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Physics

INTERACTION OF TOEPyP4 PORPHYRIN WITH A FORM OF DNA

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The peculiarities of binding the water-soluble meso-tetra-(4N-oxyethylpyridyl) porphyrin (TOEPyP4) with B-DNA and A-DNA have been studied by means of UV-VIS spectrophotometry and circular dichroism method. The binding constant (K_b) and stoichiometry (n) were determined based on the absorbance spectra for each DNA–porphyrin complex. The free energy, enthalpy and entropy of binding also were calculated using the values of K_b. A comparative analysis with TOEPyP4–B-DNA complex was performed. The obtained results show that the porphyrin interacts with B-DNA by way of intercalation at low relative concentrations ($r = C_{porf}/C_{DNA}$) and external binding at large values of r, whereas the interaction of TOEPyP4 with A-DNA occurs via the outside binding mode only.

Keywords: DNA conformation, porphyrin, thermodynamics, absorbance spectra, circular dichroism.

Introduction. Porphyrins are well studied compounds, and their chemical and photochemical properties have been widely used in medicine and biology. In particular, porphyrins are known as antiviral and anticancer therapeutic agents. The application of porphyrins in photodynamic therapy due to the quality of accumulation in tumor cells highlights the importance of comprehension of porphyrin–DNA interactions [1, 2]. For many years considerable research efforts have been made in many centers all over the world, including our department, for investigation of the interaction of porphyrins with DNA and oligo-nucleotides [3]. However, there are no data published on the mechanisms of interactions of porphyrins with A-DNA. A-DNA is fairly similar to the B-DNA form, but is characterized by a shorter and more compact helical structure. The increase in the number of base pairs per rotation and smaller rise/turn of A-DNA compared to those for B-DNA results in a deepening of the major groove. The circular dichroism (CD) spectra of poly[d(G)]poly[d(C)] is similar to that of the true A form DNA and is characterized by a dominant positive band and a negative band [4]. It is well known that during the cell division DNA is in A conformation. The investigators expressed an increasing interest in the study of binding parameters of porphyrins with A-DNA and the features of their interaction. It was established that porphyrins interact with DNA by means of three mechanisms: the intercalation, the disordered external and ordered external binding. The porphyrin–DNA binding

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mode depends on the nature of the central metal of the porphyrin and the peripheral substituents of the pyridylic ring, as well as the composition and conformation of DNA. In the present paper the binding of water soluble meso-tetra-(4N-oxyethylpyridyl) porphyrin (TOEPyP4) with A-DNA has been investigated.

Materials and Methods. The ultra-pure Calf Thymus DNA (protein<0.1%, RNA<0.1%, molecular mass ~ 30 *MDa*) used in this investigation has been isolated by prof. D.Y. Lando (Institute of Bioorganic Chemistry, Minsk, Belarus). The GC content of DNA proved to be 42%. The concentration of DNA was determined spectrophotometrically using an extinction coefficient: ε_{260} = 13860 $M^{-1}\cdot cm^{-1}$. The water soluble cationic porphyrin TOEPyP4 was synthesized and kindly provided by prof. R. Ghazaryan at the Department of Pharmaceutical Chemistry at Yerevan State Medical University. The porphyrin concentration was determined using an extinction coefficient ε_{424}=2.26·10^5 $M^{-1}\cdot cm^{-1}$. The structure of this porphyrin is shown in Fig. 1. All studies were performed in diluted solution of BPSE buffer (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 carefully stirring of the solution. In this way DNA may be dissolved in 80% alcohol without precipitation provided that the concentration of Na^+ does not exceed 10^{-3}*M*.

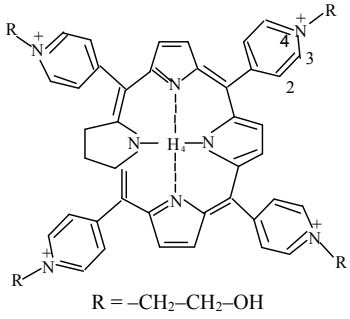


Fig. 1. Structure of porphyrin.

