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Interaction of 5, 10, 15, 20-Tetrakis (N-Benzylpyridilium-4-yl) Porphyrin (H2TBzPyP) and Its Metal Derivatives with Calf Thymus DNA

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Abstract

Interactions of 5, 10, 15, 20-tetrakis (N-benzylpyridilium-4-yl) porphyrin (TBzPyP) and its metal complexes (Cu(II), Ni(II), Co(II) and Mn(III)) with calf thymus DNA were investigated in view of thermodynamics; using UV-vis spectroscopy. The measurements were done in 5 mM phosphate buffer, pH 7.0. The optical absorption spectra of porphyrins were analyzed in order to obtain binding constants and stoichiometries using SQUAD software. The results show that the best fitting corresponds to 1:1 complex model between base pair of DNA and porphyrins. Running the measurements at various temperatures provided the completed thermodynamic analysis on basis of van't Hoff equation. The results represents the enthalpy driven of the process and the predominate role of electrostatic interaction. The following order has been obtained for binding affinity and exothermicity:

MnTBzPyP > CoTBzPyP > NiTBzPyP > CuTBzPyP > TBzPyP

This result has been interpreted on basis of the chemical structure of porphyrins and electronegativity of their central metal. The values of calculated binding constants represent the less affinity of TBzPyP and its metal derivatives to DNA in comparison with meso-tetrakis(N-methylpyridinium-4-yl)porphyrin (TMPyP). This can be related to the larger peripheral benzyl groups of TBzPyP that probably inhibits stronger intercalation binding and favors outside self-stacking and non-specific binding.

Keywords: DNA, porphyrin, SQUAD, thermodynamic of binding, optical absorption.

1. Introduction

Porphyrins and metalloporphyrins have many potential applications in biological and medicine such as photodynamic therapy[1] acting as antiviral[2, 3] and anticancer drugs[4].The interaction between DNA and cationic porphyrins has been studied intensively for its unique physicochemical properties in the interaction with nucleic acid[5–8]. Development in this area is predicated upon a detailed understanding of the porphyrin-nucleic acid binding mechanism. The cationic meso-tetrakis (N-methylpyridinium-4-yl) porphyrin (TMPyP) and some of metal derivatives such as Cu(II), Ni(II), Pd(II), Fe(III), Co(III), and Mn(III) complexes have been extensively studied[8,9]. It has been shown that these cationic porphyrins have a very high affinity to anionic DNA strands, with association constants at 10^5–10^7 M^{-1} level[6].

The three-binding mode for the porphyrin-DNA complex has been generally accepted, namely intercalation, outside self-stacking, and non-specific binding. These structures of the porphyrin-DNA complexes have been extensively characterized using a variety of physical techniques.[5, 10] Binding mode could be modulated by the nature of the metallation and the size and location of the substitute groups on the periphery of the porphyrin. Generally,

the free bases and square planar complexes such as Ni^{2+} and Cu^{2+} intercalate between DNA base pairs (to the GC site). For the porphyrin-metal complex, having axially bound ligands such as Co^{3+}, Mn^{3+}, and Fe^{3+} or those with bulky substitutes on the periphery on 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, 10] and the ionic strength of the medium, which affects self-association of the porphyrin.[11, 12] 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 pyridinium 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 basis of this importance, in the present study, the interaction between 5,10,15,20-tetrakis (N-benzylpyridilium-4-yl) prophyrin (H_2TBzPyP) and its metal complexes (Cu(II), Ni(II), Co(II) and Mn(III)) (Scheme 1) with calf thymus DNA(ct-DNA) have been investigated in view of thermodynamics; using UV-vis spectroscopy. Running the measurements at various temperatures provided the completed thermodynamic analysis on basis of van't Hoff equation. A very reasonable correlation has been done between the values of calculated thermodynamic parameters and chemical structure of studied porphyrins.

