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Energetics of Catalytic Reaction of Acetylcholinesterase (AChE) with Acetylcholine (ACh): Role of the Oxyanion Hole

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Energetics of Catalytic Reaction of Acetylcholinesterase (AChE) with Acetylcholine (ACh): Role of the Oxyanion Hole[#]

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Abstract

Motivation. Acetylcholinesterase (AChE) is one of the enzymes which synthesize the neurotransmitter acetylcholine (ACh) by the transition of acyl-groups of acetyl CoA and it is an important enzyme in biochemistry. It is also known that AChE catalyzes the hydrolysis with a high catalytic efficiency. However, details of the reaction mechanism are still in controversy. In particular, the effects of the oxyanion hole on the energetics of the catalytic reaction are not clearly understood. In the present study, potential energy diagrams for the catalytic reaction of AChE with ACh have been determined by means of *ab initio* and hybrid-DFT calculations using the model molecules of AChE and ACh. We have focused our attention mainly on the role of the oxyanion hole in the reaction.

Method. The *ab initio* and hybrid-DFT calculations were carried out at the HF/3–21G(d)//HF/3–21G(d) and B3LYP/6–311G(d,p)//HF/3–21G(d) levels of theory.

Results. The pre-complex composed of AChE and ACh, expressed by AChE–ACh, was 10.3 kcal/mol more stable than that of its separated state (AChE + ACh). The activation barrier for the formation of the tetrahedral intermediate from AChE–ACh was calculated to be 20.5 kcal/mol higher in energy than the pre-complex, and the tetrahedral intermediate is 18.0 kcal/mol higher relative to the pre-complex. In addition, the effect of the oxyanion hole was examined.

Conclusions. The activation energy was decreased from 20.5 to 10.4 kcal/mol by the interaction with the oxyanion hole. The present calculated value (10.4 kcal/mol) was in good agreement with recent experimental one (12.0 kcal/mol). The mechanism of the formation of the tetrahedral intermediate from AChE–ACh was discussed on the basis of theoretical results.

Keywords. Acetylcholine; acetylcholinesterase; catalytic triad; acylation and deacylation; effect of oxyanion hole; DFT calculation.

Abbreviations and notations

Ach, acetylcholine

DFT, density functional theory

AChE, acetylcholinesterase

[#] Dedicated to Professor Nenad Trinajstić on the occasion of the 65th birthday.

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1 INTRODUCTION

Acetylcholinesterase (AChE) is an enzymes composed of a catalytic triad in the active site consisting of three amino acids, namely serine (Ser), histidine (His), and glutamic acid (Glu). Also, AChE can synthesize the neurotransmitter acetylcholine (ACh) by the transition of acyl-groups of acetyl CoA [1–11]. ACh is mainly distributed in both cytoplasm of synaptic ending and synaptic vesicle, and it transmits impulse signal in synapse of myoneural junction.

AChE catalyzes the hydrolysis of ACh with a high catalytic efficiency. The change of ACh into choline and acetic acid by AChE proceeds in two stages, acylation and deacylation. These processes are schematically illustrated in Figure 1. In the case of human AChE, Ser200 and His440 in the active site are involved in the reaction with the substrate ACh during the AChE catalysis. The reaction proceeds after the proton transfer from Ser200 to imidazole of His440 (this is referred hereafter by the first proton transfer) and nucleophilic addition of the oxygen atom of Ser200 to the substrate ACh occurs subsequently. This is the acylation process. In the case of deacylation process, the acyl-enzyme is hydrolyzed back to its original form by water. Although the overall reactions are thus well-established, the energetics along the reaction coordinate and role of the oxyanion hole has been not clearly understood [1–11].

