In silico study of the impact of the PRKAG2-H401Q mutation on AMPK affinity for AMP and ATP

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Abstract. *Mutations in the PRKAG2 gene, which encodes the γ2 subunit of AMP-activated protein kinase (AMPK), are linked to a rare cardiomyopathy involving glycogen accumulation, left ventricular hypertrophy, and sudden death. This study investigates a novel His401Gln missense mutation in the PRKAG2 gene and its effects on AMPK γ2 subunit dynamics. Through molecular simulations and free energy analyses, we compared AMP and ATP binding affinities between the wild-type and mutant γ2 subunits. Structural modeling and simulations revealed a significant change in ATP binding at site 3 in the mutant AMPK, suggesting that the His401Gln mutation impacts protein binding behavior. This alteration may contribute to the pathological mechanisms of PRKAG2 cardiomyopathy.*

Introduction

Mutations in the PRKAG2 gene are known to cause a rare syndrome that manifests as cardiomyopathy, characterized by glycogen accumulation in cardiomyocytes, left ventricular hypertrophy, ventricular pre-excitation, arrhythmias, and sudden death [AHMAD et al. 2005; GOLLOB et al. 2001]. PRKAG2 cardiomyopathy results from mutations in the gene encoding the γ2 regulatory subunit of AMP-activated protein kinase [ARAD et al. 2002]. Although the prevalence of this disease is unknown, the number of cases has increased due to greater access to molecular diagnosis [MAGALHÃES et al. 2022].

The AMP-activated protein kinase (AMPK) is a heterotrimeric protein composed of a catalytic alpha subunit, with α1 and α2 isoforms; a regulatory beta subunit, with β1 and β2 isoforms; and a regulatory gamma subunit, with γ1, γ2, and γ3 isoforms [KOMURCU-BAYRAK et al. 2022]. AMPK monitors cellular energy status, activated in response to an increase in cellular AMP and ADP concentrations, along with a decrease in ATP [STEINBERG; HARDIE 2023]. The α subunit contains an N-terminal kinase domain, including a critical activation site (Thr172) responsible for AMPK activity and an auto-inhibitory domain. The β subunit plays a significant role in the structural organization and communication between the subunits and in regulating activity through glycogen binding. The γ subunit contains four CBS (cystathionine-β-synthase) repeats that create four potential binding sites for AMP, ADP, or ATP, responsible for the allosteric activation of AMPK, named sites 1, 2, 3, and 4. Only site 2 remains unoccupied due to the substitution of a conserved aspartic acid residue by an arginine in the α-helix [AFINANISA; CHO; SEONG 2021; BAIRWA; PARAJULI; DYCK 2016; DAY et al. 2007; XIAO et al. 2007]. The γ isoforms of AMPK are highly conserved concerning the CBS repeats; however, the AMPK γ2

subunit has a large N-terminal extension of unknown structure [ALBERNAZ SIQUEIRA et al. 2020], which is not available in the Protein Data Bank (PDB).

In a familial case study conducted by SIQUEIRA et al. in 2020, a novel missense mutation, His401Gln, in the PRKAG2 gene was reported in a family where two young adult sisters experienced sudden cardiac death. In this article, the potential effect of the mutation on the protein was illustrated in PyMOL (Schrödinger, Inc.) using a three-dimensional model of the AMPK α2β1γ1 complex (PDB 4CFE) by arbitrarily selecting glutamine rotamers. However, molecular dynamics simulations and free energy analyses should be performed to confirm this hypothesis. To investigate the effect of the PRKAG2-His401Gln variant on the structural dynamics of the protein and consequently on the disease's pathophysiology, we performed molecular dynamics simulations to identify the affinities of the wild-type AMPK γ2 subunit and the His401Gln missense mutation for the AMP and ATP ligands, using a structure of the human γ2 isoform generated by homology modeling.

Materials and Methods

The γ2 structure used was generated through homology modeling in AutoModel [DE A. FILHO; DEL REAL TAMARIZ; FERNANDEZ 2018] by aligning a murine γ1 subunit structure (PDB 2V8Q) with the human γ2 subunit amino acid sequence (NCBI NP_057287.2), showing 77% identity. According to the new numbering, the His401Gln mutation was introduced into the structure at histidine residue 146. Both ligands were numbered 303, 304, and 305, corresponding to sites 3, 1, and 4, respectively. Molecular simulations were conducted in Desmond-GPU, the molecular dynamics algorithm used by the Maestro environment (version 2022-4). The wild-type and mutant systems were solvated and equilibrated by molecular dynamics for five ns in an octahedral box with water, represented by the standard TIP3P model. The size of the solvated systems was, on average, 34,442 atoms.

To analyze the binding affinity at the allosteric site of the γ2 subunit, we used an adaptive method that forces the system out of local minima, metadynamics. We performed five repetitions for each potential AMP and ATP binding site in the wild-type and His401Gln mutant structures. The simulations were conducted at a constant temperature of 310 K and a pressure of 1 atm, with the AMP (PubChem CID6083) and ATP (PubChem CID5957) ligands, using the OPLS 2005 force field, with a simulation time of 60 ns. We collected and analyzed two variables in the hAMPK-AMP and hAMPK-ATP complexes for the wild-type and His401Gln mutant structures. The first variable collected (CV1) consists of the distance between the centers of mass of the ligand at the binding site and the outside residue, and the second variable (CV2) consists of monitoring the C-N-C-O dihedral located between the adenine and the ribose of the AMP and ATP ligands. The residues selected for the distance variable are Arg148 for the experiment with ligands at site 3 and Lys124 for the experiments with ligands at sites 1 and 4. We compared the free energy values (ΔG) obtained for the ligand-protein complexes in the initial contact zone with the protein (**CZ**) and inside the binding site (**BS**), and the simulation trajectories and metadynamics analisis were visualized in Desmond-Maestro 22-4. Structural representations and visual analysis were performed in PyMOL (https://pymol.org/2/).

Results and Discussion

The rationale for the experiment is based on the typical interaction between the AMP and ATP ligands with the AMPK regulatory subunit. Initially, both ligands interact electrostatically through their phosphate groups (Figure 1:A) before settling into their binding sites (Figure 1:B). This structural transition is accompanied by changes in the binding energy of the complex, reflecting variations in affinity during the ligand's progression from the contact zone (**CZ**, Figure 2:B) to the final binding site (**BS**, Figure 2:B).

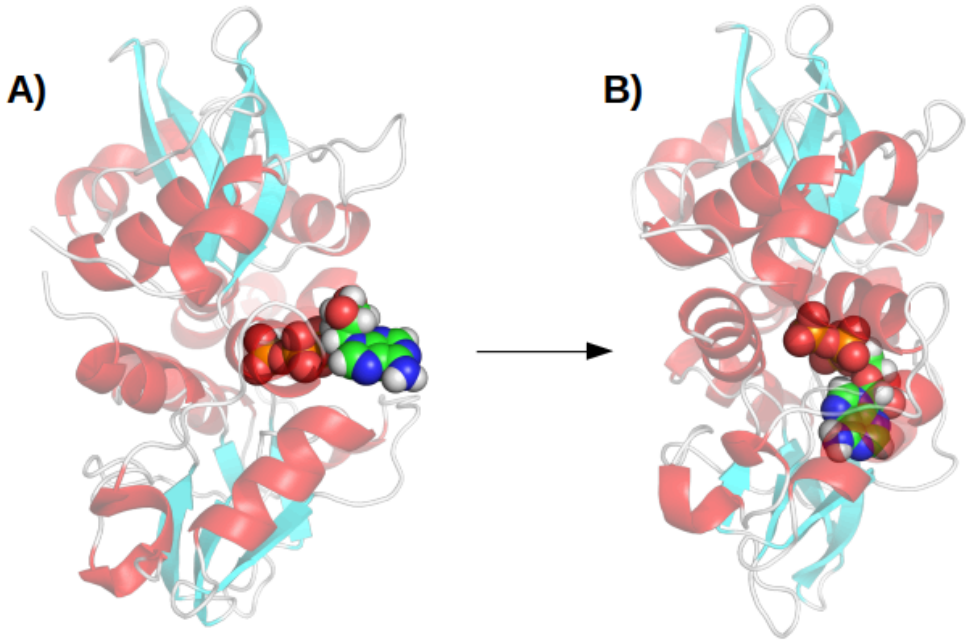

Figure 1. ATP at site 3 in A) Contact zone (CZ); and B) Binding site (BS).

The free energy values (ΔG) at the initial contact zone with the protein (**CZ**) and inside the binding site (**BS**) obtained in metadynamics experiments are presented in Table I, along with the respective standard error (SEM). For specific complexes, evaluating the free energy in the contact zone with the protein was more challenging because the ligand tends to remain inside the binding site, difficulting the capture of a **CZ** and **BS** ligand positions for all repetitions. Therefore, for these complexes, the calculation was performed with three or four repetitions, and "mut-ATP 304" showed a **CZ** position in only one of the repetitions and the others only in two of the five repetitions. Specifically, at site 3, the analysis of variance (ANOVA; $P \leq 0.001$) of the ATP binding ΔG obtained for the wild-type and mutant complexes was statistically different. The negative ΔΔG between initial **CZ** and “final” **BS** 3 of ATP in the wild-type complex suggests favorable ligand accommodation. In contrast, this value is positive in the mutant complex (Table I), indicating that ligand accommodation is energetically unfavorable.

Table I. Average free energy values (ΔG) obtained for each complex, in kcal/mol.

