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A Molecular Orbital Study on the Complex between Aspartic Acid and Histidine in the Charge Relay Structure

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α-Chymotrypsin, trypsin, elastase and acyl-α-chymotrypsin contain a charge relay structure composed of aspartate, histidine and serine or water; in trypsin and elastase, histidine forms a bifurcated hydrogen bond with aspartic acid, while on the other hand, histidine of α-chymotrypsin forms a linear hydrogen bond. First, in order to determine the origin of the charge relay structure, the interaction energy between histidine and aspartate was calculated by the *ab initio* method. Since the bifurcated structure was much less stable than the linear one, it was suggested that reactions of enzymes containing the bifurcated hydrogen bond structure will occur *via* a linear hydrogen bond. The main contributors to the stability difference were the differences of electrostatic interaction energy and charge transfer energy. Moreover, the position of the proton between histidine and aspartate was determined by geometry optimization. Since the proton took a position midway between N of histidine and O of aspartate, the complex was named "hispartic acid."

The interaction energy between water and hispartic acid was more stable than that between water and the histidine anion; this was due to the differences of electrostatic interaction energy and exchange repulsion energy. Secondly, the interaction energies between water and histidine were calculated in detail; the linear hydrogen bond structure was more stable than the bifurcated one. These energies were also decomposed in order to elucidate their origins.

Keywords——*ab initio*; structure; molecular orbital; enzyme; charge relay structure; serineprotease; complex; proton transfer; chymotrypsin; trypsin

A charge relay structure composed of serine, histidine and aspartate was found in α-chymotrypsin by Blow *et al.*[2] Subtilisin, trypsin and elastase[3] as well as α-chymotrypsin contain similar hydrogen bond structures. Aspartate in the charge relay structure greatly lowers the potential barrier for proton transfer from serine or water to histidine in general base catalysis.[4] Such charge relay structures have been studied in relation to the enzymatic reactions of α-chymotrypsin,[5] acyl-α-chymotrypsin[6] and lactate dehydrogenase.[7] In lactate dehydrogenase, L-lactate acts as a proton donor in place of serine, and, hence, the charge relay structure is composed of aspartate, histidine and L-lactate.[7,8] In the case of acyl-α-

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2) D.M. Blow, J.J. Birktoft, and B.S. Hartley, *Nature* (London), **221**, 337 (1969).
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chymotrypsin, a water molecule acts as the proton donor, and the charge relay structure is composed of aspartate, histidine and water.[9] It is very interesting from an evolutionary point of view that enzymes having different functions in biological systems contain similar charge relay structures. Scheiner *et al.* calculated the charge relay structure and tetrahedral intermediate structure in the acylation of serine proteases.[4d,4f] However, since they used X-ray data, too large an activation energy was obtained. We considered that before calculating the charge relay structure using X-ray data, optimization of the charge relay structure would be necessary. Moreover, Scheiner *et al.* used the partial retention of diatomic differential overlap (PRDDO) method. *Ab initio* calculations using an STO–3G basis set give more appropriate results than the PRDDO method.[4g] In this paper, the origin of the charge relay structure is studied from an *ab initio* quantum-chemical point of view.

Method

Molecular Orbital Calculations——All calculations were performed within the framework of the closed shell single determinant LCAO-SCF-MO theory. The GAUSSIAN 70 program of Hehre *et al.* was used as a nonempirical method.[10] STO-3G[11] and 4-31G[12] basis sets were used as primitive functions. Calculations were carried out using HITAC 8700 and 8800 computers at the Tokyo University Computer Center. The interaction energy, ΔE, was calculated as the difference between the sum of the total energies of the two isolated structures and the total energy of the complex.

$$\Delta E = E - (E_A + E_B)$$

where E represents the total energy of the complex, and E_A and E_B are the total energies of the isolated molecules A and B, respectively. The interaction energy was decomposed according to the following equation.

$$\Delta E = ES + EX + PL + CT + COP',$$

where ES represents electrostatic interaction, EX exchange repulsion, PL polarization, CT charge transfer, and COP' a coupling term. A detailed explanation of the energy decomposition has been given in other papers.[13] In addition, the coupling energy was decomposed into the following four terms.

$$COP = COP_{A_OA_VB_O} + COP_{A_OB_OB_V} + COP_{A_OA_VB_V} + COP_{A_VB_OB_V}$$

where A_O is the occupied molecular orbital (MO) set of the A molecule, A_V the unoccupied MO set of the A molecule, B_O the occupied MO set of the B molecule, and B_V the unoccupied MO set of the B molecule.

