RbgA – a prokaryotic GTPase involved in Ribosome assembly: Simulation studies

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Abstract

RbgA is an essential GTPase which participates in ribosome maturation. Depletion of RbgA in cells results in an incomplete form of 50S ribosome subunit - essentially a 45S intermediate subunit. It is therefore an important target for design of antimicrobials against bacterial pathogens. Like all GTPases, biological functions of RbgA depends on its conformational state, which is determined by the bound nucleotide. Using molecular dynamics (MD) simulation, we explore these conformational changes in RbgA bound to different guanine nucleotides-GTP, GDP, GDPNP and GTPGS compared to the apo form.

Keywords: ***GTPase, RbgA, nucleotides, molecular dynamics***

1. Introduction

RbgA, a GTP binding protein which binds and hydrolyses GTP, belongs to the GTPase super family [1, 2]. These GTPases plays a role in many biological events like protein biosynthesis, cell division, signaling and ribosome biogenesis [1, 2, 3, 4]. Conserved motifs G1-G2-G3-G4-G5 are present in all the members of this family. The loop containing the G1 motif is called P-loop; it interacts with the gamma phosphate of the GTP. Switch-I and switch-II regions are in the loops containing G2 and G3 motifs respectively and undergoes large conformational changes. G4 and G5 are plays in recognizing guanine base [2, 3]. These GTPases exists in GTP bound (ON sate), empty and GDP bound state (OFF state). Switching between these states enhances under the influence of GAPs (GTPase accelerating proteins) and GEFs (guanine nucleotide exchange factors) [1].

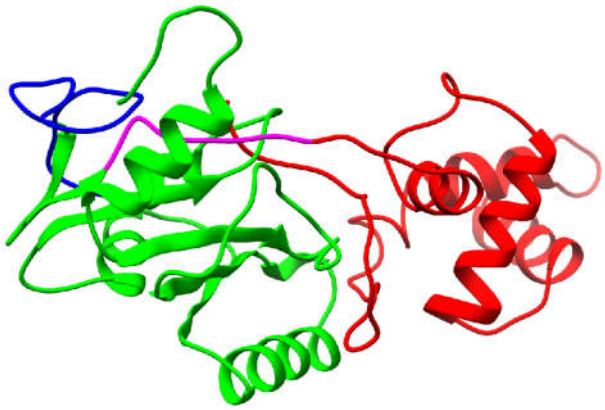

Fig. 1. Cartoon representation of RbgA. N-terminal domain represented by green color, C-terminal by red, switch-I and switch-II are highlighted with blue and magenta colors.

RbgA and its homologous GTPases are the members of the circularly permuted GTPase family, where the commonly conserved motifs G1-G2-G3-G4-G5 are in the order of G4-G5-G1-G2-G3 [5,6]. Also these belongs to the family of HAS (hydrophobic amino acid substituted) GTPases, where the hydrophobic amino acid residue substituted in the catalytic glutamine of the switch II region [7].

RbgA consists of N-terminal GTP binding domain and C-terminal RNA binding domain also termed ANTAR (AmiR NasR Transcription Anti termination Regulator domain) domain [8,9](Fig.1). RbgA and its homologs are said to be participated in ribosome maturation. Depletion or mutation in RbgA results in lack of complete 70S ribosome unit, large number of

45S intermediates instead of 50S subunit [8,10,11]. Therefore, it is inferred that RbgA is essential in maturing complete 50S ribosome subunit in turn complete 70S ribosome assembly. Association of RbgA-ribosome is stable when it is in the complex form with GTPGS (non hydrolyzable GTP analog) and less stable with GDP binding [10, 12]. This prompts us to investigate the structural changes and dynamics of RbgA in its apo form and complexes with the guanine nucleotides. In the present study 10 ns MD simulations for modeled systems (RbgA, RbgA-GTP, RbgA-GDP, RbgA-GDPNP, RbgA-GTPGS) complexes has been carried out.

2. Materials and Methods

Protein structure for the present work has taken from the protein data bank (PDB) with PDB ID-3CNO GTPase-GDP complex of *T.maritima* [9]. Different systems - RbgA, RbgA-GTP, RbgA-GDPNP, RbgA-GTPGS - are modeled with the CHIMERA [13]- package for visualization and modeling. Missing residues and atoms were fixed using the MODELLER [14] with the chimera interface.

