A first principle study on the interaction between acetylcholinesterase and acetylcholine, and also rivastigmine in alzheimer's disease case

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Abstract. The catalytic activity of acetylcholinesterase enzyme (AChE) relates to the symptom progress in Alzheimer's disease. Interaction of AChE with rivastigmine (from the medicine) can reduce its catalytic activity toward acetylcholine to decelerate the progression of Alzheimer's disease. This research attempts to study the interaction between AChE and rivastigmine, and also acetylcholine (without the presence of rivastigmine) using density functional theory by simplifying the reaction occurs in the active site, which is assumed to be $\text{C}_2\text{H}_5\text{OH}$, $\text{C}_3\text{N}_2\text{H}_3(\text{CH}_3)$, and $\text{CH}_3\text{COO}^-$. The results suggest that AChE interacts easier with acetylcholine than with rivastigmine, which implies that the medicine does not effectively reduce the catalytic activity of AChE. At this stage, no experimental data is available to be compared with the calculation results. Nonetheless, this study has shown a good prospect to understand the AChE-substrate interaction using a first-principles calculation.

1. Introduction

Acetylcholine, one of important neurotransmitters, plays a significant role in increasing attention and facilitating learning process [1]. Under cholinergic hypothesis, the deficiency of acetylcholine is responsible for memory impairment in Alzheimer's disease (AD). This deficiency occurs due to a high catalytic activity of acetylcholinesterase enzyme (AChE) to hydrolize acetylcholine into acetate and choline. The hydrolysis process of acetylcholine triggers the termination of neurotransmission in brain synapses which results in the failure of memory formation.

Therapeutic strategy that has been effective so far for enhancing cholinergic function is the use of cholinesterase inhibitors. The inhibitors increase the levels of acetylcholine in brain synapses by inhibiting AChE, preventing AChE to break acetylcholine. Theoretically, by maintaining of acetylcholine levels in brain synapses, the inhibitors assist to slow the progression of AD. The first generation cholinesterase inhibitors approved for treatment of AD are tacrine [2, 3] and carbamate physostigmine [4, 5]. However, both of them have potentially adverse side effect on hepatic and cardiovascular function [6]. The second generation cholinesterase inhibitor, rivastigmine [7], is
generally well tolerated. It is approved as an effective treatment for symptoms of AD in mild stage to moderate stage [8].

The precision mechanism of rivastigmine's action as cholinesterase inhibitors in molecular level is still unclear [8], thereby providing theoretical study is a necessity. We aim to model the complicated enzyme and substrates interaction system by utilizing density functional theory (DFT). As a first attempt, we simplify AChE as C\textsubscript{2}H\textsubscript{5}OH, C\textsubscript{3}N\textsubscript{2}H\textsubscript{3}(CH\textsubscript{3}), and CH\textsubscript{3}COO\textsuperscript{-}. Thus, in this study we focus on the interaction between AChE and rivastigmine, and also acetylcholine because both of substrates interact with AChE on the same essential unit in active site namely catalytic triad. The catalytic triad consists of Serine (Ser200), Histidine (His440) and Glutamic acid (Glu327). Previous study reveals that rivastigmine binds to Ser200 after AChE inhibition [9]. This amino acid is also the interaction key of AChE to catalyze the hydrolysis of acetylcholine [10, 11].

2. Calculation method
We firstly construct AChE by using Igarashi model [12]. Igarashi model consists of C\textsubscript{2}H\textsubscript{5}OH, C\textsubscript{3}N\textsubscript{2}H\textsubscript{3}(CH\textsubscript{3})\textsuperscript{+}, and CH\textsubscript{3}COO\textsuperscript{-} which represents serine (Ser200), histidine (His440), and glutamic acid (Glu327) respectively. The chosen substrates are acetylcholine and rivastigmine (see figure 1). We perform geometry optimization to obtain the most stable structure of AChE, acetylcholine and rivastigmine. The optimized structures then become the base for establishing interaction models, AChE-acetylcholine and AChE-rivastigmine (see figure 2 and figure 3). We further perform optimization in different C-O distances between Ser200 and substrate with interval 0.2 Å to get the minimum reaction energy path. To ensure that the maximum and minimum potential energies correspond to the right transition and bonding structures, we perform frequency calculations of the optimized structures. We carry out all DFT calculations in gas phase by using Becke-Lee-Yang-Par (B3LYP) exchange-correlation functional [13] and 6-31G(d,p) [14, 15] basis set which are implemented in Gaussian09 suite program [16].

