Quantum mechanical/molecular mechanical analysis of mechanisms of enzyme action. Human acetylcholinesterase*

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The influence of the system separation into the QM and MM parts on the result of quantum mechanical/molecular mechanical (QM/MM) modeling of acetylcholine hydrolysis in the acetylcholinesterase active site was considered. The minimum acceptable quantum subsystem that provides an adequate description of the enzymatic reaction energy profile was identified. The computed energy profiles were analyzed and possible inaccuracies in QM/MM calculations were estimated. The fairly high tetrahedral intermediate stability was demonstrated, which is in a good agreement with the newest experimental observations.

Key words: acetylcholinesterase, reaction mechanism, acetylcholine, QM/MM method, quantum subsystem, density functional theory (DFT), BB1K and B3LYP functionals, AMBER force field.

The nature of the high catalytic efficiency and selectivity of enzymes is a subject of profound interest of the modern chemical science. The unique catalytic activity that exceeds 10^{10}—10^{15} times that of traditional chemical acid-base catalysts such as hydrogen ion makes enzymes an object of keen attention of modern physical chemistry.

In recent decades, the traditional approach to the study of the catalytic effect and the selectivity of enzymatic catalysis is based on the chemical kinetic approach, which provides grounds for the understanding of the multistep nature of chemical transformations in the active sites of enzymes, identification of the elementary steps, and determination of their kinetic and energy characteristics.[1—5]

According to the modern, basically new level of investigation of the nature of enzymatic catalysis, the elementary steps of the catalytic cycle are analyzed resorting to the fundamental views of the molecular mechanics of protein molecules and results of quantum chemical calculations of the potential energy surfaces (PES) of molecular systems, which model the enzyme active sites in the catalytic transformations of substrates to reaction products. The modern development is based on fairly reliable data on the protein structures presented in the PDB database (www.pdb.org[6]). The methods for estimating the interaction energies in protein molecules by molecular mechanics[7] and advanced quantum chemical approaches[8,9] are being developed. The use of quantum chemical methods for the calculation of the elementary steps of enzymatic reactions is largely related to the application of supercomputers such as SKIF MSU "Chebyshev" and "Lomonosov."

The essence of the quantitative approach being developed is combination of molecular mechanics and quantum chemistry methods. The main bulk of a protein molecule is described by molecular mechanics methods based on classical views on the molecular structure, whereas the proper catalytic cycle taking place in the active site is analyzed by quantum chemistry methods, which allow rather reliable prediction of the behaviors of 100—200 atoms. In this approximation, methodologically the most uncertain problem is to distinguish the quantum subsystem. The combination of atoms and functional groups used for quantum chemical calculations should comprise all the significant participants of the process and as few atoms as possible to reduce the size of computations.

The main approach to distinguishing the quantum subsystem is based on the knowledge available from the literature on the properties of the item under study and the existing views on the structure of the enzyme active site and the protein participants of the catalytic process. However, it is difficult in some cases to determine all these participants.

Here we used a novel approach based on successive complication of the structures included in the quantum

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chemical calculations. When the PES extrema found (transition states and metastable intermediates) no longer depend on further inclusion of additional protein groups present in the active site environment, this may serve as evidence that the optimal complexity has been achieved. This approach is illustrated by the example of acetylcholinesterase (AChE), a human cholinesterase enzyme.

Acetylcholinestease is a key enzyme of the central nervous and neuromuscular systems that regulates the nerve signal transmission *via* fast hydrolysis of the neurotransmitter, acetylcholine. Hence, AChE is the target for organophosphorus poisons and a key target for the search for medical drugs to treat Alzheimer´s disease.[10,11] The determination of the AChE crystal structure in 1991[12] facilitated the progress in the research into the catalytic action of AChE and provided the key information on the significant amino acid residues of the active site, which was supported by subsequent site-directed mutagenesis studies and kinetic data.[13—15] The key amino acid residues are Ser203, His447, and Glu334 (catalytic triad), oxyanion hole residues, Gly121, Gly122, and Ala204, and non-catalytic Glu202 group. The interest in the cholinesterase family continuously increases due to the development of new pesticides and pharmaceuticals and to considerable role of these enzymes in the study of human molecular polymorphism.[16]

