Potential producers of biogenic magnetic nanoparticles among disease-producing microorganisms of the brain

S.V.Gorobets, O.Yu.Gorobets, Y.A.Darmenko

National Technical University of Ukraine "Igor Sikorsky Kyiv Polytechnic Institute", 37 Pereniohy Ave., 03156 Kyiv, Ukraine

In this paper, microorganisms-causative agents of brain disease have the potential to produce biogenic magnetic nanoparticles (BMN), which can accumulate in the human brain in addition to BMN, which are biomineralized in the human brain. The BMN of these microorganisms can attract particular interest for the manufacture of magnetic nanoparticles as functional materials of a wide range of applications such as nanoelectronics, targeted delivery of drugs and a contrast agent for magnetic resonance imaging.

Keywords: biogenic magnetic nanoparticles, pathogenic microorganisms, meningitis.

Установлена потенциальная восприимчивость микроорганизмов-возбудителей заболевания мозга к продукции биогенных магнитных наночастиц (БМН), которые могут накапливаться в мозге человека дополнительно к БМН, которые биоминерализуются в мозге человека. БМН этих микроорганизмов могут представлять особый интерес для изготовления магнитных наночастиц как функциональных материалов широкого спектра использования, например, для наноэлектроники, при целевой доставке лекарств, как контрастный агент для магнитно-резонансной томографии.

Потенційні продуценти біогенних магнітних наночастинок серед мікроорганізмів збудників захворювань мозку. *С.В.Горобець, О.Ю.Горобець, Є.А.Дарменко.*

Встановлено потенційна чутливість мікроорганізмів-збудників захворювання мозку до продукції біогенних магнітних наночастинок (БМН), які можуть накопичуватися у мозку людини додатково до БМН, які біомінералізуються у мозку людини. БМН цих мікроорганізмів можуть становити окремий інтерес для виготовлення магнітних наночастинок як функціональних матеріалів широкого спектру використання, наприклад, для наноелектроніки, при цільовій доставці ліків, як контрастний агент для магнітно-резонансної томографії.

1. Introduction

For several decades the interest in drug delivery systems using magnetic nanoparticles is increased. On today, the topical issues are connected with the presence of biogenic magnetic nanoparticles (BMNs) in human organs and tissues that should be considered in the design of such systems.

BMNs are revealed in many organs of the human's body. Normally BMNs locate in heart, liver, spleen [1] adrenal glands [2], ethmoid bone [3] and brain [4−7]. During inflammatory processes and pathologies, such as atherosclerotic plaques [8], the neurodegenerative (Alzheimer's, Parkinson's, Huntington's [9]) and cancer the number of BMN is considerably larger than the normal range [5].

It's important to understand how magnetic nanoparticles of artificial and natural origin that ingested as part of magnetically labeled drugs or as part of symbioses, pathogens and opportunistic bacteria may

interact with BMN that are located in human's organs and tissues [10]. Also the work [11] showed that the use of magnetic resonance imaging method (MRI) can be dangerous to the human brain with the accumulation of BMN with age [12].

Since the discovery of BMN in living organisms the BMNs have been considered as a functional material that provides magnetotaxis in bacteria [13] and magnetoreception in multicellular organisms [14]. In this investigation, for the first time, BMN is considered as a functional material of a wide range of applications.

In this paper, the meningitis — infection of the brain is considered. The main causative agents of meningitis are *Streptococcus pneumoniae, Neisseria meningitidis, Haemophilus influenzae type b, Listeria monocytogenes, Pseudomonas aeruginosa and Streptococcus agalactiae* [15–18].

The content of magnetic nanocrystals is about $5 \cdot 10^6$ per gram in the tissues of the human brain, more than 10^8 per gram in the brain membrane, 50 ng/g average. About 90 % of the particles have a size of 10−70 nm, and 10 % have a size 90−200 nm. The particles are grouped into clusters of 50 to 100 nanoparticles [4, 19].

Recent advances have shown that artificial nanoparticles are able to get into the brain, overcoming the blood-brain barrier. So if artificial nanoparticles are able to get into the brain they can interact with BMN in brain tissue. This data should be considered as the treatment of pathologies of the brain and during MRI survey especially significant for people in old age.

Extensive use of magnetic resonance in clinical practice and BMN discovery in the tissues and organs of the human body have raised new issues concerning the health risks associated with MR. It was proved by using isothermal residual magnetization and magnetic field changes that the magnetic force associated with the interaction of particles and ferromagnetic MR magnetic fields can be dangerous to sensitive tissue such as the human brain [11].

