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PEQULIARITIES OF COMPLEX-FORMATION OF ETHIDIUM BROMIDE AND HOECHST 33258 WITH DNA UNDER INFLUENCE OF NON THERMAL ELECTROMAGNETIC MILLIMETER WAVES

P.O. VARDEVANYAN[1], R.A. KARAPETYAN[2], A.P. ANTONYAN[1], V.P. KALANTARYAN[3]

[1]Yerevan State University, Department of Biophysics
[1]Yerevan State Medical University after Mkhitar Heratsi, the Second Department of Pediatry,
[2]Yerevan State University, Department of Microwave Radiophysics and Telecommunication
biophys_dep@mail.ru

The influence of electromagnetic waves of millimeter interval (MM EMW) on melting parameters – melting temperature and melting interval width of DNA as well as DNA-ligand complexes has been investigated in this work. It has been shown that irradiation of water-saline solutions of DNA and its complexes with ligands leads to water structure significant changes that is bound with DNA, which, in its turn, leads to significant changes of thermodynamic characteristics of DNA and DNA-ligand complexes. Moreover in case of non intercalator ligand – Hoechst 33258 (H33258) the irradiation results in disappearing of specificity to AT-sequences at high ionic strengths of solution. On the other hand, in case of intercalators – ethidium bromide (EtBr) the interaction mechanism changing is not observed, but the changes of thermodynamic characteristics take place.

Electromagnetic waves of millimeter interval – irradiation – DNA – Hoechst 33258 – ethidium bromide – melting parameters

Ուսումնասիրվել է միլիմետրային տիրույթի էլեկտրամագնիսական ալիքների (ՄՄ ԷՄԱ) ազդեցությունը ԴՆԹ-ի և ԴՆԹ-լիգանդ կոմպլեքսների հալման պարամետրերի՝ հալման ջերմաստիճանի և հալման միջակայքի լայնության վրա: Ցույց է տրվել, որ ԴՆԹ-ի և ԴՆԹ-ի հետ լիգանդների կոմպլեքսների ջրաաղային լուծույթների ճառագայթահարումը հանգեցնում է ԴՆԹ-ին կապված ջրի կառուցվածքի էական փոփոխություններին, ինչն էլ իր հերթին հանգեցնում է ԴՆԹ-ի և ԴՆԹ-լիգանդ կոմպլեքսների թերմոդինամիկ բնութագրիչների էական փոփոխություններին: Ավելին, ոչ ինտերկալյատոր լիգանդի՝ Hoechst 33258-ի (H33258) դեպքում ճառագայթահարումը հանգեցնում է AT-հաջորդականությունների նկատմամբ սպեցիֆիկության կորստին լուծույթի բարձր իոնական ուժերում: Մյուս կողմից, ինտերկալյատորների՝ էթիդիումի բրոմիդի (ԷԲ) դեպքում փոխազդեցության մեխանիզմի փոփոխություն տեղի չի ունենում, սակայն տեղի է ունենում թերմոդինամիկ բնութագրիչների փոփոխություն:

Միլիմետրային տիրույթի էլեկտրամագնիսական ալիքներ — ճառագայթահարում — ԴՆԹ — Hoechst 33258 — էթիդիումի բրոմիդ — հալման պարամետրեր

Исследовано влияние электромагнитных волн миллиметрового диапазона (ММ ЭМВ) на параметры плавления – температура плавления и ширина интервала плавления ДНК и ее комплексов с лигандами. Показано, что облучение водно-солевых растворов ДНК и ДНК-лиганд комплексов приводит к значительным изменениям структуры воды, связанной с ДНК, что в

свою очередь приводит к значительным изменениям термодинамических характеристик ДНК и ДНК-лиганд комплексов. Более того, в случае не интеркаляторного лиганда – Hoechst 33258 (H33258) облучение приводит к исчезновению специфичности к AT-последовательностям при высоких ионных силах раствора. С другой стороны, в случае интеркаляторов – бромистый этидий (БЭ), изменение механизма взаимодействия не наблюдается, однако происходит изменение термодинамических характеристик.

