[Chem. Pharm. Bull. 29(4) 918—925 (1981)]

Molecular Orbital Study of Proton Transfer Energetics in the Active Site of Papain by Using Methanethiol-Imidazole-Formic Acid Complex as a Model

HIDEAKI UMEYAMA* and SETSUKO NAKAGAWA

School of Pharmaceutical Sciences, Kitasato University, Shirokane 5-chome, Minato-ku, Tokyo, 108, Japan

(Received October 27, 1980)

The energetics of proton transfers in the methanethiol-imidazole-formic acid complex were analyzed from a quantum chemical point of view in order to elucidate the nature of the enzymatic reaction of papain and the electronic structure in the active site. Methanethiol, imidazole and formic acid were used as models of the side chains of Cys 25, His 159 and Asp 158. The calculations were performed within the closed-shell-LCAO-*ab initio* MO method by using a 4-31G basis set. The hydrogen-bonding structure including the anion form of the methanethiol, the cation form of the imidazole and the anion form of the formic acid was the most stable of the various hydrogen-bonding structures. This result is in good agreement with experiments on the electronic structure in the active site of papain, and it supports the mechanism of the enzymatic reaction proposed by Angelides and Fink.

Keywords——enzyme; structure; *ab initio*; thiol protease; papain; hydrogen bond; proton transfer; molecular orbital; quantum chemistry; serine protease

Cysteine plays a significant role in the enzymatic reaction of papain. The electronic structure of the significant cysteine residue, Cys 25, has been studied by many researchers. Polgar found that the alkylation of papain by haloacetamides displays double sigmoid pH-rate profiles with pK_a values (4.0 and 8.4) similar to those found in acylation reactions,[1] and demonstrated the presence of a mercaptide ion in catalytically active papain.[2] Lewis *et al.* suggested on the basis of the potentiometric difference study of papain and papain–S–CH_3 that, in the intermediate pH range, 90% of the papain exists in a form where the active site contains the thiolate-imidazole ion pair.[3] Shinitzky and Goldman indicated on the basis of observation of the quenching interaction that the pK of the histidine residue in the active site was 8.6;[4] as a result, the pK of the cysteine residue was concluded to be close to 4.2. From the emission curves of free papain and SS papain against pH, Sluyterman and De Graaf showed that in the free enzyme between pH 4.2 and 8.6 the thiolate-imidazole ion pair predominates.[5] Further, since the Hammett plot for acylation suggests that electrophilic, presumably general acid, catalysis is important in the acylation step, the imidazole cation may be significant in the enzymatic reaction.[6]

The imidazole-thiolate ion pair is also expected from a structural point of view. The imidazole group of His 159 is hydrogen-bonded to Asn 175 as determined by X-ray diffraction analyses.[7] The distances between Asn 175 O^{γ} and His 159 $N^{\delta 1}$ and between Cys 25 S^{γ} and His 159 $N^{\epsilon 2}$ are 2.6 and 3.4 Å, respectively.[7,8] Broer *et al.*,[9] Bolis *et al.*[10] and Nakagawa and Umeyama[11] described the potential energy curves of proton transfer from Cys 25 to His 159 in the hydrogen-bonding system consisting of Cys 25, His 159 and Asn 175. However, no molecular orbital (MO) calculations have yet been carried out to show that the thiolate-imidazole ion pair is more stable than the neutral form of thiol-imidazole pair. Thus, the calculated results based on the data of Drenth *et al.*[7] could not explain the experimental results indicating the presence of the thiolate-imidazole ion pair. On the other hand, the presence of intermediates additional to those shown in the scheme of the normal enzymatic reaction has been reported.[12–14] Mattis and Fruton insisted on the presence of an additional intermediate prior to amide bond scission and acyl-enzyme formation.[15] Angelides and Fink proposed that there was a movement of the imidazole of His 159 from crystallographically

