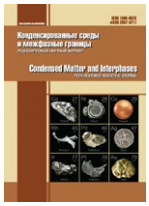

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E. coli cells as a source of biohybrid material: electron microscopy and microanalysis study

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Abstract

Objectives: The work relates to the research of *E.coli* cells formed under conditions of superproduction of the bacterioferritin protein Dps. These protein molecules are capable of forming biohybrid structures by accumulating inorganic nanoparticles of the iron-oxygen system with identical properties, including sizes within the nanometer range, in their internal cavities. Current methods of obtaining bacterioferritin Dps protein molecules rely on destroying their source: *E.coli* cells. A key issue for study and subsequent application is establishing whether it is possible to obtain these protein molecules without destroying *E.coli* cells in order to form biohybrid structures.

Experimental: E.coli cells were grown under conditions of superproduction of bacterioferritin Dps protein molecules, then deposited on a molybdenum foil substrate for electron microscopy and energy-dispersive microanalysis studies. Based on the resulting data on morphology data and elemental composition, the possibility of forming protein molecules without destroying the cells was investigated in order to create biohybrid structures based on them.

Conclusions: It has been established that under conditions of protein superproduction, *E.coli* cells produce bacterioferritin Dps molecules, with a significant amount of this protein possibly being released into the extracellular space. The morphology of *E.coli* cells themselves does not change under conditions of superproduction and protein emission. When Mohr's salt was added to the culture fluid, the released protein contains a significant amount of iron atoms, which may result from bacterioferritin Dps molecules forming biohybrid structures. These results demonstrate a simple, affordable method of forming biohybrid structures containing iron-oxygen nanoparticles for use in technologies, including the targeted delivery of nanoparticles and the functionalization of accessible surfaces.

Keywords: *E.coli* cells, Dps protein molecules, Protein superproduction, Biohybrid material, Scanning electron microscopy, Energy-dispersive microanalysis, Morphology and composition

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1. Introduction

Nature-like materials and structures are a promising object of modern scientific research and development of technological approaches in terms of low cost or efficiency and flexibility in their practical use [1–5]. A bright example of this class of objects are protein molecules, for example, bacterioferritin Dps (DNA-binding Protein from Starved cells) of *Escherichia coli* (*E. coli*) bacteria, which is a representative of bacterial ferritins [1, 4]. These natural objects are known for their ability to form inorganic iron-oxygen (Fe–O) nanoparticles of a given composition and size inside the voids of molecules [1, 4, 6–8]. Moreover, the result of the formation of a biohybrid material is the fact that each of the particles is initially, by its natural origin, packaged in an organic container, the protein molecule itself. Such objects can be stored for a long, practically unlimited time without changing their macro- and microscopic properties. Finally, considering that the storage of a molecular culture or biohybrid material takes place in a liquid medium, it is convenient to deliver such objects in a targeted manner within the framework of the technology used [9]. The source of bacterioferritin Dps molecules are *E.coli* cells. The traditional way to obtain protein here is to isolate it upon complete destruction of the source cells, followed by a sequence of complex, often costly biophysical and biochemical procedures for purification, filtration and stabilization of the molecular culture [1–2, 7, 10]. Subsequently, after the cells are destroyed and the molecular culture of bacterioferritin Dps is isolated, interaction with the decomposition products of the Mohr salt $Fe(NH_4)_2(SO_4)_2 \cdot 6H_2O$ occurs to form a biohybrid material as a source of divalent iron (Fe^{2+}) in a culture solution. The natural function of Dps molecules is to deposit or accumulate iron oxide Fe^{2+}, which is toxic to biological culture, into a nanoparticle predominantly consisting of iron Fe^{3+} bound to oxygen [4, 8]. Note that the dimensions of the outer walls of the protein molecule, according to the data obtained earlier, do not exceed ten nanometers, while the sizes of nanoparticles formed inside the molecules can be several nanometers [4, 6, 7]. One Dps protein molecule can act as a single "setup" for the controlled production of a biohybrid structure with an inorganic nanoparticle of specified properties, including sizes up to units of nanometers. An attractive development of approaches for obtaining a molecular culture or biohybrid objects based on it is an attempt to use *E.coli* cells to isolate protein without destroying them i.e. repeatedly. Then a cell, an object much more massive than a molecule, can be considered to be a kind of "factory" for the production of hybrid structures.