2. Experimental

Tetrakis (4-pyridyl) porphyrin (TPyP) was obtained from Sigma Chemical Co. and used as supplied. Synthesis of porphyrin, H_2TBzPyP was obtained from its precursor (TPyP), by reaction with benzyl bromide in DMF[13]. Passing the aqueous solution of H_2TBzPyPBr_4 over an anionic exchange resin (Dowex-1, Sigma, USA) made chloride salt of H_2TBzPyP (H_2TBzPyPCl_4). The obtained aqueous solution is lyophilized and the product dried in vacuum over P_2O_5. The metal derivatives of H_2TBzPyPCl_4 were prepared, purified by a slightly modified published procedure[14–16]. All of the synthetic complexes were characterized by UV-vis, and elemental analysis. All of the chemicals, which have been used for these syntheses, were of analytical grade and purchased from Sigma. To prepare the DNA stock solution about 2 mg of DNA was dissolved in 1mL of the phosphate buffer at 4 °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 $\varepsilon_{258nm} = 6700\ M^{-1}cm^{-1}$ for DNA[17, 18] (i.e. reported in molar base pairs). In all experiments, the porphyrins and DNA solutions were freshly prepared and protected from direct sun lights before they were inserted into the cell compartments.

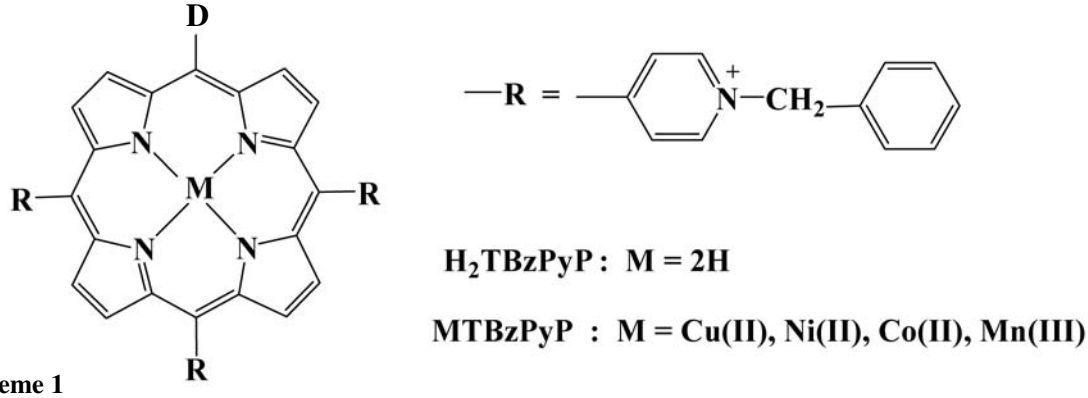
The absorption spectra were recorded on a Cary 500 double beam spectrophotometer using 1cm quartz cuvettes with thermostat cell compartment, that control the temperature around the cell within ± 0.1 °C.

Titration of porphyrin solution with DNA was performed at 20, 25, 30, 35, 40 and 45 °C in a 5 mM phosphate buffer pH 7.0. A stock solution of ct-DNA was added to the porphyrin solution stepwise and the spectrum of porphyrin was recorded at each step. The titration experiment was continued until the absorbance of the porphyrin solution in the UV-vis range remained constant. The starting volume of the porphyrin solution was 1800 μL, and the amount of DNA stock solution added in each step was 50 μL. The spectra were recorded within the range of 300–700 nm about 5 min after each addition of DNA solution. It has been checked that this maintenance time is enough to reach the equilibrium of the DNA-porphyrin reaction. The spectra were also corrected respect to dilution effect.

3. Results and Discussion

3.1. Spectral Data Analysis

A typical of the spectrophotometric titration of CuTBzPyP with DNA in 5 mM phosphate buffer, pH 7.0 at 25 °C has been shown in Figure 1. The similar situa-



Scheme 1

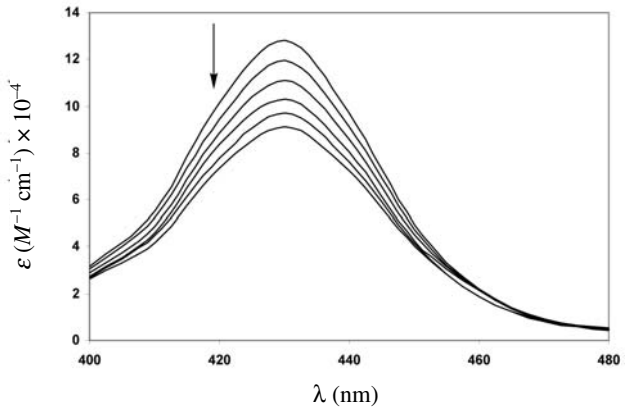


Fig. 1: The spectrophotometric titration of CuTBzPyP with DNA in 5 mM phosphate buffer, pH 7.0 and at 25 °C. The concentration of DNA increases in the direction of arrow and the concentration of porphyrin in the cell was 3.68×10^{-6} M.

