



Biolog. Journal of Armenia, 1 (70), 2018

## EFFECT OF NON-THERMAL MILLIMETER ELECTROMAGNETIC RADIATION ON THERMODYNAMIC PARAMETERS OF THE BINDING OF LIGANDS WITH NUCLEIC ACIDS

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It has been shown that intercalating (mitoxantone, doxorubicin) and non-intercalating (netropsin, Hoechst 33258) ligands form more strong complex with nucleic acids irradiated by resonant frequencies (64,5 and 50.3 GHz) for oscillations of water structures. Under the effect of the mentioned non-thermal millimeter waves a dehydration of nucleotides occurs, which results in increasing of the binding constant of ligands with irradiated double-stranded nucleic acids (NA). Calculations showed that as a result of irradiation, the enthalpy ( $\Delta H$ ) and entropy ( $\Delta S$ ) of the binding of ligands to NA has been changed. However the value of  $\Delta H$  is mainly being changed for intercalators, and the value of  $\Delta S$  - for non-intercalators.

*DNA – synthetic polyribonucleotide – mitoxantrone – doxorubicin – netropsin – Hoechst 33258*

Ցույց է տրվել, որ ինտերկալացվող (միտոքսանտրոն, դոքսորուբիցին) և չինտերկալացվող (նետրոպսին, Hoechst 33258) լիգանդները ջրի կառուցվածքների տատանումների ռեզոնանսային հաճախություններով (64.5 և 50.3 ԳՀց) ճառագայթված նուկլեինաթթուների հետ առաջացնում են ավելի ամուր կոմպլեքս: Նշված ոչ ջերմային միլիմետրային ալիքների ազդեցության ներքո տեղի է ունենում նուկլեոտիդների դեհիդրատացիա, ինչը հանգեցնում է ճառագայթահարված երկշղթա նուկլեինաթթուների (ՆԹ) հետ լիգանդների կապման հաստատունի մեծացմանը: Հաշվարկները ցույց են տվել, որ ճառագայթման արդյունքում փոխվում են ՆԹ-ի հետ լիգանդների կապման էնթալպիան ( $\Delta H$ ) և էնթալպիան ( $\Delta S$ ), սակայն ինտերկալատորների կապման արդյունքում հիմնականում փոխվում է  $\Delta H$ -ը, իսկ ոչ ինտերկալատորների համար՝  $\Delta S$ -ը:

*ՆԹ – սինթետիկ պոլիռիբոնուկլեոտիդ – միտոքսանտրոն – դոքսորուբիցին –  
նետրոպսին – Hoechst 33258*

Показано, что интеркалирующие (митоксантрон, доксорубин) и неинтеркалирующие (нетропсин, Hoechst 33258) лиганды с нуклеиновыми кислотами, облученными резонансными частотами (64.5 и 50.3 ГГц) колебаний водных структур, образуют более прочный комплекс. Под действием указанных нетепловых миллиметровых волн происходит дегидратация нуклеотидов, что и приводит к увеличению константы связывания лигандов с облученными двуспиральными нуклеиновыми кислотами (НК). Расчеты показали, что вследствие облучения изменяются энтальпия ( $\Delta H$ ) и энтропия ( $\Delta S$ ) связывания лигандов с НК, однако для интеркаляторов вследствие связывания в основном меняется  $\Delta H$ , а для неинтеркаляторов –  $\Delta S$ .

*ДНК – синтетический полирибонуклеотид – митоксантрон – доксорубин –  
нетропсин – Hoechst 33258*

Numerous physical factors of environment, playing a key role in vital processes of biological systems, have an electromagnetic nature. Electromagnetic waves of the artificial origin and various frequency ranges have a significant effect on the living organisms and possess a wide application [1, 2]. The topics connected to the possible negative impacts of electromagnetic waves on living organisms as well as medicobiological aspects of their application as therapeutic agents are actual directions of contemporary science [3-6]. It was shown in [7-9] that non-thermal millimeter electromagnetic waves (MM EMW) certainly affect the water-saline solutions of DNA in vitro. At the irradiation of DNA solutions by resonant frequencies for oscillations of water molecular structures, MM EMW penetrate deeper in water solutions [7], due to which the thermostability of DNA increases, which in turn is conditioned by dehydration of DNA molecules and  $\text{Na}^+$  ions in the solution. It is known that many biologically active compounds (particularly, drugs) penetrating into a cell, bind to nucleic acids (NA) mainly [10, 11]. Taking into consideration that the hydrate layer of NA plays an important role at the complex-formation of biologically active compounds with NA, in this paper the binding of several low-molecular compounds with NA earlier irradiated by MM EMW has been studied.

