BIOCHEMISTRY
including biophysical chemistry & molecular biology

Article

pubs.acs.org/biochemistry

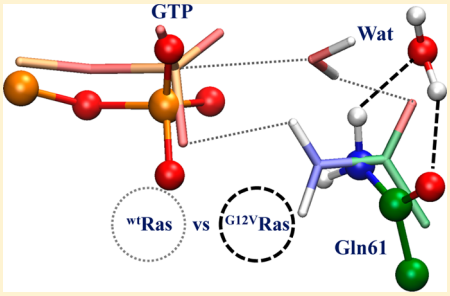
Modeling the Role of G12V and G13V Ras Mutations in the Ras-GAP-Catalyzed Hydrolysis Reaction of Guanosine Triphosphate

Maria G. Khrenova,[†] Vladimir A. Mironov,[†] Bella L. Grigorenko,[†,‡] and Alexander V. Nemukhin*[,†,‡]

[†]Department of Chemistry, M. V. Lomonosov Moscow State University, Leninskie Gory 1/3, Moscow 119991, Russian Federation
[‡]N. M. Emanuel Institute of Biochemical Physics, Russian Academy of Sciences, Kosygina 4, Moscow 119334, Russian Federation

Supporting Information

ABSTRACT: Cancer-associated point mutations in Ras, in particular, at glycine 12 and glycine 13, affect the normal cycle between inactive GDP-bound and active GTP-bound states. In this work, the role of G12V and G13V replacements in the GAP-stimulated intrinsic GTP hydrolysis reaction in Ras is studied using molecular dynamics (MD) simulations with quantum mechanics/molecular mechanics (QM/MM) potentials. A model molecular system was constructed by motifs of the relevant crystal structure (Protein Data Bank entry 1WQ1). QM/MM optimization of geometry parameters in the Ras-GAP-GTP complex and QM/MM−MD simulations were performed with a quantum subsystem comprising a large fraction of the enzyme active site. For the system with wild-type Ras, the conformations fluctuated near the structure ready to be involved in the efficient chemical reaction leading to the cleavage of the phosphorus−oxygen bond in GTP upon approach of the properly aligned catalytic water molecule. Dynamics of the system with the G13V mutant is characterized by an enhanced flexibility in the area occupied by the γ-phosphate group of GTP, catalytic water, and the side chains of Arg789 and Gln61, which should somewhat hinder fast chemical steps. Conformational dynamics of the system with the G12V mutant shows considerable displacement of the Gln61 side chain and catalytic water from their favorable arrangement in the active site that may lead to a marked reduction in the reaction rate. The obtained computational results correlate well with the recent kinetic measurements of the Ras-GAP-catalyzed hydrolysis of GTP.



Wild-type Ras proteins normally cycle between inactive GDP-bound and active GTP-bound states.[1,2] The chemical reaction of GTP hydrolysis (GTP + H_2O → GDP + P_i) dramatically accelerated upon complexation of Ras with GTPase-activating protein (GAP) is a driving force to inactivate the GTP-bound state. Point mutations, in particular, at glycine 12, glycine 13, or glutamine 61, impair intrinsic and GAP-stimulated GTP hydrolysis activity, rendering Ras persistently active and GTP-bound.[3] Elucidating the role of Ras polymorphism demands an immense amount of attention because several point mutations in Ras are related to the development of cancer.[4] Numerous investigations aim to resolve the puzzles of GTP hydrolysis in the wild-type and mutated Ras variants approaching the target from the wide range of distinctly different directions.[5] It is hardly possible to cite even the most important papers devoted to crystallography and spectroscopy studies of the mechanism of GTP hydrolysis in Ras-like GTPases; we mention only one of the recent reviews.[6]

Computer-aided molecular modeling[7] provides a sound support for these experimental studies. Simulations of hydrolysis of phosphates constitute a subject of important contributions from Warshel's research group (e.g., refs 8−16). In a series of recent works, Gerwert and co-authors systematically investigated details of the enzyme-catalyzed GTP hydrolysis mechanism using the methods of time-resolved Fourier transform infrared spectroscopy and biomolecular simulations.[17−25] Classical molecular dynamics simulations (e.g., refs 26 and 27) and semiempirical quantum calculations[28] from other groups have been reported recently, as well.

Our studies[29−32] focus on detailed computational characterization of the chemical steps of the enzyme-catalyzed GTP hydrolysis reaction, assuming that the reagents (GTP + H_2O) and the products (GDP + P_i) are trapped inside the protein cavity. Using the quantum mechanics/molecular mechanics (QM/MM) theory,[33] the mechanism of chemical transformations between these two states was simulated for Ras,[31] Ras-GAP,[30] and EF-Tu[32] protein systems. According to simulation results, the molecular events of these chemical steps include (i) cleavage of the phosphorus (P_γ)−oxygen bond in GTP upon approach of the properly aligned catalytic water molecule, (ii) formation of a new chemical bond between the P_γ phosphorus and the oxygen of water, and (iii) redistribution of protons between reacting species leading to inorganic phosphate P_i. The obtained mechanistic picture is consistent with the variety of experimental data and results of different quantum mechanics-based modeling.

Experimental kinetic studies of GTP hydrolysis in Ras variants[34−38] evidence that point mutations slow rates of the

Received: September 9, 2014
Revised: October 23, 2014
Published: October 23, 2014

dx.doi.org/10.1021/bi5011333 | *Biochemistry* 2014, 53, 7093−7099

process at different scales. A recent paper by Wey et al.[38] reports newly measured kinetic parameters for a series of mutations at the G12 and G13 positions for Harvey Ras without and with p120GAP. According to these data, replacement of Gly13 with Val slows hydrolysis in Ras-GAP: the corresponding rate constants are 18.0 s^{-1} for ^{wt}Ras-GAP and 0.1 s^{-1} for ^{G13V}Ras-GAP. Replacement of Gly12 with Val affects hydrolysis markedly by reducing the rate constant in ^{G12V}Ras-GAP to 0.01 s^{-1}.

