STATEMENT

on a dissertation for obtaining the educational and scientific degree "Doctor"

Professional direction: 4.3 Biological sciences

Doctoral program: Biophysics

Author: Vesela Vasileva Yordanova

Form of doctoral studies: Independent preparation

Topic: Membrane reorganization under oxidative stress: effect of oxidized lipids

Author of the statement: Assoc. Prof. Desislava Anri Lazarova, PhD - Department of Physics, Biophysics and Radiology, Faculty of Medicine, Sofia University "St. Kliment Ohridski"

The statement was prepared on the basis of the Law on the Development of the Academic Staff in the Republic of Bulgaria (LDASRB), the Regulations for the Implementation of LDASRB and Order № 605/10.08.2023 of the Director of the Institute of Biophysics and Biomedical Engineering of the Bulgarian Academy of Sciences, for appointment of the scientific jury for the competition.

1. Biographical data

Vesela Vasileva Yordanova was born on 27.01.1985. She graduated from with a bachelor's degree in "Biotechnology" and a master's program in "Plant Biotechnology" at the Faculty of Biology of the Sofia University "St. Kliment Ohridski" in 2009 with full honors. Since 2013, she has been working at the Bulgarian Academy of Sciences, first at the Institute of Plant Physiology and Genetics, and then (until now) at the Institute of Biophysics and Biomedical Engineering. She was enrolled as a self-study doctoral student in the "Lipid-protein interactions" section at IBPBME - BAS in 2020.

2. Actuality of the dissertation work

The dissertation work presented to me for statement, developed by Vesela Vasileva Yordanova, is dedicated to a current problem related to the study of the influence of biologically active oxidized lipids on the structural organization of the lipid bilayer of biomimetic systems. There is increasing evidence in the literature that in inflammatory, autoimmune and neurodegenerative diseases, oxidized lipids are generated and a deeper understanding of their impact on membrane architecture and related processes at the molecular level, could contribute to the development of new diagnostic and therapeutic approaches.

3. Characteristics of the dissertation work

The dissertation is 151 pages long. It is well illustrated with 64 figures, high resolution, correct and complete descriptions.

The literature review provides a detailed analysis of the structural composition of biological membranes, in particular the diversity of membrane lipids and their influence on membrane properties. Particular attention is paid to the effect of oxidized phospholipids on membrane organization and to the mechanism of action of $sPLA_2$. The literature review comprehensively reflects the theoretical and experimental knowledge of the studied problem to date, fully corresponds to the topic of the dissertation and introduces the context of the obtained results.

The goal is clearly formulated, and detailed and logical tasks are set for its clarification.

The methods and materials section include a detailed description of the lipids, markers and probes used, the methods of vesicle formation, as well as their research with fluorescence spectroscopy. Fluorescence quenching of DPH by TEMPO and Laurdan fluorescence spectroscopy in liposome suspensions were performed, providing information on the degree of lipid ordering in the membrane and the lateral structure of the membranes. The effect of oxidized lipids on the activity of phospholipase A_2 was evaluated, depending on the degree of unsaturation of fatty acids in the glycerophospholipid molecules in mixtures. From the way the experimental conditions are described and the discussion of their influence, in the research done on the dissertation work, precision, accuracy and thoroughness in planning, can be seen.

4. Experimental results, conclusions and contributions

The PhD student investigated the influence of the oxidized lipids POVPC and PGPC and the degree of unsaturation of the fatty acid at the *sn-2* position in the PC molecule on the formation and size of the raft domains at physiological temperature by means of DPH-TEMPO fluorescence spectroscopy. For this purpose, the effect of the degree of unsaturation of the fatty acids in the molecule of the glycerophospholipids POPC and PDPC on the formation of raft domains in two- and three-component control mixtures and the influence of the oxidized lipids POVPC and PGPC on the formation of raft domains and their size in three-component mixtures, model of L_o/L_d coexistence (liquid ordered (L_o) and liquid disordered (L_d) phase), has been consistently clarified.

In the subsequent experiments described in the dissertation, the influence of the oxidized lipids POVPC and PGPC and the degree of unsaturation of the fatty acid at the *sn*-2 position in the PC molecule on the degree of lipid ordering in the lipid bilayer, was investigated by means of Laurdan fluorescence spectroscopy.

In the last, third section of the experimental results, the influence of the oxidized lipids POVPC and PGPC and the degree of unsaturation of the fatty acid at the *sn-2* position in the PC molecule on the

enzymatic activity of $sPLA_2$ at physiological temperature was investigated by means of fluorescence spectroscopy using a fluorogenic substrate of the enzyme. The kinetics of $sPLA_2$ activity on one-component control vesicles composed of the monounsaturated lipid POPC or the polyunsaturated PDPC, models of the L_d phase labeled with the fluorogenic substrate PED6, were recorded and the effects of OxPCs (oxidized lipids) and cholesterol were investigated – individually and in mixtures. on the activity of $sPLA_2$ depending on the degree of fatty acid unsaturation at the *sn-2* position of the glycerophospholipids POPC and PDPC.

The obtained results are detailed and comprehensively described and discussed adequately in the context of the data published in the literature on the subject of the dissertation.

The conclusions made in the dissertation (9 in number) are correctly and clearly formulated. The contributions are well organized and emphasize on the first-time obtained data on membrane reorganization under oxidative stress.

In connection with the thesis, 3 scientific publications have been published, two of them with IF and one with SJR (in Q1, Q2 and Q3 respectively, according to data from *JCR* of *WoS* and/or *Scimago*). In all three scientific articles, the doctoral student is the first author. These scient metric indicators fully meet the national minimum requirements for awarding the ESD "Doctor" and the additional requirements laid down in the Regulations for the implementation of the LDASRB in IBPBME-BAS (the candidate must have 3 publications on the dissertation work, of which at least 1 in an international journal with IF and at least 1 article as first author).

The presented author summary is designed precisely and corresponds to the content of the dissertation.

5. Conclusion

The materials presented in connection with the dissertation work are in full compliance and exceed the required scient metric indicators according to the LDASRB, the Regulations for the implementation of the LDASRB and the Rules for the implementation of the LDASRB in IBPBME-BAS. The above analysis is a convincing reason to give my **positive assessment** and to recommend to the Scientific Jury to award the educational and scientific degree "Doctor" in professional field 4.3. Biological Sciences (Biophysics) to Vesela Vasileva Yordanova.

Assoc. Prof. Desislava Anri Lazarova, PhD

September 8, 2023

Sofia