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Towards quantum-based modeling of enzymatic reaction pathways: Application to the acetylholinesterase catalysis

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ABSTRACT

We apply computational methods aiming to approach a full quantum mechanical treatment of chemical reactions in proteins. A combination of the quantum mechanical – molecular mechanical methodology for geometry optimization and the fragment molecular orbital approach for energy calculations is examined for an example of acetylcholinesterase catalysis. The codes based on the GAMESS(US) package operational on the 'RSC Tornado' computational cluster are applied to determine that the energy of the reaction intermediate upon hydrolysis of acetylcholine is lower than that of the enzyme–substrate complex. This conclusion is consistent with the experiments and it is free from the empirical force field contributions.

1. Introduction

Modeling chemical transformations in active sites of enzymes constitutes an important step in understanding mechanisms of enzyme activity. Knowledge of details of such reactions at atomic resolution assists developments in the field of rational drug design and in creation of genetically modified proteins for potential use in medicine and biotechnology. Significance of quantum description of chemical processes in proteins should not be underestimated since re-arrangements of covalent bonds are to be described. However, a vast amount of atoms in the corresponding macromolecules complicates considerably the use of fully quantum mechanical (QM) algorithms for calculations of energies and forces acting on atoms in the system.

The quantum mechanical – molecular mechanical (QM/MM) approach [1–4] is currently being considered as a practically convenient compromise, since it allows one to apply a quantum characterization for a part of the enzyme system in which cleavage and formation of chemical bonds occur and takes into account contributions to the reaction energy profiles from the rest of the protein matrix described at the MM level. Such strategy is successfully used in many applications; however, certain inaccuracies may be introduced in QM/MM calculations. Among them, deficiencies in conventional force field parameters in the MM-subsystem, a possible mismatch between different accuracy levels in the QM and MM-parts, possible errors in treating the QM–MM interface may be mentioned. Examples are known when different QM/MM calculations applied for the same system arrived to contradictory conclusions; in such cases, use of computational algorithms which are closer to the full quantum description can help to resolve discrepancies.

In this respect, the fragment molecular orbital (FMO) electronic structure method [5–9] seems to be a very promising approach that principally enables full QM treatment of proteins. In the two-body FMO version, a series of electronic structure calculations should be performed for the fragment monomers and dimers under the environmental electrostatic potential until a consistent solution is obtained. The most recent review [9] summarizes the method developments and applications. Importantly, the FMO approach makes possible nearly linear scaling calculations of large molecular systems.

In this work we apply both QM/MM and FMO approaches in order to clarify a critical question related to one of the most requested enzymatic processes, the acetylcholinesterase (AChE) catalyzed hydrolysis of the neurotransmitter acetylcholine (ACh) [10]. This process has a tremendous value because of vital importance in nervous systems in many organisms. It also may be considered as a prototype of numerous conjugated enzymatic mechanisms with cholinesterases studied in many research fields from chemical warfare [11] to Alzheimer disease [12]. Qualitatively, the process is well characterized at the molecular level as a two-stage (acylation and deacylation) series of chemical transformations typical for serine hydrolases. Fig. 1 illustrates

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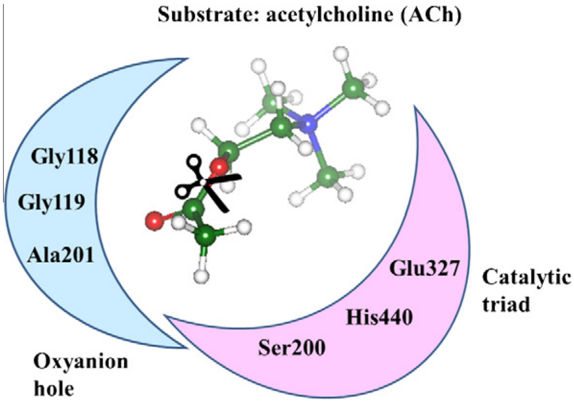

Fig. 1. Schematic illustration of the active site of acetylcholinesterase with a trapped substrate, acetylcholine. At the acylation stage, a concerted action of the residues from the catalytic triad (Ser200, His440, Glu327) assisted by contributions from the oxyanion hole residues (Gly118, Gly119, Ala201) leads to cleavage of the C–O chemical bond in the substrate. Here and in all figures carbon atoms are colored in green, oxygen in red and nitrogen in blue. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

schematically the first stage of ACh hydrolysis by the enzyme with the residue numbering consistent with the *Torpedo californica* acetylcholinesterase [13]. Proton shuttle involving serine and histidine residues from the catalytic triad, the substrate along with formation of the tetrahedral intermediates constitute essential features of the hydrolysis mechanism. However, a detailed description of energetics of the corresponding elementary stages is not yet completed.