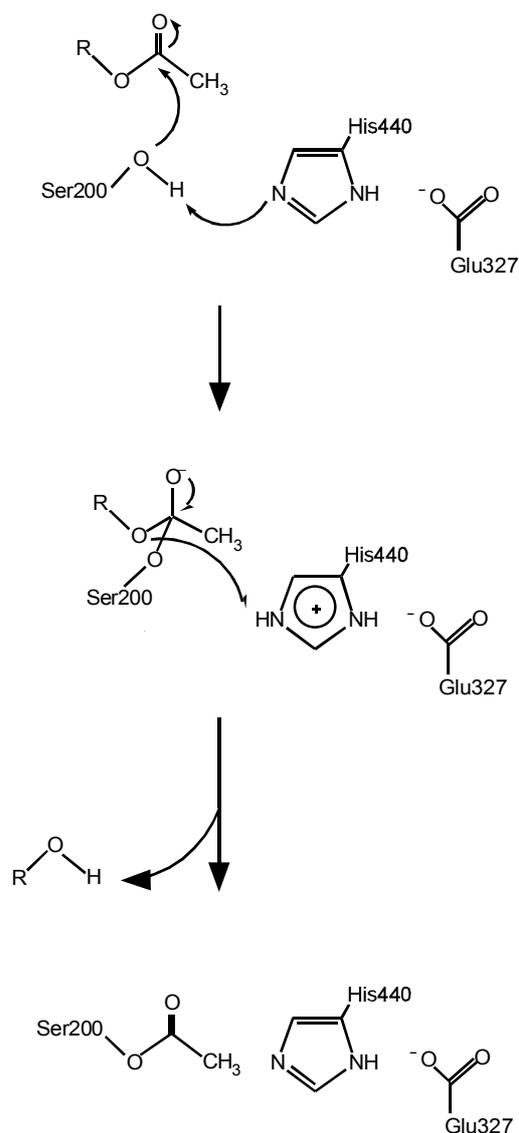
In the present study, *ab initio* molecular orbital (MO) and hybrid density functional theory (DFT) calculations were carried out for the initial step of the acylation reaction catalyzed by AChE in order to elucidate details of the reaction mechanism. In particular, we focus our attention mainly on the mechanism of the reaction and effect of the oxyanion hole on the energy diagram for the reactions.

2 Computational Section

2.1 Model of Enzyme–Substrate System

The catalytic triad in acetylcholinesterase (AChE) is composed of Ser200, His440, and Glu327. In the present study, these components were modeled by C_2H_5OH , $C_3N_2H_4(CH_3)$, and CH_3COO^- , respectively. These molecules are denoted hereafter by Ser200, His440 and Glu327, respectively, and the catalytic triad is expressed hereafter by AChE or Ser200–His440–Glu327. The structure of the substrate acetylcholine (ACh) was constructed by $C_2H_5COOCH_3$. It was known that the oxyanion hole is composed of peptidic N–H groups from Glu118, Glu119 and Ala201. In the present study, we assumed $H_2N(CO)CH_2NH_2$ molecule as a model of the oxyanion hole. This molecule, which is denoted hereafter by OAH, has two N–H groups which are possible to interact with ACh.

Acylation



Deacylation

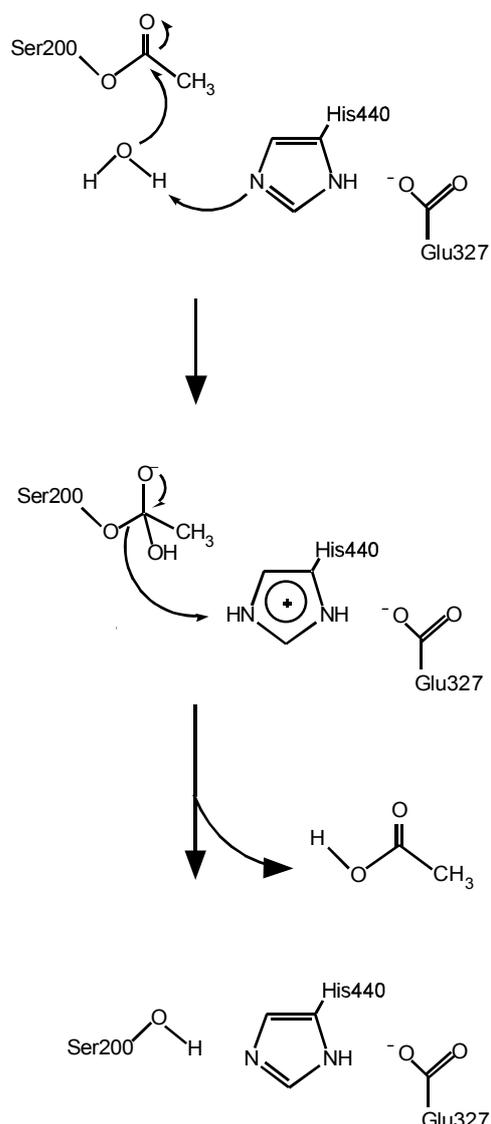


Figure 1. Reaction scheme of the hydrolysis of ACh catalyzed by AChE.

