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Original Research Article

Study on DNA conformational transition affected by extremely low frequency magnetic field

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ABSTRACT

Increased application of electrical devices and consequent expansion of electrical distribution network, expose us to extremely low frequency electromagnetic fields (ELF-EMF) more than ever. Mechanisms of action of ELF-EMFs on cells and biomolecules are not well understood. Presence of specific sequence for responsiveness of DNA to ELF-EMF exposure are demonstrated by several *in vivo* studies. In the present study, possibility of conformational transition and denaturation of double-stranded oligonucleotides with different sequences which exposed to ELF-EMF were examined by circular dichroism (CD) spectroscopy. The data obtained from this study did not provide any evidence in support of significant change in double-stranded oligonucleotides conformation.

***Keywords*:** ELF-EMF, DNA, promoter, circular dichroism, cancer, HSP 70

INTRODUCTION

Electromagnetic radiation propagates with the same frequency for magnetic and electrical radiation in the space. The spectrum of electromagnetic is a range including all possible electromagnetic frequencies from extremely low frequency (ELF) fields to greatly high frequency radiations [1]. Frequencies lower than 3000 Hz are considered ELF electromagnetic fields (EMF) [2]. World health organization

(WHO) has accounted ELF-EMFs as a possible cancerous factor (B2 category) and there are some meta-analysis studies that have proposed connection between ELF-EMFs and cancer [3-5].

HSP 70 protein families are kind of molecular chaperons in the cells. Expression of the proteins are raised in response of decreasing of oxygen concentration [6], increasing of cadmium ions and pesticides concentration, adenoviral infections [7] and several other stress factors. It is proposed that increased level of HSP 70 proteins is a strategy for resistance to chemotherapy in the cancerous cells [8].

HSP 70 expression as a consequent of heat shock puff activation in drosophila melanogaster salivary glands [9] and increased level of SSA1 expression in *saccharomyces* cerevisiae [10] that it equal to HSP 70 in human reported after exposure to ELF-EMFs. It is shown that accumulation of HSP 70 protein [11] and expression of HSP 70 gene [11,12] affected by ELF-EMF in Hela cells. A plasmid contains specific ELF-EMF responsive region of promoter of HSP 70 gene which located before luciferase gene, electrotransferred into quadriceps muscles of BALB/c mice. After exposure of the mice to ELF-EMF, luciferase gene expressed significantly [13]. It was reported that responsiveness of HSP 70 promoter of HeLa cells to ELF-MF depends on presence of C-MYC protein and three motifs CTCT at -160, -230 and -165 [14,15] of the promoter. Additionally the presence of motifs CTCT in c-myc promoter in response to ELF magnetic field (MF) was shown [16,17].

Purines and pyrimidines cycles absorb ultra violet radiation at 240-260 nm strongly. Nucleotide pairing and base-stacking perform essential roles in the ultra violet (UV) absorption [18] and consequently, sequence and conformation of DNA have significant impact on UV absorption of DNA. Circular dichroism (CD) is an extremely sensitive and valuable tool to study conformational transition of DNA molecules. Furthermore, the method is appropriate to study DNA oligonucleotides at very low concentrations with high accuracy [19].

As regards significancy of motifs CTCT for responsiveness of DNA to ELF-MF and support of theoretical hypothesis of direct interaction of ELF-MF with electrons in DNA molecules was shown [20]. In this study, effects of such fields on conformation and denaturation of double-stranded DNA oligonucleotides with different sequences were examined.

MATERIALS AND METHODS

Oligonucleotides

DNA oligonucleotides were designed with following sequences:

Double-stranded oligonucleotide A : 5'-CCCCTGGACTCTTTCTGAGC-3'

Double-stranded oligonucleotide B :5'-CGCGCGCGCGCGCGCGCGCG-3'

Double-stranded oligonucleotide C : 5'-AAAAAAAAAAAAAAAAAAA-3'

All oligonucleotides were synthesized by Shanghai Generay Biotech company and provided as lyophilized powder.

Study of oligonucleotides by CD

To prepare stock solutions, appropriate amount of Tris-EDTA (TE) buffer was added to DNA lyophilized powder. Two samples with volumes of 400 µl prepared from stock solutions for each sequence of DNA. Samples labeled A, B and C represent the sequence of oligonucleotides. Final oligonucleotide concentration for each samples were 100 µg/ml and pH 8. Both samples of each sequence were exposed simultaneously under same ELF-MF, one of them for 5 min (labeled as index 1) and the other for ten min (labeled as index 2). Samples transferred into cuvette of CD spectroscopy immediately after exposure. Cuvette were washed with distilled water before and after each experiment.

