ISSN 1070-3632, Russian Journal of General Chemistry, 2008, Vol. 78, No. 4, pp. 696–703. © Pleiades Publishing, Ltd., 2008.
Original Russian Text © A.V. Nemukhin, B.L. Grigorenko, M.S. Shadrina, 2007, published in Rossiiskii Khimicheskii Zhurnal, 2007, Vol. 51, No. 5, pp. 27–33.

Mechanisms of Enzymatic Hydrolysis of Nucleoside Triphosphates by Quantum and Molecular Mechanics

A. V. Nemukhin[a,b], B. L. Grigorenko[a], and M. S. Shadrina[a]

[a]*Chemical Department, Lomonosov Moscow State University, Leninskie Goty 1/3, Moscow, 119992 Russia*
e-mail: anem@lcc.chem.msu.ru

[b]*Emanuel Institute of Biochemical Physics, Russian Academy of Sciences, ul. Kosygina 4, Moscow, 119334 Russia*

Received August 20, 2007

Abstract—Results of simulation of the mechanism of hydrolysis of adenosine triphosphate and guanosine triphosphate in protein matrices, as well as of deprotonated methyl triphosphate in water clusters by quantum and molecular mechanics with separation of the reaction system into conformationally flexible effective fragments are discussed.

DOI: 10.1134/S1070363208040312

INTRODUCTION

Hydrolysis of nucleoside triphosphates represents essential biochemical reactions responsible, in particular, for energy transformation (hydrolysis of adenosine triphosphate, ATP) and signal transduction (hydrolysis of guanosine triphosphate, GTP) in live systems. In spite of the importance of the hydrolysis of nucleoside triphosphate (NTP), leading to nucleoside diphosphate (NDP) and inorganic phosphate (P_i):

$$NTP + H_2O \rightarrow NBP + P_{i,} \quad (1)$$

the mechanism of this essential reaction is still under discussion. The problem of whether this mechanism is dissociative or associative, which relates to different configurations of the transition state of the limiting stage, still remains unsolved. To know the mechanism of enzymatic hydrolysis [reaction (1)] is necessary for forcasting effects of point mutations in the protein matrix, that lead, in particular, to pathological changes in live systems.

In view of the exceptional importance of NTP hydrolysis, this reaction has been extensively studied at both experimental and theoretical levels. The PubMed database (URL http://www.pubmedcentral.nih.gov) contains more that 10500 papers on ATP hydrolysis and about 4000 papers on GTP hydrolysis.

In the present paper we discuss the results of simulation of the mechanisms of enzymatic hydrolysis of ATP and GTP by means of a combined quantum and molecular mechanics (QM/MM) approach.

In the QM/MM method, the energies and forces acting on atoms in a small central part of a large molecular system are calculated by quantum-chemical equations [1], and the surrounding subsystem is described in terms of molecular-mechanics force fields [2]. At present this method is the principal tool for simulation of the mechanisms of chemical reactions in protein matrices and in solutions. In applications of the QM/MM method to simulation of the mechanisms of chemical reactions, the energy in each point of the potential energy surface (PES) is combined of the energy of the quantum part in the field of the MM subsystem and the molecular mechanics energy. The mechanism of a reaction is explored by an analysis of points on the PES, i.e. geometric configurations of local minima corresponding to reagents, reaction products, and possible intermediates, as well as saddle points between local minima. Special attention is paid to calculation of energy barriers on the path from reagents to reaction products. Transition to experimentally significant values, such as reaction rate constants, is preformed in terms of the transition state theory.

The idea of the QM/MM method was formulated by Warshel and Levitt in 1976 [3], and over the

following 30 years a number of versions of computer realization of the QM/MM approach, differing in the way of accounting for interrelation of the QM and MM subsystems appeared. Far not all of these versions adequately describe the effect of protein matrix on reaction center, which explains the fairly great number of publications containing unreally high estimates for the activation barriers of enzymatic catalysis (over 20 kcal mol^{-1}). In particular, this situation is quite characteristic for simulation of ATP and GTP hydrolysis. Our used version of the QM/MM method is based on the concept of conformationally flexible effective fragments [4–9], which allows the results for the whole system (QM+MM) to approach fairly closely those obtained by nonempirical calculations. This approach is realized within the framework of a high-performance version of GAMESS [10], namely, PC GAMESS [11] (developer A. Granovsky, URL http://lcc.chem.msu.ru/gran/gamess), in combination with TINKER molecular simulation package (URL http://dasher.wustl.edu/tinker).

