

UDC 577.15, 543.55
EDN: ARDPID

PACS: 87.15.R-

Study of changes in the adsorption properties of L-asparaginase on mica under thermal action for the development of a nanopore-based detector

E. E. Vazhenkova¹, I. D. Shumov^{1*}, D. D. Zhdanov¹, V. V. Shumyantseva¹, V. S. Ziborov², A. N. Ableev¹, O. N. Afonin¹, N. V. Vaulin³, D. V. Lebedev³, A. S. Bukatin³, I. S. Mukhin³, V. Y. Tatur⁴, A. A. Lukyanitsa^{4,5}, E. A. Ponomarenko¹, A. I. Archakov¹, and Yu. D. Ivanov¹

¹ Institute of Biomedical Chemistry, Moscow, 119121 Russia

* E-mail: shum230988@yandex.ru

² Joint Institute for High Temperatures of Russian Academy of Sciences, Moscow, 125412 Russia

³ Alferov Federal State Budgetary Institution of Higher Education and Science Saint Petersburg National Research Academic University of the Russian Academy of Sciences, St. Petersburg, 194021 Russia

⁴ Foundation of Perspective Technologies and Novations, Moscow, 125315 Russia

⁵ Faculty of Computational Mathematics and Cybernetics, Moscow State University, Moscow, 119991 Russia

Received 10.03.2026; revised 24.03.2026; accepted 5.06.2026

Atomic force microscopy (AFM) is used to study the adsorption of L-asparaginase (L-ASNase) E. carotovora enzyme on mica within 25 °C to 60 °C temperature range. Thermally induced changes in the oligomeric state of L-ASNase and its adsorbability on mica are revealed. Maximum adsorption of L-ASNase in the form of individual globules is observed at 45 °C. At 55 °C, the enzyme adsorbs in the form of filamentous structures, and at 60 °C it loses its adsorbability. The results obtained are fundamentally important for the development of electrical molecular detectors based on solid-state nanopores (SSN-EDs) intended for studies of the mechanism and kinetics of enzymatic catalysis, and may also be of interest for the development of anti-cancer drugs.

Keywords: L-asparaginase, atomic force microscopy, enzyme adsorption, thermal denaturation, solid-state nanopore.

DOI: 10.51368/2949-561X-2026-3-93-98

For citation: Vazhenkova E. E., Shumov I. D., Zhdanov D. D., Shumyantseva V. V., Ziborov V. S., Ableev A. N., Afonin O. N., Vaulin N. V., Lebedev D. V., Bukatin A. S., Mukhin I. S., Tatur V. Y., Lukyanitsa A. A., Ponomarenko E. A., Archakov A. I., and Ivanov Yu. D., Applied Physics, No. 3, 93–98 (2026).

Introduction

The enzyme L-asparaginase (L-ASNase) belongs to hydrolases and catalyzes the conversion of L-asparagine to L-aspartate [1]. L-ASNase (type II) has found application in the therapy of oncological diseases – in particular, acute lymphoblastic leukemia [2]. This enzyme is also used in the food industry to prevent or reduce the formation of acrylamide in food products during thermal processing [3]. The

latter has attracted great interest to investigating the effects of elevated temperatures on the physicochemical properties of L-ASNase [3, 4].

Currently, the thermal stability of bacterial type II L-asparaginases is widely studied by spectroscopic methods [4, 5], which do not allow obtaining a signal from single molecules of the enzyme under study [6]. The study of the physicochemical properties of single enzyme molecules, in turn, allows a deeper insight into the kinetics and mechanism of enzymatic

catalysis [6]. This is done by using molecular detectors: the atomic force microscope and solid-state nanopore-based electrical detectors (SSN-ED). SSN-EDs allow recording the catalytic activity of single enzyme macromolecules [7], which is essential for studying the kinetics and mechanism of enzymatic catalysis [6, 8]. It should be noted that the key element of an SSN-ED is an inorganic membrane, which is often fabricated on the basis of silicon compounds [9] with a solid-state nanopore [7]. The adsorption of the enzyme under study onto the surface of this membrane and the enzyme behavior on this surface can fundamentally affect the results of studying its functioning with the use of an SSN-ED. Thus, an essential task is to investigate the adsorption of enzymes onto inorganic silicon-containing surfaces. Atomic force microscopy (AFM) allows the visualization of single enzyme macromolecules [10, 11] and the registration of the slightest changes in their adsorption properties. Mica, a silicon compound whose surface chemical properties are similar to those of the membrane material of the SSN-ED - silicon nitride Si_3N_4 , is often used as a substrate in AFM [12, 13].

