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# INFLUENCE OF LOW INTENSITY COHERENT ELECTROMAGNETIC MILLIMETER RADIATION (EMR) ON AQUA SOLUTION OF DNA

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**Abstract**—The thermostability and density of water-salt solutions of DNA, irradiated by non thermal coherent millimeter electromagnetic waves with frequency 64.5 GHz have been investigated using the methods of spectrophotometry and densitometry. It is shown that the thermostability of DNA and density of its solutions are increased, depending on time of irradiation. It is expected that under the influence of millimeter electromagnetic radiation the hydration of DNA and ions of $Na^+$ that are present in solution decrease. As a result, the physicochemical characteristics of DNA are changed.

## 1. INTRODUCTION

At present, the low-power electromagnetic millimeter waves (MMWs) are widely used in biology, medicine, radio- and telecommunications, in various electronic devices. For example, during last years the MMWs were used along with anticancer drugs in the chemotherapy of tumor

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of the laboratory animals. The experiments proved that without reducing the effect of anticancer drugs, it became possible to reduce adverse toxic reaction of the organism to the drugs. However, the mechanisms of low-intensity MMWs action on biological object still remain unclear [5, 6, 8].

Currently, the concept that non-thermal effect of MMWs on biological systems is determined by the effect of MMWs on water, causing changes of bound water properties is rather acknowledged. It is known that electromagnetic radiation of extremely high frequensies (EHF) of 30–300 GHz barely penetrates living organisms and biological tissues. The EHF electromagnetic radiation is nonionizing. The energy of quanta of low-power (non-thermal) EHF radiation is less than the energy of heat motion of atoms and moleculs and appreciably less than the energy of hydrogen bonds in living organisms.

Numerous effects of influence of low intensity extra high frequency electromagnetic radiation on the level of human organism and animals, bacteria, as well as on prokaryotic and eukaryotic cells are revealed [9, 13]. The existence of selective frequency dependent effects of MMWs has been shown along with occurrence of non selective, frequency independent influence [6, 11]. At present, the concept that non- thermal influence of MMWs on biosystems is being mediated by influence of water has got confirmation [7, 11]. Thus, the research of influence MMWs on structure and physical and chemical properties of water and water solutions of biological objects is essential. It is shown that rather weak physical influence including the MM-waves, impacts the properties of water solutions due to change of structure of water in an environment of the dissolved substances. Moreover, it is revealed, that functional changes of biological objects occur at irradiation of solutions of MM-waves [12].

At present, the animal and plant life is constantly being subjected to the low intensity microwave radiation (mobile phones, antennas, space communication, household appliances, etc). At the same time, the resonance frequencies of oscillations of molecular structures of biological tissues and molecular structures of water are in that frequency range too. Therefore, during their vital activity, the living organisms are constantly subjected to those electromagnetic fields influences and investigating the effect of electromagnetic waves of that range on water and biological systems could prove useful in developing certain mechanisms of protection from them.

The purpose of the work is to investigate the physical properties of DNA solutions irradiated by low-energy coherent electromagnetic MMWs with frequency of 64.5 GHz, in correspondence with resonance frequency of oscillations of hexagonal structures of water [11].

## 2. MATERIALS AND METHODS

In this work, DNAs from Calf thymus, rat liver and tumour sarcoma 45 (S-45) have been used. All samples of DNA studied in a standard citrate-salt solution 0.1 × SSC (1 × SSC = 0.15 M NaCl + 0.015 M Na-citrate), at presence of $10^{-5}$ M EDTA, pH = 7.3, ionic strength of solution is $[Na^{+}] = 0.0195$ M. DNA from a liver of rats and from tumor S-45 have been allocated at the Institute of Fine Organic Chemistry (IFOC) (Armenia). The contents of proteins in DNA preparations of a liver of healthy animals (h-DNA) was 1.5 ± 0.2%, and in DNA from S-45 (s-DNA) was 1.3 ± 0.2%, that implies to pure DNA preparation standards.

