Proc. Natl. Acad. Sci. USA
Vol. 81, pp. 6266–6270, October 1984
Biochemistry

Charge state of His-57–Asp-102 couple in a transition state analogue–trypsin complex: A molecular orbital study

(serine protease/enzymatic reaction/charge relay system/quantum chemistry/*ab initio* MO)

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Communicated by William N. Lipscomb, June 11, 1984

ABSTRACT ***Ab initio*** **molecular orbital studies have been made as a model for the deacylation step of trypsin. Ser-195 is modeled by H_2O in which one H is replaced either by $—PO_2(OH)^-$ (monoisopropyl phosphoryl, MIP) or by $—CHO(OH)^-$ (a transition state analogue, TSD). The quantum mechanical region includes imidazole$^+$ and acetate$^-$ as models for His-57$^+$ and Asp-102$^-$, two hydrogen bonds from two formamide molecules to the oxyanion MIP or TSD, and three hydrogen bonds to Asp-102. The remainder of the enzyme is treated classically as a fractional charge model. The effect of proton transfer from His-57$^+$ to Asp-102$^-$ is very similar for the MIP and TSD models, and the proton transfer is energetically unfavorable for all models that include at least the hydrogen bond from an H_2O that models Ser-214. Thus, the several hydrogen bonds to the models of the catalytic unit (substrate, Ser-195, His-57, and Asp-102) stabilize the His-57$^+$/Asp-102$^-$ salt link, and this indicates that proton transfer does not occur from His-57$^+$ to Asp-102$^-$. (Also, the similarities of energy of transfer of this proton transfer for the various models show that the model substrate analogue behaves very similarly to the MIP inhibitor.)**

Serine proteases like chymotrypsin, trypsin, etc., are characterized by the presence of a catalytic triad consisting of the precisely arranged aspartate, histidine, and serine residues. Blow *et al.* (1) proposed a "charge relay system," in which the aspartate played a role as an ultimate base holding a proton during the catalysis, from their x-ray structure analysis of α-chymotrypsin. The experiments of ^{13}C NMR (2) and infrared spectroscopy (3) had supported this hypothesis. But, the experiments of low-field ^1H NMR (4), His-57 $C^{\epsilon 1}$ ^1H NMR (5), and ^{15}N NMR (6) have not supported this hypothesis. The results of *ab initio* molecular orbital (MO) calculations for the protonated catalytic triad in β-trypsin have been in good agreement with those of the latter experiments (7, 8). The charge state of the protonated catalytic triad may be different from that of the transition state in which the serine oxygen, having negative charge, attacks the carbonyl carbon of a substrate.

In order to elucidate the charge state of the His–Asp couple in the process of the enzymatic reaction, MO calculations have been performed. Early MO calculations using semiempirical methods showed the possibility of double proton transfer in the catalytic triad (9–11). However, *ab initio* MO calculations that appeared later showed that aspartate in the triad is to stay unprotonated during catalysis. First, Hayes and Kollman (12) suggested that unprotonated aspartate functions to stabilize the histidine–serine ion pair in α-chymotrypsin. In the calculation of the potential energy of the proton transfer from Ser-195 to His-57 and from His-57 to Asp-102 (α- and β-proton transfers, respectively) in pancreatic trypsin inhibitor (PTI)-trypsin complex, the triple ion

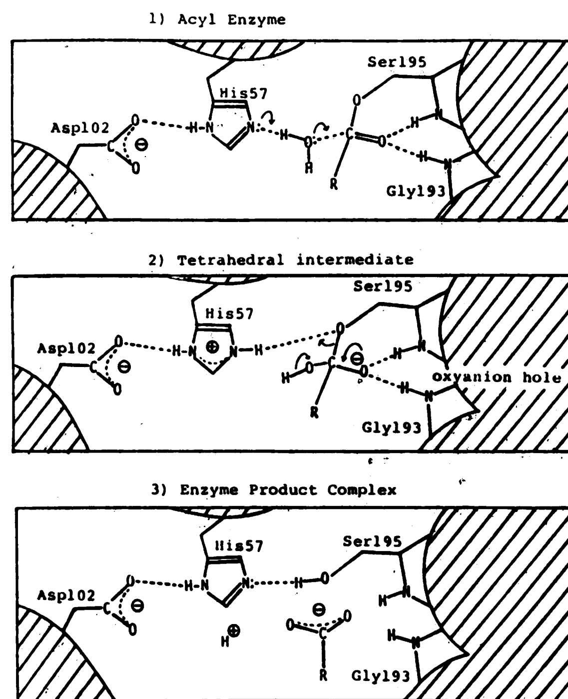

FIG. 1. Deacylation process in serine protease hydrolysis.

