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INFLUENCE OF CATIONIC PORPHYRINS ON CISPLATIN-MODIFIED DNA

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The antitumor activity of cisplatin is achieved through covalent binding to DNA. To eliminate systemic toxicity and side effects, studies of cisplatin–DNA complexes are often conducted in combination with other drugs that selectively accumulate in tumor tissues without forming covalent bonds with DNA and are easily excreted from the body. In the present study, the interactions of the cationic porphyrins H_2TOEtPyP4 and ZnTOEtPyP4 with platinated DNA were investigated in comparison with native DNA. Thermal melting curves show that the porphyrin ZnTOEtPyP4 stabilizes the platinized DNA double helix much better than H_2TOEtPyP4. UV-CD spectra show that ZnTOEtPyP4 induces structural changes in the DNA double helix similar to the B-Z transition. This twisting of the DNA molecule occurs due to an increase in the binding ability of porphyrins to DNA. The binding constants of the studied porphyrins with platinated DNA are approximately twice as large as with native DNA. However, the small value of the exclusion parameter (n<2) shows that the ZnTOEtPyP4 porphyrin molecules at high concentrations are located in greater quantities on the surface of the platinated DNA molecule and stabilize it.

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Introduction. Cisplatin (cis-diamminedichloroplatinum(II), cisPt) is a platinum-based chemotherapy drug, with manifest therapeutic effects and well-defined mechanisms of action that are widely used in the clinic [1]. Cisplatin typically binds to DNA through N7 nucleophilic sites, induces DNA cross-linking, prevents DNA replication, blocks mRNA and protein production, and activates multiple transduction pathways that ultimately lead to necrosis or apoptosis [2, 3]. However, cisplatin is causing systemic toxicity besides killing tumor cells. Platinum anti-cancer drugs have serious side effects, including dose-limiting toxicity, especially nephrotoxicity, neurotoxicity, ototoxicity, and myelosuppression [4], and long-term use of cisplatin causes serious damage to normal tissues [5]. Although cisplatin is widely used in chemotherapy, its side effects and resistance of osteosarcoma cells to cisplatin are two of the most important issues that have led

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researchers to explore alternative therapeutic approaches [6]. One of the new strategies in tumor therapy may be the combined use of cisplatin with other drugs. By combining several drugs, it is possible to minimize the individual toxicity of each of them, as well as reduce the resistance of cancer cells to these drugs [7]. Porphyrins are compounds that exhibit antitumor activity, widely used in cancer therapy and have practically no harmful side effects. The preferential localization of porphyrins in tumors, their ability to generate reactive singlet oxygen, and low dark toxicity have led to their use for therapeutic purposes, such as photodynamic therapy (PDT) of cancer [8]. Porphyrins can be simply dissolved in water or surfactants, injected intravenously and irradiated to the target area, as is done in traditional photodynamic therapy. Therefore, many types of drugs can be used in combination with porphyrins for a variety of therapeutic and imaging purposes.

Porphyrins may associate with DNA in three binding modes: intercalation, groove binding, and outside binding with self-stacking along the DNA helix [9, 10]. The aim of this study is to compare the efficiency of the interaction of water-soluble H_2TOEtPyP4 and ZnTOEtPyP4 cationic porphyrins with modified by cisplatin (platinated) DNA compared with the interaction of the same porphyrins with native DNA in order to determine the prospects for their use in anticancer therapy.

Materials and Methods.

Materials. Ultra-pure Calf Thymus DNA from "Sigma-Aldrich" was used in this work. Porphyrins were synthesized in the Chair of Pharmacological Chemistry, YSMU [11]. The stock solutions of porphyrins (10^{-3} *M*) were prepared in distilled water. Cisplatin from "Sigma-Aldrich" was dissolved in distilled water (1 *mg*/*mL*). Solutions of double-helical DNA at a concentration of 1.2 *mg*/*mL* were incubated with cisplatin in 0.01 *M* $NaClO_4$, in the dark at 37°C during 48 *h* [12–14]. The relative concentration of cisplatin to DNA base pairs is equal 0.025 throughout all experimental procedures.

Absorption Spectra were measured on a Perkin-Elmer Lambda 800 UV/VIS double-beam spectrophotometer with a 1 *cm* path-length quartz cell. Titration spectra were carried out by the stepwise addition of the platinated or native DNA stock solution to a porphyrins solution; absorption spectra were recorded in the 220–500 *nm* range at 20°C. The titration was terminated when the wavelength and intensity of the absorption band for porphyrins did not change anymore upon three successive additions of porphyrins. The calculation was performed according to the McGee and von Hippel model using Eq. (1) of Correia et al. [15]:

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

where C_f is the free porphyrins concentration in solution; $r=C_b/C_{DNA}$; C_b is the concentration of bound porphyrins; C_{DNA} is the concentration of DNA in base pairs; K_b is the binding constant; *n* is the exclusion parameter, i.e. the number of base pairs that are occupied by the interaction of the porphyrin molecules.

UV-Melting. Thermal denaturation was carried out in a quartz cuvette of 1 *cm* path length using a Perkin-Elmer Lambda 800 UV/VIS spectrophotometer equipped with a Peltier effect heated cuvette holder. A temperature range of 40–95°C was used to monitor the absorbance at 260 *nm* with a heating rate of 0.5°C/*min*. The unwinding degree of DNA was measured in versus $1–\theta$ by *T*,°C:

$$1-\theta=\frac{A-A_{\min}}{A_{\max}-A_{\min}}, \quad (2)$$

where A, A_{min} and A_{max} are the absorbencies of the experimental curves the lower baseline (helix) and the upper baseline (coil), respectively at a given temperature T°C [16]. The melting temperature (T_m) was determined as the temperature, at which half of all base pairs "melted", i.e. $1–\theta = 0.5$, and melting interval (ΔT) is equal to the temperature difference, at which the tangent at the inflection point crosses the levels $\theta = 0$ and $\theta = 1$, respectively, and calculated with formula:

$$\Delta T=\left(\frac{\partial\theta}{\partial T}\right)^{-1}_{T=T_m}. \quad (3)$$

Melting was carried out in biphosphate buffer solution 0.15 BPSE, $[Na^+] = 0.03$, pH 7.0, DNA concentration in all samples was diluted to 0.04 *mg*/*mL*.