The aim of this work was to provide a plausible explanation of these recent kinetic data for the Ras-GAP-catalyzed GTP hydrolysis at the atomistic level by using advanced molecular modeling tools. At the first stage, we applied a strategy having certain features common to that used previously by us[30] and recently by Gerwert et al.[23,24] Namely, we started from the coordinates of heavy atoms of the crystal structure of Protein Data Bank (PDB) entry 1WQ1,[39] constructed a three-dimensional full-atom model of the Ras-GAP-GTP complex, and calculated its equilibrium geometry configuration using the QM/MM unconstrained geometry optimization. Unlike all previous approaches, a very large QM subsystem comprising a large fraction of the enzyme active site was considered. Energies and forces in QM were computed at the appropriate density functional theory level.

To elucidate the role of G12V and G13V mutations in Ras, we replaced Gly with Val at positions 12 and 13 in the relaxed complex with wild-type Ras and compared results of conformational dynamics of three model systems (^{wt}Ras-GAP-GTP, ^{G12V}Ras-GAP-GTP, and ^{G13V}Ras-GAP-GTP) using MD simulations with QM/MM potentials. We focused on analysis of productive conformations responsible for efficient chemical transformations leading to the cleavage of the phosphorus (P_γ)−oxygen bond in GTP upon approach of the properly aligned catalytic water molecule. The obtained computational results show good correlation with the recent kinetics measurements of the Ras-GAP-catalyzed hydrolysis of GTP, thus providing a clear interpretation of the corresponding reaction mechanisms.

MODELS AND METHODS

A model molecular system was constructed by motifs of the crystal structure of PDB entry 1WQ1[39] followed by the QM/MM optimization of the Ras-GAP-GTP complex with a quantum subsystem comprising a large fraction of the enzyme active site: the triphosphate group of GTP, the catalytic water molecule, the side chains of Lys16, Ser17, Thr35, and Gln61, Mg^{2+} and two water molecules from the magnesium coordination shell, and the side chain of Arg789 from GAP. The quantum part included 86 atoms, while 12500 atoms in total were considered in the QM/MM scheme. For QM/MM calculations, we used the NWChem program package.[40] The customary link atom scheme to treat the boundary and the electronic embedding strategy were applied. The latter approach assumes that electrostatic properties of the MM region are taken into account during calculations on the QM region. The QM subsystem was treated in the DFT/cc-pVDZ approximation with the PBE0 functional.[41,42] The MM subsystem was modeled with the AMBER force field parameters.[43] Upon geometry optimization, QM/MM-MD calculations were conducted with the CP2K program package.[44] The QM part included the same molecular groups as in QM/MM optimization as well as the side chains (Gly or Val) at positions 12 and 13. Technically, the CP2K program operates with the mixed Gaussian and Plane Wave (GPW) basis sets, and the use of the BLYP functional in quantum subsystems is assumed in applications. In our simulations, the DZVP Gaussian basis set and the Goedecker-Teter-Hutter pseudopotentials were applied. The MM part was treated with the CHARMM force field parameters.[45] The model system was solvated in the large water box. No constraints were imposed in the treatment of the MM subsystem.

Sets of MD trajectories of 10−30 ps length each were executed in the *NVT* ensemble at 300 K with a 1 fs integration time step. Analysis of hydrogen bond patterns along MD trajectories relied on the following criteria: the hydrogen bond bonding interactions are assumed if the participating atoms are <2.2 Å apart, and the angle formed between the heavy atoms and donated hydrogen is >120°.

RESULTS

Equilibrium Geometry Configuration of the ^{wt}Ras-GAP-GTP Complex. Figure 1 illustrates a fragment of the active site obtained in the QM/MM geometry optimization. First, we emphasize that the newly obtained structure of the enzyme−substrate complex demonstrates the features common for all previously considered GTPases.[30−32] This is an important conclusion because we use here a different version of QM/MM and a different software package compared to those used in our previous treatment.[30] Among the features critical for the reaction mechanism, we point out a specific alignment of a catalytic water molecule with an almost linear arrangement of three atoms [O(Wat)−P_γ−$O_{\beta\gamma}$]. The equilibrium distance from O(Wat) to P_γ (3.22 Å) is consistent with the previous results for the enzymatic hydrolysis of GTP[30−32] and ATP.[46,47] A proper alignment of water in the Ras-GAP-GTP reactive complex is due to the hydrogen bond network formed by residues Thr35 and Gln61. All three oxygen atoms of the γ-phosphate group of GTP are tightly bound by the surrounding groups: O2 is captured by Mg^{2+}, O3 is involved in hydrogen bonding with Arg789 [the role of the arginine finger for Ras-GAP catalysis has been discussed in numerous

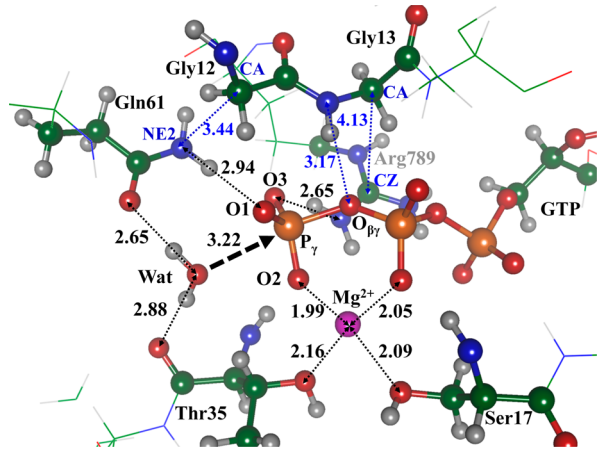


Figure 1. Equilibrium geometry configuration of the ^{wt}Ras-GAP-GTP complex. Here and in all figures, carbon atoms are colored green, oxygen atoms red, nitrogen atoms blue, phosphorus atoms orange, and magnesium atoms magenta. Distances are given in angstroms. Two water molecules from the Mg^{2+} coordination shell are not shown for the sake of clarity.