tions were observed for other systems. In order to analysis the spectral data of porphyrins at various concentration of DNA in titration experiments , the 50 wavelengths showing suitable absorbance variations upon addition of DNA were selected from spectrum of porphyrin. The values of absorbance of these selected wavelengths at various DNA concentrations were analyzed in order to calculate equilibrium formation constants using SQUAD software. This method has been recently used for analysis of DNA-porphyrazine and DNA-porphyrin spectral data.[19, 20]

This program is designed to calculate the best values for the stability constants of the proposed equilibrium model by employing a non-linear least square approach.[21, 22] This program is completely general in scope, having the capability to refine stability constants for the general complex $M_m M_l' H_j L_n L_q'$, where m,l,n,q ≥ 0 and j is positive for protons, negative (for hydroxyl ions) or zero . The algorithm employed in SQUAD and their relationships to each other have been described previously[21,23]. Our input data for analysis of porphyrin-DNA system were absorbance at 50 different wavelengths of 15 porphyrin spectra. These 15 spectra were corresponding to 15 various concentrations of DNA. The outputs are the logarithm of equilibrium formation constant, $\log K_{rs}$, for formation of $(DNA)_r (Porphyrin)_s$ is defined with respect to the eq. (2)

$$rDNA + sPorphyrin \Leftrightarrow (DNA)_r (Porphyrin)_s \quad (1)$$

$$K_{rs} = \frac{[(DNA)_r (prophyrin)_s]}{[DNA]^r [porphyrin]^s} \quad (2)$$

The values of uncertainty in $\log K_{rs}$ are also calculated by the program. The results show that the best fitting corresponds to 1:1 complex model at all studied temperatures with sum of squares of reduced error between 10^{-3}–10^{-4}. The existence of a distinct isosbestic point in titration spectra is also in concord with formation of 1:1 complex.

3.2. Thermodynamics of DNA: Porphyrin Interactions

The energetic of DNA: Porphyrin equilibrium can be conveniently characterized by three familiar thermodynamic parameters; standard Gibbs free energy, $\Delta G°$, enthalpy, $\Delta H°$, and entropy, $\Delta S°$, changes. The $\Delta G°$ can be calculated from equilibrium constant, K, of the reaction using the familiar relationship, $\Delta G° = -RT\ln K$ in which R and T referring to the gas constant and the absolute temperature, respectively. If heat capacity change of reaction is negligible, the plot $\Delta G°/T$ versus $1/T$ (van't Hoff plot) should be linear with respect to the following equation (eq. (3)).

$$\frac{\Delta G^o}{T} = \frac{\Delta H^o}{T} - \Delta S^o \quad (3)$$

The slope and Y-intercept of this equation should be equal to $\Delta H°$ and $\Delta S°$, respectively. Such plots for binding of TBzPyP and its metal complexes to DNA in the phosphate buffer are shown in Figure 2 and their calculated thermodynamic parameters with their standard errors are listed in Table 1. The Sigma Plot software was used for doing these calculations.

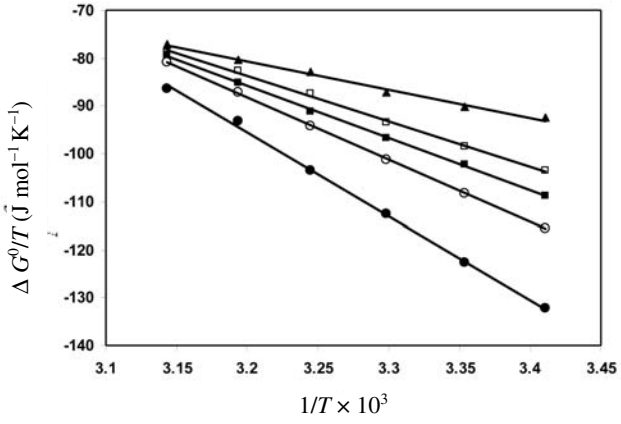


Fig. 2: The plot of $\Delta G°/T$ versus $1/T$ (van't Hoff plot) for binding of TBzPyP (▲), CuTBzPyP (□), NiTBzPyP (■), CoTBzPyP (○) and MnTBzPyP (●) to DNA in 5 mM phosphate buffer, pH 7.0.

