Magnetic Isotopes of $^{25}$Mg and $^{67}$Zn and Magnetic Fields Influence on Adenosine Triphosphate Content in *Escherichia Coli*

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**Abstract.** Studies on the magnetic isotopes effect of zinc $^{67}$Zn and magnesium $^{25}$Mg on the adenosine triphosphate (ATP) concentration in living organisms were held in this work on the example of *E. coli* bacteria. External static magnetic fields 0-100 mT were used to enhance the effect of magnetic isotopes on intracellular processes. Enrichment of microorganisms with magnesium or zinc isotopes during growth changes the ATP concentration in cells depending on the type of isotope – magnetic or non-magnetic. The effect of weak magnetic fields of 0-10 mT stimulates the bacterial cell growth and the intracellular ATP concentration change. The maximum concentration of ATP was achieved by using a magnetic isotope of magnesium $^{25}$Mg and exposure of bacteria to the static magnetic fields 70-90 mT. Non-magnetic isotopes of magnesium or zinc and magnetic fields other than the ranges 0-10 and 70-90 mT can be used to decrease the rate of ATP synthesis.

1. **Introduction**

Adenosine triphosphate, or ATP, is the main macroergic molecule in living organisms. The energy released during the splitting is used in many biological processes, including enzyme reactions, synthesis of deoxyribonucleic (DNA) and ribonucleic (RNA) acids. The possibility of controlling the intracellular concentration, or adenosine triphosphate (ATP) pool, and the process of ATP synthesis is the important problem of fundamental biology and medicine. One of the possible ways affecting ATP pool is an external magnetic field or magnetic isotopes influence. The intracellular ATP content increased to 3.5 times after one hour of exposure to the static magnetic field (SMF) (17 mT) on the *Escherichia coli* bacteria [1]. The membranes-bonded ATP synthesis is stimulated by alternative electromagnetic fields [2]. However, the mechanism of magnetic effect on the ATP production and consumption by intracellular systems are not proposed by the authors of those works.

The effects of external magnetic field and magnetic moments of atomic nuclei of chemical elements, for example, magnetic isotope $^{31}$P, $^{25}$Mg, $^{67}$Zn, on living organisms can be carried out through the elementary acts sequence of enzymatic reactions with one or more electrons transfer. The ion-radical pair (IRP) formed in such processes serves as the "primary receiver" of the magnetic field [3] and the main source of magnetic field and magnetic isotope effects [4]. Magnetic exposure can induce a conversion of IRP from the primary singlet state into the triplet one. It leads to a change of probabilities of direct and reverse enzymatic reactions and product yield. For enzymatic ATP synthesis the proposed mechanism has many experimental confirmations with the participation of ions of the magnetic magnesium $^{25}$Mg$^{2+}$, zinc $^{67}$Zn$^{2+}$, calcium $^{43}$Ca$^{2+}$ isotopes [4]. Moreover, the rate
constant of the ATP synthesis by creatine kinase is increased with addition of the external magnetic field only for the enzyme enriched with magnesium magnetic isotope $^{25}\text{Mg}$. Enrichment of the enzyme by non-magnetic isotope of magnesium $^{24}\text{Mg}$ does not change the kinetic characteristics of enzymatic reactions in an external magnetic field [5].

The magnetic isotopes efficiency in intracellular processes has been repeatedly confirmed in experiments in vivo [6, 7]. Magnetic isotope of magnesium, $^{25}\text{Mg}$, contained in the E. coli cells, affect their growth, development and vital activity, and its biological effects differ from the effects of a non-magnetic isotopes $^{24,26}\text{Mg}$ [7]. The colony-forming ability the E. coli bacteria is increased (in the range 0-15 and 76-93 mT) and intracellular elemental composition are changed as a result of combined action of the external SMF and magnetic magnesium $^{25}\text{Mg}$ and $^{67}\text{Zn}$ isotope [6, 8]. The observed effects indicate the magnetic moments of atomic nuclei of the $^{25}\text{Mg}$, $^{67}\text{Zn}$ isotopes influence on the whole organism through a sequence of intracellular enzymatic processes, including ATP synthesis.