Circular Dichroism (CD) spectra were recorded on an Olis DSM spectrophotometer in quartz cuvette (Perkin Elmer) with an optical path length of 1 *cm* at 20°C. CD titration was measured by adding multiple aliquots of porphyrin solution to a constant concentration of DNA solution. Each sample was collected from 220 *nm* to 500 *nm* at a scan speed of 200 *nm*/*min* with a response time of 0.5 *s* in a 1 *cm* quartz cell. The spectrum from a blank sample containing only buffer was used as the background (baseline) that was subtracted from the averaged data. All CD spectra were corrected with baseline and normalized for concentration and presented as molar ellipticity.

All experimental data are presented as the average of three measurements. In all experiments the statistical error was $p<0.03$.

Results.

***Absorption Spectra*.** Features of the interaction of H_2TOEtPyP4 and ZnTOEtPyP4 with native and platinated DNA duplexes were revealed by monitoring changes in absorption spectra in the Soret Region. The most convenient and informational method for determining the features of the interaction of DNA with the studied porphyrins is spectrophotometric titration. Multiple aliquots of DNA solution are sequentially added to a porphyrin solution of known concentration, and the optical parameters of the complex are measured at each stage. The addition of DNA leads to an increase in free-binding sites. Titration continues until the next addition of DNA no longer results in changes in absorption spectra, indicating saturation of binding sites. The presence of isosbestic points in the spectra indicates the predominance of one binding mode in a given concentration range. H_2TOEtPyP4 has a planar structure and behaves as a classical intercalator: large red shift ($\Delta\lambda$ = 12 *nm*) and hypochromic effect (Δh=61.3%) are observed (Fig. 1, a and b; Tab. 1). The relatively small red shift ($\Delta \lambda$ = 8 *nm*) and hypochromic effect (Δh = 57.2%) observed in absorption spectra of H_2TOEtPyP4 at addition of platinated DNA. The monitoring of absorption spectra of ZnTOEtPyP4 at complex formation with DNA and platinated DNA show the small red shift (2 *nm* and 1 *nm*) and hypochromic effect (19.6% and 22.9%, respectively) (Fig. 2, a and b). ZnTOEtPyP4 porphyrin have one axial ligand and intercalation is excluded. Hence, at interaction with DNA, they can be located only on the external surface of the DNA double helix or exhibit partial intercalation through pyridyl rings in side radicals.

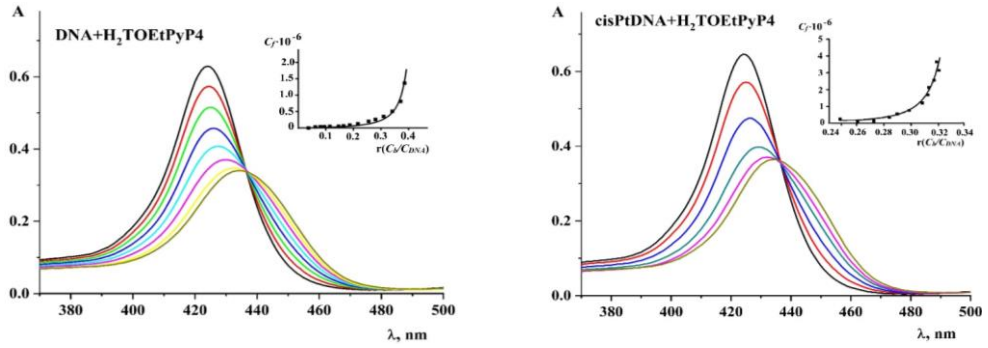
External binding occurs only at AT-rich sites and partial intercalation at GC-rich sites, where cisplatin molecules bind to DNA and form crosslinks.

The calculation of binding constants involves finding the concentrations of free and bound ligands at a known concentration of base pairs. Based on the optical characteristics, concentrations of free (C_f) and bound (C_b) ligands can be determined at each titration stage. Typically, the fraction of bound ligand at each titration step α_i is determined for this purpose:

$$\alpha_i = \frac{A_i - A_f}{A_b - A_f}, \quad (4)$$

where A_i is the optical absorption of the solution at the i-th titration step; A_f is the absorption of the porphyrin solution before titration begins, when all porphyrin molecules are in the free state; A_b is the absorption of the solution at the end of titration, when all porphyrin molecules are bound. The concentration of bound ligands $C_b{}^i$ at the i-th titration step will be equal to $C_b^i = \alpha_i C_t$, where C_t is the total concentration of the ligand in the solution. The concentration of free ligand C_f^i at the i-th titration step is determined as and the relative concentration of bound ligand $C_f^i = C_t - C_b^i$, where C_{DNA}^i is the concentration of added DNA at the i-th titration step.

The binding constant K_b and stoichiometry n were determined from binding isotherms for both complexes of porphyrins with DNA and platinated DNA. Porphyrin solutions were titrated with a stock solution of DNA at 20°C (Figs. 1, 2 and Tab. 1). As can be seen from Tab. 1, the binding constants of the studied porphyrins to platinated DNA are approximately twice as large as with DNA.



a b

Fig. 1. Visible absorption spectra and binding isotherms (insertions) of H_2TOEtPyP4 porphyrin in the Soret band with DNA (a) and platinated DNA (b) at different relative concentrations, cisPt/nucleotide molar ratio was 0.025, [Na^+]=0.03, pH 7.0.

It is known that porphyrins are capable of forming *H*-type aggregates in aqueous solutions, characterized by a weak red shift and slight hypochromism. Therefore, it can be concluded that ZnTOEtPyP4 porphyrin molecules with axial ligands can be wedged between DNA base pairs or in the minor groove by flat pyridyl rings of side radicals, but the porphyrin core is located outside the helix, occupying extended sections of the helix. Indeed, calculations show that the exclusion

parameter has a value on n = 4.9. Platination of DNA increases the binding constant almost twofold, but the exclusion parameter in this case is extremely small: n = 0.88. This circumstance indicates that cisplatin has occupied possible sites intended for partial intercalation and externally binding mechanism is mainly occurs. The presence of *H*- and *J*-aggregates cannot be excluded also. In addition, since covalent bonded mono- and difunctional adducts formed by cisplatin unwind the DNA molecule by approximately ~13° [17], the ability of porphyrins to bind to DNA helix increased.

