Combined effect of magnesium isotopes and antibiotics on morphology of *E. coli*

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**Abstract.** In this paper, the morphology of *Escherichia coli* bacteria grown in the presence of magnesium isotopes (magnetic $^{25}$Mg, non-magnetic $^{24,26}$Mg and natural Mg), and antibiotics of various groups were studied. Atomic force microscopy (AFM) was used as the main method. When the bacteria were cultured in the presence of different groups of antibiotics, the following morphological changes were detected: filamentation, cell lysis, cell adhesion, cell wall damage, and the formation of "depressions" or pores. For *E. coli* bacteria cultured on a medium with tobramycin and lincomycin, a magnetic magnesium isotope effect $^{25}$Mg in bacteria morphology was detected, which was manifested as a change in the roughness of bacterial cells. Both of these antibacterial drugs act on the same intracellular system – protein synthesis. It has also been observed that the elongation of bacterial cells, when exposed to levofloxacin and ciprofloxacin, depends on the type of magnesium isotope in the nutrient medium. The combined action of the $^{25}$Mg magnetic isotope and quinolones on bacteria makes it possible to obtain the process followed by significant elongation of cells, thereby indicating improved antibiotic effect, as compared with the nonmagnetic $^{24,26}$Mg isotopes.

1. **Introduction**

Magnetic isotopes of magnesium ($^{25}$Mg), calcium ($^{43}$Ca), and zinc ($^{67}$Zn) possessing the nuclear spin and magnetic moment respectively can have their specific effect on enzymatic ATP and DNA synthesis processes *in vitro* [1-2]. With *E. coli* cells enriched by a $^{25}$Mg magnetic isotope, their concentration is increased and colony-forming capacity is enforced and, as a result, the intracellular biochemical composition is changed [3]. Magnesium is involved in 300 enzyme systems within bacterial cells. Nuclei of isotopes with a magnetic moment can affect the enzymatic reaction realized with transfer of one or several electrons [1, 4]. As a matter of fact, changes occur not only in the product of the enzymatic reaction, but also in the physiology of organisms - e.g. bacteria-formed biofilm or antimicrobial drug susceptibility [5]. For example, on adding magnetic magnesium isotope to nutrient medium, it is possible to improve the effect induced by nalidixic acid and ciprofloxacin on *E. coli* cells [5-6].

Specific morphological features of *Escherichia coli* incubated in the presence of magnesium isotopes (magnetic $^{25}$Mg and nonmagnetic $^{24,26}$Mg) and antibiotics of various groups have been studied in this paper. The problem was formulated in order to view influence of various intracellular systems - i.e. antibiotic targets - and to identify the nuclear magnetic moment susceptibility of a magnesium isotope through visualization of bacterial cells. Atomic-force microscopy (AFM) was a basic procedure applied for making intravital study of the bacterial ultrastructure.
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, Ural branch of RAS, Orenburg, Russia), which was grown in minimum synthetic nutrient media M9: 37.4 mM NH₄Cl, 2.2 mM ²⁴,²⁵,²⁶MgSO₄, 55.5 mM glucose, 84.5 mM Na₂HPO₄, 44.1 mM KH₂PO₄, 17.1 mM NaCl (Reachem, Moscow, Russia). The media differed only by the isotope form of magnesium in the salt: nonmagnetic ²⁴Mg and, magnetic ²⁵Mg [3]. Magnesium isotopes were added to the media as sulphate MgSO₄. For the sulphates preparation, isotopically pure oxides were used: ²⁴MgO, ²⁵MgO, ²⁶MgO (Combine “Electrochempribor”, Lesnoy, Russia) with extremely high isotope enrichment. The museum strain of E. coli was pre-incubated in LB broth (Sigma-Aldrich, USA). Then bacteria were incubated at 37°C 11 h in M9 medium containing magnesium isotopes and antibiotics, and cell samples were obtained for microscopy.

2.2. Determination of the minimal growth-inhibiting concentration (MIC)
MIC was determined by the serial dilutions method. Bacteria were grown in liquid M9 medium with magnesium isotopes. The initial density of the culture (immediately after inoculation) was 10⁵ CFU/mL. To obtain cell samples, the bacteria were cultured in an M9 medium containing an antibiotic at a concentration of ½ MIC. The following antibiotics were tested in experiments: ciprofloxacin, levofloxacin, amoxicillin, gentamicin, amikacin, tobramycin, lincomycin.