The structure of the catalytic triad (Ser200–His440–Glu327) was firstly constructed on the basis of the crystal structure of AChE determined at 1.8 Å resolution by X-ray experiment for the native enzyme (*T. californica*, PDB entry 1EA5) [12]. The relative intermolecular angles, ACh–Ser200–His440–Glu327, were fixed to those of the experimental values, and then the other geometrical parameters were fully optimized at the HF/3–21G(d) level of theory. The initial position of ACh relative to Ser200–His440–Glu327 was assumed on the basis of the crystal structure of inhibitor–AChE, *m*–(*N,N,N*–trimethylammino)trifluoroaceto–phenone–AChE (PDB entry 1AMN), and then the structure and relative position was reoptimized with the HF/3–21G(d) method. A schematic illustration of the model of the AChE–substrate–(oxyanion hole) system is given in Figure 2. All calculations were carried out using GAUSSIAN98 program package [13].

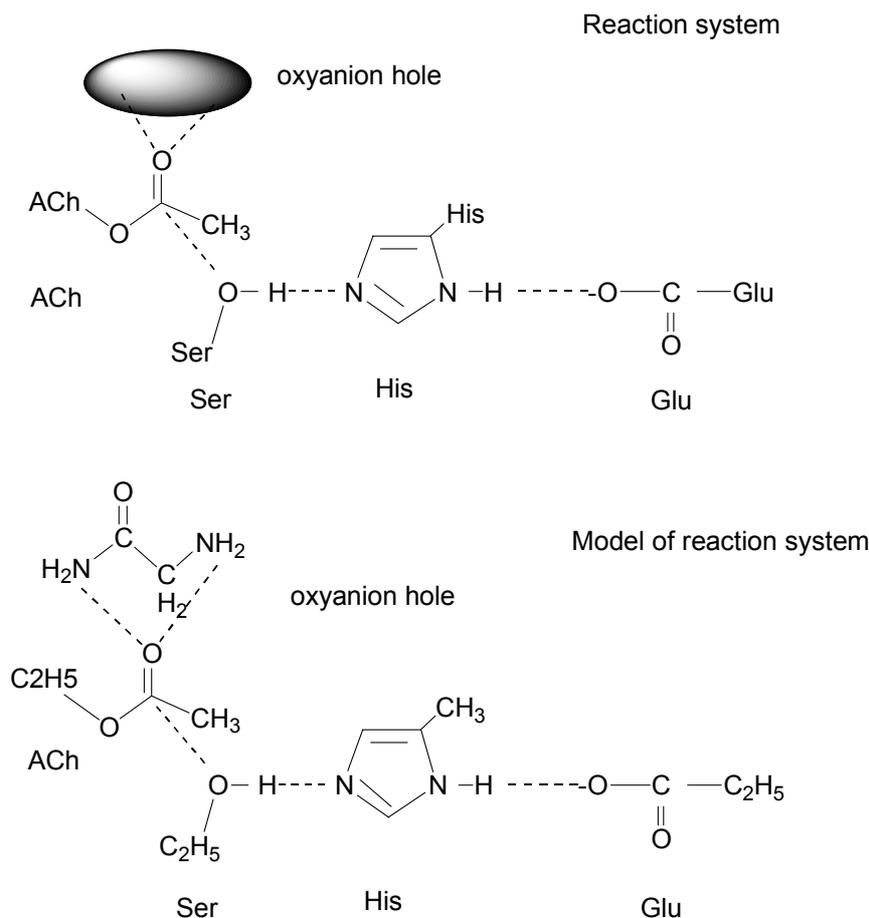


Figure 2. Model system for AChE–substrate (ACh)–(oxyanion hole) used in the present study. The catalytic triad is composed of Ser200, His440 and Glu327.

2.2 *Ab initio* Calculations

Geometries of all component molecules of AChE (Ser200–His440–Glu327), and AChE–substrate ACh, and AChE–ACh–(oxyanion hole) systems were optimized at the HF/3–21G(d) level, and then the energies of the system were recalculated at the B3LYP/6–311G(d,p) level for comparison.

3 RESULTS

3.1 Structures of Models of Enzyme (AChE) and Enzyme–substrate (AChE–ACh) Systems

The model of the catalytic triad (model of AChE) used in the present study is constructed of $\text{C}_2\text{H}_5\text{OH}$, $\text{C}_3\text{N}_2\text{H}_4(\text{CH}_3)$, and CH_3COO^- , denoted by Ser200, His440 and Glu327, respectively. First, the structure of free AChE without ACh was optimized at the HF/3–21G(d) level. The intermolecular distances for Ser200–His440 and His440–Glu327 were calculated to be 1.782 Å and 1.510 Å, respectively.