determined position, *i.e.*, hydrogen-bonded to Asn 175, to one involving electrostatic interaction with the carboxylate of Asp 158.[16] This movement could be achieved by a rotation of the C^{α}–C^{β} bond of His 159 by 79°, being accompanied by small movements of the side chains of Trp 177, Asp 158 and Cys 25.[16] Angelides and Fink called the moved form of the enzyme the "DOWN" form, in which the imidazole group is protonated and forms an ionic bond with the carboxylate side chain of Asp 158. If the "DOWN" form is an appropriate model of the enzymatic reaction, the structure consisting of the anion form of Cys 25, the cation of His 159 and the anion of Asp 158 should be the most stable form in the Cys 25-His 159-Asp 158 system. In order to determine the electronic structure of the "DOWN" form, a quantum study is necessary. However, although MO calculations on the Cys 25-His 159-Asn 175 system have been carried out,[9–11] no *ab initio* MO calculations on the Cys 25-His 159-Asp 158 system have been performed. Calculations in connection with proton transfer energetics should be carried out by a double zeta *ab initio* method, as is clear from calculations of the proton transfer energetics at the active site of β-trypsin reported by Nakagawa *et al.*[17] In the present work, therefore, double zeta *ab initio* quantum chemical calculations on proton transfer energetics including the structural changes were performed by using methanethiol, imidazole and formic acid as models of the side chains of Cys 25, His 159 and Asp 158.

Method

Calculation Method——The modified Gaussian 70 program for quantum chemical calculations according to the closed-shell LCAO MO SCF approximation was used.[18] A 4-31G basis set was employed in the *ab initio* calculations.[19]

Geometries——Methanethiol, imidazole and formic acid are used as models in place of Cys 25, His 159 and Asp 158 of the enzyme. For CH_3SH, the values used were $r(CH)=1.1039$ Å, $r(SH)=1.3291$ Å, $r(CS)=1.8177$ Å and $\angle HCS=108.629°$.[20] For CH_3S^-, the values used were $r(CH)=1.1039$ Å,[20] $r(CS)=1.8870$ Å and $\angle HCS=112.882°$; the values of $r(CS)$ and $\angle HCS$ were obtained from optimized calculations of the geometry with a 4-31G basis set. The geometrical data for imidazole except for the hydrogens were obtained from experimental data.[20] Other values were as follows: for the neutral form of imidazole, $r(CH)=1.0622$ Å and $r(NH)=0.9892$ Å;[21] for the cation of imidazole, $r(CH)=1.0622$ Å and $r(NH)=0.9949$ Å;[21] for $HCOO^-$ $r(CH)=1.1126$ Å, $r(CO)=1.2506$ Å and $\angle HCO=114.8°$;[21] for HCOOH, $r(CH)=1.0732$ Å, $r(CO)=1.1960$ Å, $r(CO)=1.3504$ Å, $r(OH)=0.9614$ Å, $\angle COH=114.6°$, $\angle OCO=124.7°$ and $\angle HCO=110.4°$.[21] The anion form of formic acid is indicated by Fo^- or $H^{\beta}C^{\gamma}O^{\delta 1}O^{\delta 2-}$; the neutral form of formic acid is shown as FoH or $H^{\beta}C^{\gamma}O^{\delta 1}O^{\delta 2}H^{\delta 2}$. The neutral form of imidazole is shown as $HN^{\delta 1}ImN^{\varepsilon 2}$, where H is covalently bonded to $N^{\delta 1}$ of the imidazole; the cation is $HN^{\delta 1}Im^{+}N^{\varepsilon 2}H$; the anion is $N^{\delta 1}Im^{-}N^{\varepsilon 2}$. The neutral form of methanethiol is shown as HMs or $H^{\gamma}S^{\gamma}C^{\beta}H_3$; the anion is Ms^- or $^-S^{\gamma}C^{\beta}H_3$. The imidazole $N^{\delta 1}$ and $N^{\varepsilon 2}$ form hydrogen bonds with the formic acid $O^{\delta 2}$ and the methanethiol S^{γ}, respectively.

Results

Complex of the Anion Form of Formic Acid, the Neutral Form of Imidazole and the Neutral Form of Methanethiol

Ab initio MO calculations on formic acid-imidazole complex formation were performed by Umeyama and Nakagawa.[22] In the optimized structure, the formic acid and the imidazole were in the anion and neutral forms, respectively; $r(O^{\delta 2}N^{\delta 1})$ and $\angle C^{\gamma}O^{\delta 2}N^{\delta 1}$ were 2.73 Å and 108.6°, respectively. Geometry optimization calculations on imidazole (neutral form)-methanethiol (neutral form) complex formation were performed. The intermolecular distance $r(N^{\varepsilon 2}—S^{\gamma})$ and the torsion angle of the imidazole carbon, the imidazole $N^{\varepsilon 2}$, the methanethiol S^{γ} and the methanethiol C^{β} were calculated to be 3.61 Å and 90°, respectively. The stabilization energy of complex formation and the total energy were calculated to be −4.1 kcal/mol and −661.65188 hartrees, respectively. Based on the optimized structure of formic acid (anion form)-imidazole (neutral form) complex, calculations of the optimized structure between the Fo^-–$HN^{\delta 1}ImN^{\varepsilon 2}$ complex and methanethiol were carried out. The intermolecular distance between $N^{\varepsilon 2}$ of the imidazole and S^{γ} of the methanethiol was calculated to be 3.36 Å. The