Электромагнитные волны миллиметрового диапазона – облучение – ДНК – Hoechst 33258 – бромистый этидий – параметры плавления

The life has been forming on Earth under the action of electromagnetic irradiation of the Sun, and any region of its spectra plays either this or another role in evolution of living organisms. On the other hand it is known that these irradiating microwaves are absorbed in air especially by water vapor. Consequently living organisms were not exposed to the effect of this irradiation and could not create protective mechanisms. The direct evidences of these assumptions are the works of academician N.D. Devyatkov's group that show either inhibition or stimulation of vital functions of organisms by microwave irradiation. It has been shown that this effect has informative but not energetic character in other words it does not depend on effect power but is determined by its parameters – frequency, penetration depth, polarization etc. Informative effects are transferred via one of the transferring systems and information treatment in organism. With development of human civilization electromagnetic fields of artificial origin were added to the electromagnetic fields of natural origin. That is why these new physical factors of environment should exhibit a significant effect on living organization of different levels and each of them should create a respective mechanism for reacting to these "new" irradiations.

Taking into account all above mentioned at present the influence of non thermal MM EMW on biological objects is being intensively investigated. One of the main conclusions of such investigations is the identity of resonance spectrum of water and human organism tissue in this frequency range. Such similarity of resonance between human organs and water in the case of extremely high frequencies electromagnetic waves points to the unique physical nature of their interaction with molecular water structures in these objects [7-9, 12]. At the same time it is shown that at low threshold of incident MM EMW there are narrow bands of resonant interaction frequencies which reflect the inner nature wave processes in these mediums [3, 4].

Since the structural and functional peculiarities of biological molecules and their interaction with each other do depend on the state of water environment, MM EMW may influence on the molecular biological processes. Particularly the degree of DNA hydration plays significant role in its interaction with ligands, because one of the stabilizing factors of the DNA-ligand complex is the hydrophobic transfer of ligand molecules from the free state to bound one [1, 2, 6, 10, 14]. Therefore the changes in the structure of the hydration shell of DNA induced by MM EMW may significantly influence on the complex-formation of classical intercalator and external binding compound with DNA. The aim of this work is to investigate the influence of MM EMW on the thermo-dynamic parameters of DNA complexes with Ethidium Bromide (EtBr) and Hoechst 33258 (H33258) at different concentrations of Na^+ of solutions (ionic strengths, μ). Obtained data showed that irradiation by MM EMW of the water-saline solutions of DNA-ligand complexes leads to the increase of thermostability of the complexes and the stabilization effect depended on the ionic strength.

Materials and methods. Calf thymus DNA and H33258 purchased from "Sigma" (USA), ethidium bromide obtained from "Serva" (Germany), HCl, NaOH, EDTA (ethylenediaminetetraacetate) were used in experiments.

All preparations were used without further purification. Concentrations were determined spectrophotometrically, using the following extinction coefficients: calf thymus DNA - ε_{260} = 6600 $M^{-1}см^{-1}$, EtBr - ε_{480} =5850 $M^{-1}cm^{-1}$, Hoechst 33258 - ε_{343}=42000 $M^{-1}cm^{-1}$. All salt-dependent experiments were conducted in citrate-phosphate buffer containing μ =0,002; 0,01; 0,02; 0.05 and 0,1 M Na^+. Doubly distilled water was used throughout.