Starting from these systems, MD simulations for 10 ns were performed using *GROMACS* package [15-21]. We have used GROMOS96 43a1 force field [22] for generating topology for protein. PRODRG [23] server was used to generate topology for ligands. Modeled systems were immersed in the simulating environment of triclinic box with the periodic boundary condition. Systems were solvated with spc water model and neutralized with Cl^- ions. Subsequently we performed energy minimization using steepest descent algorithm with the energy tolerance of 1000 kJ/mol. Following energy minimization systems were subjected to 100 ps NVT and NPT equilibration with the position restraints of heavy atoms and lincs constraints for bond vibrations. Finally 10 ns production run was performed. Equilibration and production

run was carried out under the condition of 300K temperature and 1bar pressure. V-rescale thermostat [24] and Parinello-Rahaman barrostat [25] were employed to achieve the desired temperature and pressure with coupling time constants 0.1ps and 2ps respectively and iso-thermal compressibility was set to 4.5e-5 bar^{-1}. Long range electrostatic force calculations were handled by PME (particle mesh ewald) method with interpolation order of 4 and a grid spacing of 0.12. Verlet cut-off scheme was used with cut-off value 1.4nm for both Lennard-Jones and electrostatic interactions.

Trajectory analysis was carried out using the programs inside the GROMACS package. Chimera [13] was used for visualization, generating images and superimposition of the simulated data. Plotting has been done with xmgrace.

3. Results and Discussion

For each of the studied systems (RbgA, RbgA-GTP, RbgA-GTP, RbgA-GDPNP, RbgA-GTPGS), 10 ns MD simulations were performed. To validate the stability of the systems during simulation we obtained rmsd graph (**Fig.2**) and identify the last 4ns is in the equilibration state. For further structural analysis and comparison, average of the last 4ns is considered as representative structure.

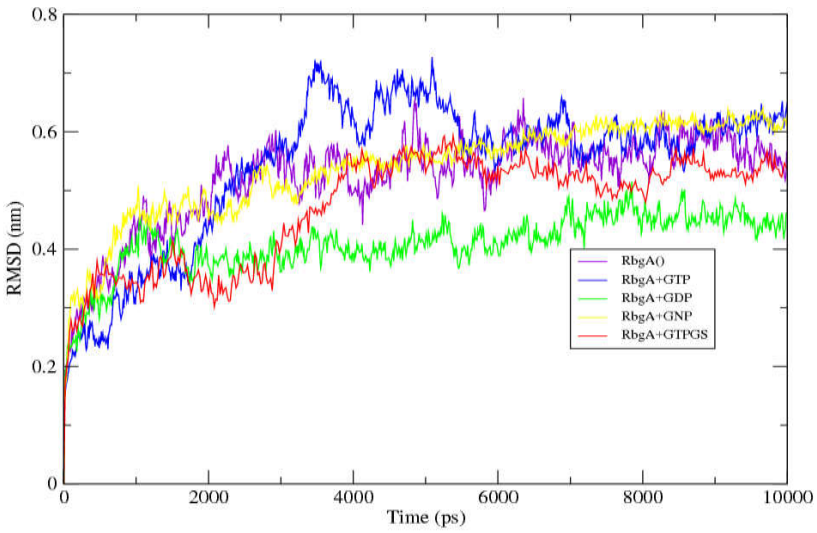

Fig. 2. RMSD plots of the backbone atoms with respect to the starting coordinates (after minimization and equilibration) during time evolution from 0 to 10 ns for five different models

Results of RMSF calculations performed for all five systems is plotted (**Fig.3**). RMSF graph indicates that there is large fluctuation in switch-I (121-138) region in all RbgA-nucleotide combinations. In all GTPases, Switch-I is known to play a critical role in sensing the guanine nucleotide bound state at the active-site. In case of RbgA too, we find that while the G-domain is largely stable, there are major conformational changes in the Switch-I loop.