dx.doi.org/10.1021/bi5011333 | *Biochemistry* 2014, 53, 7093−7099

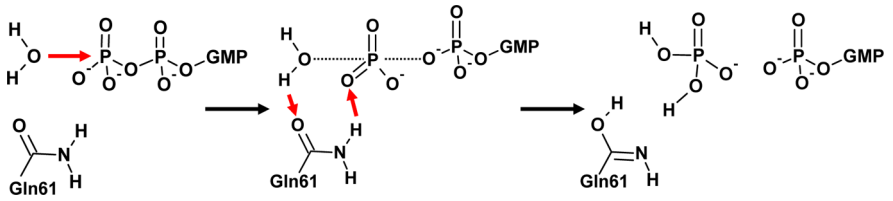


Figure 2. Chemical transformations in the active site of the Ras-Gap-GTP complex consistent with the mechanism of ref 30 and with the structure of the enzyme–substrate complex shown in Figure 1.

publications (e.g., refs 19 and 48)], and O1 forms hydrogen bonds with the protonated amine group of Lys16 (not shown in Figure 1 for the sake of clarity) and with the amide group of Gln61.

It is not instructive to compare a majority of computed geometry parameters and those from the crystal structure of PDB entry 1WQ1[39] in the immediate area of the active site, because introduction of the phosphate as a substitute for the metal fluoride molecule in the crystal disturbs the local environment. However, aside from this area, some comparison is useful: in particular, the CZ(Arg789)–CA(Gly13) distance in the model system, 4.13 Å (distinguished by the blue color in Figure 1), practically coincides with that from the crystal, 4.11 Å, thus showing that interaction of the Arg789 and Gly13 side chains is reproduced well in computations. The N(Gly13)–$O_{\beta\gamma}$ distance in the model system, 3.17 Å, correlates with the value of 2.66 Å in the crystal. Finally, the CA(Gly12)–NE2(Gln61) distance in the model system, 3.44 Å, also reasonably agrees with that in the crystal, 3.86 Å. Analysis of other structural motifs involving the magnesium shell and side chains of Thr35, Lys16, and Ser17 provides validation of the computed model structure, as well.

As shown in our previous study of the chemical step of GTP hydrolysis in Ras-GAP,[30] such a tightly bound structure of the enzyme–substrate complex is responsible for a low-barrier cleavage of the P_γ–$O_{\beta\gamma}$ bond in GTP. The subsequent chemical transformations include inversion of the $P_\gamma O_3$ plane toward the reaction intermediate followed by proton transfers as illustrated in Figure 2. As a result, the inorganic phosphate $H_2PO_4^-$ is formed in complete accord with the most recent experimental and theoretical results from Gerwert's group.[17,24] The appearance of the tautomeric Gln side chain upon GTPase-catalyzed hydrolysis of GTP (bottom side in Figure 2) was hypothesized in an earlier study by Sondek et al.[49] and characterized computationally in ref 30. A similar tautomeric isomer of the Gln side chain can be observed in distinctively different proteins.[50−52] Direct involvement of the Gln61 side chain in chemical transformations upon GTP hydrolysis in Ras-GAP is in agreement with the known critical role of this residue; its replacement should notably affect the hydrolysis reaction and prevent inactivation of Ras from the GTP-bound state.

The orientation of the attacking water molecule shown in Figure 1 corresponds to one of several possible geometric configurations of the active site. In particular, we reported previously[53] that molecular dynamics simulations with the conventional force field parameters predicted another type of orientation of the catalytic water molecule in the active site of the EF-Tu protein. Here we conducted QM/MM geometry optimization for a model system in which the water molecule formed a hydrogen bond with the oxygen atom of the γ-phosphate group of GTP. The corresponding structure is illustrated in Figure S1 of the Supporting Information. Importantly, its QM/MM energy is 7 kJ/mol higher than that of the model system shown in Figure 1.

To justify application of another approximation in the quantum subsystem [the BLYP functional with the Gaussian (DZVP) and plane wave basis sets] and other force field parameters (CHARMM) in calculations of QM/MM-MD trajectories, as compared to the QM/MM calculations of equilibrium geometry configurations with the PBE0/cc-pVDZ approximation in the QM part and the AMBER force field parameters, we located the minimum energy structure of the ^{wt}Ras-GAP-GTP complex in the QM(BLYP/GPW)/MM-(CHARMM) approach. Optimized geometry parameters of this structure, illustrated in Figure S2 of the Supporting Information, are close to those found in the QM(PBE0/cc-pVDZ)/MM(AMBER) approach (Figure 1). The major difference refers to the slightly increased distance (to 3.4 Å) between the oxygen of the catalytic water molecule and the phosphorus of the γ-phosphate group of GTP. Other critical features of the geometry configuration responsible for the efficient chemical reaction remain unchanged.

Comparison of the Dynamics of ^{wt}Ras-GAP-GTP and ^{G13V}Ras-GAP-GTP Complexes. We compared the dynamical properties of the ^{wt}Ras-GAP-GTP and ^{G13V}Ras-GAP-GTP model systems following the results of QM/MM-MD calculations. Trajectories were initiated from the QM/MM-optimized structure of the enzyme–substrate complex in the case of the wild-type Ras and from the manually constructed structure for the ^{G13V}Ras-GAP-GTP mutant. In the latter case, we used partial MM-based optimization of geometry parameters in the local area of the introduced valine side chain prior to the MD runs.