form [Asp-102(−)–His-57(+)–Ser-195(−)] has been found to be more stable than the double-proton-transfer form [Asp-102(neutral)–His-57(neutral)–Ser-195(−)] (13–17). Umeyama *et al.* (14) have proposed an "electrostatic mechanism" in which the anion form of Asp-102 plays a role in lowering the potential barrier of α-proton transfer by an electrostatic interaction. It has been shown, moreover, that the change of the charge state of the catalytic triad is greatly affected by the environment around it [the environment effect (18)].

However, it is desirable to elucidate the charge state of the His–Asp couple in the enzymatic reaction pathway using a more adequate model for the transition state. The charge state of the active center in monoisopropyl phosphoryl (MIP)-trypsin, which is a transition state analogue–enzyme complex, is similar to that in the transition state. Kossiakoff and Spencer (19) have determined the position of the hydrogen atom between His-57 and Asp-102 in MIP-trypsin by a neutron diffraction method and have shown that the His–Asp couple in MIP-trypsin is an ion-pair form.

In this paper, we study the electronic structure of the ac-

Abbreviations: MIP, monoisopropyl phosphoryl; Mip, (OH)-$PO_2^-(O^{\gamma}H)$; DIP, diisopropyl phosphoryl; TSD, transition-state analogue of deacylation; Tsd, $HCO^-(OH)(O^{\gamma}H)$; PTI, pancreatic trypsin inhibitor; PCAA, point fractional charge of amino acids outside of the quantum mechanical region; SCF, self-consistent field.

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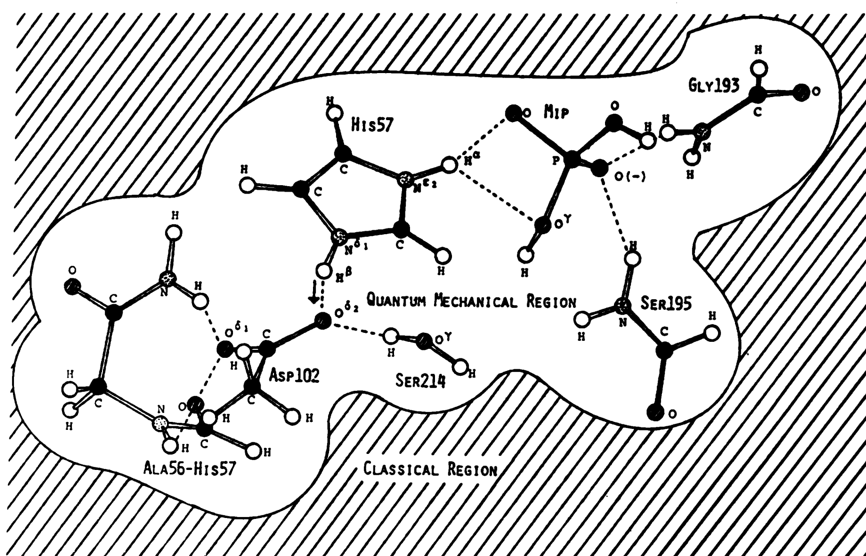

FIG. 2. Calculated model structure of MIP-trypsin complex (see text). The enzyme system is partitioned into a classical region (shaded area) and a quantum mechanical region.

tive center of MIP-trypsin, and show that the charge state of the His–Asp couple in MIP-trypsin obtained from *ab initio* calculations is consistent with that from a neutron diffraction study. Additionally, the effect of the environment on the stabilization of the His–Asp ion pair of the active center is analyzed. Furthermore, the charge state of the His–Asp couple in the transition state analogue of deacylation (TSD)-trypsin is studied. TSD-trypsin is itself a transition state analogue in the step of the deacylation reaction shown in Fig. 1.

