

UDC 57.052(045)+57.088.5(045)  
DOI: 10.18372/2306-1472.73.12189

Svitlana Gorobets<sup>1</sup>  
Oksana Gorobets<sup>2</sup>  
Oleksandr Medviediev<sup>3</sup>  
Liubov Kuzminykh<sup>4</sup>

## THE ROLE OF PATHOGENIC MICROORGANISMS IN THE ACCUMULATION OF BIOGENIC MAGNETIC NANOPARTICLES IN LUNG TISSUES

<sup>1,2,3,4</sup>National Technical University of Ukraine “Igor Sikorsky Kyiv Polytechnic Institute”  
37, Prospect Peremogy, Kiev, 03056, Ukraine  
E-mails: <sup>1</sup>pitbm@ukr.net; <sup>2</sup>gorobets.oksana@gmail.com; <sup>3</sup>will.be.psychedelic@gmail.com;  
<sup>4</sup>eugenekuz@gmail.com

### Abstract

**Purpose:** In this paper the role of pathogenic microorganisms in the accumulation of biogenic magnetic nanoparticles in lung tissues is examined. The main purpose of the present research is to prove that biogenic magnetic nanoparticles can be accumulated in lung tissues because of the pathogenic microorganisms during lung disease. **Methods:** Pairwise and multiple alignment of amino acid sequences, electron paramagnetic resonance. **Results:** The producers of biogenic magnetic nanoparticles were found among pathogenic microorganisms that cause lung disease. Biogenic magnetic nanoparticles can be accumulated in lung tissues because of these pathogenic microorganisms during relevant lung diseases. The presence of biogenic magnetic nanoparticles in lung tissues was proved using the electron paramagnetic resonance spectra analysis. **Discussion:** The results of the present research can be used to understand the possible reasons of toxic and allergic effects and even corking of vessels during uncontrolled accumulation of magnetic nanoparticles in different organs and tissues because of their magnetic dipole-dipole interaction with biogenic magnetic nanoparticles on cell membranes and to prevent it.

**Keywords:** biogenic magnetic nanoparticles; biomineralization; electron paramagnetic resonance; lung tissues; pathogenic microorganisms.

### 1. Introduction

Biogenic magnetic nanoparticles (BMN) are the subject of intense research since 1975 when they were first found in magnetotactic bacteria (MTB) which show taxis in the direction of the geomagnetic field [1]. Since then, BMN were found in many other organisms that belong to all three domains: eukaryotes, prokaryotes and archaea.

In addition, the presence of the BMN were experimentally confirmed in such organisms as: protozoa and algae [2], worms [3], termites [4], snails [5], sea turtles [6], birds [7-9], ants and butterflies [10, 11], honey bees [12], lobsters [13], tritons [14], fish [15-17], dolphins and whales [18], bats [19] and human [20-22].

BMN were detected during experimental researches in several human tissues and organs: liver, heart, spleen [23], ethmoid bone [24], adrenal glands [25] and brain [21, 22]. BMN were also found in pathologically changed tissues after or

during different neurodegenerative diseases [26], atherosclerosis [27], cancer [22, 28]. Moreover, BMN concentration is higher in the inflammation zone during cancer [22, 28] and neurodegenerative disease [29, 30] than in the same tissues in a normal state.

BMN are strong natural magnets and act as concentrators of various compounds and vesicles [31, 32]. BMN are located on cell membranes in several normal and abnormal human organs and tissues [21-26]. This may cause toxic and allergic effects and even corking of vessels during uncontrolled accumulation of magnetic nanoparticles in such organs and tissues [33] because of their magnetic dipole-dipole interaction with BMN on cell membranes [31, 32].

The problem of accumulation of magnetic nanoparticles in human organs, particular in lungs, is extremely relevant. Magnetic nanoparticles can reach organs and tissues in different ways. For example, accumulating during bacterial diseases

with pathogens in which BMN are present, during treatments with magnetic targeting drug delivery, produced as a result of biomineralization in the lung tissues or even get inside the human organism with contaminated air.

In view of the above, potential BMN producers research among different lung pathogens is important because of the possibility to prevent and cure possible corking of vessels, toxic and allergic effects during uncontrolled accumulation of magnetic nanoparticles

The main objective of this work is the detection of lung pathogens characterized by BMN biomineralization process and experimental confirmation of BMN presence in lung tissues.

## 2. Experimental part

To predict the pathogens that are potential producers of BMN, proteins homologues to the proteins of magnetotactic bacteria magnetosome island Magnetospirillum gryphiswaldense MSR-1 were found using the database and blast resource of the National center for biotechnology information.

