Ab Initio Study on the Transition State of Acylation Step of Trypsin Catalysis

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The transition state of acylation step of trypsin catalysis was determined by molecular orbital calculations. The calculations were carried out at the RHF-LCAO-SCF approximation level with double zeta basis set (plus polarization functions).

The role of His57 residue in the acylation step of the catalytic reaction of trypsin was analysed from a quantum mechanical point of view. The influences of surrounding residues, such as oxyanion hole and Asp102, and the electrostatic effect of the other regions of the enzyme were also studied.

His57 was proved to capture the proton from Ser195 side chain terminus with its lone pair and to transfer it to substrate with electrostatic assistance of Asp102 and oxyanion hole.

Keywords — trypsin; transition state; electrostatic effect; ab initio; molecular orbital; energy decomposition

Introduction

Proteins and nucleic acids are the most basic substances that play significant functional and structural roles in biochemical activities. Enzymes that play the former one are deeply involved in biochemical reactions as catalysts. Therefore, elucidating the mechanisms of enzymatic reactions is indispensable for understanding biological phenomena. Three dimensional structures of proteins such as enzymes that exist in biological systems are established by results from X-ray diffraction experiments, neutron diffraction analyses, and so on. What we can derive from those results, however, is only a guess on static aspects of the biological molecules. It is, therefore, requisite to investigate the reactions quantitatively from another standpoint rather than the static one mentioned above.

Problems related to molecules can be reduced eventually to ones on nuclei and electrons, which is also true for enzymes. Therefore, the enzymatic reactions should be studied at a molecular level as problems of nuclei and electrons. (It is needless to say that in addition to this sort of analytical approach synthetic ones should also be done.)

The final goal of the authors’ project is to clarify the mechanism of enzymatic reactions applying methodologies of quantum mechanics and molecular dynamics. Umeyama and his colleagues have been devoting their effort into studies on the enzymatic reaction mechanisms from a theoretical standpoint. Their targets were mainly serine proteases, on which many X-ray diffraction and neutron diffraction experiments had been done and reliable coordinates had been reported. These are just one group among a large number of enzymes. They hope, however, that a very close insight into these enzymes will reveal principles which are common to all enzymes. The present work came in line with all previous studies to develop and deepen the understanding of enzymatic mechanisms.

The system taken up in the present work is trypsin. Three dimensional structures of serine proteases such as trypsin have been made clear to a large extent from experiments. According to those analyses, the group of enzymes have two common structures which are necessary to exert influence on the biological activity. One of these is the active center which is called the catalytic triad: composed of Ser195, His57 and Asp102. The other is the part called the oxyanion hole consisting of N-H groups of the main

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The behavior of two protons contributing to two hydrogen bonds of the catalytic triad in the acylation step has been a controversial subject for a long period of time. At the very beginning Blow et al.\textsuperscript{11} proposed a charge relay mechanism which brings about the strong nucleophilicity of Ser195 side chain. According to their theory, one proton transfers from Ser195 to His57, and at the same time the other proton goes from His57 to Asp102. In the same year, however, Polgar et al.\textsuperscript{2} reported another theory which is opposed to Blow’s one, stating that Asp102 anion stabilized His57 imidazolium cation by making a hydrogen bond with a nitrogen atom (delta one) of His57. The difference between the two is on the role of Asp102.

As for experimental reports which had supported Blow’s theory, Hunkapiller, Smallcombe, Hunkapiller et al.\textsuperscript{3} and Koepppe et al.\textsuperscript{4} determined the $pK_\alpha$ of Asp102 to be approximately 6.8, and that of His57 to be about 3.8 from nuclear magnetic resonance (NMR) and infrared measurements, respectively. These mean that Asp102 and His57 function as a proton acceptor and a proton donor, respectively. Theoretically Umeyama et al.\textsuperscript{5} buttressed up the mechanism with CNDO/2 calculation.