Our research group[7,17—20] is traditionally engaged in the combined quantum mechanical/molecular mechanical (QM/MM) calculations of the catalytic cycles of cholinesterases.[7,17—20] The whole cycle of acetylcholine hydrolysis in the AChE active site was modeled using QM/MM in the mechanical insertion approximation.[17] The full energy profile of the reaction was constructed and the PES stationary points for acylation and deacylation steps were located.[17] Apart from the substrate, all amino acid residues of the catalytic triad, the oxyanion hole, non-catalytic glutamic acid Glu202*, Ser229, and three water molecules were included in the quantum subsystem. It was shown that deacylation is the rate-limiting step of the process; the reaction occurs by a one-proton mechanism without proton transfer from His447 to Glu334 to give a short hydrogen bond, which additionally stabilizes the imidazolium cation and, hence, the intermediate. Although these results are in good agreement with experimental data,[21—23] it was nevertheless impossible to locate the second transition state of acylation.

This reaction was considered by other researchers. The free energy profiles calculated by QM/MM methods for acylation and deacylation of acetylcholine by AChE were reported.[24] The umbrella sampling and molecular-dynamics calculations, the B3LYP/6-31G(d) method for the QM subsystem, and AMBER force field parameters for the MM subsystem were used. Only the side chains of the catalytic triad (Ser203, His447, Glu334) and the substrate were included in the QM subsystem at the acylation step. A water molecule was added at the deacylation step.

The reported[24] energy profiles show high energies of the tetrahedral intermediates (the enzyme—substrate complex and acylenzyme) compared to the initial structures, in particular, 11.3 and 16.3 kcal mol^{-1} for the two steps. The energies of the tetrahedral intermediates differ from the energies of the saddle point structures to within 1 kcal mol^{-1}. The details reported in the paper[24] are inadequate for understanding how the numbers indicated in the energy profile figures were obtained.

The overestimated energies of tetrahedral intermediates are apparently related to the lack of the side chains of the oxyanion hole residues and Glu202 in the QM subsystem. When the oxyanion hole is poorly described,[25] the energy profile has the form reported in the study cited.[24] Below we demonstrate that in some cases, point charges on the MM subsystem atoms can make up for the absence of molecular groups of the oxyanion hole in the QM part. However, in the implementation of the QM/MM technique it is often customary to eliminate the atom charges in the MM part adjoining the QM subsystem. Note once again that the inadequate description of the computation procedure, as in the work cited,[24] precludes the elucidation of the causes of discrepancies in the published results.

In a QM/MM study of the specificity (selectivity) of the AChE enzymatic catalysis[18,26] providing rather good agreement with experimental data,[27] the reactivities of a number of non-charged substrates, acetates of aliphatic alcohols, were predicted. Two QM subsystems were used, one described above that comprised only the fragments directly involved in the reaction: the substrate, Ser203, and His447. A quantitative agreement with the experiment was attained only for the extended QM subsystem. In a study of the nature of human molecular polymorphism, the results of calculations were used to interpret the role of the polymeric modification (Asp70Gly) of butyrylcholinesterase in the transfer of succinylcholine from the muscle relaxant drug category to the poison category.[20]

In this study we performed variation of the amino acid residues of the active site included in the QM subsystem and identified the optimal composition of the participants of the catalytic cycle.

Methodical Part

The influence of the choice and size of the QM subsystem on the QM/MM modeling results was analyzed in relation to the acylation step of the hydrolysis of acetylcholine by AChE. The model system was prepared based on the crystallographic structure for human AChE 1B41 (see Ref. 28) obtained with a 2.76 Å resolution. Due to the difficulty of crystallization, human AChE

* For uniformity, here and below the numbering of amino acid residues for human AChE is given.

