REVIEW

by an offical 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 speciality 1.5.2. Biophysics

Relevance

Potassium channels are present in almost all cells of living organisms. Voltage-gated potassium channels, which include KCNQ1, are sensitive to changes in the potential difference across the cell membrane and actively participate in the passage of the action potential, playing a key role in returning a depolarized cell to a resting state. KCNQ1, also known as Kv7.1, regulates gastric acid secretion, salt and glucose homeostasis, and cardiac rhythm. Its functionality is ensured by co-assembly with the beta subunits KCNE1-5. In non-excitable cells, KCNQ1 forms a complex with KCNE3, and in the heart, the KCNQ1/KCNE1 complex is involved in the regulation of cardiomyocyte excitability. Opening of the pore is regulated by the signaling lipid PIP_2. Channel dysfunction caused by mutations in the kcnq1 gene causes such human diseases as long QT syndrome, short QT syndrome, atrial fibrillation, diabetes, and deafness. Despite numerous outstanding modern studies, the details of the channel's structural organization, the molecular mechanisms of its functioning, and its interaction with biologically active compounds of pharmacological and natural origin remain poorly understood. The presented thesis is devoted to elucidating the structural features and rearrangements that occur during channel function in norm and pathology. Natural biologically active compounds are widely used in the traditional Chinese medicine. The current research also investigates their potential bindins sites and protective functions.

Main results

The dissertation yielded several significant results:

1. Differences in the regulatory effects of mallotoxin on KCNQ1 complexes with KCNE3 and KCNE1 were identified. It was shown that mallotoxin's inhibitory effect on the KCNQ1/KCNE3 complex is due to its binding at high positive electrostatic potentials within the channel pore, while its stabilizing effect on KCNQ1/KCNE1 is due to its binding at the periphery of the complex's transmembrane domain.

2. The atomic model of the structure of the flexible linker of the coiled-coil domains HC and HD of the KCNQ1 channel was obtained using molecular modeling methods combined with electron density mapping analysis.

3. Using molecular dynamics methods, the effects of pathogenic mutations D242N and R243H in the KCNQ1 subunit were studied. It was shown that, through different molecular mechanisms, they destabilize the KCNQ1/KCNE3 complex by increasing the conformational flexibility of the potential-sensing domain, with the R243H mutation causing a more pronounced structural perturbation.

4. The biological activity of several plant extracts and their mechanisms of influence on KCNQ1 channel regulation were studied using a combination of experimental and molecular modeling methods.

The reliability of the obtained results is confirmed by the use of a wide range of modern scientific approaches and methods. The results of the research were published in peer-reviewed scientific journals and were presented at several conferences in Russia and China.

Scientific novelty, theoretical and practical value

All results obtained in this dissertation are new. The proposed HC-HD linker model opens up new possibilities for studying the interaction of the KCNQ1 channel with regulatory proteins and the phosphorylation process. Studying the

D242N and R243H mutations elucidates the mechanisms of channel function and provides a basis for therapeutic strategies for the treatment of long QT syndrome (LQT1). The developed approaches to studying natural activators provide a basis for the development of selective channel modulators for the treatment of LQT1 and atrial fibrillation.

Structure of the dissertation

The dissertation follows a traditional outline. The "Introduction" chapter outlines the relevance of the work, the current state of the research topic, and the objectives of the paper, its scientific novelty, and its theoretical and practical value. The "Literature Review" introduces the current state of scientific research into the structure, regulatory function, and pathologies of the KCNQ1 potassium channel. This chapter also provides an overview of modern methods used in this field of science, such as molecular dynamics, molecular docking, and principles for assessing the antioxidant activity of natural compounds. The "Methods" chapter describes the specific methodological approaches used by the candidate. The "Results and Discussion" chapter is followed by "Conclusions."

The manuscript is 129 pages long, containing 37 figures, 3 tables, and a bibliography of 220 items.

Comments and questions

The dissertation is written in modern scientific language. The results are well-founded. Comments on the work are primarily due to the excessive brevity of some sections of the work.