$A_OA_VB_O$ represents the MO interaction energy among A_O, A_V and B_O.

$$COP_{A_OA_VB_O} = A_OA_VB_O - (CT_{B-A} + EX'_{A_O-B_O} + PLX_A),$$

where CT_{B-A} is the charge transfer energy from the B molecule to A, PLX_A is the polarization energy containing the exchange term in the A molecule induced by the B molecule, and $EX'_{A_O-B_O}$ is the exchange repulsion energy between the two occupied MO sets of the A and B molecules. Moreover,

$$COP_{A_OB_OB_V} = A_OB_OB_V - (CT_{A-B} + EX'_{A_O-B_O} + PLX_B)$$
$$COP_{A_OA_VB_V} = A_OA_VB_V - (CT_{A-B} + EX'_{A_V-B_V} + PLX_A)$$
$$COP_{A_VB_OB_V} = A_VB_OB_V - (CT_{B-A} + EX'_{A_V-B_V} + PLX_B).$$

Calculational details have been described in another paper.[14] Since COP is not equal to COP', the prime was added. When PLX is used in place of PL, COP is equal to COP'.

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Geometries——The geometry (C_{2v}) for $HCOO^-$ used in the charge relay structure was optimized using a STO-3G basis set; r(CH) = 1.1494 Å, r(CO) = 1.26693 Å, and ∠HCO = 114.9014°. The coordinates of heavy atoms for imidazole were obtained from experimental data for histidine.[15] The bond distances r(CH) and r(NH) of imidazole were optimized using a STO-3G basis set; r(CH) = 1.0791 Å for the imidazole anion; r(CH) = 1.0791 Å and r(NH) = 1.0231 Å for imidazole. For the water molecule, the experimental values of r(OH) = 0.956 Å and ∠HOH = 105.2° were used. For HCOOH, the geometry was optimized using a STO-3G basis set; r(CH) = 1.105 Å, r(CO) = 1.217 Å, r(CO) = 1.384 Å, r(OH) = 0.989 Å, ∠HOC = 106.179°, ∠OCO = 124.860° and ∠HCO = 125.174°. In comparing the MO energy levels and the AO coefficients of the nitrogen lone pair of the imidazole anion, of imidazole and of the imidazole–formate complex, only the MO energy levels having an absolute value over 0.1 for the AO coefficients of the N lone pair are considered. The X-component of the AO coefficient was used, since this component lies nearly along the lone pair direction. In the calculation of the complex between imidazole and the formic acid ion, an angle of 111.696° between the N of imidazole and the O and C of the formic acid ion was obtained from calculations on the complex between ammonia and the formic acid ion using a STO-3G basis set.

Results

Proton Affinity of Amino Acid Residues forming the Charge Relay Structure

Blow *et al.* reported negative charge transfers from Asp 102 to His 57, and from His 57 to Ser 195 in the charge relay structure of α-chymotrypsin.[1] The amino acid on which the negative charge is placed should have a larger proton affinity than the other two amino acids.[4a,4e] Proton affinities for the formate ion, the imidazole anion, the methanol anion and the hydroxyl ion are shown in Table I, in which the former three molecules were used in

Table I. Proton Affinities of the Formate Ion, Imidazole Anion, Methanol Anion and Hydroxyl Ion in kcal/mol obtained using a 4–31G Basis Set

Molecule	Proton affinity
$HCOO^-$ [a]	−359.2
Imidazole Anion[a]	−378.9
CH_3O^- [b]	−410.2
OH^- [b]	−426.0

a) Calculations using a 4–31G basis set were performed for the geometries obtained by optimization using an STO–3G basis set. For the imidazole ring, experimental values were used.
b) Reference 17.

place of aspartate, histidine and serine, respectively, in order to simplify the calculations. The order is methanol anion>imidazole anion>formate ion. Therefore the negative charge may be placed on aspartate rather than on histidine or serine; that is, the charge relay structure may be composed of the aspartate ion, histidine and serine. For the case of a charge relay structure composed of aspartate, histidine and water, the order is hydroxyl ion>imidazole ion>formate ion. The negative charge may again be placed on aspartate. On the other hand, since the proton affinity of $HCOO^-$ is close to that of the imidazole anion, the proton might be located between both molecules.