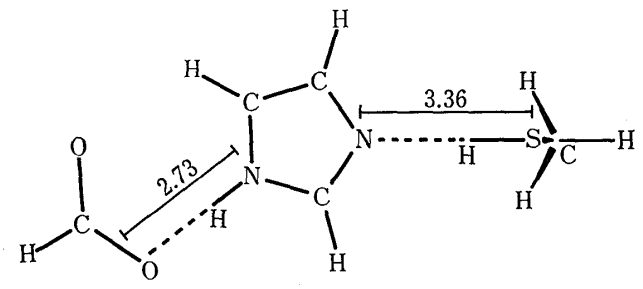

Fig. 1. The Energy-optimized Structure including the Anion Form of Formic Acid, the Neutral Form of Imidazole and the Neutral Form of Methanethiol

structure of the Fo^-–$HN^{\delta 1}ImN^{\varepsilon 2}$–HMs complex is shown in Fig. 1. The stabilization energy of complex formation between the two moieties and the total energy of the optimized structure were calculated to be −8.6 kcal/mol and −849.60942 hartrees, respectively. The intermolecular distance between $N^{\varepsilon 2}$ and S^r in the ternary complex is shorter than that in the binary complex by 0.25 Å.

Proton Transfer from Methanethiol to Imidazole in the Fo^-–$HN^{\delta 1}ImN^{\varepsilon 2}$–HMs Complex

The thiolate-imidazole ion pair has been found in the active site of papain.[1-5] If proton transfer occurs from the methanethiol to the imidazole in the Fo^-–$HN^{\delta 1}ImN^{\varepsilon 2}$–HMs complex, the form of the complex changes from the thiol-imidazole neutral pair to the thiolate-imidazole ion pair. Whether the total energy of the form Fo^-–$HN^{\delta 1}ImN^{\varepsilon 2}$–HMs or the form Fo^-–$HN^{\delta 1}$-$Im^+N^{\varepsilon 2}H$–Ms^- is more stable is important in relation to the experiments mentioned above. The barrier heights of proton transfer from the methanethiol to the imidazole in the Fo^-–$HN^{\delta 1}$-$ImN^{\varepsilon 2}$–HMs complex were calculated at various values of $r(H^rS^r)$. The results are shown in the second column of Table I. The total energies of the ternary complex relative to the structure in which the H^r proton is covalently bonded to S^r of the methanethiol were plotted against the distance $r(H^rS^r)$. The potential energy curve of the proton transfer is shown in Fig. 2. The energy at $r(H^rS^r)=1.87$ Å is maximum, and the maximum value is estimated to be 10.6 kcal/mol; the proton will be transferred easily between $N^{\varepsilon 2}$ of the imidazole and S^r of the methanethiol. The structure in which the proton is covalently bonded to $N^{\varepsilon 2}$ is more stable than the initial state. In other words, in the ternary complex including the anion form of the formic acid, the thiolate-imidazole ion pair is more stable than the thiol-imidazole neutral pair. This result is in agreement with experiment.[1-5] In order to elucidate the role of the anion form of the formic acid, the barrier heights of proton transfer from the methanethiol to the imidazole in the binary complex without including the anion form of the formic acid were calculated. These values are shown in the fourth column of Table I. The barrier heights are much higher than those in the second column. Therefore, the formic acid plays a significant role in lowering the barrier height of proton transfer from the methanethiol to the imidazole. Moreover, similar calculations in which the formic acid was included as