The aim of this study is to investigate the adsorption of type II L-ASNase onto mica under conditions of thermal exposure on the enzyme.

Materials and Methods

Experimental design. Temperature-related changes in the adsorption properties of the enzyme were studied by AFM within the temperature range from 25 to 60 °C.

Reagents and enzyme. The enzyme L-asparaginase from *E. carotovora* was obtained and purified at the Laboratory of Medical Biotechnology of the Institute of Biomedical Chemistry (IBMC) in accordance with previously published protocols [14]. The lyophilized enzyme powder was dissolved in 2 mmol/l Dulbecco's phosphate-buffered saline (PBS-D) and diluted to reach the required concentration for the experiment. PBS-D (pH 7.4; 2 mmol/l phosphate, 30 mmol/l NaCl) was prepared from a salt mixture purchased from Pierce (USA). All solutions used in the experiments were prepared using deionized ultrapure water (18.2 M Ω ·cm) obtained with a Simplicity UV system (Millipore, France).

Atomic force microscopy. Samples of the enzyme solution were first heated in a microcentrifuge tube to the required temperature, and then the enzyme was adsorbed from this solution onto mica substrates for AFM as follows.

First, a 0.1 $\mu\text{mol/l}$ solution of L-ASNase in 2 mmol/l PBS-D (pH 7.4) was placed in a thermostatted shaker Thermomixer Comfort (Eppendorf, Germany) and sequentially heated to the required temperatures (25 °C, 30 °C, 45 °C, 55 °C and 60 °C). At each of these temperatures, two 1 ml samples of the solution were taken, and heating was continued up to 60 °C. The enzyme from the samples treated as described above was then adsorbed by direct adsorption [15] onto freshly cleaved mica AFM substrates. Each 1 ml sample of the enzyme solution was pipetted into a 1.7 ml Eppendorf tube, and a rectangular muscovite mica plate (SPI, USA) sized 7×15 mm was immersed into the solution. The tube was continuously shaken at 600 rpm at room temperature (25 °C) for 10 min. After that, the AFM substrate was removed from the enzyme solution, placed in another tube with 1 ml of water, and shaken again at 600 rpm at room temperature (25 °C) for 10 min to remove buffer salts from its surface. All AFM measurements were performed using an NTEGRA PRIMA instrument (NT-MDT, Zelenograd, Russia) in the intermittent-contact mode in air under controlled conditions (temperature 25 °C, air humidity 55%). At least 25 scans sized 2 $\mu\text{m} \times 2 \mu\text{m}$ with a resolution of 256×256 points were obtained for each substrate.

AFM data analysis (including the calculation of height distributions of the AFM images of enzyme molecules $\rho(h)$ and the number of enzyme molecules normalized to an AFM substrate area 400 μm^2 , denoted as N_{400}) was performed as described previously [16].

Results and Discussion

Figure 1 shows typical AFM images of L-ASNase adsorbed onto mica from solutions incubated at temperatures of 25 °C, 30 °C, 45 °C and 55 °C, as well as typical distribution curves $\rho(h)$ obtained for enzyme samples treated at 25 °C, 30 °C, 45 °C or 55 °C. At 60 °C, no enzyme adsorption was observed.

According to the presented AFM images, in the temperature range from 25 to 45 °C, the enzyme adsorbed onto mica in the form of individual globules, whereas heating the enzyme

solution to 55 °C resulted in its adsorption in the form of filamentous structures. The latter fact indicates significant denaturation of the enzyme at 55 °C.