Absorbance versus temperature profiles (melting curves) of DNA and its complexes with ligands were measured on a spectrophotometer PYE UNICAM-SP8-100 (England). DNA-ligand solutions in 1 cm-quartz curettes with hermetically closed Teflon covers placed in a thermostatically controlled cell of the spectrophotometer. Heating was performed using a temperature controller 876 SPX Series 2 (England) at a constant speed $0{,}25^0$C/min. The data of the absorption patterns were displayed on a program calculator Hewlett Packard 97S I/O (USA). Measurements were carried out with 5-fold repetitions, after which the data were averaged. The experimental error does not exceed 10-15%. Melting curves were obtained as described in [14]. Irradiation was carried out in a special glass container. The thickness of the irradiated layer solution was ≈ 1mm. As a source of millimeter wave radiation the generator of coherent EHF-oscillations G4-142 was used, operating in range of frequencies of 53.57-78.33 GHz (made in Russia). Exposure of solution was conducted at in the far-field zone of cone-shaped antenna at a distance of 250 mm from the radiating plane of the antenna in the mode of continuous generation with incident power density (IPD) at the location of the object about 50 $\mu W/cm^2$. The bottom square of the glass container corresponded to the square of the exposed zone created by the major lobe of the antenna. To eliminate the interference in the plane of exposed object, an effective multi-layer absorbent was placed between the container and the floor; therefore, the conditions of exposure were close to the free field conditions. The output power of the generator was measured by a M5-49 thermistor and a M3-10A wattmeter (Istok, Fryazino, Russia). The frequency of output signal was controlled by a CH2-25 wavemeter (Istok, Fryazino, Russia).

Experimentally we have shown the all physical changes of the cluster structure of water and DNA and DNA-ligand water-saline solutions are completed after 90 min of exposure. Therefore all experimental samples were irradiated by MM EMW of 64,5 GHz during 90 min.

Results and Discussion. It is known that water molecules bound with DNA and ligands are in great importance for complex formation of different low-molecular compounds with DNA [6]. Therefore any changes in water structure may play a significant role in the interaction between DNA and various compounds. Early obtained experimental data showed that irradiation of water and water-saline solutions by their resonant frequencies in the range of MM EMW induces their structural changes [8,9,11,16]. Therefore it can be expected that these waves may change the stability of DNA-ligand complexes. To confirm these assumptions, the melting curves of DNA and DNA complexes with EtBr and H33258 were obtained at different ionic strengths of solution (μ) and at the experimentally chosen optimal ratio of ligand/DNA=0,04.

The melting temperature T_m and the melting interval ΔT were determined from melting curves. The change of melting temperature δT_m ($\delta T_m = T_m - T_0$, where T_0 is the melting temperature of non irradiated samples (in curves 1 - non irradiated DNA, in curves 2 – non irradiated DNA-ligand complexes), T_m is the melting temperature of irradiated samples respectively) and the change of the melting interval width $\delta\Delta T$ ($\delta\Delta T = \Delta T - \Delta 0T$, where $\Delta 0T$ is the melting interval width of non irradiated samples (in curves 1 – non irradiated DNA, in curves 2 – non irradiated DNA-ligand complexes), ΔT is the melting interval width of irradiated samples respectively) were also obtained at different ionic strengths.

Fig. 1 represents the dependences of δT_m (a) and $\delta\Delta T$ (b) of DNA and DNA-H33258 complexes on μ, which shows that the shapes of the dependences of δT_m on μ for both DNA (curve 1) and DNA-H33258 complexes (curve 2) are like each other: they monotonously

decrease up to $\mu \approx 2x10^{-2}$ M Na^+ and the further increase in μ does not influence on the values of δT_m. The irradiation stabilizes the helix structure of DNA and its complexes with H33258 (T_m of irradiated samples is greater than that of non irradiated samples (tab. 1)).

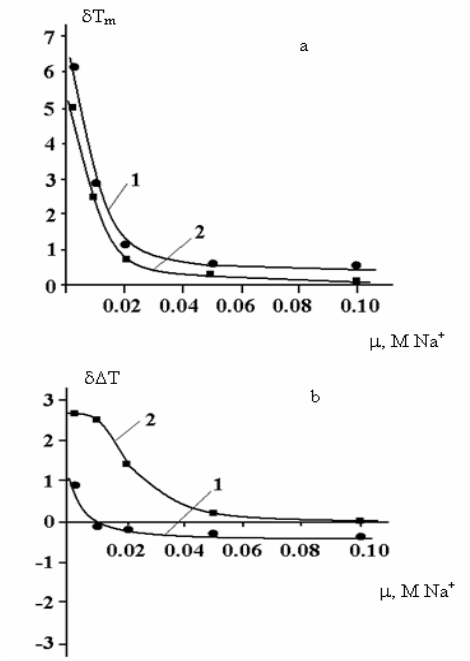

Fig.1. Dependences of δTm (a) and δΔT (b) of DNA and DNA-H33258 complexes on μ. Curves 1 correspond to DNA, curves 2 – to DNA-H33258 complexes.

