



REVIEW

on a dissertation for acquisition of the educational and scientific degree "Doctor" (PhD)

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Topic of the dissertation: "DNA DEGLYCATING ACTIVITY OF THE GLYCOLYTIC ENZYME PHOSPHOGLUCOSE ISOMERASE"

Scientific supervisor: Prof. Roumyana Mironova, PhD

Reviewer: Prof. Mariana Dimitrova Argirova, DSc

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This review is prepared in response of Order No. 111-OB/28.06.2024 issued by the Director of the Institute of Molecular Biology (IMB) "Acad. Rumen Tsanev" and the decision of the first meeting of the scientific jury, held on 12.07.2024. It complies with the requirements of the Development of the Academic Staff in the Republic of Bulgaria Act (DASRBA), the General regulations on its implementation, as well as the specific institutional requirements of the Regulations for the development of the academic staff of IMB "Acad. Rumen Tsanev" (2021).

All materials necessary for the preparation of this review were presented to me in electronic format.

1. Brief biographical data about the PhD student

Elitsa Boteva graduated from the Faculty of biology at the SU "St. Kliment Ohridski", where in 2011 she received a master's degree in genetics after defending her thesis on "Repair of Amadori products in the chromosomal DNA of Escherichia coli". In the same year, she joined the staff of IMB, first as a biologist, and then as an Assistant Professor. Since 2016, she has been a part-time doctoral student at the Institute. She specialized in scientific institutions in Sweden, the Netherlands and Japan, and with her DAAD scholarship she specialized in Germany. Elitsa Boteva is a participant in three scientific projects related to the subject of the dissertation.

2. Actuality of the topic and appropriateness of the set goals and tasks

Glycation (non-enzymatic glycosylation) is one of the most common post-translational modifications of proteins in living organisms, but this process also affects other long-lived biomolecules. Undoubtedly, this process is part of the molecular basis of the complications that develop in diabetic patients, and the suppression of glycation is part of the strategy to combat this widespread disease of the modern world. There is also a number of experimental data that the nitrogenous bases of nucleic acids, which contain free amino groups, mainly guanine, can also be the objects of glycation with various carbonyl-containing metabolites. The resulting adducts can affect the intactness and structure of DNA, its genomic integrity and could have mutagenic and carcinogenic effects.

The most commonly used therapeutic approaches to combat the detrimental biological consequences of glycation include the administration of compounds that cleave bonds in cross-linked proteins; those that inhibit the formation of advanced glycation end products (AGE) by competitive binding of reactive dicarbonyl compounds, AGE receptor antagonists (RAGE), etc. A relatively new but still understudied mechanism to prevent the harmful effects of glycation exploits the reversibility of Amadori product formation and the possibility of enzymatic recovery of native biomolecules. A limited number of enzymes that can catalyze this process, collectively known as amadoriases or deglycases, have been studied. Enzymatic deglycation is a potential and promising strategy that one day may hopefully help in preventing the consequences of glycation – delaying the onset of diabetes complications and aging. That is why I believe that the topic of the dissertation work "DNA deglycating activity of the glycolytic enzyme phosphoglucose isomerase" for the acquisition of educational and scientific degree "Doctor", the set goal and following tasks have an up-to-date scientific and practical value.

3. Knowing the problem

In general, the literature review demonstrates doctoral student's profound understanding of the problem. It is a critical look at the existing knowledge of glycation as a process, highlighting the milestones in this knowledge and the questions still to be answered. Despite the diverse and multidirectional manifestations of the Maillard reaction in foods and living organisms, in the review of the current scientific literature the doctoral student managed to present the process of interaction between amine-containing compounds and carbohydrates in a very concentrated but informative way with an emphasis on the known *in vitro* and *in vivo* evidence for DNA glycation and the mechanisms of action of known deglycating enzymes. The latest trends in glycation research are also outlined.

4. Characteristics and evaluation of the dissertation

The PhD thesis has a classic structure and a good balance between the its parts – literature review, materials and methods, results and discussion, and used literature (287 sources).

The analytically presented literature review ends with an overview of the previous experience of the team led by Prof. Rumiana Mironova on glycation in *E. coli* and its role as

a source of spontaneous chemical damage in bacterial proteins and DNA, the possibilities for enzymatic deglycation of proteins and nucleic acids, as well as the still unsolved and unanswered questions, which makes it possible to formulate the purpose of the thesis in a very sensible way.

In the Materials and Methods section, all methods used are described in sufficient details to allow reproduction, and where already validated and published methods by other authors are used, the PhD student refers to the original works.

The realization of the goal and specific tasks set in the dissertation work was carried out in several steps. For the isolation and identification of the phosphoglucose isomerase enzyme in *E. coli* lysate, the doctoral student applied subcellular fractionation of the total lysate and DNA isolation; one- and two-dimensional electrophoresis of fractions presumed to contain the desired enzyme, immunological methods and techniques, and as a final step, protein sequencing was performed to definitively prove the presence of the phosphoglucose isomerase. In the next steps, the deglycating ability of the enzyme is proven by using *in vitro* glycated oligonucleotide and double-stranded DNA, and a strain with a deleted gene for phosphoglucose isomerase was used. A number of chromatographic methods are used in various stages of the research to solve the problems – gel permeation, ion-exchange, high-performance liquid chromatography with mass detection, as well as spectrophotometric methods for quantification of some compounds. Where necessary, the obtained results are subjected to appropriate statistical evaluation.