Molecular models for calculation of the energy profiles of NTP hydrolysis reactions are constructed of atomic coordinates of protein complexes, archived in the Protein Data Bank (PDB, URL http://www.rcsb.org.pdb). By experimental conditions (X-ray diffraction or NMR studies), the structures in PDB are related to analogs of enzyme–substrate complexes, most frequently with slowly hydrolysable analogs of nucleoside triphosphates. Accordingly, the task of simulation is to reproduce a real enzyme–substrate complexes, simulate hydrolysis (1) to products, and compare the calculated energy profile of the reaction path with experimental data.

In what follows we discuss the results obtained by molecular simulation methods for certain important hydrolysis reactions (1).

Myosin-catalyzed Hydrolysis of Adenosine Triphosphate

Myosin is a protein that induces ATP hydrolysis with release of the ATP-stored energy that provides muscle contraction. In the course of ATP hydrolysis under the action of myosin the latter changes conformation, which results in directed displacement of the "head" of this protein along actin fibers. The overall process includes capture of ATP with myosin in the initial conformation (M*), hydrolysis (1), change of the protein conformation (M**), and release of products: adenosine diphosphate (ADP) and inorganic phosphate P_i. Schematically, this process can be written as follows:

$$\mathrm{M^* + ATP \rightarrow M^* \times ATP \xrightarrow{H_2O} M^{**}(ADP + P_i) \rightarrow ADP + P_i}. \quad (2)$$

This process is described by a multistage kinetic scheme [12] from which it follows that that the intrinsic activation barrier of a chemical reaction, from enzyme–substrate to enzyme–products complex, should not exceed values of about 10 kcal mol^{-1} at inconsiderable conformational changes of the protein. However, quantum-chemical calculations in terms of the so-called associative mechanism of hydrolysis result in energy profiles with at least three times higher barriers [13–15]. In [16], based QM/MM calcula-tions, we constructed a different model developing a heuristic hypothesis [17] that the reaction involves two active water molecules and found that the calculation results were fully consistent with experimental data.

For quantum-chemical calculations of a model biomolecular system we used as starting coordinates of heavy atoms those in the crystal structure of the myosin complex with $Mg^{2+} \times$ ADP $\times$ VO_4^- (vanadate substitutes in the crystal structure the γ-phosphate group of ATP, and the magnesium cation in the protein matrix is necessary for binding negatively charged ATP and ADP molecules) (PDB code 1VOM [18]). Further on, by molecular simulation we constructed a reactive molecular system in the following way: introduced the γ-PO_3^- group instead of vanadate, added hydrogen atoms lacking in the 1VOM crystal structure, and added water molecules in cavities of the myosin structure and on its surface. The QM subsystem (a total of 47 atoms) of the resulting biomolecular system included ATP phosphate groups, magnesium cation, side chains of the amino acid residues of the so-called salt bridge Arg238–Glu459, and two water molecules Wat1 and Wat2 clamped in the active site with the salt bridge (Fig. 1, the QM subsystem is depicted by balls and rods). The MM subsystem which fully enclosed the active site included 1800 atoms of the protein molecule, grouped in conformationally flexible effective fragments [4, 5] interacting with the QM subsystem. The energies and forces acting in the QM part were calculated by the Hartree–Fock method with the LANL2DZdp_ECP basis set (and pseudo-potential for phosphorus) [19]. Atomic interactions in the MM