Circular Dicroism. The characteristics of porphyrin complexation with DNA were investigated using a special technique of CD, by means of which not only the conformational changes in the DNA molecule, but also the preferred type of porphyrin binding with DNA and polynucleotides [5, 6] will be exactly determined. CD spectra of DNA duplexes with and without porphyrins were recorded by means of the Olis DSM spectrophotometer in quartz cuvettes (Perkin Elmer) at 25°*C*. CD titration was measured by adding multiple aliquots of porphyrin solution to a known amount of DNA solution, where the initial concentration of DNA is 50·10^{-3} *mg/mL*.

Visible Absorbtion Spectrophotometry. Absorbtion spectra were recorded in quartz cuvettes (Perkin Elmer) at 1 *cm* thickness using Perkin Elmer Lambda 800 UV/VIS spectrophotometer. Data were collected at 20, 25, 30, and 35°*C*. All absorbtion titrations were carried out by gradual addition of aliquots of a stock solution of DNA to a cuvette containing 2 *mL* of porphyrin solution to fixed DNA by the absorbance at 422 *nm* wavelength, which corresponds to λ_{max} of Soret region for the free porphyrin. The binding parameters of porphyrins with DNA can be obtained from the absorbtion spectra using the Correia and Chaires equation [7]:

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

where K_b is the binding constant of the porphyrin to B- and A-DNA and n is the stoichiometry of complexes.

Results and Discussion. The conformational changes of the DNA molecule may be accurately determined by means of CD method. It is known that the variation of ethanol concentration causes a change in the concentration of B- and A-DNA in a solution [8]. Taking to account the CD spectra of DNA, was supposed that in the transition range there are only two types of DNA structure: B and A.

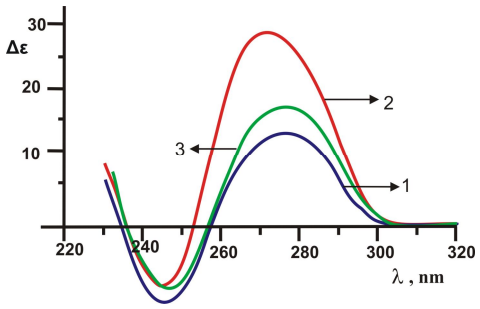
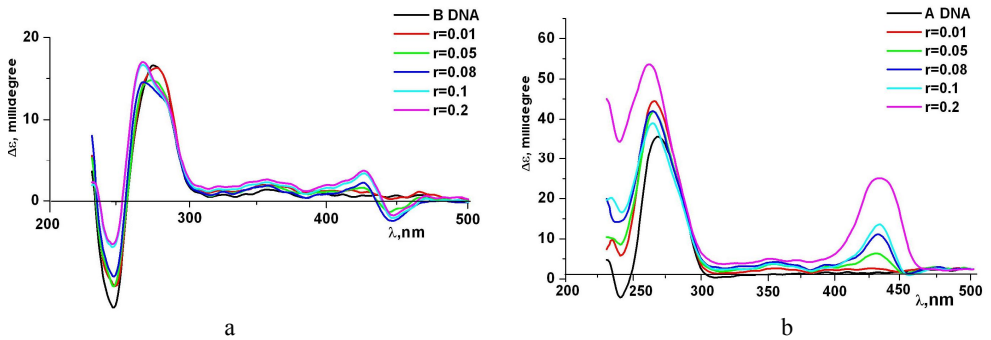


Fig. 2. CD spectra of DNA in water–alcohol solution: 1) B-DNA; 2) A-DNA in 72% alcohol; 3) A-DNA in 73% alcohol.

