Evaluation of Damage in DNA Molecules by Very Low Frequency Magnetic Fields Using Bacterial Cells with Bioluminescence Gene

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Abstract:

The effect of very low frequency magnetic field (VLFMF) on living biological cells was investigated using a highly sensitive mutagenesis assay method. A bacterial gene expression system for mutation repair (*umu* system) was used for the sensitive evaluation of damage in DNA molecules. A mutagen-sensitive bacterium that possesses a gene expression system (a *umu* operon) for a highly-sensitive SOS response to mutation, fused with the *lux A,B* gene as bioluminescence reporter genes, *Salmonella typhimurium* TA1535 /pHYUmuLuxAB was exposed to VLFMF(20kHz, 60 μ T) in a specially designed magnetic field loading chamber. The experimental results showed that the *umu* assay could be effectively applied to the evaluation of damage in DNA molecules derived by exposure to VLFMF.

Key words: mutation repairing gene, *umu* operon, very low frequency magnetic field (VLFMF), *Salmonella typhimurium* TA1535 /pHYUmuLuxAB, bioluminescence gene

1. Introduction

Recent public concern has focused on possible human health effects from exposure to magnetic fields produced by household appliances[1].

Recently, induction cooking appliances have begun to be widely used in homes and restaurants. It is estimated that over 500,000 induction cooking units will be sold/in use in Japan in the year 2003. An induction cooking appliance works like this : The core of the unit is an electric generator and an electromagnetic coil. The coil is energized by the generator and creates a magnetic field. When an iron or ferrous metal pan is placed on the glass cook top, the magnetic field flux links with the pan and causes the atoms in the pan to move rapidly, so the metal heats up. But, some magnetic flux does not link with the pan and leaks out to the surrounding space. Therefore, a cook is exposed to the leaked magnetic flux.

Moreover, the present 20kHz excitation frequency of induction cooking appliances tends to produce higher frequencies to about 100kHz in order to enhance the performance. As induction cooking appliance seem to be being used more and more widely, and the general citizen's opportunity for exposure to 20kHz-100kHz very low frequency magnetic field (VLFMF) has increased. The effect mechanism on human health of VLFMF has not yet been completely clarified, but general citizens are anxious about this effect. The key of this study is the use of living bacterial cells, which were constructed with a light emission gene (*lux*A, B) as detection system reporter genes fused with SOS repair response genes for the bacterial DNA molecule damage with the *umu* operon.

It is well known that mutation is a direct cause of cancer development, and there is a close relation between the oncogenesis of cells and the molecular damage of DNA. Recently, genetic engineering has developed remarkably, and experimental techniques using DNA analysis are now applied to many fields of science. Therefore, gene level research on the impact of VLFMF exposure on living cells can also be done using molecular biological techniques. When the DNA of a bacterium is damaged, SOS repairing genetic systems such as, a bacterial *umu* operon and a *recA* gene, which try to restore the damage rapidly, are expressed. The gene expression of genetic systems can be conveniently and quickly detected by the expression of reporter genes (such as *lux*A, *lux*B) , and the damage to the DNA can be evaluated quantitatively.

In this study, the possibility of applying the expression system of an SOS regulon to clarify the impact of magnetic field exposure on the damage to DNA molecules was examined. We exposed the bacterium *Salmonella typhimurium* TA1535/pHYUmuLuxAB (a derivative strain whose pathogenicity was deleted) to VLFMF, and the expression intensity of the activity of *lux A, B*, which are the bioluminescence genes located downstream of the *umu* SOS gene operon, was examined using the previously developed *umu* assay method with a non-exposure control[2],[3]. In this

paper, the results of an *umu* assay of the damage to DNA molecules by VLFMF exposure are reported.

2. VLFMF exposure equipment

Fig. 1 shows the VLFMF exposure equipment used in this study. It consists of Helmholtz coils for canceling the earth's magnetic field, a Solenoid coil for generating the VLFMF exposure, and a device for controlling the temperature of the bacterial culture liquid on a microplate. The details of the earth magnetic field canceling coils, the Solenoid coil for the VLFMF exposure, and the temperature-controlled incubator bottle device are illustrated in the figure.