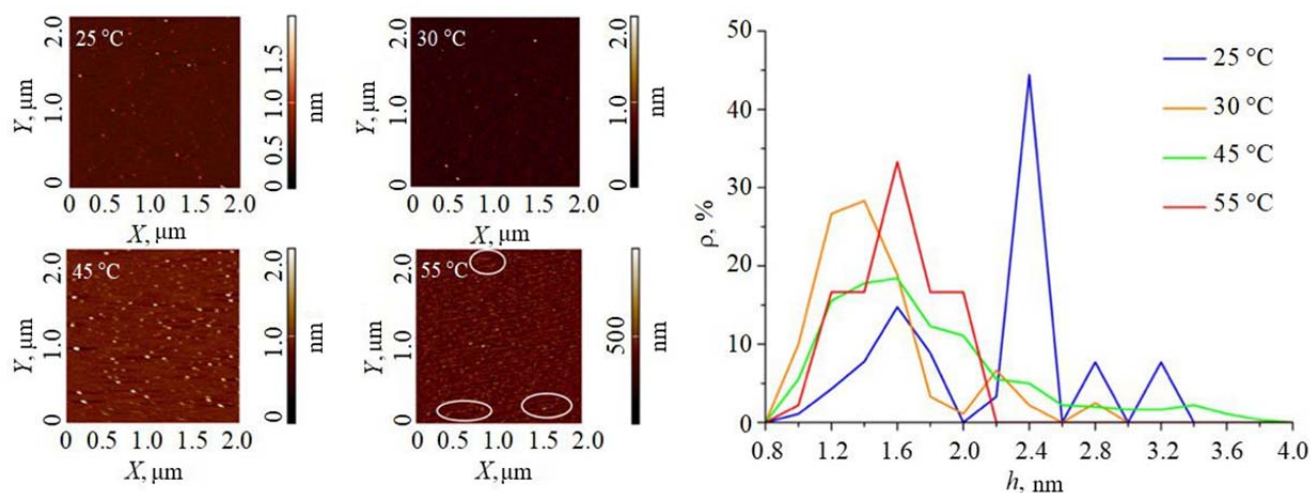


Fig. 1. Typical AFM images and height distribution curves $\rho(h)$ of L-ASNase adsorbed onto mica from solutions incubated at 25 °C, 30 °C, 45 °C or 55 °C. Ellipses mark the images of denatured enzyme structures. AFM image size: $2 \mu\text{m} \times 2 \mu\text{m}$. Experimental conditions: enzyme solution concentration $0.1 \mu\text{mol/l}$, pH 7.4

The blue curve in Fig. 1 shows that the maximum of the height distribution $\rho(h)$ for the L-ASNase sample adsorbed onto mica at 25 °C corresponds to a height value of $h_{\text{max}} = 2.4 \text{ nm}$. Previous studies of another enzyme – horseradish peroxidase (HRP), whose molecular weight (44 kDa [17]) is comparable to that of L-ASNase (which is 36 kDa [18]) showed that the maximum height of AFM images of the monomeric form of HRP on mica is 1.0 to 1.2 nm [10, 11]; while the height of AFM images of higher-order aggregates of HRP on mica is $\geq 2.4 \text{ nm}$ [11]. Based on these considerations, we conclude that after being treated at 25 °C, L-ASNase adsorbs onto mica in the form of aggregates with AFM image heights of about 2.4 nm.

Upon incubation of L-ASNase at higher temperatures (from 30 to 45 °C), h_{max} values can be observed to decrease to 1.6 and 1.4 nm (Fig. 1, orange and green curves). This fact indicates the disaggregation of L-ASNase with an increase in the relative content of the monomeric form of the enzyme as compared to the total number of particles adsorbed onto mica.

AFM images of individual L-ASNase molecules are quite distinguishable at all investigated temperatures up to 45 °C, but at a

higher temperature (55 °C), compact objects are practically not observed (Fig. 1). The absence of compact objects in the AFM images obtained in the experiments involving the enzyme incubated at 55 °C indicates that the enzyme molecules undergo significant structural changes at this elevated temperature. These changes lead to the formation of filamentous structures on mica (Fig. 1), while adsorption of the enzyme in its “normal” form is not observed. During experiments conducted at 55 °C, only filamentous structures of the denatured enzyme are visualized. The height of the AFM images of these structures ranges from 0.5 to 0.8 nm. A further increase in temperature to 60 °C leads to the complete disappearance of enzyme adsorption on mica: after enzyme treatment at such a high temperature, neither compact objects nor filamentous structures are observed. It should be noted that the count of enzyme particles N_{400} adsorbed onto mica at temperatures of 25, 30, 45, and 55 °C was 1003, 540, 1605, and 159 particles/400 μm^2 , respectively.

