REVIEW

by the official opponent of the dissertation work of 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 KCNQ1 (Kv7.1) channel plays a critical role in the regulation of heart rhythm (formation of the slow component of the delayed rectified potassium current IKs) and in a few epithelial processes. Mutations in the KCNQ1 gene cause severe hereditary diseases: long QT syndrome (LQT1), short QT syndrome, familial atrial fibrillation, and deafness (Jervell-Lange-Nielsen syndrome). However, the functional effect of the mutation is determined not only by the KCNQ1 channel itself, but also by its mandatory interaction with accessory KCNE subunits (primarily KCNE1). The KCNE1 subunit dramatically alters the kinetics, conductance, and pharmacological sensitivity of the KCNQ1 channel, transforming it from a rapidly activated channel to one with extremely slow activation and no inactivation—this is what forms the physiological IKS (Slow Delayed Rectifier Potassium Current). However, the interaction of small-molecular-weight ligands with KCNQ1-KCNE complexes is currently poorly understood, making the model of selective mallotoxin (MTX) binding proposed in this study of particular interest.

Equally important is the study of the structure of the intracellular domain of the KCNQ1 channel, as it controls channel assembly and trafficking, perceives signals from β-adrenergic receptors, and mutations in this domain cause LQT1 (Long QT Syndrome Type 1). The structure of the well-ordered regions of the intracellular domain is considered well-known, but the proposed model of a linker between the HC and HD coiled-coils opens the way to studying the poorly ordered regions involved in interactions with partner proteins.

Of note is the study's examination of the redox sensitivity of the KCNQ1 channel and the possibility of modulating this sensitivity with antioxidants, which may be important in the study of ischemia, reperfusion, and aging.

Thus, the relevance of this work is undeniable: it is at the forefront of the structural biophysics of potassium channels and has direct implications for personalized cardiology and rational drug design.

Main results obtained in the study

1. **Mallotoxin Effects:** using molecular modeling methods, we simulated the structure of the KCNQ1/KCNE1 complex and examined the molecular basis for the multidirectional effects of the low-molecular-weight compound MTX on the KCNQ1/KCNE1 and KCNQ1/KCNE3 complexes. MTX activates KCNQ1-KCNE1 but inhibits KCNQ1-KCNE3 due to differences in electrostatic potential in the channel's inner cavity.

2. **HC-HD Linker Model:** by using modern molecular modeling methods to examine previously published cryo-EM electron density maps, a new "domain-swapped" structural model was proposed for the HC-HD linker of the C-terminal domain of the KCNQ1 channel, which is critical for channel regulation.

3. **Mutations:** by using molecular dynamics modeling and principal component analysis of the KCNQ1/KCNE3 complex, we examined the effect of two KCNQ1 channel mutations (D242N and R243H) associated with LQT1 syndrome on the structural stability and dynamics of this complex. D242N causes electrostatic locking, while R243H disrupts structural coupling, both leading to long QT syndrome (LQT1).

4. **Natural Activators:** based on the results of molecular docking, potential binding sites for natural activators of the KCNQ1 channel, including tanshinone IIA and resveratrol, were hypothesized. Tanshinone IIA, quercetin, and resveratrol can bind to the ML277 activator pocket, with tanshinone IIA showing the most stable binding.

5. **Antioxidant Screening:** experimental methods were used to quantitatively evaluate the antioxidant capacity of nine plant extracts, and possible binding modes of the bioactive compounds they contain (in particular, rutin and quercetin) to the KCNQ1 channel were modeled to elucidate their potential mechanism of protection against oxidative stress. E. ulmoides extract showed the highest antioxidant activity among nine tested TCMs. Its key components, rutin and quercetin, may protect the redox-sensitive Cys214 residue in KCNQ1 from oxidative stress.

The reliability of the results obtained is confirmed using modern scientific approaches and methods. The results of the study were presented by the candidate at conferences in Russia and China.

Scientific Novelty, Theoretical and Practical Significance of the Work.

