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Synthesis of DnaK and GroEL in *Escherichia coli* cells exposed to different magnetic field signals

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Abstract

The effects of extremely low frequency magnetic field (ELF-MF)(1 mT, 50 Hz) on the heat shock protein (HSP) synthesis in *Escherichia coli* were investigated. Two magnetic field signals were studied: sinusoidal (SMF) and pulsed square wave (PMF). It was found that bacteria exposed to SMF showed a significantly higher level of DnaK and GroEL proteins as compared to sham-exposed bacteria as revealed by Western blot, whereas a lower level was observed after PMF exposure. Similar results were obtained when bacterial cells were exposed to heat shock (HS) after ELF-MF exposure: again SMF and PMF resulted in an increase and in a reduction of HSP amount in comparison with sham control, respectively. In conclusion, the MF influences the synthesis of HSPs in *E. coli* in a way that critically depends on the signal characteristics.

Keywords: *Escherichia coli*; Heat shock protein; Extremely-low-frequency magnetic field; Sinusoidal magnetic field; Square magnetic field

1. Introduction

The exposure to extremely low frequency-magnetic fields (ELF-MFs) induces a variety of biological effects including effects on gene expression [1] and cell viability [2], however whether MF exposure may be a source of cellular stress remains somewhat controversial since literature data are often conflicting [3–5].

The cell response to stress depends to some extent on the quality of the stressor and in general involves the expression of a set of highly conserved genes coding for heat shock proteins (HSPs) [6]. Among these proteins, the most highly induced is the 70 kDa protein (HSP70) which is conserved in almost all the organisms from *Escherichia coli* (DnaK) to humans [7]. Also the members of the HSP60 family show a high level of conservation across all species: indeed there is a high level of identity and homology between human HSP60 and the correspondent GroEL in *E.coli*.

HSP70 induction by exposure to ELF-MFs has been reported by some authors in bacteria, yeast, mammalian cells and *Drosophila*. These authors suggested that the cellular response to ELF-MFs is similar to the heat shock response (HSR) [8–10]. However, other authors did not observe the induction of HSPs after MF exposure thus suggesting that MFs, differently from other physical agents, are not universal stressors and do not lead to reproducible induction of HSPs [11–13]. MF effects are actually more difficult to investigate than other physical stresses since in general induce only a moderate variation of the biological functions, as the living organisms seem to tolerate a wide range of electromagnetic stimulation without major effects. Furthermore, biological effects of MFs seem to critically depend on various factors: the cell type examined, the genetic background, the duration of exposure, the magnitude of magnetic induction, the homogeneity of the field, the signal characteristics, etc., so that the results obtained by different authors are often not comparable. A systematic comparative research on different MF signals is still lacking although it

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represents a relevant topic for a better understanding of the interaction mechanisms between MFs and living organisms.

In previous works we found that different MF signals affected in an opposite way the transposition activity of a mini-Tn*10* transposon in *E. coli* cells. More specifically we found that a long exposure (58 h) to a sinusoidal magnetic field (SMF) negatively affected the transposition activity whereas exposure to a pulsed magnetic field (PMF) stimulated the transposon mobility. These effects were positively correlated to the intensity of the MF in a dose–effect manner. We also observed that cell viability was differently affected by the two MF signals [14,15]. Since it is known that transposons are very sensitive to environmental stress, these results suggested us that ELF-MFs may represent a general stress factor.

The aim of this study was to investigate the influence of MFs on the induction of HSPs in *E. coli* and to verify if PMF and SMF exposure, as previously observed for transposition activity, elicit opposite effects on this biological end-point.

2. Experimental

The exposure system consisted of two pairs of Helmholtz coils, 23 cm diameter, 40 (20+20) turns, which were double-wrapped in order to obtain wound (active coil) or counter-wound configuration. In the counter-wound configuration, the current is the same as in the active coil but the MF is zero (sham). The coils were powered by a home-made DC current amplifier connected to a signal generator (Beckman FG3A). Two MF signals were used: a) 50 Hz sinusoidal (SMF) and b) pulsed-square wave (PMF) with a duty cycle of 50% and 50 Hz repetition frequency. Fig. 1 shows the square-wave signal and its spectral analysis performed by Fast Fourier Transform

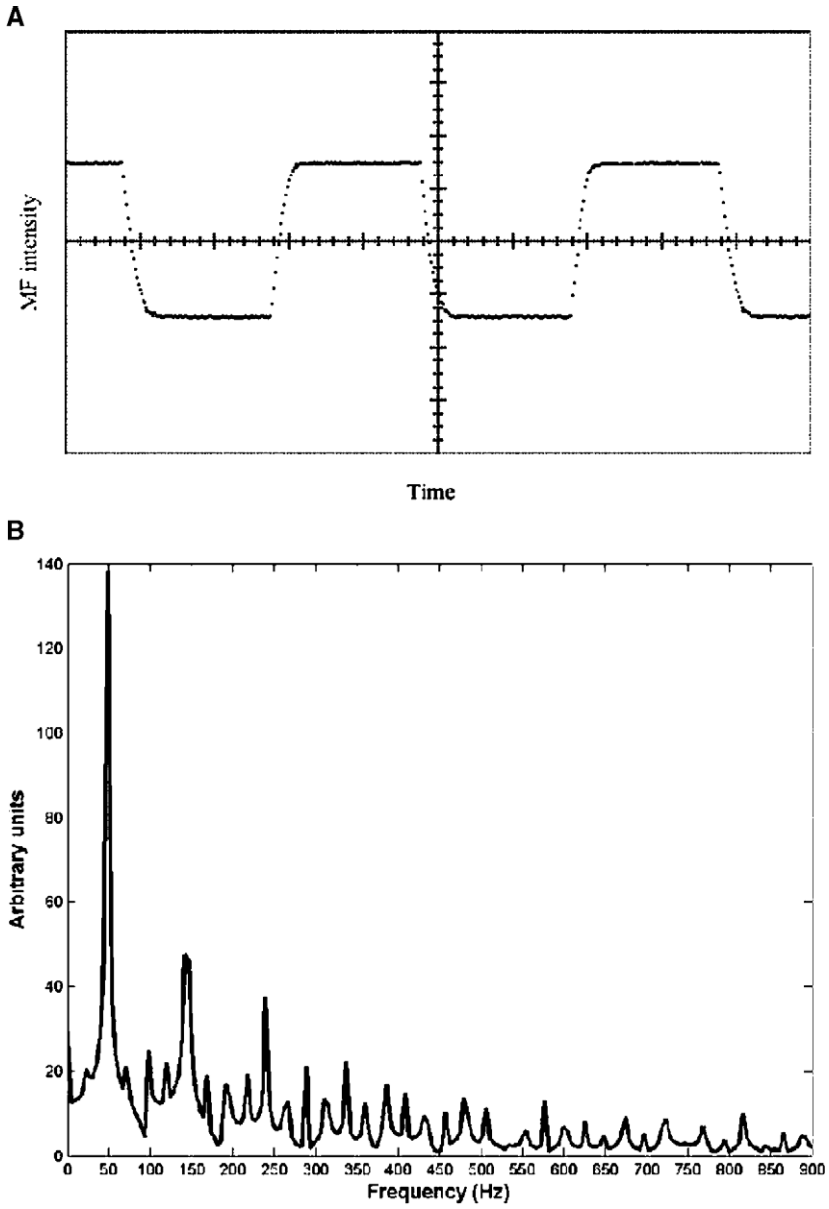

Fig. 1. Oscillogram of the square-wave magnetic signal (Panel A) and its spectral composition (Panel B) (see text for details).

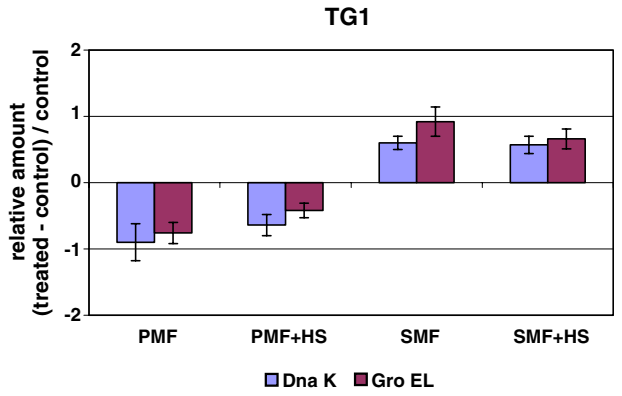

Fig. 2. Comparison of HSP gene expression evaluated by Western blot after subjecting TG1 cells to PMF (1 mT, 50 Hz, at 30 °C for 40 min) alone, SMF (1 mT, 50 Hz, at 30 °C for 40 min) alone, HS (40 min at 42 °C) after PMF exposure, HS after SMF exposure. Data are calculated with reference to respective sham-exposed controls. The results of independent experiments are combined. Each test was replicated four times.