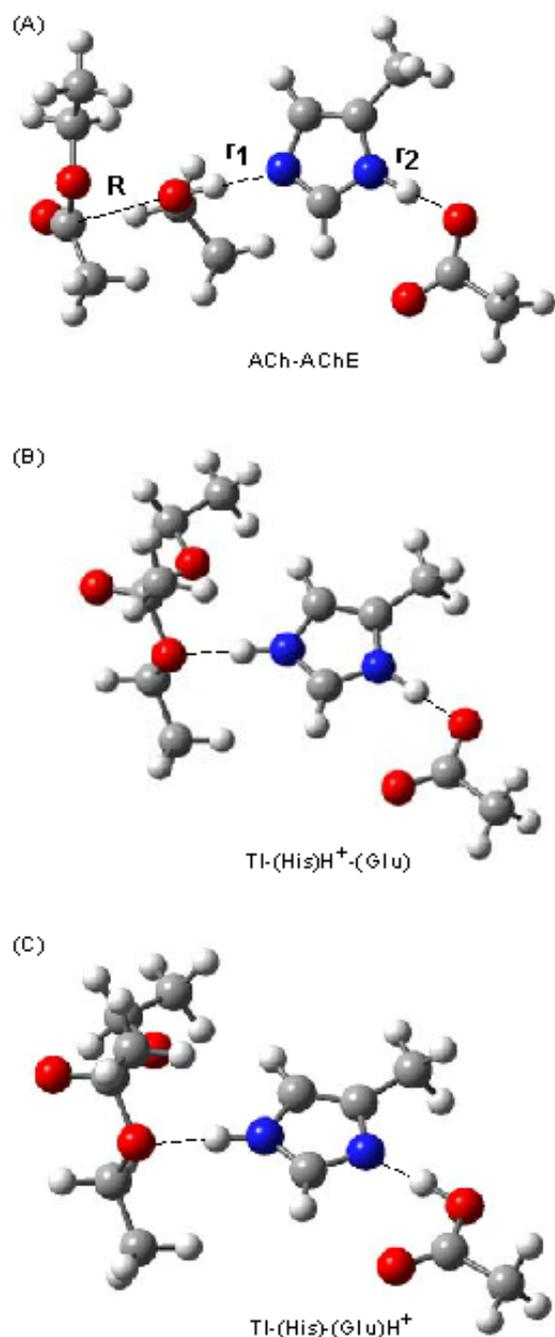


Figure 3. Optimized structures of ACh–AChE model system calculated at the HF/3–21G(d) level. (A) Structure of the pre-complex composed of catalytic triad (denoted by AChE) and substrate ACh. (B) Structure of the complex composed of tetrahedral intermediate (TI), protonated His and Glu. The proton of Ser is transferred to His (the first proton transfer). (C) Structure of the complex composed of tetrahedral intermediate (TI), His and protonated Glu. The proton of His is transferred to Glu (the second proton transfer).

Next, the structure of the catalytic triad–substrate system, expressed by AChE–ACh, was optimized at the same level of theory. The calculated structure is illustrated in Figure 3A. The intermolecular distance between substrate (ACh) and Ser200 of AChE was calculated to be $R = 2.721 \text{ \AA}$, where R denotes the distance between oxygen atom of Ser200 and carbonyl carbon of ACh. The structure of AChE was slightly changed by the interaction with ACh: $r_1 = 1.776$ and

$r_2 = 1.071 \text{ \AA}$ vs. $r_1 = 1.782$ and $r_2 = 1.069 \text{ \AA}$. The distance between Ser200 and His440 was slightly elongated by the interaction with ACh.

3.2 Structures of Tetrahedral Intermediate (TI)–(His)H⁺–Glu Complex

The acylation reaction of Ser200 with ACh leads to the product which is known as tetrahedral intermediate (TI). The product would be formed a complex composed of TI, protonated histidine (His–H⁺), and Glu. The optimized structure of the product complex expressed by TI–(His)H⁺–(Glu) is illustrated in Figure 3B. The interatomic distances for TI–(His)H⁺ and (His)H⁺–Glu were calculated to be 2.775 \AA (O–N distance between Ser200 and His440) and 2.579 \AA (N–O distance between His440 and Glu327), respectively. The distances of the proton from the residues were calculated to be $r_1 = 1.044 \text{ \AA}$ and $r_2 = 1.114 \text{ \AA}$. The proton of Ser200 was fully transferred to the imidazole ring of His440 after the formation of TI. The distance of TI from (His)H⁺ in the complex was calculated to be $R = 1.527 \text{ \AA}$, indicating that Ser200 is bound to ACh and then the covalent bond is formed.

