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Physicochemical Study of the Interaction of some Water Soluble Porphyrins with Calf Thymus DNA

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ABSTRACT

In order to shed more light on the effect of peripheral groups of porphyrin core into physicochemical properties and DNA binding behavior of porphyrins, we have chosen to investigate solution properties and calf thymus DNA binding behavior of meso-tetrakis (4-N-benzyl-pyridyl) porphyrin ($TBzP_yP$) and its Mn (III), Co (III), Ni (II) and Cu (II) complexes derivatives have been studied in thermodynamic viewpoint using Uv/Vis spectroscopy. They have been chosen because of their good solubility in physiological solution as well as moderately hydrophobic property, so that we may consider them as promising compounds for clinical applications. The measurements were done in 1mM phosphate buffer, pH 7.0 and various temperatures. 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 a 1:1 complex model between base pair of DNA and porphyrins. The estimation of binding constant at various temperatures enabled as to calculate all of the thermodynamic parameters of binding using vant Hoff equation. All of the studied porphyrins showed strong electrolyte effect and increasing of NaCl concentration induces self-aggregation of porphyrins. We also made a comparison between the properties of above-mentioned porphyrins and that of a less-hydrophobic water-soluble porphyrin [meso Tetrakis (4-N-Methyl-pyridyl) porphyrin (TMPyP). The results showed that the above-mentioned porphyrins exist as monomer at low ionic strength medium. While TBzPyP and CuTBzPyP formed ill-defined self-aggregates at high strength medium. The hypochromicity among with small red shift has been observed in Soret band of porphyrins due to increasing of DNA.

Keywords: DNA; Porphyrin; SQUAD; Thermodynamic parameters

INTRODUCTION

The interactions of cationic porphyrins with nucleic acids have received considerable at tension [1, 2]. Several of these strongest DNA binders, with association constant $10^5\ M^{-1}$ to $10^6\ M^{-1}$ [3,6], have clinical potential as anticancer agents in photodynamic therapy [7,8], 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 .One of the such compounds can be 5,10,15,20-tetrakis (4-N-bezyl–pyridyl) porphyrin ($TBzP_yP$)(scheem1) which can be coordinated with a range of transition

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metals ,this has a more hydrophobic nature with respect to its well studied amalogovs,i.e.TMP_yP. In order to shed more light on the effect of peripheral groups and metal ions of porphyrin core into DNA binding behavior of metallo porphrins we have chosen to investigate. Interaction of TB_ZP_YP and Mn (III).

Co (III), Ni (II) and Cu (II) Complexes with calf thymus DNA. These new benzyl pyridiniumyl porphyrins are very attractive from the point of view of DNA binding since they are cationic, show additional peripheral systems, and are more hydrophobic. These factors can modify the association process of macro cycles, altering formation equilibrium constants and spectroscopic photo physics properties of complexes. Recently, we have used several spectroscopic techniques. For the physicochemical characterization and portion binding behavior of TBZPyP and CuTBZPyP [9, 10], we found that these porphyrins are monomers at low ionic strength and have high affinity to Human serum albumin. In this work, Uv-Vis absorption spectroscopy was applied to study the interaction between calf thymus DNA and TBZPyP, and its (Cu (II), Ni (II), Co (III) and Mn (III) complexes and 1mM phosphate buffer pH 7.0 as low ionic strength medium at various temperatures. Thermodynamic analysis of binding process regarding, the stoichiometvy of binding and binding constant are probided. Further more, comparisons are made between the DNA-TBZPyP complexes metallo TBZPyP–DNA adducts and the results are reported here.