On the other hand, there are many papers which deny the charge relay mechanism. Experimentally Robillard et al.\textsuperscript{6} and Bachovhin et al.\textsuperscript{7} performed NMR measurements to show that the $pK_\alpha$ value of His57 is rather high, standing by for Polgar et al.\textsuperscript{2} Theoretically, Nakagawa et al.\textsuperscript{8} carried out \textit{ab initio} molecular orbital calculations based on 4-31G basis set. They drew a potential map taking degrees of transfer of two protons along two hydrogen bonds in the catalytic triad as two independent parameters, and showed that the charge relay mechanism was not energetically preferable. Umeyama et al.\textsuperscript{9} reported it was difficult for the proton bound to the nitrogen atom (delta one) of His57 ($\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\·
such as PTI (pancreatic trypsin inhibitor)-trypsin complex (Huber et al.\textsuperscript{20}) and STI (soybean trypsin inhibitor)-trypsin complex (Sweet et al.\textsuperscript{21}). Hydrogen bonds in an oxyanion hole are supposed to maintain low energy of tetrahedral structure of substrate-enzyme complex and secure an energetically preferable path of catalytic reaction (for a review, Kraut\textsuperscript{22}). Nakagawa et al.\textsuperscript{13} explained quantitatively the effect of oxyanion hole using \textit{ab initio} molecular orbital calculation at the level of 4-31G basis set, with reference to X-ray diffraction data.

As reviewed above, serine proteases have two common structural systems, catalytic triad and oxyanion hole, which are important from a functional viewpoint. The role of Asp102\textsuperscript{−} in the former structure has been made clear under the influence of enzymatic field including the latter portion.

Then what role does the lone pair of nitrogen (epsilon two) of His57 in the catalytic triad play in the catalysis? In order to solve this problem, we decided the location of the proton transferred from Ser195 side chain to the nitrogen atom of main chain of substrate in the tetrahedral adduct by determining the transition state of a model system, and established the effects of surrounding amino acid residues and the field of the whole enzyme theoretically.

**Method**

(1) Calculation — \textit{Ab initio} molecular orbital calculations were carried out based on RHF-LCAO-SCF approximation. First, the structures of transition states for model systems of His57-substrate-Ser195 were obtained. For this purpose, Gaussian 80 \textit{ab initio} molecular orbital calculation program (Binkley et al.\textsuperscript{23}) was employed with STO-3G basis functions. Molecules in the initial and final systems of the reaction were calculated for their optimal geometries. Gaussian general program (\textit{Ab initio} molecular orbital calculation program modified and generalized based on IMSPACK program (Morokuma, Kato, Kitaura, Ohmine, Sakai and Obara; program package containing Gaussian 70, HONDO, etc.) was used for calculations of energies of transition states themselves and of those under influence of surrounding amino acid residues and the electrostatic field of the enzyme. For these calculations, basis sets of 4-31G, 4-31G*, 4-31G**, 3-21G, 3-21G* and 3-21G** were employed. Energies of reactant and product molecules were estimated with 3-21G basis set. When the electrostatic field of enzyme was taken into account, point fractional charges based on \textit{ab initio} molecular orbital calculations with STO-3G basis set (Sheridan et al.\textsuperscript{24}) were placed on all the atomic nuclei of the remaining part of the enzyme other than systems which were treated explicitly quantum mechanically; His57-substrate-Ser195 catalytic triad with and without Asp102\textsuperscript{−} and Oxyanion hole. In this case, Hamiltonian is written as:

\[
H = \sum_{i} \left(-\frac{1}{2} \Delta_{i} - \sum_{\alpha} Z_{\alpha}/r_{\alpha i}\right) + \sum_{i>j} \frac{1}{r_{ij}} + \sum_{\alpha>\beta} Z_{\alpha}Z_{\beta}/R_{\alpha\beta} - \sum_{i} \sum_{\gamma} P_{\gamma}r_{\gamma i} + \sum_{\alpha} \sum_{\gamma} Z_{\alpha}P_{\gamma}/R_{\alpha\gamma} + \sum_{\gamma>\delta} P_{\gamma}P_{\delta}/R_{\gamma\delta}
\]

where \(i, j\) : electron; \(Z_{\alpha}, Z_{\beta}\) : atomic nuclear charge; \(P_{\gamma}, P_{\delta}\) : point fractional charge; \(r, R\) : distance. Coordinates for point fractional charges were based on registration number 3PTP of Protein Data Bank (Bernstein et al.\textsuperscript{25}).

Technique of energy decomposition procedure was used according to the method by Morokuma,\textsuperscript{26} and Kitaura et al.\textsuperscript{27}

All the calculations were carried out on HITAC M-200H computer at the computer center of the Institute for Molecular Sciences.