3.3 Structure of the Double Proton Transferred Product, TI–(His)–(Glu)H⁺

If the proton of Ser200 is transferred to the imidazole ring of His440, it is possible that the second proton of His440 would be transferred to Glu327. The structure of the second proton transferred product composed of TI–(His)–(Glu)H⁺ was optimized at the HF/3–21G(d) level. The result is given in Figure 3C. The positions of two protons were calculated as $r_1 = 1.036$ and $r_2 = 1.493 \text{ \AA}$.

3.4 Effects of the Oxyanion Hole on the Structures of the Reaction System

In order to elucidate the effect of oxyanion hole (OAH) on the acylation reaction, the system including the modeled oxyanion hole was calculated at the same level of theory. The optimized structure of the pre-complex, OAH–ACh–AChE, is illustrated in Figure 4A. Two N–H protons of OAH orient toward the oxygen atom of ACh by hydrogen bond. The distances between the NH protons of OAH and the oxygen atom of ACh were calculated to be 2.056 and 1.902 \AA , indicating that one of the N–H protons of OAH is more strongly bound to the oxygen atom of ACh.

In the case of tetrahedral intermediate which is denoted by OAH–TI–(His)H⁺–Glu, the distances of the protons of OAH from the oxygen atom of ACh were calculated to be 1.968 and 1.766 \AA . The structure is illustrated in Figure 4B. The proton of Ser200 was fully transferred to His440. The distances of the hydrogen bonds in OAH–ACh–AChE become shorter in OAH–TI–(His)H⁺–Glu, indicating that the hydrogen bonds in tetrahedral intermediate are stronger than initial complex OAH–AChE–ACh.

After the second proton is transferred from His440 to Glu327, the hydrogen bond of OAH become stronger: namely, the distances were more shortened to be 1.898 and 1.724 \AA . This

structure is illustrated in Figure 4C.

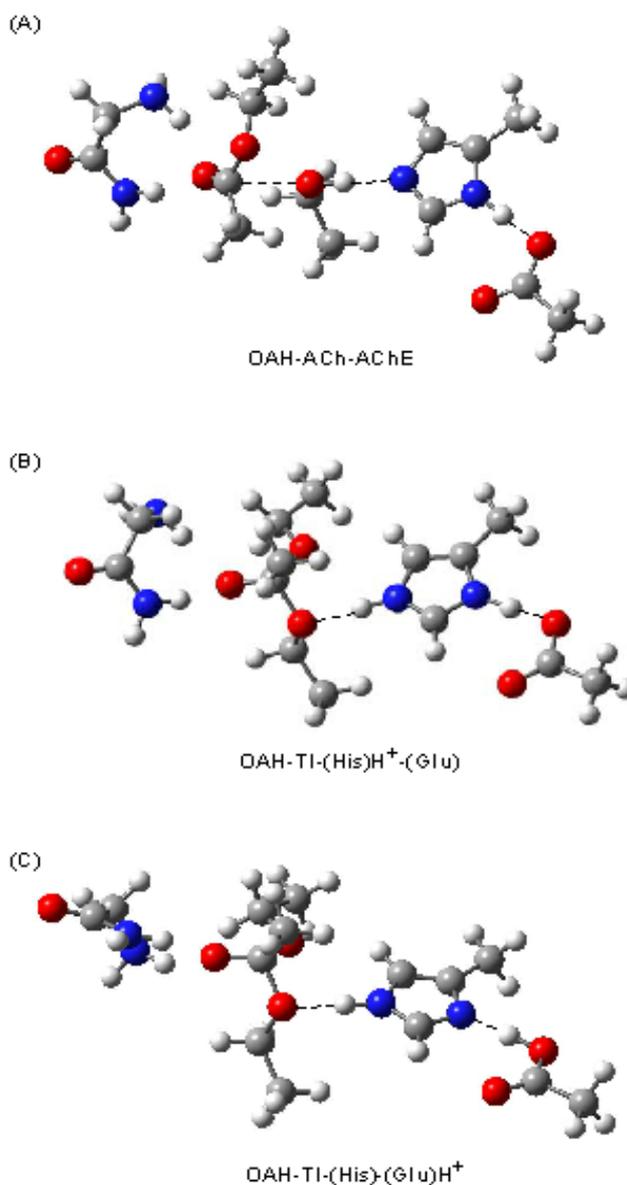


Figure 4. Optimized structures of oxyanion hole (OAH)–ACh–AChE system calculated at the HF/3–21G(d) level. (A) Structure of pre-complex composed of AChE, ACh and oxyanion hole (OAH). (B) Structure of OAH–TI–(His)H⁺–(Glu). (C) Structure of OAH–TI–(His)–(Glu)H⁺.