All results presented in this dissertation are novel. The study provides insights into KCNQ1 channel regulation, dysfunction, and therapeutic targeting.

It highlights the potential of natural compounds for treating channelopathies like LQT1 by combining channel activation and antioxidant protection.

Molecular modeling methods were used to obtain data on the structure of the KCNQ1 channel and its complexes with KCNE, as well as on structural rearrangements induced by biologically active compounds and mutations. The molecular basis for the multidirectional effects of mallotoxin on KCNQ1 complexes with KCNE1 and KCNE3 are elucidated. A model of the flexible HC-HD linker of the C-terminal domain of KCNQ1, constructed based on low-resolution data, is proposed. This linker model provides the basis for studying the structure of the KCNQ1/Yotiao/PKA complex and the phosphorylation of residues in the N-terminal domain of the KCNQ1 channel. Pathogenic mutations D242N and R243H are shown to affect channel compactness, the free energy surface area, and PIP_2 binding. Understanding the conformational and dynamic changes caused by these mutations

deepens our understanding of the mechanisms of KCNQ1 channel function and allows us to identify new approaches to treating LQT1 syndrome. Among the nine plant extracts the extract from E. ulmoides exhibited the highest antioxidant activity. Using natural biologically active substances as an example, it was shown that their binding sites can be located both on the extracellular surface of the KCNQ1 channel molecule and on the membrane-facing surface. The diversity of binding sites helps explain both the activating and antioxidant effects of quercetin on the KCNQ1 channel. An experimental study of plant extracts demonstrates their high potential as a source of natural compounds active against the KCNQ1 channel. The approaches proposed in this study for identifying the binding sites of small-molecular compounds to the KCNQ1 channel facilitate the development of new selective modulators of this channel for the treatment of long QT syndrome type 1 (LQT1) and atrial fibrillation.

Dissertation Structure

Mai Lisha's dissertation is 130 typewritten pages long and consists of an Introduction, Literature Review, Materials and Methods, Results and Discussion, Conclusions, and a Bibliography (220 references). The work contains 37 figures and 3 tables. The abstract and published articles adequately reflect the content of the dissertation.

The Introduction formulates the main goals and objectives of the study and justifies its relevance and practical significance. The Literature Review discusses the structure and function of KCNQ1 channels, some computational methods, and methods for assessing antioxidant activity. The strengths of the work include citations of recent publications

Main comments and questions.

Several criticisms of the paper, both in terms of presentation and scientific issues, can be raised. Here are some of them:

1. The text repeatedly mentions normal and prolonged QT intervals but does not report the duration of these intervals (including Figure 4 on page 17).

2. The paper is poorly illustrated. Section 1.1 lacks a figure showing the overall structure of the KCNQ1 channel and the location of the elements discussed. (Such a figure (Fig. 7) is provided much later, but Section 1.1 does not reference it.) Furthermore, figures would be useful in the sections "Structural Architecture of the KCNQ1 C-terminal Domain" and "Multifunctional Regulatory Platform", "KCNQ1-KCNE3 complex" (pp. 18-20), "2.2.3 Calmodulin-Mediated Regulation of Channel Activation" (p. 21), "2.2.5 Intrinsic Activator: ML277" (p. 25), and in many of the following sections. Furthermore, Figure 25 has very small text and is difficult to read.

3. The literature review presents molecular docking and molecular dynamics methods (Section 2.4), but says nothing about principal component analysis, potential energy surface calculations, solvent accessible surface area (SASA) calculations, or many other methods used in the work.

4. Quite a few ligands for the KCNQ1 channel are known. Why did you decide to study the mechanism of selective action of mallotoxin? Is the approach used applicable to other potassium channel ligands?

5. In the section "System Construction for MD Simulations," the composition of the systems studied should be specified, namely, the amount of each type of lipid per bilayer, the number of added ions and water molecules. In addition, the text (p. 55) indicates the membrane composition as "a lipid bilayer composed of POPC: POPG: Cholesterol (90:5:5) to reproduce the cardiac membrane environment" (POPC:POPG:cholesterol), and the caption under the figure illustrating this system (see Fig. 16) indicates the membrane composition as "a membrane bilayer composed of POPC/POPG" (only POPC:POPG, without cholesterol). What was the actual composition of the membranes in the studies? In Fig. 16, it would be desirable to highlight PIP_2 in a separate color for clarity.