(2) Molecular Structure of Models — Imidazole ring, formamide and water molecules were used for models of His57, substrate and Ser195 side chain, respectively, in His57-substrate-Ser195 structure. Since the location of the proton between His57 and substrate in the transition state is of interest here, two structures of transition states were considered. In one of those, the proton in question is in the middle among nitrogen (epsilon two) of His57, nitrogen of substrate and oxygen (gamma) of Ser195. In
the other type of transition state, the proton is bound covalently to the nitrogen (epsilon two) of His57. On determination of the former type of transition state, all the atoms were constrained to be in one plane except for oxygen and hydrogen atoms bound to carbon which took tetrahedral structure and two hydrogens of NH₂ group of the substrate. For the latter an additional constraint that the distance between the nitrogen (epsilon two) of His57 and the transferring proton was fixed to be 1.03 angstroms was imposed.

Asp102⁻ was modeled by formic acid ion. Oxyanion hole was composed of main chain part ranging from the nitrogen of Gly193 to the nitrogen of Ser195 with Asp194 side chain deleted and replaced by a hydrogen atom for the sake of computational cost. Hydrogen atoms were

a) TSa

<table>
<thead>
<tr>
<th>dihedral angles</th>
</tr>
</thead>
</table>
| C₅-N₁-C₁₁-O₁₄            | 84.348  
| C₅-N₁-C₁₁-H₁₂            | -87.826 
| N₁-C₁₁-O₁₂-H₁₆           | 107.737 
| N₁-C₁₁-N₁₂-H₁₇           | 117.679 
| N₁-C₁₁-N₁₃-H₁₈           | -116.158

b) TSb

<table>
<thead>
<tr>
<th>dihedral angles</th>
</tr>
</thead>
</table>
| C₅-N₁-C₁₁-O₁₄            | 85.367  
| C₅-N₁-C₁₁-H₁₂            | -89.189 
| N₁-C₁₁-O₁₂-H₁₆           | 118.251 
| N₁-C₁₁-N₁₂-H₁₇           | 131.427 
| N₁-C₁₁-N₁₃-H₁₈           | -118.448
added with regular bond lengths on the bonds deleted upon modeling. Coordinates of heavy atoms such as carbons, nitrogens and oxygens were taken from a Protein Data Bank entry numbered 3PTP.

Product molecules in the final state of the acylation step were represented by ammonia and formic acid molecules.

Results

(1) Structures of Transition States

![Diagram](image-url)

Fig. 1. Transition State Structure of His57 - Substrate - Ser195 Model and Embedding into the Enzyme  

a, c) The proton is free from any other specific molecules; b, d) Imidazole is protonated. a, b) The structures were determined with STO-3G basis set. Values in parentheses are distances from the plane of this sheet of paper (negative one for front of the plane). Units are angstrom and degree. c, d) Atoms in structures of a) and b) were least-square-fitted to corresponding atoms of protein data bank entry 3PTP, respectively. Thin lines show 3PTP data.
Geometries of His57-substrate-Ser195 which correspond to two transition states with different location of the proton in question are presented in Fig. 1. Figure 1 (a) is for the transition state without the additional constraint on location of the proton (TSA). Figure 1 (b) is for the other transition state with protonated imidazole ring of His57 (TSB). The two structures, TSA and TSB, which were obtained as isolated systems, were embedded into coordinates of 3PTP obtained from X-ray diffraction experiments by least-square-fitting of corresponding atoms. This situation is seen in Fig. 1 (c) and (d), where models for oxanion hole and Asp102 are also included. The thin lines indicate coordinates of 3PTP of Protein Data Bank. Geometries of TSA and TSB fit well to the X-ray data, implying the procedure of obtaining transition states was appropriate. Transition state structure of TSB is a little shrunken in comparison with that of TSA, which may be due to ionic interaction between His57+ and (substrate-Ser195−).

(2) Dependence of Transition State Energies on Basis Functions and Effect of Electrostatic Field of Enzyme

Energies of transition state structures of His57-substrate-Ser195 isolated alone (including neither oxanion hole nor Asp102−) are −468.9568 and −468.9864 a.u. for TSA and TSB, respectively, with 4-31G basis set. Structure of TSB is more stable than that of TSA by 0.0297 a.u. (18.6 kcal/mol) (Table I). Accordingly, His57 may take part in transfer of the proton in catalysis in a preferable fashion. The catalytic triad, however, does not exist by itself at all. Therefore, the electrostatic field of the enzyme other than this central region was described as a set of point fractional charges and included in the calculations. The results showed that energies of TSA and TSB were −492.8178 and −492.8590 a.u., respectively, and that the TSB structure was more stable than the other by 0.0412 a.u. (25.8 kcal/mol) (Table II). Consequently TSB is stabilized more drastically than TSA under influence of the electrostatic field of enzyme.

