REVIEW

by: Prof. Dr. Mariela Ojakova-Baytosheva, Faculty of Biology of SU "St. Kliment Ohridski", member of the scientific jury according to order No. - 111-OB dated 28.06.2024 of the Director of the IMB, BAS

for awarding the educational and scientific degree "doctor" in professional field 4.3 Biological sciences, scientific specialty "Molecular biology" PhD student Elitsa Hristova Boteva, IMB, BAS

Dissertation topic: DNA deglycating activity of the glycolytic enzyme phosphoglucoisomerase

General data about the applicant

Elitsa Boteva has a bachelor's degree in Molecular Biology (2006) and a master's degree in Genetics (2011) from the Faculty of Biology, Sofia University „ St. Kliment Ohridski".
She worked as a specialist-biologist successively in: "Prosel" Medical Center (2006-2008); Electrochemical and Cancer Institute, Hasumi International Research Foundation Tokyo, Japan (2008-2009); National Reference Laboratory for HIV/AIDS, National Reference Laboratory for HIV/AIDS (2010); IMB, BAS (2013-present, and since 2016 he has been a PhD student)

Fulfillment of the minimum state requirements and the individual plan

The results of the research are reflected in 6 out-of-print publications with a total number of points from the respective quartiles of 85 points with a required minimum of 30 points and a total impact factor of 10.012 with a minimum of 2.0, with which the doctoral student's assets significantly exceed the requirements of LDACSRB and the regulations for its implementation in BAS and IMB-BAS. In four of the publications, the PhD student is the first author, and 10 citations of these publications have been noticed so far. The results have been reported at 10 scientific national and international forums. E. Boteva has covered and significantly exceeded the number of credits required by the Training Center at the BAS for the acquisition of PhD. With a required minimum of 250 credits, a total of 822 credits were collected, i.e. more than three times as many. The research in the dissertation was carried out within the framework of two projects with the financial support of the Ministry of Education and Culture (the "Scientific Research" Fund and the "Program for supporting young scientists and doctoral students at the BAS"), which shows that the doctoral student has gained experience in teamwork and in developing of scientific research projects.

Dissertation analysis

The intensive development of molecular biology in recent decades has led to the rejection of some rigid notions such as those about the exclusively protein nature of biocatalysts and their strict specificity to a certain substrate. In addition, some enzymes perform other, non-enzymatic functions. An example of such an enzyme is the object of research in this dissertation – the glycolytic enzyme phosphoglucose isomerase (Pgi). In addition to its specific isomerase activity in glycolysis, the human enzyme, for example, acts as a cytokine and a neuroleukin. The dissertation of Elitsa Hristova Boteva fits into the light of the most modern molecular biological

research, on the one hand, expanding our understanding of the variety of functions that an enzyme can perform, and on the other hand - examining it in the context of such a significant process for human physiology and pathology as the process of non-enzymatic glycosylation or glycation.

The dissertation is written on 189 pages (including the unnumbered ones) and includes the standard sections, with the Results and Discussion sections separated into separate chapters. The Literature review and the Discussion end with brief conclusions that logically lead to the goal and summarize the results achieved. The dissertation contains rich illustrative material of 55 figures and 3 tables that document and support the reported results. The literature reference of 287 literary sources is indicative of a good familiarity of the PhD student with the state and trends of research on the topic of the dissertation. This is also evident from the literature review written in good literary language and scientific style. It examines key research issues such as the Maillard reaction with its steps and products, with an emphasis on DNA glycation and the physiological consequences of this process. Another major section of the review is devoted to defense mechanisms against glycation and in particular to the repair of glycated DNA. Thus, it is clear that data on the repair of glycated DNA are scarce and contradictory, and that there is no data at all on the repair of DNA modified with early glycoadducts. In this way, even in the literature review, the topicality and significance of the dissertation are outlined and its purpose is logically deduced.

The purpose of the dissertation is a consequence of previous studies that show that lysates from the intestinal bacterium *Escherichia coli* catalyze the removal of fructose-6-phosphate residues from DNA. More specifically, the goal is to identify and characterize this activity. To achieve the goal, two groups of tasks have been identified - the first, related to the identification of the activity, and the second - to its detailed study in bacterial and human cells with adequate molecular-biological methods and theoretical analyses.