We found that in the case of wild-type Ras, the system fluctuated near the structure that was prepared for efficient chemical transformations leading to the cleavage of the phosphorus–oxygen bond in GTP. In particular, the P_γ–O(Wat) distance fluctuated around 3.4 Å with a standard deviation of 0.2 Å. The dynamics of the system with the G13V mutant is different; it is characterized by an enhanced flexibility in the area occupied by the γ-phosphate group of GTP, the catalytic water, and the side chains of Arg789 and Gln61.

Figure 3 illustrates conformational changes in the ^{G13V}Ras-GAP-GTP model compared to the ^{wt}Ras-GAP-GTP complex. Substitution of a small amino acid residue Gly13 with large hydrophobic Val results in the twist of the guanidine plane of Arg789, leading to rotation of the $P_\gamma O_3$ fragment. In the complex with wild-type Ras, Gln61 forms a hydrogen bond with only O1 of the γ-phosphate, not with O3. Rotation of the $P_\gamma O_3$ fragment results in the formation of two hydrogen bonds, HNE(Gln61)–O1(GTP) and HNE(Gln61)–O3(GTP), thus affecting the alignment of the catalytic water molecule.

It is important to examine the distribution of P_γ–O(Wat) distances along the trajectories. As seen from the data in Table

dx.doi.org/10.1021/bi5011333 | Biochemistry 2014, 53, 7093−7099

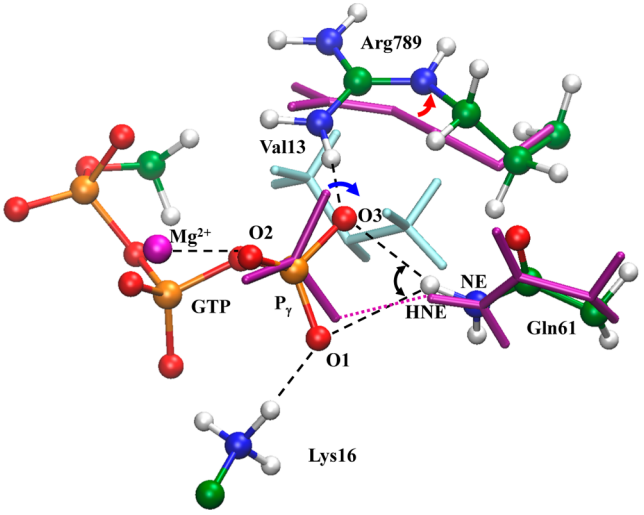


Figure 3. Illustration of conformational changes in the ^{G13V}Ras-GAP-GTP model (the balls and sticks represent the selected molecular groups) compared to the ^{wt}Ras-GAP-GTP complex. Magenta sticks specify groups of the γ-phosphate and the side chains of Gln61 and Arg789 in the ^{wt}Ras-GAP-GTP system. The orientation of the lytic water molecule is essentially the same as in the structure with wild-type Ras shown in Figure 1.

1, the fraction of conformations with a P_γ–O(Wat) distance of <3.4 Å is 53% in the system with wild-type Ras and 38% in the G13V mutant. Hydrogen bond patterns for one of the key residues, Thr35, which is responsible for a proper alignment of the catalytic water molecule, also deserve to be analyzed: in the case of wild-type Ras, the side chain of Thr35 forms a stable hydrogen bond with water (97%); in the G13V mutant, this bond is formed less readily (80%).

Dynamics of the ^{G12V}Ras-GAP-GTP Complex. QM/MM-MD calculations for the ^{G12V}Ras-GAP-GTP system demonstrated even stronger deviation of the active site from a productive conformation (Figure 1) than in the case of the ^{G13V}Ras-GAP-GTP complex. We show in Figure 4 positions of the triphosphate groups, catalytic water, and side chains of Arg789 and Gln61 in the ^{wt}Ras-GAP-GTP model system using sticks and in the ^{G12V}Ras-GAP-GTP system using balls and sticks. Apparently, the G12V mutation leads to a displacement of the Gln61 side chain apart from GTP (the hydrogen bond

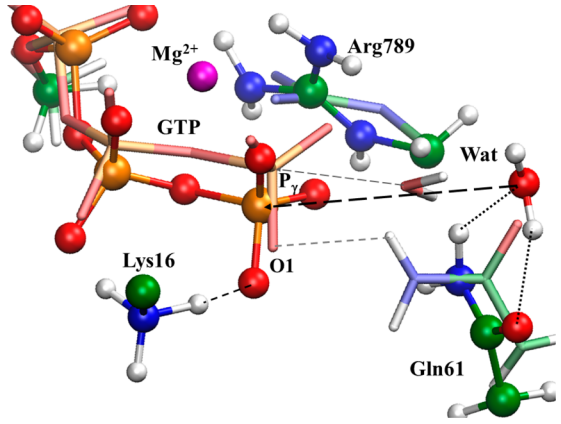


Figure 4. Fragment of the active site in the enzyme–substrate complex. The sticks illustrate an arrangement of the γ-phosphate and the side chains of Gln61 and Arg789 in the ^{wt}Ras-GAP-GTP system. Balls and sticks show the corresponding molecular groups in the ^{G12V}Ras-GAP-GTP system.

with O1 of γ-phosphate is lost) and the catalytic water molecule from their favorable arrangements in the active site.