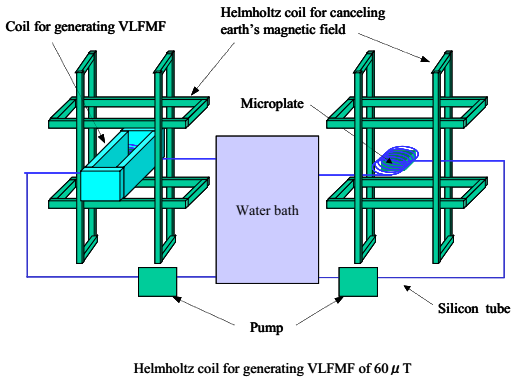


Fig. 1 Experimental system for testing bacterial DNA damage by VLFMF exposure.

2-1 Earth magnetic field canceling coils

Two square coils were placed facing each other 40 cm apart with a common axis to constitute a Helmholtz coil. Earth magnetic field canceling coils were installed in the VLFMF exposure equipment. The coils were installed using square frames with 80 cm sides, each having 15 turns. Vertical and horizontal components of the earth's magnetic field were canceled using two sets of Helmholtz coils, one for generating the VLFMF exposure and the other as a non-exposure control. The coil bobbin was made of wood, and the volt-nuts were made of brass. The probe of a magnetometer for measuring the earth's magnetic field was placed at the center of the Helmholtz coils. Direct current passes through the Helmholtz coils. The direct current intensity was adjusted in order to minimize the earth's magnetic field while reading the indication of the magnetometer. The residual earth magnetic field strength of the vertical and horizontal components was less than 10nT. Uniformity of the residual earth magnetic field strength of both the vertical and horizontal components, which was exposed to the whole bacterial culture liquid on the microplate was less than 2%.

2-2 Solenoid coil for VLFMF exposure

Solenoid coil was used for generating VLFMF exposure. The 21 turns coil is formed around a hollow and wooden rectangular column, 18cm wide, 45cm long, and 9.5cm high. The Solenoid coil for generating VLFMF exposure was installed in the earth magnetic field canceling coil. The magnetic field (20kHz, 60 μ T) applied to the whole bacterial culture liquid on the microplate was less than 2%.

2-3 Temperature control device of incubator bottle

Warm water was circulated from a controlled water bath by a pump though a silicon tube (10mm inside diameter) spirally wound around the microplate in order to control the temperature of the bacterial culture liquid. The bacterial culture liquid was filled in a microplate, 8.5cm wide, 12.5cm long and 1.5cm high, having 96 small incubator bottles. The temperature of the microplate was measured and monitored by a thermocouple.

The temperature of the water bath was set at 37℃, When a 20kHz VLFMF of 60 μ T was applied for 12 hours, the temperature change in this period was 0.1℃ or less. The temperature distribution was thus uniformly controlled with in the range of measurement error.

3. Protocol of *umu assay* method

The development protocol of the bacterial constructs used in this study was as follows. The *umuC, D* genes were inserted into plasmid pHYLuxAB, and the resulting recombinant plasmid pHYUmuLuxAB containing *umuC, D* genes and *Lux*A, B genes was transformed into *typhimurium* TA1535. The *umuC, D* are ultra-mutant repairing genes, and LuxA, B are luminescence genes from an ocean bacterium, *Vibrio harveyi*, as shown in Fig. 2. We named the transformant strain *typhimurium* TA1535 /pHYUmuLuxAB.

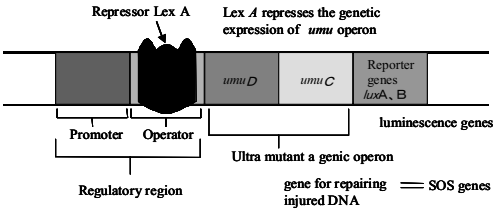


Fig. 2 Genetic constitution for *umu* assay of mutagenesis.

4. Measurement of specific luminescence intensity (SLI)

S. typhimurium TA1535/pHYUmuLuxAB is a bacterium that has *umuC, D* and *luxA, B*. If DNA-destroying substances exist, *typhimurium* TA1535/pHYUmuLuxAB generates light by the gene expression of Luciferase genes, *luxA,B*. The principle of this process is shown in Fig. 3.