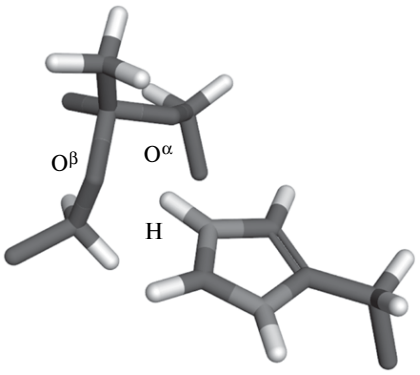

Fig. 1. Fragment of QM-subsystem intermediate.

structures with good resolution are known only for its complex with the protein toxin fasciculin-2. During the structure preparation, this protein was removed. A comparison with other AChE structures (for example, the structure of the AChE of the *Torpedo californica* fish, PDB ID 1EA5 (see Ref. 29) determined with a 1.8 Å resolution) showed that the presence of this inhibitor does not affect the active site conformation, which almost fully coincided for these two structures.

To optimize the computing effort, we chose the peptide groups of the central moiety with a ~20 Å radius around the catalytic triad. The positions of atoms located at more than 8—10 Å distances from the active site were set constant during the geometry optimization. The resulting model system contained 3533 atoms.

The hydrolysis was modeled using the combined QM/MM method. The QM part was calculated using the density functional theory (DFT) with BB1K and B3LYP hybrid functionals and 6-31G* and 6-311G** basis sets. The AMBER force field was used for the MM part.[30] Quantum chemical calculations were done by the NWChem 5.1.1 program package[31] and electron insertion method to take into account the interaction of the QM and MM subsystems.

The distance between the O atom of Ser203 and the carbonyl C atom of the substrate (O^{α}—C) was used as the reaction coordinate for the first step (from the reactants to the intermediate), while for the second step (from the intermediate to the products) this was the distance between the H atom of His447 and the substrate O atom (H—O^{β}, Fig. 1).

The geometric configuration of the transition state (TS) was found by gradually varying the chosen reaction coordinate with a 0.1 Å step. At each point, geometry optimization was performed with a constant value of the reaction coordinate. After the TS point was found, full geometry optimization was carried out (without imposing restrictions) by sloping down toward the reactants and products to obtain the reactant, TS, and product structures.

Six versions of the quantum subsystem were considered ranging from the "minimum" one, comprising only the directly reacting groups (Ser203, His477, and the substrate molecule), to the "maximum" one, which included all of the main amino acid residues of the active site (Fig. 2). The list of the amino acid residues included in the QM subsystem is shown in Fig. 3, which

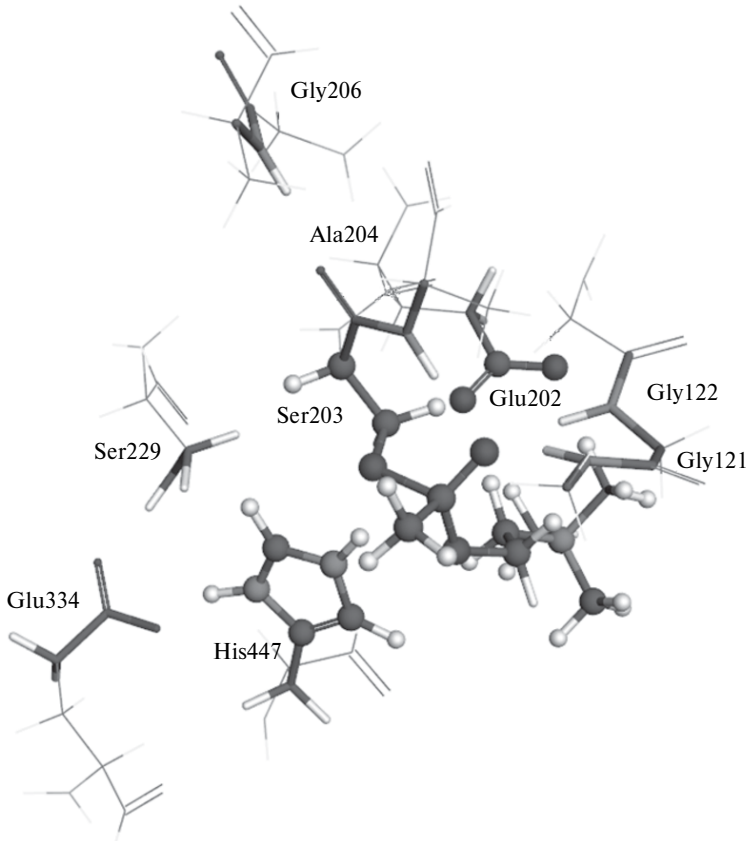

Fig. 2. Amino acid residues of the active site included in the "maximum" quanum subsystem. The rods show functional groups and the lines correspond to peptide chain fragments. For the most significant amino acid residues, the sphere-and-rod representation is used.