Consequences of such changes are clearly seen in Figure 5, demonstrating the distribution of P_γ–O(Wat) distances along a

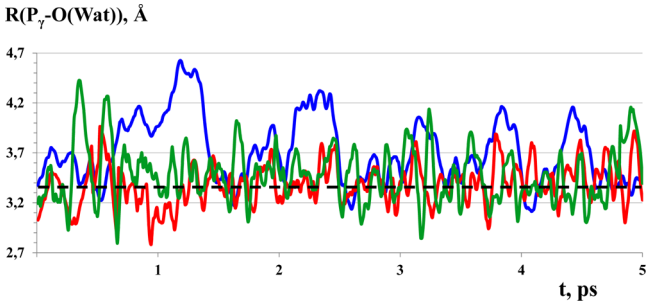


Figure 5. Distribution of P_γ–O(Wat) distances in ^{wt}Ras-GAP-GTP (red), ^{G12V}Ras-GAP-GTP (blue), and ^{G13V}Ras-GAP-GTP (green) complexes along a fragment of the QM/MM-MD trajectory.

representative fragment of the QM/MM-MD trajectory for ^{wt}Ras-GAP-GTP (red graph), ^{G12V}Ras-GAP-GTP (blue graph), and ^{G13V}Ras-GAP-GTP (green graph). This distance equals 3.2 Å in the corresponding minimum energy structure (Figure 1);

Table 1. Comparison of Dynamical Parameters along QM/MM-MD Trajectories for the ^{wt}Ras-GAP-GTP, ^{G13V}Ras-GAP-GTP, and ^{G12V}Ras-GAP-GTP Model Systems[a]

geometry parameter	statistics	^{wt}Ras-GAP-GTP	^{G13V}Ras-GAP-GTP	^{G12V}Ras-GAP-GTP
P_γ(GTP)–O(Wat)	mean (standard deviation)	3.38 (0.21)	3.56 (0.39)	3.71 (0.38)
	maximum/minimum	3.96/2.78	4.77/2.79	5.35/2.93
	%	53	38	18
HNE(Gln61)–O1	mean (standard deviation)	2.29 (0.32)	2.43 (0.29)	3.13 (0.31)
	maximum/minimum	3.40/1.56	3.32/1.72	4.23/2.24
	%	42	24	0
HNE(Gln61)–O3	mean (standard deviation)	3.11 (0.39)	2.56 (0.53)	2.43 (0.25)
	maximum/minimum	4.09/2.12	4.11/1.65	3.23/1.8
	%	<1	32	19
O(Thr35)–H(Wat)	mean (standard deviation)	1.91 (0.15)	2.05 (0.26)	1.89 (0.16)
	maximum/minimum	2.87/1.55	3.66/1.52	2.55/1.50
	%	97	80	95

[a]Mean values and standard deviations (in parentheses) are given in angstroms.

dx.doi.org/10.1021/bi5011333 | Biochemistry 2014, 53, 7093–7099

it fluctuates near 3.4 Å along the QM/MM-MD trajectory (Table 1) in the case of wild-type Ras and, to a lesser extent, in the case of ^{G13V}Ras-GAP-GTP. However, upon G12V substitution, a considerable elongation of this geometry parameter should be noted. Beyond moving apart from the γ-phosphate, the catalytic water molecule changes its orientation relative to reacting groups of GTP (Figure 4), thus reducing the frequency of conformations favorable for the fast chemical reaction.

DISCUSSION

According to recent kinetic measurements,[38] the reaction rate constant for the GTP hydrolysis by mutated ^{G13V}Ras in the complex with GAP is ∼2 orders of magnitude lower than in the case of ^{wt}Ras. The performance of the ^{G12V}Ras-GAP complex is even worse, showing a reduction in the rate constant of 3 orders of magnitude compared to that of the wild-type proteins. The mechanistic picture that can be drawn from the results of our simulations is qualitatively consistent with these observations. An enhanced flexibility in the active site in the ^{G13V}Ras-GAP-GTP complex, reflected by a displacement of the γ-phosphate group interacting with the arginine finger Arg789, should not completely inhibit the reaction but should lower its rate. In the case of the ^{G12V}Ras-GAP-GTP complex, a partial distortion of the active site occurs, affecting the critically important Gln61 side chain and the productive alignment of the catalytic water molecule (Figure 4); the consequences of such disorder should be more pronounced affecting the reaction rate to a larger extent than for the G13V mutation.

The importance of the inflexible arrangement of the glutamine side chain in the DxxGQ motif (here, Asp57-Thr58-Ala59-Gly60-Gln61) typical for small GTPases was discussed, in particular, in ref 21. In the case of Ras, cooperation of the Thr35 and Gln61 side chains assists with the favorable position of the catalytic water molecule resulting in the well-defined active site (Figure 1). Enhanced flexibility of Gln61 should lower the rate of chemical reaction of GTP hydrolysis. Following QM/MM-MD simulations, we demonstrate here that the G13V and G12V replacements in Ras are responsible for certain disruption of the active site of the Ras-GAP-GTP complex. In agreement with the recent kinetic experimental data,[38] the effect of the G12V mutation should be more pronounced.

The conclusions of this work rely on the mechanism of GTP hydrolysis in Ras-GAP, according to which Gln61 is directly involved in the chemical transformations as suggested in refs 30 and 49. It was shown previously that this mechanism is capable of interpreting the effect of mutation R789L[30] in the Ras-GAP-catalyzed reaction as well as kinetic isotope effects upon enzyme-catalyzed GTP hydrolysis.[54]

Finally, we comment on the necessity to apply a fairly expensive QM/MM-MD methodology to analyze conformational changes in the active site of Ras-GAP upon mutations. We reported earlier[32,53] that molecular mechanics or molecular dynamics simulations with the conventional force field parameters failed to reproduce the enzyme−substrate structure for GTP hydrolysis in the EF-Tu protein consistent with the results of quantum-based calculations; a proper alignment of the catalytic water molecule could not be achieved in these simulations. Calculations performed in this work provide further support for this conclusion; an analysis of conformations productive for the chemical reaction of GTP hydrolysis inside the protein cavity could not be accomplished within classical MD without additional corrections of the force field parameters. Application of the QM/MM-MD methodology guarantees more precise estimates of energies and forces in quantum subsystems.