METHODS

Molecular orbital calculations have been carried out in a closed shell LCAO-SCF (self-consistent field) approximation by the *ab initio* method. The IMSPACK program (20, *), which has been modified for a large number of point fractional charges by Nakagawa and Umeyama (21), is used for the calculations. An STO-3G and a 4-31G basis set are used here (22, 23). Since the proton affinities calculated by the latter are in fairly good agreement with the experimental values (14), a 4-31G basis set is used for the proton donor and acceptor molecules. For the other molecules, an STO-3G basis set is mainly used. The SCF calculations including point fractional charges are performed by using the following Hamiltonian:

$$H = \sum_{i}^{N}\left(-1/2\Delta_i - \sum_{\alpha}^{\nu} Z_\alpha/r_{\alpha i}\right) + \sum_{i>j}^{N} 1/r_{ij} + \sum_{\alpha>\beta}^{\nu} Z_\alpha Z_\beta/R_{\alpha\beta} - \sum_{i}^{N}\sum_{\gamma}^{\mu} P_\gamma/r_{\gamma i} + \sum_{\alpha}^{\nu}\sum_{\gamma}^{\mu} Z_\alpha P_\gamma/R_{\alpha\gamma} + \sum_{\gamma>\delta}^{\mu} P_\gamma P_\delta/R_{\gamma\delta} \quad [1]$$

where i or j is an electron; α or β, a nucleus; Z_α (or Z_β), charge of the nucleus; P_γ (or P_δ), a point fractional charge; and r (or R), distance.

The three-dimensional structure of MIP-trypsin, consisting of 223 amino acid residues (24, 25), is available from Protein Data Bank (Chambers and Stroud refer to it as DIP-trypsin, not MIP-trypsin). The model structure of MIP-trypsin used in the calculation is shown in Fig. 2. The structure of TSD-trypsin was based on that of MIP-trypsin, since the structure of the active center in MIP-trypsin is thought to be similar to that in TSD-trypsin. The model structure of TSD-trypsin is shown in Fig. 3. The position of the proton (H^α) covalently bound to His-57 $N^{\varepsilon 2}$ and the conformation of the tetrahedral-like intermediate in Fig. 3 may be shifted by a small amount in the transition state of the deacylation reaction (see Fig. 1). The structures used in the calculations are listed in Table 1. Imidazole (Im^{57}) and acetic acid (Ac^{102}) are used in the place of His-57 and Asp-102, respectively, which play a significant role in the catalysis. As a model of MIP moiety in which MIP binds to Ser-195 O^γ, $(OH)PO_2^-(O^\gamma H)$ (called "Mip") is used as shown in Fig. 2, where the superscript "γ" means Ser-195 O^γ. As a model of the tetrahedral intermediate (TSD moiety), $HCO^-(OH)(O^\gamma H)$ (called "Tsd") is used as shown in Fig. 3. The monomer structure of Tsd is determined by the geometry optimization method using an STO-3G basis set, followed by least-squares fitting of some atoms in Tsd [$H\underline{CO}^-(\underline{O}H)(\underline{O}^\gamma H)$, underlined atoms] to Mip atoms [$(OH)\underline{PO}_2^-(\underline{O}^\gamma H)$, underlined atoms] using a program "LSFIT" (26) to make up MIP-trypsin. The average deviation of the four atoms of Tsd from Mip atoms is 0.10 Å. For the side chain of Ser-214, which forms a hydrogen bond with Asp-102 $O^{\delta 2}$, a water molecule (H_2O^{214}) is used, where the superscript "214" means Ser-214.

For the peptide NH groups of Ala-56 and His-57, which form two hydrogen bonds with Asp-102 $O^{\delta 1}$, $HCONHCH_2$-$CONH_2$ (i.e., HCO^{55}—$NHCH_2CO^{56}$—NH_2^{57}) is used. For the peptide NH groups of Gly-193 and Ser-195, which form the oxyanion hole of trypsin, formamides (HCO^{192}—NH_2^{193} and HCO^{194}—NH_2^{195}) are used.

For atoms of other amino acid residues and main chain atoms that are not mentioned above, the coordinates obtained from the x-ray diffraction study of MIP-trypsin are used. Those atoms are included as point fractional charges of amino acids outside of the quantum mechanical region in the SCF calculations, which are called "PCAA," as described below.