EXPERIMENTAL

Materials

Tetra (4-pyridyl) porphyrin (TP_yP) was obtained from Aldrich and used as supplied. Synthesis of porphyrins TB_ZP_yP was obtained from its precursor (TP_yP), by reaction with benzyl bromide in DMF. Passing the aqueous solution of $TB_ZP_yPBr_4$ over an anionic exchange resin (Dowex-1, Sigma, USA) made chloride salt of TB_ZP_yP. The obtained aqueous solution is lyophilized and the product dried in vacuum over P_2O_5. The metal derivatives of TB_ZP_yP were prepared, purified and converted in to its chloride from by a slightly modified published procedure [10]. All of the synthesizes complexes were characterized by Uv-Vis, HNMR and elemental analysis. DNA from calf thymus was purchased from Sigma Chemical Co. There chemicals were of reagent grade and as supplied.

Preparation of stock solutions

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.

Absorption spectra

The absorption spectra were recorded on Cary 100 scanning spectrophotometer using 1cm quartz cuvettes, with thermostat cell compartment that control the temperature around the cell with in $\pm 0.1^{\circ}c$. Spectrophotometer titrations were carried out by adding 50 μL aliquots portions of an 80 μL stock solution of the DNA directly into a quartz cell containing 10^{-6} M. The titration experiment was continued until the absorbance of the porphyrin solution in the Uv-Vis range remained constant. The spectra were recorded with in the range of 300 to 700 nm about 3 min after each addition of DNA solution. The spectra were also corrected respect to dilution effect .The measurements were performed at 1mM phosphate buffer pH 7.0 and 6 different temperatures

(20, 25,30,35,40 and 45 $^{\circ}$c).

RESULTS AND DISCUSSION

The values of max, ε λ max and concentration range of porphyrin that the absorbance obay's Beer's law are listed in Table 6. The soret band Maximum of studied porphyrins obay's Beer's laws over an extended concentration range from this observation we can conclude that these porphyrins do not show concentration dependent aggregation. The general feature of TBzPyP, MnTBzPyP, CoTBzPyP, NiTBzPyP and CuTBzPyP spectra at various, DNA concentration at $25^{\circ}c$, were shown in Figures 1, 2, 3, 4 and 5, respectively. The spectra of these figures consist of distinct isosbestic points that can be represents a simple equilibrium. The hypochromicity among with small red shift has been observed in Soret band of porphyrins due to increasing of DNA concentration. This can be representing the out side-binding mode of porphyrins to groove of double chain of DNA. 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 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. 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. Our input data for analysis of porphyrin-DNA system were absorbance at 50 different wavelength of 15 porphyrin spectra .These 15 spectra are correspond to 15 various concentrations of DNA. 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. Our input data for analysis of porphyrin-DNA system were absorbance at 50 different wavelengths of 15 pophyrin spectra. These 15 spectra correspond to 15 various concentrations of DNA. The outputs are the logarithm of equilibrium formation constant, $\log K_{ij}$, for formation of $(DNA)_i$ $(Porphyrin)_j$ is defined with respect to the equation (2)

$$i\,DNA + j\,Porphyrin \rightleftarrows (DNA)_i (Porphyrin)_j \quad (1)$$

$$K_{ij} = \frac{[(DNA)_i (prophyrin)_j]}{[DNA]^i [porphyrin]^j} \quad (2)$$

The program also calculates the values of uncertainty in logKij. 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 $10^{-3} - 10^{-4}$. These results are in good agreement with the existence of isosbestic points that corresponds to a simple equilibrium between free prophyrin and 1:1 conjugate of DNA: Prophyrin. The estimated equilibrium constants for the formation of 1:1 complexes between DNA and TBzPyP, MnTBzPyP, CoTBzPyP, NiTBzPyP and CuTBzPyP at various temperatures are listed in Tables1, 2, 3, 4, and 5, respectively.