The transition of B to A form in the solution occurs within a narrow range of 70–80% alcohol concentration. This is a temperature independent reversible transition, and its dependence on GC content in B-DNA is insignificant. In Fig. 2 the CD spectra of DNA are shown at various concentrations of alcohol. CD spectra of the B form at 65% alcohol slightly differ from the CD spectrum of the alcohol free B form. The change in CD spectrum of the B form within the range 0–65% of alcohol is seen in Fig. 2 (lower curve) to be very small, and an increase of alcohol concentration is seen to cause a significant change in the CD spectrum of DNA (Fig. 2, upper curve). In case of further increase of alcohol concentration above 72% the amplitude of 260 *nm* decreases that may be associated with a transition of DNA to a compact form [9].

It is known that the CD spectrum of form A has an intersection point in the range of 240–250 *nm* and the amplitude of positive band is twice as large as the amplitude of B-DNA [10]. Based on literature data, we can infer that at 72% concentration of alcohol we have A conformational DNA (Fig. 2). The CD method may be used not only for determination of conformational changes in the DNA molecule, but also of the preferred type to binding porphyrin with DNA. The CD spectra (Fig. 3) were used for identification of the binding mode for DNA duplexes in the presence of porphyrins.



a b

Fig. 3. ICD spectra of complexes B-DNA (a) and A-DNA (b) with TOEPyP4.

In Fig. 3 the CD spectra for B- and A-DNA are shown for different relative concentrations (r), where $r = C_{\text{porph.}}/C_{\text{DNA}}$. It is known that intercalating molecules are well placed in minor grooves of the DNA double helix and have a negative CD

spectrum with relatively large ellipticity [11]. The CD spectra of B-DNA with TOEPyP4 porphyrin are shown at 25°*C* (Fig. 3, a).

A strongly conservative CD spectrum in the Soret region of the DNA–TOEPyP4 complex is due to the existence of two types of complexes: intercalation and outside binding. An addition of porphyrin concentration in A-DNA is seen in Fig. 3, b to lead to an appearance of positive induced CD (ICD) spectrum. In Fig. 3 is shown that TOEPyP4 porphyrin tended to intercalate into the B-DNA, rather than A-DNA, but in case of high values of relative concentration r the external binding occurs. As is seen from Fig. 3, b, TOEPyP4 interacts with A-DNA only via the external groove binding that is conditioned by A-DNA conformation.

Thermodynamics of Porphyrin–DNA Interaction. To evaluate a complexation energy of complexes the absorbtion spectra in the Soret region have been registered for porphyrin in the presence of DNA at 20, 25, 30 and 35°*C* temperatures. Fig. 4 shows the absorbtion spectra of TOEPyP4 porphyrin with B-DNA (a) and A-DNA (b) at 25°*C*.

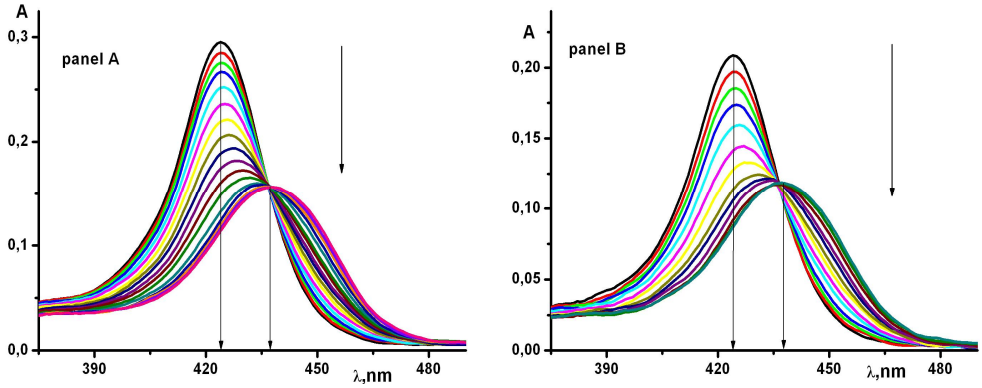


Fig. 4. Visible absorbtion spectra of porphyrin upon addition of B-DNA (a) and A-DNA (b) at 25°*C*. The direction of increasing DNA concentration is shown by the arrow.