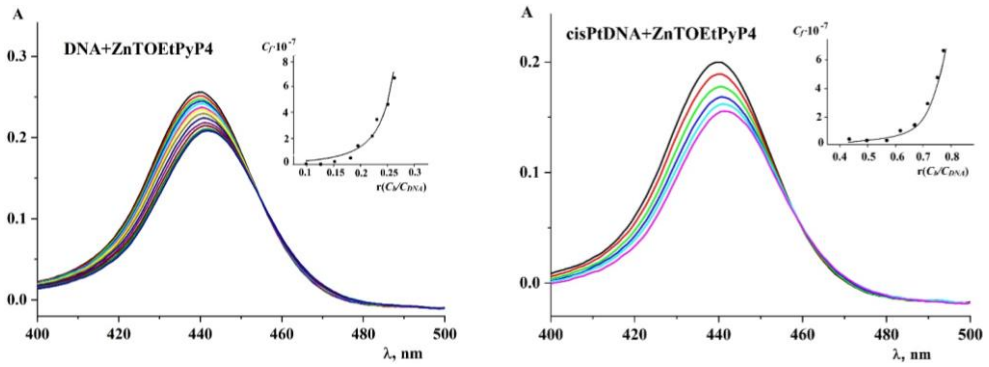


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Fig. 2. Visible absorption spectra and binding isotherms (insertions) of ZnTOEtPyP4 porphyrin in the Soret band with DNA (a) and platinated DNA (b) at different relative concentrations, cisPt/nucleotide molar ratio was 0.025, [Na^+]=0.03, pH 7.0.

Table 1

The spectral and binding parameters of H_2TOEtPyP4 and ZnTOEtPyP4 porphyrins at complex formation with DNA and platinated by cisPt DNA

Complex	Δ λ, *nm*	*h*, %	$K_b \cdot 10^7$, M^{-1}	*n*
DNA+H_2TOEtPyP4	12	61.3	1.21±0.2	2.26±0.02
cisPt DNA +H_2TOEtPyP4	8	57.2	2.14±0.3	2.87±0.04
DNA+ZnTOEtPyP4	2	19.6	1.39±0.2	4.90±0.05
cisPt DNA +ZnTOEtPyP4	1	22.9	2.70±0.1	0.88±0.04

UV-Melting. Evaluation of DNA-ligand complexes by UV thermal melting is a powerful method for determining the stability of the double helix [16]. Thermal melting experiments of DNA+H_2TOEtPyP4, cisPt DNA+H_2TOEtPyP4, DNA+ZnTOEtPyP4 and cisPt DNA+ZnTOEtPyP4 complexes at different relative concentrations of porphyrins were depicted in Figs. 3 and 4.

The cisPt/DNA relative concentration in all experiments is strongly 0.025. The melting characteristics of investigated complexes at various relative concentrations of porphyrins are summarized in Tab. 2.

Melting experiments showed, that the melting temperature (T_m = 71.5°C) of platinated DNA is lower than that of native DNA (T_m = 74.42°C). This fact is good

evidence of destabilization of cisplatin-modified DNA. At relatively low concentrations of H_2TOEtPyP4 ($r \leq 0.005$), DNA is destabilized, whereas platinated DNA at the same relative concentrations of porphyrins is stabilized. The degree of renaturation *R* (%) is also considered an important parameter for determining the binding mechanism of the studied porphyrins to DNA (Tab. 2). An increase in renaturation is observed with an increase in relative porphyrin concentration ($0.001 < r < 0.03$) for both the H_2TOEtPyP4–DNA and ZnTOEtPyP4–DNA complexes, and the highest renaturation value for both the complexes is observed at relative concentration of $r = 0.03$. The increase of DNA renaturation after the melting attests to the formation of specific interstrand cross-links. At relatively low ligand concentrations, such a change in parameters may be due to cross-linking of DNA strands. H_2TOEtPyP4 increases the renaturation of platinated DNA from 93.88% to 98.05% (Tab. 2). The addition of porphyrin H_2TOEtPyP4 almost completely restores the double helical structure of DNA, in contrast to ZnTOEtPyP4, which leads to a decrease in renaturation to 50.15%, while for DNA renaturation increases for both porphyrins H_2TOEtPyP4 and ZnTOEtPyP4 (by 3.75% and 7.05%, respectively).

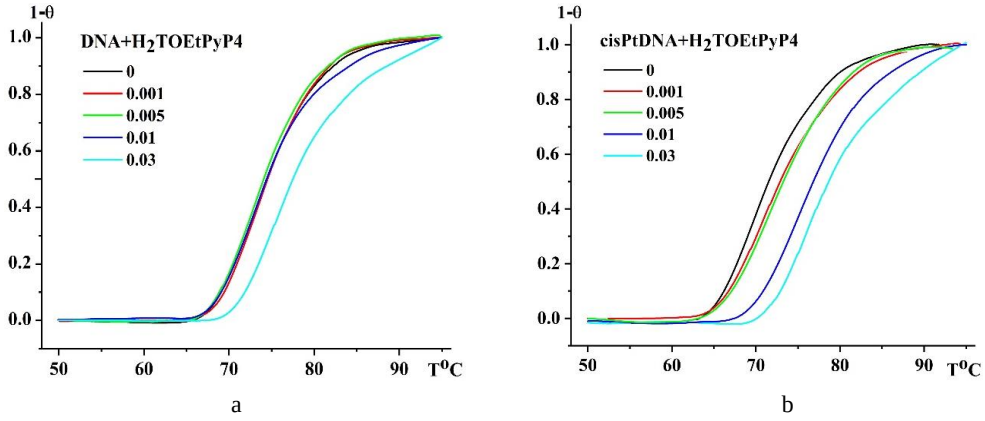


Fig. 3. The normalized melting curves of DNA (a) and platinated DNA (b) at different relative concentrations (*r*) of H_2TOEtPyP4, cisPt/DNA molar ratio was 0.025, $[Na^+] = 0.03$, pH 7.0

