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Physics

THE INTERACTION OF Ag-CONTAINING PORPHYRINS WITH DNA AT HIGH IONIC STRENGTH

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UV-VIS spectrophotometry and circular dichroism (CD) methods were applied to study the binding of DNA with three novel Ag-containing porphyrins meso-tetra(4N-allylpyridyl) (AgTAlPyP4), meso-tetra(4N-butylpyridyl) (AgTButPyP4) and meso-tetra(4N-oxyethylpyridyl) (AgTOEtPyP4) at high ionic strength. The binding constant K_b and stoichiometry n were determined from binding isotherms for each ligand–DNA complex. The Gibbs free energy, enthalpy and entropy of binding also were calculated using the values of K_b. Obtained results show that these porphyrins associate with the duplex DNA via outside binding mode.

***Keywords*:** DNA, porphyrin, ionic strength.

Introduction. The potential application of porphyrins to biology and medicine (e.g. photodynamic therapy, cancer detection and virus inhibition) highlights the importance of gaining an understanding of the porphyrin–DNA interaction [1, 2]. It is established that porphyrins association with DNA via various binding modes: including intercalation or outside groove binding (random binding or stacking) [2, 3]. The DNA-binding mode depends on the nature of central metal of porphyrins and peripheral substituents of pyridylic ring [2–4]. The insertion of metal into porphyrin core can dramatically alter the binding mode of porphyrins with DNA [5, 6].

The binding mode of porphyrins also depends on ionic strength of the medium, because it determines DNA double helix conformation. Earlier the interaction of three Ag-containing porphyrins – meso-tetra(4N-oxyethylpyridyl) (AgTOEtPyP4), meso-tetra(4N-allylpyridyl) (AgTAlPyP4) and meso-tetra(4N-butylpyridyl) (AgTButPyP4) porphyrins, with DNA and RNA duplexes at lower ionic strenght (μ=0,02) was studied by us [7, 8]. In the present work the interaction of these porphyrins with DNA duplex was studied at higher ionic strenght (μ=0,2), at which the double helix exists at more twisted conformation compared to B-form.

Materials and Methods. Ultra-pure DNA from Calf Thymus served for these experiments (protein<0,1%, RNA<0,2%, M.w.>30 *MDa*).

The water soluble cationic porphyrins AgTAlPyP4, AgTButPyP4 and AgTOEtPyP4 were synthesized as described in [9]. The structure of these por-

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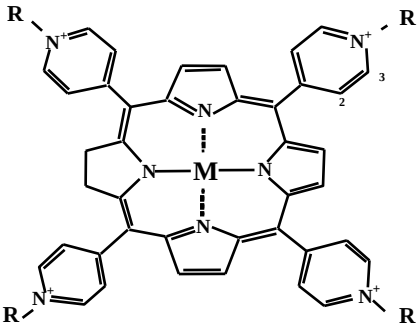
phyrins is shown in Fig. 1. Porphyrins concentrations were determined spectrophotometrically using the following extinction coefficients: $\varepsilon_{433}=3{,}69\times10^5\ M^{-1}\ cm^{-1}$ for AgTOEtPyP4, $\varepsilon_{433}=0{,}81\times10^5\ M^{-1}\ cm^{-1}$ for AgTButPyP4, $\varepsilon_{434}=1{,}73\times10^5\ M^{-1}\ cm^{-1}$ for AgTAlPyP4.

The absorbance spectra for all experiments were recorded on UV/Vis Lambda 800 spectrophotometer ("Perkin Elmer"). The Circular dichroism (CD) spectra were recorded on Roussel Jouan-II dichrograph. Initial concentration of porphyrins in solution was $\sim10^{-6}$ *M*. During titration experiments the ratio *r* (*r* = =[porphyrin]/ [nucleotide base pair]) ranged from $0{,}04< r < 0{,}8$.

All solutions were buffered in 1 BPSE, pH=7,3, μ=0,2 (1 BPSE=6 *mM* Na_2HPO_4+2 *mM* NaH_2PO_4+185 *mM* NaCl+1 *mM* EDTA).



