Energetic Analyses on the Binding between the Anionic Site of Acetylcholinesterase, ASP 72 and TRP 84, and the Quaternary Ammonium Locus of its Inhibitor, Rivastigmine
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1 Introduction

Rivastigmine, (S)-N-Ethyl-N-methyl-3-[1-(dimethylamino)ethyl]-phenyl carbamate, administered as a tartrate salt, is a reversible cholinesterase inhibitor. This compound being marketed in Europe, this is in clinical trial in U.S.A. The mechanism of rivastigmine is postulated to exert its therapeutic effect by enhancing cholinergic function, by increasing the concentration of acetylcholine (Fig. 1) through reversible inhibition of its hydrolysis by cholinesterase.

An alkylammonium-carboxylate ion-pair interaction is a common model in molecular recognition. In this work we concentrate on the reaction between the asparatic residue (ASP 72) of the active site of acetylcholinesterase (AChE) and rivastigmine. But it has been pointed out that there is no local energy minimum on the potential surface corresponding to an ion-pair.

The quarternary alkylmethyl ammonium ion (Fig.1) attracts the aromatic ring of TRP of AChE. In this report, by means of modeling a ternary complex, including ASP 72, TRP 84, and rivastigmine, energetic analyses are to be performed besides proton transfer reaction one.1

2 Method

The geometries for rivastigmine are built from standard ones. The coordinates of the two residues (ASP 72 and TRP 84) are taken from those of AChE in Protein Data Bank (PDB) (Fig. 2). The complex of rivastigmine and a carboxylate ion as the sidechain of ASP is modeled by placing them to form a straight H-bond, N−H⋯O (Fig. 3). Indole molecule as simplified for TRP is included in a ternary complex. Trimethylammonium cation replaced the ligand for simplicity (Fig. 5).

Firstly, for crude optimization, a Hartree-Fock (HF) method at 6-31G** was applied. Secondly, for higher level of calculation, optimization was performed at B3LYP/6-31G** level as well as at an MP2 method to consider electron correlation. Using final coordinates obtained here, the Natural Bond Orbital (NBO) analysis was performed at HF/6-31G** level2. The Kitaura-Morokuma (KM) energy decomposition scheme3 was applied to examine the interaction energy between the residues and rivastigmine in order to compare electrostatic energy with charge transfer one quantitatively. GaussView was used to visualize the results by the molecular orbital (MO) calculations and for molecular modeling on Windows 98. Ab initio MO calculations were performed by GAMESS (Iowa State University) and Gaussian 984 on networked Pentium (800MHz-1GHz) OpenLinux (kernel version 2.4) workstations. GAMESS was used for the KM analysis at HF/4-31G level using the coordinates that were optimized by Gaussian 98 at B3LYP/6-31G** level. Solvent effect is considered by a PCM reaction field calculation.

3 Results

3.1 H-Bond Formation of Rivastigmine with ASP 72

We assume that the quarternary ammonium group of rivastigmine binds to the carboxylate for ASP 72 of AChE, to form an ion-pair. In the strong binding through a hydrogen bond between them, proton transfer (PT) reaction is possible to occur especially in a hydrophobic environment. The PT potential functions were calculated at B3LYP/6-31G**, in vacuo or considering solvent effect. A neutral complex was finally obtained in Fig. 3.

3.2 Proton Transfer Reaction in Continuum Solvation

The potential function of PT is influenced on by the distance from the hydrogen donor atom of the ligand (N−H) to the acceptor atom of the carboxylate (O), calculated at R_{N⋯O} = 2.78 and 3.20 Å. The dielectric constant (ε) was varied in the range of ε = 1 (in vacuo), 2, and 10, designed for a more hydrophobic environment in such a active site cleft. When calculated at R_{N⋯O} = 2.78 Å and ε = 10, energy barrier (ca. 3 kcal/mol) has been found so lower that