INTERACTION OF PORPHYRINS WITH DNA

The energetic of DNA: Porphyrin equilibrium can be conveniently characterized by three familiar thermodynamic parameters: standard Gibbs free energy, ΔG° enthalpy, ΔH°, and entropy, ΔS°, changes. The ΔG° can be calculated from equilibrium constant, K, of the reaction using the familiar relationship, ΔG° = -RTlnK in which R and T referring to the gas constant and the absolute temperature, respectively. If heat capacity change of reaction is negligible, the van't Hoff equation gives a linear plot of lnK versus $\frac{1}{T}$. The ΔH° can be calculated from the slope, ΔH°/R, and the ΔS° from the intercept, ΔS°/R or from equation (4)

$$\frac{d \ln K}{d(\frac{1}{T})} = \frac{-\Delta H^\circ}{R} \quad (3)$$

$$\Delta S^\circ = \frac{(\Delta H^\circ - \Delta G^\circ)}{T} \quad (4)$$

The van't Hoff plots for binding of TBzPyP MnTBzPyP, CoTBzPyP, NiTBzPyP and CuTBzPyP to DNA in the phosphate buffer are shown in Figure 6 and their calculated thermodynamic parameters are listed in Tables 1, 2,3,4and 5, respectively.

CONCLUSIONS

All of the porphyrins studies show strong electrolyte effect and increasing of NaCl concentration induces self-aggregation of porphyrins. We also made a comparison between the properties of the above-mentioned porphyrins and that of a less-hydrophobic water-soluble porphyrin [meso Tetrakis (4-N-Methyl-pyridyl) porphyrin (TMPyP). The results showed that the above-mentioned porphyrins exist as monomer at low ionic strength medium. While TBzPyP and CuTBzPyP formed ill-defined self-aggregates at high strength medium. The hypochromicity among with small red shift has been observed in Soret band of porphyrins due to increasing of DNA.The thermodynamic parameters shows the following order for binding affinity of porphyrins at all of the studied temperatures. MnTBzPyP > CoTBzPyP > NiTBzPyP>CuTBzPyP The higher affinity of MnTBzPyP, CoTBzPyP, NiTBzPyP and CuTBzPyP can be related to formation of axial binding between metal of porphyrin and fundamental groups of DNA such as phosphate groups. This axial binding increases binding affinity. However the binding process of all porphyrins is exothermic and the order of exothermicity is the same as binding affinity. The higher affinity of MnTBzPyP can be related to its more planer structure that is related to the size of Mn ion. The hypochromicity among with small red shift has been observed in Soret band of porphyrins due to increasing of DNA concentration. This can be representing the out side-binding mode of porphyrins to groove of double chain of DNA. This kind of binding is usually occurred in AT reach of DNA chain.

ACKNOWLEDGEMENT

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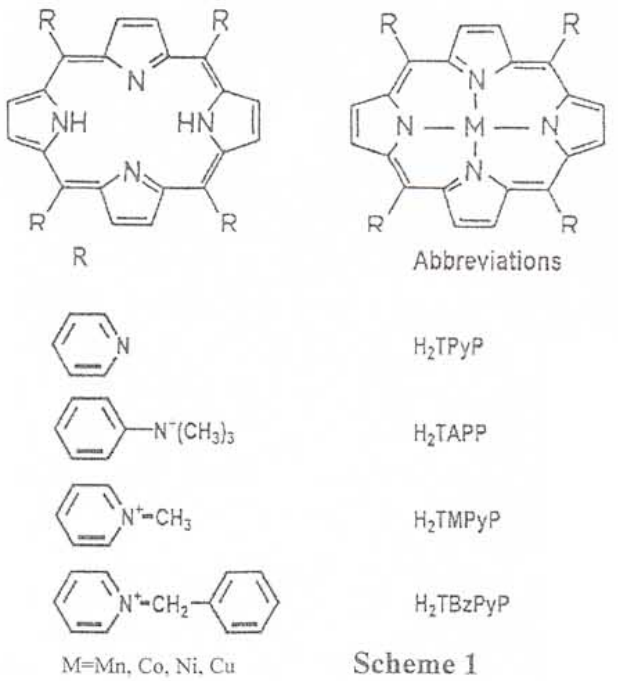