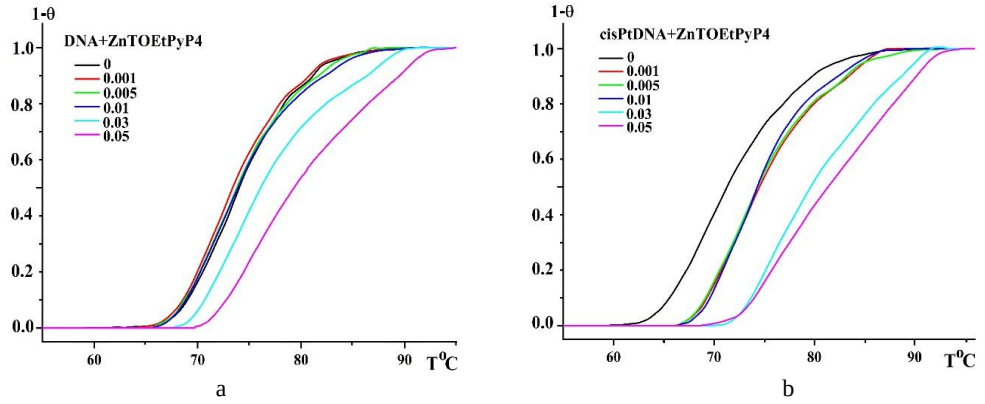


Fig. 4. The normalized melting curves of DNA (a) and platinated DNA (b) at different relative concentrations (*r*) of ZnTOEtPyP4, cisPt/DNA molar ratio was 0.025, $[Na^+] = 0.03$, pH 7.0

Table 2

The melting parameters of DNA and platinated DNA in presence of H_2TOEtPyP4 and ZnTOEtPyP4 porphyrins, cisPt/DNA molar ratio was 0.025, [Na^+]=0.03, pH 7.0

	DNA+H_2TOEtPyP4				cisPt DNA+H_2TOEtPyP4			
r	T_m, °C	Δ*T*, °C	**h*, %	***R*, %	T_m, °C	Δ*T*, °C	**h*, %	***R*, %
0	74.42	9.99	40.08	30.97	71.50	10.50	36.50	93.88
0.001	74.35	9.33	37.39	37.01	72.88	11.75	38.40	93.63
0.005	74.16	9.58	32.33	43.18	73.30	10.91	34.71	90.97
0.01	74.45	10.75	37.70	36.26	76.81	11.10	36.48	97.76
0.03	77.50	12.20	36.40	44.72	78.63	12.90	29.25	98.05
r	DNA+ZnTOEtPyP4				cisPt DNA+ZnTOEtPyP4			
0	74.42	9.99	40.08	30.97	71.50	10.50	36.50	93.88
0.001	73.39	8.98	37.72	31.64	74.52	10.59	29.34	41.98
0.005	73.73	9.48	37.45	31.73	74.35	10.50	32.11	37.07
0.01	73.87	9.93	34.32	34.06	74.34	9.30	26.56	41.05
0.03	76.10	11.80	33.56	38.49	79.64	11.90	24.79	50.15

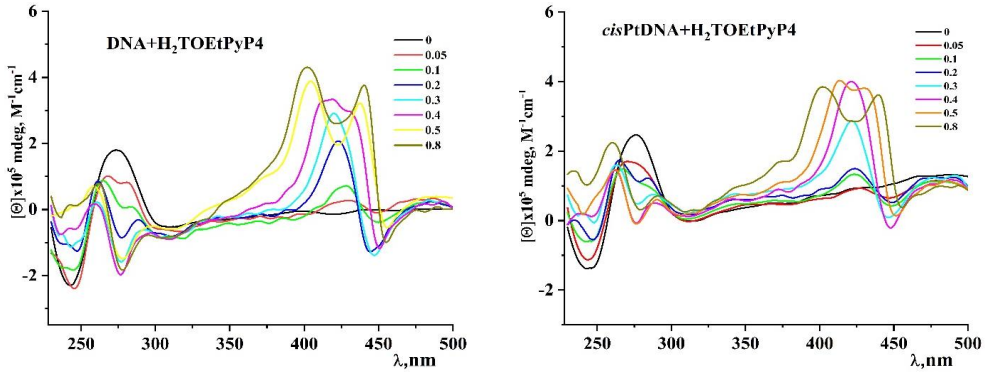
Notes: *– $h = [(A_{95}–A_{25})/A_{25}]\cdot100\%$; ** – $R = [(A_{95}–A_{R25})/(A_{95}–A_{25})]\cdot100\%$; A_{R25} – absorption after renaturation.

According to the data presented in Tab. 2, platinized DNA has a very high degree of renaturation of 93.88% due to interstrand crosslinks. With increasing concentration of H_2TOEtPyP4, an increase in the degree of renaturation of platinized DNA to 98.03% is observed. A significant decrease in the degree of renaturation is observed in platinized DNA in the presence of ZnTOEtPyP4. At the same time, a significant decrease in hypochromicity is observed, which indicates the presence of damaged, melted sites in platinated DNA.

CD Spectra. The nature of the metal ion in the cavity of porphyrins is one of the important factors determining its binding mode to DNA [18]. The porphyrins H_2TOEtPyP4 have a planar structure and intercalate at the GC-rich sites of native DNA at low *r* ratios [19]. However, the presence of one or two axial ligands on the central metal ion such as Co, Zn and Mn prevents intercalation due to the thickness of the porphyrin molecules. An induced negative CD signal in the Soret band usually indicates the presence of an intercalation binding mode, whereas a positive signal corresponds to an external binding mode. A bisignate CD spectrum in the Soret band is believed to indicate folding or stacking of the porphyrin along the DNA helix. In Fig. 5 shows the circular dichroism spectra of DNA–H_2TOEtPyP4 (a) and cisPt DNA–H_2TOEtPyP4 (b) complexes at different relative concentrations *r* (*r*=[porph]/[DNA]) of porphyrin. H_2TOEtPyP4 interacts with DNA by intercalative mode at a small porphyrin/DNA ratio ($r < 0.01$ spectra not presented) (Fig. 5, a), which gradually changes into outside stacking mode at higher *r* ratios. It is known, that H_2TOEtPyP4 have a planar structure and at lower concentrations prefer interact with DNA via intercalation binding mode [20]. On the other hand, Mn(III), Fe(III), Zn(II) and Co(II) derivatives of H_2TMPyP can bind only to the outside of the DNA duplex due to one or two axial ligands [21].