The coordinates of hydrogens are not obtainable from x-ray diffraction studies. The hydrogen atoms of Im^{57} are set in the ideal positions, respectively (8). The coordinates of hydrogens of the carboxyl group of Ac^{102}, HCO^{55}—$NHCH_2$-CO^{56}—NH_2^{57}, and HCO^{192}—NH_2^{193}, HCO^{194}—NH_2^{195} have been determined by using the optimized geometries of formic acid (27) and formamide (28). The hydrogens of the methyl group of Ac^{102} are attached to the carbon atom to

*Morokuma, K., Kato, S., Kitaura, K., Ohmine, I. & Sakai, S. (1979) "*Ab initio* Program Package Consisting of Gaussian 70 and Many Other Additional Routines," personal communication from K. Morokuma.

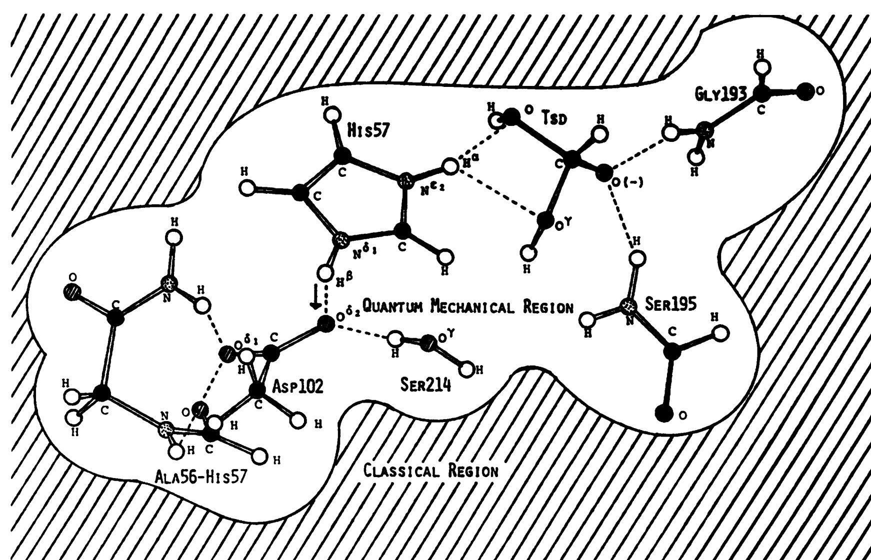

FIG. 3. Calculated model structure of TSD-trypsin (see text).

make an ideal tetrahedral form. In all model molecules, one hydrogen attached to the atom that should essentially bind with trypsin is modified to be on the line from the atom of the model to the atom of trypsin in order to reflect the connection of model molecules to trypsin—e.g., the coordinates of a hydrogen attached to O^{γ} in TSD are modified to be on the line from O^{γ} to C^{β} of Ser-195.

For O—H bonds of $(OH)PO_2^-(O^{\gamma}H)$, partially optimized bond lengths are used. The structure of $HCO^-(OH)(O^{\gamma}H)$ was determined by the geometry optimization method using an STO-3G basis set.

In the calculations including point fractional charges, the STO-3G Mulliken Net Atomic Charge Library (29) was used. However, the point charges of hydrogens of the side chains of amino acids are added to those of nearest neighbor atoms. The point charges of hydrogens of the main chain are included in the calculation. The program "PCHAIN" in which the point charges are assigned automatically on the coordinates of the protein has been developed by Nakagawa and Umeyama (unpublished data).

The relative energy, dE, of the proton transfer in MIP-trypsin or TSD-trypsin complex is defined as follows:

$$dE = E^{N} - E^{I}, \quad [2]$$

Table 1. Calculated models

Model	Molecules
I	Ac^{102}, Im^{57}, Mip or Tsd, H_2O^{214}, HCO^{55}—$NHCH_2CO^{56}$—NH_2^{57}*, HCO^{192}—NH_2^{193}*, HCO^{194}—NH_2^{195}*, PCAA†
II	Ac^{102}, Im^{57}, Mip or Tsd, H_2O^{214}, HCO^{55}—$NHCH_2CO^{56}$—NH_2^{57}*, HCO^{192}—NH_2^{193}*, HCO^{194}—NH_2^{195}*
III	Ac^{102}, Im^{57}, Mip or Tsd, H_2O^{214}, HCO^{55}—$NHCH_2CO^{56}$—NH_2^{57}*, HCO^{192}—NH_2^{193}*
IV	Ac^{102}, Im^{57}, Mip or Tsd, H_2O^{214}, HCO^{55}—$NHCH_2CO^{56}$—NH_2^{57}*
V	Ac^{102}, Im^{57}, Mip or Tsd, H_2O^{214}
VI	Ac^{102}, Im^{57}, Mip or Tsd
VII	Ac^{102}, Im^{57}

*Calculated by the use of STO-3G basis set.
†The amino acid residues of the trypsin molecule except those included in model II, which are approximated by point fractional charges in the calculation.

where E^N and E^I are total energies of the enzyme molecules which include a neutral-pair core (Im^{57n}–Ac^{102n}) and an ion-pair core (Im^{57+}–Ac^{102-}), respectively; the superscripts N, I, n, +, and − mean the neutral-pair form of the His-57–Asp-102 couple, the ion-pair form of the His-57–Asp-102 couple, neutral form, cationic form, and anionic form, respectively.

Although the SCF calculation of the total energy of a whole enzyme molecule is impossible, we can estimate the dE value. Warshel and Levitt (30) have partitioned an enzyme into quantum and classical regions, and they have decomposed the potential energy of the whole system into the following terms:

$$E = E_{\text{classical}} + E_{\text{quantum}} + E_{\text{quantum/classical}},$$

where the first two terms are the classical and quantum mechanical potential energies, respectively, and the third is a coupling term. According to this procedure, we also partitioned our enzyme system into two parts as shown in Figs. 2 and 3. One part is the quantum mechanical region consisting of the supermolecule, Ac^{102}, Im^{57}, Mip or Tsd, H_2O^{214}, HCO^{55}—$NHCH_2CO^{56}$—NH_2^{57}, HCO^{192}—NH_2^{193}, and HCO^{194}—NH_2^{195}, and the other part is the classical region (shaded area) consisting of all other amino acid residues and atoms (PCAA), which are not included in the supermolecule. (Our classical part does not include surrounding waters.) Therefore, we have

$$E^{N} = E^{N}_{\text{classical}} + E^{N}_{\text{quantum}} + E^{N}_{\text{quantum/classical}} \quad [3]$$

and

$$E^{I} = E^{I}_{\text{classical}} + E^{I}_{\text{quantum}} + E^{I}_{\text{quantum/classical}}. \quad [4]$$

As shown in Eq. 1, our Hamiltonian includes the term of point fractional charges for atoms in the classical region. The SCF calculation of the supermolecule is done in the electric field set up by these fractional charges and gives the total energy, E_{quantum}. Accordingly, among the interactions between the quantum mechanical and classical region, the E_{quantum} of our model includes the electrostatic interaction between both regions as well as the induced polarization of the quantum mechanical region. The rest of the interactions between both regions refers to the term of the $E_{\text{quantum/classical}}$.

Although some amino acid residues in the classical region

within Van der Waals distance from the quantum mechanical region affected the energy of proton transfer between His-57 and Asp-102, it was the electrostatic interaction that largely influenced the charge state in the active center (8), and so we may well consider that $E^{N}_{quantum/classical}$ is approximately equal to $E^{I}_{quantum/classical}$. $E^{N}_{classical}$ is also thought to be equal to $E^{I}_{classical}$, because the proton transfer from His-57 to Asp-102 does not seem to affect the classical region relatively remote from the active center. Accordingly, subtracting Eq. **4** from Eq. **3**, we obtain approximately

$$dE = E^{N}_{quantum} - E^{I}_{quantum}. \quad [5]$$

Thus, the relative energy, dE, of the proton transfer in the whole enzyme system can be calculated from Eq. **5**.