M=Ag, R=CH_2–CH_2–OH (AgTOEtPyP4), R=CH_2–CH=CH_2 (AgTAlPyP4), R=CH_2–CH_2–CH_2–CH_3 (AgTButPyP4).

Fig. 1. Structure of porphyrins.

Results and Discussion.

Spectral measurements. The absorbance spectra at 25^0C reveal that the consecutive addition of DNA aliquots stock solution to the constant concentration of porphyrins leads to the significant red shift (~5–8 *nm*) and large hypochromicity (46–50%) of the Soret absorption band of porphyrins (Fig. 2). Similar effects are observed at lower ionic strength [7].

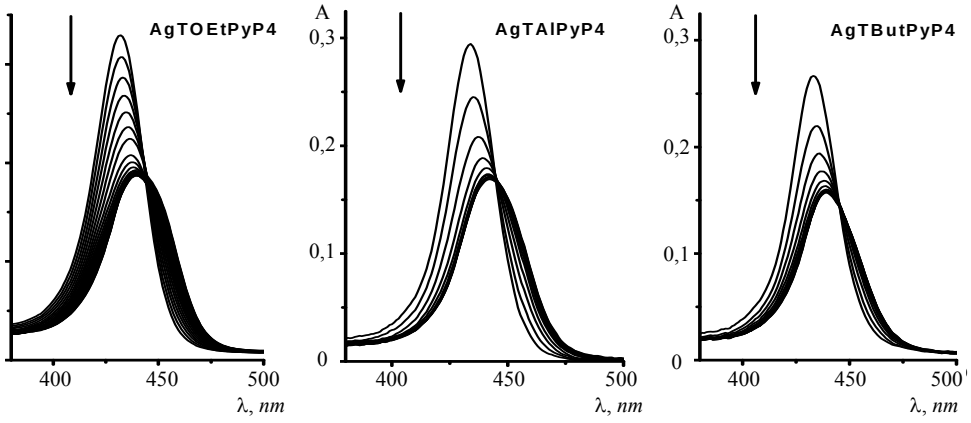


Fig. 2. Changes of Soret absorption band of porphyrins upon addition of DNA at 25^0C. The direction of increasing DNA concentration is represented by the arrow.

The large hypochromicity suggests that π-electrons of porphyrin are perturbed considerably by association with DNA. The magnitude of spectral changes depends on the type of porphyrin and its interactions with duplex DNA. Spectral changes show a single isosbestic point, typical of a simple equilibrium between free and bound porphyrin. Assumingly, the binding of all three porphyrins to DNA proceed in a single step.

CD-spectroscopy. The CD-spectra of porphyrin–DNA complexes are characterized by two regions: UV and visible (region of Soret peak). The changes of CD-spectra in visible region (CD induced by porphyrins, ICD) are more informative as they are a consequence of ordered complex formation by porphyrins on DNA matrix. It is shown that the sign and magnitude of ICD-spectra can serve as a signature for the mode of binding with DNA [4, 6]. Thus, the intercalative binding mode is characterized by negative ICD and the outside binding mode – by positive or zero ICD-spectra. The stacking mode that occurs at the DNA major groove can be classified as "extensive stacking" or "moderate stacking". In the latter form, which is favored by a low salt concentration, porphyrins stack moderately and exhibit a bisignat ICD-spectrum. The "extensive porphyrin stacking", which favors high salt concentration, is characterized by bisignal ICD-spectrum and is at least an order of magnitude larger than that of the "moderate stacking" [4, 6].