Maxima of absorption of DNA at melting are received on spectrophotometer PYE UNICAM-SP8-100 (England), in thermostatic cells. At DNA melting quartz cuvettes hermetically closed by teflon corks, by length of an optical way 1 cm were used. Melting of DNA was carried out at continuous heating DNA solutions with help of programmed device Temperature Controller SP 876 (England), with a speed of 0.25 grade/minutes. Measurements were carried out in an interval of change of temperature $25^{\circ}\text{C} \leq T \leq 95^{\circ}\text{C}$. Values of absorption were deduced on the monitor of micro calculator Hewlett Packard 97S I/O. Melting of each sample of DNA repeated 6 times. Melting curves were constructed as it is described in work [14]. Melting parameters — Temperature $T_m$ and width of melting interval $\Delta T$ of each DNA were averaged on six measurements.

On the basis of the normalized integral melting curves the method of numerical differentiation receives differential melting curves (DMC) as it is described in work [3]. Density of water, 0.1 × SSC and DNA solutions with sensitivity was determined on dencitometer DMA 4500 Anton Paar (USA), with resolutions $10^{-5}$ g/cm$^3$.

The irradiation of solutions by MM-waves was carried out in a special glass bulb. Solutions from above are closed by transparent fine chlorvinil membranula for radiation. Thickness of the irradiated sample did not exceed 1 mm. For irradiation of MMWs source of extra high frequencies was applied, generator G4-142 (Russian made), working on the basis of a back wave lamp. On an output of the generator there was a cone-shaped radiating antenna. Stability of frequency of the generator was ±0.05%. The irradiation of samples was carried out at room temperature, in a mode of amplitude modulation of frequency of 1 Hz, incident power density on a sample at frequency of 64.5 GHz was $\sim 50\,\mu\text{W/cm}^2$, the sample was placed in the far zone of antenna.

## 3. RESULTS AND DISCUSSION

The research was conducted on the exposure of MMWs on the thermostability of DNA, purified from the liver of healthy rats (h-DNA) and Sarcoma tumor 45 (S-45) in water solutions. The irradiation of the samples was carried out at room temperature, the power flux density at frequency of 64.5 GHz was $\sim$ 50 microwatt/cm$^2$. The DNA water solutions, prepared for spectrophotometer measurements, have been irradiated for 30, 40, 60, 90, 120 min. respectively. The melting curves have been obtained both, just after irradi curve of melting, magnitudes of $T_m$ and $\Delta T$ do not depend on the storing time of the irradiated DNA.

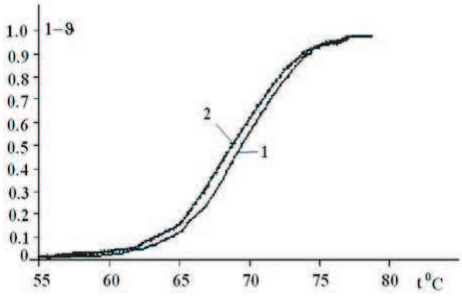


**Figure 1.** Melting curvesof DNA from sarcoma 45 irradiated for 90 min (1) and non-irradiated (2).

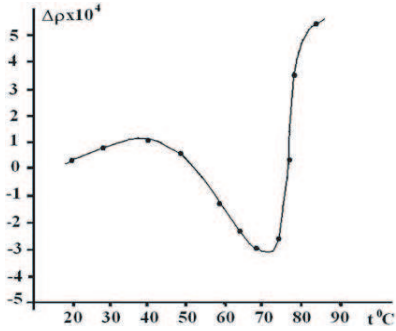


**Figure 2.** Curve of dependence of irradiated for 90 min and non-irradiated DNA densities on temperature.

Figure 1 shows melting curves of (1) irradiated during 90 min., and (2) non-irradiated S-45 DNA and h-DNA respectively. Depending on

