

EFFECT OF MILLIMETER RANGE ELECTROMAGNETIC WAVES ON
COMPLEX-FORMATION OF ETHIDIUM BROMIDE AND
HOECHST 33258 WITH DNA

M. A. SHAHINYAN ^{1*}, M. S. MIKAELYAN ^{1**}, M. A. TOROSYAN ^{2***}, A. T. KARAPETYAN ^{2****}

¹ *Chair of Biophysics, YSU, Armenia*

² *State University of Architecture and Construction, Armenia*

In this work, the effect of millimeter range electromagnetic waves on the melting parameters of DNA complexes with ethidium bromide (EtBr) and Hoechst 33258 (H33258) has been studied. It has been shown that the millimeter wave irradiation of water-saline solutions of DNA complexes with ligands leads to significant changes in the water structure, which in turn results in relevant alteration of the thermodynamic characteristics of DNA–ligand complexes. It has also been shown that the irradiation of the water-saline solutions of DNA–H33258 complexes with millimeter waves of 64.5 GHz frequency results in the manifestation of the ligand specific interaction at the high ionic strengths, which is not observed in the absence of irradiation. With the millimeter wave irradiation, as a consequence of increasing of the degree of DNA hydration, the interaction mode of EtBr with DNA is non-specific to sequences, but the intercalation mode does not change.

<https://doi.org/10.46991/PYSU:B/2020.54.2.125>

Keywords: millimeter range electromagnetic waves, irradiation, water resonant frequency, DNA–ligand complexes, melting parameters.

Introduction. Nowadays, millimeter range electromagnetic waves (MM EMW) are one of important environmental factors. These waves possess a number of important peculiarities making them interesting objects of research. Among these peculiarities, the fact should be mentioned that MM EMW affect biological objects at any level of organization [1–3]. On the other hand, MM EMW make biological effect, being of low-intensity and not having high permeability into biological tissues. MM EMW influences biological systems by various mechanisms; however, this question is still an arguable topic. In some cases, depending on frequency, they affect immediately, and indirectly in other cases, when tuned to the resonant frequencies of water [4–6]. From this point of view, the studies aimed at identifying the features of the influence of MM EMW on the binding of different low-molecular weight compounds (ligands) to DNA invoke great interest.

Studies of the interaction of various low-molecular weight compounds (ligands) with nucleic acids under different environmental conditions (presence of physical and chemical factors) are one of the key problems of contemporary

* E-mail: m.shahinyan@ysu.am

** E-mail: m.mikaelyan@ysu.am

*** E-mail: megi.torosyan@mail.ru

**** E-mail: karapetyanat@gmail.com

biophysics [7–12]. The structural and functional peculiarities of biological macromolecules are closely related to their surrounding aqueous environment. Therefore, it is natural to presume that EMW can affect molecular processes in cells involving biological macromolecules. Particularly, the degree of DNA hydration has a significant value when interacting with ligands, since one of the factors stabilizing the complexes is the hydrophobic transition of the ligand molecules from the free to the bound state. Changes in the structure of the hydration layers of DNA and ligands induced by MM EMW can particularly play a relevant role in the complexation process of ethidium bromide (EtBr) and Hoechst 33258 (H33258) with DNA [13, 14]. Therefore, the present study was aimed to investigate the effect of MM EMW on the melting parameters of DNA complexes with EtBr and H33258.

Materials and Methods. Calf thymus DNA (“Sigma”, USA), H33258 (“Sigma”, USA), EtBr (“Serva”, Germany), EDTA (ethylenediaminetetraacetate), NaCl, Na-citrate (ch.p.) were used in experiments. All preparations were used without additional purification. The concentrations of the used preparations were determined by absorption method, using the following coefficients of extinction: $\epsilon_{260} = 6600 M^{-1}cm^{-1}$ for DNA, $\epsilon_{480} = 5800 M^{-1}cm^{-1}$ for EtBr, $\epsilon_{343} = 42000 M^{-1}cm^{-1}$ for H33258. Experiments were carried out at the ionic strengths of the solution of 0.002, 0.01, 0.02, 0.05 and 0.1 M Na⁺.