Thus, the study of the thermal denaturation of L-ASNase by AFM showed that the enzyme adsorbs on mica in an aggregated form at 25 °C. An increase in temperature to 30 °C led to a

twofold decrease in the number N_{400} of enzyme particles adsorbed on mica. The latter fact indicates temperature-induced changes in the conformation of enzyme globules, which resulted in reduced enzyme adsorption. Based on N_{400} values and $\rho(h)$ curves (Fig. 1), it can be concluded that an elevated temperature promotes the disaggregation of the enzyme and the adsorption of a greater number of enzyme particles in the monomeric form. These phenomena are likely associated with the processes of thermal denaturation of the enzyme. A further increase in temperature to 45 °C promotes enzyme adsorption, which indicates that denaturation processes continue and lead to changes in the conformation of enzyme globules. This latter fact contributes to stronger binding of the enzyme to the mica surface. It should be

emphasized once again that the maximum adsorption of L-ASNase on mica is observed at 45 °C. However, a further increase in temperature to 55 °C leads to a sharp decrease in enzyme adsorption, which is accompanied by the development of filamentous structures of the denatured enzyme. At 60 °C, the enzyme completely loses its adsorption capacity, as neither compact objects nor filamentous structures are observed on mica.

Our results are critically important for the development of solid-state nanopore-based electrical detectors (SSN-ED). The pilot experiments involving L-ASNase on an SSN-ED (whose organization was described previously [7]) derived the dependence of the ionic current (I) flowing through the nanopore vs. time (t) at 25 °C (Fig. 2).

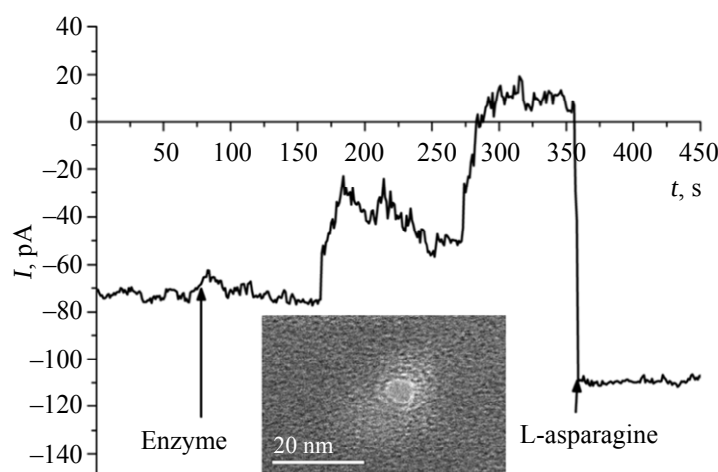


Fig. 2. Typical dependence $I(t)$ derived using an SSN-ED with an L-ASNase macromolecule embedded in the solid-state nanopore, and an image of a 6 nm nanopore formed in an inorganic membrane, obtained by transmission electron microscopy (inset)

Before the enzyme was added, a base level of I was recorded in 1 mmol/l of PBS-D (pH 7.6). After 0.1 $\mu\text{mol/l}$ was added to L-ASNase into the cis chamber of the SSN-ED measurement cell, the absolute value of I decreased, indicating partial blocking of the nanopore by the enzyme molecule. The subsequent addition of 10 $\mu\text{mol/l}$ L-asparagine solution, in turn, led to a significant increase in I , indicating the unblocking of the nanopore. In blank experiments performed using a pure buffer instead of the enzyme solution, no significant changes in I were observed. Given the substantial changes in the adsorption properties of L-ASNase under thermal exposure conditions, a detailed investigation of the kinetics of functioning of this enzyme's single molecules using an SSN-ED requires further studies.

Conclusion

The AFM method was applied to investigate the dependence of the L-ASNase adsorption properties on the thermal exposure of the enzyme solution. The height of the AFM images of L-ASNase adsorbed on mica was found to decrease from 2.4 nm at 25 °C to 1.6 and 1.4 nm in the temperature range from 35 to 45 °C. At the same time, the count of enzyme particles adsorbed on mica increases significantly, with the maximum reached at 45 °C. This fact indicates that changes in the enzyme globule structure caused by thermal denaturation in this temperature range promote its adsorption onto mica. A further increase of temperature to 55 °C leads to a sharp decrease in the adsorption

capacity of the enzyme, which is accompanied by the formation of filamentous structures on the mica surface. At 60 °C, the enzyme completely loses its ability to adsorb on mica. The obtained results are essential for the development of electrical molecular detectors based on solid-state nanopores required for an in-depth study and understanding of the mechanism and kinetics of enzymatic catalysis, and may also be useful in the development of anti -cancer drugs.

The paper has been prepared as part of the topic "The influence of compounds with geroprotective properties on single biomacromolecules, model objects and human body" with financial support from the Ministry of Science and Higher Education of the Russian Federation (Agreement No. 075-15-2024-643).