(FFT). The rise time of the square (from the base line to the peak) was about 1 ms. The root mean square (rms) of the amplitude of the square signal was the same as that of the sinusoidal signal. The intensity (rms) used both for the SMF and for the PMF was 1 mT. The MF was measured by means of a Bell gaussmeter (model 7010); the error in the magnetic flux density values was of the order of 2%. Due to the different waveshapes of the two signals the maximum dB/dt $\left(\frac{dB}{dt}\max\right)$ at 1 mT was about 0.66 and 0.14 T/s for PMF and SMF, respectively. This parameter is important, being directly related to the induced electric field within the cells and/or the Petri dish. Both the active and the sham coils were maintained in a thermostatic room at a constant temperature of 30 °C. The background field within the incubator was also measured: the static component (local geomagnetic field) was 50 μT and the A.C. component was of the order of 0.3 μT, as measured by a sensitive probe (EMDEX II, Enertech). The experiments were conducted in blind. The tubes were placed in the centre of the coil system where the field uniformity was within 1%.

The following bacterial strains were used: DH5α [F-, ϕ80d*lacZ*ΔM15, *endA*1, *recA*1, *hsdR*17 [r_k^-,m_k^+], *supE44, thi-1, gyrA96, relA1,* Δ*[lacZYA-argF]U169,* λ$^-$]; TG1 [F′, traD36, lacI, Δ*[lacZ]].* The bacterial strains were cultured overnight at 37° C in LB medium, a small amount (0.5 ml) of the culture broth was inoculated into 50 ml of fresh medium and incubated at 30 °C until the cells reached the logarithmic phase (0.22 O. D.). Then the culture was divided into four aliquots in round shaped tubes (diameter 16 mm, Sterilin bijou) and subjected for 40 min without agitation at the different conditions : incubation at 42 °C (heat shock) and at 30 °C (control), exposure to ELF-MF at 30 °C and exposure to sham control at 30 °C. After the exposure to ELF-MF and to sham, the cultures were divided into two aliquots, one of which was centrifuged and total proteins extracted, whereas the other was incubated at 42 °C (HS) for 40 min and then total proteins were extracted. Each test was replicated four times.

Total cell protein was quantified using Bradford assay reagent (Biorad) with bovine serum albumin (BSA) as standard. Gel electrophoresesis was carried out using precast polyacrylamide gels (10% Bis–Tris XT-Criterion, Biorad). For immunoblotting membranes were probed with monoclonal mouse IgG anti-DnaK, anti-GroEL (StressGen Biotechnologies Corp), IgG anti-RNA polymerase alpha subunit (an house-keeping gene product used as control) (NeoClone Biotechnology International) as primary antibodies. The immunoreactive proteins were detected using alkaline phosphatase/conjugated anti-mouse rabbit IgG (StressGen Biotechnologies Corp) then stained with NBT and BCIP (Biorad). Immunoblots were scanned and the optical density (OD) of each band was determined using a densitometer (Media Cybernetics, Image-Pro Plus Family imaging software, MD, USA).

To normalize OD values of HSP bands based on the amount of proteins loaded in each well, we took the average OD of all RNA polymerase alpha subunit bands and then calculated the normalizing factor for each lane by dividing the OD of each individual RNA polymerase alpha subunit band by the average OD of all RNA polymerase alpha subunit bands. The normalized OD of each HSP band was obtained by dividing the non-normalized OD of the HSP band by the normalizing factor. Finally, the average of the normalized OD of four HSP bands of each treatment was taken and compared.

3. Results and discussion

In order to test if ELF-MF exposure affects HSP expression and to verify if it has an influence on the normal HSR, bacterial culture were subjected for 40 min to following treatment : HS (42 °C), to ELF-MF (1 mT, 50 Hz, 30 °C) and to HS after ELF-MF exposure. The effects of the two MF signals (SMF, PMF) were carried out in two independent experiments. Since biological effects of MF seem critically depend on the genetic background of the organisms tested [16], two different strains of *E. coli* (TG1, DH5α) were used.