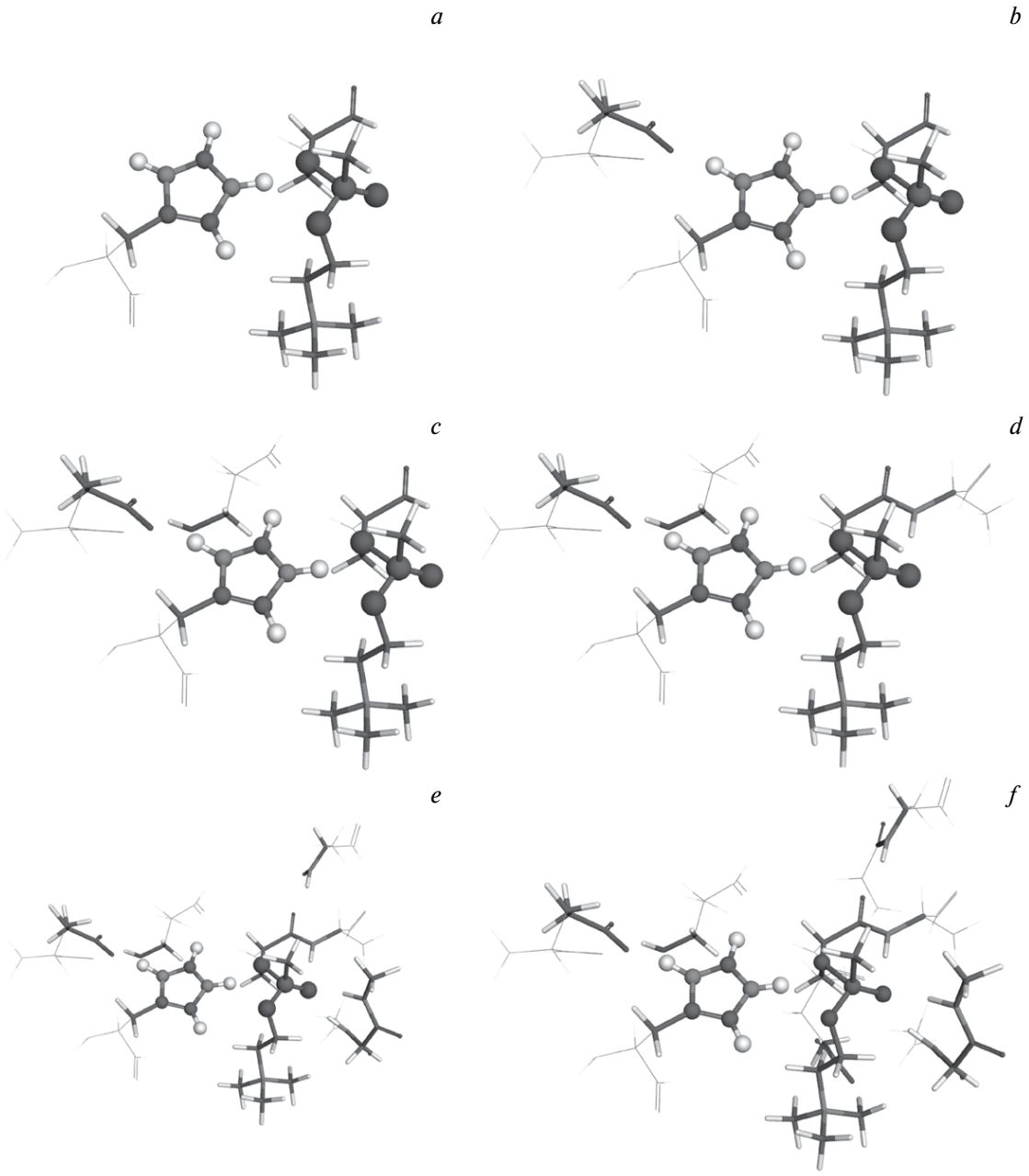

Fig. 3. Amino acid residues included (apart from the substrate) into the considered QM subsystems and graphical view of tetrahedral intermediates: (*a*) model *1*: Ser203, His447 (58 atoms); (*b*) model *2*: Glu334, Ser203, His447 (75 atoms); (*c*) model *3*: Ser229, Glu334, Ser203, His447 (88 atoms), (*d*) model *4:* Ala204, Ser229, Glu334, Ser203, His447 (98 atoms), (*e*) model *5:* Gly121, Gly122, Gly120, Ala204, Ala207, Ser229, Glu334, Ser203, His447 (131 atoms); (*f*) model *6*: Glu202, Gly206, Gly121, Gly122, Gly120, Ala204, Ala207, Ser229, Glu334, Ser203, His447 (153 atoms).

also shows their appearance in the tetrahedral intermediate configuration.

Results and Discussion