3.5 Potential energy curves along the reaction coordinate

The potential energy curve for the nucleophilic addition of Ser200 is plotted as a function of the reaction coordinate (R) in Figure 5. The geometrical parameters for the reaction system were optimized at each position along the reaction coordinate (R). The energies of the system were calculated for two cases: namely, the reaction systems with and without oxyanion hole.

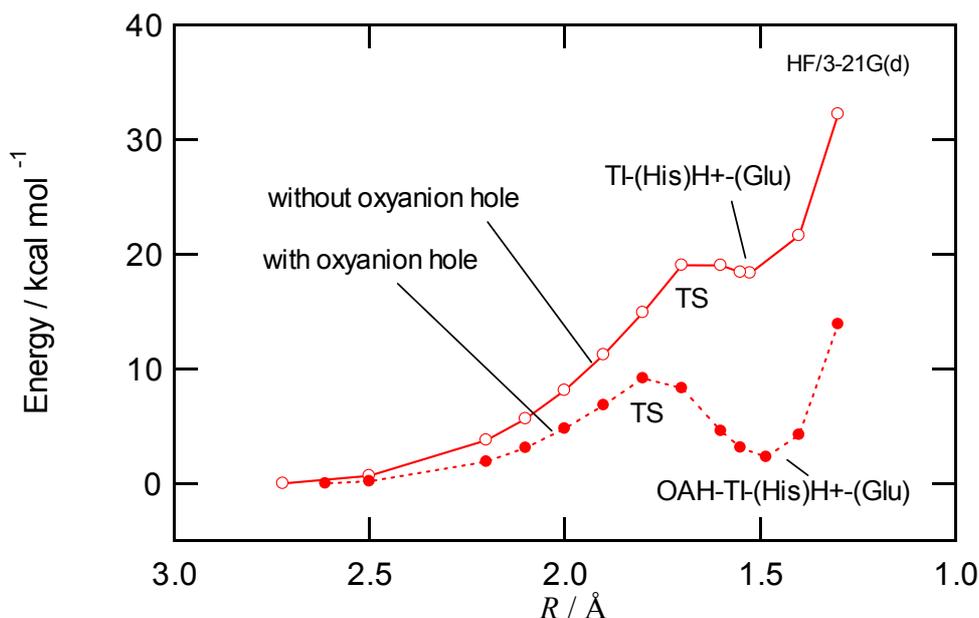


Figure 5. Potential energy curves for the formation of the tetrahedral intermediate from ACh–AChE calculated at the HF/3–21G(d) level. Real and dashed curves indicate the potential energy curves for the reaction calculated without and with the oxyanion hole (OAH), respectively.

The zero–energy level corresponds to the total energy of ACh–AChE for the reaction system without OAH. The potential energy curve for the reaction system without OAH is plotted in Figure 5 (real curve). The energy of the system was increased by approaching of Ser200 to ACh, and then it was reached to transition state (TS) at $R = 1.80 \text{ \AA}$. The barrier height was calculated to be 20.5 kcal/mol relative to ACh–AChE. More approaching of Ser200 to ACh leads to the formation of tetrahedral intermediate $\text{TI}-(\text{His})\text{H}^+(\text{Glu})$ whose energy is 18.03 kcal/mol relative to the initial state of ACh–AChE.

If OAH exists in the reaction system, the potential energy curve for the reaction is drastically changed as shown in Figure 5 (dashed curve). The barrier height under OAH was decreased to 10.4 kcal/mol which is 10.1 kcal/mol lower in energy than that of the reaction system without OAH. Also, it was found that the tetrahedral intermediate is significantly stabilized by the existence of the oxyanion hole (3.04 kcal/mol higher in energy than the initial state).