Note that with such a modification of the cell culture, which leads to the release of protein in sufficient quantities [11] and without cell destruction, the key issue is the influence of the source of iron atoms, for example, Mohr salt, necessary for the formation of biohybrid nanostructures, on the properties, primarily the morphology of (modified) *E.coli* cells. Attempts to use cells for the repeated production of molecules can lead to a disruption in the shape of such a natural source of biohybrid material. Therefore, an important and necessary task is to study the morphology and elemental composition of a modified cell culture.

Scanning electron microscopy (SEM) is one of the most in-demand methods for a variety of objects diagnostics, including nanoscale structures and biomaterials. The method's capabilities make it possible to study the morphological features of various origins objects, including natural ones, with high lateral resolution [3, 9, 12–14]. One of the most useful additions to the scanning electron microscopy method is the possibility of conducting energy-dispersive X-ray microanalysis of an object being studied in an electron microscope. The use of this method, within the framework of a single experiment with the study of morphology, by means of scanning electron microscopy, allows us to obtain information about the presence of an element in the composition of a microscopic area of the studied object. The present work is dedicated to the research of modified *E.coli* cells as a multiple source of molecular material or biohybrid structures using SEM and microanalysis methods.

2. Experimental

To achieve protein superproduction conditions, *E. coli* BL21*(DE3) cells pre-grown

to an optical density of 0.4 (λ = 600 nm), were washed with a buffer solution containing 0.1M Tris-HCl (pH 8.0) from nutrient residues and exposed to $CaCl_2$, according to the procedure described in [15] to form perforations in the cell membrane. Then, a pre-prepared aqueous solution of circular pGEM*dps* plasmid DNA (pDNA) molecules containing a full-size copy of the *dps* gene were added to the cell suspension. Based on the saturating concentration of pDNA during transformation equal to 10 μg/ml, 0.5 μl of their solution was added to the cells, followed by half an hour incubation on ice, then exposed to heat at 42 °C for 2 minutes. The tubes were then placed in an ice bath for 2 minutes, after which the cells were transferred to 750 μl of a SOC solution containing trypton (20 g/l), yeast extract (5.5 g/L), NaCl (10 mM), KCl (2.5 mM), $MnCl_2$/$MgSO_4$ (10 mM), glucose (20 mM) and incubated for 1 hour, at 37 °C in a thermostat with constant stirring at 180 rpm. After that, the cell suspension was centrifuged, and the resulting precipitate with a volume of about 50 μl was sown onto Petri dishes with LB-agar (pH 7.4) containing trypton (10 g/l), yeast extract (5 g/l), NaCl (10 g/l), bactoagar (15 g/l) and ampicillin (20 μg/ml). The growth of the obtained cells was carried out at 37 °C in the presence of ampicillin (20 μg/ml) for 16 hours in the presence of 0.02 mM IPTG. The presence of the recombinant Dps protein was controlled by electrophoresis in 12.5% denaturing polyacrylamide gel using the modified Davis method. Next, the cells were harvested by centrifugation at 10,000 rpm, washed twice from the medium with a cold buffer containing 0.1M Tris-HCl (pH 8.0), and used for research. A freshly prepared solution of Mohr salt was used as an iron source, which was added to the protein solution until an iron ion concentration of 0.25 mM was reached and incubated for 15 minutes, after which the same portion of Mohr salt was added and incubation was repeated. The resulting sample was used in studies. Obtained samples of cell culture were deposited to pre-purified molybdenum foil by layering.

A Carl Zeiss ULTRA 55 scanning electron microscope equipped with an energy dispersive microanalysis unit was used. The mode of secondary electrons registration with low values of accelerating voltages up to 5 kV was used, which is necessary for studying biostructures. The Image J software package was used to estimate the areas occupied by the cells and to estimate the spread of their shape. An energy dispersion (X-ray) spectrum was obtained from a surface area of ~ 15×20 μm for which the element distribution was mapped.