The aim of this work is the search for experimental evidence of the joint effects of weak SMFs and the magnetic moments of magnesium $^{25}\text{Mg}$ and zinc $^{67}\text{Zn}$ isotopes nuclei on the intracellular ATP concentration in E. coli. Numerous physiological and biochemical factors which affect the ATP synthesis rate in multicellular organisms are not allow studying the mechanisms of magnetic fields influence and basic acts of physic-chemical processes in such complex organisms. Thus we choose Escherichia coli bacterial cells as the object of the research. The phases of its growth and ATP concentration, both the extracellular and intracellular, are closely linked.

2. Materials and methods

2.1. Cultivation conditions

The study object was the E. coli cells culture, viz., a museum strain K12 TG1 (from collection of Institute of Cellular and Intercellular Symbiosis, Urals branch of RAS, Orenburg, Russia), which was grown in minimum synthetic nutrient media M9: 37.4 mM NH$_4$Cl, 2.2 mM $^{24,25,26}\text{MgSO}_4$ or 25 μM $^{64,66,67}\text{ZnSO}_4$, 55.5 mM glucose, 84.5 mM Na$_2$HPO$_4$, 44.1 mM KH$_2$PO$_4$, 17.1 mM NaCl (Reachem, Moscow, Russia). The media differed only by the isotope form of magnesium/zinc in the salt: nonmagnetic $^{24,26}\text{Mg}/^{64,66,67}\text{Zn}$ and, magnetic $^{25}\text{Mg}/^{67}\text{Zn}$, and natural isotope Mg/Zn [7]. Magnesium or zinc isotopes were added to the media as sulphate MgO/ZnSO$_4$; the concentration was rigidly monitored for all sulphates. For the sulphates preparation, isotopically pure oxides were used: $^{24}\text{MgO}$, $^{25}\text{MgO}$, $^{26}\text{MgO}$, $^{64}\text{ZnO}$, $^{66}\text{ZnO}$, and $^{67}\text{ZnO}$ (Combine “Electrochempribor”, Lesnoy, Russia) with extremely high isotope enrichment.

The museum strain of E. coli was pre-incubated in LB broth (Sigma-Aldrich, USA) for 16 h at 37°C. Then the cells of E. coli were re-sown into the media M9 containing magnesium or zinc isotopes. The nutrient medium LB without isotopes was used in control experiments. Then 200 μl of the medium with E. coli cells was added to each well of a 96-well plate (Apexlab, Moscow, Russia); volume of each well was 346 μl.

All the samples were placed in SMF, which was induced by the electromagnet with an iron yoke (TR-309, Takeda Riken, Tokyo, Japan) with the cooling system, and the temperature was continually monitored for 7 h to ensure a constant temperature of 37°C [6]. Aerobic cultivation conditions were provided by placing samples with growing bacterial culture on the ST-3 ELMI shaker (ST-3, ELMI, Riga, Latvia) every hour for 5 min. (rotation velocity of the platform 200 rpm). The ranges of the chosen weak magnetic fields were 0.8-98 mT in experiments with magnesium isotopes and 2.2-78 mT with zinc isotopes. The bacteria were cultivated simultaneously in 96 control points (for each of the four zinc isotopes) corresponding to 21 stationary magnetic fields, whose values were measured by a milliteslameter (TP2-2U, Fela-conrol, St. Petersburg, Russia). The magnetic field fluctuations at each point were monitored throughout the experiment and did not exceed 0.1 mT.

