Table 2. The binding parameters of porphyrins–DNA complexes at different ionic strength ( $\mu$ ). The values of  $\Delta G$  relate to 25 °C.

Obtained data reveal that  $K_b$  varies between  $1.2 \times 10^5$  M<sup>-1</sup> and  $6.6 \times 10^5$  M<sup>-1</sup>, a range typical for porphyrin-nucleic acid interactions [12–14]. It is apparent that the binding is somewhat stronger at lower ionic strength. This effect is apparently due to increased shielding of porphyrin and DNA charges at higher ionic strength which leads to decrease in electrostatic attraction of complexes. The increase in ionic strength also leads to increase of exclusion parameter *n*, which is a consequence of change of binding mode and/or amplification of selectivity. Essentially high values of n at high ionic forces testify in favor of realization semi-intercalation binding mode.

Gibbs free energy change was determined from  $\Delta G = RT^* \ln K_b$ , where  $K_b$  is the binding constant at the reference temperature  $T^* = 25^\circ$  and R is the gas constant. Indirect Van't Hoff method was used to obtain thermodynamic parameters because direct measurements (such as ITC) cannot be accomplished due to aggregation and fibril formation of DNA at high concentrations needed for such experiments. Hence thermodynamics of the porphyrins–DNA interaction was studied in terms of the values of binding constants determined at different temperatures at which DNA keeps its double helix structure. This approach provides a good means to indirectly determine the thermodynamic parameters of porphyrin–DNA interaction by the Van't Hoff plot over a temperature range (Fig.4).



Fig. 4. Temperature dependences of the binding constant for the porphyrins complexes.

Linear Van't Hoff plots indicate that no change in heat capacity occurs over the temperature range 18–45 °C. Thus, values for enthalpy change,  $\Delta H$ , upon complexation was determined from the slope of interpolated line and entropy change was calculated from  $\Delta G = \Delta H - T^* \Delta S$ . Calculated parameters are also presented in Table 2.

It has been revealed that the binding of all porphyrins to the DNA is clearly entropically driven endothermic reaction. The unfavorable enthalpy is indicative of the removal of bound water molecules into bulk solvent. We assume that the favorable entropy term results from the liberation of water and/or counterions from interacting surfaces upon binding of positively charged ligands. It is apparent that this effect would be less pronounced in case of intercalation because it leads to relatively moderate perturbations on DNA molecule surface compared to outside interaction modes. Such vision is consistent with obtained  $\Delta S$ 's.

Finally, we can conclude that new Fe-porphyrins bind via different binding modes depending on ionic strength which results in different absorbance, CD and thermodynamic profiles. Since the intercalation of these compounds having two axial ligands is impossible, outside binding and semi-intercalation is proposed as possible binding mechanisms. In the second mode the large negative sign on CD spectra can be a result of peripheral ring intercalation between DNA base pairs. The decrease in ionic strength of solution leads to decrease in the number of binding sites of porphyrins to DNA and increase in the efficiency of their binding.

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