CONCLUSION

We describe the results of QM/MM and QM/MM-MD simulations with a large fraction of the active site molecular groups included in the quantum subsystem aiming to clarify the role of mutations G12V and G13V in Ras in the Ras-GAP-catalyzed hydrolysis of GTP. The newly obtained equilibrium geometry configuration of the enzyme−substrate complex ^{wt}Ras-GAP-GTP is consistent with the previously established mechanism of chemical transformations inside the protein cavity. Computed dynamical behavior of the enzyme−substrate complexes with the mutated variants is consistent with the recent kinetic experimental data. The ^{wt}Ras-GAP-GTP model system fluctuates near the energy-minimized structure from which the cleavage of the phosphorus (P_γ)−oxygen bond in GTP occurs upon an approach of the properly aligned catalytic water molecule. The dynamics of the system with the G13V mutant is characterized by an enhanced flexibility in the area occupied by the γ-phosphate group of GTP, the catalytic water, and the side chains of Arg789 and Gln61, which should somewhat hinder fast chemical steps. The conformational dynamics of the system with the G12V mutant demonstrated considerable shifts of the Gln61 side chain and catalytic water from their favorable arrangement in the active site, thus slowing the hydrolysis reaction.

ASSOCIATED CONTENT

Supporting Information

Illustration of the active site of the ^{wt}Ras-GAP-GTP complex corresponding to a local minimum energy configuration with an alternative orientation of the catalytic water molecule (Figure S1) and a comparison of the equilibrium geometry configurations of the active site of the ^{wt}Ras-GAP-GTP complex optimized in QM/MM calculations (CP2K program suite) with the BLYP functional with the Gaussian (DZVP) and plane wave basis sets in the quantum part and the CHARMM force field parameters in MM and QM/MM calculations (NWChem program suite) with the PBE0 functional with the cc-pVDZ basis sets in the quantum part and the AMBER force field parameters in MM (Figure S2). This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*Department of Chemistry, M. V. Lomonosov Moscow State University, Leninskie Gory 1/3, Moscow 119991, Russian Federation. Phone: +7-495-939-10-96. E-mail: anemukhin@yahoo.com or anem@lcc.chem.msu.ru.

Funding

This study was supported by the Russian Science Foundation (Project 14-13-00124).

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We acknowledge the use of supercomputer resources of the M. V. Lomonosov Moscow State University[55] and of the Joint Supercomputer Center of the Russian Academy of Sciences.