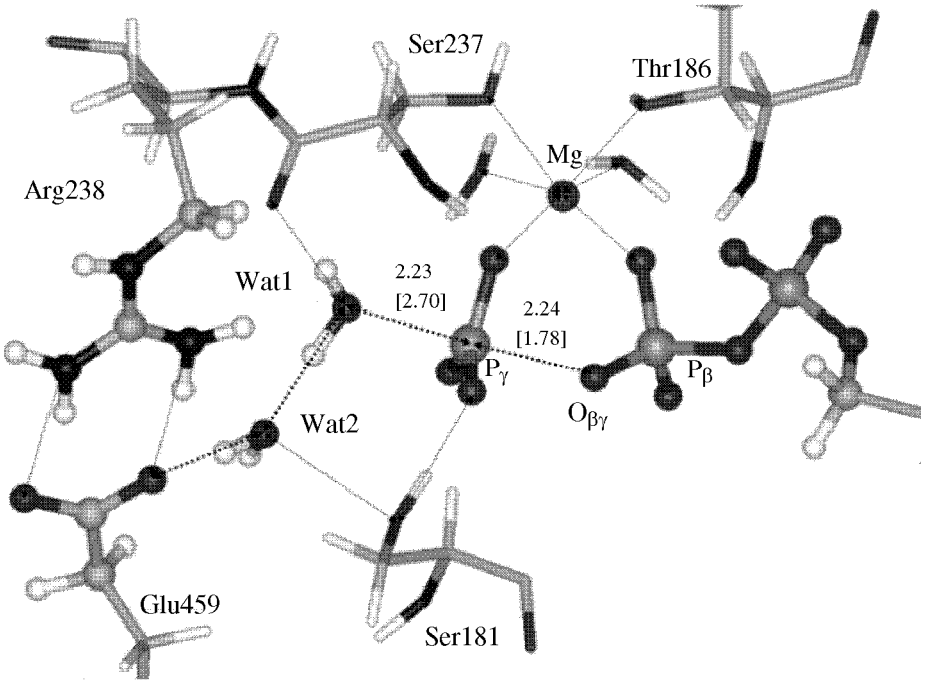

Fig. 1. Calculated geometric configuration of the active site on the top of the potential barrier for ATP hydrolysis by myosin. Distances (in Å) shown without brackets relate to the transition state and those shown in brackets, to the enzyme–substrate complex.

subsystem were described in terms of the AMBER force field [20]. The equilibrium configuration of the enzyme–substrate complex, obtained by full geometry optimization, fits well its crystal structure [16].

For the reaction coordinate we chose the distance between P_γ and the oxygen atom in the Wat1 molecule directed by side chains of the protein to a position appropriate for nucleophilic attack. This distance is gradually diminished from the starting value of 2.70 Å in the enzyme–substrate complex, and all the other geometric parameters are optimized in each point. Proton displacements along the oriented chain of hydrogen bonds Wat1 → Wat2 → Glu459, which are barrierless in this case [21], complete the process.

The calculated energy of product formation is –3.5 kcal mol^{-1} with respect to the enzyme–substrate complex. Thus, chemically, ATP hydrolysis reaction (2) at a constant global myosin conformation M* occurs in one stage with a single transition state shown in Fig. 1. Protonation of the Glu459 residue creates prerequisites for cleavage of the Arg238—Glu459 and considerable conformational transformations in myosin. By the QM/MM method we calculated the atomic configuration on the top of the potential barrier (see Fig. 1). The height of the activation barrier in this point of the transition state was estimated at 5 kcal mol^{-1}. The γ-phosphate group separated from ADP is planar in the transition state. During motion over the potential surface to reaction products the PO_3^- group undergoes stereochemical inversion to adopt a configuration favorable for bond formation between the hydroxyl of the nucleophilic Wat1 molecule and the P_γ atom and, correspondingly, for formation of the inorganic phosphate HPO_4^{2-}.

Hydrolysis of Guanosine Triphosphate, Catalyzed by the RAS Protein

Guanosine triphosphate plays an important role in the transduction of regulatory signals from cell membrane receptors to cell nuclei. The human enzyme $p21^{ras}$ (hereinfter, Ras) having a GTP molecule in the active site acts as switch in signal transduction. If RAS binds GTP, then signals coming to receptors activate cell, and it thus gains the capacity to grow and change. Hydrolysis converts GTP into guanosine diphosphate (GDP), thus transferring the physiological system into the "switched-off" position and blocking signal transduction. Note that about 30% of human tumor cells were found to contain mutated RAS proteins