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1molecule@pop12.ohm.ne.jp


4Gaussian 98 ver. A.9 and GaussView ver. 2.1 are programs obtained from Gaussian, Inc. The former program was compiled and linked by a gcc compiler in our laboratory.
a neutral complex is assumed more favorable by 6 kcal/mol, as depicted in Fig. 4a. On the other hand, at \( R_{N...O} = 3.20 \) Å, higher energy barrier values were calculated, ca. 10 kcal/mol (Fig 4b), where an ion-pair complex might hardly change to a neutral one. This result is consistent to the previous report that the trimethylamine-formic acid complex in continuum solvation is more favored by ca. 9 kcal/mol at \( R_{N...O} = 2.842 \) Å, than its ion-pair, by a high level \textit{ab initio} method MP4SDQ.\textsuperscript{5}

### 3.3 Energetic Analyses of the Interaction

A ternary complex was built, which contains N-acetyl-L-aspartate-N-methylamide for ASP 72 (denoted by unit 1), indole for TRP 84 (unit 2) and trimethylammonium ion (unit 3) for rivastigmine, as depicted in Fig. 5. This structure was optimized in energy by HF and then, DFT(B3LYP) methods at 6-31G**. Kitaura and Morokuma (KM) analysis was followed at HF/4-31G//B3LYP/6-31G** level of theory, using GAMESS. The calculated polarization (PL) and CT energies were still significant, listed in Table 1. CT energies between the units were also estimated by the deletion method of Natural Bond Orbital (NBO) analysis at HF/6-31G**//B3LYP/6-31G**, using Gaussian 98 package (Table 2).

### 4 Conclusion

This energy decomposition analysis has shown that the CT and PL energies are not negligible, although the ES interaction energy plays a significant role. The PT reaction was examined between ASP 72 and rivastigmine at B3LYP/6-31G** level. As a result, a neutral complex is more favored at \( R_{N...O} = 2.78 \) Å by 5–12 kcal/mol according to the \( \epsilon \) (the barrier is less than 3 kcal/mol). The height of the barrier has become higher (Fig. 4) by a PCM reaction field calculation using the polarizable dielectric model, \( \epsilon = 2 \sim 10 \) at \( R_{N...O} = 2.78 \) and 3.20 Å. As a result of this type of stable binding, rivastigmine has shown a potent inhibitory activity to AChE.

**Fig. 1** Acetylcholine (left), Quaternary Ammonium Cation (center) and Structure of Rivastigmine (Dicationic form is not considered) (right).

**Fig. 2** N-acetyl-L-asparatate-N-methy lamide for ASP 72 (R=CH$_2$COO$^-$) (left) and Indole for TRP 84 without the backbone atoms (right).

**Table 1.** Kitaura and Morokuma Analysis of Interactions between the Units.

<table>
<thead>
<tr>
<th>ENERGY (kcal/mol)</th>
<th>unit 1 ↔ unit 3</th>
<th>unit 2 ↔ unit 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>ELECTROSTATIC (ES)</td>
<td>-31.54</td>
<td>-10.51</td>
</tr>
<tr>
<td>EXCHANGE REPULSION (EX)</td>
<td>29.64</td>
<td>7.38</td>
</tr>
<tr>
<td>POLARIZATION (PL)</td>
<td>-5.27</td>
<td>-1.27</td>
</tr>
<tr>
<td>CHARGE TRANSFER (CT)</td>
<td>-10.70</td>
<td>-2.47</td>
</tr>
<tr>
<td>HIGH ORDER COUPLING (MIX)</td>
<td>-1.24</td>
<td>-0.51</td>
</tr>
<tr>
<td>TOTAL INTERACTION (DELTA-E)</td>
<td>-19.10</td>
<td>-7.38</td>
</tr>
</tbody>
</table>

**Table 2.** NBO Analysis of CT Energies between the Units.

<table>
<thead>
<tr>
<th>ENERGY (kcal/mol)</th>
<th>unit 1 ↔ unit 3</th>
<th>unit 2 ↔ unit 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>CHARGE TRANSFER (CT)</td>
<td>-47.878</td>
<td>-2.081</td>
</tr>
</tbody>
</table>

**Fig. 3** Optimized Structure of acetate and cationic Rivastigmine at B3LYP/6-31G**, showing the H-Bond.

A proton has been transferred from the ligand.
Fig. 4a (left) and 4b (right). Proton transfer transfer potential (Rivastugmine—Carboxylate)

Fig. 5  Optimized Structure of Methylamine, ASP and TRP at B3LYP/6-31G**, drawing the H-Bond by a thin line.