Table I. Barrier Heights in kcal/mol of Proton Transfers from S of Methanethiol to N of Imidazole in the Forms Fo^-–$HN^{\delta 1}ImN^{\varepsilon 2}$–HMs and $HN^{\delta 1}ImN^{\varepsilon 2}$–HMs

r (HS) Å	Barrier heights in the form		
	Fo^-–$HN^{\delta 1}ImN^{\varepsilon 2}$–HMs	(Fo^-)–$HN^{\delta 1}ImN^{\varepsilon 2}$–HMs[a]	$HN^{\delta 1}ImN^{\varepsilon 2}$–HMs
1.329	0[b]	0[c]	0[d]
1.587	4.2	4.4	7.4
1.845	10.5	11.3	19.9
2.102	4.6	6.0	21.8
2.360	−1.4	0.6	21.9

a) $HCOO^-$ is included in the calculations as fractional point charges. The values for H, C and O are −0.0196, 0.5952 and −0.7878, respectively.
b) Total energy of this form is −849.60942 hartrees.
c) Total energy of this form is −661.71055 hartrees, but the interaction energies among fractional point charges are not included in this value.
d) Total energy of this form is −661.65112 hartrees.

fractional point charges were performed.[23] The results are shown in the third column of Table I. The barrier heights are very similar to those obtained from the calculations on the structure consisting of formic acid, imidazole and methanethiol. Due to the electrostatic interaction, therefore, the formic acid lowers the barrier height of proton transfer from the methanethiol to the imidazole.

The Fo^-–$HN^{\delta 1}Im^+N^{\varepsilon 2}H$–$Ms^-$ Complex

In the preceding section, the potential energy curve of proton transfer from the methanethiol to the imidazole was described, and the form Fo^-–$HN^{\delta 1}Im^+N^{\varepsilon 2}H$–$Ms^-$ was calculated to be more stable than the form Fo^-–$HN^{\delta 1}ImN^{\varepsilon 2}$–HMs. However, as the proton transfers from the methanethiol to the imidazole, structural changes of the methanethiol and the imidazole will occur. To investigate the intramolecular structure change,[24] energy optimization of the structure consisting of the anion form of the imidazole, the cation form of the imidazole and the anion form of the methanethiol was performed. First, the stabilization energies between the anion form of the formic acid and the cation form of the imidazole were calculated for various structures to determine the optimized structure. The distance between $O^{\delta 2}$ of formic acid and $N^{\delta 1}$ of imidazole and the angle of the formic acid C^{γ}, the formic acid $O^{\delta 2}$ and the imidazole $N^{\delta 1}$ were estimated to be 2.53 Å and 103.1°, respectively; the molecular plane of the formic acid was estimated to coincide with that of the imidazole. Based on the geometry of the Fo^-–$HN^{\delta 1}Im^+N^{\varepsilon 2}H$ complex, stabilization energies between the Fo^-–$HN^{\delta 1}$-$Im^+N^{\varepsilon 2}H$ complex and the anion form of methanethiol upon ternary complex formation were calculated for various structures to determine the optimized structure. The distance between $N^{\varepsilon 2}$ of the imidazole and S^{γ} of the methanethiol and the angle of the imidazole $N^{\varepsilon 2}$, the methanethiol S^{γ} and the methanethiol C^{β} were estimated to be 3.13 Å and 100.3°, respectively. The stabilization energy in the optimized structure and the total energy were calculated to be −47.5 kcal/mol and −849.61773 hartrees, respectively. The structure is shown in Fig. 3.[25]

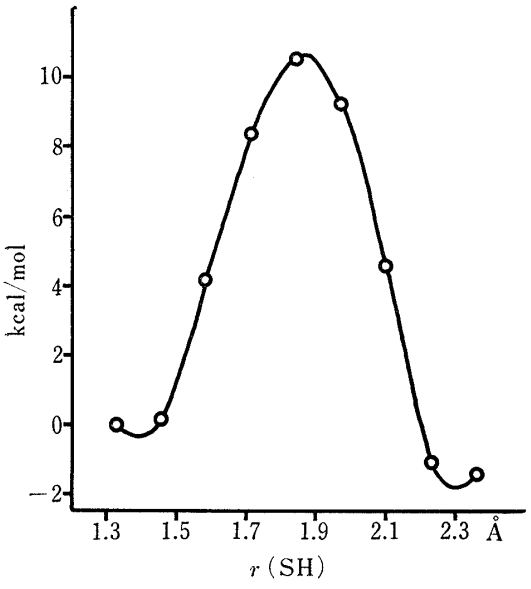

Fig. 2. The Potential Energy Curve of the Proton Transfer against r(SH) in the Formic Acid (anion form)-Imidazole (neutral form)-Methanethiol (neutral form) Complex

