Analyzing Switch Regions of Human Rab7a and Rab10 by Molecular Dynamics Simulations

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***Abstract*— Rab7a and Rab10 are small GTPases that regulates cellular processes by alternating between its GDP-bound inactive and the GTP-bound active states. Studies have shown that functional deficiencies in the pathways of these enzymes are implicated in ciliophaties, cancer and neurodegenerative diseases. Thus, the modulation of the activity of these targets may represent an interesting strategy in drug discovery for the treatment of several human diseases. In order to identify potential Rab7a and Rab10 inhibitors, we studied the mobility of the switch1-interswitch-switch2 surface to understand the active "ON" and inactive "OFF" states of these enzymes. We use molecular dynamics simulations to investigate the atomic movements of the switch regions of these enzymes associated with GDP and GTP nucleotides. We found noticeable differences in the local flexibility of switch 1 when these Rab GTPases were bound to GDP. However, the deterministic method used was not able to successfully differentiate the flexibility of switch 2 region. We hypothesized that the flexibility of the switch 1 region can be used as an indicator of in silico studies that search potential competitive inhibitors based on nucleotides against these targets. Furthermore, the present study can be useful for research that involves the description on-to-off process of other target proteins.**

Keywords-Small GTPases; structural flexibility; in silico.

I. INTRODUCTION

Rab7a and Rab10 are small monomeric enzymes that belong to the Rab GTPase family. They are responsible for regulating intracellular traffic in various pathways of different cellular sublocations, having roles in the endoplasmic reticulum, trans-Golgi network, endosomes, lysosomes, and primary cilium [1]. Several Rab GTPases are believed to be involved in cellular processes such as biogenesis, transport, and tethering of membrane-bound organelles/vesicles. However, functional dysregulations in the pathways of these enzymes are implicated in a few human diseases, such as ciliopathies, cancer, and neurodegenerative diseases [2]. For example, studies have shown that Rab10 and Rab7a have a relevant role in Alzheimer's disease (AD), where Rab10 helps in the amyloidogenic processing of the amyloid precursor protein (APP) while Rab7a is involved in the hyperphosphorylation of the Tau protein [3] [4]. Furthermore, there are studies that correlate the inhibition of these enzymes with the induction of apoptosis in cancer cells [5]. Experiments show that changes in the expression of these enzymes can activate signaling pathways for cancer cells growth and survival, leading to cancer progression. Such evidence paves the way for the application of new drug targeting strategies for the treatment of various human diseases. Since Rab7a and Rab10 are associated with several human diseases, the modulation of the activity of these enzymes using small molecules may represent a promising alternative to delay the progression of these diseases, making them potential therapeutic targets.

Rab GTPases regulates cellular processes by alternating the nucleotides guanosine triphosphate (GTP) and Guanosine diphosphate (GDP). When bound to GTP, they interact with a series of effector proteins promoting downstream signaling events. On the other hand, the hydrolysis of GTP results in conformational changes in the G domain of these enzymes, inactivating them [6]. The differences between the conformations of the G domain linked to GDP and GTP suggest that after the hydrolysis of GTP the switch 1 and switch 2 regions show a high degree of flexibility and disorder. In contrast, these regions are stabilized in the active state, which favors their recognition by effector proteins [7]. This occurs due to interactions with the phosphate binding motifs (PMs) of these enzymes, where GTP interacts with PM1-3 while GDP only with PM1 [8] [9].

In 2017, PYLYPENKO and collaborators conducted a study to obtain insights into the functional diversity of Rab GTPases. In this study, the authors analyzed, using in silico tools, 44 representatives of the subfamilies of human Rab GTPases, to obtain information on the primary sequences of these enzymes with partner proteins in the context of binding specificity and provide results functions of their interactions in the cell. In this study, the authors detailed precision the motives of the Rab GTPases that interact with GDP and GTP; however, it was not analyze the "on-to-off" process to understand the modifications conformational patterns of these enzymes when activated and inactivated.

The present study aimed to detail the structural flexibility of the switch regions of Rab7a and Rab10, considering 200 ns molecular dynamics (MD) simulations. In 2020, we performed MD simulations to investigate Rab10's internal movements in its activated and inactivated state. These results showed noticeable differences in local switch I flexibility when Rab10 was associated with GDP [10]. Thus, we hypothesize that the flexibility of the switch1 region can be used as an indicator of in silico studies that aim to identify potential competitive inhibitors against Rab10. In order to verify if the conformational change in the on-to-off process

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of Rab10 was not by chance, we extended our study to consider the Rab7a. Our new findings strengthen our hypothesis that the flexibility of the switch I region can be used as an indicator for studies aimed at identifying potential inhibitors of these enzymes.

The rest of this work is organized as follows. Section II describes the methods used in each step of this study. Section III addresses the results and discussion, while Section IV describes the conclusion and next steps of this research. The acknowledgements close the article.

II. METHODS

In this section, the in silico approaches used during the development of this work.

A. Molecular docking

The structure of Rab7a (ID: 1T91), Rab10 (ID: 5SZJ) and GDP and GTP nucleotides were obtained from the Protein Data Bank (PDB) [11]. Modeller software v9.23 [12] was used to fill the missing atoms of Rab10. The addition of hydrogen in each structure, considering the protonation state of the atoms at physiological pH, was performed using the Open Babel 3.0.0 software [13]. The Autodock Vina 1.1.2 software [14] was used to docking the nucleotides at the active site of Rab10. The grid box was defined by the mean of the Cartesian coordinates of the nucleotide GTP and phosphoaminophosphonic acid guanylate ester (GNP) co-crystallized in Rab7a and Rab10, respectively. The co-crystallized compounds were submitted to redocking to validate the docking study. The poses of each nucleotide were chosen by means of the lowest binding energy and the highest number of intermolecular bonds. The interactions between the ligands and receptor were calculated using the Maestro 12.3 interface [15].

B. Molecular dynamics

The GROMACS package version 2019.3 [16] was used in the MD simulations of complexes with GDP and GTP. The force field used was CHARMM36 [17]. The ligand parameters were obtained by the CGenFF server [18]. The complexes were centralized in cubic boxes, where the distance between the solute and the edge was 14 Å. The molecules were solvated with TIP3P water molecules and neutralized by adding the appropriate number of Na+Cl- ions considering the ionic concentration of 0.15 M. The energy minimization was performed using the steepest descent method with a maximum force of 1000 Kj/mol.nm. After minimization, the systems were equilibrated in two stages: a canonical NVT set (number of particles, volume, and temperature) followed by an isothermal-isobaric NPT set (number of particles, pressure, and temperature). The NVT equilibrium was performed with a constant temperature of 300 K for 500 ps. The NPT equilibrium was performed with a constant pressure of 1 bar and a constant temperature of 300 K for 500 ps. The production step was carried out at 300 K for 200 ns and the trajectories were saved every 10 ps. The tools of the root mean square deviation (RMSD), root mean square fluctuation (RMSF), radius of gyration (Rg) and solvent accessible surface area (SASA) were used for the trajectory analysis.

III. RESULTS AND DISCUSSION

The results and discussion of this study are described in subsequent sections.

A. Molecular docking study

The lowest energy values for each test were grouped and their molecular interactions were analyzed. The most promising poses of each ligand are described in Table 1. The comparison between the co-crystallized ligands poses with the docking poses indicated RMSD $\leqslant$ 2.00 Å, suggesting that the docking protocol was validated. Thus, GDP and GTP nucleotides were successfully anchored in the active site of Rab7a and Rab10. These complexes showed notable intermolecular interactions. When in interaction with GDP, there are 6 hydrogen bonds with the residues located in PM1, while when interacting with GTP there are more hydrogen bonds.

TABLE 1. SCORE BY THE VINA AND THE NUMBER OF INTERACTIONS CALCULATED BY MAESTRO

Complex	Score (Kcal/mol)	Salt Bridge	HBonds	Stacking
Rab7a-GDP	-10.3	3	6	2
Rab7a-GTP	-11.1	3	9	2
Rab10-GDP	-10.7	3	6	2
Rab10-GTP	-11.5	3	10	2

This occurs due to the presence of γ-phosphate in GTP, which interacts with the PM2 and PM3 motifs and can even increase the stability of the switch regions of these enzymes. Furthermore, the binding modes found for each complex are consistent with the interactions found in the Rab7a and Rab10 crystals [11] [12]. This indicates that the docking protocol was able to successfully reproduce the experimentally determined binding mode for the co-crystallized ligands.

B. Molecular dynamics study

To verify whether Rab7a and Rab10 stabilized throughout the MD simulation, the RMSD of the G domain of these enzymes was calculated. Based on our results, the trajectories reached stability after 40 ns of simulation (Fig. 1a). In the case of Rab7a, the backbone RMSD of the complexes associated with GDP and GTP stabilized close to 0.23 and 0.24 nm, respectively. In relation to Rab10, the trajectories stabilized close to values of 0.30 nm for the inactive state and 0.25 nm for the active state. Through analysis of the trajectories, it was possible to observe that the complexes associated with the GTP have more restricted movements, since the trajectories showed predominantly lower peaks than the complexes linked to GDP.

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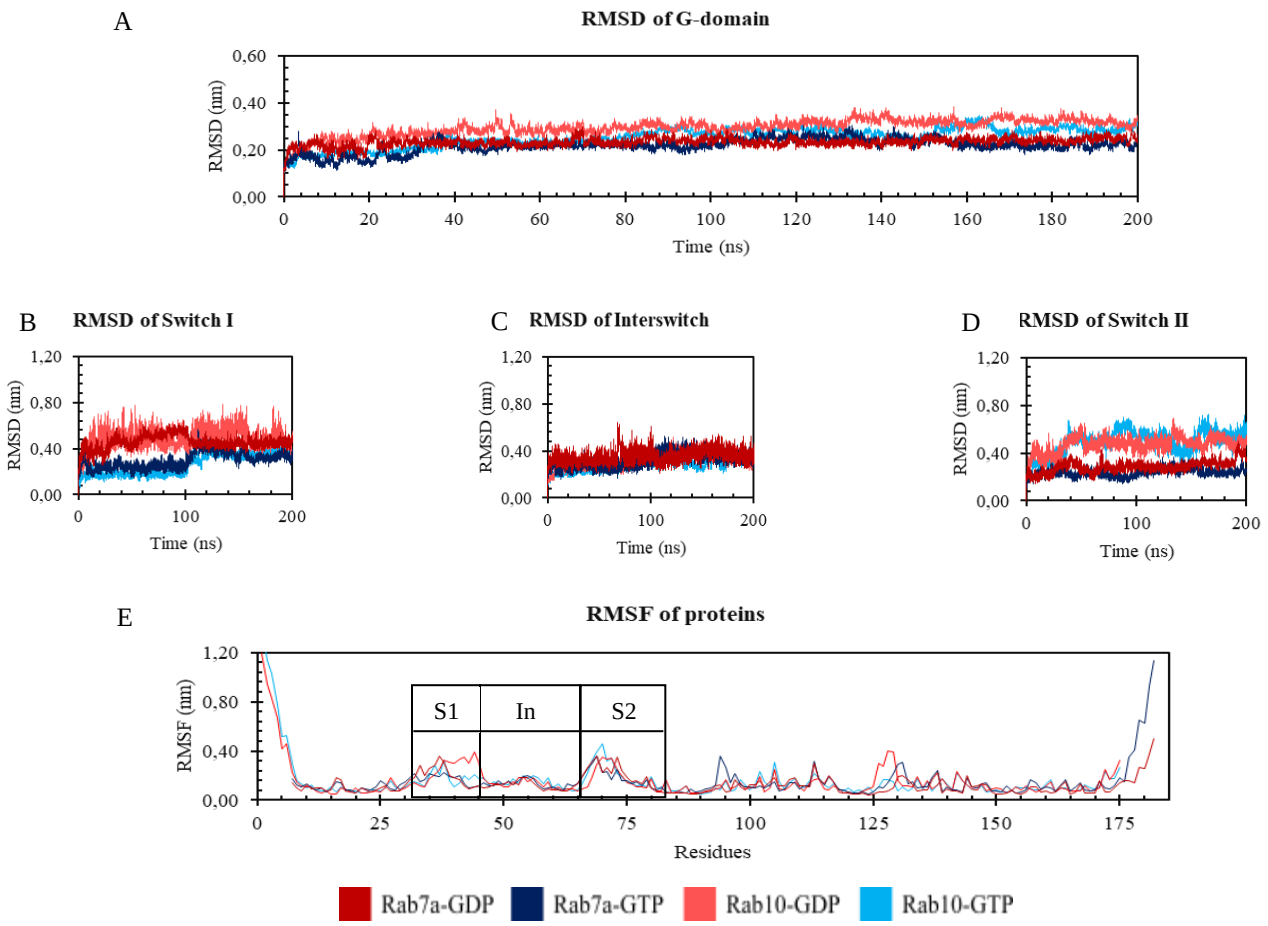

Figure 1. Analysis of the trajectories obtained in the MD simulation. (A) RMSD of G-domain backbone. (B) RMSD of switch I. (C) RMSD of interswitch. (D) RMSD of switch II. (E) RMSF of the amino acids residues. Switch 1 (S1) region is defined by positions 31-44, while interwitch (In) and Switch 2 (S2), 45-65, 66-82, respectively.

Local RMSD analysis was required to assess the inherent flexibility of switch regions. Fig. 1b shows the RMSD of the switch I region, where it is possible to visualize differences in the backbone when the enzymes analyzed were associated with the nucleotide GDP and GTP. Here, systems linked to GDP showed greater fluctuations compared to those linked to GTP. This can be explained because the phosphate portion of GDP contains only α and β-phosphate, causing switch I to suffer greater conformational flexibility due to the existence of smaller intermolecular interactions involving the PM2 and PM3 motifs. On the other hand, the presence of γ phosphate in GTP provides less flexibility in switch I due to the existence of greater intermolecular interactions with these motifs. The interswitch region was chosen as a control because it does not show conformational differences in both the activated and inactivated states of the Rab GTPases (Fig. 1c). However, the switch II region showed no noticeable RMSD differences (Fig. 1d). This result may be associated with the presence of an α-helix in switch II, which can generate motions more restrictive with varying time, as each successive turn of the α-helix is held by adjacent turns by three or four hydrogen bonds, which gives a significant stability in relation to other secondary structures of these enzymes. Fig. 1e shows the residues that make up the entire extension of the Rab7a and Rab10 enzymes, where it is possible to visualize the regions where the greatest fluctuations occur. These results show subtle differences in the flexibility of switch I, however, in all complexes where Rabs were inactivated, the fluctuation for this region was greater.

Rg trajectories indicate that the G domain of Rab7a and Rab10 entered conformational equilibrium. Fig. 2a shows that the Rg values are constant, suggesting that the folded structures of these enzymes are stable. However, the Rg trajectories of the inactivated systems showed higher values than the activated state. While the difference in these values is subtle, it does indicate that the inactivated state is subject to decompression processes throughout the simulation. Furthermore, SASA analysis was performed to evaluate the molecular surface of these Rabs. Fig. 2b shows a slight increase in SASA for inactivated states, which can be explained due to greater mobility in the switch region I.

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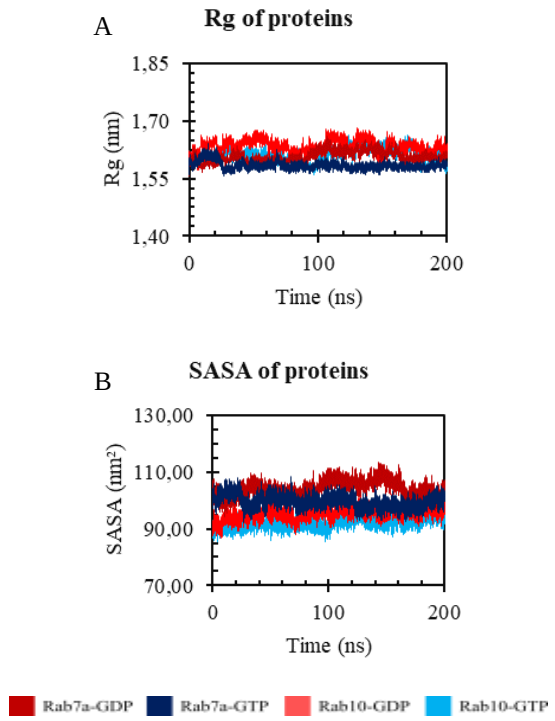

Figure 2. Analysis of compression and SASA. (A) Rg of the G-domain. (B) SASA of the G-domain.

IV. CONCLUSIONS AND FUTURE WORK

In short, the MD simulations used in this study were able to obtain notable differences in the switch 1 region of Rab7a and Rab10, enabling the identification of its active "ON" and inactive "OFF" states. However, the classical mechanics method was unable to predict the disordered movements of the switch 2 region. Our new findings strengthen our hypothesis that the flexibility of the switch I region can be used as an indicator for studies aimed at identifying potential inhibitors of these enzymes. Furthermore, the data discussed here may be useful for research involving the description of the on-to-off process of other proteins. The next steps of this research is to extend the analyzes to evaluate the on-to-off process of 44 representatives of human Rab GTPases, to understand the conformational modifications of these enzymes when activated and inactivated. Thus, it is expected that this study proposal provides useful information about the dynamics of these enzymes, allowing regions switches are used as indicators to select which putative drugs have the potential to inhibit or activate these enzymes.

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