A Molecular Dynamics Study of the DoubleDominant Negative Mutation W809E/T935E in Ras-GEF Complex

Gianluca Santarossa, Luigi Roggia, Luca De Gioia, Piercarlo Fantucci

Department of Biotechnology and Biosciences, University of Milano – Bicocca, P.zza della Scienza 2, 20126 Milan, Italy

piercarlo.fantucci@unimib.it

Keywords. Molecular Dynamics, Ras, GEF

Introduction

Ras proteins are guanine nucleotide binding enzymes, with intrinsic low GTP-ase activity, involved in the control of cell growth and cell differentiation. They act as molecular switches, cycling between active GTP-bound state and inactive GDP-bound state. Ras activation state is regulated by the competing activity of GTPase activating proteins (GAPs) and guanine nucleotide exchange factors (GEFs), the latter promoting the activation of Ras catalysing the exchange of GDP with GTP. In most tumors the activity of Ras proteins is altered, resulting in hyperactive GTP-bound forms of Ras, either because of a reduced GTPase activity or because of an increased GDP/GTP exchange. GEF mutant W809E/T935E (GEF*mut*) results in a dominant negative GEF, catalitically inactive, which binds to Ras with great affinity and forms a stable complex in the presence of excess nucleotide [1]. By means of Molecular Dynamics (MD) simulations we compared different trajectories of Ras-GEF*wt* and Ras-GEF*mut* systems and analyzed them in terms of both energetic and structural parameters, to correlate the conformational differences of *wt* and *mutant* GEFs during their interaction with Ras with the observed modifications in Ras biological activity.

Results

Ras-GEF*wt* and Ras-GEF*mut* molecular dynamics simulations were performed in explicit solvent, by generation of a water shell 6Å thick around Ras-GEF complexes. Simulations were based on CHARMM27 force field using NAMD [2] program suite, applying PME algorithm and periodic boundary conditions for non-bonded terms of the potentials. After a period of equilibration, the systems evolved in the productive phase for 1100 ps. Trajectories were analyzed using Xmgrace [3] and VMD [4]. Analysis of general properties, such as temperature, pressure and potential energy on both systems allowed us to assume that equilibrium conditions were reached during production. The effective conformational changes involved during the simulations were carried out on the basis of the RMSD values. Ras structure did show a far greater mobility in complex with GEF*wt* than with GEF*mut* *(fig 1)*. The principal configuration transformations are concentrated in SwitchI (Q25-Y40) region and its surrounding segments, including the p-loop (G10-S17). Mutations introduced in GEF two acidic residues instead of an hydrophobic one (W809) and a polar one (T935), so that local rearrangements and modifications of the patterns of hydrogen bonds and VdW interactions are expected. GEF*wt* W809 is located in a hydrophobic pocket involved in hydrogen bonds and VDW interactions with aliphatic residues both intramolecular and with Ras SwitchII, in such a way that GEF*wt* perfectly preserves its secondary and tertiary structure. On the other hand GEF*mut* segment V799-P819, containing W809E mutation, undergoes a relevant conformational change during the first 400 ps of dynamics, due to the unfavorable environment for the mutated residue E809, deeply modifying the hydrogen bonds patterns of GEF*wt*. Morover, GEF*mut* T935E mutation leads to the formation of a new strong electrostatic interaction with K939 and Ras D57 (fig. 2) stabilizing the otherwise weak interaction between K939 and D57.

Conclusions

It is well known that Ras-GEF interaction has to be sufficiently strong to displace the bound GDP nucleotide and sufficiently weak to allow disruption of the binary complex. Mutational events may alter this fine-tuned equilibrium, originating mutants with different properties. RMSD data show that the double mutation W809E/T935E in GEF*mut* causes the stabilization of Ras conformation. This can lead to a diminished affinity of Ras-GEF*mut* complex for the incoming nucleotide, which is one of the mechanisms causing the typical properties of its dominant-negative behaviour, accordingly to experimental data. W809 in GEF*wt* holds the V799-P819 loop structure in a conformation otherwise unfavorable, while E809 in GEF*mut*the loop reach a final conformation far more random coiled than in GEF*wt*. The role of this mutation in affecting Ras-GEF stability is not completely understood. We think that attention should be focused both on loss of W809 hydrophobic stabilization and on indirect increase of hydrophilic interactions lead by local rearrangement. On the other hand, effect of T935E mutation is clear. E935 favours the Ras-GEF interaction by stabilization of the interaction between Ras switchII D57 residue and GEF K939 residue, contributing to the correct displacement of SwitchII during Ras-GEF interaction.

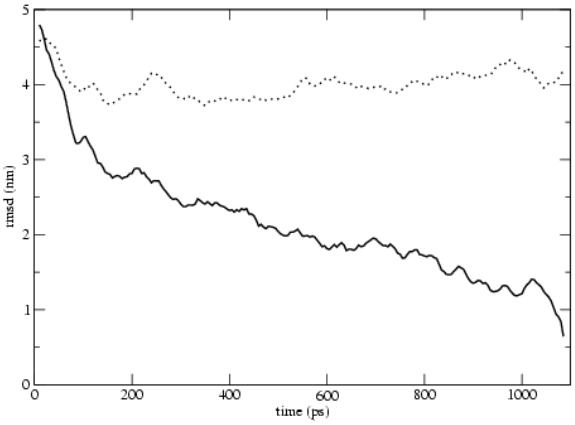

Fig. 1 RMSD of Ras in Ras-GEF*wt* (continuos line) and Ras-GEF*mut*(dotted line) complexes. Every trajectory frame was referred to last frame of Ras-GEF*wt* complex.

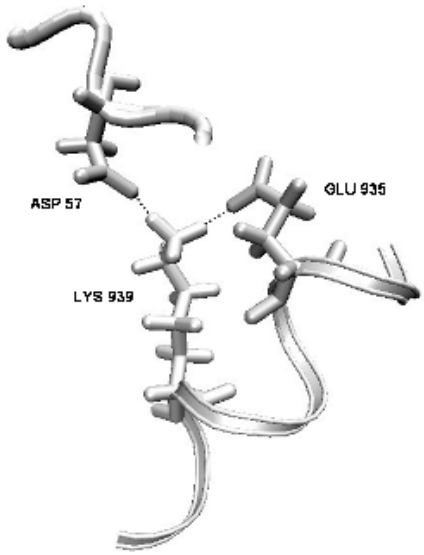

Fig. 2 Electrostatic interaction between Ras D57 and K939 is stabilized by E935 in RasGEF*mut* - complex.

References

[1] Vanoni, M., Bertini, R., Sacco, E., Fontanella, L., Rieppi,M., Colombo, S., Martegani, E., Carrera, V., Moroni, A., Bizzarri, C., Sabbatini, V., Cattozzo, M., Colagrande, A., Alberghina, L., *J Biol Chem*, **274 (51)**: 36656-36662, 1999.
[2] http://www.ks.uiuc.edu/Research/namd/
[3] http://plasma-gate.weizmann.ac.il/Grace/
[4] http://www.ks.uiuc.edu/Research/namd/