In order to make sure of the quality of the basis set functions, polarization functions were added onto atoms of the second series in the periodic table to make 4-31G*. With this basis

<table>
<thead>
<tr>
<th>Basis set</th>
<th>TSA (a.u.)</th>
<th>TSB (a.u.)</th>
<th>TSA – TSB (a.u.)</th>
<th>TSA – TSB (kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-31G</td>
<td>−468.9568</td>
<td>−468.9864</td>
<td>0.0297</td>
<td>18.6</td>
</tr>
<tr>
<td>4-31G*</td>
<td>−469.1873</td>
<td>−469.2038</td>
<td>0.0164</td>
<td>10.3</td>
</tr>
<tr>
<td>4-31G**</td>
<td>−469.2208</td>
<td>−469.2346</td>
<td>0.0138</td>
<td>8.7</td>
</tr>
<tr>
<td>3-21G</td>
<td>−467.0497</td>
<td>−467.0662</td>
<td>0.0165</td>
<td>10.3</td>
</tr>
<tr>
<td>3-21G*</td>
<td>−467.3868</td>
<td>−467.3926</td>
<td>0.0059</td>
<td>3.7</td>
</tr>
<tr>
<td>3-21G**</td>
<td>−467.4241</td>
<td>−467.4300</td>
<td>0.0059</td>
<td>3.7</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Basis set</th>
<th>TSA (a.u.)</th>
<th>TSB (a.u.)</th>
<th>TSA – TSB (a.u.)</th>
<th>TSA – TSB (kcal/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-31G</td>
<td>−492.8178</td>
<td>−492.8590</td>
<td>0.0412</td>
<td>25.8</td>
</tr>
<tr>
<td>4-31G*</td>
<td>−493.0470</td>
<td>−493.0765</td>
<td>0.0295</td>
<td>18.5</td>
</tr>
<tr>
<td>4-31G**</td>
<td>−493.0809</td>
<td>−493.1075</td>
<td>0.0267</td>
<td>16.7</td>
</tr>
<tr>
<td>3-21G</td>
<td>−490.9100</td>
<td>−490.9381</td>
<td>0.0280</td>
<td>17.6</td>
</tr>
<tr>
<td>3-21G*</td>
<td>−491.2455</td>
<td>−491.2647</td>
<td>0.0192</td>
<td>12.0</td>
</tr>
<tr>
<td>3-21G**</td>
<td>−491.2831</td>
<td>−491.3023</td>
<td>0.0191</td>
<td>12.0</td>
</tr>
</tbody>
</table>

The values in the columns of TSA and TSB contain contribution of point charge — point charge interaction energy (−23.8055 a.u.). This contribution is constant for all the cases calculated in the electrostatic field of enzyme. If this value is subtracted, energy of TSA (4-31G), for instance, becomes −469.0123 a.u. in accordance with Fig. 2.
set, energies of isolated TSa and TSb were obtained to be $-469.1873$ and $-469.2038$ a.u., respectively, and those under influence of electrostatic field were $-493.0470$ and $-493.0765$ a.u., respectively. The differences in energy are $10.3$ kcal/mol in vacuum and $18.5$ kcal/mol in electrostatic field.

Calculations with more additional polarization functions set at hydrogen atomic nuclei (4-31G**) showed $-469.2208$ and $-469.2346$ a.u. for isolated TSa and TSb, respectively, and $-493.0809$ and $-493.1076$ a.u. for those in the enzymatic electrostatic field. The differences are $8.7$ kcal/mol and $16.7$ kcal/mol without and with the field, respectively.

From these calculations it is made clear that the structure of TSb is more stable than that of TSa in all the cases and the difference in energy becomes large when the effect of the electrostatic field is taken into account (Fig. 2 (a)). Other series of calculations with 3-21G, 3-21G* and 3-21G** revealed the same trend of energies for the transition states (Tables I and II, and Fig. 2 (b)).