For the QM/MM modeling of the acylation step of acetylcholine hydrolysis by AChE, the use of B3LYP and BB1K gave identical values for energy barriers. In each of the considered subsystems, the PES stationary points were located for the enzyme—substrate complex (ES), the first and the second TS (TS1 and TS2), the tetrahedral intermediate (TI), and acylenzyme (AE) and the energy barriers were calculated.

The calculated energy profiles for the six considered models of the QM subsystem are presented in Fig. 4. It can be seen that the energy characteristics obtained for the

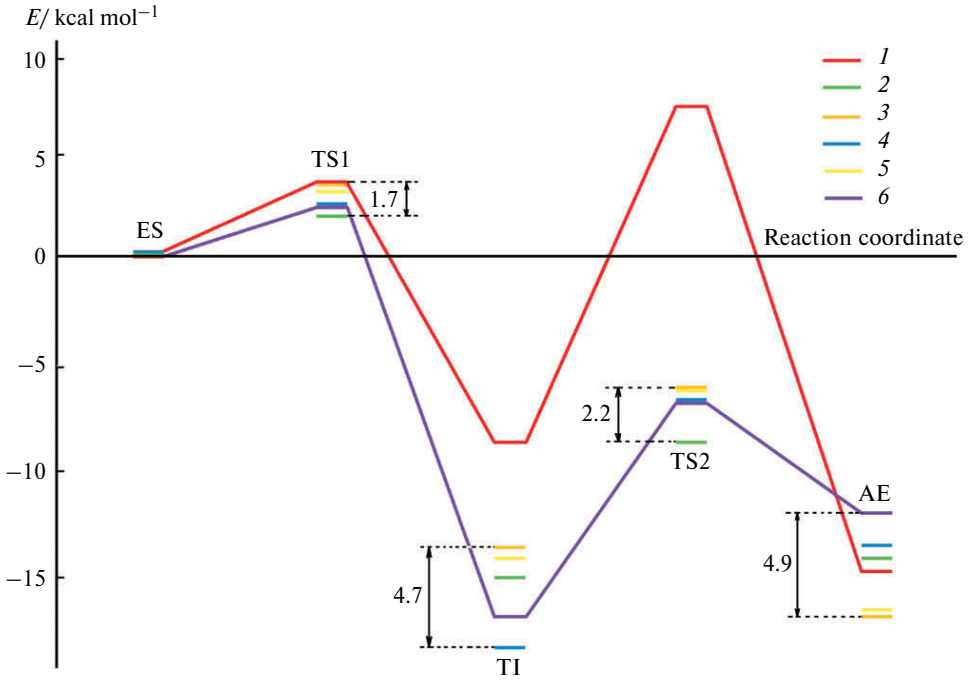

Fig. 4. Calculated energy profiles for the six models *1*—*6* of QM subsystem (see Fig. 3). The lines connect the energy levels for the "maximum" (violet) and "minimum" (red) subsystems. Figure 4 is available in full color in the on-line version of the journal (http://www.springerlink.com/fulltext.pdf).