Magnetic field exposure system

Helmholtz coil designed and composed of lacquered wire wound 34 times around a circle form 7.5 cm diameter and 5 cm height. The coil connected to a 12-volt power transformer from one side and to a 5.1-ohm resistor from other side. Power transformer connected to an AC voltage source (220 V, 50 Hz). According to calculation based on magnetic field equation ($B = (\mu\, I\, N)/2a$), the magnetic field is delivered as a continuous sinusoidal wave at an amplitude of 680 µT and an frequency of 50 Hz.

RESULTS

Comparative spectra represented sample A1 and A2 before and after exposure to the ELF-MF for 5 and 10 min were depicted in figures 1 and 2, respectively. The comparative spectra illustrated no significant difference, CD spectra were decreased near 190 nm after 10 min exposure to the ELF-MF.

Figures 3 and 4 depicted comparative spectra reflect samples B1 and B2 that exposed to ELF-MF about 5 and 10 min, respectively. The spectra of exposed samples remained without any significant alteration compare with the spectrum of blank sample.

Sample C1 exposed to ELF-MF for 5 min, sample C2 for 10 min and the comparative spectra of the samples have depicted in figures 5 and 6, respectively. Although there

were slightly alteration between spectra of sample C1 before and after exposure to ELF-MF at regions near 220 nm and 240 nm, significant alteration did not observed.

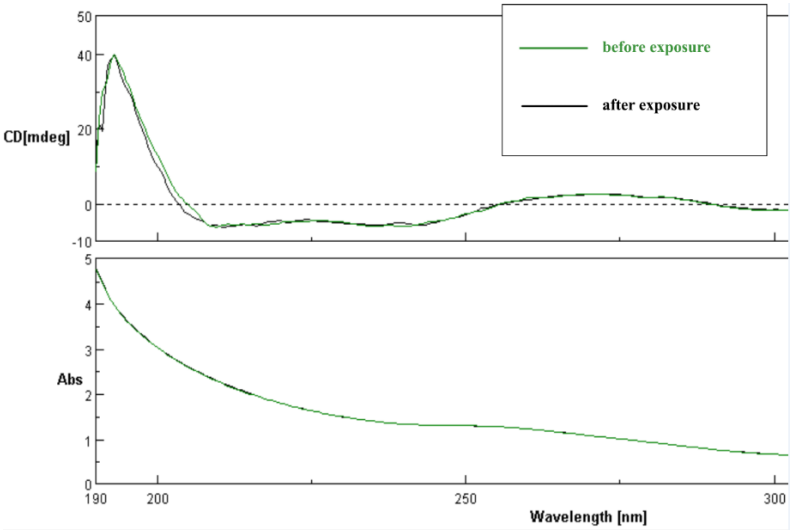

Figure 1. The spectra of sample A1 which was exposed to ELF-MF for 5 min.

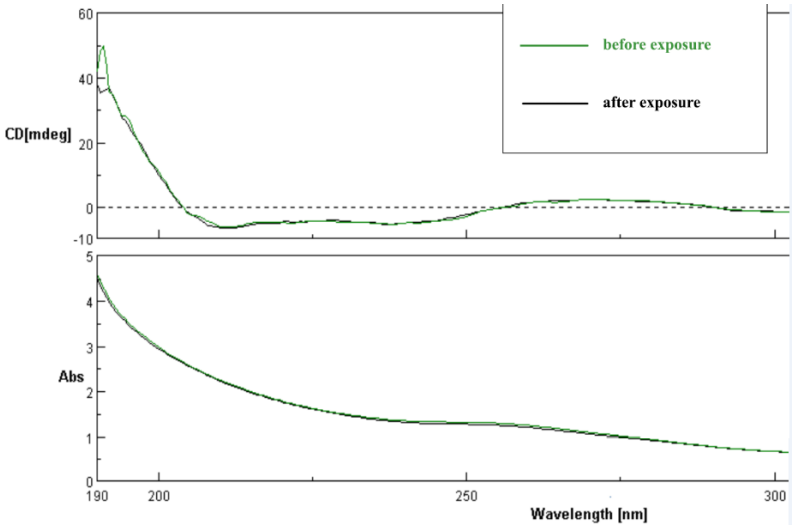

Figure 2. The spectra of sample A2 which was exposed to ELF-MF for 10 min.