**Materials and methods.** Calf thymus DNA, synthetic double-stranded polyribonucleotides poly(A)-poly(U) and poly(G)-poly(C), mitoxantrone (MTX), netropsin (Nt), doxorubicin (DX) and Hoechst 33258 (H33258) were used in experiments. All preparations were purchased from "Sigma" and used without further purification. Measurements were carried out in water solution, containing 0.01M Tris buffer and 0.1 M NaCl, pH 7.4.

Concentration of the preparations was determined using the following extinction coefficients ( $\text{M}^{-1} \cdot \text{cm}^{-1} \cdot \text{L}$ ):  $\epsilon_{260}(\text{p})=6550$  for calf thymus DNA,  $\epsilon_{260}(\text{p})=7140$  for poly(A)-poly(U),  $\epsilon_{260}(\text{p})=7900$  for poly(G)-poly(C),  $\epsilon_{659}=25090$  for MTX,  $\epsilon_{296}=21500$  for Nt,  $\epsilon_{480}=11500$  for DX and  $\epsilon_{343}=42000$  for H33258.

Spectrophotometric titration was carried out using PYE Unicam SP-8-100 spectrophotometer (England). Measurements were carried out in thermostating cells using quartz cuvettes with hermetically closing caps. For spectrophotometric titration the concentration of MTX was approximately equal to  $C_0 \approx 3 \cdot 10^{-6}$  M;  $C_0 \approx 7 \cdot 10^{-5}$  M for DX;  $C_0 \approx 2 \cdot 10^{-5}$  M for Nt;  $C_0 \approx 3 \cdot 10^{-5}$  M for H33258. It was shown that at the mentioned concentrations the self-arbitrary associates of the ligand molecules can be neglected [12,13].

For the irradiation of the solutions G4-141 and G4-142 generators were used. Irradiation was carried out in the glassy dishes. Solutions were covered by transparent for irradiation thin chloride-vinilic layer as described in details [7, 13].

**Results and Discussion.** It was shown [7, 9] that under the effect of MM EMW the density of DNA water-saline solutions and DNA thermostability increase, though the change is the biggest at the irradiation with 90 min duration and 64.5 and 50.3 GHz frequencies that coincide with resonant ones of oscillations of water structures. That is why the binding of ligands with NA previously irradiated by resonant (64.5 and 50.3 GHz) and non-resonant (48.3 GHz) frequencies and 90 min duration was studied.

Interaction of ligands with NA is reflected in alteration of the absorption spectra in visible and UV regions of the spectrum. The interaction of ligands with NA via the absorption spectra change character in visible (for MTX and DX) and distant UV (for Nt and H33258) regions at the complex-formation was studied. NA do not absorb in distant UV ( $\lambda > 320$  nm) and visible regions that is why (at complex-formation of NA-ligand) the character of ligand binding to NA can be studied by the absorption spectra change in the mentioned regions and the binding thermodynamic parameters can be determined.

The absorption spectra of non-irradiated and irradiated calf thymus DNA and polyribonucleotides were obtained at some concentrations of ligands. Experiments show

that the absorption spectra of the complexes and the character of their alteration at titration are almost similar for non-irradiated and irradiated NA. From the absorption spectra the concentration of free and bound ligand in the solution was determined and the binding isotherms in Scatchard's coordinates by the early described method were constructed [12, 13]. The binding isotherms were described by non-linear formula, provided by McGhee and von-Hippel [14], which more precisely describes the adsorption of ligands on NA. It was shown [12,13,15] that in the studying conditions MTX and DX interact with non-irradiated and irradiated NA by one intercalating mode; Nt and H33258 interact with NA by non-intercalating mode, localizing in the minor groove of NA and forming the ordered complex. It should be mentioned that the double-stranded polyribonucleotides poly(A)·poly(U) and poly(G)·poly(C) are in A-form at approximate physiological conditions [16]; the double helix parameters, hydration of nucleotides and charge density along the chain strongly differ from those of DNA in analogous conditions (B-form).