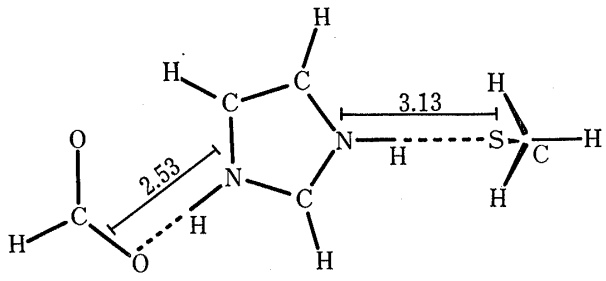

Fig. 3. The Energy-optimized Structure including the Anion Form of Formic Acid, the Cation Form of Imidazole and the Anion Form of Methanethiol

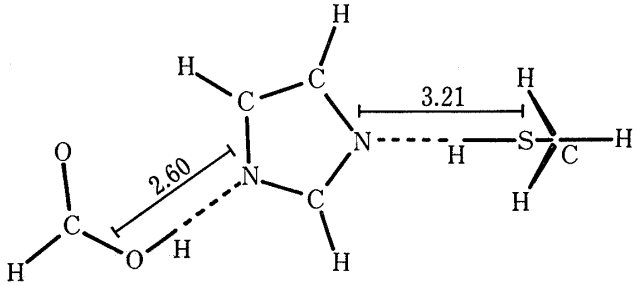

Fig. 4. The Energy-optimized Structure including the Neutral Form of Formic Acid, the Anion Form of Imidazole and the Neutral Form of Methanetiol

The distance between $N^{\varepsilon 2}$ of the imidazole and S^{γ} of the methanethiol is shorter than that in the Fo^{-}–$HN^{\delta 1}ImN^{\varepsilon 2}$–HMs complex by 0.23 Å. As the proton transfers from the methanethiol to the imidazole in the ternary complex, the intermolecular distance between the methanethiol and the imidazole becomes shorter. If the positions of atomic nuclei other than the transferred proton are unchanged, the structure is more unstable by 3.8 kcal/mol than that in which the positional changes are considered.

The FoH-$N^{\delta 1}Im^{-}N^{\varepsilon 2}$-HMs Complex

The energetics of proton transfer between the imidazole and the formic acid in the binary complex having an integral charge of −1 were studied by Umeyama and Nakagawa.[26] The distance between $N^{\delta 1}$ of the imidazole and $O^{\delta 2}$ of the formic acid in the imidazole (anion form)-formic acid (neutral form) complex was estimated to be 2.60 Å, and the optimized structure was used in the calculations of the stabilization energies of the ternary complex formation. The stabilization energies between the FoH–$N^{\delta 1}Im^{-}N^{\varepsilon 2}$ complex and the neutral form of the methanethiol were calculated at various intermolecular distances to determine the optimized structure. The distance between $N^{\varepsilon 2}$ of the imidazole and S^{γ} of the methanethiol was estimated to be 3.21 Å. The stabilization energy upon complex formation and the total energy of the optimized structure were calculated to be −12.5 kcal/mol and −849.58189 hartrees, respectively. The structure is shown in Fig. 4. When the methanethiol was omitted from the structure, as shown in Fig. 4, the energy-optimized structure consisting of the anion form of the imidazole and the neutral form of the formic acid was more unstable by 17.5 kcal/mol than that consisting of the neutral form of the imidazole and the anion form of the formic acid.[26] Similarly, the energy-optimized structure including the neutral form of the methanethiol, the anion form of the imidazole and the neutral form of the formic acid is more unstable by 17.3 kcal/mol than that including the neutral form of the methanethiol, the neutral form of the imidazole and the anion form of the formic acid. Accordingly, the energetics of proton transfer between the imidazole and the formic acid in the binary complex having an integral charge of −1 are unaffected by methanethiol.