Spectrophotometric measurements were carried out on a PYE Unicam-SP-8-100 spectrophotometer (England). Heating of the preparation solutions was performed in a PYE Unicam (England) spectrophotometer equipped with an SP 876 Series 2 temperature programme controller. For spectrophotometric measurements, quartz cuvettes with hermetically sealed Teflon caps, an optic path length of 10 mm and a volume of 3 mL were used. The absorbance values of the samples at a wavelength of 260 nm were processed and visualized in LabVIEW 6.0 program software. The melting curves were constructed as described in [15].

As a source of extremely high frequency signals, a generator G4-142 was used, operating on the basis of a reversible wave lamp, on the exit point of which there is a cone-shaped irradiating antenna. The frequency stability of the generator signal was equal to $\pm 0.05\%$, and the frequency deviation of the output signal in the continuous generation mode did not exceed 6 MHz. The irradiation of the samples was carried out at room temperature with a frequency of 64.5 GHz and a power flux density of $\sim 50 \mu W/cm^2$.

Results and Discussion. As shown earlier, the effect of MM EMW set to the resonant frequencies of water leads to changing in the structurization of water-salt solutions of DNA [16]. This means that these waves can affect the thermostability of DNA complexes with ligands, changing the state of water bound to the functional groups of these molecules. To maintain the aforementioned, the thermal melting of irradiated and non-irradiated complexes of EtBr and H33258 with DNA was carried out at various ionic strengths of the solution and a fixed optimal ratio $r = [\text{ligand}]/[\text{DNA}]$, namely $r = 0.04$ (Fig. 1). From the melting curves of non-irradiated and irradiated DNA and its complexes with ligands at different ionic strengths of the solution, the melting parameters were determined and the increments of both the melting temperature (δT_m) and the width of the melting interval ($\delta \Delta T$) were obtained (Table). The increments were calculated as follows: $\delta T_0 = T_0^{\text{irrad.}} -$

$T_0^{\text{non-irrad.}}$, where $T_0^{\text{irrad.}}$ and $T_0^{\text{non-irrad.}}$ are the melting temperatures of irradiated and non-irradiated DNA–ligand complexes, respectively. On the other hand, $\delta\Delta_0T = \Delta_0T^{\text{irrad.}} - \Delta_0T^{\text{non-irrad.}}$, where $\Delta_0T^{\text{irrad.}}$ and $\Delta_0T^{\text{non-irrad.}}$ are the widths of the melting intervals of irradiated and non-irradiated DNA–ligand complexes, respectively.

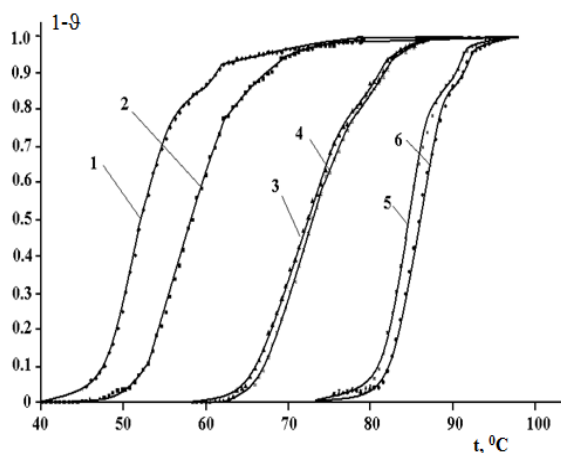


Fig. 1. Melting curves of non-irradiated and irradiated DNA at an ionic strength of 0.002 M (1 and 2), complexes of DNA with EtBr at the ionic strength 0.02 M (3 and 4), complexes of DNA with H33258 at the ionic strength 0.05 M (5 and 6), $r = 0.04$. Curves 1, 3, 5 correspond to non-irradiated and 2, 4, 6 to irradiated samples.