Starting from porphyrin concentration r = 0.05 the induced spectra of H_2TOEtPyP4 became bisignate with λ_{max} = 426 *nm* and λ_{min} = 450 *nm*, suggesting that outside stacking of porphyrin molecules is the dominant binding mode. The spectra in Soret range split starting at concentrations r=0.1 with two maximums and one minimum correspondly at λ_1 = 408 *nm*, λ_2 = 438 *nm* and negative band with a minimum at λ_3 = 422 *nm*. This suggests that the porphyrin molecules are stacked differently and may appear arrangements such as side-by-side or face on face, which corresponds to different peaks in the spectrum. Similar changes in CD spectra occur during complex formation of H_2TOEtPyP4 with cisPt DNA, only relatively less pronounced. Significant changes occur in UV-range of spectra. An increase in porphyrin concentration leads to inversion of shape DNA CD spectra (Fig. 5, a). The presence of isobestic points in CD spectra indicates the predominance of one binding mode in a certain concentration range. This effect is relatively weakly expressed during the interaction of porphyrin H_2TOEtPyP4 molecules with platinated DNA (Fig. 5, b).

ZnTOEtPyP4 porphyrin molecules have one axial ligand, are about 5–7 Å thick, and exhibit standard external binder behavior. As can be seen from Fig. 6, a, at r = 0.05, a positive band with a maximum at λ = 425 *nm* is observed in the visible part of the spectrum, which indicates an external ordered binding. The positive band grows with increasing porphyrin concentration, and starting from the value of r = 0.2, the spectrum splitting, two positive bands appear with maximum at λ_1 = 432 *nm* and λ_2 = 454 *nm* and one negative band with a minimum at λ_3 = 440 *nm*. In the UV range of the spectrum, isosbestic points are clearly observed at wavelengths λ = 262 *nm* and λ = 300 *nm*, which proves the presence of only two absorbing substances in equilibrium with overlapping absorption bands and indicate the predominance of one binding mode. The first, smallest amount of ZnTOEtPyP4 porphyrin already causes quite large structural changes in the DNA spectrum. With further addition of porphyrin concentration, the spectrum is split and the positive DNA band at λ = 275 *nm* is compressed.



a b

Fig. 5. CD spectra of DNA (a) and platinated DNA (b) at different relative concentrations of H_2TOEtPyP4, cisPt/nucleotide molar ratio was 0.025, [Na^+] = 0.03, pH 7.0.

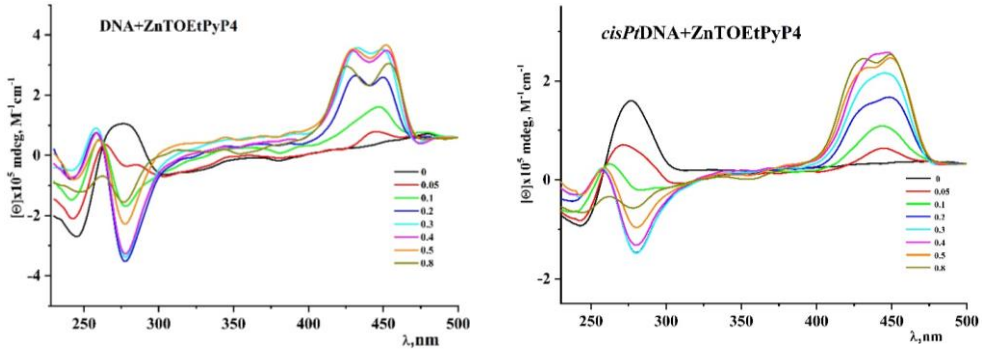


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Fig. 6. CD spectra of DNA (a) and platinated DNA (b) at different relative concentrations of ZnTOEtPyP4, cisPt/nucleotide molar ratio was 0.025, $[Na^+]$ = 0.03, pH 7.0.

The positive band decreases and negative band gradually increases. As a result, an inversion of the spectrum occurs: the negative band of the DNA CD spectrum becomes positive, and conversely. It is known that such a spectrum corresponds to the left-handed Z-conformation of DNA. At the same time, CD spectra are very helpful in interpreting the behavior of melting curves at very low concentrations. The decrease in thermal stability at r = 0.001 means that DNA destabilization is indeed a consequence of structural distortions. The interaction of ZnTOEtPyP4 with DNA leads to a pronounced splitting of the spectra in the visible region and a pronounced inversion of the spectrum in the UV region, which is characteristic of Z-DNA.

Discussion. Cisplatin is an anticancer drug widely used in cancer therapy. The main target of these drugs is the DNA molecule. Platinum (II) drugs bind to the major groove of B-form DNA while simultaneous widening of the minor groove. These perturbations lead to a combination of A- and B-form around the platination site. As a result the N7 sites of neighboring purines are closer, the major groove becomes narrower, the minor groove wider. The nucleotide at the C5′ to the platination site shows a C3′-end conformation which is found in A-form helices. Additionally, a hydrogen bond between the O6 of a guanine and the amine group of cisplatin is observed [22]. The formation of cisPt DNA crosslinks structurally distorts the DNA, which is due by formation covalently bonded mono- and bifunctional adducts [23]. It has been shown that cisPt DNA complexation is accompanied by coordination of cisPt with N7 of guanine, consequently cisplatin prefers the GC-rich sites of DNA. Planar porphyrins also prefer GC-sites for binding to DNA at low concentrations, because intercalation occurs only at GC-sites [20]. Since some of these sites are occupied by cisPt molecules, the DNA helix is more stabilized. Cisplatin includes two cis-amine non-leaving ligands and two labile chloride leaving groups. After slow dissociation of the Cl-ions in buffer solution, binding of cisplatin moiety to DNA occurs. About 90% of bound cisplatin forms intrastrand crosslinks between neighboring purines of the same strand. Cisplatin also forms interstrand crosslinks and monofunctional adducts [24–27]. It is known that intrastrand and interstrand crosslinks distort the double helix. A decrease in the melting temperature for