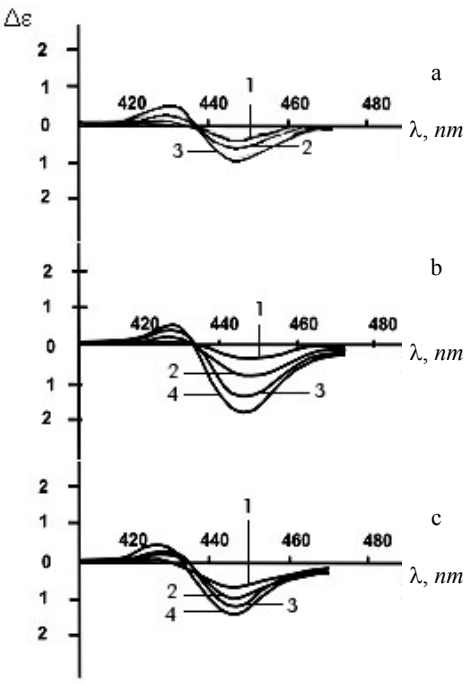


Fig. 3. ICD-spectra of complexes of DNA with: a) AgTButPyP4, r = 0,26 (cur. 1); 0,57 (cur. 2); 0,91 (cur. 3); b) AgTAlPyP4, r = 0,08 (cur. 1); 0,25 (cur. 2); 0,57 (cur. 3); 0,75 (cur. 4); c) AgTOEtPyP4, r = 0,1 (cur. 1); 0,4 (cur. 2); 0,6 (cur. 3); 0,95 (cur. 4) at 25^0C in 1 BPSE.

The ICD-spectra of Ag-containing porphyrins–DNA complexes are shown in Fig. 3. The aliquots of porphyrin were added to a fixed concentration of DNA solution and the CD-spectra from 380 *nm* to 480 *nm* were recorded at 25^0C. Upon addition of porphyrin the ICD-spectra magnitude increased consequently reaching its saturation.

The analysis of Fig. 3 allows to conclude that all three porphyrins interact with DNA duplex by intercalative mode at small porphyrin–DNA ratio, which consequently changes into outside stacking mode at higher ratios r. These results are somehow controversial to the single step binding mode concluded from absorbance data. To determine the actual binding mode of studied porphyrins with DNA thermodynamic profiles for each binding event were obtained.

Thermodynamics of the porphyrin–DNA interaction. The spectroscopic data were used to determine the binding constant K_b and stoichiometry n for each porphyrin–DNA binding event under study. The equation proposed by Correia et al. [10] was used to determine K_b and n. The calculated data are presented in Table.

The data reveal that porphyrin–DNA binding constants vary between 10^5 and $4{,}0\times 10^5\ M^{-1}$, a range is typical for porphyrin–nucleic acid interactions [8].

Thermodynamic parameters of porphyrins interactions with DNA duplex in 1 BPSE buffer, pH 7,3. All values are reported at 25^0C.

Porphyrins	$K_b\times 10^5$	n	ΔG, $kJ\ mol^{-1}$	ΔH, $kJ\ mol^{-1}$	ΔS, $J\ mol^{-1}K^{-1}$
AgTButPyP4	4,0±0,4	1,1±0,01	–9,0±0,1	9,0±1,9	60,2±6,4
AgTAlPyP4	2,5±0,8	2,3±0,1	–8,7±0,2	5,1±1,3	46,2±3,9
AgTOEtPyP4	1,1±0,1	5,3±0,1	–8,2±0,03	3,5±0,8	39,4±2,5

The free energy of complexation was calculated at 25^0C, using the following equation: ΔG_b=–RTlnK_b. Binding enthalpy ΔH_b and entropy ΔS_b were also calculated for each binding event. The results are summarized in Table. These results were obtained from Van't Hoff equation, using the temperature dependences of K_b. The binding constants were determined at four different temperatures: 18, 25, 35 and 45^0C. The linear Van't Hoff plots (Fig. 4) indicates that no change in heat capacity occurs over the temperature range 18–45^0C. Thus, ΔH_b was calculated as the slope of the line fitted to experimental points. The binding entropy then was calculated from equation $\Delta S_b=(\Delta H_b-\Delta G_b)/T$.

