Communications to the Editor

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CHARGE STATE OF HIS 57-ASP 102 COUPLE IN β-TRYPSIN: A MOLECULAR ORBITAL STUDY

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The charge state of the His 57-Asp 102 couple in β-trypsin in the acid pH range was studied using the ab initio molecular orbital method. The result was in agreement with the NMR experiments which do not support the charge relay hypothesis proposed by Blow et al. It is remarkable that the charge state of His 57-Asp 102 couple is greatly affected by the environment around the catalytic triad.

KEYWORDS——trypsin; serine protease; enzyme; reaction; mechanism; pKa; quantum chemistry; ab initio; structure; MO

Trypsin-like enzymes include the hydrogen-bonding system consisting of Asp 102, His 57 and Ser 195 in the active site. In relation to the reaction mechanism, Blow et al. proposed a charge relay mechanism, in which Asp 102 behaves as a base in the active site of α-chymotrypsin.[1] Many experiments concerning the basisity of the catalytic triad have been performed. However, the experiments with low-field ^{1}H NMR,[2] $C^{\varepsilon 1}H$ NMR[3] and ^{15}N NMR[4] have demonstrated that, due to the first protonation in the acid pH range, His 57 behaves as a normal imidazole with respect to basisity. In order theoretically to elucidate the charge state of His 57 in the acid pH range, ab initio molecular orbital (MO) calculations including the environment effect around the His 57-Asp 102 couple of β-trypsin were carried out. The Gaussian 70 program[5] was originally used, and it was modified in order to perform calculations up to 200 basis sets. The basis set used was 4-31G.[6]

In bovine β-trypsin, Ser 195 O^{γ} slightly rotated in the protonation of the His 57-Asp 102 couple to improve the His 57 $N^{\varepsilon 2}$-Ser 195 O^{γ} hydrogen bond in the low pH form.[7] This hydrogen bond is 3.26 Å and rather bent at pH 8, while it is 2.90 Å and more linear at pH 5.[7] Although the coordinates of β-trypsin molecule at pH 5 should be used, they are unpublished. Since the hydrogen-bond length in the BPTI-β-tryspin comples is more similar to that in β-trypsin at pH 5 than to that at pH 8,[7] coordinates of β-trypsin molecule of the complex[8] were used for the calculations. Atomic coordinates of the complex (except the hydrogens, which were determined using the method of Umeyama et al.[9]) were obtained from the Brookhaven Protein Data Bank.[10] For the side chain of His 57, the cation (Im 57^{+}) and neutral (Im 57^{n}) forms of imidazole were used in place of His 57^{+} and His 57^{n}, where the superscripts "+" and "n" mean the cation and neutral form of the charge state, respectively. For the side chain of Asp 102, the anion (Fo 102^{-}) and neutral (Fo 102^{n}) forms of formic acid were used in place of Asp 102^{-} and Asp 102^{n}, respectively, where the superscript "-" means the anion form. For the peptide group of Ala 56 and the side chain of Ser 214 forming hydrogen bonds with Asp 102, NH_3 and CH_3OH, respectively, were used. For the disulfide bridge of Cys 42-Cys 58, the side chain of Ala 55, the peptide group of Ser 214-Trp 215 and the peptide group of Val 213-Ser 214 interacting with the His 57-Asp 102 couple near van der Waals' distances, CH_3SSCH_3, CH_4, $HCONH_2$ and $HCONH_2$, respectively, were used. For the ionic amino acid residues and the main chain of β-trypsin, point fractional charges were approximately used. Integral charges of -1 or +1 were assigned to 4 Asp, 3 Glu, C-terminus, 2 Arg,

14 Lys, 2 His and N-terminus in β-trypsin. For the point fractional charges of the main chain residues, Mulliken populations were used.