Scheme 1

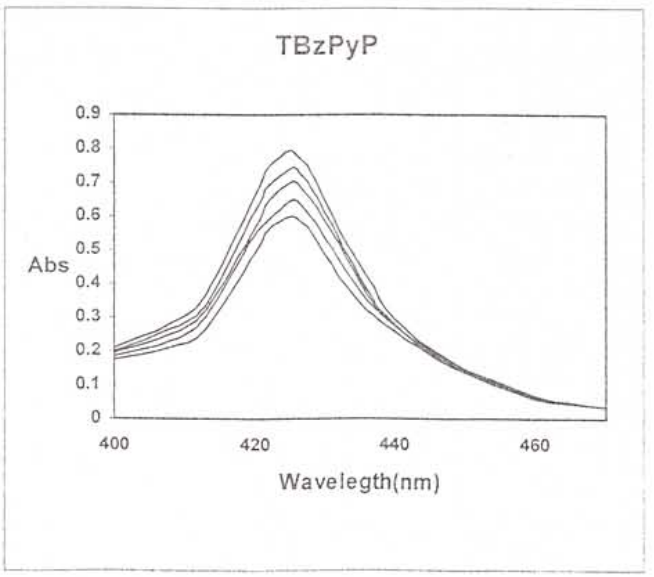

Fig.1. Corrected absorption spectra of TBzPyP upon titration with DNA in 1mM phosphate buffer. pH7.0 0 at 25 $^{\circ}C$.

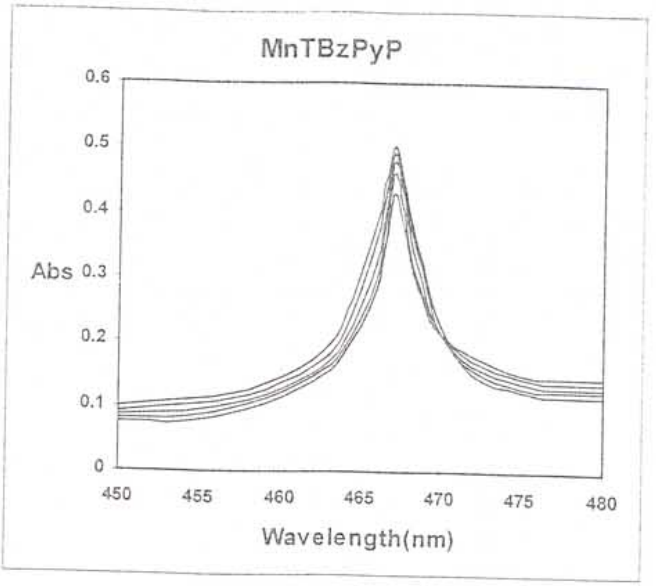

Fig.2. Corrected absorption spectra of MnTBzPyP upon titration with DNA in 1mM phosphate buffer, pH7.0 0 at 25 $^{\circ}C$.

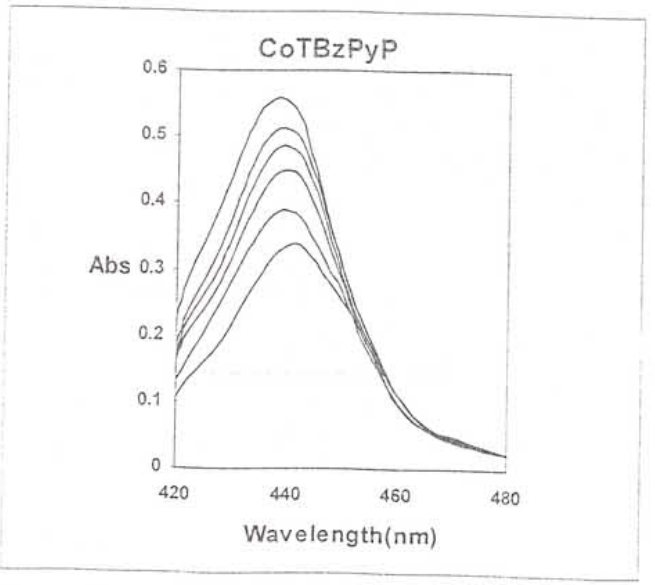

Fig.3. Corrected absorption spectra of CoTBzPyP upon titration with DNA in 1mM phosphate buffer, pH7.0 0 at 25 $^{\circ}C$.