REFERENCES

(1) Sprang, S. R. (1997) G proteins, Effectors and GAPs: Structure and Mechanism. *Curr. Opin. Struct. Biol. 7*, 849−856.
(2) Wittinghofer, A. (1998) Signal Transduction via Ras. *Biol. Chem. 379*, 933−937.
(3) Ahmadian, M. R., Zor, T., Vogt, D., Kabsch, W., Selinger, Z., Wittinghofer, A., and Scheffzek, K. (1999) Guanosine Triphosphatase Stimulation of Oncogenic Ras Mutants. *Proc. Natl. Acad. Sci. U.S.A. 96*, 7065−7070.
(4) Downward, J. (2003) Targeting RAS Signalling Pathways in Cancer Therapy. *Nat. Rev. Cancer 3*, 11−22.
(5) Goitre, L., Trapani, E., Trabalzini, L., and Retta, S. F. (2014) The Ras Superfamily of Small GTPases: The Unlocked Secrets. *Methods Mol. Biol. 1120*, 1−18.
(6) Li, G., and Zhang, X. C. (2004) GTP Hydrolysis Mechanism of Ras-like GTPases. *J. Mol. Biol. 340*, 921−932.
(7) Frushicheva, M. P., Mills, M. J., Schopf, P., Singh, M. K., Prasad, R. B., and Warshel, A. (2014) Computer Aided Enzyme Design and Catalytic Concepts. *Curr. Opin. Chem. Biol. 21C*, 56−62.
(8) Glennon, T. M., Villa, J., and Warshel, A. (2000) How Does GAP Catalyze the GTP Reaction of Ras?: A Computer Simulation Study. *Biochemistry 39*, 9641−9651.
(9) Shurki, A., and Warshel, A. (2004) Why Does the Ras Switch "Break" by Oncogenic Mutations? *Proteins 55*, 1−10.
(10) Kläbn, M., Rosta, E., and Warshel, A. (2006) On the Mechanism of Hydrolysis of Phosphate Monoesters Dianions in Solutions and Proteins. *J. Am. Chem. Soc. 128*, 15310−15323.
(11) Rosta, E., Kamerlin, S. C., and Warshel, A. (2008) On the Interpretation of the Observed Linear Free Energy Relationship in Phosphate Hydrolysis: A Thorough Computational Study of Phosphate Diester Hydrolysis in Solution. *Biochemistry 47*, 3725−37235.
(12) Kamerlin, S. C., Florián, J., and Warshel, A. (2008) Associative Versus Dissociative Mechanisms of Phosphate Monoester Hydrolysis: On the Interpretation of Activation Entropies. *ChemPhysChem 9*, 1767−1773.
(13) Kamerlin, S. C., and Warshel, A. (2009) On the Energetics of ATP Hydrolysis in Solution. *J. Phys. Chem. B 113*, 15692−15698.
(14) Plotnikov, N. V., Prasad, B. R., Chakrabarty, S., Chu, Z. T., and Warshel, A. (2013) Quantifying the Mechanism of Phosphate Monoester Hydrolysis in Aqueous Solution by Evaluating the Relevant Ab Initio QM/MM Free-Energy Surfaces. *J. Phys. Chem. B 117*, 12807−12819.
(15) Prasad, B. R., Plotnikov, N. V., and Warshel, A. (2013) Addressing Open Questions about Phosphate Hydrolysis Pathways by Careful Free Energy Mapping. *J. Phys. Chem. B 117*, 153−163.
(16) Prasad, B. R., Plotnikov, N. V., Lameira, J., and Warshel, A. (2013) Quantitative Exploration of the Molecular Origin of the Activation of GTPase. *Proc. Natl. Acad. Sci. U.S.A. 110*, 20509−20514.
(17) Kötting, C., Blessenohl, M., Suveyzdis, Y., Goody, R. S., Wittinghofer, A., and Gerwert, K. (2006) A Phosphoryl Transfer Intermediate in the GTPase Reaction of Ras in Complex with its GTPase-Activating Protein. *Proc. Natl. Acad. Sci. U.S.A. 103*, 13911−13916.
(18) te Heesen, H., Gerwert, K., and Schlitter, J. (2007) Role of the Arginine Finger in Ras·RasGAP Revealed by QM/MM Calculations. *FEBS Lett. 581*, 5677−5684.
(19) Kötting, C., Kallenbach, A., Suveyzdis, Y., Wittinghofer, A., and Gerwert, K. (2008) The GAP Arginine Finger Movement into the Catalytic Site of Ras Increases the Activation Entropy. *Proc. Natl. Acad. Sci. U.S.A. 105*, 6260−6265.
(20) Xia, F., Rudack, T., Kötting, C., Schlitter, J., and Gerwert, K. (2011) The Specific Vibrational Modes of GTP in Solution and Bound to Ras: A Detailed Theoretical Analysis by QM/MM Simulations. *Phys. Chem. Chem. Phys. 13*, 21451−21460.
(21) Gavriljuk, K., Gazdag, E. M., Itzen, A., Kötting, C., Goody, R. S., and Gerwert, K. (2012) Catalytic Mechanism of a Mammalian Rab·RabGAP Complex in Atomic Detail. *Proc. Natl. Acad. Sci. U.S.A. 109*, 21348−21353.
(22) Rudack, T., Xia, F., Schlitter, J., Kötting, C., and Gerwert, K. (2012) Ras and GTPase-Activating Protein (GAP) Drive GTP into a Precatalytic State as Revealed by Combining FTIR and Biomolecular Simulations. *Proc. Natl. Acad. Sci. U.S.A. 109*, 15295−15300.
(23) Rudack, T., Xia, F., Schlitter, J., Kötting, C., and Gerwert, K. (2012) The Role of Magnesium for Geometry and Charge in GTP Hydrolysis, Revealed by Quantum Mechanics/Molecular Mechanics Simulations. *Biophys. J. 103*, 293−302.
(24) Xia, F., Rudack, T., Cui, Q., Kötting, C., and Gerwert, K. (2012) Detailed Structure of the $H_2PO_4^-$-Guanosine Diphosphate Intermediate in Ras-GAP Decoded from FTIR Experiments by Biomolecular Simulations. *J. Am. Chem. Soc. 134*, 20041−20044.
(25) Kötting, C., and Gerwert, K. (2013) The Dynamics of the Catalytic Site in Small GTPases, Variations on a Common Motif. *FEBS Lett. 587*, 2025−2027.
(26) Prakash, P., and Gorfe, A. A. (2013) Lessons from Computer Simulations of Ras Proteins in Solution and in Membrane. *Biochim. Biophys. Acta 1830*, 5211−5218.
(27) Sharma, N., Sonavane, U., and Joshi, R. (2014) Probing the Wild-Type HRas Activation Mechanism Using Steered Molecular Dynamics, Understanding the Energy Barrier and Role of Water in the Activation. *Eur. Biophys. J. 43*, 81−95.
(28) Martín-García, F., Mendieta-Moreno, J. I., López-Viñas, E., Gómez-Puertas, P., and Mendieta, J. (2012) The Role of Gln61 in HRas GTP Hydrolysis: A Quantum Mechanics/Molecular Mechanics Study. *Biophys. J. 102*, 152−157.
(29) Topol, I. A., Cachau, R. E., Nemukhin, A. V., Grigorenko, B. L., and Burt, S. K. (2004) Quantum Chemical Modeling of the GTP Hydrolysis by the RAS-GAP Protein Complex. *Biochim. Biophys. Acta 1700*, 125−136.
(30) Grigorenko, B. L., Nemukhin, A. V., Topol, I. A., Cachau, R. E., and Burt, S. K. (2005) QM/MM Modeling the Ras-GAP Catalyzed Hydrolysis of Guanosine Triphosphate. *Proteins: Struct., Funct., Bioinf. 60*, 495−503.
(31) Grigorenko, B. L., Nemukhin, A. V., Shadrina, M. S., Topol, I. A., and Burt, S. K. (2007) Mechanisms of Guanosine Triphosphate Hydrolysis by Ras and Ras-GAP Proteins as Rationalized by Ab Initio QM/MM Simulations. *Proteins: Struct., Funct., Bioinf. 66*, 456−466.
(32) Grigorenko, B. L., Shadrina, M. S., Topol, I. A., Collins, J. R., and Nemukhin, A. V. (2008) Mechanism of the Chemical Step for the Guanosine Triphosphate (GTP) Hydrolysis Catalyzed by Elongation Factor Tu. *Biochim. Biophys. Acta 1784*, 1908−1917.
(33) Warshel, A., and Levitt, M. (1976) Theoretical Studies of Enzymic Reactions: Dielectric, Electrostatic and Steric Stabilization of the Carbonium Ion in the Reaction of Lysozyme. *J. Mol. Biol. 103*, 227−249.
(34) Gibbs, J. B., Schaber, M. D., Allard, W. J., Sigal, I. S., and Scolnick, E. M. (1988) Purification of Ras GTPase Activating Protein from Bovine Brain. *Proc. Natl. Acad. Sci. U.S.A. 85*, 5026−5030.
(35) Krengel, U., Schlichting, I., Scherer, A., Schumann, R., Frech, M., John, J., Kabsch, W., Pai, E. F., and Wittinghofer, A. (1990) Three-Dimensional Structures of H-ras p21 Mutants: Molecular Basis for their Inability to Function as Signal Switch Molecules. *Cell 62*, 539−548.
(36) Gideon, P., John, J., Frech, M., Lautwein, A., Clark, R., Scheffler, J. E., and Wittinghofer, A. (1992) Mutational and Kinetic Analyses of the GTPase-Activating Protein (GAP)-p21 Interaction: The C-Terminal Domain of GAP is not Sufficient for Full Activity. *Mol. Cell. Biol. 12*, 2050−2056.
(37) Chung, H. H., Benson, D. R., and Schultz, P. G. (1993) Probing the Structure and Mechanism of Ras Protein with an Expanded Genetic Code. *Science 259*, 806−809.
(38) Wey, M., Lee, J., Jeong, S. S., Kim, J., and Heo, J. (2013) Kinetic Mechanisms of Mutation-Dependent Harvey Ras Activation and their Relevance for the Development of Costello Syndrome. *Biochemistry 52*, 8465−8479.
(39) Scheffzek, K., Ahmadian, M. R., Kabsch, W., Wiesmuller, L., Lautwein, A., Schmitz, F., and Wittinghofer, A. (1997) The Ras-