The aim of the work is to investigate the BMN in the brain as a functional material whose biosynthesis is programmed at the genetic level, both in regions of the human brain [19] and in microorganisms causing pathogens of the brain. The task is to identify potential producers of BMN among the microorganisms of pathogens of the brain and to show that these microorganisms can lead to the additional accumulation of BMN

in brain (for example, due to the magnetic dipole interaction of the BMN of these microorganisms with the brain BMN [10]). Therefore, these microorganisms are perspective for use in attenuated form (with reduced virulence) as a contrast agent for magnetic resonance imaging (MRI) [20], as magnetically controlled vectors with natural ferrimagnetism properties for target drug delivery to the brain [21], moreover microorganisms are powerful for the *in vivo* and *in vitro* production of magnetic nanoparticles for nanoelectronics [22].

2. Experimental

Bioinformatics methods allow to compare a query protein with a specific set of proteins from a database by sequence alignment of amino acid residues. Statistically significant alignments, or matches, between the compared sequences are used for finding homologs, i.e., proteins descending from a common ancestor and having the same function.

The study standard methods used pairwise alignment with a free access program "BLAST" National Center for Biotechnology Information [23]. Two indicators Ident and E-value are considered to estimate the degree of similarity.

Ident (%) is the number of identical amino acid residues in proteins that is compared with the optimal alignment [24]. Ident value should be greater than 18 % [25]. E-value is a parameter that reflects the statistical significance of alignment reducing the value of which indicates a lower level of rate of coincidence display at amino acid residues of proteins compared. The value of this parameter changes with increasing amounts of information in the database [24]. Today, the recommended threshold E-value, to search for homologous proteins in the NCBI database, should be <0.05 [26]. The research takes into account the length of the alignment. To assert that these results are not accidental coincidence length of alignment should be > 100 amino acid residues.

Comparison of the amino acid sequences of Mam proteins, indispensable in biomineralization BMN *Magnetospirillum gryphiswaldense* MSR-1 (for which the process of biomineralization BMN is studied genetically in detail [27−29]), is carried out with proteome of *Streptococcus pneumoniae, Neisseria meningitidis, Listeria monocytogenes, Pseudomonas aeruginosa and Streptococcus agalactiae*.

Table 1. The results of alignment of amino acid sequences of protein that is essential for biomineralization of magnetite in *Magnetospirillum gryphiswaldense* MSR-1 and proteins of meningitis pathogens

The strain of microorganism	Comple-teness of genome [23]	E-value /Ident/ Length					
		Proteins of *Magnetospirillum gryphiswaldense* MSR-1					
		MamA	MamB	MamM	MamO	MamE	MamK
Streptococcus pneumoniae GA41410	25% of genome is known	0.018	5e-28	6e-24	7e-04	2e-26	**0.062**
		25%	29%	24%	24%	40%	**45%**
		109	262	261	159	174	331
Neisseria meningitidis Z2491	genome is completely sequenced	*0.002*	0.038	**3.0**	2e-10	9e-34	0.57
		23%	34%	**32%**	29%	43%	46%
		124	149	72	170	161	345
Listeria mono-cytogenes sero-type 4b str. F2365	genome is completely sequenced	5e-08	9e-36	2e-32	3e-05	1e-04	5e-14
		23%	28%	32%	33%	32%	27%
		107	271	254	246	104	325
Streptococcus agalactiae LMG 14747	25 % of genome is known	**0.15**	4e-27	9e-23	0.02	7e-26	**0.028**
		25 %	26 %	26 %	26 %	39 %	**27 %**
		120	271	264	170	185	103
* *Pseudomonas aeruginosa* M18	25 % of genome is known	**0.037**	6e-12	2e-11	1e-07	6e-34	**0.042**
		32 %	23 %	25 %	24 %	40 %	**34 %**
		81	263	281	184	197	95

* — the synthesis of BMN was confirmed experimentally [46]. In Table 1, the function shown in Table 2 do not coincide for proteins with the bold values of E-value and Ident. The functions are not known (hypothetical protein) for the proteins (Table 2) with italics values of E-value and Ident.

3. Results and discussion

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 [30–32]. Biosynthesis of BMNs from inorganic iron compounds is of particular interest because of the magnetic properties of BMNs. To date, the genetic control of the synthesis of BMNs has only been studied experimentally in magnetotactic bacteria (MTB) [33–35], where it appears to be a strict regulation of the properties and structural organization of the BMNs.