REFERENCES

1. Batool T., Makky E.A., Jalal M., Yusoff M.M. Appl Biochem Biotechnol. **178**, 900–923 (2016).
2. Aguayo A., Cortes J., Thomas D., Pierce S., Keating M., Kantarjian H. Cancer **86** (7), 1203–1209 (1999).
3. Jia R., Wan X., Geng X., Xue D., Xie Z., Chen C. Microorganisms **9**, 1659 (2021).
4. Dobryakova N., Zhdanov D., Dumina M. et al. Catalysts **13** (5), 832 (2023).
5. Upadhyay A.K., Singh A., Mukherjee K.J., Panda A.K. Front. Microbiol. **5**, 486 (2014).
6. Xie X.S., Lu H.P. J. Biol. Chem. **274** (23), 15967–15970 (1999).
7. Ivanov Y.D., Vinogradova A.V., Nevedrova E.D. et al. Int J Mol Sci. **25**, 10864 (2024).
8. Galenkamp N.S., van den Noort M., Maglia G. RSC Chem. Biol. **6**, 1686 (2025).
9. Dekker C. Nature Nanotech. **2**, 209–215 (2007).
10. Ivanov Y.D., Pleshakova T.O., Shumov I.D. et al. Sci Rep. **10**, 9022 (2020).
11. Ivanov Y.D., Shumov, I.D., Kozlov A.F. et al. Micromachines **14**, 1024 (2023).
12. Shinohara K., Makida Y. Sci. Rep. **8**, 13982 (2018).
13. Antoniou M., Tsounidi D., Petrou P.S., Beltsios K.G., Kakabakos S.E. Med. Devices Sens. **3**, e10072 (2020).
14. Papageorgiou A.C., Posypanova G.A., Andersson C.S., Sokolov N.S., Krasotkina J. FEBS J. **275** (17), 4306–4316 (2008).
15. Kiselyova O.I., Yaminsky I.V., Ivanov Yu.D., Kanaeva I.P., Kuznetsov, V.Y., Archakov A.I. Arch Biochem Biophys. **371** (1), 1–7 (1999).
16. Pleshakova T.O., Kaysheva A.L., Shumov I.D. et al. Micromachines **10**, 129 (2019).
17. Welinder K.G. Eur J Biochem. **96**, 483–502 (1979).
18. Michalska K., Jaskolski M. Acta Biochim Pol. **53** (4), 627–640 (2006).

About authors

Vazhenkova Ekaterina Evgenyevna, Junior Researcher Institute of Biomedical Chemistry (Bld. 8, 10 Pogodinskaya st., Moscow, 119121, Russia). E-mail: katyajustflash@gmail.com SPIN code: 5558-6468, AuthorID: 1262654, ORCID: 0009-0001-4224-8907.

Shumov Ivan Dmitrievich, Candidate of Biological Sciences, Senior Researcher, Institute of Biomedical Chemistry (Bld. 8, 10 Pogodinskaya st., Moscow, 119121, Russia). E-mail: shum230988@yandex.ru SPIN code: 3827-1620, AuthorID: 484732, ORCID: 0000-0002-9795-7065.

Zhdanov Dmitry Dmitrievich, Doctor of Biological Sciences, Head of Laboratory Institute of Biomedical Chemistry (Bld. 8, 10 Pogodinskaya st., Moscow, 119121, Russia). E-mail: zhdanovdd@gmail.com SPIN code: 3845-2544, AuthorID: 971666, ORCID: 0000-0003-4753-7588.

Shumyantseva Viktoria Vasilyevna, Doctor of Biological Sciences, Professor, Head of Laboratory Institute of Biomedical Chemistry (Bld. 8, 10 Pogodinskaya st., Moscow, 119121, Russia). E-mail: viktoriashumyantseva@ibmc.msk.ru SPIN code: 1104-2003, AuthorID: 46223, ORCID: 0000-0002-1509-7218.

Ziborov Vadim Serafimovich, Candidate of Physical and Mathematical Sciences, Senior Researcher Joint Institute for High Temperatures of Russian Academy of Sciences (Bld. 2, 13 Pogodinskaya st., Moscow, 125412, Russia). E-mail: ziborov.vs@yandex.ru AuthorID: 31839, ORCID: 0000-0001-7942-3337.

Ableev Alexander Narimanovich, Lead Engineer, Institute of Biomedical Chemistry (Bld. 8, 10 Pogodinskaya st., Moscow, 119121, Russia). E-mail: ableev@mail.ru SPIN code: 1193-5666, AuthorID: 1293547, ORCID: 0009-0004-3096-107X.

