



STATEMENT

Regarding: *Awarding of the educational and scientific degree "Doctor" in the professional field 4.3 Biological Sciences, scientific speciality "Molecular Biology" to part-time PhD student Elitsa Hristova Boteva, IMB, BAS*

Dissertation Topic:

"DNA deglycation activity of the glycolytic enzyme phosphoglucoseisomerase"

Scientific Supervisor: Prof. Romyana Mironova, PhD

By

Prof. Milena Georgieva Vasileva, PhD

Laboratory of Molecular Genetics, Epigenetics, and Longevity, Institute of Molecular Biology "Acad. R. Tsanev", Bulgarian Academy of Sciences

This statement is prepared concerning the defence of the dissertation work of Elitsa Hristova Boteva for awarding the educational and scientific degree "Doctor" based on Order No. 111-OB/28.06.2024 of the Director of the Institute of Molecular Biology "Acad. Rumen Tsanev", BAS, and the decision of the first meeting of the scientific jury held on 10.07.2024.

The content of the dissertation fully complies with the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria (ZRASRB) and the internal regulations of IMB-BAS for its application.

SHORT BIOGRAPHICAL OVERVIEW OF THE PHD STUDENT

Elitsa Boteva is a researcher with experience in the field of molecular biology. In 2016, she began her PhD in "Molecular Biology" at the Institute of Molecular Biology "Rumen Tsanev" at the Bulgarian Academy of Sciences (IMB-BAS), where she currently works as an assistant. She earned her master's in genetics in 2011 and her bachelor's degree in molecular biology in 2006 from the Faculty of Biology at Sofia University "St. Kliment Ohridski."

Her professional career includes experience in various institutes and scientific centres such as the Institute of Molecular Biology "Rumen Tsanev" (BAS), the Institute of Electrochemistry and Oncology at the Hasumi Foundation in Tokyo, Japan, and the National Reference Laboratory for HIV/AIDS at the NCIPD. She has also participated in international training at the Karolinska Institute in Sweden and Utrecht University in the Netherlands.

Elitsa has experience with various molecular biology techniques, including cell cultures, electrophoretic and immunological methods, PCR, chromatographic and mass spectrometric techniques.

RELEVANCE OF THE TOPIC

The dissertation topic, "DNA deglycation activity of the glycolytic enzyme phosphoglucose isomerase," addresses a highly relevant scientific question related to post-translational modifications such as glycation and its effects on biomolecules, including DNA. Experimental data have shown that DNA glycation leads to significant mutagenic and carcinogenic effects, which necessitates the search for effective mechanisms for deglycation. Studying phosphoglucose isomerase as a potential enzyme with DNA-deglycation activity represents an innovative approach by which the PhD student contributes to expanding the knowledge of the molecular mechanisms of DNA repair.

EVALUATION OF THE KNOWLEDGE OF THE PROBLEM AND LITERATURE REVIEW

The evaluation of the knowledge of the problem and the literature review reveals a deep understanding of the chemical nature of the glycation process, also known as the Maillard reaction. The review thoroughly examines the stages of glycation, the sugars involved, and the biological significance of the final products. A primary focus is placed on DNA glycation and its consequences for cellular stability, discussing physiological effects and the potential of glycated nucleotides as diagnostic markers. The review highlights the importance of research that uncovers the complexity of cellular interactions and the need for new approaches to cellular regulation.

CHARACTERISTICS OF THE DISSERTATION

Elitsa Boteva's dissertation is structured following the traditional scientific format, including the following sections: literature review, objectives and tasks, materials and methods, results, discussion of the results, conclusions, contributions, scientific publications, scientific forums, and references. The total volume of the dissertation is 177 pages, containing 3 tables, 55 figures, and 287 literature sources, reflecting the problem's relevance. The detailed structure of the work includes an introduction, a literature review (41 pages), objectives and tasks (1 page), materials and methods (26 pages), results and discussion (90 pages), a conclusion (1 page), conclusions and contributions (1 page), and appendices with information on publications and participation in scientific forums (4 publications with impact factor, 1 without IF, and 1 book chapter; 10 participations in scientific forums – 2 pages). All presented materials, including the abstract, meet the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria (ZRASRB).