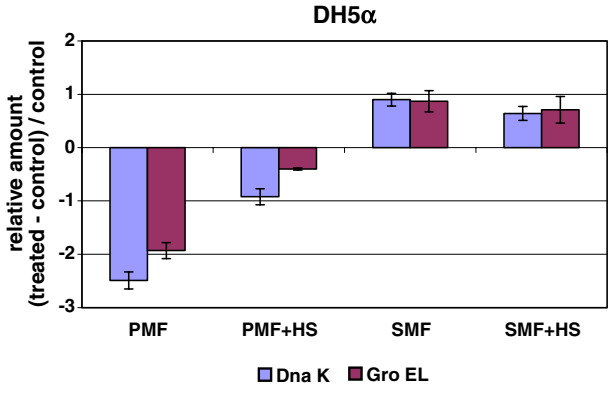

Fig. 3. Comparison of HSP gene expression evaluated by Western blot after subjecting DH5α cells to PMF (1 mT, 50 Hz, at 30 °C for 40 min) alone, SMF (1 mT, 50 Hz, at 30 °C for 40 min) alone, HS (40 min at 42 °C) after PMF exposure, HS after SMF exposure. Data are calculated with reference to respective sham-exposed controls. The results of independent experiments are combined. Each test was replicated four times.

After HS an increase in DnaK and GroEL protein levels was observed, as expected, in both Tg1 (about 2 folds) and DH5α cells (about 5 folds) (data not shown).

In Fig. 2 the results about TG1 cells exposed to ELF-MF are reported: exposure of the cells to PMFs alone gave a reduction of both DnaK and GroEL amounts as compared with sham-exposed controls. This reduction was observed also when HS followed PMF treatment (PMF+HS). On the contrary, an increase of about 1,5 fold of HSP, as compared with sham-exposed samples, was observed when TG1 cells were exposed to SMF and to HS after SMF (SMF+HS) exposure.

The data about DH5α generally mirrored those observed for TG1 strain and are reported in Fig. 3. It can be noted that PMF exposure induced a marked reduction of HSPs (about 4 folds) as compared with controls (sham-exposed), while SMF gave an increase of about 2 fold amount as compared with sham.

Therefore in the tested strains we found a decrease of the HSP synthesis in PMF exposed bacteria as compared to sham control and a increase of the HSP synthesis after SMF exposure as compared to sham exposure. No significant differences were detected between DnaK and GroEL expression.

The SMF effect is consistent with numerous other results from literature, regarding various biological models exposed for short time (30–60 min) to 50/60 Hz SMF of low magnetic flux density (8–2300 μT) [8,10,17,18]. At variance, when using MFs with higher magnetic flux densities (8–300 mT) or longer exposure times (4–24 h) some authors did not find any effect on HSP synthesis [11–13,19].

A decrease of HSP70 levels was observed when a repeated exposure was tested [20,21]. Therefore the period of exposure and the magnetic flux density seem to play an important role in eliciting biological effects that cannot modeled as a simple dose–response curve on the type usually obtained in toxicological studies of chemicals; indeed, prolonging the time of exposure or increasing the magnetic flux density does not always correspond to an intensified effect, but may result in a weaker or a different biological response.

To date, few papers have reported results about comparison between exposure to PMF and SMF signals [22,23]. In all the tested strains we found a decrease of the HSP synthesis in PMF exposed bacteria as compared to sham control. In *E. coli* HSPs are abundant under all physiological conditions and during exponential growth are present at a level of approximately 1% of total protein. During HS response, the HSP induction transiently peaks at 5–15 min after temperature upshift and then drops to a new steady-state level of synthesis [24]. However, the response may differ quantitatively according to the different strains used. We observed that after 40 min of PMF exposure the DnaK and GroEL levels decrease as compared to sham control. This reduction might be a drop after a peak or an inhibition of the constitutive expression. The kinetics of the cellular response to ELF-MF exposure is not known and it might be that the shutoff of the heat shock induction occur with different timing in response to PMF and to SMF. Work is in progress to clarify this point.

In conclusion we observed that 50 Hz ELF-MF elicits a cellular response involving the HSPs in a way depending on the MF signal characteristics, even if further work is needed to elucidate the mechanisms involved in such different responses.

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