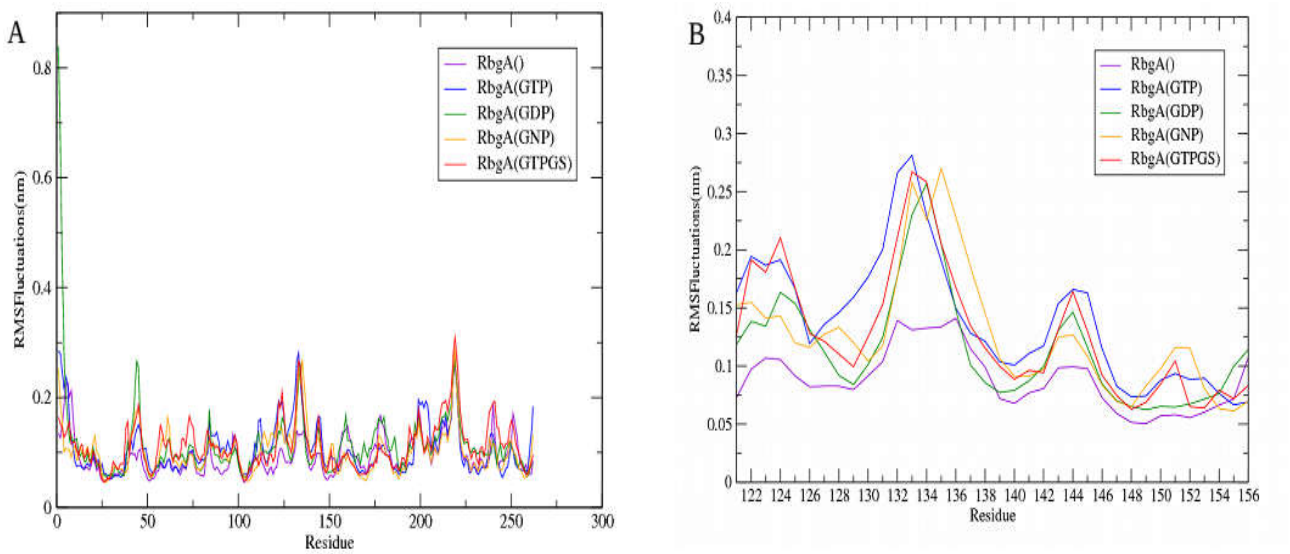

Fig. 3. RMSF plots (A) for entire protein (B) for switch regions (switch I(121-138) switch II(149-156))

In switch II (149-156) fluctuations are relatively more in GTP, GTPGS and GDPNP bound states than apo and GDP bound form. For further structural analysis, superimposition of apo-form with nucleotide binding forms has been done by aligning N-terminal GTP binding domains. Superposition of the structures revealed significant domain movements of the ANTAR domain, relative to the G-domain. The calculated distance between C-alpha atoms of the superposed structures gives quantitative information of the same phenomenon. From these results, it appears that the nucleotide bound state of the G-domain indeed controls the overall conformation of RbgA by inducing changes in the orientation of the ANTAR domain. Structural analysis reveals that the Switch-I and Switch-II loops are directly linked to the ANTAR domain.

Our data shows that the changes in the conformation of the Switch-I and Switch-II loop directly influences the orientation of the ANTAR domain.

Essential dynamics or principal component analysis was perfumed to understand essential movements in the structures in different combinations. Maximal and minimal projections along the largest eigen vectors to the average structure was obtained and superimposed. Distance between C-alpha atoms of the superimposed structures were extracted and plotted. The results again reiterate our findings that there are significant changes in RbgA conformation in response to the guanine nucleotide bound state.

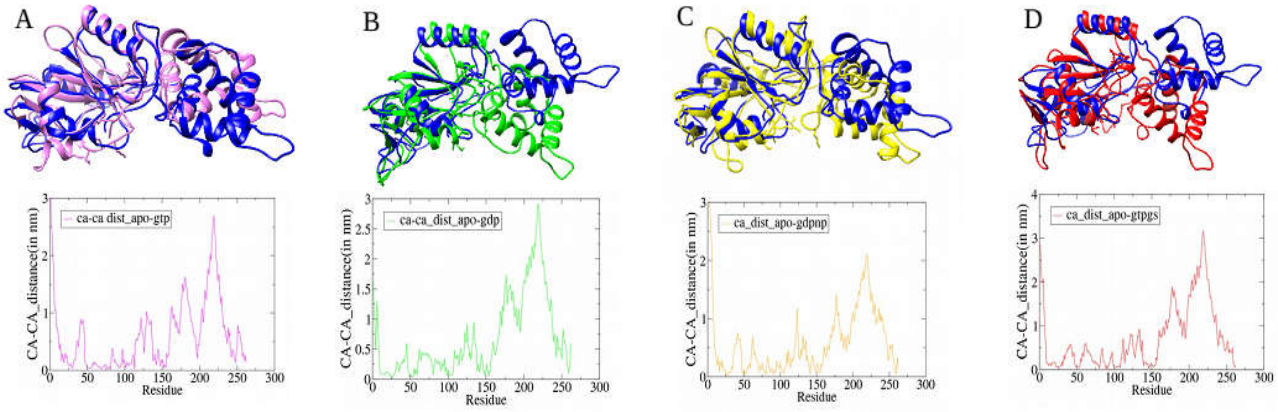

Fig. 4. Superimposing of RbgA by aligning GTPase domain of (A) APO-GTP (B)APO-GDP (C)APO-GDPNP (D)APO- GTPGS; at the bottom c-alpha c-alpha distance of the superposed structure with respect to residues is plotted.

