Magnetic field exposure enhances DNA repair through the induction of DnaK/J synthesis

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Abstract In contrast to the common impression that exposure to a magnetic field of low frequency causes mutations to organisms, we have demonstrated that a magnetic field can actually enhance the efficiency of DNA repair. Using *Escherichia coli* strain XL-1 Blue as the host and plasmid pUC8 that had been mutagenized by hydroxylamine as the vector for assessment, we found that bacterial transformants that had been exposed to a magnetic field of 50 Hz gave lower percentages of white colonies as compared to transformants that had not been exposed to the magnetic field. This result was indicative that the efficiency of DNA repair had been improved. The improvement was found to be mediated by the induced overproduction of heat shock proteins DnaK/J (Hsp70/40).

Key words: Magnetic field; DNA repair; Heat shock protein; DnaK; Hydroxylamine

1. Introduction

The potentially hazardous influence of electric and magnetic fields on genetic stability has long been a concern. Research in this regard generally shows that frequency-power fields do not induce any genetic changes or chromosomal aberrations (for review, [1]). However, there are occasions when mutagenic effects or DNA breakage can be observed (e.g. [2,3]), and there is also a suspicion that magnetic fields do not act on the DNA directly but rather enhance the effects of other mutagenic agents [4].

In many of these electric and magnetic field exposure studies, assessments were generally based on cultured mammalian cells or bacterial systems, i.e. cultures were exposed to the power-frequency fields, and changes in mutation rates were then analyzed. This approach has its methodological limitations. The establishment of a mutation point on the DNA molecule involves two phases: modification of the nucleotide, and leakage of the process of DNA repair. If the frequency-power fields do not modify nucleotides nor interact with DNA molecules at all but rather modify protein structures/functions and accordingly impair the process of DNA repair or some other DNA-related reactions, the conventional approach will be unable to ascertain the hazardous effect of the frequency-power fields on DNA molecules. Actually, it is already known that some protein molecules are susceptible to electric and magnetic field modulations; for instance, the charges within the ion pump enzyme Na^+/K^+-ATPase can be altered by a magnetic field, and the enzyme behaves differently when subject to magnetic field exposure [5].

As the conventional approach has its limitations, it is necessary to make use of methods that can specifically illustrate the effects of frequency-power fields on DNA-related activities. In this regard, we have designed an experiment to address the effect of magnetic field exposure on the process of DNA repair of *Escherichia coli*. Unexpectedly, we found that exposure to the magnetic field not only failed to produce mutations in the tested system, but even improved the efficiency of the process of DNA repair. In this communication, we report our discovery that heat shock proteins are overproduced when *E. coli* cultures are exposed to a low frequency magnetic field, and the heat shock proteins DnaK/J (Hsp70/40) are involved in improving the efficiency of the process of DNA repair.

2. Materials and methods

2.1. Bacterial strain and plasmids

The bacterial strain used in this study was *E. coli* XL-1 Blue (Stratagene). This strain was grown in LB medium. Plasmids pOFXtac-KJ1 and pOFXtac-SL1, which were used for the inductive overproduction of DnaK/J and GroEL/ES respectively, and pOFX-tac1, which was used as the negative control, were provided by Dr. O. Fayet [6]. XL-1 Blue cells carrying any one of the above plasmids were grown in LB medium supplemented with kanamycin (50 μg/ml). Plasmid pUC8, which when induced with IPTG can complement XL-1 Blue to produce blue coloration in the presence of X-gal, was used as the indicator for the assessment of the establishment of mutations in XL-1 Blue.

2.2. In vitro mutagenesis of plasmid pUC8

Plasmid pUC8 was mutagenized by hydroxylamine treatment under in vitro conditions. The procedures adopted were similar to those suggested by J. Miller [7]. In a small glass test tube, 0.4 ml of 0.5 M potassium phosphate (pH 6) and 5 mM EDTA was mixed with 0.8 ml double distilled water and 0.8 ml of 1 M hydroxylamine (pH 6). An amount of 2 μl of CsCl-purified pUC8 (1 μg/μl) was added to the mixture and the tube was capped. The mixture was incubated at 37°C for 30 h. The mutagenized plasmid was recovered by the glass milk method, and the procedures used were the same as those suggested by the Geneclean kit supplier.

