REVIEW

On a doctoral thesis for the acquisition of the scientific and educational degree "doctor" in the professional direction "4.3. Biological Sciences", PhD program Biophysics

of Vesela Vasileva Yordanova, doctoral student of self training at the "Lipid-Protein Interactions" Section at the Institute of Biophysics and Biomedical Engineering at the Bulgarian Academy of Sciences

on the subject:

"MEMBRANE REORGANIZATION UNDER OXIDATIVE STRESS: EFFECT OF OXIDIZED LIPIDS"

With Scientific Consultant Prof. Dr. Galya Staneva, Section "Lipid-Protein Interactions" at the Institute of Biophysics and Biomedical Engineering at the Bulgarian Ac ademy of Sciences

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Data on the doctoral student and the doctoral program.

Vesela Vasileva Yordanova graduated with a Bachelor's degree in Biotechnology at the Faculty of Biology of Sofia University "St. Kliment Ohridski" in 2007 and master's degree in Plant Biotechnology at the same University in 2009. In 2020, she was enrolled as a self training doctoral student in Doctoral program Biophysics, professional direction 4.3. Biological Sciences, at the "Lipid-Protein Interactions" Section of Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences on the basis of Art. 67 of the Law

on the Development of the Academic Staff in the Republic of Bulgaria, Art. 11 of the Regulations for the Implementation of the Law on the Development of the Academic Staff in the Republic of Bulgaria, and the Regulations for the Conditions and Procedures for Acquiring Scientific Degrees and Holding Academic Positions at the Institute of Biophysics and Biomedical Engineering at the Bulgarian Academy of Sciences. For the duration of the doctoral studies, Vesela Yordanova successfully passed all the necessary exams and completed the tasks according to the approved individual plan. On 27.07.2023, an open sectional meeting of the section "Lipid-Protein Interactions" was held for the deduction and pre-defense of her doctoral thesis, at which a preliminary discussion of the dissertation was made and a decision was made to direct it to an official defense. All deadlines and minimal national requirements have been met, as the presented documentation fully meets the requirements of the Law on the development of the academic staff in the Republic of Bulgaria, and the dissertation work and the resume have successfully passed the plagiarism prevention check. There are no established violations in the implementation of the procedures for preparation, deduction and preliminary discussion of the dissertation work.

In addition to her work on the dissertation, Vesela Yordanova has increased her scientific capacity by being a participant in a total of 7 scientific projects and the leader of an youth project related to the field of her dissertation topic, which were financed by the Scientific Research Found of the Ministry of Education and Culture and under the COST action program or a bilateral agreement with Egypt. Outside of her dissertation work, doctoral student Yordanova is also a co-author in 11 scientific publications in the field of biomedical research and biophysics.

Dissertation data.

The thesis topic "Membrane Reorganization under Oxidative Stress: Effect of Oxidized Lipids" fully reflects the essence of the work, which is in a rapidly developing field of biomedical sciences. The main goal of the topic is to elucidate the mechanisms of membrane reorganization under oxidative stress. These studies logically fit into the general work plan of the team of the "Lipid-Protein Interactions" Section, as a complex study, including methods from the field of physicochemical and biophysical studies of membrane organization, which leads to results with both fundamental and applied meaning.

The main parts of the dissertation follow the plan generally accepted for such work and include: Introduction – 2 pages, Literature review – 41 pages, Aim and tasks – 2 pages, Materials and methods – 9 pages, Results and discussion – 48 pages, Conclusions – 2 pages and Contributions – 1 page. A total of 30 figures and 4 tables were used for illustration. The dissertation is well balanced, and the discussion of the obtained results is well done in view of the physicochemical parameters affecting the formation of membrane domains and their importance in interpreting the data in the different model systems. In total, the dissertation contains 151 pages.

The literature review contains seven points dealing with the structure of biological membranes and the development of their models, membrane lipids and the concept of lipid rafts, types of biomimetic membrane models and their use in studying membrane changes under oxidative stress and signal transduction involving phospholipases. The review is made in accessible language, concise and clear and is illustrated with 16 figures, which are duly cited. Special attention is paid to modern methods that provide better opportunities for studying the domain organization of membranes and, in particular, the plasmalemma.

The aim of the dissertation is to investigate the influence of biologically active oxidized lipids on the membrane lateral organization and the activity of sPLA2 in model systems with different degrees of unsaturation of fatty acids at the sn-2 position. presented, and to it logically and systematically formulated seven tasks, outlining the logic and the plan according to which the experimental part of the dissertation work was carried out, as for each of the tasks, the main method by which it will be carried out is noted. Tasks include the creation of comprehensive model systems dependent on lamellarity, phase states and transitions that have been used to study the influence of lipid unsaturation on the formation of raft domains and the activity of secretory phospholipases, the size of rafts in the presence of oxidized lipids, and analysis of enzyme reaction rates in membrane systems modeling the liquid-disordered phase state (Ld) and the heterogeneous Lo/Ld state.