RasGAP Complex: Structural Basis for GTPase Activation and its Loss in Oncogenic Ras Mutants. *Science 277*, 333−338.
(40) Valiev, M., Bylaska, E. J., Govind, N., Kowalski, K., Straatsma, T. P., van Dam, H. J. J., Wang, D., Nieplocha, J., Apra, E., Windus, T. L., et al. (2010) NWChem: A Comprehensive and Scalable Open-Source Solution for Large Scale Molecular Simulations. *Comput. Phys. Commun. 181*, 1477−1489.
(41) Perdew, J. P., Burke, K., and Ernzerhof, M. (1996) Generalized Gradient Approximation Made Simple. *Phys. Rev. Lett. 77*, 3865−3868.
(42) Adamo, C., and Barone, V. (1999) Toward Reliable Density Functionals without Adjustable Parameters: The PBE0 Model. *J. Chem. Phys. 110*, 6158−6170.
(43) Cornell, W. D., Cieplak, P., Bayly, C. I., Gould, I. R., Merz, K. M., Ferguson, D. M., Spellmeyer, D. C., Fox, T., Caldwell, J. W., and Kollman, P. A. (1995) A Second Generation Force Field for the Simulation of Proteins, Nucleic Acids, and Organic Molecules. *J. Am. Chem. Soc. 117*, 5179−5197.
(44) VandeVondele, J., Krack, M., Mohamed, F., Parrinello, M., Chassaing, T., and Hutter, J. (2005) Quickstep: Fast and Accurate Density Functional Calculations Using a Mixed Gaussian and Plane Waves Approach. *Comput. Phys. Commun. 167*, 103−128.
(45) MacKerell, A. D., Bashford, D., Bellott, M., Dunbrack, R. L., Evanseck, J. D., Field, M. J., Fischer, S., Gao, J., Guo, H., Ha, S., et al. (1998) All-Atom Empirical Potential for Molecular Modeling and Dynamics Studies of Proteins. *J. Phys. Chem. B 102*, 3586−3616.
(46) Grigorenko, B. L., Rogov, A. V., Topol, I. A., Burt, S. K., Martinez, H. M., and Nemukhin, A. V. (2007) Mechanism of the Myosin Catalyzed Hydrolysis of ATP as Rationalized by Molecular Modeling. *Proc. Natl. Acad. Sci. U.S.A. 104*, 7057−7061.
(47) Grigorenko, B. L., Kaliman, I. A., and Nemukhin, A. V. (2011) Minimum Energy Reaction Profiles for ATP Hydrolysis in Myosin. *J. Mol. Graphics Modell. 31*, 1−4.
(48) Bourne, H. R. (1997) G Proteins. The Arginine Finger Strikes Again. *Nature 389*, 673−674.
(49) Sondek, J., Lambright, D. G., Noel, J. P., Hamm, H. E., and Sigler, P. B. (1994) GTPase Mechanism of G Proteins from the 1.7-Å Crystal Structure of Transducin α-GDP-AIF-4. *Nature 372*, 276−279.
(50) Domratcheva, T., Grigorenko, B. L., Schlichting, I., and Nemukhin, A. V. (2008) Molecular Models Predict Light-induced Glutamine Tautomerization in BLUF Photoreceptors. *Biophys. J. 94*, 3872−3879.
(51) Khrenova, M. G., Domratcheva, T., Schlichting, I., Grigorenko, B. L., and Nemukhin, A. V. (2011) Computational Characterization of Reaction Intermediates in the Photocycle of the Sensory Domain of the AppA Blue Light Photoreceptor. *Photochem. Photobiol. 87*, 564−573.
(52) Khrenova, M. G., Nemukhin, A. V., and Domratcheva, T. (2013) Photoinduced Electron Transfer Facilitates Tautomerization of the Conserved Signaling Glutamine Side Chain in BLUF Protein Light Sensors. *J. Phys. Chem. B 117*, 2369−2377.
(53) Shadrina, M. S., Grigorenko, B. L., and Nemukhin, A. V. (2008) Structure of the Enzyme-Substrate Complex for Guanosine Triphosphate Hydrolysis by Elongation Factor EF-Tu: Comparison of Quantum Mechanics/Molecular Mechanics and Molecular Dynamics Results. *Moscow Univ. Chem. Bull. (Engl. Transl.) 63*, 321−323.
(54) Nemukhin, A. V., Shadrina, M. S., Grigorenko, B. L., and Du, X. (2009) Simulated ^{18}O Kinetic Isotope Effects in Enzymatic Hydrolysis of Guanosine Triphosphate. *Biochemistry (Moscow) 74*, 1044−1048.
(55) Voevodin, Vl. V., Zhumatiy, S. A., Sobolev, S. I., Antonov, A. S., Bryzgalov, P. A., Nikitenko, D. A., Stefanov, K. S., and Voevodin, Vad. V. (2012) Practice of "Lomonosov" Supercomputer. *Open Systems Journal (Moscow) 7*, 36−39.