4. Discussion

Figure 3 shows typical absorbance spectra of the compounds. The values of ε_{max}, λ_{max}, and the concentration range of porphyrin that the absorbance obeys Beer-Lambert law are listed in Table 2. In these concentration ranges, porphyrins do not show concentration dependent aggregation.

The most commonly observed spectroscopic consequence of porphyrin nonplanarity is a red shift in the π–π* absorption bands in the UV-vis spectrum[24]. Shift in the Soret or B band, typically 400 nm, of as much as 50 nm have been observed as a result of nonplanar distortion[25–27].

Table 1: Calculated thermodynamic parameters for binding of studied porphyrins to DNA in 5 mM phosphate buffer, pH 7.0. The values of $\Delta G°$ have been reported at 25 °C.

Porphyrin	$\Delta G°$ (kJ mol^{-1})	$\Delta H°$ (kJ mol^{-1})	$\Delta S°$ (J mol^{-1} K^{-1})
H_2TBzPyP	–26.90 ± 0.50	–59.07 ± 2.51	–108.4 ± 8.22
CuTBzPyP	–29.34 ± 0.19	–94.77 ± 2.09	–219.60 ± 6.86
NiTBzPyP	–30.48 ± 0.12	–108.6 ± 1.27	–261.6 ± 4.16
CoTBzPyP	–32.25 ± 0.06	–129.8 ± 0.91	–327.2 ± 2.98
MnTBzPyP	–36.59 ± 0.01	–174.5 ± 3.12	–462.9 ± 10.24

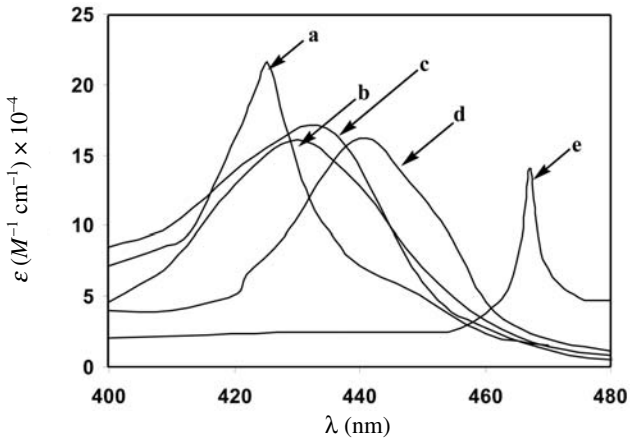


Fig. 3. The spectral feature of TBzPyP (a), CuTBzPyP (b), NiTBzPyP (c), CoTBzPyP (d) and MnTBzPyP (e) in the Soret region.

Thus the observed red shift in the spectrum of M^{n+}TBzPyP in comparison with TBzPyP can be related to the extend of distortion from planarity. On basis of this interpretation, the MnTBzPyP must have the most and CuTBzPyP the least distortion in contrast with TBzPyP.

The results of thermodynamic analysis represent that the associative DNA-porphyrin interactions are essentially enthalpy driven with the following order for binding affinity and exothermicity:

MnTBzPyP > CoTBzPyP > NiTBzPyP > CuTBzPyP > TBzPyP

The negative entropy values are the characteristic of associative reaction with little changes in heat capacity change. On basis of these results it can be concluded that the nature of interaction forces is predominately electrostatic not hydrophobic. However, relatively small values of $\Delta G°$ compared with $\Delta H°$ indicate that entropic opposition is not negligible with enthalpic driving force, hence, hydrophobic interaction even if not dominating should be considered. This conclusion can also be explained with respect to chemical structure of porphyrins. In fact, the strength of electrostatic interaction should be related to positive charge density of peripheral groups of these tetracationic porphyrins that directly related to electronegativity of central metal of porphyrin. The order of electronegativity of M^{n+} ions that were used in this study are as the same as binding affinity and exothermicity of DNA-porphyrin association reaction that mentioned above.[28] By the way, increasing of electro negativity of central metal increases the positive charge density of peripheral groups that consequently increases the electrostatic interactive forces, binding affinity and exothermicity of DNA-porphyrin interaction. A slight observed red shift in the porphyrin absorption region due to its interaction with DNA is also certified the existence of a simple non-covalent interaction.[25]