Interaction between Aspartate and Histidine

Aspartate forms a linear hydrogen bond with histidine in α-chymotrypsin. In trypsin or subtilisin, on the other hand, the hydrogen bond between aspartate and histidine has a bifurcated structure. It is interesting, therefore, to calculate whether the linear or bifurcated hydrogen bond structure is more stable. Table II shows the total energies of the bifurcated

15) L.E. Sutton and B. Phil, "Interatomic Distances," published by the Chemical Society, London, 1958.

and linear hydrogen bond structures shown in Fig. 1. The potential energies were calculated for rotation of the formate ion around the linear hydrogen bond as shown in Table II. The plane of imidazole coincides with that of the formate ion in the most stable structure,

Table II. Total Energy of the Bifurcated and Linear Hydrogen Bond Structures between Formate and Imidazole[a)]

Distance[b)] (Å)	Total energy of the bifurcated structure (a.u.)[c)]
3.0	−407.48325 (0.1)[d)]
3.0433[e)]	−407.48344 (0.0)
3.1	−407.48315 (0.2)
3.2	−407.48155 (1.2)
3.3	−407.47908 (2.7)
Distance[f)] (Å)	**Total energy of the linear structure (a.u.)[g)]**
2.28	−407.50908 (1.7)
2.35	−407.51154 (0.1)
2.38[h)]	−407.51172 (0.0)
2.41	−407.51150 (0.1)
2.48	−407.50970 (1.3)
2.58	−407.50500 (4.2)
Angle[i)] (Degrees)	**Total energy of the linear structure (a.u.)[g)]**
30	−407.51128 (1.7)
60	−407.51044 (1.0)
90	−407.51017 (1.1)
180	−407.51203 (−0.0)

a) STO-3G basis set.
b) Distance between N in imidazole and C in the formate ion.
c) The N–H bond in imidazole and C–H bond in the formate ion lie on the same straight line.
d) Relative energy(kcal/mol) for the local stable structure.
e) This value, 3.0433, is the length obtained from a parabolic fit among three values, 3.0, 3.1 and 3.2 Å.
f) Distance between N and O in the linear hydrogen bond.
g) The N–H bond is on the N–O line.
h) This value, 2.38, is the length obtained from a parabolic fit among three values, 2.35, 2.38 and 2.41 Å.

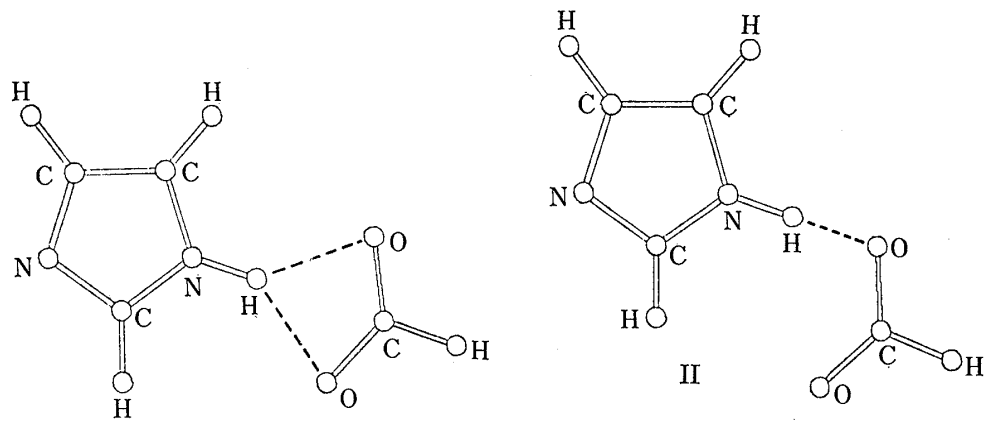


Fig. 1. Optimized Bifurcated Hydrogen Bond Complex (I) and Linear Hydrogen Bond Complex (II) between Neutral Imidazole and/Formate Ion

as shown in Fig. 1. The linear structure was most stable for a distance of 2.38 Å between N of imidazole and O of the formate ion. The bifurcated structure was most stable for a distance of 3.04 Å between N of imidazole and C of the formate ion (Fig. 1). The bifurcated hydrogen bond structure was less stable than the linear one by 17.9 kcal/mol. The bifurcated structure found in trypsin and subtilisin is thus much less stable than the linear hydrogen bond found in α-chymotrypsin. This result means that the bifurcated structure in trypsin and subtilisin may change to a linear hydrogen bond during enzymatic reactions.