Table 1. The values of T_m (^{0}C) and ΔT (^{0}C) of irradiated and non irradiated DNA and its complexes with H33258 at different ionic strengths of solution (μ_{Na+})

μ_{Na+}	DNA				DNA-Hoechst 33258			
	non irradiated		irradiated		non irradiated		irradiated	
	T_m	ΔT	T_m	ΔT	T_m	ΔT	T_m	ΔT
0.002	51.5	13.2	58,5	15.7	57.5	23.3	62.5	26
0.01	63	13.5	66.8	14	68.5	12.5	70	15
0.02	69	14.5	70.9	14.8	70	14.8	71.6	16.2
0.05	76.8	14.9	78.2	13.7	78.1	12.6	78.6	12.8
0.1	82.5	13	82.9	13	84	10	85.5	10

These experimentally obtained results made it possible to suggest that the irradiation changed the water cluster structure of hydrate shell of both DNA and DNA-H33258 complexes increasing H33258 stabilizing effect of double-stranded (ds-) structure of DNA. This assumption supported by the fact that δΔT becomes negative only at μ=0.05 and 0.1M Na^+ ionic strengths in the case of irradiated samples, while for non irradiated samples it occurs in the range of $\mu \geq 0{,}01$M Na^+ [18].

On the other hand, it is shown that at $\mu \leq 0.002$ M Na^+ the specificity of H33258 to AT sequences does not occur and possible mode of binding is intercalation: the ligand is non-specifically redistributed from melted to double strand sites in the melting interval and, therefore, ΔT increases [13].

Similar investigations were carried out for DNA-EtBr complexes. Fig. 2 shows the dependence of δT_m (a) and $\delta\Delta T$ (b) of DNA (curve 1) and DNA-EtBr complexes (curve 2) on μ and the corresponding experimental values of T_m and ΔT are presented in tab. 2.

Table 2. The values of T_m (^{0}C) and ΔT (^{0}C) of irradiated and non irradiated DNA and their complexes with EtBr at different ionic strengths of solution (μNa^+)

μ_{Na+}	DNA				DNA-EtBr			
	non irradiated		irradiated		non irradiated		irradiated	
	T_m	ΔT	T_m	ΔT	T_m	ΔT	T_m	ΔT
0.002	52.1	13.2	58,5	13.1	55.3	25.1	61.2	24.6
0.01	63.9	13.5	66.8	13.3	65.2	24.8	67.6	24.3
0.02	69.8	14.5	70.9	14.3	72.2	18.5	73.0	18.0
0.05	77.1	14.9	78.2	14.6	79.1	12.9	79.3	12.3
0.1	81.9	13	82.9	12.6	82.6	12.2	83.7	11.6

The comparison of the data presented in tab. 1 and 2 shows that δT_m of DNA-ligand complexes decreases compared with δT_m of DNA. Molecules of ligand destroy partially the hydrate shell of DNA at binding. Hence we assume that involved water molecules in hydrate shell of DNA are more ordered compared with DNA-ligand complexes, by which the greater change of Tm of DNA is conditioned. During irradiation the degree of DNA hydration increases and water molecules compete with ligand molecules in the formation of hydrogen bonds with nucleotide pairs, due to of which H33258 preferably binds by intercalation mode. In case of EtBr binding to DNA the increase of the macromolecule hydration degree leads to decreasing of entropy of complexes during irradiation, hence the main mode of ligand binding with DNA is intercalation. As a result, the melting temperature of complexes at irradiation increases compared with non irradiated samples, because at increasing of degree of hydration the contribution of hydrophobic effect at intercalation of EtBr molecules into DNA increases, that leads to the best ligand molecules are shielded from water.