2.3. Magnetic field application

The magnetic field was generated by dual filament wound 15 cm radius Helmholtz coils connected to an AC low voltage power supplier (022-317, Unilab, Blackburn, UK) of 50 Hz. The magnetic field so produced was in the vertical direction. Cultures for magnetic field exposure were contained in Eppendorf tubes (size 1.5 ml) and fixed at the center of the coil diameter. The peak field strength exerted on the tested culture was determined by a Hall effect probe magnetometer (F.W. Bell, Model 9640, Orlando, FL, USA). For control assessments, the bacterial cultures were fixed in the same position as the tested ones except that the power supply was turned off. Mu shielding

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was used to prevent magnetic interference from other laboratory facilities. Both the magnetic field exposure and control assessments were conducted at room temperature under good ventilation. No temperature difference between the tested and control cultures was observed.

2.4. Assessment of the efficiency of DNA repair

Mutagenized pUC8 was used to transform competent cells of XL-1 Blue. During the phenotypic expression stage of transformation, cultures were kept in 1.5 ml Eppendorf tubes and fixed in the magnetic field. After 1 h of expression at room temperature, cells were seeded on LB agar plates supplemented with ampicillin (125 μl/ml), IPTG (10 μl of 0.1 M stock per plate) and X-gal (50 μl of 10% stock in DMF per plate). The seeded agar plates were incubated at 37°C for 20 h. The numbers of blue and white colonies that had appeared on the agar plates were scored. For the investigation of the effect of overproduction of heat shock proteins on DNA repair efficiency, XL-1 Blue competent cells already carrying one of the plasmids pOFXtac-1, pOFXtac-SL1, or pOFXtac-KJ1 were used for transformation. During the phenotypic expression stage, IPTG was added to the desired concentrations to the cultures suspended in LB medium. After 1 h of incubation at 37°C, cells were seeded on LB agar plates supplemented with ampicillin, IPTG and X-gal in the aforementioned concentrations. The agar plates were incubated at 37°C for 20 h and the numbers of blue and white colonies grown were scored.

2.5. Protein gel electrophoresis and immunoblot analysis

Samples of cell extracts were resolved by SDS–PAGE on the Bio-Rad minigel system. The Bradford method [8] was used to monitor the amount of proteins used in all samples. Monoclonal antibodies for GroEL (SPA-870) and for DnaK (SPA-880) were purchased from StressGen (Canada). The procedures of using the monoclonal antibodies for DnaK and GroEL detection were essentially as reported earlier [9].

3. Results and discussion

In this DNA repair assessment, colonies appearing with a blue coloration indicated that the plasmid pUC8 established in the host cells either had not been mutagenized or had been mutagenized but was repaired, and white colonies were indicative that the mutagenized plasmid had not been repaired. The occurrence of a higher percentage of white colonies among the transformant colonies under the tested conditions implies that the repair of mutagenized DNA was less efficient.

The results of the effect of magnetic field exposure on DNA repair are presented in Fig. 1. The percentages of white colonies derived from the magnetic field-exposed cultures were lower than those derived from the control. For cultures exposed to a magnetic field (50 Hz) strength of 0.4, 0.8 or 1.2 mT, the corresponding percentages were similar and amounted to about 0.9% while the culture exposed to the sham treatment scored about 1.2%. There was more than 20% improvement in these three treatments. Exposure of cultures to a magnetic field strength of 0.1 mT also showed improvement but at a more modest rate. When cells were transformed by pUC8 that had not been mutagenized, no white colonies could be scored at any of the magnetic field exposures nor in the sham treatment (data not shown). This observation not only echoes previous investigations that concluded exposure to low frequency magnetic fields does not cause genetic changes, it also implies that magnetic field exposure could actually improve the efficiency of DNA repair.