In order to determine why the linear structure is more stable than the bifurcated one, energy decomposition analyses were carried out for the complex structures. Table III shows

Table III. Energy Decomposition Analysis of the Stable Structures of the Bifurcated and Linear Hydrogen Bonds in kcal/mol[a]

	Bifurcated structure	Linear structure		Difference
ΔE	−30.9	−48.8	$\Delta\Delta E$	−17.9
ES	−25.1	−44.1	ΔES	−19.0(34%)
EX	16.1	53.7	ΔEX	37.2
PL	−3.9	−5.0	ΔPL	−1.1(2%)
CT	−14.3	−33.4	ΔCT	−19.0(34%)
COP'	−3.6	−19.7	$\Delta COP'$	−16.1(29%)

a) STO-3G basis set.

the results. The interaction energy of the linear hydrogen bond is attributable to ES and CT. The CT energy is almost equal to the sum of CT_{A-B} and CT_{B-A} (A and B represent imidazole and the formate ion, respectively), which are −0.3 and −33.0 kcal/mol, respectively. Therefore charge transfer from the occupied MO set of the formate ion to the unoccupied MO set of imidazole was significant in the total CT energy. The COP' term, −19.7 kcal/mol, is large. The coupling terms are shown in Table IV. The coupling term

Table IV. Energy Decomposition Analysis for the Coupling Term of the Interaction Energy of the Linear Hydrogen Bond Structure in kcal/mol[a]

Term[b]	Energy[c]
COP	−17.8
COP_{AoAvBo}	−15.9
COP_{AoBoBv}	0.2
COP_{AoAvBv}	−0.0
COP_{AvBoBv}	−2.0

a) STO-3G basis set.
b) The A and B molecules are neutral imidazole and the formate ion, respectively.

COP is a little different from COP', as mentioned in the "Method" section. COP is due almost entirely to COP_{AoAvBo}, which is the coupling interaction energy among the occupied and unoccupied MO sets of imidazole and the occupied MO set of the formate ion. For the bifurcated structure, on the other hand, ES is the main contributor, and CT is next most important. COP' and PL contribute little. The differences between the bifurcated and linear hydrogen bond structures are shown in Table III. Thus, the linear structure is more stable than the bifurcated one because of ΔES, ΔCT and $\Delta COP'$.

The hydrogen bonded to the nitrogen of imidazole was fixed at the covalent bond distance for the above calculations. In another series of calculations, the hydrogen was translated

along the line of the linear hydrogen bond of N and O to find a potential minimum. Table V shows the results. The structure was most stable at a distance of 1.26 Å from the nitrogen. The hydrogen was placed at a distance of 1.12 Å from the oxygen of the formate ion. The most stable structure after the movement of the hydrogen is called "hispartic acid" in this paper. The structure is shown in Fig. 2.

TABLE V. Total Energy of Proton Transfer from Imidazole to the Formate Ion in the Stable Linear Hydrogen Bond Structure[a)]

Distance[b)]	Total energy[c)]
1.0231	−407.51199
1.1151	−407.52496
1.2071	−407.53081
1.2644[d)]	−407.53189
1.2993	−407.53144
1.3910	−407.52423

a) STO-3G basis set.
b) Distance between the nitrogen in imidazole and the hydrogen bonded to the nitrogen. The hydrogen-bonded N---O distance was fixed at 2.38 Å.
c) Atomic units.
d) Distance obtained from a parabolic fit.