**Table 1.** Values of the binding constant (K) and saturation stoichiometry (n) determined for the complexes of ligands with non-irradiated and irradiated NA

Ligand type	T, K	Non-irradiated	Irradiated, frequency			n
			50.3 GHz	64.5 GHz	48.3 GHz	
		Binding constant, M <sup>-1</sup>				
DNA						
MTX	298.15	(5.1±0.1)·10 <sup>5</sup>	(10.0±0.2)·10 <sup>5</sup>	(8.7±0.2)·10 <sup>5</sup>	(5.2±0.1)·10 <sup>5</sup>	2.4±0.2
	303.15	(4.1±0.1)·10 <sup>5</sup>	(7.5±0.1)·10 <sup>5</sup>	(6.9±0.3)·10 <sup>5</sup>	(4.2±0.2)·10 <sup>5</sup>	2.5±0.2
	308.15	(3.35±0.05)·10 <sup>5</sup>	(5.1±0.2)·10 <sup>5</sup>	(4.8±0.2)·10 <sup>5</sup>	(3.4±0.1)·10 <sup>5</sup>	2.3±0.2
DX	290.15	(6.2±0.1)·10 <sup>5</sup>	(64.5±0.2)·10 <sup>5</sup>	(62.0±0.2)·10 <sup>5</sup>	(6.9±0.1)·10 <sup>5</sup>	4.0±0.1
	300.15	(5.1±0.1)·10 <sup>5</sup>	(50.2±0.2)·10 <sup>5</sup>	(48.1±0.2)·10 <sup>5</sup>	(5.9±0.1)·10 <sup>5</sup>	4.1±0.2
	310.15	(4.5±0.2)·10 <sup>5</sup>	(39.4±0.2)·10 <sup>5</sup>	(38.2±0.1)·10 <sup>5</sup>	(5.1±0.1)·10 <sup>5</sup>	4.0±0.1
Nt	293.15	(5.0±0.2)·10 <sup>8</sup>	(36.1±0.3)·10 <sup>8</sup>	(38.4±0.2)·10 <sup>8</sup>	(6.9±0.2)·10 <sup>8</sup>	5.9±0.2
	303.15	(3.0±0.1)·10 <sup>8</sup>	(21.1±0.2)·10 <sup>8</sup>	(22.3±0.3)·10 <sup>8</sup>	(4.1±0.2)·10 <sup>8</sup>	6.0±0.2
	313.15	(1.9±0.2)·10 <sup>8</sup>	(12.8±0.2)·10 <sup>8</sup>	(13.0±0.2)·10 <sup>8</sup>	(4.1±0.2)·10 <sup>8</sup>	6.0±0.2
H33258	298.15	(5.5±0.5)·10 <sup>7</sup>	(23.1±0.5)·10 <sup>7</sup>	(24.2±0.4)·10 <sup>7</sup>	(6.1±0.5)·10 <sup>7</sup>	6.0±0.1
	303.15	(8.5±0.5)·10 <sup>7</sup>	(40.3±0.4)·10 <sup>7</sup>	(50.5±0.5)·10 <sup>7</sup>	(9.2±0.3)·10 <sup>7</sup>	6.0±0.2
	308.15	(10.5±0.2)·10 <sup>7</sup>	(72.1±0.5)·10 <sup>7</sup>	(81.7±0.5)·10 <sup>7</sup>	(12.8±0.5)·10 <sup>7</sup>	6.0±0.2
poly(G)·poly(C)						
MTX	308.15	(9.5±0.3)·10 <sup>4</sup>	(1.8±0.1)·10 <sup>5</sup>	(2.1±0.1)·10 <sup>5</sup>	(9.6±0.4)·10 <sup>4</sup>	6.0±0.3
	323.15	(5.7±0.2)·10 <sup>4</sup>	(1.1±0.1)·10 <sup>5</sup>	(1.25±0.1)·10 <sup>5</sup>	(5.9±0.3)·10 <sup>4</sup>	5.8±0.4
	333.15	(4.2±0.2)·10 <sup>4</sup>	(0.75±0.08)·10 <sup>5</sup>	(0.81±0.1)·10 <sup>5</sup>	(4.2±0.3)·10 <sup>4</sup>	5.9±0.3
DX	290.15	(4.4±0.1)·10 <sup>5</sup>	(9.3±0.2)·10 <sup>5</sup>	(8.8±0.2)·10 <sup>5</sup>	(4.9±0.1)·10 <sup>5</sup>	6.0±0.2
	300.15	(2.2±0.1)·10 <sup>5</sup>	(4.8±0.2)·10 <sup>5</sup>	(3.3±0.1)·10 <sup>5</sup>	(2.3±0.1)·10 <sup>5</sup>	5.9±0.2
	310.15	(1.7±0.1)·10 <sup>5</sup>	(3.4±0.1)·10 <sup>5</sup>	(2.8±0.1)·10 <sup>5</sup>	(1.8±0.2)·10 <sup>5</sup>	6.0±0.1
poly(A)·poly(U)						
Nt	293.15	(1.2±0.02)·10 <sup>8</sup>	(3.9±0.1)·10 <sup>8</sup>	(4.2±0.1)·10 <sup>8</sup>	(1.71±0.1)·10 <sup>8</sup>	8.0±0.2
	303.15	(0.72±0.03)·10 <sup>8</sup>	(2.3±0.1)·10 <sup>8</sup>	(2.5±0.1)·10 <sup>8</sup>	(1.02±0.1)·10 <sup>8</sup>	8.1±0.2
	313.15	(0.44±0.02)·10 <sup>8</sup>	(1.4±0.1)·10 <sup>8</sup>	(1.5±0.2)·10 <sup>8</sup>	(0.63±0.1)·10 <sup>8</sup>	8.0±0.2
H33258	298.15	(2.1±0.3)·10 <sup>7</sup>	(11.0±0.4)·10 <sup>7</sup>	(11.3±0.5)·10 <sup>7</sup>	(2.8±0.3)·10 <sup>7</sup>	6.2±0.2
	303.15	(3.3±0.4)·10 <sup>7</sup>	(15.2±0.5)·10 <sup>7</sup>	(16.8±0.4)·10 <sup>7</sup>	(3.4±0.2)·10 <sup>7</sup>	6.5±0.2
	308.15	(5.0±0.5)·10 <sup>7</sup>	(23.1±0.5)·10 <sup>7</sup>	(23.0±0.5)·10 <sup>7</sup>	(4.9±0.4)·10 <sup>7</sup>	7.1±0.2