The energy value, dE_{CORE}, of the proton transfer from Im 57 $N^{\delta 1}$ to Fo 102 $O^{\delta 2}$ in Im 57-Fo 102 couple (CORE) is defined as

$$dE_{CORE} = E^{N}_{CORE} - E^{I}_{CORE} \quad (1)$$

where E^{N}_{CORE} and E^{I}_{CORE} are total energies of neutral CORE(Im 57^{n}-Fo 102^{n}) and ion-pair CORE(Im 57^{+}-Fo 102^{-}), respectively. On the other hand, the energy value, dE, of the proton transfer in β-trypsin is defined as

$$dE = E^{N} - E^{I} \quad (2)$$

where E^{N} and E^{I} are total energies of β-trypsin molecule which include the His 57^{n}-Asp 102^{n} and His 57^{+}-Asp 102^{-} forms, respectively. If the value of dE is positive, the enthalpy of the ion-pair structure is lower than that of the neutral structure. Since the entropy change due to the proton transfer may be small in the His 57-Asp 102 couple, the free energy of the ion-pair structure will be lower than that of the neutral one. When G is defined as the effect of the environment around the His 57-Asp 102 couple for the proton transfer, we have

$$G = dE - dE_{CORE} \quad (3).$$

Since the calculation of the total energy of the β-trypsin molecule is impossible, we calculate approximately the G value. The environment of the Im 57-Fo 102 couple is composed of various moieties of the enzyme. Each of the various moieties affects the electronic structure of the Im 57-Fo 102 couple, and the environment effect is shown by the following equation in an assumption of additivity of the contributions of the various moieties:

$$G = G_{\alpha} + G_{\beta} + G_{\gamma} + G_{\delta} + G_{\varepsilon} + G_{\zeta} \quad (4)$$

where α to ζ show the structures consisting of CORE and one of the various moieties as shown in Table I. G_{ζ} is assumed to be zero.

Table I. Various Moieties which Affect the Charge State of the His 57-Asp 102 Couple, and the Energies(G_{α} to G_{ε})

Structure	Moiety in addition to Im 57-Fo 102 couple	Energy in kcal/mol
α	Peptide moieties of His 57 and Asp 102	5.9
β	Amino acid residues hydrogen-bonding to CORE	10.5
γ	Amino acid residues interacting with CORE near van der Waals' distances	7.3
δ	Side chain moieties of ionic amino acid residues, N-terminus and C-terminus	3.8
ε	Main chain from 2nd to 222th except moieties in the structures of α to δ	2.7
ζ	Moieties in which the structures of α to ε do not include	-

When the environment (α to ε) of the Im 57-Fo 102 couple is included in the calculation of β-trypsin, the total effect for the proton transfer was calculated to be 30 kcal/mol (G). When dE_{CORE} was added to G, the potential energy of the proton transfer was 17 kcal/mol (dE), and, hence, the ion-pair His 57^{+}-Asp 102^{-} form will exist in β-trypsin. Since solvent molecules do not form a hydrogen bond with the His 57-Asp 102 couple,[11] the effect of solvent molecules such as Water 702 and Water 710 is thought to be very small. The values of G_{α} to G_{ε} are shown in Table I.

It is remarkable that, in comparison with the neutral His 57^{n}-Asp 102^{n} form, the ion-pair His 57^{+}-Asp 102^{-} form is greatly stabilized by the environment in β-trypsin. Moreover, the calculated charge state of His 57 agrees with that of His 57 reported from the NMR experiments.

Nakagawa et al.[12] calculated the potential energy changes for two proton transfers from Ser 195 to His 57 and from His 57 to Asp 102, and the Ser 195^{-}-His 57^{+}-Asp 102^{-} form was more stable than the Ser 195^{-}-His 57^{n}-Asp 102^{n} form. It was concluded that Asp 102 plays a significant role in lowering the barrier height of the proton transfer from Ser 195 to His 57 due to the electrostatic interaction.[9] Nakagawa et al.[12] and Umeyama et al.[9] performed the calculations in the process of the enzymatic reaction, and insisted on the significance of the ion-pair His 57^{+}-Asp 102^{-} form in the transition state. In this communication, we reported the significance of the ion-pair His 57^{+}-Asp 102^{-} form in the acid pH range, and our conclusion is in agreement with the NMR experiments which do not support the charge relay mechanism.

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