2.2. Measurements of intracellular ATP

Bioluminescence method was used for the measurement of intracellular ATP in the E. coli bacteria. It was performed by the luminometer "LUM-01" (LUM-01, Lumtek, Moscow, Russia). The ATP
concentrations were measured using the Lumtek set (measurement of the total concentration of ATP in extracts of cells and tissues) (Lumtek, Moscow, Russia). The set includes 4 solutions: the ATP-reagent Lumtek, lyophilized [9]; the solution for reconstruction of the ATP-reagent; ATP-control, lyophilized; the solution for cell destruction. After measuring the ATP concentration of the bacterial population was calculated by the formula [6]. To calculate the intracellular ATP pool in single bacteria the ATP concentration measured by using the bioluminescent method was divided by the number of bacterial cells measured using the CFU method.

2.3. CFU measurements
The colony-forming ability of bacteria, which was measured after 7 h of cell incubation (at the end of logarithmic phase of growth) in an external SMF, was chosen as the main indicator of growth. The method of serial dilutions was applied for measuring CFU: three dilutions of the medium containing E. coli cells in the physiological solution [10-11].

The cell concentration in each sample was determined photometrically by using the SOLAR CM2203 spectrofluorimeter according to the calibration curve. Equal amounts of E. coli cells grown on the media M9 with magnetic and nonmagnetic zinc isotopes diluted in the corresponding concentrations were sowed on the solid nutrient medium LB agar in Petri dishes. The CFU values were calculated after 16 h of incubation at 37°C.

2.4. Statistical Analysis
Data were expressed as mean ± SD. The Shapiro-Wilk normality test was used to determine whether experimental data were drawn from a normally distributed population. Student’s tests were used to determine statistical differences by Origin 8.0 software (Version 8.0; Microcal Software Inc., Northampton, MA, USA). Differences between groups were considered as statistically significant when p<0.05. Values of sample size n and degrees of freedom df are indicated in the figure legend.

3. Results and discussion
The dependence of the intracellular ATP concentration in bacteria E. coli on the external SMF for control samples is presented in Figure 1. There were 6 experimental series; each was repeated twice or three times. The average ATP content in single bacterial cell in the selected growth conditions was 10^{-19} mol, which was consistent with the literature data [9]. Two characteristic ranges of the magnetic-field dependencies on the ATP content in E. coli were observed: 0-10, and 15-95 mT.

In the first range the ATP pool reaches the maximum value, twice times higher than ATP concentration for bacteria cultivated in geomagnetic field (the first point on the curve). A large number of studies wrongly focused on the detection of magnetic field effects in strong magnetic fields, much larger than the Earth’s magnetic field [12-13]. The effects usually detected in such cases cannot explain the magnetic sensitivity of living organisms in the weak magnetic field of a technogenic nature and their reaction to variations of this field. Moreover, those results can hardly be used to understand a mechanism of magnetic control of intracellular enzymatic process. The combined effects of external SMFs and magnetic moments of atomic nuclei can be observed and registered exactly in weak magnetic fields, the strength of which is less than the value of hyperfine interactions constants. The range of magnetic fields for observing such effects should be 0-10 mT according to theoretical calculations [3]. In this range we expected the bright magnetic-field effects in living organisms. According to the theory of enzymatic magnetosensitivity, the primary receiver of an external magnetic field is a spin-dependent stage of elementary acts of enzymatic processes. The magnetosensitivity of these stages is due to the participation of particles with nuclear magnetic moments, such as magnetic isotopes 1H, 13C, 39K and their hyperfine interaction with the electronic spin and the external magnetic field. These isotopes are presence in all living organisms independent of growths conditions. A required condition of such magnetosensitive enzyme reactions is the electron transfer and the formation of an IRP. The nuclear magnetic moments of stable isotopes and an external SMF are to induce the transition of ion-radical pairs from the initial singlet state into a triplet one [3]. The
probabilities of the forward and reverse electron processes depend on the total spin state of such pair. For example, the singlet-triplet conversion of the pair “magnesium ion 25Mg+ – ADP radical” in the active site of ATP-synthase [4] leads to increase the probability of a direct reaction (ATP formation). The magnetic field effects, which were obtained in the range 0-10 mT, are the most interesting effects accordingly to the theoretical predictions [3].