Calculations show that the studied ligands with more hydrated B-form form more solid complex, than with less hydrated A-form (tabl. 1). It should be mentioned that the value of the binding parameters (binding constant (K) and parameter n which characterizes a stoichiometry of NA-ligand complex at the saturation and is equal to number of base pairs occupied by one bound ligand molecule) correlate with the data for

the binding of ligands with DNA obtained by the other authors [17, 18]. The value of the binding constant of the ligands (DX and Nt) with polynucleotide in A-form is almost an order higher at the irradiation of the latter by resonant frequencies of water structures – 64.5 and 50.3 GHz; in the case of MTX and H33258 it is higher several times, while at binding to A-form it is higher several times as well (tabl. 1). At the irradiation of the solutions of DNA and polyribonucleotides by non-resonant frequency (for example, 48.3 GHz), the thermodynamic parameters, characterizing the complex-formation of double-stranded NA with ligands in the error frameworks are unchangeable [tabl. 1 and 2]. In the work [7] at the explanation of the obtained experimental data it was assumed that at the irradiation by resonant frequencies for water structures, the hydration of both  $\text{Na}^+$  ions existing in the solution and base pairs of double-stranded DNA decreases. Consequently, it is probable that at the irradiation of NA by 50.3 and 64.5 GHz the stronger dehydration in more hydrated B-form (DNA) occurs due to which the binding of ligands with NA becomes possible to be more solid, which in turn leads to the enhancement of the binding constant at unchanged n.