The FoH-$N^{\delta 1}ImN^{\varepsilon 2}$H-$Ms^{-}$ Complex

The structure of the form Fo^{-}–$HN^{\delta 1}Im^{+}N^{\varepsilon 2}$H–$Ms^{-}$ was calculated to be more stable by 5.2 kcal/mol and 22.5 kcal/mol than the structures of the forms Fo^{-}–$HN^{\delta 1}ImN^{\varepsilon 2}$–HMs and FoH–$N^{\delta 1}Im^{-}N^{\varepsilon 2}$–HMs, respectively. The following question then arises: when the proton covalently bonded to $N^{\delta 1}$ in the Fo^{-}–$HN^{\delta 1}Im^{+}N^{\varepsilon 2}$H–$Ms^{-}$ complex transfers from the imidazole to the formic acid, which complex, Fo^{-}–$HN^{\delta 1}Im^{+}N^{\varepsilon 2}$H–$Ms^{-}$ or FoH–$N^{\delta 1}ImN^{\varepsilon 2}$H–$Ms^{-}$, is more stable and why? First, energy optimization in the structure consisting of the neutral form of the formic acid and the neutral form of the imidazole was performed. The distance between $O^{\delta 2}$ of the formic acid and $N^{\delta 1}$ of the imidazole and the torsion angle of the formic acid C^{γ}, the formic acid $O^{\delta 2}$, the imidazole $N^{\delta 1}$ and the imidazole C^{γ} chain were estimated to be 2.72 Å and 0°, respectively. Based on the energy-optimized geometry of the imidazole (neutral form)-formic acid (neutral form) complex, the stabilization energies between the FoH–$N^{\delta 1}ImN^{\varepsilon 2}$H complex and the anion form of the methanethiol upon ternary complex formation were calculated for various structures to determine the energy-optimized structure. The distance between the imidazole $N^{\varepsilon 2}$ and the methanethiol S^{γ} and the angle of $N^{\varepsilon 2}$, S^{γ} and the methanethiol C^{β} were estimated to be 3.21 Å and 100.3°, respectively. The stabilization energy upon complex formation and the total energy of the energy-optimized structure were calculated to be −33.3 kcal/mol and −849.61379 hartrees, respectively. The structure is shown in Fig. 5. The structure of the form FoH–$N^{\delta 1}ImN^{\varepsilon 2}$H–$Ms^{-}$ is more unstable than that of the form Fo^{-}–$HN^{\delta 1}$-$Im^{+}N^{\varepsilon 2}$H–Ms^{-} by 2.5 kcal/mol. In the calculations without the anion form of the methanethiol, the structure consisted of the neutral form of the formic acid and the neutral form of the imidazole is more stable than that consisting of the anion form of the formic acid and the cation form of the imidazole by 11.7 kcal/mol. Therefore the anion form of the methanethiol stabili-

zes the structure including the formic acid-imidazole ion pair by 14.2 kcal/mol more than that including the formic acid-imidazole neutral pair.

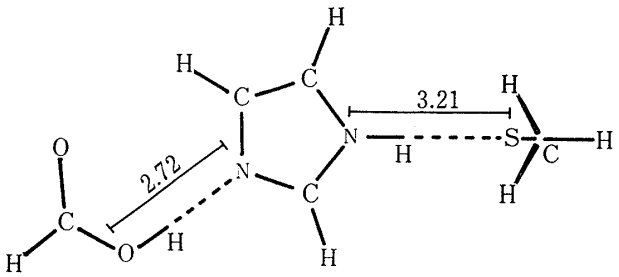

Fig. 5. The Energy-optimized Structure including the Neutral Form of Formic Acid, the Neutral Form of Imidazole and the Anion Form of Methanethiol

TABLE II. Energies in kcal/mol of Various Forms Relative to the Form Fo^{-}–$HN^{\delta 1}Im^{+}N^{\varepsilon 2}H$–$Ms^{-}$

Form	Structure	Relative energy
Fo^{-}–$HN^{\delta 1}ImN^{\varepsilon 2}$–HMs	A	5.2
Fo^{-}–$HN^{\delta 1}Im^{+}N^{\varepsilon 2}H$–$Ms^{-}$	B	0[a]
FoH–$N^{\delta 1}Im^{-}N^{\varepsilon 2}$–HMs	C	22.5
FoH–$N^{\delta 1}ImN^{\varepsilon 2}H$–$Ms^{-}$	D	2.5

a) Total energy is −849.61773 hartrees.