A comparison of the amino acid sequences of the Mam group proteins, without which the BMN biomineralization in *Magnetospirillum gryphiswaldense* MSR-1 is impossible, was conducted using pairwise and multiple alignment of amino acid sequences methods with the proteins of the following bacteria *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Mycobacterium tuberculosis*, *Mycobacterium bovis* and some other microorganisms of the following families: *Enterobacteriaceae* and *Peptostreptococcus spp.* as a pathogens with the inflammation zone and disease localization in the lung tissues.

In the present research the samples of *Sus domestica* lung, liver and heart tissues were used as a model, which is genetically close to the human organism and the mechanism of BMN biomineralization is the same for all the organisms of archaea, prokaryotes and eukaryotes domains. It is based on a set of homologous proteins to MTB proteins, without which the biomineralization of magnetic nanoparticles is impossible

Electron paramagnetic resonance (EPR) was used as a method for the presence of magnetic nanoparticles detecting in lung tissues as more suitable for biological samples analysis. Electron paramagnetic resonance is a method for studying materials with unpaired electrons. The basic concepts of EPR are analogous to those of nuclear magnetic resonance, but it is electron spins that are

excited instead of the spins of atomic nuclei. EPR spectroscopy is particularly useful for studying metal complexes or organic radicals. BMN was experimentally found in the human heart and liver [23]. So, the samples of *Sus domestica* liver and heart were used for comparison of the EPR signal values (as the control samples).

Tissue samples were prepared at the Laboratory of magnetic nanotechnologies in biology and medicine of the chair of bioinformatics National Technical University of Ukraine "Igor Sykorsky Polytechnic Institute". 5 *Sus domestica* lung tissue samples were taken for the research. 4 *Sus domestica* liver tissue samples and 4 *Sus domestica* heart tissue samples were taken as a control. All the samples were chiseled with a ceramic knife to prevent the ingestion of external iron particles. All the samples were dried to a constant mass in the drying cabinet at 105° C. 0,01 g of the dried substance of every sample was taken to determine the EPR spectrum.

The presence, quantity and character of magnetic nanoparticles in the investigated tissues were determined using the EPR spectroscopy method with dried samples. The samples were reused to determine the magnetic response after thermal treatment at 250° C.

The results obtained using EPR are usually presented through the first derivative of the absorption spectrum. Possible errors were taken into account in the calculations. Results were interpreted as for 1 g of raw tissues. Also, the average content of magnetite in each investigated tissue per 1 g of raw substance was determined. The diagrams of the dependence of the first derivative energy of adsorption of electromagnetic radiation from the magnetic field induction of dried and thermally treated samples of *Sus domestica* lung, heart and liver tissues are presented at the figures 1, 2 [34].

## 3. Results and discussion

As a result of the conducted bioinformatical analysis the following microorganisms were determined as synthesizers of crystalline biogenic magnetic nanoparticles: *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Legionella pneumophila*, *Salmonella enterica*, *Yersinia pestis*, *Yersinia enterocolitica*. This is evidenced by the value of statistical numbers that showed the homology and common functions between the proteins of the investigated microorganisms and the proteins of MTB magnetosome island (MI), without which the biomineralization of magnetic nanoparticles is impossible.

The conducted bioinformatical analysis of the homology between MI MTB proteins and the proteins of pathogenic human microorganisms has shown that such microorganisms (pathogens of lung diseases) as *Staphylococcus aureus*, *Staphylococcus aureus* RF122, *Staphylococcus aureus subsp. aureus* ST228, *Klebsiella pneumoniae* RYC492, *Klebsiella pneumoniae* 342, *Legionella pneumophila*, *Pseudomonas aeruginosa*, *Pseudomonas aeruginosa* M18, *Enterobacteriaceae*, *Salmonella enterica*, *Yersinia pestis*, *Yersinia enterocolitica*, *Yersinia enterocolitica* LC20, *Peptostreptococcus sp.* MV1, *Peptostreptococcus anaerobius*, *Peptostreptococcus anaerobius* VPI 4330, *Peptostreptococcus stomatis*, may be potential producers of amorphous BMN because mamA protein functions in this microorganism are different from the functions of the same MTB protein. In this case MamA can take part in the forming of BMN crystalline structure. For the intracellular crystalline BMN, that are localized in a cell as a chain, biomineralization, it is necessary to have all the homologues of the MI MTB proteins and the homologues of the mamK protein, that are responsible for the formation of BMN chains.