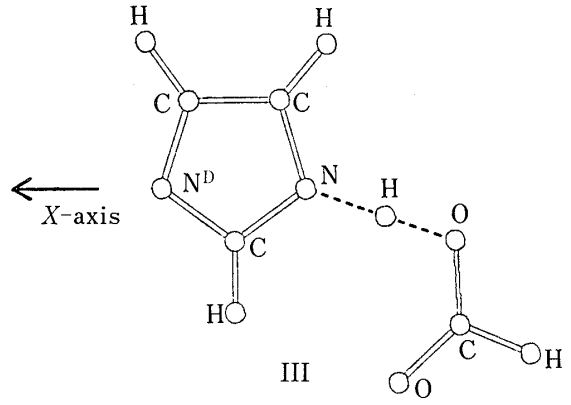


Fig. 2. Optimized Linear Hydrogen Bond Structure (III) in which Proton Transfer Occurs

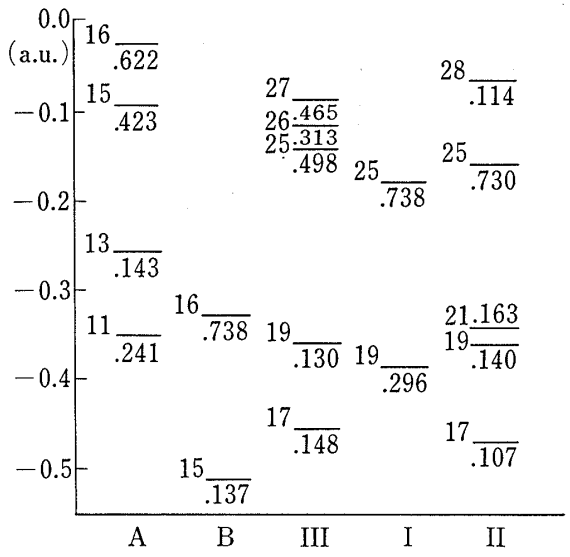


Fig. 3. MO Levels of the Imidazole Anion (A), Neutral Imidazole (B) and Various Complexes (I, II, and III) between Imidazole and Formate

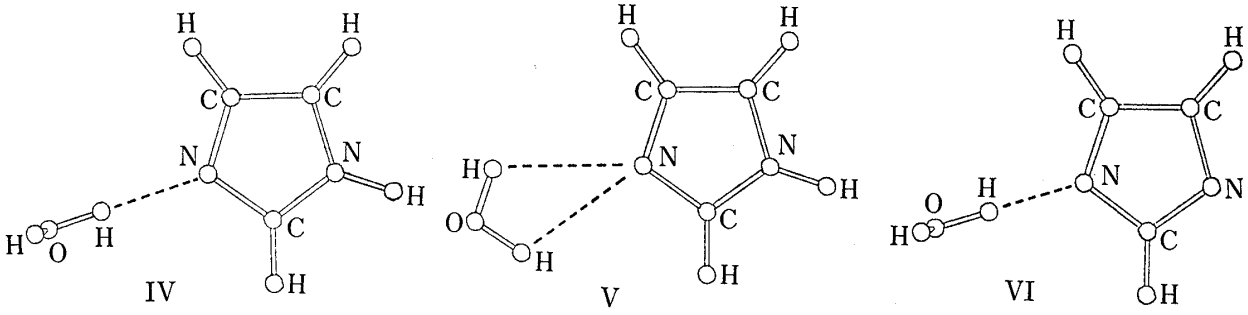


Fig. 4. Linear Hydrogen Bond Complex (IV) and Bifurcated Hydrogen Bond Complex (V) between Water and Neutral Imidazole

The linear hydrogen bond complex(VI) between water and imidazole anion, is also shown.

The MO energy levels of the hispartate anion and AO coefficients of the lone pair of the nitrogen (N^{δ}) were compared with those of the imidazole anion and imidazole, as shown in Fig. 3. The hispartate anion is similar to the imidazole anion. Accordingly, N^{δ} of the hispartate anion may be able to interact strongly with a water molecule or serine. On the other hand, the imidazole part of the bifurcated structure is similar to isolated imidazole with respect to the highest occupied MO (HOMO) in the σ MO set of the bifurcated structure and the AO coefficient of the N^{δ} lone pair.