Discussion

The optimized structures of the four ternary complexes,

Fo^{-}–$HN^{\delta 1}ImN^{\varepsilon 2}$–HMs (A)

Fo^{-}–$HN^{\delta 1}Im^{+}N^{\varepsilon 2}H$–$Ms^{-}$ (B)

FoH–$N^{\delta 1}Im^{-}N^{\varepsilon 2}$–HMs (C)

FoH–$N^{\delta 1}ImN^{\varepsilon 2}H$–$Ms^{-}$ (D)

were obtained as shown in Figs. 1,3,4 and 5; these four structures are called "A", "B", "C" and "D", respectively. The energies of three complexes (A, C and D) relative to the complex B, whose structure was the most stable, are shown in Table II. The most stable B structure of the form Fo^{-}– $HN^{\delta 1}Im^{+}N^{\varepsilon 2}H$–$Ms^{-}$ includes the imidazole-methanethiol ion pair. This result is in good agreement with experiment.[1-5] The theory of the enzymatic reaction of papain proposed by Angelides and Fink,[16] that there is a movement of His 159 to a structure involving electrostatic interaction with the carboxylate of Asp 158, is supported by the results in Table II. If the enzymatic reaction of papain happens according to the "DOWN" model proposed by Angelides and Fink,[16] the B structure should be present in the enzymatic reaction rather than the A structure. The result that the B structure is more stable than the A structure by 5.2 kcal/mol supports the "DOWN" model.

In the present work, we have considered the enzymatic reaction of papain in terms of the hydrogen-bonding system consisting of Cys 25, His 159 and Asp 158 and in terms of model systems in which the three-dimensional structure of papain is not taken into account. As the next step, similar calculations will be carried out based on the simulated structure determined from X-ray diffraction analyses. Nevertheless, the energetics of proton transfers among the models of Cys 25, His 159 and Asp 158 are significant, because results such as those indicating the presence of the imidazole-thiolate ion pair are obviously relevant to the mechanism of the enzymatic reaction.

Thiolsubtilisin can be regarded as a model thiol protease, although it can only catalyze the hydrolysis of active esters.[27] The structures of subtilisin and thiolsubtilisin are identical within experimental error, except that the sulfur replaces an oxygen atom;[28] this means that there is a catalytic triad comprised of an aspartic acid residue, a histidine residue and a cysteine residue. Polgar and Halasz found that at around neutral pH there was an interaction between the thiol and imidazole groups.[29] They concluded that a mercaptide imidazolium ion-pair was formed in thiolsubtilisin;[28] *i.e.*, a hydrogen-bonding structure consisting of the anion form of aspartic acid residue, the cation form of histidine residue and the anion form of cysteine

residue is present in the active site of thiolsubtilisin. Therefore the results obtained on the energetics of the proton transfers in the formic acid-imidazole-methanethiol complex are relevant to the electronic structure in the active site of thiolsubtilisin as well as that of papain.

In the calculations, formic acid was used as the model for the aspartic acid residue. If the C^{α} carbon of the peptide moiety is included in the form of $C^{\alpha}H_3C^{\beta}H_2C^{\gamma}O^{\delta 1}O^{\delta 2-}$, the model might be more appropriate. However, the proton affinity of $HCOO^-$, 345 kcal/mol, is very close to that of $CH_3CH_2COO^-$, 347 kcal/mol.[30] This similarity suggests that the use of formic acid in the enzyme model is valid. Moreover, the calculated values of the proton affinity of $HCOO^-$ were 360 and 479 kcal/mol by using double zeta (4-31G) and single zeta (STO-3G) basis sets, respectively. The value calculated by using the 4-31G basis set is in very good agreement with the experimental value mentioned above. Therefore, the calculated results based on the 4-31G basis set for the present enzyme model should reflect the situation in the enzyme itself reasonably well.

Acknowledgement The authors are grateful to Professor I. Moriguchi of this university for his support. Numerical calculations were carried out with HITAC M-180 and M-200 computers at the Computer Center at the Institute for Molecular Science.

The authors are also grateful for a private communication from Prof. Kollman,[31] who calculated similar proton transfer energetics by using a STO-3G basis set. He did not obtain the imidazole-thiolate ion pair structure, however, presumably because such a small basis set is not appropriate in relation to proton transfer energetics.[17]