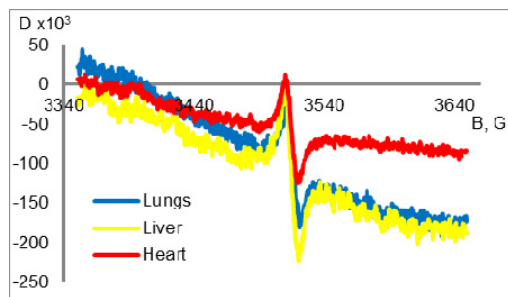


Fig. 1. EPR spectra: dependence of the first derivative of the electromagnetic microwave radiation absorption energy D on magnetic field flux density B for dried samples of *Sus domestica* lung, heart and liver tissues.

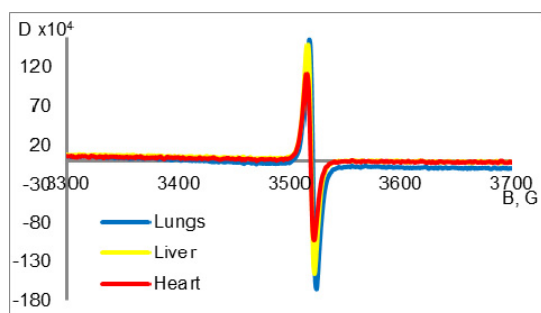


Fig. 2. EPR spectra: dependence of the first derivative of the electromagnetic microwave radiation absorption energy D on magnetic field flux density B for thermally treated samples of *Sus domestica* lung, heart and liver tissues.

At the same time, the functions of the MamK protein homologies in such organisms as *S. aureus*, *S. aureus subsp. aureus* ST228, *S. suis* BM407, *Enterobacter aerogenes* KCTC 2190, *Klebsiella pneumoniae* 342, *Legionella pneumophila*, *P. aeruginosa*, *S. enterica*, *Y. enterocolitica*, *Y. enterocolitica* LC20, *E. coli*, *M. tuberculosis complex*, *M. tuberculosis avium* are different from the functions of the same protein in MTB, which suggests a possible lack of BMN chain formation in these microorganisms and the localization of BMN in the cytoplasm (not on the membrane) due to the magneto-dipole interaction force.

56% of all analyzed microorganisms may be potential producers of BMN and 44% have no ability to magnetotaxis because of the MI MTB proteins lack.

Fig. 1, 2 shows dependences of the first derivative of adsorption energy of electromagnetic radiation (D) on the induction of a magnetic field (B, G) for dried and thermally treated samples. Figure 1 shows a diagram for all dried tissue samples: lungs, liver and heart. Figure 2 shows a diagram for all thermally treated tissue samples: lungs, liver and heart. As a result, the thermally treated samples show greater magnetic response than the dried samples.

In the present research the presence of the BMN in *Sus domestica* lung tissue was experimentally confirmed using analysis of EPR spectra of thermally treated and dried lungs, liver and heart samples. Moreover, BMN can be accumulated in lung tissues because of the above mentioned pathogenic microorganisms during relevant lung diseases. The BMN biomineralization process is the same for eukaryotes, prokaryotes and archaea. *Sus domestica* is a genetically close to human model organism, so we can assume, that the results of the present research are fair for the human lung tissues.

The results of the present research can be used to understand the possible reasons of toxic and allergic effects and even corking of vessels during uncontrolled accumulation of magnetic nanoparticles in different organs and tissues because of their magnetic dipole-dipole interaction with BMN on cell membranes and to prevent it.

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**С.В. Горобець<sup>1</sup>, О.Ю. Горобець<sup>2</sup>, О.В. Медведєв<sup>3</sup>, Л.В. Кузьмініх<sup>4</sup>**

**Роль патогенних мікроорганізмів у накопиченні біогенних магнітних наночастинок у тканинах легень**

<sup>1, 2, 3, 4</sup>Національний технічний університет України "Київський політехнічний інститут імені Ігоря Сікорського", проспект Перемоги, 37, Київ, Україна, 03056

E-mails: <sup>1</sup>pitbm@ukr.net; <sup>2</sup>gorobets.oksana@gmail.com; <sup>3</sup>will.be.psychedelic@gmail.com;