6. Section "4.1.1 Structural Insights and Electrostatic Characteristics" (p. 60) states: "ESP maps computed using the same visualization scale and solver settings" – but what were these parameters? The "Methods" section does not contain this information. Nor does it provide any information on how the molecular systems were prepared for electrostatic potential calculations.

7. As the author rightly points out: "full channel gating transitions occur on microsecond to millisecond timescales," the chosen time of 100 ns for studying dynamics seems somewhat short and may be insufficient for studying conformational changes in the protein that arise from the rearrangement of ligand-binding pockets (pp. 56-57). Furthermore, only a portion of the complex is embedded in the membrane in this study. To what extent can its behavior without the surrounding parts of the system be applicable to describing the characteristics of the entire system?

8. The work indicates (pp. 23-25, section "2.2.4 Yotiao Scaffolding and Phosphorylation in the IKs Complex") that the Yotiao protein is important for channel function and likely binds specifically to the HC-HD linker. However, this binding is not considered in the work from a molecular perspective. Why?

9. Why were the integral parameters Rg and SASA chosen to analyze the influence of local amino acid mutations? Perhaps it would be more productive to focus on the local environment of residues 242 and 243?

10. In Section 4.2.1, the author puts forward an interesting idea of domain swapping to resolve discrepancies between the molecular structure of the binding site and the existing electron density map obtained by cryo-electron microscopy (EMD-20966, for a closed pore without PIP2). However, the text does not explain how well the developed model corresponds to the possibility of channel opening.

11. Do components PC1 and PC2 have a physical meaning? Can we be sure that these components have the same meaning for the three systems (WT, D242N, and R243H)? If not, how could we compare the properties of these components?

12. As shown in Figure 29A (p. 85), tanshinone IIA, resveratrol, and quercetin can share the same binding site as ML277. Is there any experimental evidence that these ligands bind at the same site as ML277? Because any docking algorithm can place any ligand at any site determined by the algorithm's settings.

13. From Fig. 23A (see text on p. 71: "both D242N and R243H mutations exhibit increased hydrogen bond fluctuations and variability"), no noticeable fluctuations or inconsistency of hydrogen bonds are visible. It would be desirable to further illustrate this point using a probability plot (violin diagram, etc.).

14. How can the influence of the Cys214 residue on channel tetramerization be explained if, according to Fig. 37, it is in the voltage-sensing domain at the periphery of the channel, far from the interaction interfaces of the channel subunits?

15. In Figure 26, the two-dimensional maps of the first principal components are U-shaped, which may indicate an insufficient data set on the dynamics of the corresponding systems. It seems that the author is bold in interpreting diagrams of this type in terms of the influence of introduced mutations, rather than a simple insufficiency of the data set. It would be advisable to study each system in several replicates and compare the resulting two-dimensional maps PC1-PC2 for a range of molecular dynamics trajectories.

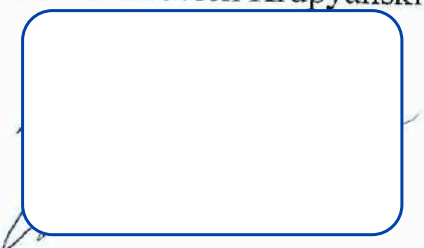
Conclusion

These comments do not detract from the significance of this dissertation. The research advances understanding of KCNQ1 structure and function, offering a foundation for developing targeted treatments for cardiac arrhythmias. This dissertation meets the requirements established by Lomonosov Moscow State University for works of this kind. The dissertation's content complies with specialty 1.5.2. Biophysics, as well as the criteria defined in paragraphs 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. Therefore, applicant Mai Lisha deserves to be awarded the degree of Doctor of Philosophy in Biology in specialty 1.5.2. Biophysics.

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