As is shown in Fig. 4, each ligand–DNA binding is characterized by large hypochromicity (40–50%) and significant red shift (8–10 *nm*) of the Soret maximum. Another important feature demonstrated in Fig. 4 is the presence of isosbestic points $0.2< r <0.4$ for B-DNA (a) and $0.2< r <0.5$ for A-DNA (b), where $r = C_{\text{porph.}} / C_{\text{DNA}}$, that suggests the existence of one binding mode. The modes of porphyrin binding to nucleic acids can be explained based on characteristic changes in visible absorbance and induced CD spectra in the Soret region. The analyses of Fig. 3 and Fig. 4 allow us to conclude that TOEPyP4 interacts with B-DNA in the intercalative mode at small porphyrin–DNA ratio, that consequently changes into the outside stacking mode at a higher r ratio, that is confirmed by an exhibition of a bisignal ICD spectrum in Soret region (Fig. 3, a). Whereas at the interaction of the same porphyrin with A-DNA only a positive ICD band is exhibited that is an evidence in favor of the fact that the outside groove binding mode occurs.

The spectroscopic data were used to determine the binding constant K_b and stoichiometry n for each studied porphyrin-DNA complexes. For determination of K_b and n the equation proposed by Correia and Chaires (1) was used, the binding

constants were determined at four different temperatures: 20, 25, 30, 35°*C*. The binding enthalpy was obtained by the means of van't Hoff equation, using the temperature dependences of K_b. The binding free energies of complexation (ΔG_b) and entropies (ΔS_b) were calculated, using the following equations:

$$\Delta G_b = -RT \ln K_b \quad \text{and} \quad \Delta S_b = \left(\Delta H_b - \Delta G_b\right)/T.$$

The dependences of binding constants ($\ln K_b$) versus reciprocal temperature ($1/T$) for the association of the TOEPyP4 with B- and A-DNA are shown on Fig. 5.

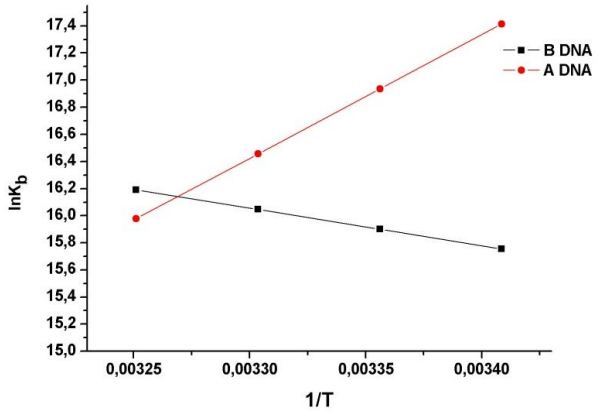


Fig. 5. Temperature dependences of the binding constant for TOEPyP4 complexes with B-DNA (a) and A-DNA (b).

The linear van't Hoff plots indicate that no change in heat capacity occurs over 20–35°*C* temperature range. 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 entropies then were calculated by equation $\Delta S_b = (\Delta H_b - \Delta G_b)/T$. The thermodynamic parameters for association of porphyrins with B-DNA and A-DNA at 25°*C* are given in Table.

Thermodynamic parameters complexes of TOEPyP4–B-DNA and TOEPyP4–A-DNA

Porphyrin	DNA	K_b, $10^6\ M^{-1}$	n	ΔG_b, *kcal/mol*	ΔH_b, *kcal/mol*	ΔS_b, *cal/mol·K*
TOEPyP4	B	8.5	2.2	–9.45	5.53	50.3
	A	18	1.7	–9.89	–18.13	–27.65