"minimum" quantum subsystem (model *1*) are in discrepancy with those calculated for other versions, being especially distinguished by very high energy barrier for TS2 (15.6 kcal mol^{-1}, Fig. 5, *a*). This is attributable to the fact that the third unit of the catalytic triad, Glu334, and the Gly121, Gly122, and Ala204 oxyanion hole residues were not included in the system. The inclusion of Glu334 reduced the energy barrier by 8.3 kcal mol^{-1} (Fig. 5, *b*) and additionally stabilized the TI (5.3 kcal mol^{-1}).

A serious stabilizing factor is also the inclusion of the Gly121 and Gly122 amino acid residues of the oxyanion hole into the QM subsystem. Figure 6 shows the energy profiles for models *4* and *5* of the QM subsystem. It can be seen that inclusion of these amino acid residues entails stabilization of the TI by 3.3 kcal mol^{-1}.

The unusually high stability of TI in the cholinesterase hydrolysis of acetylcholine was experimentally studied in detail.[23,32] The study of the secondary isotope effect showed that AChE hydrolysis of acetylcholine is accompanied by accumulation of stable TI, and its hydrolysis is the rate-limiting step,[23] which is in good agreement with our results.

It is significant that the considered models of the QM subsystem, except for the "minimum" one, produce qualitatively similar results. All of the energy profiles presented in Fig. 4 are in line with experimental data, reflecting the low barrier for transition from the ES to the fairly stable TI and a noticeably higher barrier for TI dissociation to give AE, which has a higher energy. The energy scatters for TS1 and TS2 do not exceed 1.7 kcal mol^{-1} and 2.2 kcal mol^{-1}, respectively (see Fig. 4), *i.e.*, within the framework of the QM/MM approach, they can be considered insignificant. For different models of the QM subsystem, the energy differences of TI and AE reach 4.7 and 4.9 kcal mol^{-1}, respectively, since they are much more sensitive to various stabilization effects that are described in different ways depending on the size of the QM subsystem.

Thus, using the QM/MM method, it is possible to obtain qualitative data on the regularities of enzymatic catalysis without imposing strict requirement on the number of amino acid residues included in the QM subsystem. However, the quantitative characteristics are extremely sensitive to this number.

A more objective selection of the QM subsystem may be based on bioinformatics techniques, which allow one to compare proteins of different structures in order to distinguish the invariance of the amino acids that constitute the catalytic site of the enzyme.[33—38] This is done by automated computer comparison of the amino acid sequences of proteins that have a certain degree of homology and catalyze the same reaction. The results are statistically reliable if the sampling comprises more than 100 proteins (multiple alignment of the amino acid sequences of proteins taking into account insertions and deletions).

It was surprisingly found that amino acids that form the enzyme catalytic site occupy invariant positions in the related protein sequences and have zero information en-