2.3. Determination of the morphological features by AFM
The samples subject to testing for morphology were prepared for carrying out the AFM procedure as follows: medium was thoroughly washed off, 2-3 µL of bacterial suspension was placed on a metal washer used as substrate for a mica fragment of approximately 8x8 mm. The probe microscope CMM-2000 (manufacturer: PROTON-MIET JSC, Russia) was used for atomic-force scanning of the samples in the air. The microscope MSCT-AUNM cantilevers (Veeco Instruments Inc., USA) with the beam stiffness of 0.03 N/m and with about 10 nm radius of needle curvature were used for testing. The common microscope software was used for obtaining quantitative description of scans upon morphometric analysis (length, width, height, and roughness).

2.4. Statistical Analysis
Data were expressed as mean ± SD. Student’s tests were used to determine statistical differences by Origin 8.0 software (Version 8.0; Microcal Software, USA). Differences between groups were considered as statistically significant when p<0.05. A sample size of each group was n=10, a degree of freedom was df=18.

3. Results and discussion
The AFM-images of bacterial E. coli cells cultured in M9 medium with magnesium isotopes and antibiotics were obtained and studied. Table 1 shows control test sample morphometric measurement results - i.e. E. coli incubated in M9 medium with magnesium isotopes only. The results for various groups (²⁴Mg, ²⁵Mg, and ²⁶Mg) display no significant differences. When cultivating bacteria in the presence of antibiotics of various groups, the following morphological changes were found: filamentation (Figure1a), formation of "depressions" or pores (Figure1b), cell agglomeration (Figure1c), cell wall damage (destroyed peptidoglycan layer) (Figure1d), cell lysis (Figure1e). Need to say that no statistical discrepancy was found for amoxicillin, gentamycin, and amikacin when they acted on bacteria E. coli cultured in the presence of various magnesium isotopes. Table 2 shows results of morphological changes in bacteria exposed to ciprofloxacin, levofloxacin, tobramycin, and lincomycin. All data are listed in respect to the control test results (Table 1) and given in %.
Table 1. Morphometric indicators of *E. coli* bacteria cultured in M9 medium with magnesium isotopes $^{24}$Mg, $^{25}$Mg or $^{26}$Mg without antibiotics (control), $n=10$, $df=18$

| Mg isotope | Length, $\mu$m | Width, $\mu$m | Height, nm | Roughness, % |
|------------|----------------|--------------|------------|--------------|
| $^{24}$Mg  | 2.01±0.12      | 0.82±0.08    | 250±15     | 72.52±8.25   |
| $^{25}$Mg  | 1.95±0.14      | 0.90±0.06    | 240±14     | 82.29±6.89   |
| $^{26}$Mg  | 2.12±0.18      | 0.90±0.07    | 250±15     | 91.58±9.02   |

Figure 1. The AFM images of bacterial *E. coli* cells - examples of morphological changes:
- a) filamentation (cultured in M9 medium with $^{25}$Mg and levofloxacin);
- b) formation of "depressions" or pores (cultured in M9 medium with $^{25}$Mg and tobramycin);
- c) cell agglomeration (cultured in M9 medium with $^{26}$Mg and lincomycin);
- d) cell wall damage (cultured in M9 medium with $^{26}$Mg and lincomycin);
e) cell lysis (cultured in M9 medium with $^{25}$Mg and tobramycin);
f) control samples of bacteria (cultured in M9 medium with $^{25}$Mg without antibiotics).

Table 2. Morphometric indicators of E. coli bacteria cultured in M9 medium with magnesium isotopes $^{24}$Mg, $^{25}$Mg or $^{26}$Mg and antibiotics*

| Mg isotope in M9 | Ciprofloxacin | Levofloxacin | Tobramycin | Lincomycin |
|-----------------|---------------|--------------|------------|------------|
| Concentration of antibiotic, µg/ml | 0.38 | 0.33 | 0.94 | 19 |
| **Length, %** | | | | |
| $^{24}$Mg | >298.5±50.3 | >398.0±29.1 | 80.1±10.2 | 106.9±7.8 |
| $^{25}$Mg | >461.5±41.2b | >512.8±53.2b | 89.7±5.1 | 97.4±9.5 |
| $^{26}$Mg | >283.0±42.9 | >330.2±47.5 | 96.2±9.8 | 108.5±10.3 |
| **Width, %** | | | | |
| $^{24}$Mg | 106.2±9.4 | 111.1±12.9 | 104.9±5.6 | 100±4.5 |
| $^{25}$Mg | 152.2±12.1b | 136.7±10.1b | 107.8±6.3 | **114.4±5.3**a |
| $^{26}$Mg | 102.2±8.2 | 98.9±5.6 | 101.1±12.1 | 106.9±3.7 |
| **Height, %** | | | | |
| $^{24}$Mg | 84.0±5.6 | 76.0±6.8 | 84.0±8.2 | 72.0±7.8 |
| $^{25}$Mg | 41.7±7.2b | 58.3±5.7b | 95.8±5.3 | 83.3±8.1 |
| $^{26}$Mg | 80.0±8.4 | 72.0±7.3 | 88.0±3.4 | 72.0±9.2 |
| **Roughness, %** | | | | |
| $^{24}$Mg | –d | –d | 90.9±5.6 | 88.5±9.1 |
| $^{25}$Mg | –d | –d | **39.9±9.3**b | **63.5±5.1**a |
| $^{26}$Mg | –d | –d | 69.1±10.5 | 72.2±8.3 |
| **Morphological changes** | $^{24}$Mg | filamentation | filamentation | cell wall damage | formation of "depressions" or pores |
| | $^{25}$Mg | filamentation, cell lysis, cell agglomeration | filamentation (figure 1a), cell lysis, cell agglomeration | cell agglomeration, formation of "depressions" or pores (figure 1b), cell lysis (figure 1c), formation of "depressions" or pores |
| | $^{26}$Mg | filamentation | filamentation | cell wall damage (figure 1d), formation of "depressions" or pores |