### Table III. Effect of Adjacent Residues and Electrostatic Field of Enzyme on Transition State Energy

<table>
<thead>
<tr>
<th>System</th>
<th>TSa</th>
<th>TSb</th>
<th>TSa - TSb</th>
</tr>
</thead>
<tbody>
<tr>
<td>H + S + O + A + P</td>
<td>$-1144.1142$ (a.u.)</td>
<td>$-1144.4177$ (a.u.)</td>
<td>$-0.0175$ (a.u.)</td>
</tr>
<tr>
<td>(H, S) + O + A + P</td>
<td>$-1144.5930$</td>
<td>$-1144.6095$</td>
<td>$0.0165$</td>
</tr>
<tr>
<td>(H, S, O) + A + P</td>
<td>$-1144.6147$</td>
<td>$-1144.6477$</td>
<td>$0.0330$</td>
</tr>
<tr>
<td>(H, S, O, A) + P</td>
<td>$-1144.6777$</td>
<td>$-1144.7392$</td>
<td>$0.0616$</td>
</tr>
<tr>
<td>(H, S, O, A, P)</td>
<td>$-1144.9326$</td>
<td>$-1144.9906$</td>
<td>$0.0581$</td>
</tr>
</tbody>
</table>

H, His57* (modeled by imidazolium cation); S, substrate - Ser195 (modeled by NH$_2$CH(O-)OH (Fig. 1)); O, oxyanion hole (modeled by NH$_2$CH$_2$CONHCH$_2$CONH$_2$ (Fig. 1)); A, Asp102* (modeled by formic acid); P, point fractional charge field.

Parentheses stand for complex or system which is treated at the same time quantum chemically. Calculated with 3-21G basis set.
(3) Effects of Adjacent Residues and Electrostatic Field of the Remaining Residues of Enzyme upon Transition State Energies

To examine closely the results obtained in the preceding section, residues adjacent to His57-substrate-Ser195, i.e. oxyanion hole and Asp102\(^{-}\), were included into calculation one by one, and the other region of enzyme was also considered as the electrostatic field.

Behavior of energies upon inclusion of these additional effects are tabulated in Table III, and illustrated in Fig. 3. Basis set used was 3-21G. All the energies of TSb systems are lower than those of corresponding TSa systems. If oxyanion hole is considered, the energy difference goes from 10.3 kcal/mol up to 20.7 kcal/mol. Further consideration of Asp102\(^{-}\) residue brings quite a large change to the difference, from 20.7 kcal/mol up to 38.6 kcal/mol. Taking into account the electrostatic field of the remaining region does not affect the difference so much (from 38.6 kcal/mol to 36.4 kcal/mol). The effects of oxyanion hole and Asp102\(^{-}\) residue are proved to be significant.

(4) Interaction between His57-Substrate-Ser195 System and Oxyanion Hole-Asp102\(^{-}\) System

Since in the preceding section oxyanion hole and Asp102\(^{-}\) residue were found to play important roles in stabilization of transition states of His57-substrate-Ser195, interaction energy between His57-substrate-Ser195 and oxyanion hole-Asp102\(^{-}\) was studied by energy decomposition technique, regarding each group of molecules as a supermolecule. The results are shown in Table IV. Total interaction energies are very different (−39.6 kcal/mol for TSa and −69.7 kcal/mol for TSb), which is mainly due to difference in exchange repulsion (27.1 kcal/mol). Difference in electrostatic interaction is secondly significant (6.1 kcal/mol).

Discussion

Substrate-Ser195 part including the transferring proton in the TSa structure, His57-substrate-Ser195, which was treated collectively in the present work, resembles the transition state structure (1) where His57 is ignored and Ser195 is displaced by methanol (Koizumi et al.\(^{28}\));

\[
\begin{align*}
\text{H} & \quad \text{O} \quad \text{CH}_3 \\
\text{H} & \quad \text{O} \quad \text{CH}_3 \\
\text{H} & \quad \text{O} \quad \text{CH}_3 \\
\text{H} & \quad \text{O} \quad \text{CH}_3 \\
\text{H} & \quad \text{O} \quad \text{CH}_3 \\
\text{H} & \quad \text{O} \quad \text{CH}_3 \\
\text{H} & \quad \text{O} \quad \text{CH}_3 \\
\text{H} & \quad \text{O} \quad \text{CH}_3 \\
\text{H} & \quad \text{O} \quad \text{CH}_3 \\
\end{align*}
\]