3. Results and discussion

Fig. 1 shows a surface morphology obtained by the SEM method for the cellular array formed on the molybdenum foil. Data on the shape and size of cells and their arrays (accumulations) are shown in Fig. 1a, information on the shape and size of a single cell is given in Fig. 1b, and section "c" of the same figure represents the result of estimating the size of cells in the surface area of Fig. 1a, made according to the cell areas. It should be noted that the size of *E.coli* cells grown under protein superproduction conditions is about three micrometers long and one micrometer wide, which corresponds to the selected cell type [12]. They are generally oval in shape with clear boundaries, the observation of which suggests that the height of the cell is comparable to its width, including after vacuuming of the formed cell array in the electron microscope chamber. It should also be noted that, as a result of layering, it is possible to form a sufficiently dense layer of cells distributed over the surface of the foil in "islands" one cell high, incompletely covering the bearing surface of molybdenum. The conditions of protein superproduction during the formation of a cell array do not lead to significant cell morphology disorders. Some of the cells, however, are smaller (~ 1×1 μm) or larger (~ 5×1 μm) than indicated above (Fig. 1c). This is the result of ongoing cell division at the time of layering on the substrate. In addition to the forming cell arrays and uncovered sections of molybdenum foil, there are a number of elements indicated in Fig. 1a with a white dotted line. The size of these elements is comparable to the size of a cell, they have random contours, uneven morphology and are adjacent directly to individual cells or are located within the cell array. Such elements are not observed on the surface of cell arrays themselves and are not located on individual areas of the substrate surface that are not coated with *E. coli*. At the same time, there are

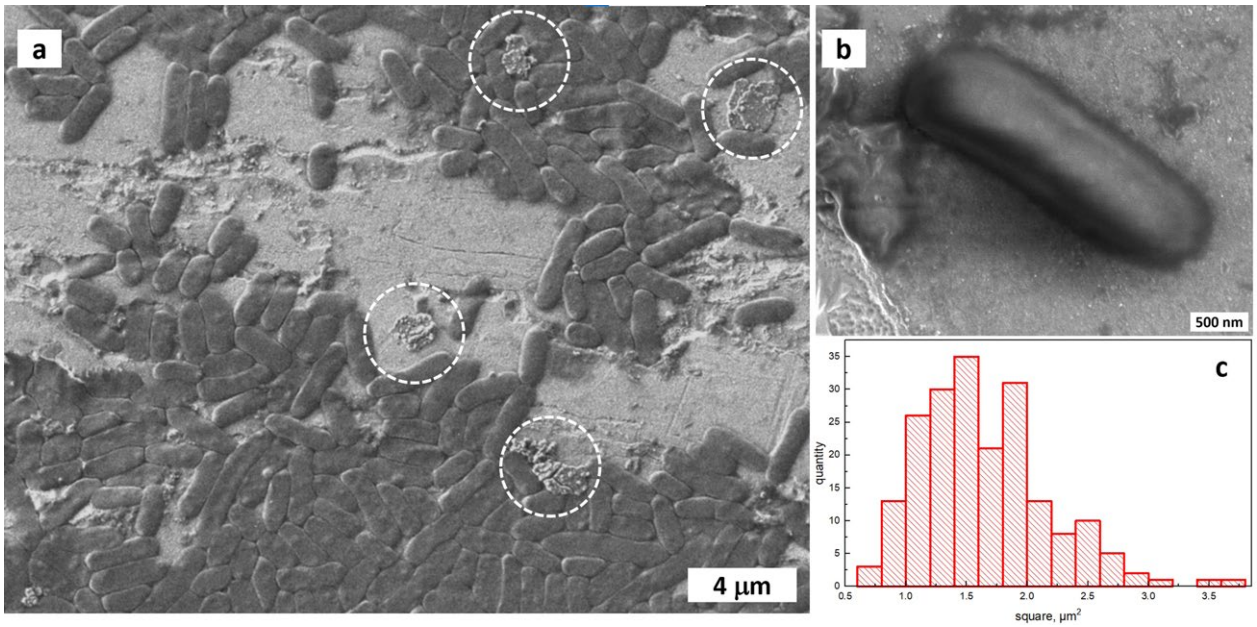

Fig. 1. Scanning electron microscopy of the *E.coli* cells on a molybdenum foil substrate. a: cells array with white dotted lines showing areas of protein release; b: single *E. coli* cell image; c: distribution of cells by their surface areas

no noticeable variations of the shape and size of cells adjacent to these morphological elements. It should be noted that the modified *E. coli* cells were formed under conditions of the superproduction of bacterioferritin Dps protein molecules, placed after layering on the substrate surface in a scanning electron microscope chamber, and then evacuated. Thus, the origin of these arrays may be related to the release of excess protein into the extracellular media, including during the transition of vacuum conditions.

