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THERMODYNAMICS OF INTERACTIONS OF CoTOEPyP4 PORPHYRIN WITH A-DNA

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We have employed UV light absorption and circular dichroism methods to study the binding of water-soluble Co(II)-containing meso-tetra-(4N-oxyethylpyridyl) porphyrin (CoTOEPyP4) with B- and A-DNA from Calf Thymus. The binding constant K_b we determined from binding isotherms for each DNA–porphyrin complexes and calculated binding free energy ΔG, enthalpy ΔH and entropy ΔS. Our results suggest that CoTOEPyP4 porphyrin preferably binds via outside self-stacking mode with B- and A-DNA.

Keywords: DNA conformation, Co-porphyrin, alcohol solution, absorbance spectra, circular dichroism.

Introduction. Water-soluble porphyrins and their derivatives continue to be a subject of considerable interest in biochemistry due to their binding affinity to synthetic or natural nucleic acids and the ability to selectively cleave DNA [1, 2]. The application of photodynamic therapy due to the quality of the porphyrins accumulation in tumor cells highlights the importance of understanding porphyrin–DNA interactions [3, 4]. It has been established that porphyrins may associate with DNA in three binding modes that include intercalation, groove binding, and outside binding with self-stacking along the DNA helix [5, 6]. The study of cationic porphyrin–DNA interaction in solution shows that intercalation of porphyrin into DNA requires the existence of planar conformation of porphyrin molecule (i.e., it must have a limited effective thickness). External binding is typical for porphyrins that cannot fit between nucleotides due to sterical factors, i.e. porphyrins bearing bulky peripheral substituents or axial ligands on the central ion [7]. The following assumption can be made explaining the observed differences of binding preferences of TOEPyP4 and its Co-containing derivative. It is known that some metal ions, such as Zn, Fe, Mn, extend their coordination number to five or six by cation interaction with H_2O or other molecules as axial ligands [8]. The porphyrin–DNA binding mode depends on the chemical features of porphyrin, on the nature of the central metal and the peripheral substituents of the pyridylic ring, as well as the composition and conformation of DNA. It is well known, that DNA is in A- conformation during cell division. The interest to the study of binding

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parameters of porphyrins with A-DNA and the features of their interaction is increasing. Thus, in this paper the interaction of the Co(II)-containing water-soluble meso-tetra-(4N-oxyethylpyridyl) porphyrin (CoTOEPyP4) with A-DNA are investigated and classified, comparing with previous results for TOEPyP4. The circular dichroism (CD) method can accurately determine the conformational changes of DNA helix. As it is known, the variation of ethanol concentration in the solution causes change of conformation of B- to A-DNA [9]. We obtained A-conformation of DNA at 72% concentration of alcohol [10] and we can use CD method to determine the preferred type of binding mode of CoTOEPyP4 with DNA.

Materials and Methods. In this investigation we used ultra-pure Calf Thymus DNA (protein<0.1%, RNA<0.1%, molecular mass ~30 *MDa*), isolated by prof. D.Y. Lando (Institute of Bioorganic Chemistry, Belarus). The concentration of DNA was determined spectrophotometrically using an extinction coefficient $\varepsilon_{260}=1.39\cdot10^5\ M^{-1}\cdot cm^{-1}$ for DNA. Water soluble cationic porphyrin CoTOEPyP4 was synthesized, which kindly was provided by prof. R. Ghazaryan at the Department of Pharmaceutical Chemistry at YSMU. Porphyrin concentration was determined using an extinction coefficient of CoTOEPyP4 $\varepsilon_{431} = 6.95 \cdot 10^5\ M^{-1}\cdot cm^{-1}$. The structure of this porphyrin is shown in Fig. 1. All spectroscopic measurements were performed in buffer solution (BPSE = 6 *mM* Na_2HPO_4 + 2 *mM* NaH_2PO_4 + +185 *mM* NaCl+0.1 *mM* EDTA), pH 7.0, ionic strength $[Na^+]=10^{-3}\ M$. The A- form of DNA in solution was prepared in the following way: the required quantity of alcohol was added step by step, each time with careful stirring [11]. In this way DNA can be dissolved in 70–80% alcohol without precipitation provided that Na^+ concentration does not exceed $10^{-3}\ M$.

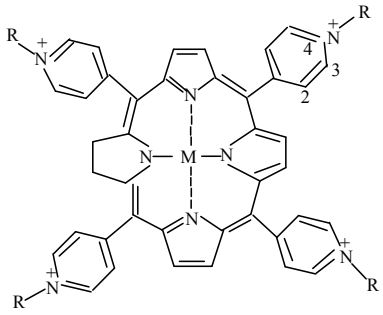


Fig. 1. Structure of porphyrin.