**Table 2.** Thermodynamic parameters of the binding of ligands with non-irradiated and irradiated double-stranded nucleic acids

Thermodynamic parameter	Non-irradiated	Irradiated, frequency (GHz)		
		50.3	64.5	48.3
DNA-mitoxantrone				
-ΔG, kcal/mol	7.8	8.0	8.1	7.8
-ΔH, kcal/mol	7.6	8.4	8.5	7.7
ΔS, cal/mol·K	0.8	1.2	1.4	1.2
poly(G)-poly(C)-mitoxantrone				
-ΔG, kcal/mol	7.1	7.5	7.6	7.1
-ΔH, kcal/mol	6.7	7.3	7.4	6.8
ΔS, cal/mol·K	1.3	2.2	2.1	1.2
DNA-doxorubicin				
-ΔG, kcal/mol	7.8	11.3	10.8	7.9
-ΔH, kcal/mol	2.8	4.3	4.1	2.9
ΔS, cal/mol·K	16.6	20.0	18.3	16.7
poly(G)-poly(C)-doxorubicin				
-ΔG, kcal/mol	7.7	6.9	7.7	7.7
-ΔH, kcal/mol	2.6	2.7	2.8	2.6
ΔS, cal/mol·K	15.1	15.3	15.2	15.1
DNA-netropsin				
-ΔG, kcal/mol	11.8	12.9	13.0	11.9
-ΔH, kcal/mol	9.1	9.5	9.6	9.2
ΔS, cal/mol·K	8.6	11.2	11.2	8.9
poly(A)-poly(U)-netropsin				
-ΔG, kcal/mol	10.9	11.6	11.6	11.1
-ΔH, kcal/mol	9.1	9.3	9.3	9.1
ΔS, cal/mol·K	5.9	7.6	7.6	6.2
DNA-Hoechst 33258				
-ΔG, kcal/mol	11.0	11.5	11.5	11.1
ΔH, kcal/mol	6.0	6.5	6.4	5.9
ΔS, cal/mol·K	56	61	63	58
poly(A)-poly(U)-Hoechst 33258				
-ΔG, kcal/mol	10.5	11.0	11.1	10.2
ΔH, kcal/mol	8.5	9.2	9.3	8.6
ΔS, cal/mol·K	62	71	72	64

Thermodynamic parameters of the binding: changes in Gibbs free energy ( $\Delta G$ ), enthalpy ( $\Delta H$ ) and entropy ( $\Delta S$ ) were determined from analysis of Want-Hoff dependence of  $K$  on temperature. For this aim the adsorption isotherms were obtained at three temperatures, where it becomes known from the melting curves of NA-ligand complexes that NA is in double-stranded state. For non-irradiated and irradiated complexes the dependence of  $\ln K$  on  $1/T$  was constructed. In each plot through three experimental points a direct line was passed using the least square method and from this curve the values of  $\Delta H$  and  $\Delta S$  were determined [12, 13]. Values of the binding thermodynamic parameters for the investigating systems are presented in tabl. 2.

As it follows from tabl. 2, at complex-formation of intercalating and non-intercalating ligands with double-stranded NA irradiated by resonant frequencies, the enhancement of absolute value of the thermodynamic parameters of the binding ( $\Delta G$ ,  $\Delta H$  and  $\Delta S$ ) is observed as compared to non-irradiated complexes: the ligands form stronger complex with irradiated NA. Despite the fact that at the binding to irradiated NA the absolute values of  $\Delta H$  and  $\Delta S$  are being increased, the value of  $\Delta H$  is mainly being changed for intercalators (MTX and DX), and the value of  $\Delta S$  - for non-intercalators.

The obtained results indicate the perspective of elaboration of new optimal regimes of the application of anti-tumorous preparations in combination with millimeter waves.

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*Received on 09.11.2017*