RESULTS AND DISCUSSION

In each model shown in Table 1, the energies of two charge states, E^{N} and E^{I}, are calculated by the *ab initio* MO method, and the relative energy, dE, is obtained from Eq. **5**. First, Mip is used in all of the models shown in Table 1. For model I, which represents whole MIP-trypsin complex, the relative energy is

$$dE_{I} = E^{N}_{I} - E^{I}_{I},$$

where E^{N}_{I} and E^{I}_{I} are the total energies of the quantum mechanical region of MIP-trypsin in which the His-57–Asp-102 couple is the neutral-pair form and the ion-pair form, respectively; the subscript I means model I. The dE_{I} is calculated to be 11.5 kcal/mol. This value represents the energy of the proton transfer from His-57 to Asp-102 in the MIP-trypsin molecule. Because of the magnitude of dE_{I}, such a transfer is not expected to occur. The result, the β proton is covalently bonded to His-57 $N^{\delta 1}$, is consistent with that from the neutron diffraction study. Thus, this level of theory supports the ion-pair form for the His-57–Asp-102 couple in MIP-trypsin. We define the G value,

$$G = dE_{I} - dE_{VII},$$

as the environment effect around the His-57–Asp-102 couple on the change of the charge state. Here, dE_{VII} is the energy of the proton transfer from Im^{57} to Ac^{102} in the isolated Im^{57}–Ac^{102} system (model VII in Table 1). The G value is calculated to be 25.6 kcal/mol. This large value means that the environmental effect around the His-57–Asp-102 couple plays an important role in changing the charge state from the neutral-pair to the ion-pair form. Moreover, in order to estimate the effects of various moieties around the His-57–Asp-102 couple, the total energies of the proton-transferred state and the initial state are calculated by the use of models II–VI shown in Table 1. The values of dE_{I} to dE_{VII} are plotted as the solid line in Fig. 4.

The effects of PCAA, HCO^{194}—NH_2^{195}, HCO^{192}—NH_2^{193}, HCO^{55}—$NHCH_2CO^{56}NH_2^{57}$, H_2O^{214}, and Mip are estimated from the equations, $G_{I} = dE_{I} - dE_{II}$, $G_{II} = dE_{II} - dE_{III}$, $G_{III} = dE_{III} - dE_{IV}$, $G_{IV} = dE_{IV} - dE_{V}$, $G_{V} = dE_{V} - dE_{VI}$, and $G_{VI} = dE_{VI} - dE_{VII}$, respectively. These values are shown in Table 2.

The MIP moiety is the largest contributor to the stabilization of the ion-pair form of the His-57–Asp-102 couple. The side-chain moiety of Ser-214 forming a hydrogen bond with Asp-102 $O^{\delta 2}$ makes a large contribution. The moieties of Ala-56 and His-57 forming hydrogen bonds with Asp-102 $O^{\delta 1}$ and PCAA contribute nearly equally. On the other hand, the contributions of both peptide NH groups of Gly-193 and Ser-195 forming the oxyanion hole of substrate are negative and, hence, are slightly destabilizing. As noted above, the calculation of the charge state of the active center in MIP-trypsin gives a result consistent with that from the experiment of Kossiakoff and Spencer (19). However, MIP-trypsin is the inhibited enzyme system, although the triad structure consisting of Asp-102, His-57, and MIP moiety is similar to that of the transition state of the enzymatic reaction shown in Fig. 1. If a better transition state analogue–enzyme complex such as TSD-trypsin (shown in Fig. 3) were studied in connection with the charge state of the His-57–Asp-102 couple, such a study would verify the conclusion drawn from the MIP-trypsin system elucidating the enzymatic reaction mechanism. The similar calculations are performed in TSD-trypsin by using models I–VII in Table 1, where Tsd is used in the place of Mip.