	Wild-type			*Mutant*		
	Site 1	Site 3	Site 4	Site 1	Site 3	Site 4
CZ-AMP	-10.0±2.0	-8.2±0.4	-7.7±0.9	-8.0±0.6	-12.0±0.8	-9.5±2.5
BS-AMP	-14.4±1.3	-12.3±0.5	-11.8±1.4	-12.2±0.9	-14.0±1.3	-11.4±1.4
CZ-ATP	-8.5±1.5	-8.7±0.8	-7.0±0.8	-10.0	-10.9±1.9	-7.4±0.6
BS-ATP	-14.4±1.0	-16.2±0.6*	-10.3±1.0	-18.4±0.5	-8.6±0.9*	-9.7±1.6

*P ≤ 0.001

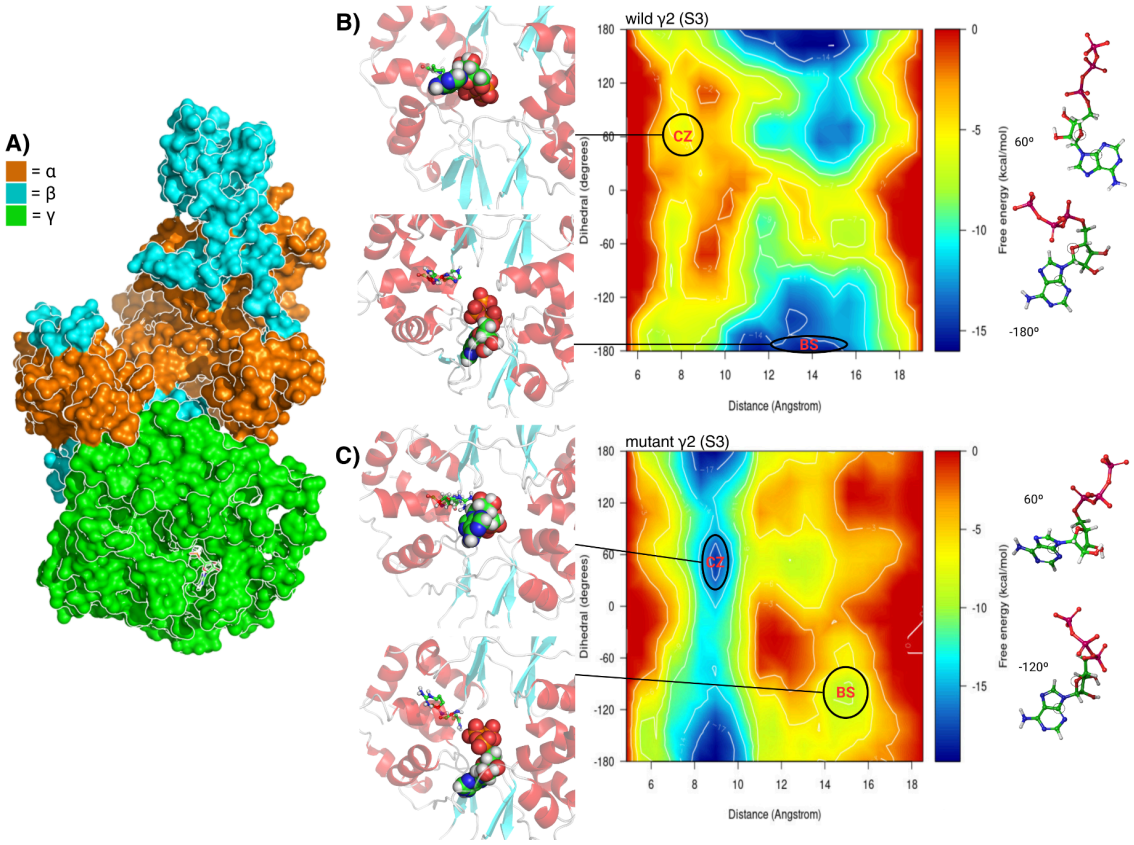

Figure 2. Trajectory analysis of ATP at site 3 of the wild-type and mutant AMPK gamma 2 subunit. A) Heterotrimeric structure of AMP-activated protein kinase a2b2g1 PDB 6B2E; B) ATP trajectory in the wild-type complex; C) ATP trajectory in the mutant complex.

The ΔΔG values obtained for ATP binding to the His401Gln mutant strongly suggest that the ligand initially interacts electrostatically with the AMPK gamma subunit via its phosphate groups but fails to properly accommodate in binding site 3.

The loss of this interaction between ATP and site 3 in the mutant may lead to impaired AMPK function.

Conclusions

The study's findings reveal the significant impact of the His401Gln mutation in the PRKAG2 gene on the structural dynamics and ligand-binding properties of the AMPK γ2 subunit. The differences in free energy (ΔΔG) for ATP binding at site 3 between the wild-type and mutant proteins suggest that this mutation disrupts normal protein function, potentially contributing to the pathological features of PRKAG2 cardiomyopathy. Specifically, the unfavorable energetics of ATP binding in the mutant complex may impair AMPK's ability to regulate cellular energy homeostasis, thus promoting disease manifestation.

These insights underscore the critical role of molecular dynamics simulations in unraveling the mechanistic effects of genetic mutations in rare diseases. The His401Gln mutation's disruption of ATP binding presents a potential therapeutic target to restore AMPK function and slow disease progression. Future research should explore the essential dynamics of the heterotrimeric protein to better understand how this mutation affects AMPK's overall conformation and functionality. This study enhances our understanding of the molecular mechanisms underlying PRKAG2 cardiomyopathy and opens the door to developing targeted treatment strategies.

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