1. In Section 2.4.2, the variables included in equations (1) – (3) are not explained.

2. In Section 3.2.2, the description of the molecular dynamics method lacks a detailed description of the atomic models themselves — separately for the subsequent Sections 4.3 and 4.4. The number of atoms contained in each model

component, the total number of atoms in the model, and the cell size are not included. The water model used in the calculations is also not specified.

3. In Chapter 4.1, the applicant models the transmembrane portion of the KCNE1 subunit based on the known atomic structure of 6V01, the KCNQ1/KCNE3 complex, replacing residues 53–92 of the known structure with residues 39–78 of the target structure. However, no quantitative justification is provided for choosing this particular sequence comparison.

4. How exactly were the electrostatic potential (EP) maps calculated?

5. How high are the "high values" of the ESP? It would be helpful to indicate the maximum values and the level of the equipotential surface shown in Figure 17.

6. The mallotoxin docking process, including the starting positions and the selection of the most probable configurations, is not described in sufficient detail.

7. In the Discussion, it would be appropriate to provide a more detailed comparison (3D geometry of the transmembrane portion of the beta subunit; position of PIP2) of the proposed model with recent publications:

Cui et al. Mechanisms of KCNQ1 gating modulation by KCNE1/3 for cell-specific function. (2025) *Cell Res.* 35, 876–886.

Zhong et al. Secondary structure transitions and dual PIP2 binding define cardiac KCNQ1-KCNE1 channel gating. (2025) *Cell Res.* 35, 887–899.

8. In Section 4.2, it is not entirely clear how the two turns of the alpha helix of the HC-HD linker were completed.

9. Were these two turns stable or unwound during the 100 ns calculations described in the next section?

10. How exactly was the electron density of the HC-HD linker's atomic model quantitatively compared with available experimental electron density maps and how was the best variant selected?

11. If the HC-HD linker is dynamically mobile, then it would seem that the electron density map should correspond not to a single specific structure, but to the result of averaging over a sufficiently long MD trajectory?

12. When specifying the pathogenicity of the D242N and R243H mutations selected for study, it would be appropriate to reference their classification and current status in the ClinVar database.

13. With both amino acid substitutions considered in the study, the solvent-accessible surface area (SASA) increased compared to the wild-type (Figure 22C). In the discussion, the increase in this value with the R243H substitution, which replaces a positive charge with a neutral one, is explained by the applicant as a weakening of the electrostatic field. How can the increase in SASA with the D242N substitution be explained?

14. Was the calculation time of 100 ns sufficient? In Figure 22A, the RMSD does not reach a plateau.

15. To what extent do the activity estimates of natural compounds obtained in this study coincide with their activity estimates in traditional Chinese medicine?

16. In the caption to Figure 6, the six-pointed star is called a five-pointed star.

All comments made are for clarification purposes only and do not detract from the scientific significance of the dissertation research.

Conclusion

The dissertation meets the requirements established by Lomonosov Moscow State University for the works of this kind. The content of the dissertation corresponds to the speciality 1.5.2. Biophysics (biological sciences), as well as the criteria defined in paragraphs 2.1-2.5 Regulations on the awarding of academic degrees at the 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 academic degree of Doctor of Philosophy in Biology in the specialty 1.5.2. Biophysics.

Official opponent:
Leading Researcher
Institute of Mechanics,
Lomonosov Moscow State University
Doctor of Sciences in Physics and Mathematics

Koubassova Natalia Alekseevna

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Contact information:
Tel.: 7 (495) 939-12-52; e-mail: natalia@imec.msu.ru
Specialty in which the official opponent defended the dissertation:
03.01.02. Biophysics (physical and mathematical sciences)

Work address:
119192, Moscow, Michurinsky Prospekt, Bldg. 1,
Institute of Mechanics, Lomonosov Moscow State University
Tel.: 7 (495) 939-12-52; E-mail: natalia@imec.msu.ru