<sup>4</sup>eugenekuz@gmail.com

**Мета:** У цій роботі досліджується роль патогенних мікроорганізмів у накопиченні біогенних магнітних наночастинок у тканинах легень. Основна мета даного дослідження – довести, що біогенні магнітні наночастинок можуть потрапляти у тканини легень разом з патогенними мікроорганізмами та накопичуватись там при відповідних захворюваннях. **Методи дослідження:** Попарне та множинне вирівнювання амінокислотних послідовностей, електронний парамагнітний резонанс. **Результати:** Продукти біогенних магнітних наночастинок були знайдені серед патогенних мікроорганізмів, що є збудниками захворювань легень. Біогенні магнітні наночастинок можуть потрапляти у тканини легень разом з патогенними мікроорганізмами та накопичуватись там при відповідних захворюваннях. Наявність біогенних магнітних наночастинок у тканинах легень була експериментально доведена в ході аналізу спектра електронного парамагнітного резонансу. **Обговорення:** Результати даного дослідження можуть бути використані для запобігання та розуміння можливих причин токсичного, алергічного впливу і закупорки судин під час неконтрольованого накопичення магнітних наночастинок в різних органах та тканинах через їх магнітні диполь-дипольні взаємодії з біогенними магнітними наночастинок на клітинних мембранах.

**Ключові слова:** біогенні магнітні наночастинок; біомінералізація; електронний парамагнітний резонанс; патогенні мікроорганізми; тканини легень.

**С.В. Горобец<sup>1</sup>, О.Ю. Горобец<sup>2</sup>, А.В. Медведев<sup>3</sup>, Л.В. Кузьминых<sup>4</sup>**

**Роль патогенных микроорганизмов в накоплении биогенных магнитных наночастиц в тканях легких**

<sup>1,2,3,4</sup> Национальный технический университет Украины "Киевский политехнический институт имени Игоря Сикорского", проспект Победы, 37, Киев, Украина, 03056

E-mails: <sup>1</sup>pitbm@ukr.net; <sup>2</sup>gorobets.oksana@gmail.com; <sup>3</sup>will.be.psychedelic@gmail.com;  
<sup>4</sup>eugenekuz@gmail.com

**Цель:** В данной работе исследуется роль патогенных микроорганизмов в накоплении биогенных магнитных наночастиц в тканях легких. Основная цель исследования – доказать, что биогенные магнитные наночастицы могут попадать в ткани легких вместе с патогенными микроорганизмами и накапливаться там при соответственных заболеваниях. **Методы исследования:** Парное и множественное выравнивание аминокислотных последовательностей, электронный парамагнитный резонанс. **Результаты:** Продуценты биогенных магнитных наночастиц были обнаружены среди патогенных микроорганизмов, которые являются возбудителями заболеваний легких. Биогенные магнитные наночастицы могут попадать в ткани легких вместе с патогенными микроорганизмами и накапливаться там при соответственных заболеваниях. Наличие биогенных магнитных наночастиц в тканях легких была экспериментально подтверждена в ходе анализа спектра электронного парамагнитного резонанса. **Обсуждение:** Результаты данного исследования могут быть использованы для предотвращения и понимания возможных причин токсического, аллергического эффектов и закупорки сосудов во время неконтролируемого накопления магнитных наночастиц в различных органах и тканях вследствие их магнитных диполь-дипольных взаимодействий с биогенными магнитными наночастицами на клеточных мембранах.

**Ключевые слова:** биогенные магнитные наночастицы; биоминерализация; патогенные микроорганизмы; ткани легких; электронный парамагнитный резонанс.

**Gorobets Svitlana.** Doctor of Technical Sciences. Professor.

Chief of chair of Bioinformatics, Faculty of Biotechnology and Biotechnics, National Technical University of Ukraine "Igor Sikorsky Kyiv Polytechnic Institute", Kyiv, Ukraine.

Research area: bioinformatics, nanostructured materials, magnetic nanoparticles

Publications: 278

E-mail: pitbm@ukr.net

**Gorobets Oksana.** Doctor of Physical and Mathematical Sciences. Professor.

Chair of Bioinformatics, Faculty of Biotechnology and Biotechnics, National Technical University of Ukraine "Igor Sikorsky Kyiv Polytechnic Institute", Kyiv, Ukraine.

Research area: bioinformatics, nanostructured materials, nanomagnetic materials.

Publications: 184

E-mail: gorobets.oksana@gmail.com

**Medvediev Oleksandr.** Graduate student.

Chair of Bioinformatics, Faculty of Biotechnology and Biotechnics, National Technical University of Ukraine "Igor Sikorsky Kyiv Polytechnic Institute", Kyiv, Ukraine.

Research area: bioinformatics, magnetic nanoparticles.

Publications: 4

E-mail: will.be.psychedelic@gmail.com

**Kuzminykh Liubov.** Student.

Chair of Bioinformatics, Faculty of Biotechnology and Biotechnics, National Technical University of Ukraine "Igor Sikorsky Kyiv Polytechnic Institute", Kyiv, Ukraine.

Publications: 1

E-mail: 8eugenekuz@gmail.com