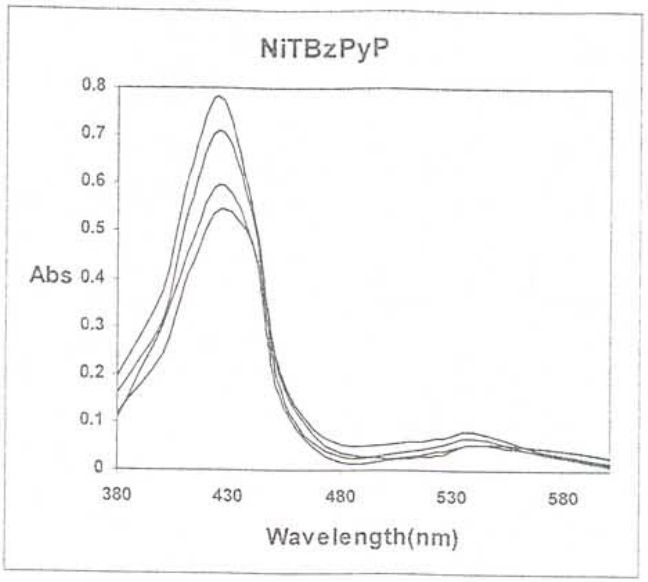

Fig.4. Corrected absorption spectra of NiTBzPyP upon titration with DNA in 1mM phosphate buffer, pH7.0 0 at 25 $^{\circ}C$.

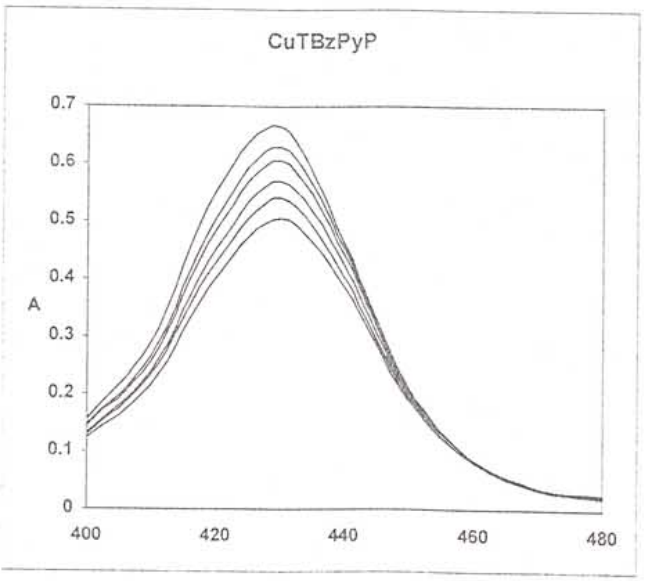

Fig.5. Corrected absorption spectra of CuTBzPyP upon titration with DNA in 1mM phosphate buffer, pH7.0 0 at 25 $^{\circ}C$.

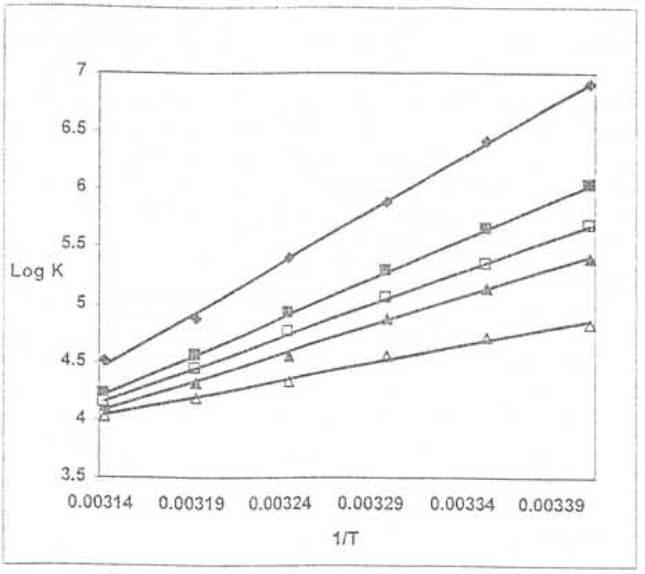

Fig.6.The Van't Hoff plots for binding of MnTBzPyP (◆), CoTBzPyP (■), NiTBzPyP (□), CuTBzPyP (▲), and TBzPyP (△) to DNA in 1mM phosphate buffer, pH7.00 at various temperatures.

