

Ab initio model study on acetylcholinesterase catalysis: potential energy surfaces of the proton transfer reactions

Hiroto Tachikawa ^{a,*}, Manabu Igarashi ^b, Jun Nishihira ^b, Teruo Ishibashi ^b

^a Division of Molecular Chemistry, Graduate School of Engineering, Hokkaido University, Sapporo 060-8628, Japan

^b Graduate School of Medicine, Hokkaido University, Sapporo 060-8628, Japan

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Abstract

Ab initio molecular orbital (MO) and hybrid density functional theory (DFT) calculations have been applied to the initial step of the acylation reaction catalyzed by acetylcholinesterase (AChE), which is the nucleophilic addition of Ser200 in catalytic triads to a neurotransmitter acetylcholine (ACh). We focus our attention mainly on the effects of oxyanion hole and Glu327 on the potential energy surfaces (PESs) for the proton transfer reactions in the catalytic triad Ser200–His440–Glu327. The activation barrier for the addition reaction of Ser200 to ACh was calculated to be 23.4 kcal/mol at the B3LYP/6-31G(d)//HF/3-21G(d) level of theory. The barrier height under the existence of oxyanion hole, namely, Ser200–His440–Glu327–ACh–(oxyanion hole) system, decreased significantly to 14.2 kcal/mol, which is in reasonable agreement with recent experimental value (12.0 kcal/mol). Removal of Glu327 from the catalytic triad caused destabilization of both energy of transition state for the reaction and tetrahedral intermediate (product). PESs calculated for the proton transfer reactions showed that the first proton transfer process is the most important in the stabilization of tetrahedral intermediate complex. The mechanism of addition reaction of ACh was discussed on the basis of theoretical results.

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1. Introduction

Acetylcholinesterase (AChE) is one of the enzymes which is composed of catalytic triad in the active site consisting of the amino acids serine (Ser), histidine (His), and glutamic acid (Glu), and it can synthesize neurotransmitter acetylcholine (ACh) by the transition of acetyl groups of acetyl CoA [1–4]. 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 Fig. 1. In the case of human AChE, Ser200 and His440 in the active site are involved in the reaction with 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 deacylation, the acyl enzyme is hydrolyzed back to its original form by water.

Although the overall reactions are thus well established, the details of the reaction mechanism is still controversial [4–11]. In particular, the catalytic role of the third residue of the catalytic triad, Glu327, and also role of oxyanion hole has been not clearly understood. Also,

* Corresponding author. Tel.: +81 11706 6750; fax: +81 11706 7897.
E-mail address: hiroto@eng.hokudai.ac.jp (H. Tachikawa).

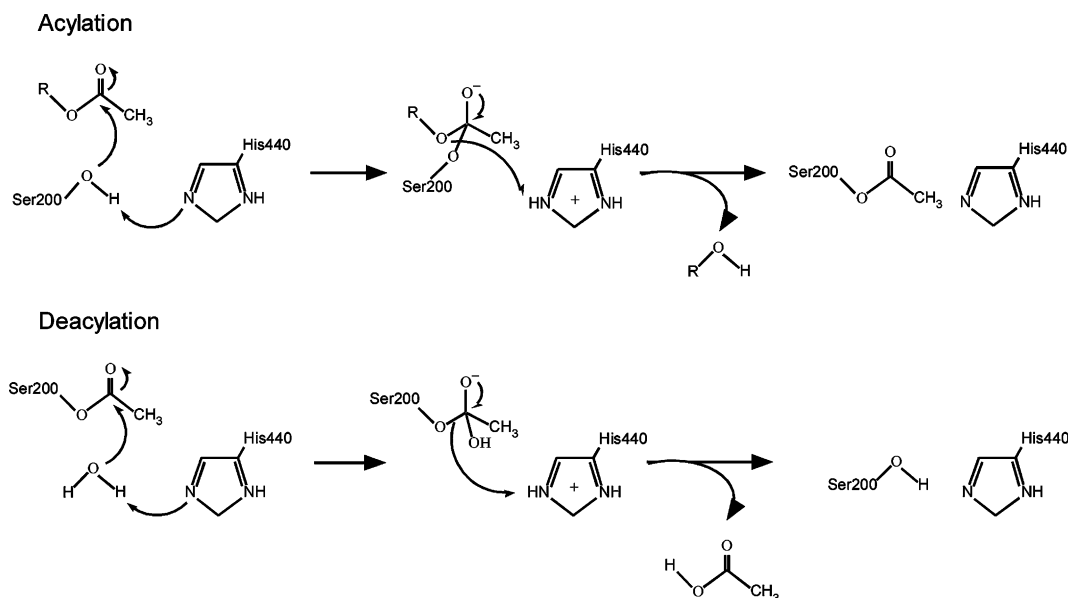


Fig. 1. Reaction scheme of the hydrolysis of ACh catalyzed by AChE.

the question whether the second proton transfer from His440 to Glu327 occurs or not is still unclear.

From the previous works, the role of Glu327 has been investigated on the basis of theoretical calculations. The first is “charge-relay” mechanism in which two protons (namely, both first and second protons) are transferred from Ser to His and from His to Glu327, respectively [5,6]. A positive charge is transferred from Ser to Glu by the movement of protons. The second model is “single proton transfer mechanism” that the electrostatic interaction from Glu327 to imidazole cation of His stabilizes the transition state in the reaction and also the energy of tetrahedral intermediate complex [7,8]. Only the first proton is transferred, while the second proton is still retained in His440. The third model is “low-barrier hydrogen bond” mechanism proposed on the basis of proton NMR experiment [4,9]. Also, role of oxyanion hole is still in controversy.

Li et al. [10] investigated proton transfer processes in a model triad composed of the simplest three components, formate–imidazole–water system, using *ab initio* calculations. They focused on the proton transfer processes: namely, charge relay mechanism or single proton-transfer mechanism in the formate–imidazole–water system. From their calculations, it was suggested that both mechanisms are energetically comparable with each other, although the charge relay mechanism is slightly lower in energy than that of simple proton transfer mechanism. On the other hand, recent work carried out by Zhang et al. [11] showed that the second proton transfer needs higher activation energy for the reaction to proceed.

In the present study, *ab initio* molecular orbital (MO) and hybrid density functional theory (DFT) calculations

are 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 on the mechanism for the proton transfer processes in Ser200–His440–Glu327 during the addition reaction of the oxygen atom of Ser200 to ACh. Therefore, two dimensional potential energy surfaces for the two-proton transfers along the reaction coordinate of the addition reaction are calculated. The role of Glu327 and the role of the oxyanion hole are also investigated in the present study.

2. Computational

2.1. Model of catalytic triad–substrate system

Component molecules of catalytic triad in AChE, Ser200, His440, and Glu327, are modeled by C₂H₅OH, C₃N₂H₄(CH₃), and CH₃COO[−], respectively. This catalytic triad is expressed hereafter by Ser200–His440–Glu327 or AChE. The structure of substrate ACh was constructed by C₂H₅COOCH₃. The oxyanion hole is composed of peptidic N–H groups from Glu118, Glu119 and Ala201. This has three N–H groups interacting with Ach. Among them, two N–H groups are close to ACh. In the present study, we assumed the H₂N(CO)CH₂NH₂ molecule as a model of the oxyanion hole.

The structures of AChE and oxyanion hole are constructed on the basis of the crystalline structure of AChE determined at 2.5 Å resolution by the X-ray experiment for native enzyme [12], and then those are fully optimized at the HF/3-21G(d) level of theory:

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