3.6 Energy Diagram of the Reaction

Potential energy diagram for the nucleophilic addition of Ser200 to ACh catalyzed by AChE is given in Figure 6. The energy of the initial state with zero–level corresponds to summation of total energies of ACh and AChE. If the substrate ACh is bound to AChE, the energy of the system was decreased by 10.29 kcal/mol. This state is represented hereafter by pre–complex ACh–AChE. The transition state (TS) is 20.50 kcal/mol higher in energy than that of pre–complex. After TS is left, tetrahedral intermediate (TI) is formed, which is expressed by $(\text{TI})-(\text{His})\text{H}^+(\text{Glu})$. This state is 2.47 kcal/mol more stable than TS. If the second proton transfer takes place from His to Glu, the next

complex, TI–His –(Glu)H⁺, is formed as product. This energy is comparable to that of the first proton transfer product (TI)–(His)H⁺–Glu, although the second proton transferred state is slightly higher in energy than the first proton transferred state. The activation barrier from the first to second proton transfer products was estimated to be 3.15 kcal/mol.

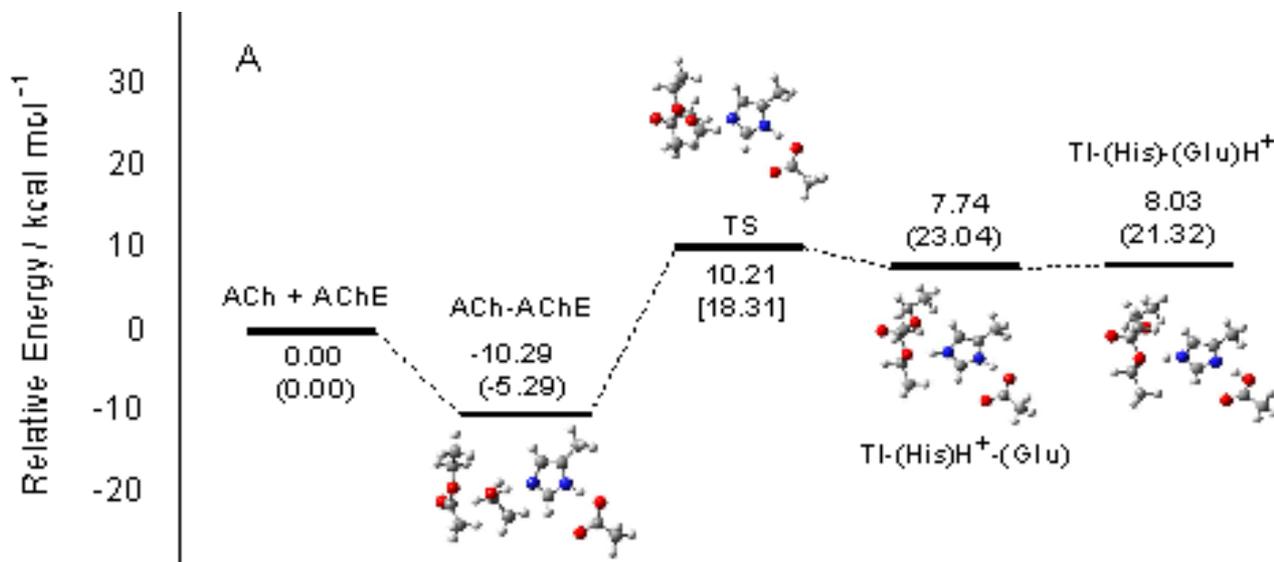


Figure 6. Potential energy diagram for the initial step of acylation of Serine catalyzed by AChE calculated at the HF/3–21G(d) level. B3LYP/6–311G(d,p)//HF/3–21G(d) values are given in parenthesis.

The corresponding values calculated at the B3LYP/6–311G(d,p)//HF/3–21G(d) are given in parenthesis of Figure 6. The tendency obtained was essentially similar to that of HF/3–21G(d). However, the energy of TS becomes lower than those of (TI)–(His)H⁺–Glu and TI–His –(Glu)H⁺. This is due to the fact that the geometry of TS is assumed to that of HF/3–21G(d). More accurate calculation is required to obtain more realistic energetics. Such calculations are now in progress.