Afonin Oleg Nikolaevich, Candidate of Technical Sciences, Senior Researcher, Institute of Biomedical Chemistry (Bld. 8, 10 Pogodinskaya st., Moscow, 119121, Russia). E-mail: sunweb@mail.ru SPIN code: 1767-5835, AuthorID: 167545, ORCID: 0009-0008-7947-3674.

Vaulin Nikita Vasilyevich, Laboratory Assistant (Bld. 3, 8 Khlopina st., letter A, Saint Petersburg, 194021, Russia). E-mail: nikitavaylin@mail.ru SPIN code: 7466-5599, AuthorID: 1212356, ORCID: 0000-0001-6080-0729.

Lebedev Denis Vladimirovich, Candidate of Physical and Mathematical Sciences, Senior Researcher, Alferov Federal State Budgetary Institution of Higher Education and Science Saint Petersburg National Research Academic University of the Russian Academy of Sciences (Bld. 3, 8 Khlopina st., letter A, Saint Petersburg, 194021, Russia). E-mail: denis.v.lebedev@gmail.com SPIN code: 8079-9286, AuthorID: 751908, ORCID: 0000-0001-5389-2899.

Bukatin Anton Sergeevich, Candidate of Physical and Mathematical Sciences, Senior Researcher, Associate Professor Alferov Federal State Budgetary Institution of Higher Education and Science Saint Petersburg National Research Academic University of the Russian Academy of Sciences (Bld. 3, 8 Khlopina st., letter A, Saint Petersburg, 194021, Russia). E-mail: antbuk.fiztek@gmail.com SPIN code: 4604-0330, AuthorID: 744078, ORCID: 0000-0002-5459-1438.

Mukhin Ivan Sergeevich, Doctor of Physical and Mathematical Sciences, Professor, Head of the Laboratory, Alferov Federal State Budgetary Institution of Higher Education and Science Saint Petersburg National Research Academic University of the Russian Academy of Sciences (Bld. 3, 8 Khlopina st., letter A, Saint Petersburg, 194021, Russia). E-mail: imukhin@yandex.ru SPIN code: 3242-8122, AuthorID: 546023, ORCID: 0000-0001-9792-045X.

Tatur Vadim Yurievich, Executive Director Foundation of Perspective Technologies and Novations (Bld. 1, 8 2-nd Ambulatory Proyezd, Moscow, 125315, Russia). E-mail: v_tatur@mail.ru SPIN code: 5383-2035, AuthorID: 1292871, ORCID: 0000-0002-6415-5189.

Andrei Alexandrovich Lukyanitsa, Doctor of Technical Sciences, Leading Researcher Foundation of Perspective Technologies and Novations (Bld. 1, 8 2-nd Ambulatory Proyezd, Moscow, 125315, Russia); Faculty of Computational Mathematics and Cybernetics, Moscow State University (Bld. 52, GSP-1 Ulitsa, Leninskiye Gory, Moscow, 119991, Russia). E-mail: andrei_luk@mail.ru SPIN code: 6172-6061, AuthorID: 4162, ORCID: 0000-0002-0517-0602.

Ponomarenko Elena Alexandrovna, Corresponding Member of the Russian Academy of Sciences, Doctor of Biological Sciences, Director Institute of Biomedical Chemistry (Bld. 8, 10 Pogodinskaya st., Moscow, 119121, Russia). E-mail: 2463731@gmail.com SPIN code: 8427-5490, AuthorID: 180406, ORCID: 0000-0001-8527-6003.

Archakov Alexander Ivanovich, Academician of the Russian Academy of Sciences, Doctor of Biological Sciences, Professor, Scientific Supervisor Institute of Biomedical Chemistry (Bld. 8, 10 Pogodinskaya st., Moscow, 119121, Russia). E-mail: alexander.archakov@ibmc.msk.ru SPIN code: 9412-0222, AuthorID: 78435, ORCID: 0000-0002-2290-8090.

Ivanov Yuri Dmitrievich, Doctor of Biological Sciences, Professor, Head of Laboratory Institute of Biomedical Chemistry (Bld. 8, 10 Pogodinskaya st., Moscow, 119121, Russia). E-mail: yurii.ivanov.nata@gmail.com SPIN code: 7935-0461, AuthorID: 78445, ORCID: 0000-0001-5041-1914.