The Materials and Methods section lists the lipids used to create the models, giving their structural formulas and molecular masses, and also presents the structural formulas of the fluorescent probes DPH, TEMPO, and Laurdan used to assess the degree of order of lipids in model membranes and the fluorogenic substrate PED6 to assess phospholipase activity. The methods are described in detail, with a complete composition of the buffers and solutions used, as well as a sufficiently clear theoretical justification for their adequacy. In total, this part is illustrated with 14 figures. The formulas used to calculate the size of the raft domains

in the case of DPH/TEMPO quenching; the general Laurdan polarization and the way of interpreting the fluorescence spectroscopy data in determining the enzymatic activity of phospholipases are indicated. The methods are sufficiently well described to be repeatable.

The results are well explained and illustrated, strictly following the tasks set. Along with the explanation of the results, a brief discussion of each of them is made. During the preparation of the model systems for the study of lipid organization, it was found that the pure PC vesicles, a model of the Ld phase, are characterized by the lowest values of order and, accordingly, of DPH quenching, and the SM/Chol mixture is characterized by the highest (50:50), Lo phase model. All other mixtures, bi- and three-component, exhibiting Lo/Ld coexistence fall between these values. In contrast to the binary mixtures, in the three-component systems a greater degree of order was observed in the polyunsaturated membranes compared to the monounsaturated ones. Oxidized lipids increase the capacity to form raft domains, which is also confirmed by other authors, but the mechanisms for this have not been clarified. The dissertation takes a step in this direction by comparing mixtures of varying complexity and estimating the size of the resulting shelves as a function of the degree of lipid hydration. The PhD student proves that due to the high sensitivity of ω-3 docosahexaenoic fatty acid to autoxidation processes, the experimental conditions are of great importance, including the protocol used for mixing and hydration of the lipids and, more specifically, the temperature at which the liposomes are formed, as well as the time of the measurements. The results of these studies lead to a reasoned proposal for the optimization of a protocol widely used in scientific research, which would avoid additional oxidation of lipids in model membranes and, from there, misinterpretation of the obtained data. Studies of changes in the activity of secretory phospholipases showed suppressed activity in the presence of cholesterol in monocomponent membranes and POVPC in all Lo/Ld membranes tested. Nine conclusions were drawn from the obtained results, which correspond to the presented data and analyses.

Scientific apparatus.

A total of 417 sources are cited, of which only 2 are in Bulgarian. All sources are directly related to the researched topic, which shows the excellent awareness of the PhD student. Citations are made in compliance with established standards for citing scientific literature.

Applications

There are no specially indicated Appendices to the dissertation and the author's abstract, but as such a List of used abbreviations can be accepted, which is useful and facilitates the reading of the dissertation work.

The resume

The resume contains 53 pages and correctly reflects the content of the doctoral thesis. The main results are correctly presented, illustrated with a total of 22 figures. The Introduction and Materials and Methods parts are presented in the most abbreviated form to allow for a more complete presentation of Results, Conclusions and Contributions. A list of publications and participation in scientific forums related to the dissertation is also presented.

Publications.

The results of the dissertation, as well as parts of the literature review, were presented at 6 national and international scientific forums and were included in three articles, published in journals with impact factor and quartiles (respectively Q1, 2 and 3) - International Journal of Molecular Sciences, Comptes rendus de l'Académie bulgare des Sciences and Oxidation Communications. These publications fully cover and exceed the minimum national requirements for the defense of a dissertation for the scientific and educational degree Doctor in Science 4.3. Biological sciences, according to Appendix 1 of the Law on the development of the academic staff in the Republic of Bulgaria.

Scientific and applied contributions.

The contributions of the dissertation work are three in total and are of a scientific-applied and fundamental nature. One of the contributions relates to the improvement of the protocol for the hydration and mixing of lipids in the formation of model systems composed of polyunsaturated and oxidized glycerophospholipids. The other two are obtaining new data related to lateral membrane organization induced by oxidized lipids POVPC and PGPC in biomimetic systems, as well as changes in secretory phospholipase A2 activity depending on the type of oxidized lipid and the degree of fatty acid unsaturation at sn-2 position in the glycerophospholipid molecule.

Conclusion.

The work submitted for review is a study of the effect of oxidative stress on membrane lipid organization. The dissertation is focused on the effect of oxidized lipids and polyunsaturated acids on the raft domains and activity of secreted phospholipase A. This topic would help to elucidate the changes occurring in the plasmalemma under oxidative stress and how cell signaling is modulated in relation to this. Doctoral student Vesela Yordanova has fulfilled the goals and tasks set in the dissertation and fully meets the requirements of Appendix 1 of the Law on the development of the academic staff in the Republic of Bulgaria regarding the number of publications.

In conclusion, I consider that the doctoral candidate fully meets the requirements of the Law on the development of the academic staff in the Republic of Bulgaria for awarding the scientific and educational degree "Doctor" and I give my positive assessment Vesela Vasileva Yordanova to be awarded with the scientific and educational degree "Doctor" in scientific direction 4.3. Biological Sciences, scientific field Biophysics.

09/06/2023 Prepared the review: assoc. prof. Tanya Topouzova-Hristova