platinated DNA molecules is in accordance with this fact [28]. The melting temperature of native DNA decreases slightly at porphyrin concentrations of $r \leq 0.01$, and increases starting from $r > 0.01$. Meanwhile, in the case of platinated DNA stabilization of the complexes observed at al investigated porphyrin concentrations. Binding of cisplatin to DNA locally distorts the double helix. Intrastrand crosslinks, which account for 90% of all damage caused by cisplatin, distort the double helix at their locations [26–29].

However, besides local influence on the double helix stability, interstrand crosslinks give rise additional long-range stabilizing effects as the formation of additional loops in melted regions [30, 31]. Neither interstrand crosslinks nor monofunctional adducts causes such effects. Besides local destabilizing distortions of the double helix, all cisplatin adducts decrease the DNA charge density and in this way increase DNA stability. Galyuk E.N. and others showed that the melting temperature of DNA when interacting with positively charged protamines, histones and polylysine is noticeably higher than that of free DNA, since Na^+ ions, which shield DNA, are competitively displaced by the compounds under study [14]. The increase in thermal stability observed at interaction of DNA with positively charged porphyrins H_2TOEtPyP4 and ZnTOEtPyP4 can be explained in the same way.

ZnTOEtPyP4 demonstrate the external binding mode at complex formation with platinated DNA: positive bands were observed in the visible range of induced CD spectrum (Fig. 6, b). Changes in the positive band of CD spectrum in UV-range of native DNA are observed starting from $r = 0.1$. An increase in porphyrin concentration leads to a more pronounced inversion of the spectrum than in the case of platinated DNA. This fact can be explained by the fact that ZnTOEtPyP4 molecules cannot exert their helix-unwiding effect on DNA due to already existing interstrand and intrastrand cross-links of cisPt. ZnTOEtPyP4 molecules, regardless of the presence of platinum, are located on the external surface of DNA helix, that is, the penetration of these molecules between base pairs is initially excluded due to the axial ligand. However, starting from a certain concentration, the positively charged ZnTOEtPyP4 porphyrin molecules are so densely arranged around the DNA helix that each subsequent molecule tries to be closer to the negatively charged DNA macromolecule. With such competition, porphyrins with a thickness of approximately 5–7 Å can penetrate into grooves or between base pairs with peripheral planar pyridyl rings, which ultimately leads to unwinding of the DNA double helix and the formation of a left-handed helix. In such cases, it is customary to talk about so-called partial intercalation. The partial intercalation, as an intercalation, can be realized in GC-rich sites. The case of penetration of pyridyl rings in the minor groove of DNA is also considered a case of partial intercalation. The splitting, observed in the visible range of the spectrum in the high concentration range is precisely the result of the penetration of pyridyl rings into the uncoiled parts or minor grooves of the DNA. In other words, it can be argued that in the region of high concentrations, there are two binding mechanisms: an externally ordered mode and partial intercalation.

Studying the feutures of the interaction of porphyrins H_2TOEtPyP4 and ZnTOEtPyP4 with platinated DNA may have a promising direction in tumor therapy, also taking into account the fact that the studied porphyrins selectively accumulate in tumor cells and induce tumor apoptosis [32].

Conclusion. In this study were investigated the peculiarities of interaction of H_2TOEtPyP4 and ZnTOEtPyP4 with DNA and modified by cisplatin DNA using UV-visible and circular dichroism spectra and melting curves. From the melting curves it follows that ZnTOEtPyP4 porphyrin stabilize the double helix of platinated DNA better than metal-free porphyrin H_2TOEtPyP4. Consequently, the destabilizing effect of cisplatin on the DNA molecule is reduced at presence ZnTOEtPyP4 porphyrins. The high degree of renaturation of platinated DNA (93.88%), which is caused by covalent platinum bonds, is almost halved when interacting with ZnTOEtPyP4 (50.15%) compared to H_2TOEtPyP4. CD spectra of complexes of platinized DNA with zinc porphyrin show that with increasing of porphyrin concentration, structural changes in the double helix occur. This fact is confirmed by the inversion of the CD spectra in the UV region of the spectrum, which is similar to the B-Z transition. With such twisting of the DNA molecule, the binding ability of porphyrins should increase. The binding constants of the studied porphyrins to platinated DNA are approximately two times greater than to native DNA. But the small value of the exclusion parameter *n* shows that the ZnTOEtPyP4 molecules are stacked on the outer surface of the DNA molecule, which more stabilizes the platinated molecule.

The authors declare that they have no conflict of interest.

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REFERENCES

1. Qi L., Luo Q., et al. Advances in Toxicological Research of the Anticancer Drug Cisplatin. *Chem. Res. Toxicol.* **32** (2019) 1469–1486. https://doi.org/10.1021/acs.chemrestox.9b00204
2. Ciccarelli R.B., Solomon M.J., et al. *In vivo* Effects of cis- and trans-Diamminedichloroplatinum (II) on SV40 Chromosomes: Differential Repair, DNA-Protein Cross-Linking, and Inhibition of Replication. *Biochemistry* **24** (1985) 7533–7540. https://doi.org/10.1021/bi00347a005
3. Jordan P., Carmo-Fonseca M. Molecular Mechanisms Involved in Cisplatin Cytotoxicity. *Cell Mol. Life Sci.* **57** (2000) 1229–1235. https://doi.org/10.1007/PL00000762
4. Wlodarczyk M.T., Dragulska S.A., et al. Platinum (II) Complex-Nuclear Localization Sequence Peptide Hybrid for Overcoming Platinum Resistance in Cancer Therapy. ACS *Biomater. Sci. Eng.* **4** (2018), 463–467. https://doi.org/10.1021/acsbiomaterials.7b00921
5. Mjos K.D., Orvig C. Metallodrugs in Medicinal Inorganic Chemistry. *Chem. Rev.* **114** (2014), 4540–4563. https://doi.org/10.1021/cr400460s
6. Wong E., Giandomenico C.M. Current Status of Platinum-Based Antitumor Drugs. *Chem Rev.* **99** (1999), 2451–2466. https://doi.org/10.1021/cr980420v

7. Kaiser J. Combining Targeted Drugs to Stop Resistant Tumors. *Science.* **331** (2011), 1542–1545. https://doi.org/10.1126/science.331.6024.1542
8. Huang H., Song W., et al. Emerging Applications of Porphyrins in Photomedicine. *Front. Phys.* **3** (2015), 23. https://doi.org/10.3389/fphy.2015.00023
9. Barkhudaryan V.G., Ananyan G.V., et al. Development of Viscometric Methods for Studying the Interaction of Various Porphyrins with DNA. Part I: Meso-tetra-(4N-hydroxyethylpyridyl) porphyrin and its Ni-, Cu-, Co- and Zn-Containing Derivatives. *Journal of Porphyrins and Phthalocyanines* **18** (2014), 594–599. https://doi.org/10.1142/S1088424614500357
10. Ananyan G.V., Dalyan Y.B., et al. Interaction of MnTOEtPyP4 Porphyrin with DNA. *Journal of Biomolecular Structure and Dynamics.* **41** (2023), 7290-96. https://doi.org/10.1142/S1088424614500357
11. Ghazaryan R.K., Sahakyan L.A., Tovmasyan A.G. *Derivatives of Meso-tetra-4-N-pyridylporphyne and their Metallocomplexes*. Patent of the Republic of Armenia, N 1714 A2 (2006).
12. Zaludová R., Kleinwächter V., Brabec V. The Effect of Ionic Strength on Melting of DNA Modified by Platinum(II) Complexes. *Biophys Chem.* **60** (1996), 135–142. https://doi.org/10.1016/0301-4622(96)00010-5
13. Nováková O., Vrána O., et al. DNA interactions of Antitumor Platinum(IV) Complexes. *Eur. J. Biochem.* **228** (1995), 616–624. https://doi.org/10.1111/j.1432-1033.1995.tb20301.x
14. Galyuk E.N., Lando D.Y., et al. Na_2CO_3 Influence on DNA Double Helix Stability: Strong Anion Destabilizing Effect. *J. Biomol. Struct. Dyn*. **20** (2003), 801–809. https://doi.org/10.1080/07391102.2003.10506896
15. Correia J.J., Chaires J.B. Analysis of Drug-DNA Binding Isotherms: A Monte Carlo Approach. *Methods in Enzymology* **240** (1994), 593–614.
16. Mergny J.L., Lacroix L. Analysis of thermal melting curves. *Oligonucleotides* **13** (2003), 515–537. https://doi.org/10.1089/154545703322860825
17. Bellon S.F., Coleman J.H., Lippard S.J. DNA Unwinding Produced By Site-Specific Intrastrand Cross-Links Of The antitumor drug cis-Diamminedichloroplatinum(II). *Biochemistry* **30** (1991), 8026–8035. https://doi.org/10.1021/bi00246a021
18. Huang X., Nakanishi K., Berova N. Porphyrins and Metalloporphyrins: Versatile Circular Dichroic Reporter Groups for Structural Studies. *Chirality* **12** (2000), 237–255. https://doi.org/10.1002/(SICI)1520-636X(2000)12:4%3C237: AID-CHIR10%3E3.0.CO;2-6
19. Gong L, Bae I., Kim S.K. Effect of Axial Ligand on the Binding Mode of M-*meso*-Tetrakis(N-methylpyridinium-4-yl)porphyrin to DNA Probed by Circular and Linear Dichroism Spectroscopies. *J. Phys. Chem. B* **116** (2012), 12510–12521. https://doi.org/10.1021/jp3081063
20. Pasternack R.F., Gibbs E.J. Porphyrin and Metalloporphyrin Interactions with Nucleic Acids. *Met. Ions Biol. Syst.* **33** (1996), 367–397.
21. Tjahjono D.H., Mima S., Akutsu T., Yoshioka N., Inoue H.J. Interaction of Metallopyrazoliumyl-Porphyrins with Calf Thymus DNA. *Inorg. Biochem.* **85** (2001), 219–228. https://doi.org/10.1016/S0162-0134(01)00186-6
22. Malinina L, Tereshko V., et al. Structural Variability and New Intermolecular Interactions of Z-DNA in Crystals of d(pCpGpCpGpCpG). *Biophys. J.* **74** (1998), 2482–2490. https://doi.org/10.1016/s0006-3495(98)77956-1
23. Zaludova R., Zakovska A., et al. DNA Interactions of Bifunctional Dinuclear Platinum(II) Antitumor Agents. *Eur. J. Biochem.* **246** (1997), 508–517. https://doi.org/10.1111/j.1432-1033.1997.00508.x
24. Coste F., Malinge J.M., et al. Crystal Structure of a Double-Stranded DNA Containing a Cisplatin Interstrand Cross-Link at 1.63 A Resolution: Hydration at the Platinated Site. *Nucleic Acids Res.* **27** (1999), 1837–1846. https://doi.org/10.1093/nar/27.8.1837
25. Takahara P.M., Rosenzweig A.C., et al. Crystal Structure of Double-Stranded DNA Containing the Major Adduct of the Anticancer Drug Cisplatin. *Nature* **377** (1995), 649–652. https://doi.org/10.1038/377649a0