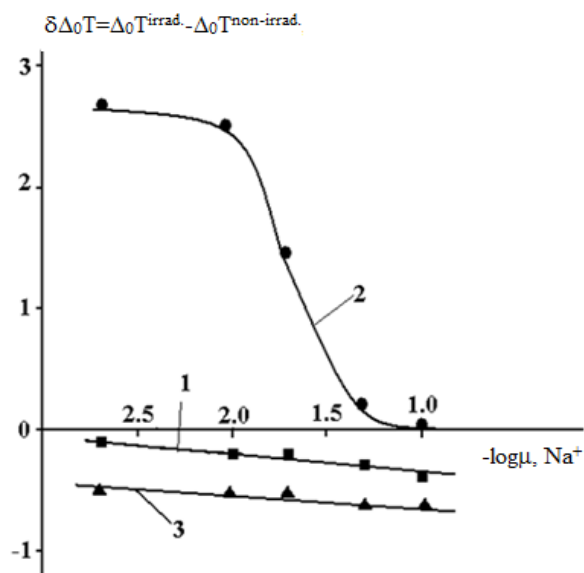
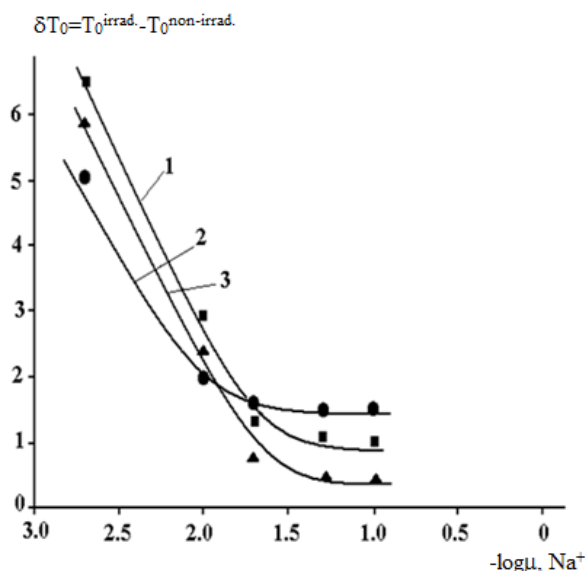
The dependencies of the increments in the melting temperature of DNA and complexes of DNA with H33258 and EtBr on the negative logarithm of the solution ionic strength at $r = 0.04$ are presented in Fig. 2.

Values of the increments in the melting temperature and the width of the melting interval of irradiated and non-irradiated DNA and its complexes with EtBr and H33258 at various ionic strengths of the solution

Ionic strength, M	DNA		DNA–EtBr		DNA–H33258	
	δT_0	$\delta\Delta_0T$	δT_m	$\delta\Delta T$	δT_m	$\delta\Delta T$
0.002	6.5	–0.1	5.9	–0.5	5.0	2.7
0.01	2.9	–0.2	2.4	–0.5	1.5	2.5
0.02	1.1	–0.2	0.8	–0.5	1.6	1.4
0.05	1.1	–0.3	0.7	–0.6	1.5	0.2
0.1	1.0	–0.4	0.4	–0.6	1.5	10.0

As seen from Fig. 2, all curves behave in the same way: they decrease monotonically with an increase in the ionic strength up to 0.0004 M , but the further increase in the salt concentration does not affect the value of δT_0 . MM EMW irradiation of water-salt solutions leads to an enhancement of T_0 of DNA and its complexes with H33258 as compared to those of non-irradiated samples (Table). This is a consequence of the stabilization of the samples under the influence of MM EMW. Most probably, this is due to the fact that MM EMW irradiation of water-salt solutions of DNA and DNA–ligand complexes results in change in water structure. In the case of H33258, among the factors, stabilizing DNA ds-structure, mention may be made of hydrogen bonds that are formed between nucleotide bases and ligand molecules. Upon irradiation, the degree of DNA hydration increases, and water

molecules start to compete with ligand molecules to form hydrogen bonds with nucleotide pairs, due to which H33258 binds preferentially by intercalation. Consequently, one can consider the increase in T_0 of the irradiated samples as resulting from the enhancement of the water structurization in the local surrounding of DNA and its complexes with H33258. This leads to an increase in the size of the hydration shell of the macromolecule, which, in turn, results in the rising of the stabilizing effect of H33258 on the DNA ds-structure.