The unexpected effect of magnetic field exposure on DNA stability prompted us to further our investigation in order to find out the mechanism involved. In this regard, research of Cairo et al. [10] showing that when *E. coli* cultures are exposed to a magnetic field (60 Hz) of 1.2 mT, the expression of δ^{32}, a transcription factor that guides RNA polymerase to

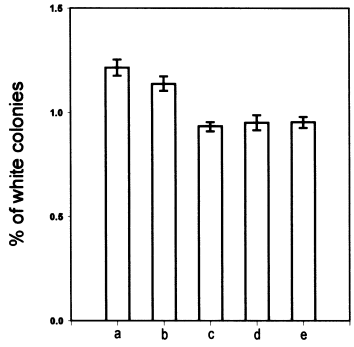

Fig. 1. Effect of magnetic field exposure on the occurrence of white colonies. Hydroxylamine-mutagenized pUC8 was used to transform *E. coli* XL-1 Blue cells. During the phenotypic expression stage, cells were exposed to a magnetic field (50 Hz) of different field strengths (a, 0.0 mT; b, 0.1 mT; c, 0.4 mT; d, 0.8 mT; e, 1.2 mT) at room temperature. After 1 h of expression, cells were seeded on agar plates supplemented with ampicillin, IPTG and X-gal. The seeded plates were incubated at 37°C for 20 h. The grown colonies, blue or white, were scored. The percentages presented were calculated from the number of white colonies/total number of blue and white colonies. Data shown represents means ± S.D. ($n = 4$) and in each treatment, the total number of transformant colonies scored per experiment was in the range of 9000–12 000.

interact with a variety of stress gene promoters, would be enhanced directed us to consider the potential role of heat shock proteins. As a matter of fact, there were three lines of research results that had inspired us. Firstly, promoters regulating heat shock protein production are responsive to the transcription factor δ^{32}. In other words, when cells are exposed to the stress of a magnetic field, the whole spectrum of heat shock proteins is likely to be produced to cope with the stress (for review, [11]). In this regard, hsp70 has already been shown to be overproduced when *Drosophila melanogaster* salivary glands, cultured human cells, yeast culture, etc. are exposed to weak magnetic fields (for review, [12]). Secondly, in a study on *groES*$^-$, *groEL*$^-$ and *dnaK*$^-$ mutants of *E. coli*, Ogata et al. [13] discovered that these mutants exhibited impaired homologous recombination efficiency as compared with the wild-type, and they hypothesized that the functional heat shock proteins corresponding to these genes might be involved in the RecF pathway of damaged DNA repair. Thirdly, Zou et al. [14] were able to show that in the presence of DnaK, UvrA was able to undergo more cycles of UvrB loading and eventually led to an increase in DNA repair efficiency.

As shown in Fig. 2, we were able to repeat the observation of DnaK overproduction in *E. coli* when the bacterial cells were subjected to low frequency magnetic field exposure. The immunoblots of protein profiles indicate that both DnaK and GroEL, corresponding to Hsp70 and Hsp60 respectively,

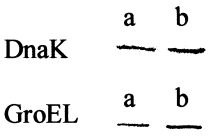

Fig. 2. Detection of DnaK and GroEL accumulated in differentially treated *E. coli* cultures. Cell extracts derived from either a control culture (a) or a culture that had been magnetic field-stressed (50 Hz, 1.2 mT) (b) were blotted and detected by antisera of DnaK and GroEL.

