REVIEW

by an official opponent of the dissertation by Mai Lisha «Molecular Modeling and Structural Insights into KCNQ1 Channel Regulation by Bioactive Compounds and Mutations» submitted for the degree of Doctor of Philosophy in Biology in specialty 1.5.2. Biophysics

Relevance

The functioning of ion channels has been known since the mid-20th century from the experiments of Alan Lloyd Hodgkin and Andrew Huxley. For their model describing the electrical mechanisms of nerve signal generation and transmission involving ion channels, the authors were awarded the Nobel Prize. Since then, the Nobel Prize has been awarded twice more for research on ion channels. The value of scientific knowledge about ion channels is high because the operation of ion channels sustains life (ensuring irritability, metabolism, homeostasis, and regulating cellular function). Without exaggeration, research on ion channels is relevant and critically important for medicine, yet there is currently no "universal drug" based on the action of active compounds on ion channels. Humanity continues to suffer from cardiovascular and other diseases associated with ion channel dysfunction. Thus, the subject of Mai Lisha's dissertation, "Molecular Modeling of Structural Rearrangements of the KCNQ1 Channel Induced by Bioactive Compounds and Mutations," is relevant and promising.

The main results

The value of Mai Lishi's scientific work lies in the combination of experimental work with molecular modeling methods. Mai Lishi's experimental work with sources of biologically active compounds – medicinal plants (*Dendrobium officinale, Ganoderma lucidum, Gastrodia elata, Cornus officinalis, Eucommia ulmoides, Cistanche deserticola, Astragalus membranaceus, Panax quinquefolius, Codonopsis pilosula*) allowed us to identify the most significant active substances from the extract of *Eucommia ulmoides*: rutin and quercetin (related plant bioflavonoids having powerful antioxidant and cardioprotective

effect). A possible pocket of binding of these plant bioflavonoids near the redox-sensitive residue of cysteine-214 of the KCNQ1 channel was characterized by the method of molecular modeling (docking). Cysteine-214 is located in a loop between S3 and S4 helices of the potential-sensitive domain of the channel (localization from the outside of the cell). Mai Lishi's work presents the hypothesis that rutin and quercetin prevent the oxidation of cysteine-214 by reactive oxygen species and contribute to the normal operation of the potassium channel under conditions of oxygen stress. Normally, a change in the potential of a cardiomyocyte leads to a shift in the S3 and S4 helices - the potassium channel opens the flow of potassium from the cell. Thus, at the molecular and cellular levels, the function is preserved: the completion of myocardial contraction and preparation for the next heartbeat. According to the results of Mai Lishi's work, the activation of the KCNQ1 channel by other natural compounds (tanshinone IIA from *Salvia miltiorrhiza,* resveratrol from grapes, blueberries and other plants, mallotoxin from *Mallotus oppositifolius*) occurs due to their action on the loops between S3 and S4 spirals of the potential-sensitive domain. The blocking effect of mallotoxin on the KCNQ1/KCNE3 complex is explained by binding in the pore region of the channel. Mai Lisha's work shows the effect of pathological substitutions in the region of the potential-sensitive domain of the KCNQ1 channel on global conformational changes in the functional complex of the potassium channel, consisting of the KCNQ1 subunit, the regulatory KCNE3 subunit, and the phospholipid PIP2 by molecular dynamics.

Scientific novelty, theoretical and practical value

In addition to new information about the functional complexes of the KCNQ1 channel with natural activators, blockers, and in the presence of pathological substitutions, the novelty of Mai Lisha's dissertation work consists in presenting a molecular model of the KCNQ1 channel with the missing intramembrane part of the channel (residues 535-625), constructed in accordance with cryo-electron microscopy data. The model opens up the possibility for studying the KCNQ1

channel in combination with intracellular regulatory proteins, and has prospects for obtaining practically significant results.