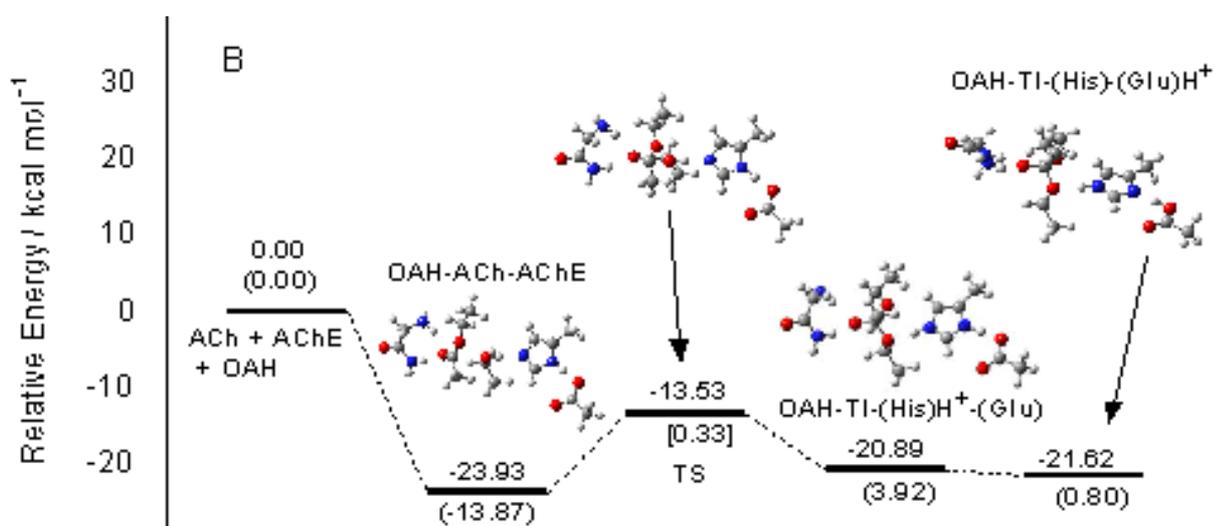


Figure 7. Effects of oxyanion hole on the potential energies for the reaction.

3.7 Effect of the Oxyanion Hole on the Energy Diagram of the Reaction

To elucidate the effect of oxyanion hole (OAH) on the nucleophilic addition, potential energy diagram for the OAH–ACh–AChE system was calculated and the results are given in Figure 7. The pre-complex in the OAH–ACh–AChE system was 23.93 kcal/mol more stable in energy than the separated state (ACh + AChE + OAH). Activation barrier for the reaction was calculated to be 10.4 kcal/mol relative to the pre-complex. The energy of the tetrahedral intermediate is 3.04 kcal/mol higher than that of the pre-complex. The second proton transferred state was energetically favorite than the first-proton transferred state. The activation barrier from the first to second proton transfer products was estimated to be 2.56 kcal/mol, which is slightly lower in energy than that of the proton transfer reaction without OAH. Thus, the present calculations indicated strongly that the oxyanion hole affects significantly the stabilization of TS and the product TI.

4 DISCUSSION

A. Summary and Conclusion

In the present study, *ab initio* MO and hybrid DFT calculations have been carried out for the reaction of ACh with AChE to form tetrahedral intermediate complex. The reaction needs an activation energy to proceed to the product. The reaction without oxyanion hole, the barrier height is estimated to be 20.5 kcal/mol at the HF/3–21G(d)//HF/3–21G(d) level. If the oxyanion hole is located in the vicinity of ACh, this energy is changed to 10.4 kcal/mol, meaning that the barrier height for the nucleophilic addition of Ser200 to ACh is significantly lowered by the existence of oxyanion hole interacting with ACh. The present calculated value for the activation barrier obtained by 10.4 kcal/mol is in good agreement with the recent experimental value (12.0 kcal/mol) [1, 6].

B. Comparison with previous theoretical studies

The activation barrier for the addition reaction of Ser203 to ACh was calculated by Zhang *et al.* by means of *ab initio* quantum mechanical/molecular mechanical (QM/MM) approach [11]. The barrier height obtained by them is 10.5 kcal/mol, which is in good agreement with the present calculated value (10.3 kcal/mol). They also showed that the effect of environment around the active site causes energy lowering of the barrier height, and the oxyanion hole interacts with ACh using two hydrogen bonds. This result is consistent with the present calculations.

Very recently, Zhang *et al.* have carried out trajectory calculation of ACh–AChE complex to generate the structures of ACh–AChE, and then activation barriers for first step of acylation reaction using selected structures generated by the trajectory calculations [20]. The average of the activation barrier was calculated to be 13.0 kcal/mol, which is also in good agreement with the present value.

C. Additional comments

In the present study, we assumed the HF/3–21G(d) optimized geometries for the several molecules at the stationary points throughout. In previous papers, we investigated several large molecular systems at the HF/3–21G(d) level of theory [14–19]. The results obtained at the HF/3–21G(d) calculations would give a reasonable feature for the structures. Therefore, the level of theory used in the present calculation would be adequate to discuss qualitatively the reaction of AChE with ACh. However, more accurate wave functions may provide deeper insight in the reaction mechanism. Such calculation is now in progress.

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