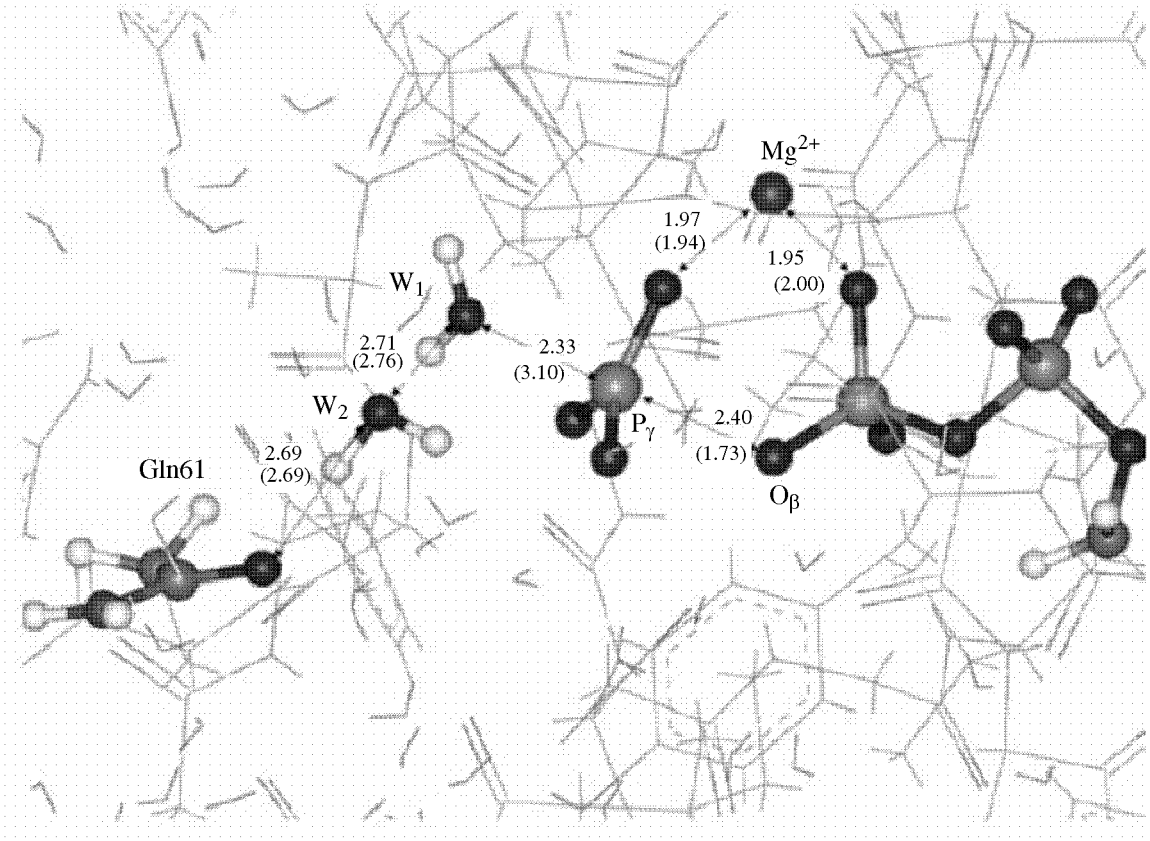

Fig. 2. Calculated geometric configuration of the active site on the top of the potential barrier for GTP hydrolysis by RAS. Distances (in Å) shown without brackets relate to the transition state and those shown in brackets, to the enzyme–substrate complex.

blocked in the active state. They are incapable to switch off (terminate) signal transduction, which can result in tumor formation.

The theoretical approaches to RAS-catalyzed GTP hydrolysis were realized in the framework of a specially parametrized empirical scheme of the valence bond method [22–24], as well as quantum-chemical [25], and molecular dynamics methods [26, 27]. The resulting data proved to be controversial and give no way for unambiguous conclusions concerning the mechanism of hydrolysis in the protein matrix.

In [28], for simulation the hydrolysis of GTP by RAS we made use of a strategy similar to that described above for the ATP–myosin system. For the starting coordinates of heavy atoms we considered the structure of the GTP (GTP) × Mg^{2+} × RAS complex (PDB code 1QRA) corresponding to the prehydrolysis state of the system at low temperatures [29]. The position of the substrate molecule in the protein was refined by means of molecular docking [30]. Lacking hydrogen atoms and water molecules were added. The configuration of the enzyme–substrateoro complex was determined by unconditional energy minimization in the framework of the QM/MM method, with separation into conformationally flexible effective fragments. The calculations were performed by the Hartree–Fock method with the LANL2DZdp_ECP basis set (and pseudo-potential for phosphorus) [19] and the AMBER force field [20]. The quantum subsystem included phosphate groups of GTP, magnesium ion, side chain of the Gln61 amino acid residue whose key role was noted in experimental studies, and two water molecules W1 and W2 (Fig. 2, the quantum subsystem is depicted by balls and rods).

Figure 2 shows the configuration of the active site on the top of the potential barrier, obtained on the motion along the reaction coordinate chosen as the distance between P_γ and the oxygen atom of the water molecule oriented by hydrogen bonds with

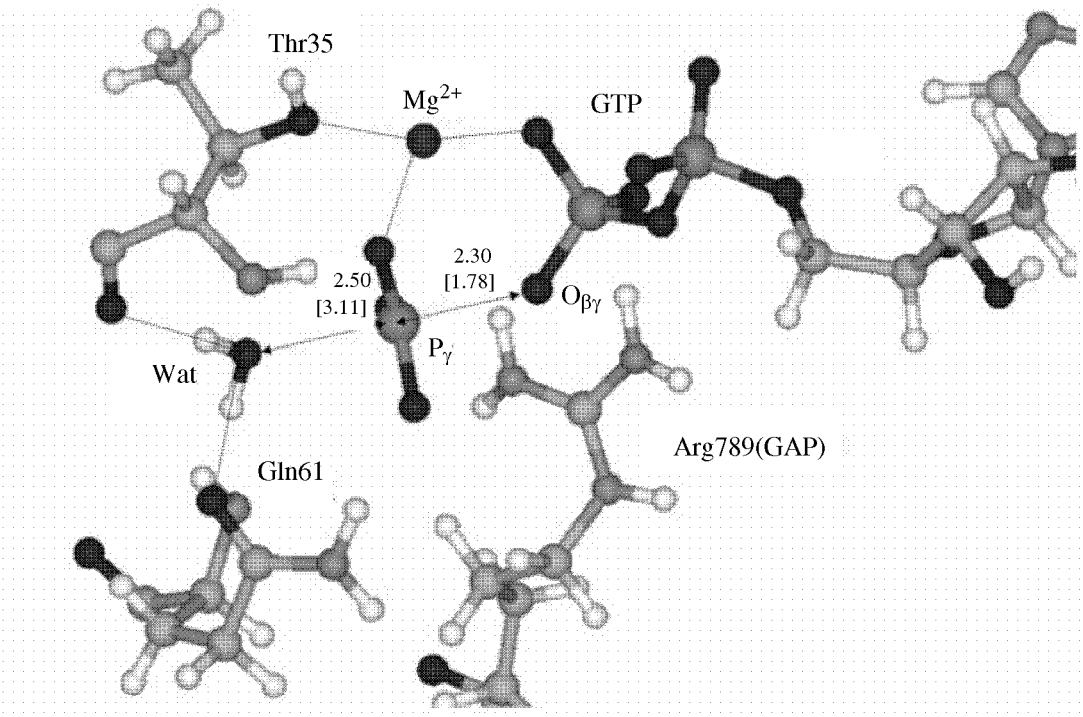

Fig. 3. Calculated geometric configuration of the active site on the top of the potential barrier for GTP hydrolysis by RAS–GAP. Distances (in Å) shown without brackets relate to the transition state and those shown in brackets, to the enzyme–substrate complex.

surrounding molecular groups to a position appropriate for nucleophilic attack.

Comparison of the structures in Figs. 1 and 2 shows that the transition states of ATP hydrolysis by myosin and of GTP hydrolysis by RAS are fairly similar to each other. Unlike the former reaction, the second reaction occurs in two stages. The first stage involves cleavage of the γ-PO_3^- group under the action of the surrounding of the active site of the protein and water molecules. As this takes place, the phosphate group undergoes stereoinversion. In the following stages, the water molecule reacts with the γ-phosphate group, which is accompanied by two proton transfer: from the reacting to its neighboring water molecule and further to the Gln61 residue. In the intermediate stage, HPO_4^{2-} formation occurs. Finally, proton transfer from Gln61 to HPO_4^{2-} over a chain of hydrogen bonds occurs to form the reaction product $H_2PO_4^-$. The activation barriers of the first (γ-phosphate cleavage) and second (formation of inorganic phosphate) stages, calculated by the QM/MM method, are 16 and 5 kcal mol^{-1}, respectively [28].

Hydrolysis of Guanosine Triphosphate, Catalyzed by the RAS–GAP Protein Complex

The rate of GTP hydrolysis in live systems is much increased (by five orders of magnitude) is RAS acts as a complex with another protein, specifically $p120^{GAP}$ (GAP), which is classed with GTPase promoter. In the RAS–GAP complex, a substrate molecule is clamped by peptide chains inside the cavity of the complex rather than resides on the surface of solvated protein, like in GTP hydrolysis catalyzed by free RAS. As a result, the active site of the enzyme (protein complex) can accommodate only one nucleophilic water molecule (Fig. 3). There has been much experimental and theoretical work [31–36] on the mechanism of GTP hydrolysis in this system, but no unambiguous conclusions could be drawn.

In our works [37–39], we performed simulation of RAS–GAP-catalyzed hydrolysis of GTP in a real protein surrounding by the QM/MM method with separation of conformationally flexible effective fragments.

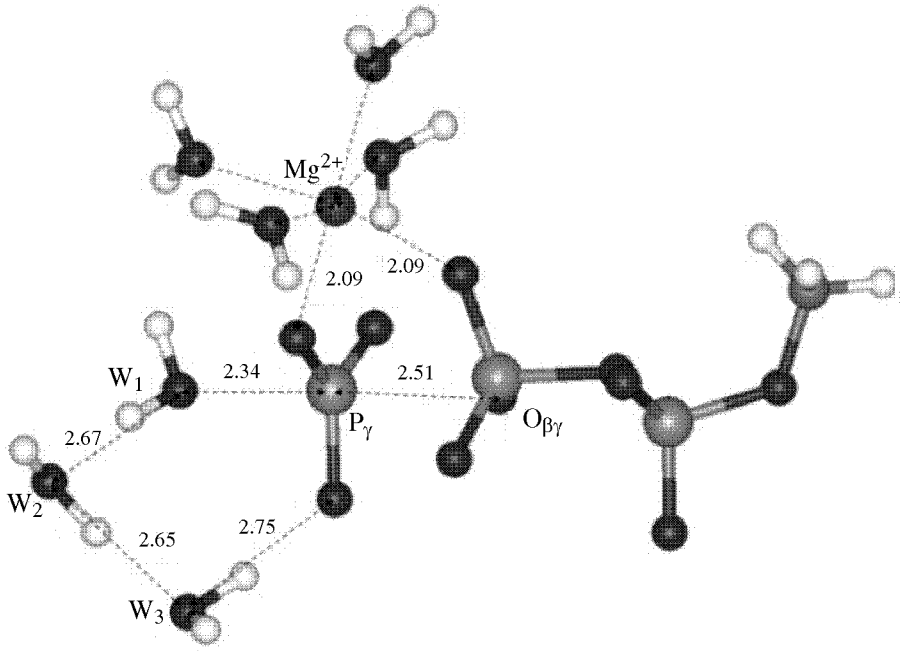

Fig. 4. Calculated geometric configuration of the active site on the top of the potential barrier for methyl triphosphate hydrolysis in water clusters. Interatomic distances are given in Å.

The molecular model for QM/MM calculations was constructed in the basis of atomic coordinates for the RAS–GAP complex including an analog of GTP, namely, GDP with an AlF_3 fragment instead of g-phosphate (PDB code 1WQ1) [40]. This model structure is considered [40] to correspond to the transition state of hydrolysis reaction (1), since the planar AlF_3 group that replaces γ-phosphate in the crystal structure mimics the suggested configuration of the GTP γ-phosphate group on the top of the energy barrier. In [41] we suggested that the more probable mimicking group in PDBID:1WQ1 is MgF_3^-, but this does not affect quantitative interpretation of X-ray diffraction data.

In our model, the quantum subsystem (43 atoms) includes the GTP phosphate groups, water molecule, magnesium cation, Gln61 fragment of Ras, and Arg789 fragment of GAP promoter (so-called "arginine finger"). It is these amino acid residues that are considered key components in functioning the whole enzyme complex. The calculations were performed by the Hartree–Fock method with the LANL2DZdp_ECP basis set (and pseudo-potential for phosphorus) [19] and the AMBER force field [20]. Figure 3 depicts the geometric configuration of the transition state of the first reaction stage: γ-phosphate cleavage. The hydrolysis of GTP, catalyzed by the RAS–GAP complex, involves the same stages as with free RAS, except that here the active part in the proton redistribution for forming inorganic phosphate belongs to the Gln61 residues.

The QM/MM calculations predict the activation barriers in the first (γ-phosphate cleavage) and second (formation of inorganic phosphate) stages of 4 and 10 kcal mol^{-1}, respectively, which is completely consistent with the degree of acceleration of the RAS–GAP-catalyzed hydrolysis compared to the RAS-catalyzed reaction. Heuristically, such mechanism was suggested by Sondek et al. [42]. The role of the key amino acid residues Gln61(RAS) and Arg789(GAP) is quite clear, and, therefore, it is unquestionable that mutations over these positions should increase the activation barrier or completely alter the catalysis mechanism.

Hydrolysis of Methyl Triphosphate in Water

Analysis of the hydrolysis of the fully deprotonated methyl triphosphate $[CH_3–O–(PO_3)_3]^{4–}$ in aqueous solutions provides reference data for assessing the catalytic effect of protein matrices in enzymatic conversions of ATP and GTP. The hydrolysis rate

constants of methyl monophosphate, measured at various pH [43], made it possible to estimate the activation barriers on the free energy surface (27–31 kcal mol^{-1}) [35, 44], but allowed no conclusions on the reaction mechanism. A Car–Parinello molecular dynamics simulation of the mechanism of methyl triphosphate hydrolysis in water clusters $(H_2O)_n$, which resulted in highly overestimated activation barriers, was described [45].

In our works [46, 47] we calculated of the energy profile of the hydrolysis of methyl triphosphate (MTP^{4-}) to methyl diphosphate (MDP^{3-}) in water:

$$MTP^{4-} + H_2O \rightarrow MDP^{3-} + H_2PO_4^- . \quad (3)$$

We considered two molecular systems differing from each other in that one contained and the other did not contain the magnesium cation. The QM subsystem in model I included the four-charged methyl triphosphate anion and five water molecules. The MM subsystem included 75 water molecules described in terms of effective fragments. The QM part in model II included MTP^{4-}, Mg^{2+}, and seven water molecules, and the MM part, 73 water molecules (effective fragments). These mole-cular clusters were constructed by consecutively adding water molecules to the reacting species $MTP^{4-} + H_2O$; therewith, the geometry of the whole system was optimized in each step, so as to maintain the hydrogen-bond net. The chosen number of water molecules (a total of 80) was large enough for the reacting species $MTP^{4-} + H_2O$ to be completely surrounded by solvent molecules.

The energies and energy gradients in the QM subsystem were calculated by the Hartree–Fock (with the LANL2DZdp_ECP basis set and pseudo-potential for phosphorus) and DFT/B3LYP methods using the PC GAMESS program suit [11].

In model I, the Hartree–Fock activation barriers for the first stage the first (γ-phosphate cleavage) and second (formation of inorganic phosphate) stages were 20 and 14 kcal mol^{-1}, respectively. These data provide clear evidence for the catalytic effect of the protein matrices (myosin, RAS, and RAS–GAP) in ATP and GTP hydrolysis: The activation barriers of these reactions are lower compared with the reaction in water clusters.

The geometric configuration of the transition state, calculated in model II (Fig. 4), resembles the transitions states for ATP and GTP hydrolysis, implying a common reaction mechanism in all the cases.

CONCLUSIONS

Molecular simulation is gaining increasing recognition as a tool for studying biomolecular processes, including enzymatically catalyzed reactions. Computation-oriented research provides support for experimental works by substantially reducing time and material expenses for projects. Moreover, reliable results of molecular simulation allow one to unequivocally establish and visualize the mechanism of chemical transformations in enzyme systems. Experimental approaches to research into mechanisms of intricate chemical transformations all provide important but indirect data on fine details of reaction paths, and simulation gives additional and very essential information. In the present review we used the example of hydrolysis of nucleoside triphosphates to demonstrate advances in contemporary molecular simulation on the basis of quantum theory.

Further progress in computer simulation may be associated with development and application of a combined quantum mechanics–molecular dynamics (QM/MD) approach. This approach will allow accounting for more profound conformational transformations in the course of chemical reactions in protein systems. First steps in this direction have already been made: The QM/MD method has been used to success in the present work.

Another promising route forward in molecular simulation is creation of databases of intermediates and transition states of elementary states of enzymatic catalysis. The accumulated experience in simulation of nucleoside triphosphate hydrolysis under the action of various enzymes allows one to make first steps in this direction.

ACKNOWLEDGMENTS

In this review we used the results of research supported by the Russian Foundation for Basic Research (project no. 07-03-00060) and Russian Academy of Sciences (Program no. 10, Department of Chemistry and Materials Sciences).

REFERENCES

1. Minkin,V.I., Simkin, B.Ya., and Minyaev R.M., *Teoriya stroeniya molekul* (Theory of Molecular Structure), Rostov-on-Don: Feniks, 1997.