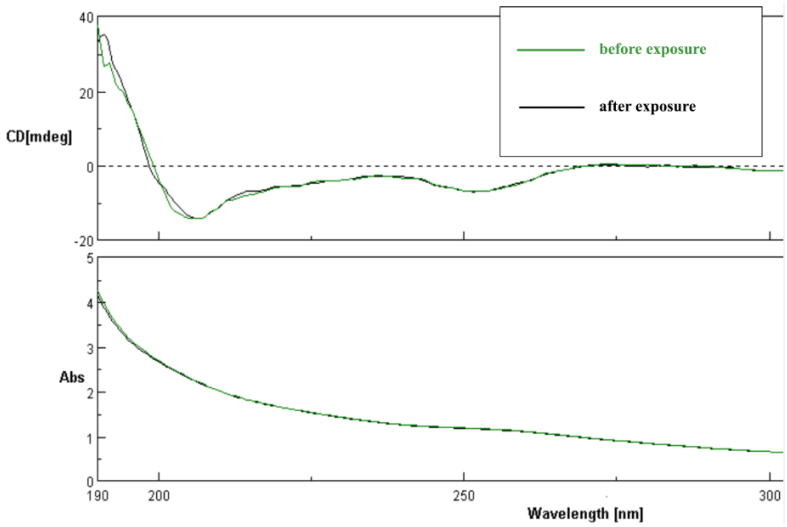

Figure3. The spectra of sample B1 which was exposed to ELF-MF for 5 min.

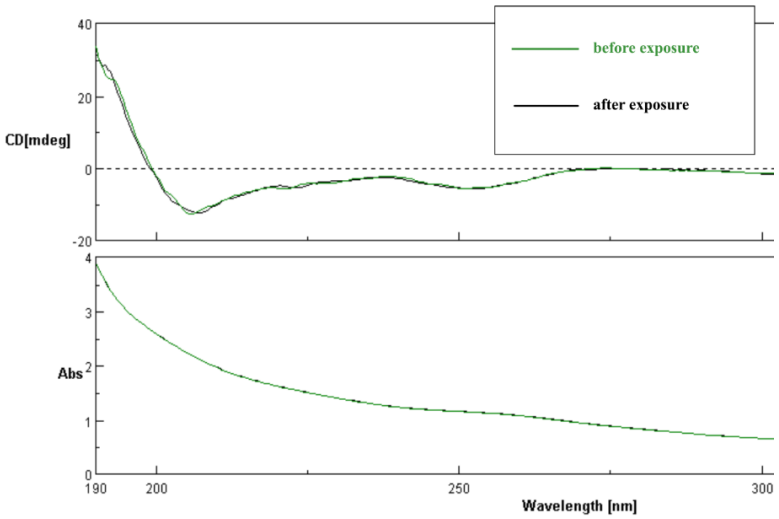

Figure 4. The spectra of sample B2 which was exposed to ELF-MF for 10 min.

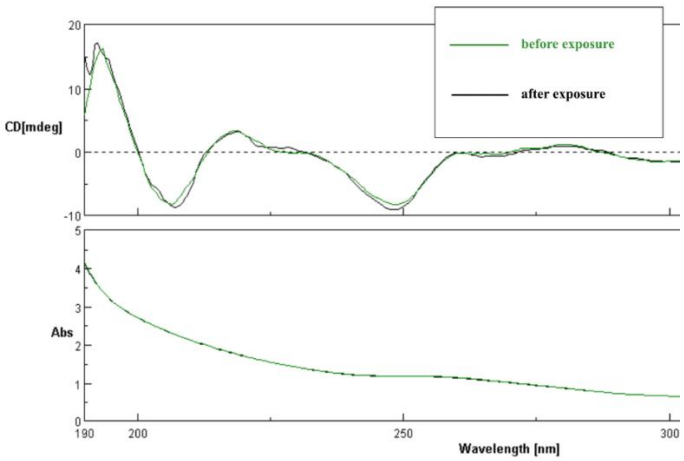

Figure 5. The spectra of sample C1 which was exposed to ELF-MF for 5 min.