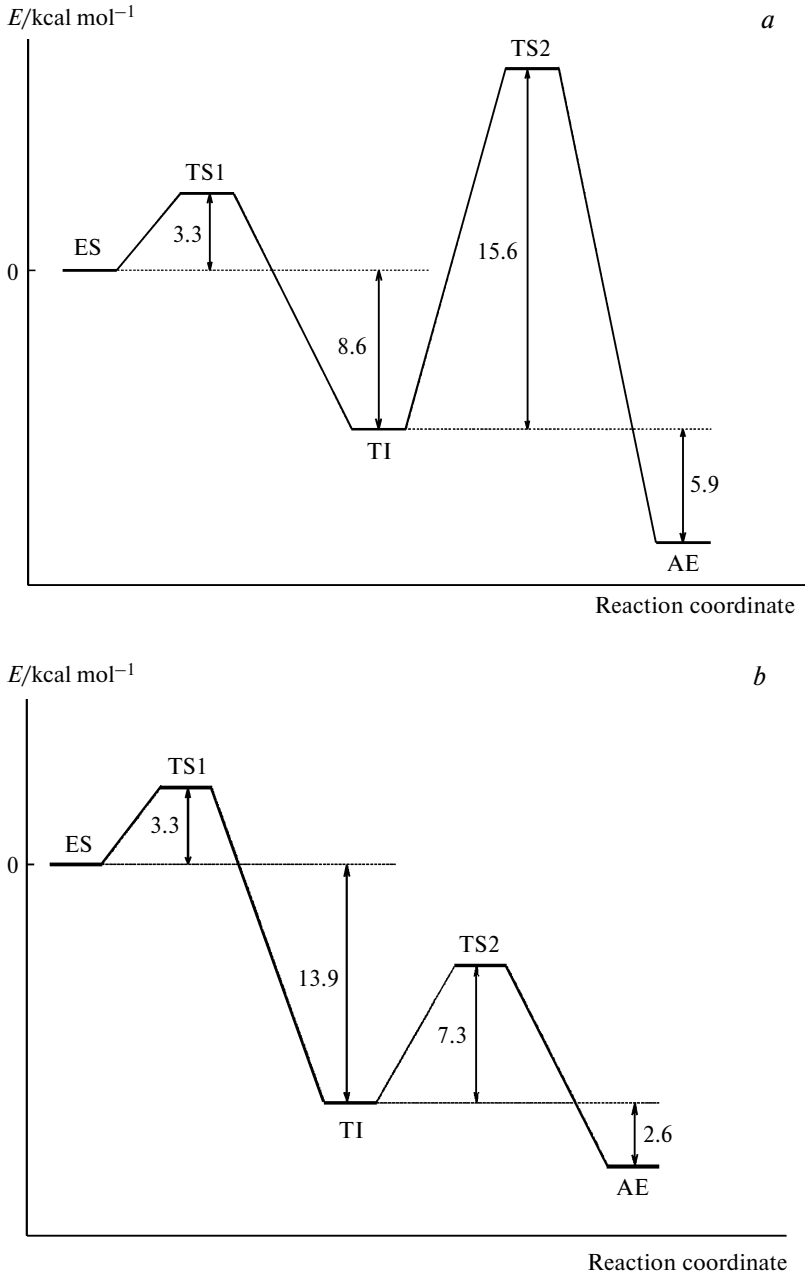

Fig. 5. Energy profiles of the acylation step of hydrolysis of acetylcholine by AChE for the "minimum" QM subsystem (model *1*) (*a*), for the subsystem including Glu334 (model *2*) (*b*).

tropy.[37,38] As a rule, these are amino acids bearing acid-base or charged groups and included in the top five most conserved amino acids (Asp, His, Glu, Arg, Ser). This approach is suitable to identify the groups that form the enzyme catalytic site. The elimination of any of these amino acids from the QM subsystem has a crucial effect on the calculation results (see Fig. 4).

The enzyme catalytic site can be identified using known 3D structure of the protein by constructing the structural template of the active site.[38] Using the geometric characteristics of the 3D structures of enzymes for which the active site structure (characteristic distances between the key atoms and angles between the planes) has been rather strictly determined, it is possible to construct the template and search for it through the whole set of proteins included in the PDB. This allows one to identify the configuration of the atoms that form the active site and use it in quantum chemical calculations. The application of this bioinformatic analysis[35] to AChE shows that conserved amino acid residues are those involved in the catalytic