This subject presents a relevant example when different QM/MM computational protocols result in qualitatively different conclusions [14–17]. More specifically, a position at the energy scale of the reaction intermediate, the tetrahedral intermediate (TI), relative to the level of the enzyme–substrate (ES) complex at the acylation stage of the hydrolysis process is a matter of a dispute. Following results of ab initio type QM/MM approach, Zhang et al. [14,15] concluded that the energy of TI should be 40–50 kJ/mol *higher* than that of ES, while another group [16,17] calculated this energy 50 kJ/mol *lower* than the level of ES. Variation of technical details of both QM/MM variants, in particular, as mentioned in Ref. [15], did not resolve discrepancies.

The question on the energy difference between ES and TI is important not only because a dispute on performance of QM/MM approaches, but mostly because of its relation to the results of recent experimental studies of kinetic isotope effects [18]. The latter are interpreted as indirect evidences of accumulation of the tetrahedral intermediate in acetylcholinesterase catalysis; thus a high energy level of TI relative to ES is hardly consistent with these observations [18]. Another important goal of this work is to estimate efficiency of modern computer platforms to execute the FMO algorithms. In this work we use the ‘RSC Tornado’ computer recently installed at the Moscow Institute of Physics and Technology I-SCALARE laboratory (URL http://rscgroup.ru/en/technologies/, accessed on August 17, 2012). It was designed for computationally intensive life science applications, in particular, for simulations of large molecular systems of biological importance.

2. Computational protocol

2.1. The protein model

The crystal structure of *Torpedo californica* acetylcholinesterase complexed with a nonhydrolyzable substrate analogue, 4-oxo-N,N,N-trimethylpentanaminium iodide, [13] served as a source of initial coordinates of heavy atoms for our model system. It is believed that this crystal structure (PDB ID: 2VJA) resembles the configuration of the TI state. We restored manually the true substrate, acetylcholine, by motifs of its analogue, added hydrogen atoms by assuming conventional protonation states of polar amino-acid residues (Arg, Lys, Asp, Glu) and performed optimization of geometry parameters by using the QM/MM procedure. The entire system for numerical simulations including 79 water molecules contained 2570 atoms in total.

2.2. FMO calculations

For FMO calculations, the system was divided into 65 FMO units (fragments). Each fragment from the entire set of 2333 atoms typically comprised two–three amino-acid residues ($\sim$30–40 atoms). In addition 79 water molecules described as effective fragments [19] contributed to this model. After trial and error calculations we had to collect to a single fragment (called here a primary fragment) the molecular groups involved in transformations on the reaction route from ES to TI conformations. Specifically, the ACh moiety, the side chains of the catalytic triad members, Ser200, His440, Glu327 and of the oxyanion hole residues Gly118, Gly119, Ala201 contributed to the primary fragment. Several other decompositions have been analyzed; we show below the results of a partitioning when the primary fragment constitutes the minimal allowed subsystem, namely, the substrate ACh, and the side chains of Ser200 and His440. The energies in the FMO approximation were mainly computed in the RHF/6–31G* approximation; an additional check was performed by using the B3LYP/6–31G* approach. The GAMESS(US) codes [20,21] performing the FMO algorithms were executed on the ‘RSC Tornado’ computer.

It is prohibitively expensive to find equilibrium geometry configurations for such model systems by using the FMO algorithms. Therefore, we restricted here an application of FMO for single point energy calculations, while the structures were optimized by using the QM/MM approach.

2.3. QM/MM calculations

To locate minimum energy configurations corresponding to the ES and TI structures of AChE with ACh we used here the flexible effective fragment variant [22,23] of the effective fragment potential (EFP) QM/MM theory [19,24,25]. In this method, the MM subsystem is represented by a collection of effective fragments contributing their electrostatic potentials expanded up to octupoles to the quantum Hamiltonian. The exchange-repulsion potentials which are combined with the electrostatic terms are created in preliminary quantum calculations. In our approach [22,23], the fragment–fragment interactions are computed beyond the framework of the EFP formalism [19,24,25], but instead are estimated with the help of conventional force field parameters of molecular mechanics. Therefore, all empirical parameters are entirely within the MM subsystem.

Two options to select the QM-subsystem have been considered. The basic variant comprising 143 atoms refers to the QP-part composed of the substrate, ACh, the side chains of the catalytic triad members, Ser200, His440, Glu327, of the oxyanion hole residues Gly118, Gly119, Ala201 and of the residues assisting in proper binding and orientation of ACh, Glu199 and Gly441. The reduced variant refers to a smaller QM composition with an assignment of the oxyanion hole groups, Gly118, Gly119, Ala201, to the MM-part. We note that in the EFP-based approaches [19,24,25] the electrostatic contributions from the MM groups to the quantum Hamiltonian are properly taken into account. The Hartree–Fock approximation RHF/6-31G* was used in the QM-subsystem.