Table 1. Thermodynamic parameters for binding of TBzPyP to DNA in 1mM phosphate buffer, pH7.0 at various temperatures

$t^{\circ}c$	$(K \pm \Delta K)\times 10^{-4}$	$\Delta G^{\circ} \pm \Delta\Delta G^{\circ} kJmol^{-1}$	$\Delta H^{\circ} \pm \Delta\Delta H^{\circ} kJmol^{-1}$	$\Delta S^{\circ} \pm \Delta\Delta S^{\circ} JK^{-1}mol^{-1}$
20	6.771 ± 1.045	-27.110 ± 0.107	-58.908 ± 0.124	-108.470 ± 0.293
25	5.159 ± 1.039	-26.898 ± 0.097	-58.908 ± 0.124	-107.362 ± 0.228
30	3.556 ± 1.028	-26.411 ± 0.071	-58.908 ± 0.124	-107.198 ± 0.279
35	2.142 ± 1.052	-25.325 ± 0.131	-58.908 ± 0.124	-108.983 ± 0.273
40	1.552 ± 1.054	-25.124 ± 0.135	-58.908 ± 0.124	-107.884 ± 0.266
45	1.073 ± 1.049	-24.549 ± 0.126	-58.908 ± 0.124	-107.996 ± 0.259

Table 2. Thermodynamic parameters for binding of MnTBzPyP to DNA in 1mM phosphate buffer, pH7.0 at various temperatures

$t^{\circ}c$	$(K \pm \Delta K)\times 10^{-4}$	$\Delta G^{\circ} \pm \Delta\Delta G^{\circ} kJmol^{-1}$	$\Delta H^{\circ} \pm \Delta\Delta H^{\circ} kJmol^{-1}$	$\Delta S^{\circ} \pm \Delta\Delta S^{\circ} JK^{-1}mol^{-1}$
20	815.384 ± 1.025	-38.786 ± 0.061	-174.386 ± 0.025	-462.562 ± 0.727
25	257.666 ± 1.033	-36.592 ± 0.079	-174.386 ± 0.025	-462.163 ± 0.707
30	76.071 ± 1.029	-34.131 ± 0.099	-174.386 ± 0.025	-462.659 ± 0.768
35	25.756 ± 1.039	-31.919 ± 0.073	-174.386 ± 0.025	-462.330 ± 0.669
40	7.431 ± 1.049	-29.201 ± 0.124	-174.386 ± 0.025	-463.628 ± 0.851
45	3.244 ± 1.054	-27.475 ± 0.140	-174.386 ± 0.025	-461.823 ± 0.735

Table 3. Thermodynamic parameters for binding of CoTBzPyP to DNA in 1mM phosphate buffer, pH7.0 at various temperatures

$t^{\circ}c$	$(K \pm \Delta K)\times 10^{-4}$	$\Delta G^{\circ} \pm \Delta\Delta G^{\circ} kJmol^{-1}$	$\Delta H^{\circ} \pm \Delta\Delta H^{\circ} kJmol^{-1}$	$\Delta S^{\circ} \pm \Delta\Delta S^{\circ} JK^{-1}mol^{-1}$
20	107.410 ± 1.029	-33.846 ± 0.077	-129.733 ± 0.038	-327.092 ± 0.558
25	44.775 ± 1.025	-32.254 ± 0.062	-129.733 ± 0.038	-326.946 ± 0.544
30	19.544 ± 1.038	-30.706 ± 0.093	-129.733 ± 0.038	-326.660 ± 0.529
35	8.336 ± 1.045	-29.030 ± 0.112	-129.733 ± 0.038	-326.799 ± 0.515
40	3.556 ± 1.028	-27.282 ± 0.073	-129.733 ± 0.038	-327.163 ± 0.520
45	1.663 ± 1.033	-25.708 ± 0.085	-129.733 ± 0.038	-326.969 ± 0.539