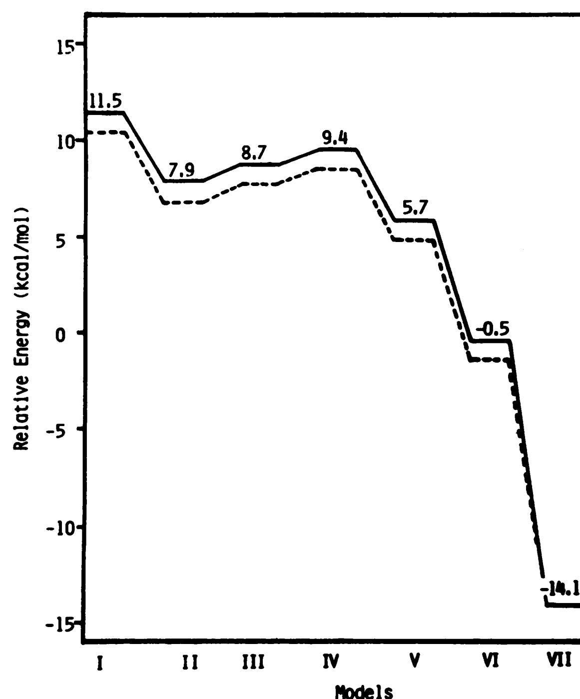

FIG. 4. Energies of β-proton transfer from His-57^{+} to Asp-102^{-} in each of models I–VII. As summarized in Table 1, model I is the most complete, while model VII refers to an isolated His–Asp couple. Values connected by a solid line refer to the proton transfer energies for MIP-trypsin system, and those connected by a broken line for TSD-trypsin system.

The values of dE_{I} to dE_{VII} are plotted by the broken line in Fig. 4. dE_{I} is calculated to be 10.4 kcal/mol. This shows that the proton transfer from His-57 to Asp-102 does not occur in TSD-trypsin. In the transition state of the enzymatic reaction, the β proton between His-57 and Asp-102 is on the side

Table 2. Effect of moieties on proton transfer

Environmental effect	Moiety	Calculated G value, kcal/mol	
		MIP-trypsin	TSD-trypsin
G_{I}	PCAA	3.6	3.6
G_{II}	HCO^{194}—NH_2^{195}	−0.8	−0.9
G_{III}	HCO^{192}—NH_2^{193}	−0.7	−0.7
G_{IV}	HCO^{55}—$NHCH_2CO^{56}$—NH_2^{57}	3.7	3.7
G_{V}	H_2O^{214}	6.2	6.2
G_{VI}	Mip or Tsd	13.6	12.6

Effects of various moieties around the His-57–Asp-102 couple on the β-proton transfer from His-57 to Asp-102 in MIP-trypsin and TSD-trypsin. If the G value is positive, the change of the charge state of the His-57–Asp-102 couple from the neutral-pair form to the ion-pair form is accelerated by the moiety.

of His-57 $N^{\delta 1}$. Accordingly, the charge relay system, in which Asp-102 accepts the proton from His-57 in the process of the enzymatic reaction, is not supported. The G (= $dE_I - dE_{VII}$) value, indicating the environment effect around the His-57–Asp-102 couple, is calculated to be 24.6 kcal/mol in the TSD-trypsin system. The environmental effect plays an important role in stabilizing the ion-pair form more than the neutral-pair form in the TSD-trypsin molecule as well as in the MIP-trypsin molecule. The effects of various moieties of the environment on the proton transfer from His-57 to Asp-102 in TSD-trypsin are also listed in Table 2. These values, G_I to G_V corresponding to the effects of PCAA, HCO^{194}—NH_2^{195}, HCO^{192}—NH_2^{193}, HCO^{55}—$NHCH_2$-CO^{56}—NH_2^{57}, and H_2O^{214}, respectively, are in almost complete agreement with the values in MIP-trypsin.

CONCLUSION

The charge state of the His-57–Asp-102 couple in MIP-trypsin is greatly influenced by the environment around it, and the His-57–Asp-102 couple exists in the ion-pair form in MIP-trypsin. The calculated results are consistent with the result from the neutron diffraction study of MIP-trypsin. Moreover, it is expected from the calculations of TSD-trypsin that the His-57–Asp-102 couple is in the ion-pair form in the transition state. In other words, the proton transfer from His-57 to Asp-102 is not thought to occur in the enzymatic reaction of trypsin. Our results confirm the results by Hayes and Kollman (12), our previous works (7, 8, 13, 14, 17, 18), and Allen (15). The charge relay system is not supported in the catalytic reaction of serine proteases from *ab initio* MO calculations.

The authors are grateful to Prof. I. Moriguchi of this university for his support. Moreover we thank Prof. W. N. Lipscomb of Harvard University for his helpful discussions and Dr. R. R. Mohr of Harvard University for his linguistic advice. Numerical calculations have been carried out with a HITAC M-200H computer at the computer center of the Institute for Molecular Sciences (Okazaki, Japan).

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