This is also indicated by the fact that the dependence of $\delta\Delta T$ does not show any relevant changes at the ionic strengths of 0.05 and 0.1 M in the case of the irradiated samples (Fig. 3, curve 2), while for the non-irradiated samples the values of $\delta\Delta T$ are negative at 0.01 M (Fig. 1, curves 3, 4). Analogous studies were carried out for the DNA–EtBr complexes as well. The comparison of the data presented in Table shows that, upon irradiation, the value of δT_0 for the DNA–EtBr complexes increases, as in the case for H33258. Apparently, it is due to the fact that, upon binding of EtBr to DNA and irradiation with MM EMW, an increase in the degree of DNA hydration leads to a decrease in the entropy of the complexes, and that is why the intercalation is the main mode of complex-formation, due to which $\delta\Delta T$ practically does not change (Fig. 3, curve 3). It was shown earlier that $\delta\Delta T$ of the non-irradiated complexes of DNA with EtBr decreases with increasing ionic strength. This is explained by the fact that at low ionic strength, in comparison with cases with high ionic strength, the DNA helix is in a more untwisted state, and the distances between the planes of base pairs are greater [17, 18]. As a result of this fact, the ligand stabilizes the ds-structure, being inserted into base pairs of ds-DNA. Under MM EMW irradiation at the given values of the ionic strength, the degree of hydration of DNA molecule increases, which makes the melting interval of the complexes independent of the ionic strength of the solution.

Conclusion. Thus, it can be concluded that the millimeter wave irradiation of water-saline solutions of DNA complexes with ligands leads to relevant alterations in water structure, which in turn lead to significant changes in the thermodynamic characteristics of DNA–ligand complexes. The irradiation of the water-saline solutions of DNA–H33258 complexes with millimeter waves of frequency 64.5 GHz results in the manifestation of the ligand specific interaction at high ionic strengths, which is not observed in the absence of the irradiation. This is connected with the structural changes in the water environment of AT-regions in the minor groove. MM EMW irradiation with a frequency of 64.5 GHz does not affect the width of the melting interval of DNA–EtBr complexes. As a result of an increase in the degree of DNA hydration and a decrease in the entropy of the complexes, the interaction mode of EtBr with DNA is not sequences specific, but the intercalation mode does not change.

Received 09.03.2020

Reviewed 23.07.2020

Accepted 27.07.2020

REFERENCES

1. Zhang M., Li X., Bai L., et al. Effects of Low Frequency Electromagnetic Field on Proliferation of Human Epidermal Stem Cells: An *in vitro* Study. *Bioelectromagnetics* **34** (2013), 74–80. <https://doi.org/10.1002/bem.21747>