**Table 1.** Temperature and range of DNA melting obtained from of healthy rats liver and tumor sarcoma 45.

| Time of irradiation, min. | h DNA | | S-45 DNA | |
|---|---|---|---|---|
| | $T_m$, °C | $\Delta T$, °C | $T_m$, °C | $\Delta T$, °C |
| 0 | 69.4 ± 0.1 | 7.2 ± 0.2 | 68.8 ± 0.2 | 7.9 ± 0.2 |
| 30 | 69.4 ± 0.1 | 7.2 ± 0.2 | 68.9 ± 0.1 | 7.9 ± 0.2 |
| 40 | 69.5 ± 0.2 | 7.1 ± 0.2 | 69.0 ± 0.1 | 7.8 ± 0.2 |
| 60 | 69.9 ± 0.1 | 7.0 ± 0.2 | 69.8 ± 0.1 | 7.8 ± 0.2 |
| 90 | 70.3 ± 0.2 | 7.2 ± 0.2 | 70.2 ± 0.2 | 7.6 ± 0.2 |
| 120 | 70.4 ± 0.2 | 7.0 ± 0.2 | 70.2 ± 0.2 | 7.5 ± 0.2 |

the time of irradiation the thermostability of DNA is being increased, and this is more obvious for S-45 DNA (Figure 1).

Upon irradiation of 90 min. the $T_m$ of a solution of hDNA is being increased up to $\sim 1°C$ and $T_m$ of S-45 DNA approximately up to 1.5°C; while the changes of $\Delta T$ remain within experimental errors ($\Delta T$ is being slowly decreased as a result of irradiation). The data on $T_m$ and $\Delta T$ of DNA from the liver of healthy rats and S-45, without treatment as well as irradiated during 90 minutes is shown in Table 1 [2].

The stronger changes of melting parameters observed for DNA of S-45 DNA is probably based on the structural changes of this DNA in comparison with hDNA, as a result of which the hydration of S-45 DNA in certain hypermethylated parts may significantly differ from the hydration of other parts of DNA [1, 4, 10].

Structural changes of bound water may be explored also by measuring of changes of DNA solution density as a result of irradiation. The experiments showed that as a result of irradiation of a buffer and DNA solution by frequency 64.5 GHz resonant for water, the buffer density and DNA solutions tend to increase.

The dependence of density of solution DNA on temperature at an irradiating duration of 90 and 120 minutes is shown on Figure 2 in order to revealing changes in water environment at an irradiating dependant on temperature. It is shown that as temperature increases the density irradiated and non-irradiated DNA decreases. However, there is essential difference between solutions irradiated and non-irradiated DNA. Dependence of changes of density-$\Delta\rho$ on $T$ where $\Delta\rho$ is a difference of density of solutions irradiated and non-irradiated DNA is shown on Figure 2.

The dependence of poorly increases in an temperature interval $20 \leq T \leq 40°C$ while sharp decrease of $\Delta\rho$ is observed in an interval $40 < T \leq 70°C$. Sharp decrease of density of DNA upon radiation occurred, whereas density of non-radiated DNA does not change significantly. At $T > 70°C$ density of radiated DNA increased that is caused by the rises of dependence curve of $\Delta\rho$.

The changes in values of radiated and non-radiated DNA's densities are summarized in Table 2.

Usually, upon research of influence of different factors on base pairs of DNA, it is necessary to study the influence of the external factor on DNA from different sources with different GC content. In a number of cases these differences may be insignificant and are within experimental uncertainty.

To avoid systematic errors, one may consider the influence of the studied factor with different GC content on the certain parts of the same DNA, and only after that one may guess about electoral influence

**Table 2.** Magnitude of solutions density before and after exposure of MMEMW.

| Time of irradiation, min. | Buffer | Buffer + DNA |
|---|---|---|
| 0 | 0.999201 ± 0.000005 | 0.999232 ± 0.000004 |
| 30 | 0.999220 ± 0.000005 | 0.999242 ± 0.000005 |
| 60 | 0.999241 ± 0.000004 | 0.999269 ± 0.000004 |
| 90 | 0.999253 ± 0.000004 | 0.999291 ± 0.000005 |

of the given factor.

Figure 3 shows the Differential Melting Curve (DMC) of calf thymus DNA and its dissolution into 5 Gauss components.