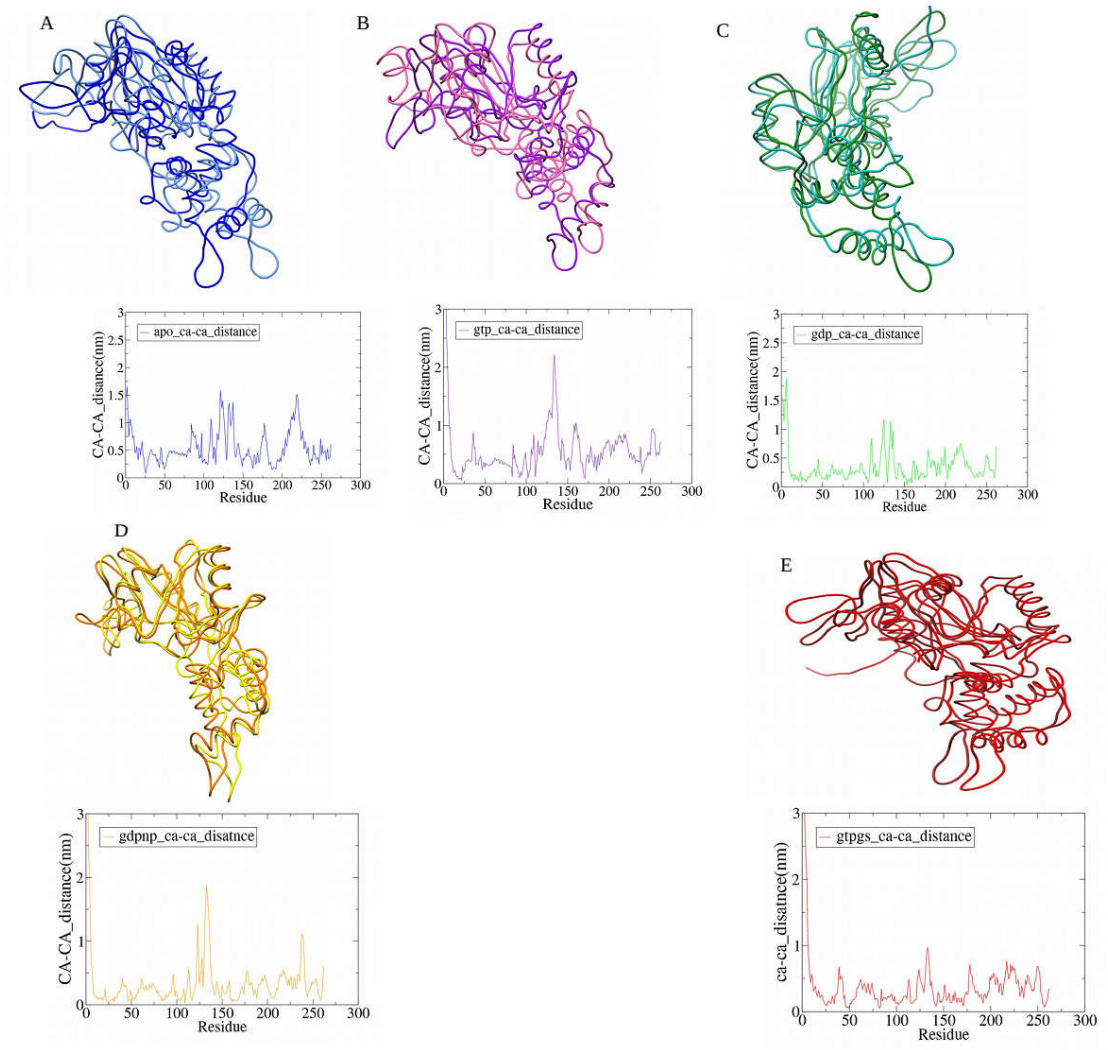

Fig. 5. Superimposed structures of maximal and minimal projections along the largest eigen vector. Bottom graphs gives the quantitative information of essential movements by calculating CA-CA distances.

4. Conclusion

MD simulations was carried out for modelled systems of RbgA, RbgA+Guanine nucleotides (GTP, GDP, GDPNP,GTPGS). Analysis of the results infers that conformational changes are maximum in all guanine nucleotide binding cases with subtle variations than in apo form. Further investigations are delineate the molecular interactions that underpin the regulation of RbgA conformation. Simulations of RbgA and its effector protein or ribosomal RNA as a single system need to be conducted to identify the protein surface (and residues) that stabilize RbgA-ribosome interactions.

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