26. van Boom S.S., Yang D., et al. Structural Effect of Intra-Strand Cisplatin-Crosslink on Palindromic DNA Sequences. *J. Biomol. Struct. Dyn.* **13** (1996), 989–998. https://doi.org/10.1080/07391102.1996.10508913
27. Paquet F., Pérez C., et al. NMR Solution Structure of a DNA Decamer Containing an Interstrand Cross-Link of the Antitumor Drug cis-Diamminedichloroplatinum (II). *J. Biomol. Struct. Dyn.* **14** (1996), 67–77. https://doi.org/10.1080/07391102.1996.10508930
28. Zaludová R., Kleinwächter V., Brabec V. The Effect of Ionic Strength on Melting of DNA Modified by Platinum(II) Complexes. *Biophys. Chem.* **60** (1996), 135–142. https://doi.org/10.1016/0301-4622(96)00010-5
29. Coste F., Malinge J.M., Et Al. Crystal Structure Of A Double-Stranded DNA Containing a Cisplatin Interstrand Cross-Link at 1.63 A Resolution: Hydration at the Platinated Site. *Nucleic Acids Res.* **27** (1999), 1837–1846. https://doi.org/10.1093/nar/27.8.1837
30. Lando D.Y., Fridman A.S., et al. Melting of Cross-Linked DNA: II. Influence of Interstrand Linking on DNA Stability. *J. Biomol. Struct. Dyn.* **15** (1997), 141–150. https://doi.org/10.1080/07391102.1997.10508953
31. Lando D.Y., Fridman A.S, et al. Melting of Cross-Linked DNA. III. Calculation of Differential Melting Curves. *J. Biomol. Struct. Dyn.* **16** (1998), 59–67. https://doi.org/10.1080/07391102.1998.10508227
32. Karapetyan N.H., Torosyan L.V., et al. DNA Damage and Anti-Tumor Activity Induced by Zn, Ag and Co Containing meso-tetra-(4-N-oxyethylpyridyl)porphyrins *in vivo*. *J. Porphyrins Phthalocyanines.* **14** (2010), 1–5. https://doi.org/10.1142/S1088424610002100

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ԿԱՏԻՈՆԱՅԻՆ ՊՈՐՖԻՐԻՆՆԵՐԻ ԱԶԴԵՑՈՒԹՅՈՒՆԸ ՑԻՍՊԼԱՏԻՆՈՎ ՄՈԴԻՖԻԿԱՑՎԱԾ ԴՆԹ-Ի ՎՐԱ

Ցիսպլատինի հակաուռուցքային ակտիվությունը պայմանավորված է ԴՆԹ-ի հետ կովալենտ կապվելու հատկությամբ: Համակարգային թունավորումը և կողմնային ազդեցությունները բացառելու նպատակով հաճախ ցիսպլատին-ԴՆԹ կոմպլեքսների ուսումնասիրություններն իրականցվում են այնպիսի դեղամիջոցների հետ համատեղ, որոնք ընտրողաբար կուտակվում են ուռուցքային հյուսվածքներում, ԴՆԹ-ի հետ կովալենտ կապեր չեն առաջացնում և հեշտությամբ արտազատվում են օրգանիզմից: Այս հոդվածում կատարվել է նատիվ ԴՆԹ-ի և ցիսպլատինով մոդիֆիկացված ԴՆԹ-ի հետ H2TOEtPyP4 և ZnTOEtPyP4 կատիոնային պորֆիրինների փոխազդեցությունների համեմատական հետազոտություն: Ջերմային հալման կորերը ցույց են տվել, որ ZnTOEtPyP4 պորֆիրինը զգալիորեն ավելի լավ է կայունացնում ցիսպլատինով մոդիֆիկացված ԴՆԹ-ի կրկնակի պարույրը, քան H2TOEtPyP4-ը: ՈւՄ տիրույթում ՇԴ սպեկտրներից երևում է, որ ZnTOEtPyP4-ը ԴՆԹ-ի կրկնակի պարույրում առաջացնում է կառուցվածքային փոփոխություններ, որոնք նման են B-Z անցմանը: ԴՆԹ-ի մոլեկուլի նման ոլորումը տեղի է ունենում պորֆիրինային մոլեկուլների՝ ԴՆԹ-ի հետ կապելու կարողության մեծացման հետևանքով: Պլատինացված ԴՆԹ-ի հետ պորֆիրինների կապման հաստատունները մոտավորապես երկու անգամ գերազանցում են նատիվ ԴՆԹ-ի հետ կապման հաստատուններին: Սակայն, բացառման պարամետրի

փոքր արժեքը (n<2) ցույց է տալիս, որ ZnTOEtPyP4 պորֆիրինի մոլեկուլները հարաբերական կոնցենտրացյի մեծ արժեքների դեպքում ավելի մեծ քանակությամբ են տեղակայվում պլատինացված ԴՆԹ մոլեկուլի մակերեսին և կայունացնում են այն:

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ВЛИЯНИЕ КАТИОННЫХ ПОРФИРИНОВ НА МОДИФИЦИРОВАННУЮ ЦИСПЛАТИНОМ ДНК

Противоопухолевая активность цисплатины достигается за счет ее ковалентного связывания с ДНК. Для исключения системной токсичности и побочных эффектов исследования комплексов цисплатина–ДНК (цисPt-ДНК) часто проводятся в сочетании с другими препаратами, которые селективно накапливаются в опухолевых тканях, не образуя ковалентных связей с ДНК, и легко выводятся из организма. В статье исследовалось взаимодействие катионных порфиринов H2TOEtPyP4 и ZnTOEtPyP4 с модифицированной цисплатиной ДНК по сравнению с нативной ДНК. Кривые плавления показывают, что порфирин ZnTOEtPyP4 стабилизирует спираль цисPt-ДНК значительно лучше, чем H2TOEtPyP4. УФ-КД-спектры показывают, что ZnTOEtPyP4 вызывает структурные изменения в двойной спирали ДНК, аналогичные B-Z-переходу. Такое скручивание молекулы ДНК происходит за счет увеличения связывающей способности порфиринов с ДНК. Константы связывания исследованных порфиринов с модифицированной цисплатиной ДНК примерно вдвое больше, чем с нативной. Однако малое значение параметра исключения (n<2) свидетельствует о том, что молекулы порфирина ZnTOEtPyP4 при высоких относительных концентрациях в основном располагаются на поверхности платинированной молекулы ДНК и стабилизируют ее.