First, when mutagenic substances damage the DNA in *S. typhimurium* TA1535/pHYUmuLuxAB, RecA protein binds to the damaged DNA. Second, RecA protein binds to the damaged DNA decomposed LexA protein, repressor protein of *umu* gene expression. After the repressor detaches from the *umu* operator region, the *umuC, D* genes begins to repair the damaged DNA. Finally, *the luxA, B* genes placed downstream of *umuC, D* are also expressed to emit light as reporter genes.

In this study, using this *umu* gene expression system, we report the results of evaluations of damage to DNA molecules in bacterial cells by VLFMF exposure.

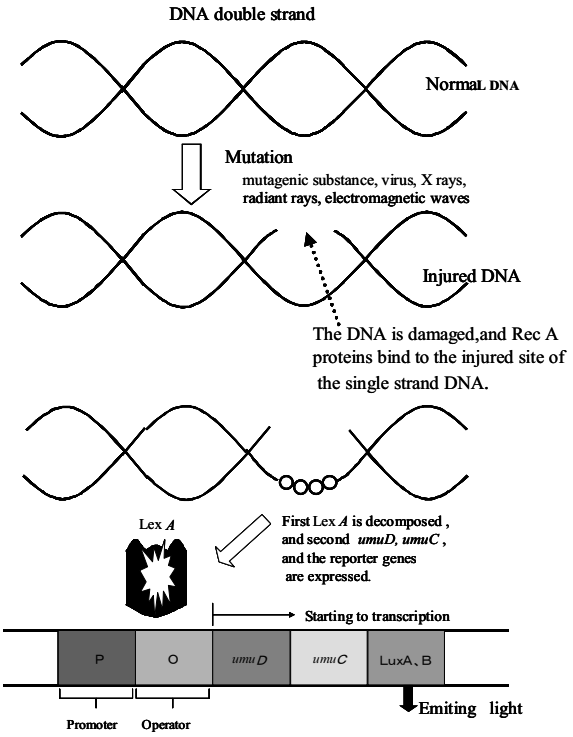


Fig. 3 The principle of light emission due to mutant substances damaging part of the DNA

Measured photometric counts of the emitted light were divided by the amount of bacterial cells measured as OD_{600}, optical densities of bacterial culture liquid measured at light wavelength 600nm, the value of which we named SLI (Specific Luminescence Intensity). SLI was determined as the value of (measured photometric value) / (amount of bacterium measured as OD_{600}). SLI was used to evaluate the intensity of damage in the bacterial DNA molecules caused by VLFMF exposure.

5. Positive control of *umu* assay

In this study, the positive control test was performed as follows, from cultivation of bacteria to measurement of SLI. A ten micro liter culture of *S. typhimurium* TA1535/pHYUmuLuxAB was cultivated in 10ml of LB culture medium[4] (at 100ppm ampicilin, 37℃, 18 hours, on a rotating shaker) and then in 10ml of LB culture medium for 4 hours (at 100ppm ampicilin, 37℃, 18 hour's on a rotating shaker). Then, *S. typhimurium* TA1535/pHYUmuLuxAB was transferred from the LB culture medium to TF II culture medium [5] (containing 100ppm of ampicilin). The final culture volume was 10ml.

Ethidiumbromide (EtBr) is well known as a mutagenic substance. We prepared three kinds of TF II cultures : without EtBr, with 10ppm EtBr, and with 100ppm EtBr respectively. Then, we added 290 μ l of these solutions onto the microplate.

Measurement was done for 12 hours, luminescence photons were counted and the amount of bacterial cells at OD_{600} was measured every hour. To measure the luminescence photon count, 10 μ l of decanal was added to 290 μ l of bacterial culture. After shaking for 3 seconds, the luminescence photons were counted by a Lumat9507 (Berthold, Germany). OD_{600} was measured by a CS-9000 Spectrophotometer (Shimadzu Co., Japan).

Fig. 4 shows the SLI caused by EtBr exposure. The SLI increased in proportion to the concentration of EtBr. Therefore, we considered it suitable to use *S.typhimurium* TA1535/pHYUmuLuxAB to evaluate the DNA damage caused by exposure to VLFMF.