The free binding energies (ΔG_b), enthalpies (ΔH_b) and entropies (ΔS_b) have been evaluated from data on temperature dependence of binding constants. An analysis of data in the Table shows that our determined porphyrin–DNA binding constants vary between $6.7 \cdot 10^6$ and $4.6 \cdot 10^7 M^{-1}$. This range is typical for porphyrin–nucleic acid interactions [12]. A favorable change in enthalpy upon porphyrin intercalation is consistent with a picture, in which the bound porphyrin forms strong intermolecular interactions with DNA. The analysis of the Table shows that the binding of TOEPyP4 porphyrin to A-DNA is accompanied by a favorable enthalpy change and an unfavorable entropy change. The strong electrostatic interactions between the positively charged pyridinium groups of the porphyrin and DNA phosphates result in a negative change in enthalpy –18.13 *kcal/mol* and

negative change in entropy of –27.65*cal/mol·K*. The nature of TOEPyP4–A-DNA interaction essentially enthalpical. On the other hand, when TOEPyP4 porphyrin interacts with B-DNA we have an unfavorable enthalpy change and a favorable entropy change as is seen in Table. The unfavorable changes in enthalpy, ΔH_b = 5.53 *kcal/mol*, that accompany external groove porphyrin binding may, in part, reflect dehydration of some polar and charged groups of DNA. Interaction of TOEPyP4 with the B-DNA is accompanied with highly positive change in entropy of 50.3 *cal/mol·K*. The nature of TOEPyP4–B-DNA interaction is entropical. One can conclude based on these results that the nature of interaction forces is predominately hydrophobic.

As a whole, our results suggest that the thermodynamic profile of porphyrin–DNA binding correlates with the binding mode. This correlation reflects the differential nature of the molecular forces that stabilize/destabilize the two modes of binding intercalation versus external self-stacking along DNA. In general, thermodynamic results presented here with thermodynamic data of porphyrin–A-DNA may be useful in developing a rational design of porphyrin-based ligands with predictable affinity and specificity.

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REFERENCES

1. **Vicente M.G.** Porphyrin-Based Sensitizers in the Detection and Treatment of Cancer: Recent Progress. // Curr. Med. Chem. Anti-Cancer Agents, 2001, v. 1, p. 175–194.
2. **Marzilli L.G.** Medical Aspects of DNA-Porphyrin Interactions. // New J. Chem., 1990, v. 14, p. 409–420.
3. **Pasternack R.F., Gibbs E.J.** In: Metal Ions in Biological Systems (Eds. Sigel A., Sigel H.). New York: Marcel Dekker, 1996, p. 367–397.
4. **Chang Y.-M., Cammy K.-M., Hou Ch., Hou M.-H.** Conformational Changes in DNA Upon Ligands Binding Monitored by Circular Dichroism. // Int. J. Mol. Sci., 2012, v. 13, p. 3394–3413.
5. **Gibbs E.J., Pasternack R.F.** Interaction of Porphyrins and Metalloporphyrins with Nucleic Acids. // Semin Hematol, 1989, v. 26, № 2, p. 77–85.
6. **Pasternack R.F.** Circular Dichroism and the Interactions of Water Soluble Porphyrins with DNA. // Chirality, 2003, v. 15, p. 329–332.
7. **Correia J.J., Chaires J.B.** Analysis of drug-DNA Binding Isotherms: a Monte Carlo Approach. // Methods in Enzymology, 1994, v. 240, p. 593–614.
8. **Ivanov V.I., Minchenkova L.E., Minyat E.E., Frank-Kamenetski M.D., Schyolkina A.K.** The B to Ā Transition of DNA in Solution. // J. Mol. Biol., 1974, v. 87, p. 817–833.
9. **Geiduschek E.P., Gray I.** // J. Amer. Chem. Soc., 1962, v. 84, p. 3843–3852.
10. **Usatyi A.F., Shlyakhtenko L.S.** Melting of DNA in Ethanol–Water Solutions. // Biopolymers, 1974, v. 13, p. 2435–2446.
11. **Uno T., Hamasaki K., Tanigawa M., Shimabayashi S.** Binding of Meso-tetrakis(N-methylpyridinium-4-yl) porphyrin to Double Helical RNA and DNA. RNA Hybrids. // Inorg. Chem., 1997, v. 36, p. 1676–1683.
12. **Pasternack R.F., Gibbs E.J., Villafranca J.J.** Interactions of Porphyrins with Nucleic Acids. // Biochem., 1983, v. 22, p. 2406–2414.