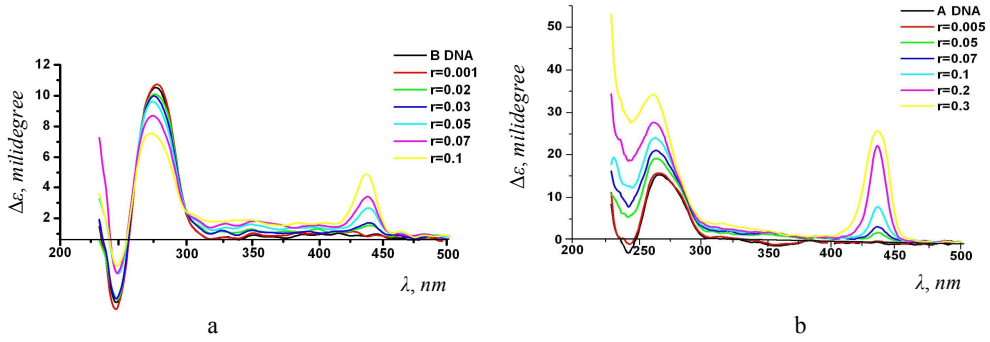
Visible Absorption Spectrophotometry. Visible absorption spectra of the complexes in the Soret region were collected at 20, 25, 30 and 35°*C* using Perkin Elmer Lambda 800 UV/VIS spectrophotometer and an optical cuvette with 1 *cm* path length. All absorption titration experiments were carried out by stepwise addition of a stock solution of DNA to a cuvette containing 2 *mL* of porphyrin solution with a concentration of $\sim10^{-6}$ *mol*/*mL*. The initial DNA concentration was $3\cdot10^{-4}$ *mol* base pairs/*mL*. The porphyrin concentrations were corrected at each point of titration for dilution effects resulting from the change in volume upon each DNA addition. The binding parameters of porphyrins with DNA can be obtained from the absorption spectra using the Correia and Chaires equation [12]:

$$\frac{r}{C_f} = K_b\left(1-nr\right)\left[\frac{1-nr}{1-\left(n-1\right)r}\right]^{n-1}, \qquad (1)$$

where K_b is the binding constant of porphyrin to B- and A-DNA and n is the stoichiometry of complexes. The quantity r in Eq. (1) represents the binding ratio and is given by $r = (C_t–C_f) / (C_{DNA})$, where C_t and C_f represent the total and free concentrations of porphyrin, *mol* /*mL*; C_{DNA} is total concentration of DNA, *mol* base pairs/*mL*.

Circular Dicroism. The characteristics of porphyrin complexation with DNA were investigated using a special technique of CD, by means of which not only conformational changes in DNA molecule, but also the preferred type of binding of porphyrin with DNA and polynucleotides [13, 14] can be exactly determined. CD spectra of DNA duplexes with and without porphyrins were recorded at 25°*C* using an Olis DSM spectrophotometer in quartz cuvettes (Perkin Elmer). CD titration was measured by adding aliquots of the porphyrin solution to the known amount of DNA solution. The initial DNA concentration was $4.5 \cdot 10^{-5}$ *mol* base pairs/*mL*. Results were averaged taking into account their standard errors.

Results and Discussion. *Modes of Binding*. We use CD spectra to identify the binding mode for DNA duplexes in the presence of porphyrins. The aliquots of porphyrin were added on fixed concentration of DNA solution and the CD spectra from 220 *nm* to 500 *nm* were recorded at 25°*C*. Pure porphyrins do not display CD spectra in the UV and visible range. Adding of porphyrin to DNA leads to the appearance of positive ICD spectrum in Sore region (400–500 *nm*). The CD spectra of porphyrin–DNA complexes are displayed in Fig. 2.



a b

Fig. 2. CD spectra of complexes of CoTOEPyP4 with B- (a) and A-DNA (b).

The induced CD spectra of porphyrins with nucleic acids provides more information about the binding mode: a positive induced CD band is indicative of external groove binding, and a negative induced CD band is present upon intercalation [15]. As it can be seen from Fig. 2, CoTOEPyP4 porphyrin interacts with B- and A-DNA only through external groove binding, while TOEPyP4 interact with B-DNA by intercalative mode at small porphyrin–DNA ratio, which consequently changes into outside stacking mode at higher rations *r* [10]. It reaches saturation adding of porphyrin at $r = 0.3$.

Binding Thermodynamics of Porphyrin–DNA Interaction. The interaction of studied porphyrins with B- and A-DNA was monitored with changes in Soret region of absorbance spectra. Fig. 3 shows the absorption spectra of CoTOEPyP4 porphyrin with B- (a) and A-DNA (b) at 25°*C*.

The addition of aliquots of DNA stock solution to the fixed concentration of porphyrin leads to the red shift and small hypochromicity of the Soret maximum. The values for the hypochromicity and the bathochromic shift appeared to be smaller for CoTOEPyP4 ($\Delta\lambda \sim +2$ *nm*, $\Delta h \sim 19\%$), then the ones obtained for TOEPyP4 ($\Delta\lambda \sim +10$ *nm*, $\Delta h \sim 40\%$) at addition of B- and A-DNA. The titration

experiments were continued until no further change in the Soret band was observed, which indicated that saturation was reached that is all porphyrins were already bound. Another important feature, shown in Fig. 3, is the presence of isobestic points $0.4 < r < 0.7$ for CoTOEPyP4–DNA complexes, which suggests the existence of one binding mode. From spectroscopic data we can determinate the binding constant K_b and stoichiometry n for each porphyrin–DNA complexes using Eq. (1). The binding parameters for each porphyrin–DNA complexes obtained at 25°*C* and summarized in Table.