(1)
Moreover it is similar to transition states 2 proposed by Koizumi et al., 28) Oie et al., 29,30) and Oie et al. 31);

\[
\begin{align*}
\text{H} & \quad \text{O} \quad \text{H} \\
\text{H} & \quad \text{O} \quad \text{H} \\
\text{H} & \quad \text{N} \quad \text{C} \quad \text{H} \\
\text{H} & \quad \text{H} \quad \text{O} \\
\text{N} & \quad \text{C} \quad \text{H} \\
\text{H} & \quad \text{O} \\
\text{H} & \quad \text{N} \quad \text{C} \quad \text{H} \\
\end{align*}
\]

\[ \text{(2)} \]

Since Ser195 was modeled by H_2O in our treatment, the part of TSA in question corresponds to 2.

According to Koizumi et al., 28) activation energies for reactions (1) and (2) are practically the same. Therefore, participation of Ser195 beta carbon in the catalysis does not have a significant meaning energetically. They said in their report that other factors such as His57 were needed to be considered to explain the acceleration of the reaction. Actually Oie et al. 29,30) showed that activation energies for systems of ammonia — formic acid and ammonia — glycine were lowered by NH_3 catalyst. In the present case, we included His57 explicitly in the calculations, and we got activation energies for structure TSA and TSB (Fig. 4 and Table V), which are even lower than those for structure (2) obtained by Oie et al. 29,30) with the same basis set, i.e. 3-21G.

<table>
<thead>
<tr>
<th>Species</th>
<th>Energy (a.u. (kcal/mol))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Im</td>
<td>-223.5465</td>
</tr>
<tr>
<td>Fm</td>
<td>-167.9818</td>
</tr>
<tr>
<td>Wa</td>
<td>-75.5836</td>
</tr>
<tr>
<td>Fc</td>
<td>-187.6964</td>
</tr>
<tr>
<td>Am</td>
<td>-55.8678</td>
</tr>
<tr>
<td>Re=Im+Fm+Wa</td>
<td>-467.1121</td>
</tr>
<tr>
<td>Pr=Im+Fc+Am</td>
<td>-467.1108</td>
</tr>
<tr>
<td>TSA-Re</td>
<td>0.0624 (39.2)</td>
</tr>
<tr>
<td>TSB-Re</td>
<td>0.0459 (28.8)</td>
</tr>
<tr>
<td>Pr-Re</td>
<td>0.0013 ( 0.8)</td>
</tr>
</tbody>
</table>

Calculated with 3-21G. Im, imidazole; Fm, formamide; Wa, water; Fc, formic acid; Am, ammonia; Re, reactants; Pr, products; TSA and TSB, see Fig. 1.

Accordingly, His57 works in favor of the reaction. Since the energy of TSB structure is lower than that of TSA (Tables I, II and V, and Figs. 2 and 4), the transferring proton is considered to be bound to His57 covalently. His57 is, therefore, thought to join the catalysis as follows:

\[
\begin{align*}
\text{H} & \quad \text{N} \quad \text{C} \\
\text{H} & \quad \text{O} \\
\text{H} & \quad \text{N} \quad \text{C} \\
\text{H} & \quad \text{O} \\
\text{H} & \quad \text{N} \quad \text{C} \\
\text{H} & \quad \text{O} \\
\text{H} & \quad \text{N} \quad \text{C} \\
\text{H} & \quad \text{O} \\
\text{H} & \quad \text{N} \quad \text{C} \\
\text{H} & \quad \text{O} \\
\end{align*}
\]

Fig. 4. Energy Diagram of the Catalysis

3-21G basis set was used. Energy of TSB is lower than that of TSA by 10.3 kcal/mol.
The results are consistent within the basis sets and approximations used here, even if an other part of the enzyme is taken into consideration (Table III and Fig. 3).

The transition state TSb was determined under an additional constraint that N-H length was 1.03 angstroms, as is mentioned in the Method section. Therefore, the transition state TSb should be considered in a subspace of the space to which TSa belongs and should not be discussed at the same level as TSa; If TSa belongs to N-dimensional space, TSb belongs to (N-1)-dimensional space. Furthermore, the geometries were obtained with STO-3G basis set, and there is no guarantee that the potential energy surface is consistent with those obtained with other basis sets.