The dissertation presents a thorough study aimed at identifying and characterizing the deglycation activity in *Escherichia coli*, which is responsible for removing fructose-6-phosphate residues in DNA. The primary goal is realized through a series of strictly formulated tasks, including enzymological and kinetic characterization of the phosphoglucose isomerase (PGI) enzyme. The study employs an interdisciplinary approach, using analytical, biochemical, immunological, and molecular biological methods. The results demonstrate a new deglycation function of PGI and suggest its role in DNA repair processes, opening new horizons in biochemical research on the multifunctionality of enzymes.

The PhD student demonstrates high scientific competence and the ability to work with complex methods in molecular biology. She utilizes various techniques, from electrophoresis and chromatographic techniques to modern bioinformatics approaches such as molecular modelling and docking. This allows the identification of a previously unknown DNA-deglycation activity

of the enzyme phosphoglucose isomerase, which is part of the scientific contributions of the PhD candidate.

CONTRIBUTIONS OF THE DISSERTATION

The main scientific contributions of Elitsa Boteva's dissertation include:

- Discovery of a new DNA-deglycation activity of the glycolytic enzyme phosphoglucose isomerase.
- Identification of the Amadoriase activity of this enzyme reveals potential therapeutic opportunities for restoring glycated DNA molecules.
- Conducting the first molecular dynamics simulation of the interaction between phosphoglucose isomerase and glycated DNA.
- The discovery of nuclear localization of two isoforms of human phosphoglucose isomerase has expanded the understanding of its involvement in cellular processes.

PUBLICATIONS AND PRESENTATION OF RESULTS

The results of the dissertation work have been presented in 6 scientific articles, 4 indexed in Web of Science, with one in the first quartile and three in the third quartile. In four of the publications, Elitsa is the first author. This demonstrates a significant level of scientific contribution by the PhD candidate. Additionally, the results have been presented at 10 national and international scientific conferences, showing active scientific communication and engagement with the international scientific community.

The total number of points from the mandatory minimum (30 points) according to the IMB-BAS regulations for the application of ZRASRB, achieved as a result of the scientific work of the PhD candidate, is 85 points. These are detailed in the provided documents and reflect well-structured research work. It is clear that they far exceed the required minimum and guarantee the candidate's readiness for independent scientific work.

CONTENT AND QUALITY OF THE ABSTRACT

The abstract is well-structured and concisely reflects the content of the dissertation. Within 40 pages, the objectives and tasks of the research, the methods used, and the results and their discussion are presented. The novelty of the research conducted, the conclusions drawn, and the dissertation's contributions are highlighted. The bibliographic data of the six publications based on the dissertation material and the scientific forums where the research results were presented are included.

RECOMMENDATIONS

Elitsa Boteva's dissertation is impressive, as is the detailed biochemical, molecular biological, and bioinformatic research the PhD candidate conducted during her studies. It is written well academically, logically structured, and well-illustrated. Some terminological corrections could be made in places, but they do not affect the overall quality of the work. The questions regarding Elitsa's research work were asked during the internal defence and answered and defended by PhD candidate Elitsa Boteva very adequately and successfully. I strongly recommend that Elitsa Boteva continue and expand her scientific work through participation in international projects, focusing on publications in prestigious journals and additional bioinformatics training. Active

participation in conferences and developing interdisciplinary approaches would contribute to a deeper understanding and application of her scientific research in practice.

CONCLUSION

After a careful review of the dissertation and the accompanying publications, I confirm that they meet all the requirements for obtaining the educational and scientific degree "Doctor" in the professional field 4.3 Biological Sciences (Molecular Biology) following the criteria of ZRASRB, BAS, and the IMB-BAS regulations for obtaining the degree "Doctor." The scientific and applied contributions described by the candidate are significantly original and have the potential for future development. Therefore, I recommend the respected Scientific Jury to award Elitsa Boteva the educational and scientific degree "Doctor" in the professional field 4.3 Biological Sciences, scientific speciality Molecular Biology.

25.09.2024

Prepared by: _____

Sofia

/Prof. Milena Georgieva, PhD, IMB-BAS/