Interaction between Histidine and Water

The charge relay structure in acyl-α-chymotrypsin is composed of aspartate, histidine and water molecules. The interaction energy between histidine and water is calculated in this section. Imidazole was used in place of histidine in order to simplify the calculations. Histidine is neutral before the proton transfers from histidine to the aspartate ion. First, the interaction energies between water and imidazole were calculated. The linear hydrogen bond (Fig. 4) was most stable with a distance of 2.89 Å between N^{δ} of imidazole and O of water, as shown in Table VI. The energy decomposition analysis at the optimal distance is shown in Table VII. The interaction energy was −6.1 kcal/mol. The *ES* and *CT* terms contributed to this. CT_{A-B} (A and B are imidazole and water, respectively) was −6.479 kcal/mol. *CT* from the occupied σ MO set in the valence MO's of imidazole to water was −6.463 kcal/mol. *CT* from σ_{15} and σ_{16} to water was −6.323 kcal/mol. Moreover *CT* from σ_{16} to water was −6.322 kcal/mol. Therefore *CT* from σ_{16} (HOMO in the σ MO set of imidazole) to water was the most significant factor. On the other hand, the bifurcated hydrogen bond structure (V in Fig. 4), in which the most stable location was with a distance of 3.04 Å between N^{δ} and O, was less stable than the linear one by 2.4 kcal/mol. The interaction

Table VI. Total Energies for the Interactions between Neutal Imidazole and Water, and Between the Imidazole Anion and Water
The basis set is STO-3G.

Distance (Å)	Linear structure (IV)[a] (a.u.)
2.83	−296.95010
2.88	−296.95021
2.88846	−296.95022
2.93	−296.95016
3.03	−296.94970
	Bifurcated structure (V)[b]
3.0	−296.94633
3.0373	−296.94635
3.1	−296.94631
3.2	−296.94609
	Linear structure (VI)[c]
2.4	−296.25522
2.5	−296.26059
2.6	−296.26267
2.65	−296.26287
2.7	−296.26265

a) Linear hydrogen bond structure between neutral imidazole and water (IV).
b) Bifurcated hydrogen bond structure between neutral imidazole and water (V).
c) Linear hydrogen bond structure between the imidazole anion and water (VI).

Table VII. Energy Decomposition Analyses for Interactions between Neutral Imidazole and Water, and between the Imidazole Anion and Water in kcal/mol[a)]

	IV	V	VI		VI−IV	V−IV
ΔE	−6.1	−3.7	−17.8	$\Delta\Delta E$	−11.7	2.4
ES	−8.0	−4.1	−19.3	ΔES	−11.3	3.9
EX	9.3	1.7	22.7	ΔEX	13.4	−7.6
PL	−0.5	−0.1	−2.3	ΔPL	−1.7	0.4
CT	−6.6	−1.1	−14.9	ΔCT	−8.4	5.4
COP'	−0.3	−0.0	−4.0	$\Delta COP'$	−3.6	0.3

a) STO-3G basis set.

energy, −3.7 kcal/mol, was attributed to the large ES and smaller CT terms. Since CT_{A-B} was −1.1 kcal/mol, CT was almost completely from imidazole to water. The bifurcated structure was less stable than the linear one due to ΔCT and ΔES. In the charge relay structure, therefore, the conformation between histidine and the water molecule will involve a linear hydrogen bond. Since histidine becomes an anion after proton transfer to the aspartate ion, the interaction energy between the imidazole anion and the water molecule was calculated. The structure was most stable with a distance of 2.65 Å between N^{δ} and O, as shown in Table VI. The total interaction energy, −17.8 kcal/mol, originated from the large ES and CT terms. CT_{A-B} (A and B are the imidazole anion and water, respectively) was −14.796 kcal/mol. CT from the occupied σ MO set in the valence MO's of the imidazole anion to the unoccupied MO set of water was −14.820 kcal/mol. CT from σ_{12}, σ_{13}, σ_{15} and σ_{16} shown by the A structure in Fig. 3 to water was −15.0 kcal/mol. CT from σ_{15} and σ_{16} to water was −14.9 kcal/mol. CT from σ_{16} to water was −9.3 kcal/mol. Therefore σ_{15} and σ_{16} in the valence MO's of the imidazole anion were the most significant. Moreover, since COP_{AoBoBv} was −4.7 kcal/mol, the MO interactions among the occupied MO set in the imidazole anion, and the occupied and unoccupied MO sets in water will be the main contributors to the coupling terms. In comparison with the linear hydrogen bond structure between imidazole and water, the large increase of the interaction energy is due to ΔES and ΔCT. It was also shown that the intermolecular distance decreases from 2.89 to 2.65 Å when histidine changes from the neutral form to the anion.