Both the transition structures, however, gave assumptive geometries for our purpose, i.e., to investigate the role of His57. Vibrational analysis was not performed, but it was ascertained from Gaussian 80 calculation that only one negative eigen value for Hessian matrix was obtained.

One should pay attention to electron correlation effect when one treats unnaturally distorted structures like transition states. Electron correlation generally stabilizes transition states. Therefore, strictly speaking, the stabilization effect should be estimated quantitatively.

Oie et al. treated similar systems (Eq. (2)) to ours (Eq. (3)), and evaluated the electron correlation effect at MP2/6-31G** level. According to them, correlation and polarization effects were in opposite directions, but both affected each transition state that they studied in the same order.

Therefore, cancellation of the two effects brings about qualitatively the same result at the double zeta Hartree-Fock calculation level as that by a more sophisticated treatment such as MP2 or MP4 with polarization functions.

We concentrated ourselves on comparison between two kinds of transition states, TSa and TSb, and did not choose to take the correlation problem up, with good reason in the light of the situation mentioned above.

Asp102 has the largest effect on stabilization of the energy of the transition state, while the other portions have smaller ones (Table III and Fig. 3). This gives support to the theory by Polgar et al., which says Asp102 stabilizes His57 imidazolium cation. The oxyanion hole stabilizes the alkoxy anion of the substrate further. These stabilizing effects are larger for TSb than for TSa. This is reasonable, because electrostatic interaction between Asp102 and His57 is enhanced and at the same time hydrogen bonds between anionic oxygen of the substrate and the oxyanion hole is strengthened, if the transferred proton is near His57 and distant from the substrate. The remaining part of enzyme other than these two effective sites causes similar stabilization energies to both the transition states.

When the interaction energy between His57-substrate-Ser195 system and Asp102-oxyanion hole system is decomposed into components, electrostatic interaction is found to be significant (Table IV). The second biggest contribution to the interaction energy is due to the exchange repulsion. The difference of the total interaction energy between TSa or TSb and the surrounding system also depends mainly on the exchange repulsion term.

A comment on basis set superposition error (BSSE) is made briefly here. Nakagawa et al. performed an energy decomposition analysis of the stabilization energy between the reactive site and Asp102. According to the report, the effect of BSSE on the energy change from the initial to transition state is only ~0.6 kcal/mol. They also did the same analysis of the energy between the reactive site and the oxyanion hole. The result showed the BSSE is 2.1 kcal/mol. The value of BSSE of the present system, in which Asp102 and the oxyanion hole are treat-
ed as a supermolecule, is estimated to be the sum of the above two values, namely $-0.6 + 2.1 = 1.5$ kcal/mol. This magnitude of value can not alter the result discussed above.

A possible problem here is the premise to embed the geometries of His57-substrate-Ser195 obtained as they are isolated in space, into enzyme. Precisely speaking, the transition states should be considered on energy surface for all the atoms of the enzyme. A possible procedure taken for the purpose may be molecular mechanics. However, the accuracy of parameters of the method is quite questionable when discussion at the molecular or electronic level is done. Therefore, a quantum mechanical point of view is inevitable in order to follow the essence of chemical reactions. Actually, approaches from this aspect are already reported (Koizumi et al., 28) Oie et al., 29,30 Oie et al. 31). But they did not include explicit consideration of the effects of surroundings. In such a situation, the procedure taken in the present work is the best policy to attack this sort of problem at the present time. However, it remains open how to take into account adjacent residues electrostatically or to describe those as an effective potential, upon determination of a transition state quantum chemically. After these improvements, and in view of degrees of freedom and suppleness of surrounding residues in three dimensional space, exchange repulsion of $T_{sa}$ will become smaller and approach that of $T_{sb}$.

Another possible question is on the basis set dependence of the determined transition state geometries. In the present work STO-3G basis set was used to obtain the transition state structures, for the sake of computational cost. If another basis set, say 4-31G, is used, the calculation may give somewhat different structures. But we believe that they would not change the trend that transition state $T_{sb}$ is more stable than $T_{sa}$, as is indicated in Tables I—IV.

In conclusion, His57, which is involved deeply in the acylation step of catalysis, is proved to capture the proton from Ser195 side chain terminus with its lone pair and to transfer it to the substrate with electrostatic assistance of Asp102$^-$ and the oxyanion hole.

References


14. H. Umeayama and S. Nakagawa: The $pK_a$ Value of His57-Asp102 Couple in the Active Site of Bovine Pan-