The Materials and Methods section of the dissertation impresses with the variety and detailed description of the materials used (strains and cell lines, chemicals, consumables and antibodies) and methods. The arsenal of methods used is extremely rich and includes chromatographic, electrophoretic, immunological, mass-spectral and quantitative methods, enzyme and mutation tests, statistical and bioinformatic analyses, molecular modeling, docking and molecular dynamics. Undoubtedly, this contributed to the excellent methodical preparation of the doctoral student on the one hand, and on the other - to successfully solving the tasks and achieving the goal of the dissertation.

The results are presented in five chapters and impress with the logical sequence of planned and conducted experiments. Chapter 1 describes the identification of phosphoglucose isomerase as the most likely carrier of the observed DNA deglycase activity. In Chapter 2, the observed DNA deglycase activity in bacteria is characterized in detail. The investigations in this chapter conclude with the determination of the kinetic constants of the enzyme as a DNA deglycase and an investigation of its physiological role as a possible DNA repair enzyme. The putative DNA-repair function of the enzyme is supported by the observed significantly higher frequency of spontaneous mutations in the Pgi gene-deleted strain. Another merit of the dissertation is its scope. Research in it is not limited to bacteria, but based on the high conservatism of the enzyme, they are also transferred to human cells. In Chapter 3, the presence of Pgi in the nuclei of normal and cancer

human cells is convincingly demonstrated by several independent methods, confirming literature data and indicating yet another reason for the nuclear localization of the enzyme. In Chapter 4, the experimental evidence for DNA deglycase (reparative) activity of Pgi is convincingly supported by extensive bioinformatic analyses, which identify the enzyme as a bona fide DNA repair enzyme. On the other hand, the molecular dynamics studies in Chapter 5 well explain the experimental data for a lower deglycase activity of the enzyme compared to its isomerase activity.

The discussion of the results demonstrates Elitsa Boteva's maturity and ability to thoroughly analyze the results of her research in the context of the world scientific literature, as well as to realistically assess their significance and prospects for future research. A pleasant impression is made in the conclusion of the dissertation, emphasizing the biological significance of the newly discovered enzymatic activity of Pgi, which in one catalytic act repairs DNA and recycles sugar for economical use in metabolic pathways. The conclusions drawn and the assessment of the scientific contributions are short, logical and accurately reflect the results of the conducted research. 4 scientific contributions of an original and 1 of a confirmatory nature have been formulated, which are credible and with which I fully agree.

The abstract is a suitably abridged version of the dissertation, in which it has lost none of its merits, which is why I accept the way it is framed and presented to the reader.

I have the following question: In Fig. 20C in Chapter 2 of Results, data on glucose-6-phosphate content in *E. coli* are presented in micromoles per cell count. Could you please specify what molar concentrations this data corresponds to so that we can get a clearer idea of the physiological cellular concentrations of glucose-6-phosphate, which according to the data from the thesis appears to be a potential mutagen.

CONCLUSION

I have excellent impressions of the dissertation submitted for my opinion. Elitsa Boteva presents an extensive and in-depth study on a previously unknown function of a long-known and highly conserved enzyme. I am impressed by the scale and quality of the research. As a result of the conducted research, significant scientific results were obtained, mainly of an original nature. During the preparation of the dissertation, the doctoral student has mastered an extremely large number of state-of-the-art experimental and theoretical methods and has gained experience in the development of scientific research projects. Research results have been published in refereed international journals with a high overall impact factor and citations have already been noticed. With her scientometric indicators, the E. Boteva fully meets and significantly exceeds the criteria of the BAS and IMB-BAS for obtaining the title of "Doctor". Everything that has been said so far gives me reason to confidently give my positive vote for awarding the PhD to Elitsa Hristova Boteva in Professional field 4.3. Biological Sciences, Science Major "Molecular Biology".

25.09.2024: /prof. M. Odjakova/