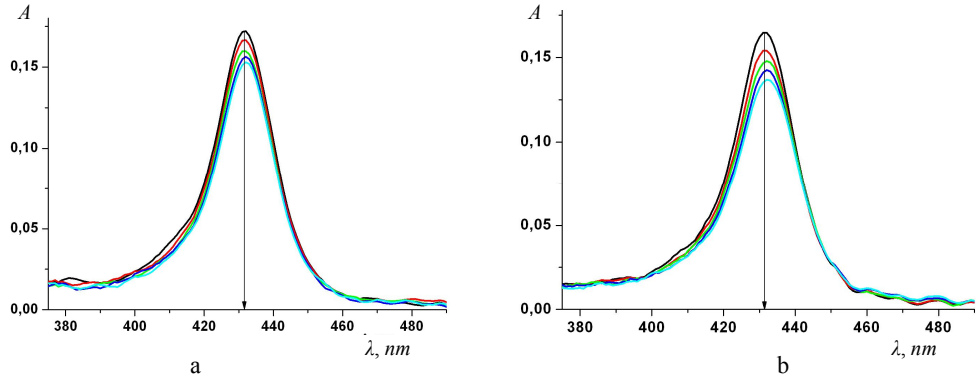


Fig. 3. Visible absorbtion spectra of CoTOEPyP4 with B- (a) and A-DNA (b) at 25°*C*.

Thermodynamics of interaction of porphyrins with DNA duplexes was investigated in terms of difference in K_b values determined at various temperatures. This approach provide to determine indirectly the thermodynamic parameters by the van't Hoff plot over a certain temperature range. The binding enthalpy we obtained by the van't Hoff equation, using the temperature dependences of K_b. We have registered the absorption spectra of complexes at 20, 25, 30 and 35°*C* temperatures. Fig. 4 plots the logarithms of the binding constants ($\ln K_b$), against reciprocal temperature ($1/T$) for the association of porphyrins with B- and A-DNA respectively.

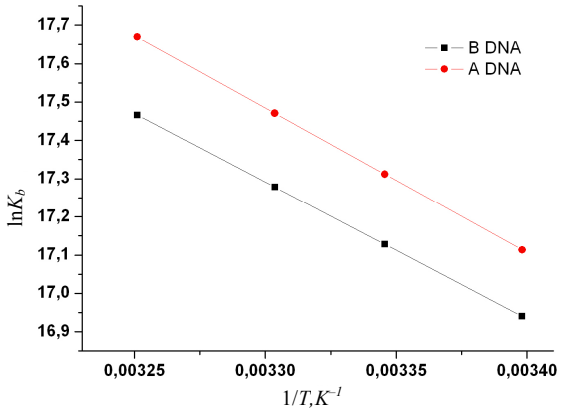


Fig. 4. Temperature dependences of the binding constant for the complexes of CoTOEPyP4 with B- and A-DNA.

These experimental dependences were approximated by linear functions; the slopes of these functions yield the binding enthalpies:

$$\Delta H_b = -R[\partial \ln K_b / \partial(1/T)]\big|_{P=\text{const}}.$$

The binding free energies of complexation (ΔG) and entropies (ΔS) were calculated, using the following equations $\Delta G = -RT \ln K_b$ and $\Delta S = (\Delta H - \Delta G)/T$.

Table presents our evaluated thermodynamic parameters for the association of CoTOEPyP4 with B- and A-DNA. For comparison the thermodynamic parameters for TOEPyP4 are given in the Table.

Thermodynamic parameters of CoTOEPyP4 and TOEPyP4 porphyrins with B- and A-DNA

Porphyrin	DNA	K_b, 10^6 M^{-1}	n	ΔG, *kcal/mol*	ΔH, *kcal/mol*	ΔS, *cal/mol·K*
CoTOEPyP4	B	3.8	1.00	–10.34	7.06	57.70
	A	2.6	1.04	–10.12	7.25	58.70
TOEPyP4	B	8.5	2.20	–9.45	5.53	50.30
	A	18.0	1.70	–9.89	–18.13	–27.65

Data in Table reveal that determined porphyrin–DNA binding constants range between ~10^6 to ~10^7 M^{-1} is a range typical for porphyrin–nucleic acid interactions [16]. As it is seen from Table, the value of binding constant K_b for CoTOEPyP4 is ten times more than for TOEPyP4. The conformational changes B- to A-form of DNA have a slight effect on binding parameters (K_b and n) for CoTOEPyP4, while for TOEPyP4 K_b is twice more for A- than B-DNA.

The analysis of Table shows that the binding free energies of complexation of both porphyrins are almost similar for A- and B-DNA. The binding of CoTOEPyP4 are accompanied by unfavorable changes in enthalpy values are positive and favorable changes in entropy with both B- and A-DNA. On basis of these results it can be concluded that the nature of interaction forces is predominately hydrophobic. This result complies with conclusion made on the basis of CD-spectra of complexes.

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