ROLE OF LOCAL INDUCED-FIT OF SER 195 IN β-TRYPSIN

A molecular orbital study

Setsuko NAKAGAWA and Hideaki UMEYAMA
School of Pharmaceutical Sciences, Kitasato University, Shirokane, Minato-ku, Tokyo 108, Japan

Received 17 December 1981

1. Introduction

The following kinetics of β-trypsin–BPTI complex formation were proposed in [1,2]:

$$I + E \rightleftarrows L \rightleftarrows C \rightleftarrows L^* \rightleftarrows I^* + E$$

where I is the inhibitor, E the native enzyme, L the loose and non-covalent complex, and C the stable complex; * = the modified structure with a susceptible peptide-bond split. Formation of L is very fast, but dissociation rates C → L are extremely slow at neutral pH. Formation of C may involve slight conformational changes, allowing optimal filling of both molecules accompanied by a favorable enthalpy contribution [3]. Upon complex formation of the BPTI–trypsin complex (complex trypsin) corresponding to the above C form, there is little induced-fit movement in which O^{γ} of Ser 195 in the active site of β-trypsin rotates around C^{β}–C^{α} bond ($\chi_1 = -60°C$ to $-83°C$ [3]. The hydrogen bond between Ser 195 O^{γ} and His 57 $N^{\epsilon 2}$ is thus improved considerably in the complex, becoming linear with a length of 2.7 Å, compared to the long and bent bond in free bovine trypsin at pH 8 [3]. Accompanied with the improvement of the hydrogen bond, it is expected that the barrier height of the proton transfer from Ser 195 O^{γ} to His 57 $N^{\epsilon 2}$ is lowered and that the nucleophilicity of Ser 195 O^{γ}, which is approximately perpendicular to the Lys 15(I)–Ala 16(I) susceptible peptide plane above the carbonyl carbon [3], is enhanced.

Abbreviations: BPTI, bovine pancreatic trypsin inhibitor; LCAO–SCF, linear combination of atomic orbitals–self-consistent field; MO, molecular orbital; DIP, diisopropyl-fluorophosphate

Many molecular orbital calculations [4–8] have been done in relation to a 'charge relay mechanism' [9]. From the potential energy change for the 2-proton transfer in the catalytic triad model, Asp 102 was reported not to accept a proton in the enzymatic reaction [10–12]; the 'charge relay mechanism' was thus not supported. An 'electrostatic mechanism' was proposed in the role of Asp 102 [13].

Here, we have studied the role of the induced-fit movement of Ser 195 upon complex formation between bovine trypsin and a substrate model, from quantum chemical calculations using X-ray data of the native trypsin and the complex trypsin [14–16] as a Michaelis-Menten model.

2. Methods

Molecular orbital calculations have been done within the closed-shell LCAO–SCF approximation using the ab initio MO method. The IMSPACK program [17,18], modified for the large number of point charges, was used for the calculations. The basis sets used were 4-31G [19] and STO-3G [20].

The co-ordinates for native β-trypsin and the complex trypsin were taken from [14] and [15], respectively, [14,15], which are available from the protein data bank [16]. Fig.1 shows the model structure of the active site consisting of the catalytic triad and its environment of complex trypsin used for the calculations. In the case of the native trypsin, the calculated model structure is almost similar to that of the complex except for Ser 195 and the substrate model (Sub); Ser 195 O^{γ} forms the bent and long hydrogen bond (3.1 Å) with His 57 $N^{\epsilon 2}$ and there is no Sub part. The positions of hydrogens in fig.1 were deter-

Published by Elsevier Biomedical Press
00145793/82/0000–0000/$02.75

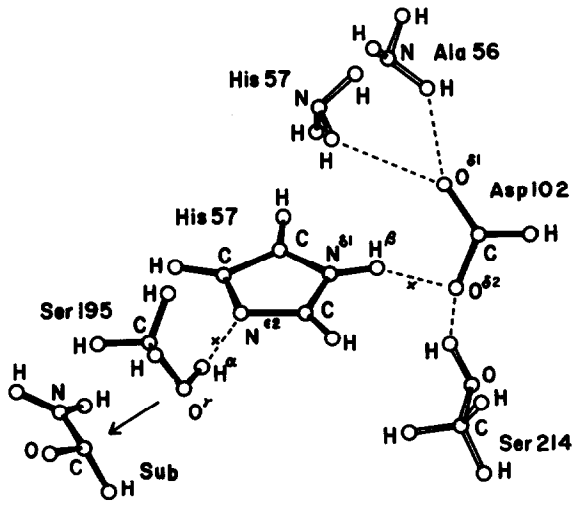

Fig.1. The model structure of active site used in the calculations of the complex trypsin.

