

## Opinion

**by a member of the scientific jury:** Albena Georgieva Jordanova, Professor of biochemistry at the Faculty of Medicine, Sofia University "St. Kliment Ohridski", appointed as a member of the scientific jury by order 605/10.08.2023 of the director of the Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences

**For the acquisition of the educational and scientific PhD degree,** in the field of higher education: 4. "Natural sciences, mathematics and informatics"; Professional field: 4.3. "Biological Sciences"; Scientific specialty "Biophysics"

**Author of the dissertation:** Vesela Vasileva Yordanova

**Dissertation topic:** Membrane reorganization under oxidative stress: effect of oxidized lipids

**Scientific consultant:** Prof. Galya Staneva, Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences

In the PhD thesis "*Membrane reorganization under oxidative stress: effect of oxidized lipids*" by PhD student Vesela Vasileva Yordanova submitted to me for opinion were analyzed the effects of the degree of unsaturation of the fatty acid in the *sn*-2 position in the phospholipid molecules 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine/POPC (OA, 18:1) and 1-palmitoyl-2-docosahexaenoyl-*sn*-glycero-3-phosphocholine/PDPC (DHA, 22:6  $\omega$ -3). In addition, two modified by oxidation products: 1-palmitoyl-2-(5'-oxo-valeroyl)-*sn*-glycero-3-phosphatidylcholine/POVPC and 1-palmitoyl-2-glutaryl-*sn*-glycero-3-phosphatidylcholine/PGPC of the containing in its molecule arachidonic acid 1-palmitoyl-2-arachidonoyl-*sn*-glycero-3-phosphocholine/PAPC on the membrane structural organization and activity of secretory phospholipase A<sub>2</sub>/sPLA<sub>2</sub> in biomimetic systems modeling different phase states.

The PhD thesis contains 151 pages and is illustrated with 64 figures, most of which contain several panels. 417 scientific publications are cited, but the most recent citations are from the 2021, which is one of my main notes regarding the dissertation. The experimental work was carried out in the laboratories of the sections "Lipid-protein interactions" and "Photoexcitable membranes" at the Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences.

The *Literature review* of the PhD thesis describes thoroughly the structure and properties of biological membranes, the structural diversity and phase behavior of membrane

lipids and lipid rafts, the role of polyunsaturated fatty acids and omega-3 fatty acids in the membrane phospholipids. Modern model membrane systems used to study and analyze biological membranes *in vitro* are described in detail, as well as the role and impact of oxidative stress, phospholipase enzyme systems, as well as some secretory isoforms of phospholipase A<sub>2</sub>, synthesized by different species, on morphology, the phase state and the lateral membrane organization.

In the section *Aim and tasks*, the set goal is clearly formulated: to study the influence of biologically active oxidized lipids on the membrane lateral organization and the activity of sPLA<sub>2</sub> in model systems with different degrees of unsaturation of fatty acids at the *sn-2* position, as well as the specific seven main tasks for its implementation. The materials used and modern methods for the experimental studies are described and reported clearly, accurately and in details.

Results and discussion obtained by the PhD student are presented on 54 pages. In three main sections, the results obtained in the study of 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 raft domains at physiological temperature, by DPH-TEMPO fluorescence spectroscopy. The obtained results show the effect of the degree of unsaturation of fatty acids in the molecule of glycerophospholipids POPC and PDPC on the formation and sizes of raft domains in two- and three-component POPC/Chol and POPC/SM/Chol mixtures;
- the degree of lipid order in the lipid bilayer by Laurdan fluorescence spectroscopy. The effect of the degree of fatty acid unsaturation in the glycerophospholipids POPC and PDPC on the degree of lipid ordering in model membranes was studied. The obtained results demonstrate the effect of oxidized lipids POVPC and PGPC on single-component POPC and PDPC vesicles and on cholesterol-containing PC vesicles. The change in the degree of ordering in the two-component SM/Chol vesicles (cell membrane raft model) upon increasing the temperature, as well as the effect of the oxidized lipids POVPC and PGPC (at concentrations of 10 and 30 mol %) on the phase state of the membranes, were investigated. In addition, three-component lipid mixtures POPC/SM/Chol and PDPC/SM/Chol were investigated in different ratios in the presence and absence of the oxidized lipids POVPC and PGPC, selecting lipid ratios between SM and Chol corresponding to their composition in cell rafts domains;