In the charge relay structure in acyl-α-chymotrypsin, the hydrogen covalently bonded to water transfers to N^{δ} of the hispartate anion. The potential energies of proton transfer were calculated for the structure VI (Fig. 5). The distance between O and N^{δ} was fixed at 2.65 Å. As shown in Table VIII, the potential energy becomes less stable upon increasing the OH distance of the water. In the enzymatic reaction, the proton transfer from water to the hispartate anion should have a double well potential.[16)] However, the above result

Table VIII. Proton Transfer From Water to the Imidazole Anion in Structure VI[a)]

Distance[b)]	Total energy[c)]
0.956	−296.26287
1.156	−296.25103
1.356	−296.21258
1.556	−296.17024

a) STO-3G basis set.
b) Distance between hydrogen and oxygen in water.
c) Atomic unit.

16) M.L. Bender, G.E. Clement, F.J. Kezdy, and H. D'A Heck, *J. Am. Chem. Soc.*, **86**, 3680 (1964).

showed a monotonic increase of the potential curve. In order to obtain a double well potential for proton transfer, the participation of the substrate should be considered, as stated in the discussion section.

Interaction between Water and Hispartic Acid

In the charge relay structure, the hispartate anion, composed of aspartate and histidine, plays a significant role as a general base. The MO levels and the AO coefficients of N^{δ} of the hispartate anion were very similar to those of the imidazole anion. Therefore the interaction energies were calculated between water and the hispartate anion. The structure was most stable with a distance of 2.71 Å between O of water and N^{δ} of the hispartate anion (Table IX). This distance is longer than that obtained for the interaction between water and the imidazole anion by 0.06 Å. However, the interaction energy, −26.3 kcal/mol, was more stable than the latter by 8.5 kcal/mol as shown in Table X. This result was clarified by energy decomposition analysis as shown in Table X. The interaction energy between water and the hispartate anion is attributable mainly to the *ES* term and, secondly, to the *CT* term. The structure VII shown in Fig. 5 was more stable than the structure VI due to ΔES and ΔEX. The result that the hispartate anion interacts more strongly with water than

Table IX. Total Enrgy for Various Separations between the Hispartate Anion and Water[a]

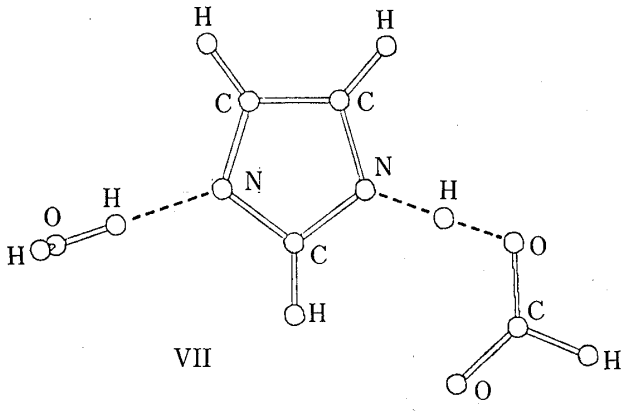
Distance[b]	Total energy[c]
2.65	−482.51605
2.70	−482.51630
2.7127	−482.51630
2.75	−482.51622

a) STO-3G basis set.
b) Distance between the oxygen in water and N^{δ} in the hispartate anion as shown in Figure 5.
c) Atomic units.

Table X. Energy Decomposition Analysis of the Interaction Energy between Water and the Hispartate Anion in kcal/mol[a]

	VII[b]		VII–VI[c]
ΔE	−26.3	$\Delta\Delta E$	−8.5
ES	−28.2	ΔES	−8.9
EX	18.0	ΔEX	−4.7
PL	−1.6	ΔPL	0.7
CT	−12.0	ΔCT	2.9
COP'	−2.4	$\Delta COR'$	1.6

a) STO-3G basis set.
b) The distance between O in water and N^{δ} in the hispartate anion is 2.7127 Å.
c) The distance between O in water and N^{δ} in the imidazole anion is 2.65 Å.



VII

Fig. 5. Optimized Charge Relay Structure composed of Water and the Hispartate Anion (Imidazole–formate Complex)

with the imidazole anion indicates greater stability of the charge relay structure. As a result, water approaches N^{δ} to interact with the hispartate anion. At the same time, N^{δ} of the hispartate anion acts as a strong general base. The hydrogen covalently bonded to O of water is easily transferred from water to the hispartate anion.

Discussion

The values of the proton affinities of amines, alcohols and ethers calculated using a 4–31G basis set were in good agreement with the experimental values.[13e] Moreover, the basis set was also used to elucidate the order ($OH^- > CH_3O^- > (H_2O)OH^-$) of proton affinities for water and methanol.[17] Therefore the basis set was applied for calculations of the proton affinities of the imidazole anion and the aspartate ion. The order methanol anion>imidazole anion>formate ion should be noted, since experimental workers have generally considered that the proton affinity of the histidine anion will be large in comparison with that of the aspartate ion or the serine anion.

Umeyama and Morokuma performed energy decomposition analyses of the linear and bifurcated hydrogen bond structures for the water dimer, and attributed the greater stability of the linear hydrogen bond to the *ES* and *CT* terms.[13h] Similarly, the linear structure in the interaction between imidazole and water was more stable than the bifurcated structure by 2.4 kcal/mol. This difference was due to *CT* and *ES*. In the interaction between the aspartate ion and imidazole, on the other hand, the linear structure was much more stable than the bifurcated one. The large value of 17.9 kcal/mol was due to the large *ES* and *CT* terms.

In the optimized structure between $HCOO^-$ and imidazole, the proton was located between O of the aspartate ion and N of imidazole. This structure is very similar to [FHF^-], which is symmetric, with an HF distance of 1.146 Å. Since the optimized bond distance for isolated HF was found to be 0.922 Å, the hydrogen is between both fluorines. There was no barrier between the reactants ($F^- + HF$) and the product ([$FHF]^-$). Energy decomposition analysis along the reaction path between HF and F^- showed large contributions from the *ES* and *CT* terms,[13d] and, hence, the formation of hispartic acid may be attributed to the *ES* and *CT* terms.

In the interaction structure between water and the imidazole anion, the proton transfer potential from water to the imidazole anion became unstable upon increase of the OH distance in water. A water molecule interacts with a serine ester in the enzymatic reaction of acyl-α-chymotrypsin, and the hydroxyl group interacts with the carboxyl carbon of the serine ester after release of the proton from water by general base catalysis.[6] When HCOOH was used in place of the ester model, the interaction energies between HCOOH and H_2O and between HCOOH and OH^- were −1.1 and −35.2 kcal/mol, respectively.[6] Therefore, when the substrate is included in the calculations, the total stabilization will be increased by the interaction between the OH group of water and the ester carbon with proton transfer. As a result, the double well potential curve necessary to explain the observed deuterium effects in the enzymatic reaction will be obtained from the calculations.[4d,4f]

Ab initio calculations for the charge relay structure were carried out using a STO–3G basis set. In calculating the proton transfer energy between two amino acids, an increase of the basis set or the inclusion of configuration interaction will give improved results.

Conclusion

1. The linear hydrogen bond structure in the histidine–aspartate complex is more stable than the bifurcated structure. Accordingly, it is suggested that enzymatic reactions such as those of subtilisin and trypsin with the bifurcated structure may occur through a linear

17) H. Umeyama and S. Nakagawa, *Chem. Pharm. Bull.* (Tokyo), **25**, 1671 (1977).

hydrogen bond. 2. The proton bonded to N of histidine in the linear hydrogen bond complex is midway between O of aspartate and N of histidine. This complex is called "hispartic acid". 3. The hispartate anion is similar to the histidine anion, as judged from the MO levels and the AO coefficients of the nitrogen lone pair. 4. The hispartate anion interaction with hydroxyl groups in molecules such as water and serine gives larger ES and CT contributions than the analogous histidine interaction does. Moreover, the hispartate anion interacts with water more strongly than the histidine anion does, as shown by the contributions of ΔES and ΔEX.

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