Suitably, the doctoral student has combined the results with their discussion, because this allows to follow the logic of the experiments and justifies the use of each method. Very often, to prove a given hypothesis, the doctoral student uses two different experimental approaches, which make the presented results of the conducted genetic and immunological studies very convincing and confirm that DNA deglycation is due to the authentic isomerase activity of phosphoglucose isomerase.

In her dissertation, the doctoral student demonstrates the ability to work with the tools of modern bioinformatics using various libraries with databases, as well as applying molecular modeling, molecular dynamics and molecular docking as additional proof of the hypothesis of deglycating action of phosphoglucose isomerase.

The thesis does not overlook some results that cannot be explained at this stage, but open up broad possibilities for future research.

The author's desire to stick to terminology in Bulgarian is impressive, but not necessary everywhere, since in Bulgarian scientific literature some of the foreign terms are already generally accepted.

5. Contributions and scientific significance of the thesis

At the end of the dissertation, the doctoral student systematizes the main conclusions from the conducted experiments, which to a significant extent provide an answer to the set goal and tasks. It outlines the main contributions of the dissertation, formulated and summarized in four main points:

- A hitherto unknown activity of the glycolytic enzyme phosphoglucose isomerase was discovered.
- For the first time DNA deglycase with amadoriasis activity and an isomerizing mechanism is described.
- The presence of a nuclear localization signal in two of the isoforms of human phosphoglucose isomerase is shown.
- The first molecular dynamic simulation of the interaction between the glycolytic enzyme phosphoglucose isomerase and glycated DNA was made.

The contributions have an original character and are novel in the scientific literature.

6. Evaluation of publications on the dissertation work

The results obtained by the doctoral student have been published in 6 scientific articles, which include a significant part of the results presented in the dissertation. All of them have been reviewed by experts in the field. Four of them have a Journal Citation Index on the Web of Science database. One of them is in the first quartile, one in the second and two of them in the third quartile. Thus, the doctoral student has a total of 75 points for published articles in publications that are indexed in world-renowned databases and exceeds the minimum requirements under indicator G of the Regulations for the implementation of DASRBA. The PhD student is the first author of three of the presented publications. A specific requirement of the Regulations for the implementation of DASRBA in IMB - BAS is that two of the publications be in journals with an impact factor of 2, which requirement was also fulfilled by the doctoral student. A published chapter of a book co-authored by the PhD student is also presented, which adds 15 points under indicator G of the Regulations.

The substantial scientific value of the published articles is also supported by their citability – they are cited by more than 10 foreign researchers within 2-3 years after their publication. Research results have been presented to the scientific community at 10 national and international conferences.

I hope that a publication including the state-of-the-art *in silico* methods of molecular modeling and molecular docking is in preparation, as it would undoubtedly be of interest to specialists in the field.

I recommend that the PhD student, in future publications on the topic to take on the responsibility of corresponding author, as this is an invariable part of the professional growth of any researcher.

7. Summary of the dissertation

The Summary follows in a brief form the content of the reviewed dissertation and in a volume of 40 pages presents the set goal and tasks, the methods used, the obtained own results and their adequate discussion, emphasizing the novelties in the conducted research, the conclusions and contributions of the dissertation. The bibliographic data of the six publications on the content of dissertation and the scientific forums where the results of the research have been presented are also included.

8. Notes and recommendations

The dissertation is written in very good scientific language, concise, follows the logical sequence of the experiments, their interpretation, and this makes it very convincing. Its graphic layout is perfect. It impresses with the multitude of learned methods and a considerable amount of data presented in 55 figures and 3 tables. There are minor terminological inaccuracies in places (p. 4 autoxidation and autoxidation in one paragraph; p. 72 deglycase activity was *eluted* mainly in fractions 28 – 35 instead of recorded; gel filtration instead of gel permeation chromatography, etc.).

I have no critical remarks about the dissertation, but it raised several questions for me:

- How are the conditions for glycation of double-stranded DNA chosen (p. 47)? Under physiological conditions, hemoglobin forms an Amadori product with glucose in about 1 week, and as the author points out, glucose-6-phosphate is far more reactive than glucose. The selected long period and the use of a high concentration of carbohydrate favor the formation of AGEs, which leads to decrease in the amount of the substrate of the studied reaction – fructosamine.
- Which form of the Amadori product of guanine was used in the docking modeling – five- or six-membered ring, as this is difficult to see from the figures? Unlike fructose, a significant proportion of Amadori products also exist as a six-membered ring.

Conclusion

According to DASRBA, "Doctor" is an educational and scientific degree. The content of the dissertation convincingly shows that the doctoral student Elitsa Boteva has not only built on the knowledge obtained in the master's degree and covered the educational component of the "Doctor" degree, but also possesses in-depth theoretical knowledge and experimental skills in the field of molecular biology. The doctoral student has mastered a number of analytical and instrumental methods; she is able to critically discuss the results obtained and present them in writing.

Based on the analysis of the significance of the research conducted, the dissertation work developed and the publications presented, I confidently give my positive assessment and recommend to the respected members of the scientific jury to award the educational and scientific degree "Doctor" to ELITSA HRISTOVA BOTEVA in scientific field 4. Natural sciences, mathematics and informatics, professional direction 4.3. Biological Sciences with a scientific specialty Molecular Biology.

September 23, 2024

Reviewer: