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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

M. S. Shadrina, B. L. Grigorenko, and A. V. Nemukhin

Department of Physical Chemistry
e-mail: anemukhin@yahoo.com

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Abstract—The equilibrium geometric configurations of the enzyme–substrate complex for guanosine triphosphate hydrolysis by elongation factor EF-Tu calculated using two theoretical approaches, a combined quantum mechanics/molecular mechanics (QM/MM) method and a molecular dynamics method, are compared. The reaction complex geometry determined by the QM/MM method is consistent with the accepted reaction mechanism, whereas, in the enzyme–substrate structure predicted by the molecular dynamics method with the CHARMM force field, the relative positions of the nucleophilic reagent (water molecules) and the base (a histidine side chain) do not correspond to the optimal reagent arrangement.

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Study of mechanisms of enzyme catalysis, including elementary chemical transformations at the active sites of protein systems, is required for gaining knowledge of functioning living cells and for solving practical problems of pharmaceutics and bioengineering [1]. Experimental methods used for studying enzyme reactions, including routine kinetic measurements (often in combination with gene engineering techniques), as well as X-ray crystallographic study of enzyme complexes with substrate or inhibitor analogues, provide very important information on the mechanisms of complex transformations in proteins. Computer-aided calculations by modern molecular simulation methods, namely, molecular mechanics (MM), molecular dynamics (MD), quantum chemistry, and combined quantum mechanics/molecular mechanics (QM/MM) methods, can complement experimental studies, provide new information, and ensure visualization of enzyme catalysis processes with atomic resolution.

The MM and MD methods, which are based on the use of empirical and semiempirical force fields for describing atom–atom potentials, are currently the most accepted tools for simulation of biomolecular systems since they can be used for computer-aided calculations of structures with hundreds and thousands of atoms. For decades, the force field parameters have been determined and refined from experimental data; in recent years, they have also been found from quantum-chemical computational data. Nevertheless, the question of whether simulation of biomolecules with force fields is adequate is constantly debated in the literature. It is evident that methods based on quantum equations for interaction potentials are preferable for describing chemical reactions, i.e., chemical bond breakage and formation. However, even in problems dealing with the structural aspects of biomolecules, difficulties can arise when empirical force fields are used.

In this paper, we discuss the structure of the enzyme–substrate complex of one of the most important enzyme catalysis systems, the EF-Tu protein in the EF-Tu–ribosome complex with guanosine triphosphate (GTP), which was determined by the QM/MM method and from MD simulation with the CHARMM force field.

Elongation factor EF-Tu is of crucial importance in protein biosynthesis. The process involves rapid GTP hydrolysis to guanosine diphosphate and inorganic phosphate in the ternary complex EF-Tu · GTP · ribosome. Kinetic measurements showed that the critical amino acid of the enzyme is His85 [2]. For theoretical study of GTP hydrolysis, we constructed a model based on the crystal structure of protein EF-Tu with a GTP analogue (protein data bank code 1EFT). The model contains a nonprotonated GTP molecule, 210 water molecules (including the reactive molecule), a magnesium cation, and the amino acid residues of EF-Tu in a radius of about 20 Å from the phosphorus atom of the γ phosphate group of GTP. The quantum subsystem comprised 36 atoms, including two terminal CH_3 groups. The MM part consisted of 1811 atoms combined in

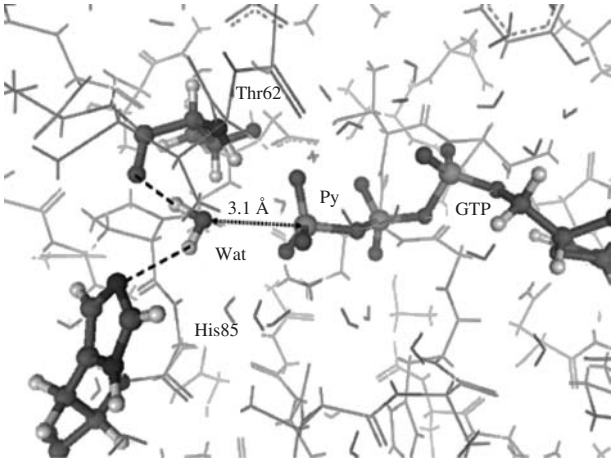

Fig. 1. Structure of the enzyme–substrate complex for GTP hydrolysis in the EF-Tu active site calculated by the QM/MM method.

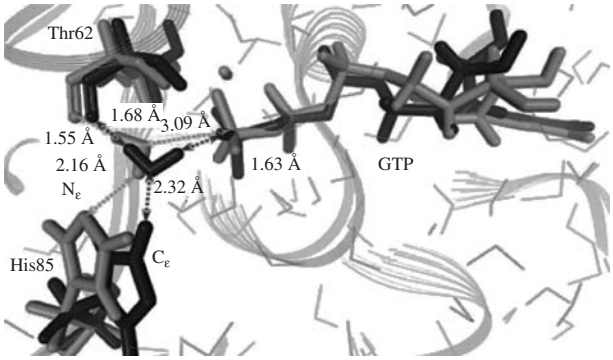

Fig. 2. Superposition of the structures of the enzyme–substrate complex for GTP hydrolysis in the EF-Tu active site. The QM/MM structure is shown in gray, and the structure obtained by MD simulation is shown in black.

556 effective fragments. The energies and forces in the QM subsystem were calculated by the Hartree–Fock method with the LANL2DZdp basis set and corresponding pseudopotential for the phosphorus atom. The energies and forces in the MM subsystem were determined with the use of the AMBER force field parameters.

The equilibrium geometry of the enzyme–substrate complex (Fig. 1) was determined from the results of calculations by the flexible effective fragment QM/MM method [3, 4].

It is important that the reactive water molecule (denoted as Wat in Fig. 1) has the optimal orientation of the hydrogen bonds involving the His85 and Thr62 side chains for nucleophilic attack at the P_γ atom of the terminal phosphate group of the GTP molecule. A similar orientation of the reactive water molecule and a distance from the water oxygen atom to P_γ of 3.1 Å are typical of the dissociative mechanism of enzyme hydrolysis of nucleoside triphosphate molecules. According to this mechanism, the transition state that appears upon the rupture of the P_γ–$O_{\beta\gamma}$ bond induced by the protein environment corresponds to the planar configuration of the $P_\gamma O_3^-$ group. The nucleophilic attack of the reactive water molecule occurs at the P_γ atom with proton transfer until the formation of inorganic phosphate. Rather realistic activation barriers have been reported for this mechanism [5–7].

To verify the efficiency of classical MD methods for simulation of the enzyme–substrate structure (Fig. 1), we carried out a computational experiment with the use of the NAMD2 program package [8] with the CHARMM force field. Previously, the geometry of the enzyme–substrate complex was determined by the QM/MM method and the coordinates of all atoms of the model system were optimized to correspond to an energy minimum with the given force field parameters. Then, from these atomic positions, a molecular dynamic trajectory lasting for 4 ns was calculated and the new coordinates of the system were again optimized to achieve minimum energy. As a result, a new enzyme–substrate structure was obtained, which is consistent with the interaction potentials of the CHARMM force field.

Figure 2 shows a superposition of the structures of the enzyme–substrate complex for GTP hydrolysis in the EF-Tu active site found by two methods. As a whole, these structures are not very different, which is consistent with the widely accepted conclusion that the MD method adequately describes structures. However, concerning the orientation of the reactive water molecule with respect to the γ-phosphate group of the GTP molecule and the His85 side residue, the structure of the active enzyme–substrate site obtained by the MD method does not allow one even to propose a scheme of the hydrolysis reaction. It is evident that attempts to simulate the nucleophilic attack of the water molecule at the γ-phosphate group of GTP beginning with this configuration will lead to significantly overestimated activation barriers as compared with the reaction path beginning with the structure shown in Fig. 1.

Thus, our simulation results show that MD predictions with the use of classical force fields should be treated with care even if the stages of chemical bond breakage and formation are not considered.

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