Fig. 2 contains the results of the elemental composition microanalysis for a surface area covered with a cells array. Fig. 2a shows an electron microscopic image of a surface area from which a mapping of iron and nitrogen atoms distribution was obtained (Fig. 2b) and spectral information was recorded (Fig. 2c) containing a signal from all sorts of atoms of the studied sample area measuring about 15×20 microns. The choice of Fe and N atoms to obtain a map of the elements distribution is caused by the fact that nitrogen atoms are contained in *E. coli* cells only and are not located on the surface of the foil.

At the same time, iron atoms, as a result of the dissolution of the Mohr salt in the culture media, can be found both in the cells themselves and around them. The molecules of the bacterioferritin

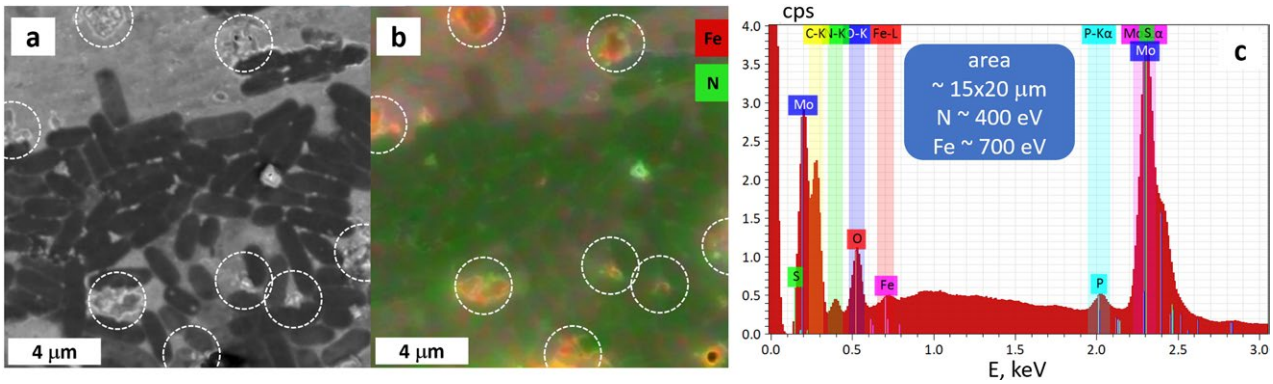

Fig.2. Energy-dispersive microanalysis of the surface area with *E. coli* cells array on a molybdenum foil substrate. a: SEM image with white dotted lines showing areas of protein release; b: distribution mapping for atoms of iron (red) and nitrogen (green); c: energy-dispersive X-ray spectrum

Dps protein are able to accumulate inside their cavities the result of dissolution of the Mohr salt, forming a nanoparticle of the iron-oxygen system consisting of iron atoms ions in different charge states of Fe^{2+} and Fe^{3+} [4, 8]. We assumed the release of bacterioferritin Dps protein molecules into the extracellular space as a result of studying the SEM data (see Fig. 1a) for *E.coli* cells formed under conditions of this protein superproduction. The signal from the iron atoms is confirmed by the spectral information obtained from the observed microarray of the surface (Fig. 2b, peak at 700 eV). The mapping data (Fig. 2b) show that the signal from the iron atoms contributes most to the areas indicated by the dotted white line in Figs 1a, 2a and 2b. We associate these sites with the "release" of the Dps protein by modified *E.coli* cells into the extracellular space.

4. Conclusions

Thus, based on the results of the morphological and elemental composition studies, the following can be stated. During superproduction and growth of *E. coli* cells, bacterioferritin Dps molecules are produced in sufficient quantities to allow this protein to be released into the extracellular space without altering the shape or size of *E. coli* cells. The released protein contains a significant number of iron atoms, which may result from bacterioferritin Dps molecules forming biohybrid structures without destroying their source, i.e. *E. coli* cells. This approach to forming biohybrid materials using protein molecules of cellular origin is promising for producing iron-oxygen nanoparticles with specific properties, including those intended for long-term storage or use in technologies such as targeted nanoparticle delivery and surface functionalization.

Contribution of the authors

The authors contributed equally to this article.

Conflict of interests

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

References

1. Nikandrov V. V. Inorganic semiconductors in biological and biochemical systems: biosynthesis, properties, and photochemical activity. *Advances in biological chemistry*. 2000;40: 357–396.

