OPINION

of a dissertation for obtaining the scientific and educational degree "Doctor"
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topic: MEMBRANE REORGANIZATION UNDER OXIDATIVE STRESS: EFFECT OF OXIDIZED LIPIDS
scientific consultant: Prof. Dr. Galya Staneva

In the work submitted for my opinion, the composition, structure and behavior of vesicles mimicking the membrane organization of native cell membranes with included "rafts" domains are considered. The gradual complication of lipid mixtures, by adding new components, methods of vesicle formation, the influence of temperature and the mechanisms of occurrence and influence of oxidative modification of polyunsaturated fatty acids caused by reactive oxygen species, as well as their influence on the enzymatic activity of phospholipase A_2 have been extensively studied and described. The topic is current and significant.

The study of the processes of lipid peroxidation leading to structural and functional changes of the lipids that make up the membrane is essential for the development of biological science. The newly formed structures play an important role in a number of pathological conditions, which makes the elucidation of the processes leading to their reorganization particularly relevant. The main methods used in the present study are: formation of multilamellar vesicles, fluorescence spectroscopy related to determining the degree of arrangement of lipids in the bilayer, calculation of membrane fluidity, as well as for determining the enzymatic activity of phospholipase A_2.

The dissertation is written in 151 standard pages. It includes introduction, literature review, aim and objectives, materials and methods, results and discussion, conclusion, contributions and references.

In the literature review, the biological membranes, their essence, the development of the models describing them, the components that make them up are examined in great detail, and the structure and function of each one of them is described, as well as the role it plays in the organization of the membrane. Lipid phases and domains, the origin of the phase distribution and the so-called "rafts" regions of the membranes are also described in detail. Oxidative stress inducing phopholipid oxidation and the effect it has on membrane organization are also examined. The general characteristics and mechanism of action of the enzyme phopholipase are described.

The aim of the present work is to investigate the influence of biologically active oxidized lipids on the membrane lateral organization and activity of sPLA $_2$ in model systems with different degrees of unsaturation of fatty acids at the sn-2 position.

To achieve this goal, seven tasks are clearly and precisely formulated. The methods used in the dissertation work are up-to-date and adequate to the set goals and objectives.

In the results and discussion chapter, the influence of oxidized lipids on the formation and dimensions of raft domains at physiological temperature is successively considered; their influence on the degree of ordering of lipids in the bilayer, as well as their influence on the enzymatic activity of phospholipase A_2.

The obtained results are presented in 15 figures. The studies described in the dissertation are multi-layered and diverse, and in the course of their development, the doctoral student has mastered a wide range of high-tech biophysical and biochemical methods.

The more important results obtained can be summarized as follows: Polyunsaturated fatty acids, in contrast to monounsaturated fatty acids, promote the formation of larger domains of the "rafts" type. Oxidized lipids alter the lateral organization of the membrane.

OxPCs change the fluidity of the membrane, and the effect depends both on the composition and the ratio of the components that make it up, as well as on the temperature.

$sPLA_2$ has higher activity in single component monounsaturated membranes.
POVPC inhibits enzyme activity, the effect of PGPC depends on the lipid mixing and hydration protocol.
$sPLA_2$ activity is suppressed upon addition of cholesterol to monocomponent membranes. In vesicles representing a cell membrane model (coexistence of Lo/Ld phases),
$sPLA_2$ was most active in the mixtures with the lowest cholesterol and shingomyelin content. POVPC inhibited $sPLA_2$ activity in all models, whereas the effect of PGPC was dependent on cholesterol and sphingomyelin content in the models.
Contributions are clearly and precisely formulated and represent new data for science.
In conclusion: Vesela Vasileva Yordanova's dissertation is written in correct and clean Bulgarian language, it is balanced and very well designed. The dissertation student has completed a large volume of work.
The content of the abstract and the dissertation correspond.
Three scientific publications are attached to the dissertation work, in all of which the doctoral student is the first author. Two of the articles are in journals with an impact factor, and one of the journals has an impact factor of 5.6. A list of participations in scientific conferences is also attached. From the attached documents, it is clear that the doctoral student has fulfilled all the requirements of the law on the development of the academic staff of the Republic of Bulgaria (successfully passed exams, preliminary examination of the dissertation work at an advanced scientific seminar, publications with an impact factor in connection with the dissertation work). Everything that has been said so far, as well as my personal impressions of the doctoral student, gives me reason to confidently recommend to the scientific jury to propose to the Scientific Council of IBFBMI-BAN to award Vesela Vasileva Yordanova the educational and scientific degree "Doctor".

Sofia
04.09.2023 (prof. Biliana Nikolova-Lefterova, PhD)