2. *Molecular Mechanics*, Burkert, U. and Allinger, N.L., Eds., Washington, DC: ACS, 1982.
3. Warshel, A. and Levitt, M., *J. Mol. Biol*., 1976, vol. 103, p. 227.
4. Nemukhin, A.V., Grigorenko, B.L., Epifanovskii, E.M., and Moskovskii A.A., *Ross. Khim. Zh*., 2006, vol. 50, p. 45.
5. Grigorenko, B.L., Nemukhin, A.V., Topol, I.A., and Burt, S.K., *J. Phys. Chem. A*, 2002, vol. 106, p. 1066.
6. Nemukhin, A.V, Grigorenko, B.L., Topol, I.A., and Burt, S.K., *J. Comp. Chem*., 2003, vol. 24, p. 1410.
7. Nemukhin, A.V., Grigorenko, B.L., Bochenkova, A.V., et. al., *Struct. Chem*., 2004, vol. 15, p. 3.
8. Nemukhin, A.V., Grigorenko, B.L., and Knyazeva, M.A., *Zh. Fiz. Khim*., 2004, vol. 78, p. 1028.
9. Nemukhin, A.V, Grigorenko, B.L., Rogov, A.V., et al., *Theor. Chem. Acc*., 2004, vol. 111, p. 36.
10. Schmidt M.W., Baldridge K.K., Boatz J.A. , et al., *J. Comput. Chem*., 1993, vol. 14, p. 1347.
11. Nemukhin, A.V., Grigorenko, B.L., and Granovskii, A.A., *Vestn. Mosk. Univ., Ser. 2: Khim*., 2004, vol. 45, p. 75.
12. Trentham, D.R., Eccleston, J.F., and Bagshaw, C.R., *Quart. Rev. Biophys*., 1976, vol. 9, p. 217.
13. Okimoto, N., Yamanaka, K., Ueno, J., et al., *Biophys. J*., 2001, vol. 81, p. 2786.
14. Li, G. and Cui, Q., *J. Phys. Chem. B*, 2004, vol. 108, p 3342.
15. Schwarzl, S., Smith, J.C., and Fisher, S., *Biochemistry,* 2006, vol. 45, p. 5830.
16. Grigorenko, B.L., Rogov A.V., Topol I.A. , et al., *Proc. Natl. Acad. Sci. USA*, 2007, vol. 104, p. 7057.
17. Onishi, H., Mochizuki, N., and Morales, M.F., *Biochemistry*, 2004, vol. 43, p. 3757.
18. Smith, C.A. and Rayment, I., *Ibid.,* 1996, vol. 35, p. 5405.
19. Hay, P.J. and Wadt, W.R., *J. Chem. Phys*., 1985, vol. 82, p. 270.
20. Cornell, W.D., Cieplak, P., Bayly, C.I., et al., *J. Am. Chem. Soc*., 1995, vol. 117, p. 5179.
21. Nemukhin, A.V., Topol, I.A., Grigorenko, B.L., and Burt, S.K., *J. Phys. Chem. B.*, 2003, vol. 107, p. 2958.
22. Langen, R., Schweins, T., and Warshel, A., *Biochemistry*, 1992, vol. 31, p. 869.
23. Schweins, T., Langen, R., and Warshel, A., *Nature Struct. Biol*., 1994, vol. 1, p. 476.
24. Schweins, T. and Warshel, A., *Biochemistry*, 1996, vol. 35, p. 14232.
25. Futatsugi, N., Hata, M., Hoshino, T., and Tsuda, M., *Biophys. J*., 1999, vol. 77, p. 3287.
26. Futatsugi, N. and Tsuda, M., *Ibid*., 2001, vol. 81, p. 3483.
27. Cavalli, A. and Carloni, P., *J. Am. Chem. Soc*., 2002, vol. 124, p. 3763.
28. Grigorenko, B.L., Shadrina, M.S., Topol, I.A. , et al., *Proteins: Struct. Funct. Bioinf*., 2007, vol. 66, p. 456.
29. Scheidig, A.J., Burmester, C., and Goody, R.S., *Structure*, 1999, vol. 7, p. 1311.
30. Shadrina, M.S., Rogov, A.V., Bravaya, K.B., and Nemukhin, A.V., *Vestn. Mosk. Univ., Ser. 2: Khim.,* 2005, vol. 46, p. 363.
31. Vetter, I.R. and Wittinghofer, A., *Science*, 2001, vol. 294, p. 1299.
32. Kosloff, M. and Selinger, Z., *Trends Biochem. Sci.,* 2001, vol. 26. p. 257.
33. Li, G. and Zhang, X.C., *J. Mol. Biol*., 2004, vol. 340, p. 921.
34. Wittinghofer, A., *Trends Biochem. Sci*., 2006, vol. 31, p. 20.
35. Glennon, T.M., Villa, J., and Warshel, A., *Biochemistry*, 2000, vol. 39, p. 9641.
36. Shurki, A. and Warshel, A., *Proteins*, 2004, vol. 55, p. 1.
37. Topol, I.A., Cachau, R.E., Nemukhin, A.V. , et al., *Biochem. Biophys. Acta*, 2004, vol. 1700, p. 125.
38. Grigorenko, B.L., Nemukhin, A.V., Topol, I.A. , et al., *Proteins: Struct. Funct. Bioinf*., 2005, vol. 60, p. 495.
39. Grigorenko, B.L., Rogov, A.V., Knyazeva, M.A., Isaeva, E.V., and Nemukhin, A.V., *Vestn. Mosk. Univ., Ser. 2: Khim.*, 2005, vol. 46, p. 19.
40. Scheffzek, K., Ahmadian, M.R., Kabsch, W. , et al., *Science*, 1997, vol. 277, p. 333.
41. Grigorenko, B.L., Nemukhin, A.V., Cachau, R.E. , et al., *J. Mol. Simulation* , 2005, vol. 11, p. 503.
42. Sondek, J., Lambright, D.G., Noel, J.P. , et al., *Nature,* 1994, vol. 372. p. 276.
43. Bunton, C.A., Llewellyn, D.R., Oldham, K.G., and Vernon, C.A., *J. Chem. Soc.*, 1958, p. 3574.
44. Hu, C.H. and Brinck, T.J., *J. Phys. Chem. A*, 1999, vol. 103, p. 5379.
45. Akola, J. and Jones, R.O., *J. Phys. Chem. B*, 2003, vol. 107, p. 11774.
46. Grigorenko, B. L., Rogov, A.V., and Nemukhin, A.V., *Ibid*., 2006, vol. 110, p. 4407.
47. Rogov, A.V., Grigorenko, B.L., Bochenkova, A.V., et al., *Vestn. Mosk. Univ., Ser. 2: Khim*., 2007, vol. 48, p. 152.