2. Behrens S. S. Synthesis of inorganic nanomaterials mediated by protein assemblies. *Journal of Materials Chemistry*. 2008;18: 3788–3798. https://doi.org/10.1039/B806551A

3. *Biological and Bio-inspired Nanomaterials. Properties and Assembly Mechanisms*. Sarah Perrett, Alexander K. Buell, Tuomas P. J. Knowles (eds.). Springer, Singapore; 2019. 440 p. https://doi.org/10.1007/978-981-13-9791-2.

4. Parinova E. V., Antipov S. S., Belikov E. A., ... Turishchev S. Yu. TEM and XPS studies of bio-nanohybrid material based on bacterial ferritin-like protein Dps. *Condensed Matter and Interphases*. 2022;24(2): 265–272. https://doi.org/10.17308/kcmf.2022.24/9267

5. Nazarovskaia D. A., Turishchev S. Yu., Titova S. S., Shatov A. A., Tyurin-Kuzmin P. A., Osminkina L. A. Photoluminescent properties of porous silicon nanoparticles: synthesis, characterization, and cellular imaging. *Condensed Matter and Interphases*. 2025;27(3): 422–432. https://doi.org/10.17308/kcmf.2025.27/13181

6. Antipov S. S., Pichkur E. B., Praslova N. V., ... Turishchev S. Yu. High resolution cryogenic transmission electron microscopy study of Escherichia coli Dps protein: first direct observation in quasinative state. *Results in Physics*. 2018;11: 926–928. https://doi.org/10.1016/j.rinp.2018.10.059

7. Antipov S., Turishchev S., Purtov Yu., ... Ozoline O. The Oligomeric Form of the Escherichia coli Dps Protein Depends on the Availability of Iron Ions. *Molecules*. 2017;22(11):1904. https://doi.org/10.3390/molecules22111904

8. Turishchev S. Yu., Antipov S. S., Novolokina N. V., ... Domashevskaya E. P. A soft X-ray synchrotron study of the charge state of iron ions in the ferrihydrite core of the ferritin Dps protein in Escherichia coli. *Biophysics*. 2016;61(5): 705–710. https://doi.org/10.1134/S0006350916050286

9. Parinova E. V., Antipov S. S., Sivakov V., ... Turishchev S. Yu. Photoluminescent properties of porous silicon nanoparticles: synthesis, characterization, and cellular imaging. *Condensed Matter and Interphases*. 2023;25(2): 207–214. https://doi.org/10.17308/kcmf.2023.25/11102

10. Antipov S. S., Tutukina M. N., Preobrazhenskaya E. V., ... Ozoline O. N. The nucleoid protein Dps binds genomic DNA of Escherichia coli in a non-random manner. *PLOS ONE*. 2017;12(8): 1–27. https://doi.org/10.1371/journal.pone.0182800

11. Hudson A. J., Andrews S. C., Hawkins C., ... Gues J. R. Overproduction, purification and characterization of the Escherichia coli ferritin. *European journal of biochemistry*. 1993;218(3): 985–995. https://doi.org/10.1111/j.1432-1033.1993.tb18457.x

12.Turishchev S.Yu., Marchenko D., Sivakov V., ... Antipov S.S. On the possibility of PhotoEmission Electron Microscopy for E. coli advanced studies. *Results in Physics*. 2020;16: 102821-1-3. https://doi.org/10.1016/j.rinp.2019.102821

13. Alvear-Daza J. J., García-Barco A., Osorio-Vargas P., Gutierrez-Zapata H. M., Sanabria J., Rengifo-Herrera J. A. Resistance and induction of viable but non culturable states (VBNC) during inactivation of E. coli and Klebsiella pneumoniae by addition of H2O2 to natural well water under

simulated solar irradiation. *Water Research*. 2021;188(116499): 1–11. https://doi.org/10.1016/j.watres.2020.116499

14. He Q., Liu D., Guo M., ... Guo M. Antibacterial mechanism of ultrasound against Escherichia coli: Alterations in membrane microstructures and properties. *Ultrasonics Sonochemistry*. 2021;73(105509): 1–12. ttps://doi.org/10.1016/j.ultsonch.2021.105509

15. Sambrook J. *Molecular cloning: a laboratory manual* J. Sambrook, E. F. Fritsch, T. Maniatis (eds.). Cold Spring Harbor Laboratory Press; 1989. 1546 p.

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