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Biogenic magnetic nanoparticles in lung, heart and liver

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The main purpose of this research is to find new human organs and tissues where BMN biomineralization can take place to prevent BMN accumulation, which can lead to side effects during drug delivery.
Keywords: biogenic magnetic nanoparticles, biomineralization, magnetic nanocomposites.

Основной целью исследования является поиск новых органов и тканей, в которых возможен процесс биоминерализации БМН для предотвращения накопления БМН, что может привести к возникновению побочных эффектов при целевой доставке лекарственных препаратов.

Передбачення та підтвердження наявності БМН у легенях, серці та печінці. *С.В.Горобець, О.Ю.Горобець, О.В.Медведєв, В.О.Голуб, Л.В.Кузьміних*.
Основною метою даного дослідження є пошук нових органів та тканин, у яких можливий процес біомінералізації БМН для запобігання накопичення БМН, що може призвести до побічних ефектів під час цільової доставки лікарських препаратів.

1. Introduction

Living organisms have a genetically programmed ability to synthesize a wide spectrum of minerals and other inorganic substances in a process known under the general name of biomineralization [1, 2]. Biosynthesis of so-called biogenic magnetic nanoparticles (BMNs) from inorganic iron compounds is of particular interest because of the magnetic properties of BMNs.

Biogenic magnetic nanoparticles were found in many organs and tissues of the human body. They can be accumulated there during lifetime [3, 4]. BMN were found in human's heart, liver, spleen [5], adrenal glands [6], ethmoid bone [7] and brain [8–11] in the normal state.

In recent years, the use of magnetically nanomaterials is promising for solving some urgent medical and biological problems. Based on these nanocomposites with unique hierarchical architecture were created. They have the ability to recognize objects in microbiological biological fluids, deposit medicines in target organs, possibility to be used in the diagnosis and treatment of diseases at the cellular level, adsorption and removal of products of cellular decay under the influence of a magnetic field.

However remain relevant issues related to the influence of magnetic nanoparticles on living cells and organisms, and toxicological aspects of magnetic nanomaterials. Not been fully elucidated mechanisms of the damaging effects of nanomaterials on organisms and cell structures, processes biode-

gradation of nanoparticles in biological environments, the dependence of the toxicity of nanoparticles on their concentration, physical and chemical properties of the coating.

Relevance of work due to the fact that magnetic nanoparticles biogenic origin present in many organs of the human body are considered as functional materials, and are likely to accumulate them throughout their lives. It is therefore important to understand how artificial magnetic particles that enter the body composed of magnetically drugs or accumulate as a result of diseases interact with those already present in the body. In addition it should be noted that the forces of interaction of drugs with magnetic nanoparticles may be greater than the strength of antigen-antibody interactions.

Nowadays magnetically controlled vector-mediated drug delivery is widely spreading [12]. It is important to find organs where BMN biomineralization can take place to prevent BMN accumulation, which can lead to side effects during drug delivery [13]. It is also important to study the mechanisms of accumulation and interaction of magnetic nanoparticles entering in the human body as a part of magnetically controlled drugs, symbionts, bacteria with BMN in organs and tissues of the human body [14].

2. *Experimental*

Gene expression levels analysis. Gene expression levels are represented by quantitative measure of genes that are used in the synthesis of a functional gene product. The process of BMN biomineralization is mainly studied for magnetotactic bacteria (MTB), where magnetic nanoparticles are situated in special membrane structures — magnetosomes. According to [15] magnetite biomineralization of these bacteria is possible when the level of oxygen is below 2 kPa (2 %). There is a straightforward correlation between extracellular oxygen level and magnetite concentration in MTB Magnetospirillum gryphiswaldense. The largest quantity of BMN in magnetosomes was found at 25 Pa (0.025 %). The oxygen level higher than 2 kPa completely inhibits the formation of BMN [15].

It is known that analogous mechanism of BMN biomineralization takes place for archaea, prokaryotes and eukaryotes [16]. It is based on a set of proteins that are homologs of original MTB proteins. Without these proteins, BMN biomineralization is impossible [17, 18]. Some proteins, which are essential for BMN biomineralization in MTB and their homologues in the human proteome (the entire set of proteins expressed by a genome, cell, tissue, or organism at a certain time) are presented in Table [19]. Human tissues and organs where BMN biomineralization is possible were prognosticated based on analysis of human proteins homologs of original MTB proteins.

Homologs were found using amino acid sequences pairwise and multiple alignment methods. These methods are used to identify regions of similarity that may indicate functional, structural and/or evolutionary relationships between two biological sequences. The most studied proteome of M. gryphiswaldense MSR-1 [17, 18] was used as a microorganism to obtain original sequences of mam proteins, as researched. MamA, mamB and mamM proteins were chosen because their expression levels were experimentally obtained for M. gryphiswaldense MSR-1 cultured under oxygen deficiency conditions ($-O_2$) as well as under microaerobic conditions (std O_2) [15].