![Figure 1. Structure model of substrate. (a) Acetylcholine and (b) Rivastigmine.](image1)

![Figure 2. Model system of AChE-acetylcholine.](image2)
3. Result and discussion

3.1. Initial calculation
We establish the interaction models based on the optimization geometry calculation. As for the ground state, the geometry optimizations give singlet spin configuration for all systems. Table 1 and table 2 present the selected parameters obtained by these calculations together with experimental data for comparison. These tables show that the results are in good agreement with X-ray diffraction results [11, 17]. This implies that B3LYP exchange correlation functional and 6-31G(d,p) are appropriate for the present systems and further calculations.

Table 1. Optimized geometry for acetylcholine.

| Parameter | DFT | Exp.\(^a\) | Error (%) |
|-----------|-----|------------|-----------|
| C7-C6     | 1.50| 1.49       | 0.74      |
| C6-O2     | 1.21| 1.18       | 2.13      |
| C6-O1     | 1.38| 1.38       | 0.21      |
| O1-C5     | 1.46| 1.45       | 0.76      |
| C5-C1     | 1.53| 1.47       | 3.92      |
| C1-N1     | 1.53| 1.49       | 2.41      |
| N1-C2     | 1.51| 1.50       | 0.67      |
| Angle (°) |     |            |           |
| N1-C1-C5  | 115.4| 116.5     | 0.94      |
| C1-C5-O1  | 103.3| 111.6     | 7.44      |
| C5-O1-C6  | 113.7| 115.7     | 1.73      |
| O1-C9-O2  | 121.1| 122.8     | 1.36      |
| O1-C6-C7  | 111.1| 111.3     | 0.18      |
| O2-C6-C7  | 121.8| 125.9     | 3.26      |

\(^a\)Experimental value taken from Ref. [17].

Table 2. Optimized geometry for acetylcholinesterase.

| Parameter | DFT | Exp.\(^a\) | Error (%) |
|-----------|-----|------------|-----------|
| O\(_S\)-N\(_{hi1}\) | 2.78| 3.10       | 10.32     |
| N\(_{hi2}\)-O\(_S\) | 2.60| 2.52       | 3.17      |

\(^a\)Experimental value taken from Ref. [11].
3.2. Potential energy curves

Figure 4 shows calculated minimum-energy paths of both systems. The plotted potential energy curve is a function of the C-O atomic distance ($R_{C-O}$). The formation of C-O bond between substrate (rivastigmine or acetylcholine) and AChE proceeds from right to the left in the potential energy curves. Figure 4 shows that the potential energy of both systems rises when substrate approaches to the Ser200, and then it drops until it reaches a minimum.

![Potential energy curve of both systems](image)

**Figure 4.** Potential energy curve of both systems, acetylcholinesterase with acetylcholine (line with circle mark) and acetylcholinesterase with rivastigmine (line with square mark). The reaction proceeds from the right to the left.

The maximum potential energy in potential energy curves indicates the transition state of C-O bond formation for both systems. The results confirm that the optimized structures are transition structures, which are indicated by one imaginary frequency in their vibrational modes. The potential reaches its maximum at $R_{C-O} = 1.48$ Å for AChE-acetylcholine system and at $R_{C-O} = 1.78$ Å for AChE-rivastigmine system.