Figure 1. Magnetic-field dependence of the ATP pool in E. coli bacteria cultivated in LB media. The range of magnetic fields of 0.8-98 mT. *The differences between the mean values for the magnetic fields 6-9 mT and magnetic fields 0.8-5, 10-95 mT are statistically significant at p<0.05. A sample size of each group was n=14, a degree of freedom was df=26.

The changes in bacterial growth of E. coli were found in the same range of SMF [6]. An increase of colony-forming ability and growth rate were observed for bacteria exposure to the SMF 0-10 mT regardless of the magnetic isotopes presence in nutrient medium. These magnetic field effects indicate validity of the theoretical predictions and the presence of magnetically sensitive enzymatic reactions occurring in the ion-radical mechanism. No magnetic field effects with statistical validity were registered for ATP pool in the second range 15-95 mT.

3.1. Combined effects of external SMF and magnetic isotope 25Mg on ATP content of E. coli

The dependence of the intracellular ATP concentration in E. coli bacteria on the external magnetic field and magnesium-isotopic content (24Mg, 25Mg, 26Mg, Mg) in media are presented in Figure 2. Three characteristic ranges of the magnetic-field dependencies on the ATP content in E. coli were observed: 0.8-16, 16-70, 70-98 mT. In the first range, the magnetic field and magnesium isotope effects were registered for all bacteria. No statistically significant effects of intracellular ATP changes in bacterial cells were detected in the range from 16 to 70 mT. The combined effect of the external SMF and magnesium magnetic isotope 25Mg on the ATP pool was discovered in the range 70-98 mT.

The ATP pool peaks in the range from 0 to 16 mT were observed for all bacteria, enriched with the magnetic 25Mg and nonmagnetic isotopes 24,26Mg, and the natural magnesium. Presumably this was the result of different efficiencies of the magnesium magnetic and nonmagnetic isotopes as intracellular
elements. Several effects such as magnetic, mass-dependent isotope effects and magnetic field effects can be summed up. The observed effects are of special interest because of the selected weak magnetic fields range [3]. It is correlated with magnetic field effects in ATP content for control experimental groups (Figure 1). Similar dependences were obtained by us earlier [6] in growth of E. coli cells.

![Figure 2. Magnetic-field dependence of the ATP pool in E. coli bacteria cultivated in M9 media with the content of the magnesium isotopes 24Mg, 25Mg, 26Mg, Mg. The range of magnetic fields of 0.8-98 mT. *The differences between the mean values for the magnetic magnesium isotope 25Mg and nonmagnetic magnesium isotopes 24Mg and 26Mg at the same magnetic field are statistically significant at p<0.001 (n=12, df=22).](image)

The combined effect of the magnesium magnetic isotope ion 25Mg2+ and SMF was detected in the ATP pool of E. coli bacteria in the range 70-98 mT. The intracellular ATP concentration increases by 2-3 times in these fields for bacteria, enriched with magnesium magnetic isotope 25Mg. Interestingly, even 10%-enrichment of the nutrient medium by the magnetic isotope 25Mg (it is the content of magnetic isotope in natural magnesium) allows us to register the ATP pool increase. These data correlated with the magnetic fields effects on the colony-forming ability [6]. The key role in this strongest magnetic field effect belongs, most likely, to an increase in the rate of ATP synthesis because of the participation of the 25Mg nuclear spin in the enzymatic radical ion reaction and induction of the singlet-triplet conversion due to the hyperfine interaction mechanism. This is also confirmed by the results of experiments in the external magnetic field with isolated enzymes enriched with magnetic 25Mg and nonmagnetic 24Mg in vitro [4-5].