The value of $\delta\Delta T$ of DNA-EtBr complexes remains practically unchanged (curve 2, fig. 2, b), while in case of DNA, this parameter decreases sharply in the range of $0.004 \leq \mu \leq 0.01$ M Na^+ (curve 1, fig. 2, b) and undergoes a slight change with further increase of ionic strength of solution ($\mu > 0.01$M Na^+). It was shown earlier that $\delta\Delta T$ of non irradiated complexes of DNA-EtBr decreases with the increase of ionic strength of solution [14, 15]. This fact takes place because at low ionic strength DNA helix is more unwound and the distance between the planes of the base pairs is greater than at high ionic strength [5, 17], making the molecule ligand easier inserted between base pairs of double stranded DNA. Under irradiation in a mentioned interval changes of ionic strength of solution the degree of hydration of DNA molecule increases, leading to unwinding of the DNA-helix and increasing the distance between base pairs along the chain, which in turn leads to alleviate the intercalation of EtBr and leveling of melting interval of complexes at different ionic strengths of solution.

Theoretical and experimental data at present, concerned to physical-chemical properties of water solutions of DNA, do not thoroughly clarify the existing discrepancy of role of water in the formation of any conformational state of nucleic acids. From this point of view the represented data reveal, that irradiation of water-saline solutions of DNA complexes with ligands leads to significant changes in water structure that is bound

with DNA, which, in its turn, leads to significant changes in the thermodynamic characteristics of DNA-ligand complexes.

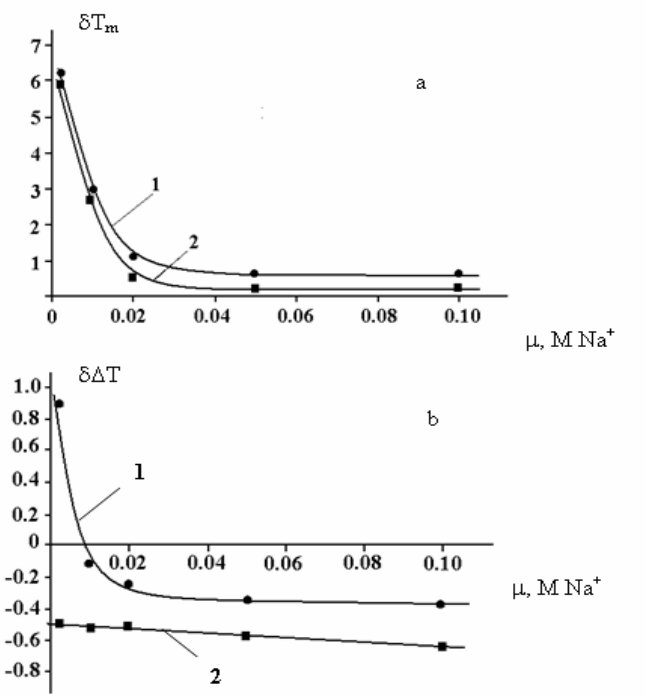

Fig. 2. Dependences of δTm (a) and δΔT (b) of DNA and DNA-EtBr complexes on μ. Curves 1 correspond to DNA, curves 2 – to DNA-EtBr complexes.

Furthermore, the effect of thermostability rising at irradiation of solutions of DNA complexes with various ligands depends on ionic strength of solution and weakens with the increasing of concentration of Na^+ ions. the irradiation of water-saline solutions of DNA-H33258 (non intercalator ligand) complexes with MM EMW results in disappearing of specificity to at-sequences at higher ionic strengths due to the changes of surrounding water structure of these sequences in DNA minor groove, which is not observed in case of non irradiated samples. However, the specificity of similar compounds to certain DNA bases may be changed not only by changing of ionic strength of solution, but also through the irradiation by MM EMW. Compared to the non intercalators, in case of intercalators (particularly EtBr) inversion of bin- ding mode is not revealed while the changes in thermodynamic characteristics are observed, that is due to the influence of water on the structure of complexes.

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