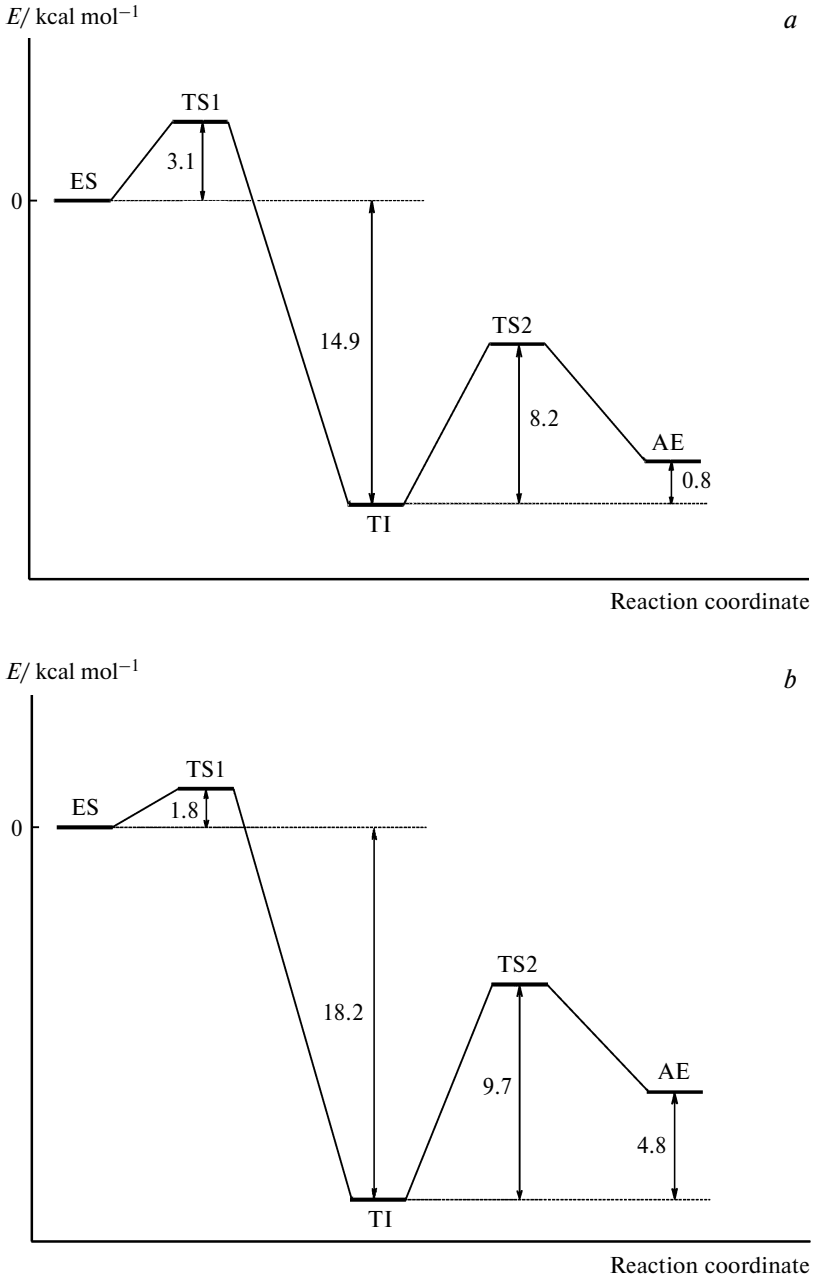

Fig. 6. Energy profiles of the acylation step of hydrolysis of acetylcholine by AChE for the model *4* of the QM subsystem (*a*), for the subsystem including oxyanion Gly121 and Gly122 residues (model *5*) (*b*).

triad, Ser203, His447, and Glu334. The inclusion of these residues in the QM subsystem is strictly obligatory, which is shown in this work by relevant calculations.

Thus, using the chemical transformations in the acetylcholinesterase active site as examples, we demonstrated that it is possible to identify a minimum QM subsystem for adequate calculations of the energy profiles of the elementary steps of enzymatic catalysis. According to the results of bioinformatic analysis, in this case, the QM subsystem must include all the amino acid residues of the catalytic triad (Ser, His, Glu) and the substrate. Failure to meet this condition results in a qualitatively wrong energy profile (see Fig. 4, model *1 cf.* models *2—6*). By comparing the energy profiles, it is possible to estimate the possible errors of QM/MM calculations. It is important that in this example, the scatter of the energy barrier heights depending on the (qualitatively correct) model is ~2 kcal mol^{-1}, while that of the energies of the intermediates is ~5 kcal mol^{-1}. It is reasonable to compare these estimates with the generally accepted characteristic error

of the relative energy calculations by quantum chemistry methods, which is ~1 kcal mol^{-1}.

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