Water distribution within GTP bound NRas in complex with GAP arginine binding loop & implications for its GTPase activity

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Context

Enzymatic reactions tailor processes necessary for cell proliferation and survival [13]. For instance, proteins from the Ras family regulate the transmission of external cell growth signals to the inner of the cell through the binding and subsequent hydrolysis of guanosine triphosphate (GTP) [4, 27, 5]. Indeed, when GTP-bound, these proteins activate numerous pathways leading to cell replication. The hydrolysis reaction of this nucleotide into guanosine diphosphate (GDP), leads to a GDP-bound state that, due to conformation changes undergone by the protein, is unable to engage in the interactions necessary for activating these same pathways. As such, the transmission of upstream cell growth signals is terminated.

The precise timing between Ras active/inactive states is crucial for the organism survival. Indeed, mutations found within members of this family of small GTPases are associated with an amplification of cell growth signals that contributes to the dissemination of cancerous tissues [3]. Indeed, such mutations hinder the protein capability to catalyze the hydrolysis reaction, thereby leaving it in a GTP-bound active state [12, 20]. As Ras catalyzing mechanisms of GTP hydrolysis and the extent to which they are hindered by point mutations remain to be fully elucidated, several non-mutated/mutated protein-ligand complexes were studied at the atomic level employing hybrid QM/MM molecular dynamics.

Methodology

In order to access NRas *dynamic* properties, Molecular Dynamics (MD) simulations of *NRas-GTP-Gap arginine binding loop* complexes were carried out employing a hybrid Quantum Mechanics/ Molecular Mechanics (QM/MM) [26] description of the interatomic interactions. Indeed, bond formation/breakage expected during GTP hydrolysis, requires a quantum treatment. Yet, the size of the entire system prevents the use of a full QM description. Hence, a hybrid approach appears as the most appropriated. In this manner, the reactants (GTP + water molecules) together with Ras residues (Gly 12, Gly 13, Tyr 32, Thr 35, Gly 60, Glu 61) and a Mg^{2+} ion identified as being essential for catalyzing the reaction [10, 19] were treated at the quantum level, thereby allowing charge redistribution, while the influence of the rest of the protein and the solvent were accounted for employing a fixed charge classical approach.

Results

Crystallographic data of the holoprotein has been unable to determine unequivocally the number of water molecules present in the active site *i.e.* those that would be placed within a reasonable distance from the nucleotide terminal group to react [14, 17]. Hence, to begin the atomistic study of NRas-GTP-GAP arginine binding loop complex, the positioning of the reactants was investigated within both non-mutated (referred to as the *wild-type*) and Gln 61 mutated NRas proteins. In particular, water molecules positioning was determined from 2D water probability densities calculated through a 2D RDF algorithm designed for this study [22].

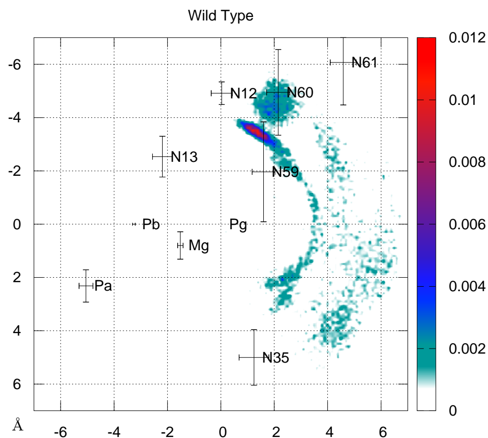

Figure 1: 2D RDF of water molecules within the active site of WT $p21^{N-ras}$. The average coordinates of nitrogen backbone atoms from residues 12, 13, 35, 59, 60 and 61 have also been projected as well as GTP phosphorus atoms and Mg^{2+} ion. The corresponding standard deviation values are represented with error bars.

As depicted in figure 1, water molecules are precisely positioned within the wild-type (WT) protein-ligand complex. Indeed, an *arch* of water presence extends from residue 12 to residue 35 such that two high amplitude peaks clearly emerge. The first one, *i.e.* the highest amplitude density peak, is located between residues 12, 59 and 60. The second one, arises in the vicinity of residue 35, a positioning analogous to that of a crystallographic water molecule. Concerning Gln 61 mutated proteins (see figure 2), this precise water positioning is lost. On the one hand, water molecules appear to be *delocalized* within four of the six studied mutants (*i.e.* Q61E, Q61P, Q61L and Q61R) while they appear to be *overlocalized* within the two remaining (Q61H and Q61K). These findings suggest that *i)* the positioning of water molecules contributes more to catalysis than their amount within the active site, *ii)* two water molecules in two distinct regions of the active site are necessary for an efficient GTP hydrolysis [17, 15, 21, 2, 9, 16]. The precise role an chemical implication in the reaction of each water molecule remains to be determined.