The highest affinity of MnTBzPyP can also be explained by less number of its d-orbital electrons that provides favorable conditions for the formation of both σ-bonds and additional dative M ← N π-bonds. This improves the electron-accepting properties of the metalloporphyrin macrocyclic and facilities interaction with the π–donors. MnTBzPyP (either in d^4, $t_{2g}^3e_g^1$ (HS) or $t_{2g}^4e_g^0$ (LS)) can form both σ-bonds and additional dative M ← N π-bonds while the other M^{2+} TBzPyP complexes due to more d-orbital electrons ($Co^{2+}(d^7)$, $Ni^{2+}(d^8)$, $Cu^{2+}(d^9)$) can not.

Finally, the results represent the association binding constants of TBzPyP and its metal derivatives are at 10^4–10^6 M^{-1} level that less than corresponding values for TMPyP (10^5–10^7 M^{-1})[6, 29]. This less affinity can be related to larger size of peripheral benzyl groups in TBzPyP that

Table 2: UV-vis spectral characteristic of TBzPyP and its metal derivatives in 5 mM phosphate buffer, pH 7.0 and at 25 °C.

Porphyrin	λ_{max}(nm)	ε_{max}(M^{-1} cm^{-1})	Concentration range that obeys from Beer's law(M)
TBzPyP	425	2.21×10^5	$1.31 \times 10^{-6} - 8.90 \times 10^{-5}$
Cu(II)TBzPyP	430	1.61×10^5	$1.21 \times 10^{-6} - 9.80 \times 10^{-5}$
Ni(II)TBzPyP	436	1.57×10^5	$1.20 \times 10^{-6} - 1.41 \times 10^{-4}$
Co(II)TBzPyP	438	1.52×10^5	$1.18 \times 10^{-6} - 1.39 \times 10^{-4}$
Mn(III)TBzPyP	467	1.41×10^5	$1.11 \times 10^{-6} - 1.12 \times 10^{-4}$

probably inhibited stronger intercalation binding and favors outside self-stacking, and non-specific binding. Moreover, the spectral data analysis using well define SQUAD software and the existence of isosbestic point in absorption spectra indicate the binding of these porphyrins is homogeneous. In the other word, it seems in contrast of N-methyl analogues of the studied porphyrins that are able to form at least three structurally distinct complexes with DNA[5, 10], these studied porphyrins predominantly form single complex species with DNA. This fact can also be clearly related to huge size of peripheral benzyl groups of these porphyrins.

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Povzetek

Z uporabo UV-vis spektroskopije smo raziskovali termodinamiko interakcije 5, 10, 15, 20-tetrakis (N-benzilpiridinijev-4-il) porfirina (TBzPyP) in njegovih kovinskih kompleksov (Cu(II), Ni(II), Co(II) and Mn(III)) z DNA telečjega timusa. Meritve smo izvedli v 5 mM fosfatnem pufru (pH 7.0) pri različnih temperaturah. Z analizo absorpcijskih spektrov porfirina s pomočjo SQUAD programskega paketa smo določili konstante in stehiometrijo vezanja. Rezultati kažejo na največjo verjetnost nastanka 1:1 kompleksa med porfirinom in DNA. Z uporabo van't Hoffove enačbe smo izvedli celotno termodinamsko analizo procesov in ugotovili, da gre za entalpijsko voden proces s prevlado elektrostatskih interakcij. Glede na afiniteto vezanja in eksotermnost procesa nastanka komplekse razvrstimo v: MnTBzPyP CoTBzPyP NiTBzPyP CuTBzPyP TBzPyP, kar lahko razložimo s kemijsko strukturo porfirina in elektronegativnostjo centralnega kovinskega iona. Izračunane konstante vezanja kažejo na manjšo afiniteto vezanja TBzPyP in njegovih kovinskih derivatov na DNA v primerjavi z mezo-tetrakis(N-metilpiridinijev-4-il)porfirin (TMPyP), kar kaže, da daljša periferna benzilna skupina v TMPyP verjetno ovira močnejše vezanje.