Comparison of gene expression levels is conducted using an average of FPKM (Fragment per Kilobase of Exon per Million Fragments Mapped). These data was obtained using Human Protein Atlas database.

Human lungs, heart and liver were selected as objects for expression levels comparison.

Detecting of BMN

The next step of the research was the experimental confirmation of the results of BMN presence in *Sus domestica* lungs which is a model organism that is genetically close to the human.

There are several methods for experimental detecting of BMN. A Scanning SQUID Microscope is a sensitive near-field imaging system for the measurement of weak magnetic fields by moving a Superconducting Quantum Interference Device (SQUID) across an area. The microscope can map out buried current-carrying wires by measuring the magnetic fields produced by the currents, or can be used to image fields produced by magnetic materials. By mapping out the current in an integrated circuit or a package, short circuits can be localized and chip designs can be verified to see that current is flowing where expected. So a scanning SQUID microscopy is better suits for detection of current-carrying wires.

The magnetic force microscope (MFM) is a variety of atomic force microscope, where a sharp magnetized tip scans a magnetic sample; the tip-sample magnetic interactions are detected and used to reconstruct

Table. MTB proteins, human homologs and functions

MTB protein	MTB protein functions	Human homologous protein	Human homologous protein functions
mamA	Contains TPR domen, that takes part in protein-protein interactions, chaperone functionality, cell cycle, transcription and protein transport	Pex-5	Pex-5 required for the assembly of functional peroxisomes. It is involved in the peroxisomal import of proteins
mamB	Transporter of cations Co^{2+}, Zn^{2+}, Cd^{2+}	Slc30a9 Slc9a4 Slc39a3	Transporter of cations Zn^{2+}
mamM	Transporter of cations Co^{2+}, Zn^{2+}, Cd^{2+}	Slc30a9 Slc39a4	Transporter of cations Zn^{2+}

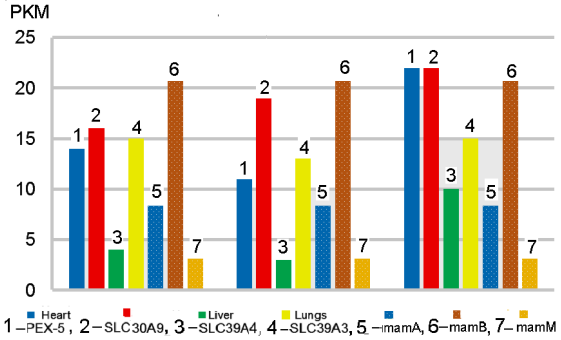

Fig. 1. Gene expression levels of homologous proteins of MTB (mamA, mamB, mamM) in the proteome of human lung, heart and liver and gene expression levels of *Magnetospirillum gryphiswaldense MSR-1* (cultured under microaerobic conditions (std O_2)).

the magnetic structure of the sample surface. Many kinds of magnetic interactions are measured by MFM, including magnetic dipole-dipole interaction. MFM scanning often uses non-contact AFM mode. This method is better for BMN localization detecting.

In this research electron paramagnetic resonance (EPR) was used as a method for BMN detecting 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 [5]. So the samples of *Sus domestica* liver and heart were used for comparison of the EPR signal values (as the control samples).

5 samples of *Sus domestica* lung tissue was taken for the experiments. 4 samples of *Sus domestica* liver tissue and 5 samples of *Sus domestica* heart tissue were also used for the control. All the samples were preliminary dried in the oven at 105°C. Experimental studies were conducted using ELEXSYS E500 EPR spectrometer (Bruker BioSpin GmbH, Germany, X-band). The same samples after heat treatment at 250°C were used for measurement of the EPR spectra. The weight of each sample was 0.01 g. The spectra were recorded at the temperature T = 293 K at the frequency of 9.86 GHz. The modulation amplitude was 0.3 E, power — 20 mW (decrease in power and modulation amplitude did not lead to a change in the shape and position of the line).

3. Results and discussion

Histogram (Fig. 1) shows the result of gene expression levels of homologous proteins of MTB in the proteome of human lung, heart and liver and gene expression levels of *Magnetospirillum gryphiswaldense MSR-1* (cultured under microaerobic conditions (std O_2) for homologue proteins. It is shown, that gene expression levels of homologous proteins of MTB in the proteome of human lung, heart and liver are enough for the BMN biomineralization process in these tissues.

EPR spectra of dried and thermally treated tissue samples of *Sus domestica* lungs, heart and liver are presented in the Fig. 2–3. Dependence of the first derivative of the absorption energy of electromagnetic microwave radiation D on magnetic field flux density B for thermally treated and fried samples of lung, heart and liver of *Sus domestica* and yeast is shown. The appearance of narrow peak in the EPR spectrum between approximately 3500 and 3540 means biogenic magnetic nanoparticle presence in the studied samples [20].