2. Zhao R., Zhang S., Xu Z., et al. Studying Gene Expression Profile of Rat Neuron Exposed to 1800 MHz Radiofrequency Electromagnetic Fields with cDNA Microassay. *Toxicology* **235** (2007), 167–175.
<https://doi.org/10.1016/j.tox.2007.03.015>
3. Kalantaryan V.P., Babayan Y.S., Gevorgyan E.S., et al. Influence of Low Intensity Coherent Electromagnetic Millimeter Radiation (EMR) on Aqua Solution of DNA. *Prog. Electromagn. Res. Lett.* **13** (2010), 1–9.
<https://doi.org/10.2528/PIERL09110605>
4. Reshetnyak S.A., Shcheglov V.A., Blagodatskikh V.I., et al. Mechanisms of Interaction of Electromagnetic Radiation with a Biosystem. *Laser Physics* **6** (1996), 621–653.
5. Shahinyan M.A., Antonyan A.P., Kalantaryan V.P., Mikaelyan M.S., Vardevanyan P.O. Study of the Influence of Millimeter Range Electromagnetic Waves on Methylene Blue Complexes with Human Serum Albumin. *J. Electromagn. Waves Appl.* **33** (2019), 2317–2327.
<https://doi.org/10.1080/09205071.2019.1677508>
6. Devyatkov N.D., Golant M.B., Betskii O.V. Millimeter Waves and Their Role in the Processes of Vital Activity. Radio i Svyaz, Moscow, 1991, 168 p. (in Russian).
7. Vardevanyan P.O., Antonyan A.P., Parsadanyan M.A., et al. Peculiarities of Interaction of Synthetic Polyribonucleotide Poly(rA)-Poly(rU) with Some Intercalators. *J. Biomol. Struct. Dyn.* **36** (2018), 3607–3613.
<https://doi.org/10.1080/07391102.2017.1402708>
8. Antonyan A.P., Parsadanyan M.A., Torosyan M.A., Karapetyan A.T. Hoechst 33258 Compound Interaction with Different GC-content DNA. *Proc. YSU B: Chem. Biol. Sci.* **53** (2019), 107–112.
<https://doi.org/10.46991/PYSU:B/2019.53.2.107>
9. Parsadanyan M.A. Comparative Study of Ethidium Bromide Interaction with Synthetic Polynucleotides ds-Poly(rA)-Poly(rU) and ds-Poly(dA)-Poly(dT), *Proc. YSU B: Chem. Biol. Sci.* **53** (2019), 175–179.
<https://doi.org/10.46991/PYSU:B/2019.53.3.175>
10. Żurek-Biesiada D., Waligórski P., Dobrucki J.W. UV-induced Spectral Shift and Protonation of DNA Fluorescent Dye Hoechst 33258. *J. Fluoresc.* **24** (2014), 1791–1801.
<https://doi.org/10.1007/s10895-014-1468-y>
11. Vekshin N. Binding of Hoechst with Nucleic Acids Using Fluorescence Spectroscopy. *J. Biophys. Chem.* **2** (2011), 443–447.
<https://doi.org/10.4236/jbpc.2011.24052>
12. Streltsov S.A., Zhuze A.L. Hoechst 33258–poly(dG-dC)·poly(dG-dC) Complexes of Three Types. *J. Biomol. Struct. Dyn.* **26** (2008), 99–113.
<https://doi.org/10.1080/07391102.2008.10507228>
13. Kiser J.R., Monk R.W., Smalls R.L., Petty J.T. Hydration Changes in the Association of Hoechst 33258 with DNA. *Biochemistry* **44** (2005), 16988–16997.
<https://doi.org/10.1021/bi051769x>
14. Kashpur V.A., Khorunzhaya O.V., Pesina D.A., et al. Hydration Effects Accompanying the Formation of DNA Complexes with Some Ligands. *Biophysics* **62** (2017), 31–37.
<https://doi.org/10.1134/S0006350917010092>
15. Vardevanyan P.O., Antonyan A.P., Hambardzumyan L.A., et al. Thermodynamic Analysis of DNA Complexes with Methylene Blue, Ethidium Bromide and Hoechst 33258. *Biopolym. Cell* **29** (2013), 515–520.
<http://doi.org/10.7124/bc.000843>
16. Babayan Y.S., Hakobyan S.N., Ghazaryan R.S., et al. Some Physicochemical Properties of DNA, Irradiated by Low-energetic, Millimeter Range, Coherent Electromagnetic Waves.. *Biomedical Technologies & Radio Electronics* (2006), no.11, 64–68. (in Russian)
17. Saenger W. Principles of Nucleic Acid Structure. Springer-Verlag, New York (1984), 556 p.
<https://doi.org/10.1007/978-1-4612-5190-3>
18. Cantor C.R., Schimmel P.R. Biophysical Chemistry. W.H. Freeman & Co., San Francisco (1980), 3 Volumes: Part I, 341 p.; Part II, 503 p.; Part III, 522 p.