The transition structure of both systems undergoes the bond length alteration of H-N$_{H1}$ in AChE structure. This alteration indicates the possibility of proton transfer from Ser200 to His440 when AChE interacts with substrates. The hydrogen atom tends to move from Ser200 to His440 such that the bond length of H-N$_{H1}$ is shortened 0.75 Å and 0.30 Å, for AChE-acetylcholine and AChE-rivastigmine systems, respectively.

Table 3 lists the calculated potential energy barrier and bonding energy. The results show that the potential energy barrier of AChE-acetylcholine is 0.42 eV higher than the energy barrier (shown in parenthesis) which estimated from the experimental value of $k_{cat}$ by simple transition theory [10, 18]. This difference may exist because we do not include the oxyanione hole in our models. The previous study shows that oxyanione hole has a significant role to stabilize the transition state, so the calculated potential energy barrier becomes less and closer to the experimental value [12]. Another reason, the differences in the calculated barrier energy relate to the structural fluctuations of AChE-substrate as reported by Zhang et al [19].
Table 3. The calculated energy barrier and bonding energy in eV.

| System                | Energy barrier  | Bonding energy |
|-----------------------|-----------------|----------------|
| AChE-achetylcholine   | 0.94 (0.52\textsuperscript{a,b}) | 0.26           |
| AChE-rivastigmine     | 1.20            | 0.43           |

\textsuperscript{a}Experimental value taken from Ref. [10].
\textsuperscript{b}Experimental value taken from Ref. [18].

The C-O bond formation between AChE and substrates occurs when potential energy reaches its minimum. The potential energy of AChE-acetylcholine reaches its minimum at $R_{C-O} = 1.34$ Å. The formation of C-O bond involves the breakdown of acetylcholine into acyl which binds to Ser200 and free choline, it is possibly a mark of a beginning hydrolysis process as explained in Ref. [20]. In AChE-rivastigmine system, rivastigmine breaks into two parts, namely the carbamyl moiety and the leaving group of rivastigmine or NAP (see figure 5). The carbamyl moiety binds covalently with Ser200 within 1.36 Å, which has a good agreement with the X-ray crystallography results (1.39 Å) [9].

![Figure 5. The bonding structure of AChE-rivastigmine system. Rivastigmine is detached into carbamyl moiety and NAP. Rivastigmine is rendered as a ball and stick model with colors, with carbon atoms colored yellow, oxygen atoms colored red, and nitrogen atoms colored purple.](image)

We further investigate the role of rivastigmine as cholinesterase inhibitor drug from calculated energy point of view. As shown in table 3, AChE-rivastigmine system has bonding energy 0.17 eV higher than that of AChE-acetylcholine. The results imply that AChE-rivastigmine system is more stable than AChE-acetylcholine system in gas phase. In contrast, the calculated energy barrier shows that AChE prefer to react with acetylcholine than with rivastigmine because the energy barrier of AChE-acetylcholine is 0.26 eV less than that of AChE-rivastigmine. This contradiction leads to a conclusion that rivastigmine is less effective to prevent AChE hydrolizes acetylcholine. However, some studies show that rivastigmine significantly improve cognitive function in Alzheimer’s disease patients, especially in the given high dose [21-27]. The high number of rivastigmine molecules probably gives a better possibility to interact with AChE due to the bonding competition with acetylcholine.

4. Conclusion

We have shown the prospect of DFT calculation for studying enzyme-substrate interaction, but our simplified models of interaction still need some refinements. Our model overestimates the energy barrier of the AChE-acetylcholine. The differences of calculated energy barrier with experimental value are reasonable since we do not include oxyanion hole in AChE model. The environmental effect should also give effect in the AChE-substrate interaction. Our results demonstrate the contradictive prediction due to the role of rivastigmine as cholinesterase inhibitor drug. The prediction gives the less
effective of rivastigmine due to the energy barrier comparison of both systems. However, at this stage our models need more experimental data to confirm.

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