Heat treatment up to 250°C leads to the decomposition of organic compounds. As a result, the relative concentration of nanoparticles in the samples increases

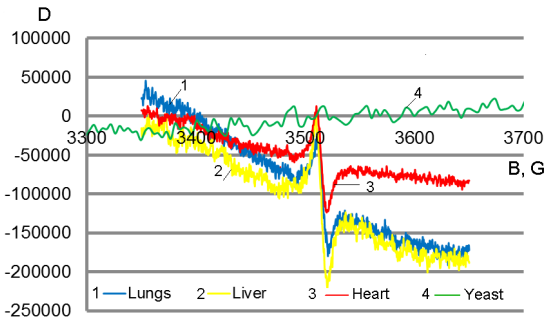

Fig. 2. EPR spectra: dependences of the first derivative of the absorption energy of electromagnetic microwave radiation D on magnetic field flux density B for dried samples of lung, heart and liver of *Sus domestica* and yeast.

sharply in comparison with the samples in Fig. 2. Since in all experiments the mass of the samples was constant, this leads to a sharp increase in the electron spin resonance signal.

So the lung, liver and heart samples contained BMN resulting in the appearance of narrow peak in the EPR spectrum [20].

4. Conclusions

The biomineralization of BMN in human lungs was confirmed based on the results of the present research and the information about the BMN presence in human heart and liver.

This confirmation was experimentally proven using analysis of EPR spectra of dried and thermally treated *Sus domestica* lungs, liver and heart samples. Taking into account that the mechanism of BMN biomineralization is the same for archaea, prokaryotes and eukaryotes [14] and *Sus domestica* is a model rganism that is genetically close to the human, we can assume that the BMN biomineralization take place in relevant organs of the human body.

References

1. F.C.Meldrum, H.Colfen, *Chem. Rev.*, **108**, 4332 (2008).
2. H.Eherlich, *Acta Biomaterialia*, **10**, 3183 (2014).
3. H.A.Lowenstam, S.Weiner, On Biomineralization, Oxford University Press, Oxford (1989).

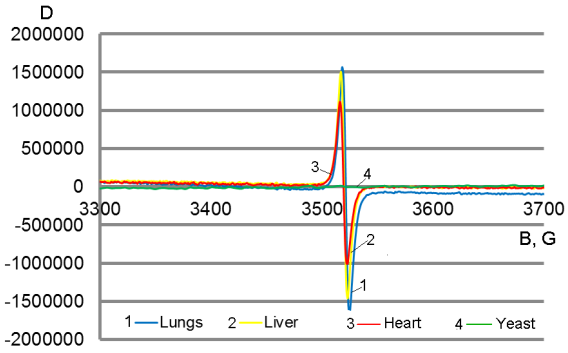

Fig. 3. EPR spectra: dependences of the first derivative of the absorption energy of electromagnetic microwave radiation D on magnetic field flux density B for burned samples of lung, heart and liver of *Sus domestica* and yeast.

4. R.Giere, *PNAS*, **113**, 11986 (2016).
5. P.P.Grassi-Schultheiss et al., *Ibid*, **10**, 351 (1997).
6. J.L.Kirschvink, *J. Exp. Biol.*, **92**, 333 (1981).
7. R.R.Baker, J.G.Mather, J.H.Kennaugh, *Nature*, **301**, 78 (1983).
8. J.L.Kirschvink, A.Kobayashi-Kirschvink, B.J.Woodford, *PNAS*, **89**, 7683 (1992).
9. A.Kobayashi, N.Yamamoto, J.L.Kirschvink, *J. Jpn Soc. Powder Metall.*, **44**, 294 (1997).
10. F.Brem, A.Hirt, M.Winklhofer et al., *J. R. Soc. Interface*, **3**, 833 (2006).
11. J.F.Collingwood, R.K.Chong, T.Kasama et al., *J. Alzheimers. Dis.*, **14**, 45 (2008).
12. R.K.Upadhyay, BioMed Research International, 2014 (2014).
13. D.H.Kim, E.A.Rozhkova, I.V.Ulasov et al., *Nature Mater.*, **9**, 165 (2010).
14. S.V.Gorobets, O.Yu.Gorobets, Yu.M.Chyzh et al., *Biophysics*, **58**, 379 (2013).
15. S.Schubbe, C.Wurdemann, J.Peplies et al., *Appl. Environ. Microbiol.*, **72**, 65 (2006).
16. O.Yu.Gorobets, S.V.Gorobets, Yu.I.Gorobets, Dekker Encyclopedia of Nanoscience and Nanotechnology, CRC Press, New York (2014).
17. M.Richter, M.Kube, D.A.Bazylinski et al., *J. Bacteriology*, **189**, 4899 (2007).
18. F.Abreu, M.E.Cantao, M.F.Nicolas et al., *The ISME Journal*, **5**, 1634 (2011).
19. S.V.Gorobets, O.Yu.Gorobets, Yu.I.Gorobets, *Bulletin of the KPI*, **3**, 28 (2013).
20. A.B.Brik, A.P.Shpak, V.L.Karbovskiy et al., *Mineralogical Journal*, **1**, 5 (2005).