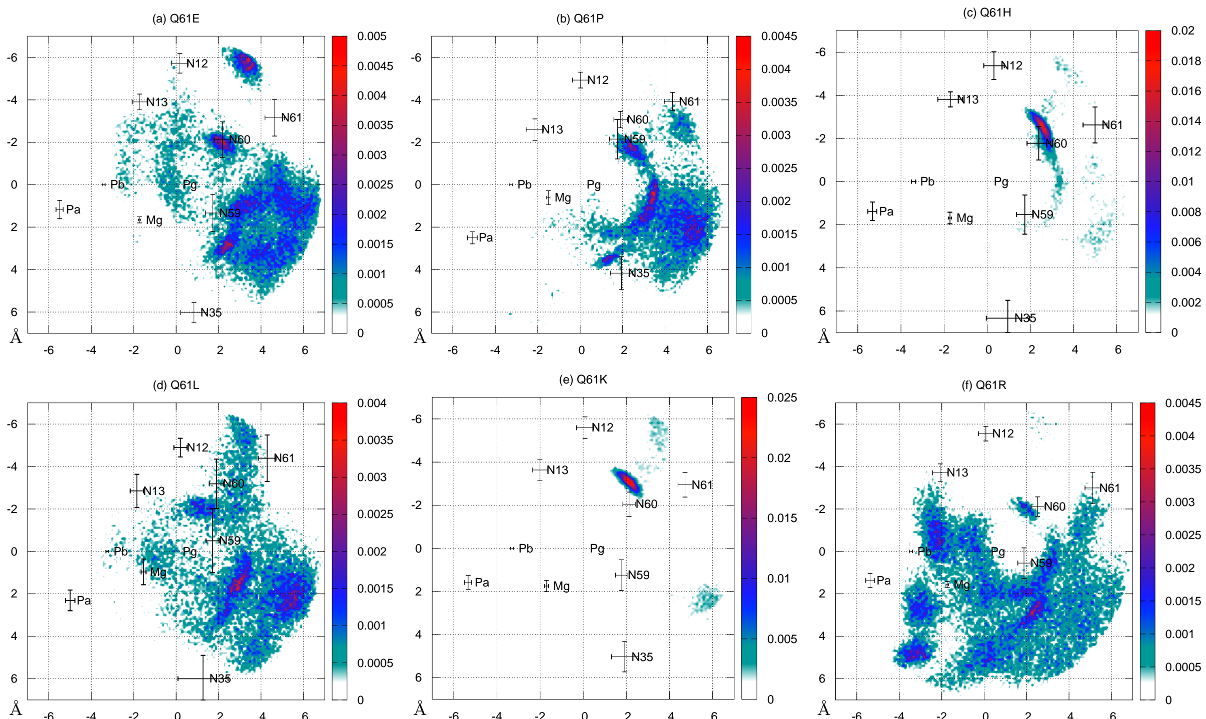

Figure 2: 2D RDF of water molecules within the active site of (a) Q61E, (b) Q61P, (c) Q61H, (d) Q61L, (e) Q61K and (f) Q61R mutants.

From this simulations [22], it appears that residues 12 and 60, highly conserved within GTPases, play an important role in accelerating the reaction by positioning water molecules. Indeed, they maintain the highest amplitude water density peak by hydrogen bonds formed through their nitrogen backbone atoms. In this scenario, Gln 61 would play an indirect role, ensuring an op-

	WT$_{QM/MM}$	WT$_{MM}$	Q61E	Q61P	Q61H	Q61L	Q61K	Q61R
$\gamma\, PO_4^{2-}$	-0.50	-0.81	-0.76	-0.77	-0.65	-0.74	-0.86	-0.77
$\beta\, PO_4^{-}$	-0.57	-0.66	-0.64	-0.59	-0.61	-0.70	-0.63	-0.57
$\alpha\, PO_4^{-}$	-0.78	-0.82	-0.75	-0.74	-0.76	-0.69	-0.82	-0.70
Mg^{2+}	-0.33	-0.18	-0.02	0.12	-0.04	-0.13	-0.04	-0.29

Table 1: Löwdin reduced charges (in a.u.) for WT NRas and six Gln 61 mutants.

timal conformation of the protein active site for catalyzing GTP hydrolysis, as previously proposed [7, 11, 24, 18].

The characterization of the protein active site issued from QM/MM dynamics reveals that both active site rearrangements and water distribution within mutant proteins hinder Ras major catalyzing effect which consists in promoting charge transfers to stabilize a product-like state [6, 25, 8, 1]. Indeed, only within the active site conformation of the WT protein, GTP charge distribution is GDP-like *i.e.* the β phosphate group holds more electronic charge than the γ phosphate (see table 1). Within the six Gln 61 mutated proteins the opposite is observed *i.e.* ${PO_4^{2-}}_\beta < {PO_4^{-}}_\gamma$ as in solution [23].

Conclusion

A QM/MM atomistic study of the GTP bound form of NRas active site in complex with GAP arginine binding loop appears to be crucial for unveiling the protein mechanisms that catalyze the hydrolysis reaction of GTP. Indeed, qualitatively, only within the WT active site conformation obtained from QM/MM molecular dynamics simulations, GTP charge distribution resembles that inferred from infrared (IR) spectroscopy studies. Now, this conformation allows water molecules to occupy very specific positions within the active site such that two water molecules appear to be suitably located to participate in the reaction. Hence, the implication of a second water molecule is supported. The precise role of this second water molecule, as well as wether its presence contributes to catalysis, remain to be determined.

NRas Gln 61 mutations have a direct impact on the active site structure such that the precise positioning of water molecules, observed within the WT, is destroyed. As a direct consequence from this structural rearrangements, GTP charge distribution is not accommodated to a GDP-like state as within the WT anymore. Because this electrostatic effect is considered to be Ras major catalyzing effect, the reduced hydrolysis rate measured within oncogenic mutants could be due to this major loss induced by both active site re-structuration and water distribution.

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