* The concentrations of the antibiotics were 50% of their MIC

a The differences between the mean values are statistically significant at p<0.05 (n=10, df=18)
b The differences between the mean values are statistically significant at p<0.001 (n=10, df=18)
c All data are represented as percentages relative to the control groups (Table 1)
d The roughness measurement was not possible due to abnormal bacterial cell length

As for antibiotics from the quinolone/fluoroquinolone group (ciprofloxacin, levofloxacin) which mechanism of action is related to suppression of a DNA synthesis process, elongated cells filamentation with the length exceeding that of normal bacteria 3-5 times typically appear. The presence of constrictions is noticeable on AFM-images of such bacteria (Figure 1a). This means that
the process of cell division is disrupted, and several bacteria form a single strand. The combined effect of the magnetic magnesium isotope $^{25}\text{Mg}$ and quinolones on bacteria leads to a significant elongation of cells as compared with the nonmagnetic $^{24,26}\text{Mg}$ isotopes, thereby indicating increased antibiotic effect. Bacteria E. coli cultured with magnetic magnesium exhibit their altered width and height as compared with control test ones and bacteria cultured with nonmagnetic isotopes. It indicates significant destruction of cells and lysis and proves that combined action of the $^{25}\text{Mg}$ magnetic isotope and quinolones results in negative effect. Ciprofloxacin and levofloxacin inhibit DNA gyrase and IV type topoisomerase thereby producing some disturbance of DNA synthesis [7]. The magnetic isotope also suppresses DNA synthesis reducing DNA polymerase efficiency [8]. This is to say in support of the previously obtained results, should magnetic magnesium and quinolones be used in combination, they improve these antimicrobial drug susceptibility [5-6].

Exposure to tobramycin (a representative of aminoglycosides) led to damage to the cell wall and cell-to-cell adhesion, which may be a consequence of the mechanism of action of tobramycin affecting protein synthesis. A characteristic morphological feature of bacteria incubated in the medium M9 with magnetic magnesium isotope $^{25}\text{Mg}$ as opposed to the nonmagnetic $^{24,26}\text{Mg}$ isotopes was the formation of "depressions" or pores (Figure 1b). With the roughness profile studied statistically, the combined effect of tobramycin and magnetic magnesium produced in the E. coli morphology (Table 2) can be proved: such bacteria have more heterogeneous structure than the control test ones and bacteria cultured with $^{24,26}\text{Mg}$. Insignificant changes in roughness of bacteria incubated in medium with the $^{25}\text{Mg}$ magnetic magnesium isotope was also found in lincomycin (lincosamides). This antibiotic has the mechanism similar to that of tobramycin since it disturbs a protein synthesis process blocking a 50S ribosome subunit. Consequently, protein synthesis in bacteria can be specified as a spin-susceptible process that has a different effect for magnetic and nonmagnetic magnesium isotopes.

4. Conclusion

On studying the combined effect of magnesium isotopes and antibiotics of various groups on morphology of Escherichia coli by means of AFM, two types of magnetic isotope effects were identified. One of them is related to changes in the roughness profile and to formation of "depressions" in bacteria incubated with magnetic magnesium and antibiotics capable to depress a protein synthesis process - i.e. tobramycin and lincomycin. The other magnesium-induced magnetic isotope effect is caused by quinolone group antibiotics - i.e. ciprofloxacin and levofloxacin - that blocks DNA synthesis in the bacterial cell. The results obtained provide for application of a magnetic magnesium isotope in order to improve the effect of antibiotic - i.e. its potentiation. The above study shows that the AFM procedure can be used for recording magnetic isotope effects produced by magnesium and other chemical elements as an independent and additional method.

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