Proteins of biomineralization of BMN in MTB can be divided into two functional classes: proteins indispensable for the biomineralization process of BMNs, and regulatory proteins that carry out the genetic control of size, shape, and localization of magnetite crystals in MTB. The first class of proteins indispensable for the process of biomineralization of magnetite in-

cludes proteins such as MamA, MamB, MamM, MamE, MamO [36–39]. Other proteins of biomineralization of MTB belong to regulatory proteins [37, 40]. A homologue of the MamK protein, which is responsible for the formation of the chains of BMN [37], is found in humans.

Proteins of biomineralization of BMN in MTB is organized mainly in four clusters of genes in MTB *Magnetospirillum gryphiswaldense*: mms6, mamAB, mamGFDC, and mamXY encoding all known magnetosome membrane proteins. Magnetosome membrane proteins were named Mam, Mme, Mms, Mtx [41].

The loss of proteins of biomineralization in MTB leads to nonmagnetic phenotype of MTB demonstrating its key role in biogenesis of BMNs [33, 42–44]. Thus, the proteins of the Mam group can be used to synthesize nanoparticles and control their physicochemical properties and morphology. This research opens perspectives in the manufacture of functional materials.

Table 2. Comparison of the functions of proteins in MTB and proteins in human pathogens

Proteins of biomineralization in MTB and their functions	Pathogens proteins and their functions
MamA — contains the TPR domain, which is involved in protein-protein interactions, functions of chaperones, cell cycle, transcription, transport of proteins	TPR- is a structural motif. TPR-domain involved in protein- protein interactions.
MamB — Transporter of Co, Zn, Cd cations	MMT1 — Transporter of Co, Zn, Cd cations
MamM — Transporter of Co, Zn, Cd cations	
MamO — Serine protease	HtrA2-Serine protease
MamE — Serine protease. PDZ domain of trypsin-like serine protease is involved in the response to heat shock, chaperone function, apoptosis	HtrA-Serine protease.

As shown in this paper [8, 45], the presence of homologs of *Magnetospirillum gryphiswaldense* MSR-1: MamA, MamB, MamE, MamO, and MamM proteins is sufficient for the formation of intracellular crystalline BMN in the microorganisms under investigation, and the presence of homologs of the MTB *Magnetospirillum gryphiswaldense* MSR-1 proteins is required to form intracellular amorphous BMN: MamM, MamB, MamE, MamO.

In alignment MTB *Magnetospirillum gryphiswaldense* MSR-1 with proteome pathogens, found that *Streptococcus pneumoniae, Neisseria meningitidis, Listeria monocytogenes, Pseudomonas aeruginosa and Streptococcus agalactiae* are potential producers of BMN (Table 1). Homology of biomineralization proteins and microorganisms pathogens of meningitis and MTB confirmed the same functions (Table 2). Lack of homolog of protein MamA in *Streptococcus agalactiae* and *Pseudomonas aeruginosa* indicates that these organisms may be potential producers of amorphous intracellular BMN. This ability to synthesize amorphous intracellular BMN in *Pseudomonas aeruginosa* confirms experimentally [46].

Neisseria meningitidis and *Streptococcus pneumonia* are potential producers of crystal BMN (Table 1). Moreover, availability of protein homologues MamK in these organisms indicates that the BMN are associated with the cell membrane [45]. Despite the fact that the E-value of the protein homolog MamK in *Neisseria meningitides* is greater than 0.05 the function of these proteins are the same. Thus it can be argued that they are homologous [25]. Lack of protein homolog to MamA in *Streptococcus agalactiae* could be associated with the fact that the genome of the microorganism is not fully sequenced.

4. Conclusions

Alignment of proteins amino acid sequences of magnetotactic bacteria *Magnetospirillum gryphiswaldense* MSR-1 and proteins of microorganisms pathogens of meningitis are conducted using comparative genomics methods. It is shown that *Streptococcus pneumoniae, Neisseria meningitidis, Streptococcus agalactiae, Pseudomonas aeruginosa and Listeria monocytogenes* are potential producers of BMN. Results of this research open new possibilities for therapy and treatment of bacterial meningitis in adults and children. In addition, these microorganisms can attract a particular interest for the manufacture of magnetic nanoparticles as functional materials of wide range of applications.

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