Table 4. Thermodynamic parameters for binding of NiTBzPyP to DNA in 1mM phosphate buffer, pH7.0 at various temperatures

$t^{\circ}c$	$(K \pm \Delta K)\times 10^{-4}$	$\Delta G^{\circ} \pm \Delta\Delta G^{\circ} kJmol^{-1}$	$\Delta H^{\circ} \pm \Delta\Delta H^{\circ} kJmol^{-1}$	$\Delta S^{\circ} \pm \Delta\Delta S^{\circ} JK^{-1}mol^{-1}$
20	47.863 ± 1.049	-31.882 ± 0.117	-108.509 ± 0.193	-261.392 ± 0.611
25	21.878 ± 1.043	-30.465 ± 0.104	-108.509 ± 0.193	-261.761 ± 0.634
30	11.221 ± 1.035	-29.312 ± 0.086	-108.509 ± 0.193	-2617.247 ± 0.683
35	5.754 ± 1.031	-28.087 ± 0.074	-108.509 ± 0.193	-260.983 ± 0.711
40	2.755 ± 1.026	-26.621 ± 0.066	-108.509 ± 0.193	-261.498 ± 0.725
45	1.413 ± 1.028	-25.279 ± 0.071	-108.509 ± 0.193	-261.606 ± 0.698

Table 5. Thermodynamic parameters for binding of CuTBzPyP to DNA in 1mM phosphate buffer, pH7.0 at various temperatures

$t^{\circ}c$	$(K \pm \Delta K)\times 10^{-4}$	$\Delta G^{\circ} \pm \Delta\Delta G^{\circ} kJmol^{-1}$	$\Delta H^{\circ} \pm \Delta\Delta H^{\circ} kJmol^{-1}$	$\Delta S^{\circ} \pm \Delta\Delta S^{\circ} JK^{-1}mol^{-1}$
20	25.756 ± 1.036	-30.366 ± 0.085	-94.702 ± 0.34	-219.464 ± 0.427
25	13.828 ± 1.039	-29.342 ± 0.097	-94.702 ± 0.34	-219.219 ± 0.416
30	7.604 ± 1.045	-28.327 ± 0.111	-94.702 ± 0.34	-107.951 ± 0.406
35	3.639 ± 1.029	-26.906 ± 0.074	-94.702 ± 0.34	-218.009 ± 0.394
40	2.093 ± 1.025	-25.903 ± 0.065	-94.702 ± 0.34	-220.699 ± 0.386
45	1.291 ± 1.042	-25.039 ± 0.110	-94.702 ± 0.34	-218.963 ± 0.376

Table 6.Uv-Vis spectral characteristic of TBzPyP and metal derivates in 1mM phosphate buffer, pH 7.0 and 25 $^{\circ}C$

Porphyrins	λ max(nm)	$\varepsilon(\lambda \max) M^{-1} cm^{-1}$	Concentration rang that obeys from Beer's law
TB_ZP_yP	425	2.21×10^{5}	1.31×10^{-6}-8.9×10^{5}
Cu(II) TB_ZP_yP	430	1.61×10^{5}	1.21×10^{-6}-9.8×10^{5}
Ni(II) TB_ZP_yP	436	1.57×10^{5}	1.20×10^{-6}-1.41×10^{-4}
Co(III) TB_ZP_yP	438	1.52×10^{5}	1.18×10^{-6}-1.39×10^{-4}
Mn(III) TB_ZP_yP	467	1.41×10^{5}	1.11×10^{-6}-1.12×10^{-4}

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