On the DMC of DNA each gauss can be presented as determined parts of DNA with an average GC-content. Thus, upon the research of the behavior of these parts under the influence of studied factors, based on the received data, one may consider the selective effect on AT and GC-nucleotide pairs of DNA. As a result of a large overlapping the maximums of first two components as per temperature scale ($T$) cannot be determined. That is why even insignificant change of DMC (within experimental errors) can lead to the dissolution with maximums under other temperatures. The divided third, fourth and fifth components can be used as parts with quasi-random distribution of nucleotide pairs with an average GC-content. One may get the average of GC-content ($x$) of the given part of DNA by using the following formula

$$T_m = 82.1 + X \cdot (40 - 1.4 \cdot \lg[\mathrm{Na}^+] + 17.3 \cdot \lg[\mathrm{Na}^+])$$

and taking into consideration melting temperature ($T_m$) of the components. The calculations prove that for the mentioned peaks the average GC-content is equal accordingly to:

$$X_3 = 0.5; X_4 = 0.58 \text{ and } X_5 = 0.66$$

The method of dissolution of the DMC DNA is used to determine exposure of MMWs of low rate on the base pairs of DNA. It was proved that the exposure of MMWs on DNA leads to the stability of DNA. The MMWs of 64.5 GHz have been absorbed into water, as a result of which the hydration of nucleotide pairs has been changed. Figure 4 shows dependence of studied components $T$ on the time of irradiation. As seen from the Figure 4.

$T$ increases, which is more for AT-rich blocks. Consequently, by changing the structure of bound water the MMWs can differently affect the changes of thermostability of AT- and GC-nucleotide pairs.

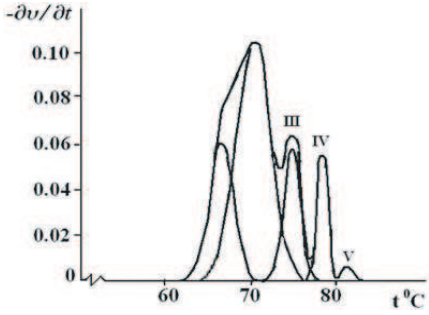


**Figure 3.** Differential Melting Curve (DMC) of calf thymus DNA and its dissolution into 5 Gauss components.

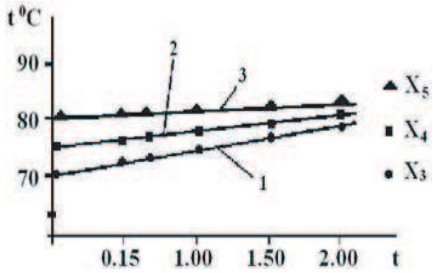


**Figure 4.** Dependence of melting temperature on 3rd (1), 4th (2) and 5th (3) components of DMC of DNA on time of irradiation.

By summarizing the above mentioned data one may assume that MMWs selectively affects nucleotide pairs. The proposed approach has certain advantages compared with other methods, since it does not require experimenting with DNA of different GC content. Just upon the direct experiment of DNA of the given organism it is possible to estimate the contribution of this or that factor to the changes of thermostability of different nucleotide pairs.

## 4. CONCLUSION

On the bases of obtained results following conclusions were made:

It has been shown that stronger change of melting parameters of S-45 DNA in comparison with h-DNA is probably based on the structural changes of this DNA, as a result of which the hydration of S-45 DNA in certain hypermethylated parts can significantly differ from the hydration of other parts of DNA.

It has been shown that the density of irradiated during denaturation DNA sharply increased in the range of temperature $40 \leq T \leq 75°\mathrm{C}$ comparing with non-irradiated DNA while at $T > 75°\mathrm{C}$ this parameter for irradiated DNA sharply increase.

It has been obtained that melting temperature of AT-rich blocks of irradiated DNA — increases more than for GC-blocks. Consequently, by changing the structure of bound water the MMWs can differently affect the changes of thermostability of AT- and GC-nucleotide pairs.

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