Մ. Ա. ՇԱՀԻՅԱՆ, Մ. Ս. ՄԻՔԱՅԵԼՅԱՆ, Մ. Ա. ԹՈՐՈՍՅԱՆ, Ա. Թ. ԿԱՐԱՊԵՏՅԱՆ

ՄԻԼԻՄԵՏՐԱՅԻՆ ՏԻՐՈՒՅԹԻ ԷԼԵԿՏՐՈՄԱԳՆԻՏԱԿԱՆ ԿՈՄՔԵՍՆԵՐԻ
ԱԶԴԵՑՈՒԹՅՈՒՆԸ ԴՆԹ-Ի ՀԵՏ ԷԹԻԴԻՈՒՄԻ ԲՐՈՄԻՍՏՈՒ ԵՎ
HOECHST 33258-Ի ԿՈՄՊԼԵՔՍԱԳՈՅՑՄԱՆ ՎՐԱ

Աշխատանքում ուսումնասիրվել է միլիմետրային տիրույթի էլեկտրամագնիսական ալիքների ազդեցությունը ԴՆԹ-ի հետ էթիդիումի բրոմիդի (ԷԲ) և Hoechst 33258-ի (H33258) կոմպլեքսների հալման պարամետրերի վրա: Ցույց է տրվել, որ ԴՆԹ-ի հետ լիզանդների կոմպլեքսների ջրա-աղային լուծույթների ճառագայթահարումը միլիմետրային ալիքներով հանգեցնում է ջրի կառուցվածքի էական փոփոխությունների, ինչն իր հերթին բերում է ԴՆԹ-լիզանդ կոմպլեքսների թերմոդինամիկական բնութագրերի զգալի փոփոխությունների: Ցույց է տրվել նաև, որ ԴՆԹ-H33258 կոմպլեքսների ջրա-աղային լուծույթների ճառագայթահարումը 64.5 ԳՀց հաճախությամբ միլիմետրային ալիքներով բարձր իոնական ուժերի պարագայում հանգեցնում է լիզանդի սպեցիֆիկ փոխազդեցության ի հայտ գալուն, ինչը չի դիտվում ճառագայթահարման բացակայության դեպքում: Միլիմետրային ալիքներով ճառագայթահարման դեպքում, ԴՆԹ-ի հիդրատացման աստիճանի բարձրացման արդյունքում ԴՆԹ-ի հետ ԷԲ-ի փոխազդեցության եղանակը ոչ սպեցիֆիկ է հաջորդականությունների նկատմամբ, իսկ ինտերկալացիան չի փոխվում:

М. А. ШАГИНЯН, М. С. МИКАЕЛЯН, М. А. ТОРОСЯН, А. Т. КАРАПЕТЯН

ВЛИЯНИЕ ЭЛЕКТРОМАГНИТНЫХ ВОЛН МИЛЛИМЕТРОВОГО
ДИАПАЗОНА НА КОМПЛЕКСООБРАЗОВАНИЕ БРОМИСТОГО
ЭТИДИЯ И НОЕЧНСТ 33258 С ДНК

В работе исследовано влияние электромагнитных волн миллиметрового диапазона на параметры плавления комплексов ДНК с бромистым этидием (БЭ) и Hoechst 33258 (H33258). Показано, что облучение миллиметровыми электромагнитными волнами водно-солевых растворов комплексов ДНК с лигандами приводит к существенным изменениям в структуре воды, что, в свою очередь, приводит к значительным изменениям термодинамических характеристик комплексов ДНК-лиганд. Показано также, что облучение водно-солевых растворов комплексов ДНК-H33258 миллиметровыми волнами с частотой 64.5 ГГц приводит к появлению специфического взаимодействия лиганда при высоких ионных силах, что не наблюдается в отсутствие облучения. При облучении миллиметровыми волнами, вследствие увеличения степени гидратации ДНК, способ взаимодействия БЭ с ДНК является неспецифическим к последовательностям, однако интеркаляция не меняется.