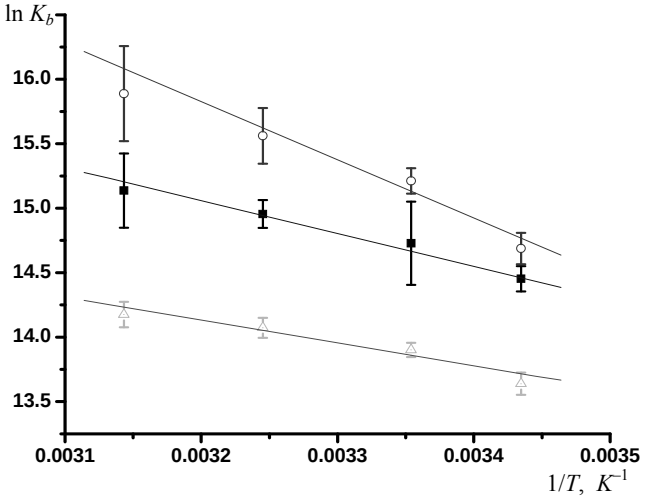


Fig. 4. Temperature dependences of the binding constant for the complexes of porphyrins with DNA: 1 – AgTButPyP4, 2 – AgTAlPyP4, 3 – AgTOEtPyP4.

The analysis of Table shows that the binding of all three porphyrins to DNA is accompanied by "unfavorable" enthalpy change and "favorable" entropy change. Assumingly the "unfavorable" change in enthalpy arises from electrostatic repulsion of positively charged peripheral groups of stacked porphyrins. Therefore, DNA–porphyrin interactions are essentially entropically driven. On basis of these results it can be concluded that the nature of interaction forces is predominately hydrophobic. We propose that the major favorable contribution to the binding entropy comes from dehydration of peripheral groups of porphyrin and DNA, and counterions release upon their association. This behavior of ligands is typical for

outside stacking on DNA and can be used as footprint for determination of binding mode of ligands. This result complies with conclusion made on the basis of CD-spectra analysis.

The comparison with our previous results shows that the increasing of ionic strength results in slight increase of binding constant and decrease of enthalpy and increase of entropy [7]. The addition of ions into the media leads to the increase screening of charged group of porphyrins and DNA. This would lead to decrease of repulsion between stacked porphyrins resulting in decrease of "unfavorable" enthalpy. Slight increase of entropy probably is connected with the increase of twist of DNA double helix, which leads to additional release of counterions and/or water molecules.

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Ag պարունակող պորֆիրինների փոխազդեցությունը ԴՆԹ-ի հետ բարձր իոնական ուժի դեպքում

ՈՒՄ և տեսանելի սպեկտրալուսաչափական և շրջանային դիքրոիզմի մեթոդներով ուսումնասիրվել է Ag պարունակող երեք նոր պորֆիրինների (մեզո-տետրա(4N-ալիլպիրիդիլ), AgTAlPyP4, մեզո-տետրա (4N-բութիլպիրիդիլ), AgTButPyP4, և մեզո-տետրա (4N-օքսիէթիլպիրիդիլ), AgTOEtPyP4) կապվելը ԴՆԹ-ի հետ բարձր իոնական ուժի դեպքում: Յուրաքանչյուր ԴՆԹ–լիգանդ կոմպլեքսի համար կլանման իզոթերմերից հաշվված են կապման հաստատունը (Kb) և ստեխիոմետրիան (n): Կապման հաստատունի արժեքների միջոցով հաշվված են Գիբսի ազատ էներգիան, կապման էնթալպիան և էնտրոպիան: Ստացված արդյունքները ցույց են տալիս, որ բարձր իոնական ուժի դեպքում այս պորֆիրիններն ավելի ուժեղ են կապվում ԴՆԹ-ի հետ:

Взаимодействие Ag-содержащих порфиринов с ДНК при высокой ионной силе

Методами УФ и видимой спектроскопии и кругового дихроизма было изучено связывание с ДНК трех новых Ag-содержащих порфиринов – мезо-тетра(4N-алилпиридил) (AgTAlPyP4), мезо-тетра(4N-бутилпиридил) (AgTButPyP4) и мезо-тетра(4N-оксиэтилпиридил) (AgTOEtPyP4) порфиринов – при высокой ионной силе. По изотермам адсорбции были рассчитаны константа связывания и стехиометрия (K_b и n) для каждого комплекса ДНК–лиганд. По значениям K_b были рассчитаны свободная энергия Гиббса, энтропия и энтальпия связывания. Полученные результаты указывают на то, что эти порфирины взаимодействуют с ДНК внешним способом.