The structure of the dissertation work

Mai Lisha's dissertation work follows a standard plan and consists of an Introduction, a review of the literature, Materials and Research Methods, Results and their discussion, Conclusions, Conclusions and a List of references. The work contains 37 figures, 3 tables, the list of references includes 220 sources.

In the Introduction, the main goals and objectives of the research are formulated, and its relevance and practical significance are substantiated. The literature review, which makes up a third of the dissertation text, is devoted to the analysis and detailed discussion of the latest data on the structure and function of KCNQ1 channels. The review allows us to understand the current state of the issue and the adequacy of the experiments planned by the author. The advantages of the review include the author's good orientation in publications of the most recent years.

The following can be noted as comments:

1. The introduction lacks a clear visual definition of the object of study, the KCNQ1 channel: speaking of structural details, the author does not provide a link to the image. The only reference in the text to the drawing in the introduction does not correspond sufficiently to the content of the text of the drawing.

2. In the Introduction, when discussing the methods that were used in the performance of the work, computational methods of molecular docking and molecular dynamics (Section 2.4), as well as experimental methods related to the determination of antioxidant activity (Section 2.5) are considered. However, in the work itself (Chapter Methods), an important place is occupied by methods for modeling the structure of proteins, including using low-resolution electron density map data.

3. The information in the review is extremely concise, and insufficiently structured, lacking logical coherence of presentation.

4. Much attention is paid to the binding of various low molecular weight ligands to the KCNQ1 channel and its complexes with additional subunits. This raises two questions. 1) Has an independent computational assessment of the activity of the compounds in question been performed with respect to this channel? 2) If the mechanism of selective action of mallotoxin is so well explained by its electric charge, how universal is the proposed mechanism for other substances interacting with KCNQ1?

5. The chapter "Structural Architecture of the KCNQ1 C-terminal Domain" is shown without a picture, this is an omission.

6. Has the calculation of molecular dynamics been carried out for the obtained HC-HD intracellular domain model? How stable is this model?

7. How reliable are the results of the analysis of the main components obtained along a single trajectory for each complex (WT, D242N, R243H)? Is it possible to give some biophysical meaning to the components PC1 and PC2?

8. The designations of the residues in Fig. 25 are very small, which makes it very difficult to perceive information.

9. On page 16 there is no transcription of "IQ motifs", later PAG motif – there is little information about conservative motives and their role.

10. The description of the methods lacks information about the length of the calculated trajectories and the convergence of calculations.

11. Frequent mention of demonstrative pronouns and many complex sentences make it difficult to understand, an example of a complex phrase:

"This selective effect strongly reveals that AC9 is the dominant cAMP producer within this compartmentalized signaling environment, which is essential for localizing PKA activation and secondary KCNQ1 phosphorylation, and for effectively combining these modifications to upstream receptor pathways"

12. Latin names: *ex vivo, in vivo, in vitro* – often designed without italics

13. The text contains information repetitions in parts 1.1 and 1.2.

14. There are few symbols in the drawings (for example, Figures 19, 20), and there is no decoding of the semantic meaning of color. Structural elements, spirals, which are discussed in the text, are often not marked in the models of structures. The location of the HC and HD domains is not clear from Figure 19.

However, these remarks do not detract from the significance of the dissertation research. The dissertation meets the requirements established by the Lomonosov Moscow State University for works of this kind. The content of the dissertation corresponds to the specialty 1.5.2. Biophysics, as well as the criteria defined in clauses 2.1-2.5 of the Regulations on Awarding Academic Degrees at Lomonosov Moscow State University. The dissertation research has been prepared in accordance with the requirements of the Regulation on the Council for the Defense of Dissertations for the Academic Degree of Doctor of Philosophy, for the Academic degree of Doctor Habilitatus at Lomonosov Moscow State University.

Thus, the applicant Mai Lisha deserves to be awarded the degree of Doctor of Philosophy in Biology in the specialty 1.5.2. Biophysics

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