In this range the combined effects of SMF and magnetic moments of atomic nuclear of 25Mg and 67Zn in content of Na, Ca, Mg, P bacteria were found previously [14]. Magnesium ions are effective participants of ion-radical reactions of the ATP enzymatic synthesis [4]. It is important to note that all of these elements Na, Ca, Mg, P are associated with the work of bacterial ATP-ases [15]. Changes in their intracellular content confirm the influence of external SMF and magnetic moments of atomic nuclei on the enzymatic processes of the ATP synthesis in the range of 70-98 mT.
3.2. Combined effects of external SMF and magnetic isotope $^{67}$Zn on ATP content of E. coli

As a result of 10 experimental series, we obtained the experimental magnetic-field dependences of the intracellular ATP content in the bacteria cultured in zinc isotope-containing media. The results are shown in Figure 3. Three ranges of magnetic fields (2.2–8, 25–35, and 60–78 mT) with characteristic features can be distinguished in the figure. In the first range, the intracellular ATP content increased regardless of zinc isotope type as well as in control groups and in experiments (Figure 1) with magnesium isotopes (Figure 2). The similar dependences in this range were obtained for CFU number and growth rate constants of E. coli cells cultured in M9 media with zinc isotopes [16]. In this range, the intracellular ATP content increased for all bacteria. However, in the bacteria grown on medium with the magnetic isotope $^{67}$Zn in the range of 2.2–4.2 mT, the ATP concentration was 2–3.5 times higher. This indicated the combined effect of external SMF and magnetic nuclear moments of $^{67}$Zn on enzymatic synthesis of ATP. In the second SMFs range (25–35 mT), the concentration of ATP increased slightly but was not significantly different from that of other test groups of bacteria. This means that the combined effects of SMF and magnetic zinc isotope on the CFU and the growth rate constants found in this range [16] is associated with other enzymatic processes rather than ATP synthesis.

![Figure 3. Magnetic-field dependence of the ATP pool in E. coli bacteria cultivated in M9 media with the content of the zinc isotopes $^{64}$Zn, $^{66}$Zn, $^{67}$Zn, $^{*}$Zn. The range of magnetic fields of 2.2-78 mT. **The differences between the mean values for the magnetic zinc isotope $^{67}$Zn and nonmagnetic zinc isotopes $^{64}$Zn and $^{66}$Zn at the same magnetic field are statistically significant at p<0.05 (n=10, df=18).](image)

In the third SMFs range (60–80 mT), we found a slight increase in the intracellular concentration of ATP for bacteria cultured in the medium containing magnetic zinc isotope $^{67}$Zn. A similar effect was observed by us earlier when studying the combined effect of magnetic field of this range and the magnetic magnesium isotope $^{25}$Mg (Figure 2). The majority of enzymatic reactions of ATP synthesis proceed through the formation of an ion–radical pair with the involvement of Mg$^{2+}$ [4, 5]. Probably, in absence of magnesium ions in medium, divalent zinc ions Zn$^{2+}$ may replace it in various reactions [17], including ATP synthesis. Metabolisms of Mg$^{2+}$, and Zn$^{2+}$ ions depend on each other, including, and on the principle of "mimicry" [18], well-known in microbiology. In the case of biological unavailability of the necessary bivalent ions, intracellular molecular complexes are able to use the available ions of the same valence in a molecular environment. So many enzymatic systems using magnesium ions in their work are able to function with zinc ions and vice versa. However, the
effect of zinc ions was significantly smaller, and the detected combined effect of external SMF and the magnetic zinc isotope was low (not higher than 15%).

4. Conclusion
All observed effects of an external magnetic field and the magnetic zinc isotope $^{67}$Zn are consistent with the theory of magnetosensitivity of living organisms. Magnetic field effects in the range of 0.8-16 mT, registered for all bacteria regardless of magnesium-isotope enrichment of the medium, indicate the sensitivity of intracellular processes to weak magnetic fields.

ATP pool in the bacteria *Escherichia coli* is a magnetically dependent indicator of the microorganism life. It depends on the magnitude of external SMF and the existence of the nuclear magnetic moment of the magnesium isotope added to the growth medium. The combined effect of the magnetic field of 70-95 mT and the magnetic isotope of magnesium $^{25}$Mg on the bacteria *E. coli* leads to a significant increase in the intracellular concentration of ATP. This combined effect confirms the possibility of magnetic control of intracellular enzymatic ATP synthesis.

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