- the degree of order of the lipids in the lipid bilayer by Laurdan fluorescence spectroscopy, and the effect of the degree of unsaturation of the fatty acids in the molecule of the glycerophospholipids POPC and PDPC, as well as the presence of the oxidized lipids POVPC and PGPC, on the activity of sPLA<sub>2</sub>, was investigated. Additionally, the effect of cholesterol on sPLA<sub>2</sub> activity was analyzed depending on the degree of unsaturation of fatty acids in POPC and PDPC, as well as sPLA<sub>2</sub> activity in PC/SM/Chol mixtures in the absence and presence of the oxidized lipids POVPC and PGPC.

The conclusions from the highly informative experiments are formulated clearly and precisely in 9 main directions and three scientific contributions to the realization of the PhD work are also described. The results of the dissertation work were published in 3 scientific articles in the period 2020-2023 - in scientific journals with Q1, Q2 and Q3, with a total impact factor of 5.943, and the PhD student being the first author in all three publications. The results were reported at 8 national and international scientific forums (with 6 posters and two reports) in the period 2018-2022, with Vesela Yordanova being the first author in all participations, which is proof of her leading role in the implementation of the experiments in the PhD thesis.

The presented abstract of Vesela Yordanova fully corresponds to the content of the PhD thesis, providing excellent information about the experimental work performed, the results obtained and the conclusions and contributions made.

I have the following remarks and questions for the PhD student:

1. On page 17 of the PhD thesis it is written that "cholesterol is synthesized in the endoplasmic reticulum", but in fact the initial steps in its synthesis are in the cytosol and only the conversion of lanosterol to cholesterol is located in the endoplasmic reticulum.
2. In my opinion, it would be clearer to understand using not the mole ratios in the two- and three-component mixtures (e.g. not 100:50:50, 100:100:50 and 100:100:100 mol/mol), but directly ratios 1:0.5:0.5, 1:1:0.5 and 1:1:1 (as mentioned on p. 111).
3. Is the role and mechanism of action of the different isoforms of the cytosolic and secretory phospholipase A<sub>2</sub> in the apoptosis process known?
4. Multiple publications have recently reported overexpression of the gene encoding cytosolic phospholipase A<sub>2</sub> in patients with various cancers: breast cancer, hepatocellular carcinoma, cervical, prostate, and lung cancer. Is there a therapeutic approach to treat these cancers through PLA<sub>2</sub> inhibitors?

5. Do you foresee further studies of membrane phospholipids with different geometries to determine the effect of degree of fatty acid unsaturation on membrane lateral organization, morphology and phase state?

From the research work carried out, the PhD thesis presented, the author's abstract, scientific publications and participation in national and international conferences, it can be summarized that Vesela Yordanova is a young scientist who can plan and conduct in-depth scientific research, analyze and discuss the obtained results, as well as to compare them with published scientific articles. With the presented publications and participation in scientific forums, she meets the requirements of the Law on the Development of the Academic Staff in the Republic of Bulgaria, the Regulations for its Implementation and the Regulations for the Development of the Academic Staff of the Institute of Biophysics and Biomedical Engineering, Bulgarian Academy of Sciences.

**All this gives me reason to recommend to the respected members of the Scientific Jury to vote positively for awarding the educational and scientific PhD degree to the PhD student Vesela Vasileva Yordanova in field of higher education: 4. "Natural sciences, mathematics and informatics"; Professional field: 4.3. "Biological Sciences"; Scientific specialty "Biophysics".**

Sofia

**Author of opinion:**

06.09.2023

(Prof. Albena Jordanova, PhD)