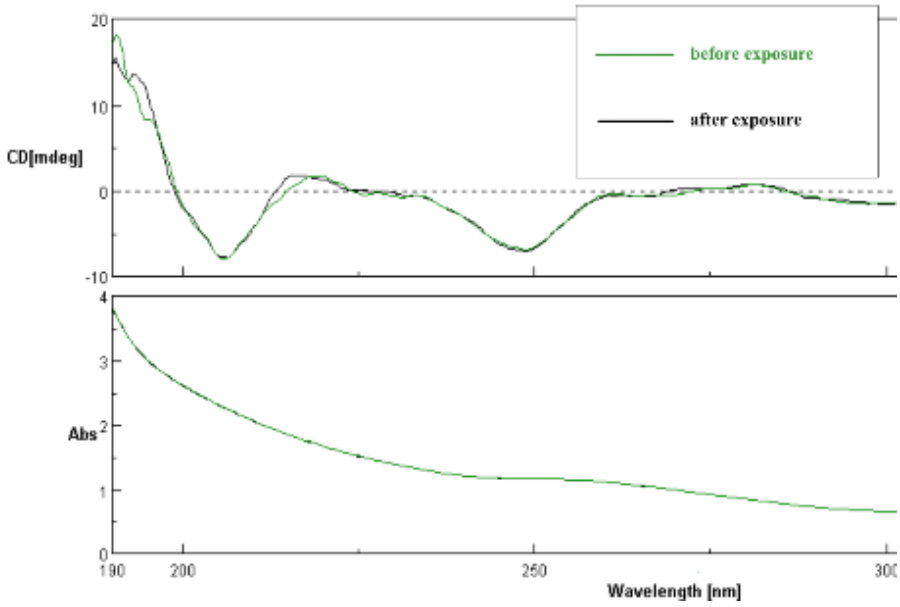

Figure 6. The spectra of sample C2 which was exposed to ELF-MF for 10 min.

DISCUSSION

According to direct interaction of electromagnetic fields with moving particles model [21], ELF-EMFs affect electrons more than other particles, due to minute mass to charge ratio of electrons [20]. Although DNA does not demonstrate a high level of conductivity for electrons, as wire does, there are quick charge transportation among local nucleobases [22]. The sequence and the distance between nucleobases account as an important factors for enhance or minimize the electrons transportations. Therefore, based on the model, sequence and conformation of DNA are great factors on responsiveness of DNA to ELF-MFs.

Sample A provided an opportunity to study a sequence contain motif CTCT. The sequence of sample A was part of hspA2 promoter which is member of HSP 70 gene families. Any significant alteration was not observed represent DNA conformational change according to CD spectra of sample A1 and A2 exposed 5 and 10 min to the ELF-MF, respectively.

Samples B represented Z-DNA conformation [23,24]. The spectrum of exposed samples demonstrated no significant alteration represented possibility of DNA conformational transition. Although CD spectra of samples B is not exactly corresponded with standard CD spectrum of

Z-DNA, probably due to low concentration of salt in the solution , it showed a profound difference from CD spectrum belong to B form of DNA.

Poly (A) tract is an essential factor for the mobilization of L1 retrotransposons in humans [25] and there are evidences to association of the tracts with promoter regions [26]. Samples C1 and C2 represent poly (dA) tract and there were no indications of conformational transition in the CD spectrum after exposure to ELF-MF.

It has been proposed that decreased of absorption at 190 nm is an evidence of DNA denaturation in CD spectrum [27]. However, absence of significant alteration in CD spectra at other regions rejected the possibility of denaturation of DNA in all samples.

DNA molecules in physiological conditions in the cells were associated with diverse proteins and many other molecules and consequently microenvironment, structure and dynamic of DNA exposed to ELF-MFs *in vivo* studies though with the same sequence, was intensely different compared with the naked DNA in present study. Nevertheless if a specific DNA sequence was supposed to be main responsive factor to ELF-MFs, it was expected to observe the effects of ELF-MFs on naked DNA as well. None of the oligonucleotides examined in the present study demonstrated conformational transition after exposure to ELF-MF, however.

It was proposed that a group of the responsive molecules to ELF-EMF were histone families, histone 3 particularly [28]. In spite of evidences of conformational change of chromatin exposed to ELF-EMF [29,30], invariable position of naked DNA, in electrophoresis gel after exposure to ELF-EMF [30], and the results provided by the present study, it was shown that presence of particular motif in DNA was not sufficient factor to detectable responsiveness of DNA to ELF-ELF.

CONCLUSION

Three double-stranded oligonucleotides with different sequences, each one represents one of CTCT motif, Z-DNA or poly (A) tract, were exposed to magnetic field 50 Hz and calculated amplitude of 680 μT to study effects of ELF-MF on dynamic of conformation of DNA. The oligonucleotides were examined by CD spectroscopy before and after exposure to ELF-MF. Provided results did not propose significant conformational transition in any of the oligonucleotides.

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