References and Notes

1) L. Polgar, *Eur. J. Biochem.*, **33**, 104 (1973).
2) L. Polgar, *FEBS Lett.*, **47**, 15 (1974).
3) S.D. Lewis, F.W. Johnson, and J. Shafer, *Biochemistry*, **15**, 5009 (1976).
4) R. Shinitzky and R. Goldman, *Eur. J. Biochem.*, **3**, 139 (1967).
5) L.A.AE. Sluyterman and M.J.R. De Graaf, *Biochim. Biophys. Acta*, **200**, 595 (1970).
6) G. Lowe, *Phil. Trans. Roy. Soc. Lond.*, **B257**, 237 (1970).
7) *a*) J. Drenth, J.N. Jansonius, R. Koekoek, H.M. Swen, and B.G. Wolthers, *Nature* (London), **218**, 929 (1968); *b*) J. Drenth, J. N. Jansonius, R. Koekoek, and B. G. Wolthers, "Enzymes," 3rd Ed. 3, 1971, p. 485.
8) However, it should be noted that the x-ray crystallographic studies were done at high pH (9.3) where the imidazole may exist primarily as the free base. Since the cysteine residue may be ionized at pH 9.3, other groups such as Asp 158 may be displaced due to the electrostatic repulsion between the anion form of Asp 158 and the anion form of Cys 25.
9) R. Broer, P. Th. Van Duijnen, and W.C. Nieuwpoort, *Chem. Phys. Letters*, **42**, 525 (1976).
10) G. Bolis, M. Ragazzi, D. Salvaderi, D.R. Ferro, and E. Clementi, *Gazzetta Chimica Italiana*, **108**, 425 (1978).
11) S. Nakagawa and H. Umeyama, *Chem. Pharm. Bull.*, **25**, 909 (1977).
12) A.W. Lake and G. Lowe, *Biochem. J.*, **101**, 402 (1966).
13) M.R. Holloway and M.J. Hardman, *Eur. J. Biochem.*, **33**, 1537 (1973).
14) A.L Fink and C. Gwyn, *Biochemistry*, **13**, 1190 (1974).
15) J. Mattis and J.S. Fruton, *Biochemistry*, **15**, 2191 (1976).
16) K.J. Angelides and A.L. Fink, *Biochemistry*, **17**, 2659 (1978).
17) S. Nakagawa, H. Umeyama, and T. Kudo, *Chem. Pharm. Bull.*, **28**, 1342 (1980).
18) W.J. Hehre, W.A. Lathan, R. Ditchfield, M.D. Newton, and J.A. Pople, 1974, Gaussian 70, Quantum Chemistry Program Exchange, Indiana University.
19) W.J. Hehre, R.F. Stewart, and J.A. Pople, *J. Chem. Phys.*, **51**, 2657 (1969).
20) L.E. Sutton and B. Phil, "Tables of Interatomic Distances and Configuration in Molecules and Ions," published by the Chemical Society, London, 1958.
21) S. Nakagawa, H. Umeyama, K. Kitaura, and K. Morokuma, *Chem. Pharm. Bull.*, **29**, 1 (1981).
22) H. Umeyama and S. Nakagawa, *Chem. Pharm. Bull.*, **28**, 1342 (1980).
23) The point fractional charges of H, C and O of formic acid are −0.020, 0.595 and −0.788, respectively. In the SCF calculations of the structure consisting of the imidazole and the methanethiol, formic acid was included by approximation as point fractional charges.
24) The aromatic ring of the imidazole was assumed to be unchanged by the ionization, since an aromatic ring having six π-electrons is stable.

25) Since the energy optimization at various distances between $O^{\delta 2}$ of the formic acid and $N^{\delta 1}$ of the imidazole upon ternary complex formation was not carried out repeatedly, the optimized structure is approximate.
26) H. Umeyama and S. Nakagawa, *Chem. Pharm. Bull.*, **28**, 2292 (1980).
27) L. Polgar and M.L. Bender, *Biochemistry*, **6**, 610 (1967).
28) R.A. Alden, C.S. Wright, F.C. Westall, and J. Kraut, "Structure-Function Relationships of Proteolytic Enzymes," ed. by P. Desnuele, H. Neurath and M. Ottesen, Munksgaad, Copenhagen, 1970, p. 173.
29) L. Polgar and P. Halasz, *Eur. J. Biochem.*, **39**, 421 (1973).
30) *a*) R. Yamdagni and P. Kebarle, *Can. J. Chem.*, **52**, 861 (1974); *b*) E.M. Arnett, L.E. Small, R.T. McIver, Jr. and J.S. Miller, *J. Am. Chem. Soc.*, **96**, 5638 (1974); *c*) J.B. Cumming and P. Kebarle, *Can. J. Chem.*, **56**, 1 (1978).
31) P.A. Kollman and D.M. Hayes, private communication.