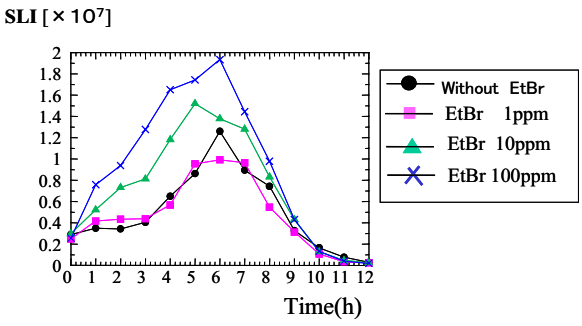
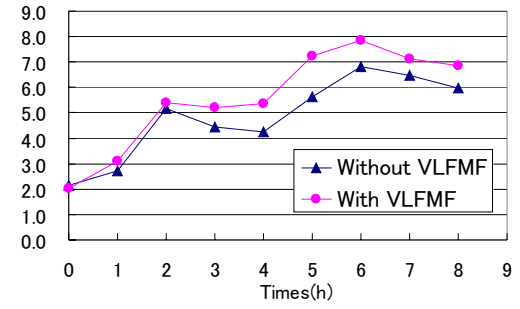


Fig. 4 SLI caused by EtBr exposure

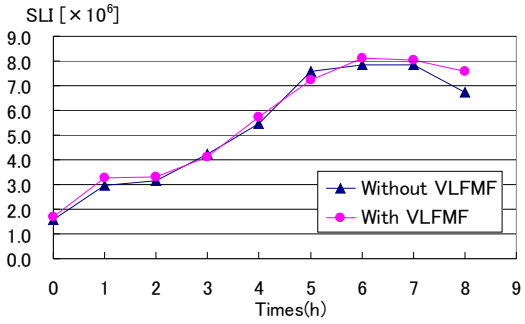
6. Measurement of DNA damage by exposure to VLFMF

The bacterial culture of *Salmonella typhimurium* TA1535/pHYUmuLuxAB was exposed to an VLFMF of

60 μ T for 8 hours. Using the *umu* assay method, the DNA damage was quantitatively evaluated. The incubation



(a)



(b)

Fig. 5 Time course of SLI caused by exposure to 20kHz magnetic field of 60 μ T

temperature of the bacteria was 37℃. The protocol of measuring the DNA destruction by exposure to VLFMF was as follows.

10 μ l of *S. typhimurium* TA1535/pHYUmuLuxAB was cultivated in 10ml of LB culture medium (at 100ppm ampicilin, 37℃, 18 hours, on a rotating shaker) and then in 10ml of LB culture medium for 4 hours (at 100ppm ampicilin, 37℃, 18hours, on a rotating shaker). Then *S. typhimurium* TA1535/pHYUmuLuxAB was transferred from the LB culture medium to the TF II culture medium (at 100ppm ampicilin). The final culture volume was 10ml.

Two microplates were set in the Helmholtz coil equipment for exposure to VLFMF or no VLFMF exposure. Measurement was done for 0 to 8 hours, luminescence cells photons were counted and the amount of bacterial cells at OD_{600} was measured every hour. To measure the luminescence photon count, the same equipment and methods that were employed in the positive control assay were applied.

Fig. 5(a) and (b) shows the SLI caused by exposure to VLFMF of 60μT for 8 hours. In this study, the experimental results are the average values of triplicated measurements. Meaningful difference were found in the measured SLI of exposure to VLFMF of 60 μ T compared with the control from the 1st hour till the 8th hours(Fig.5(a)). On the other hand, no significant difference was found either between the measured SLI values for exposure to VLFMF of 60 μ T and the values without VLFMF exposure(Fig.5(b)).

From these experiments, some data show that exposure to VLFMF causes DNA damage and other data don't. Since only a few experiments were performed, it is difficult at this point to come to a conclusion on the question of whether exposure to VLFMF causes DNA damage. Therefore, further systematic experiments need to be carried out to clarify whether exposure to VLFMF has a significant effect on DNA damage.

7. Conclusions

The *umu* assay method, which is used to detect DNA damage, was applied to evaluate the direct impact of exposure to VLFMF for DNA destruction gene mutation.

The VLFMF magnetic fields of 60 μ T at 20kHz induced bacterial *umu* DNA repair response in one experiential case. However, to know the effects of exposure to 20kHz magnetic fields on damage in DNA molecules more precise data must be gathered.

Further study is now underway.

Acknowledgment

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