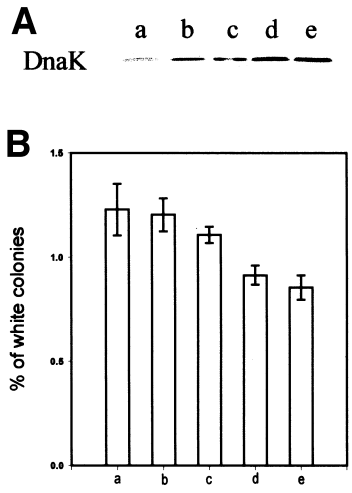

Fig. 3. Correlation of DnaK overproduction and occurrence of white colonies. A: Detection of DnaK induction in *E. coli* XL-1 Blue cells carrying pOFXtac-KJ1. The accumulation of DnaK, as detected by antiserum of DnaK, in cultures was induced by IPTG of the following concentrations: a, 0 μM; b, 5 μM; c, 10 μM; d, 30 μM; e, 100 μM. B: Percentages of white colonies derived from cultures that had been induced with IPTG at different concentrations. The percentages were determined as in the legend of Fig. 1; a, b, c, d and e refer to the concentrations of IPTG used in DnaK induction as in A. Percentages are means ± S.D. ($n = 4$) and the range of colonies scored was the same as that shown in Fig. 1.

would be induced to accumulate to higher amounts in cells that had been magnetic field-stressed as compared to cells that had been exposed to the sham treatment.

Since the inductive overproduction of DnaK and GroEL in magnetic field-stressed bacterial culture could be confirmed, we tested if these proteins were involved in improving the repair of hydroxylamine-mutagenized DNA. Fig. 3 shows the percentages of white colonies derived from pOFXtac-KJ1 carrying cells that had been induced by IPTG at different concentrations. The involvement of DnaK/J in the improvement of DNA repair is very clear in this comparison because the culture that had been induced by 100 μM of IPTG obviously yielded a lower percentage of white colonies as compared to the control, and cultures induced by IPTG of concentrations lower than 100 μM exhibited percentages of white colonies between the two extremes tested. When our investigation was extended to the XL-1 Blue cells that carried either pOFXtac-SL1 or pOFXtac-1, no similar change in percentage of white colonies could be observed (data not shown). The contribution of GroEL and GroES in the magnetic field-induced improvement of DNA repair should be negligible, if any.

In our experiment, the plasmid pUC8 used for transformation was mutagenized by hydroxylamine. This mutagen binds specifically to cytosine to generate N^4-hydroxycytosine. If unrepaired, the modified nucleotide can pair with adenine and eventually leads to a G:C to A:T transition mutation [7]. The rectifying of the modified cytosine in this instance should be through the methyl-independent mismatch repair mechanism. Interestingly, this mismatch repair mechanism involves the *recF* gene product [15], and products of *uvrA*, *B* and *C* are also known to induce incisions on a very broad spectrum of DNA damages [16,17]. The inspiring results of Ogata et al. [13] that DnaK is involved in the RecF pathway of DNA repair and Zou et al. [14] that DnaK can improve the activity of UvrA promptly explain our observation that magnetic field-exposed cells exhibited better DNA repair efficiency. That is, magnetic field exposure brings stress to *E. coli* cultures and the bacterial cells respond by overproducing heat shock proteins. One of the heat shock proteins accumulated, DnaK, confers improved activities to some of the elements involved in the process of DNA repair.

In the study of Ogata et al. [13], it was shown that both GroEL and GroES were also important for DNA repair but this observation could not be repeated in our investigation. The difference could have been related to the adoption of different approaches in these two investigations. Ogata et al. [13] employed defective mutants to compare DNA repair efficiency and we correlated protein overproduction with DNA repair efficiency for assessment. It could be that the presence of basal levels of GroEL and GroES is essential but overproduction of these heat shock proteins brings no additional advantage to DNA repair.

Since UvrA is involved in inducing DNA incision in quite a number of DNA repair mechanisms [16,17] and RecF is also known to participate in excision repair of several DNA-related activities [15,18–20], it is likely that through the induction of DnaK accumulation, magnetic field exposure can provide a good means to repair DNA lesions caused by a variety of damaging agents.

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