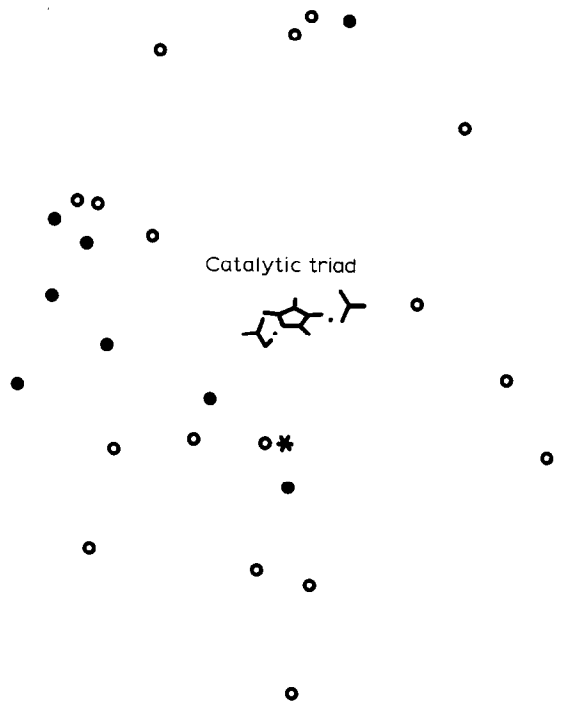

Fig.2. The model structure of the catalytic triad including all ionic amino acid residues and Lys 15(I) side chain of substrate in complex trypsin as integral charges.

mined as in [13]. The proton transfer from Ser 195 to His 57 is called α proton transfer, and that from His 57 to Asp 102 is called β proton transfer. The α proton was moved along a line between $H(O^{\gamma})$ of Ser 195 and $H(N^{\epsilon 2})$ of His 57. During α proton transfer, the conformations of His 57 and Ser 195 were assumed to be unchanged. The β proton was moved along a line between $H(N^{\delta 1})$ of His 57 and $H(O^{\delta 2})$ of Asp 102. Similarly, during β proton transfer, the conformations of Asp 102 and His 57 were assumed to be unchanged. Here, amino acid residues will be symbolized as Asp 102^{-}, His 57^{n} and Ser 195^{n}. '$^{-}$' and '^{n}' indicate the anion and neutral forms, respectively.

There are ionic amino acid residues consisting of 2 Arg, 14 Lys, 4 Asp, 3 Glu, the N-terminus and the C-terminus in β-trypsin in addition to the amino acid residues in fig.1 [14,15]. The former amino acid residues are replaced by integral charges (± 1). In the native trypsin Ca^{2+} was included as an integral charge of +2. Since the coordinate of Ca^{2+} in the BPTI–trypsin complex was not reported in the protein data bank [16], an integral charge of Ca^{2+} was not included in the calculations of the complex. The side chain of Lys 15(I) is inserted into the pocket of the trypsin like the substrate. Lys 15(I) is also replaced by integral charge in the calculations of the complex. In fig.2 anionic (●) and cationic (○) charges of the complex are indicated. (*) in fig.2 indicates the integral charge of the side chain of the substrate model, Lys 15(I). In the native trypsin the integral charge shown by the asterisk was not included because the native trypsin does not interact with the substrate.

The calculated model system (model I and II) are summarized in table 1. Formic acid, imidazole, methanol and formamide as the models of Asp 102,

Table 1
Calculated models[a]

Model		Proton transfer
I[b]	Asp 102^{-}, His 57^{n}, Ser 195^{n}, Ser 214, NH_3 56, NH_3 57, #IAA	α,β
II[c]	Asp 102^{-}, His 57^{n}, Ser 195^{n}, Ser 214, NH_3 56, NH_3 57, #IAA', Sub	α,β

[a] The fragment labeled by # is approximated by integral charges in the calculations; IAA, 25 ionic amino acid residues in addition to Ca^{2+}; IAA', 25 ionic amino acid residues in addition to Lys 15(I); NH_3 56, the peptide moiety of Ala 56; NH_3 57, the peptide moiety of His 57; Sub, the peptide moiety of Lys 15(I)–Ala 16(I)

[b] Native trypsin; [c] complex trypsin with the substrate model

His 57, Ser 195 and the substrate, respectively, were calculated by using the 4-31G basis set. Methanol, ammonia and ammonia as the models of Ser 214, Ala 56 (peptide moiety) and His 57 (peptide moiety), respectively, were calculated by using the STO-3G basis set. Ionic amino acid residues (25) and Ca^{2+} in the native trypsin were included in the SCF calculations as the integral charges (IAA). Ionic amino acid residues (25) and the side chain of Lys 15(I) in the complex trypsin were included in the SCF calculations as the integral charges (IAA′). The amino acid residues and Ca^{2+} approximated by the integral charge are labeled as '#' in table 1.

3. Results

The barrier height of the proton transfer from Ser 195 O^{γ} to His 57 $N^{\epsilon 2}$ is connected with the nucleophilicity of Ser 195 O^{γ}, since α proton transfer is coupled with the nucleophilic reaction of Ser 195. The curves of the potential energy change for α proton transfer in models I and II are shown in fig.3. The potential curve in model I was found to be a double well, and the barrier height was 67 kcal/mol at the proton-transfer distance of 0.97 Å. The barrier height in model II was 23 kcal/mol. By comparison between models I and II, the barrier height in model I is greatly higher than that in model II (higher by 44 kcal/mol). This result shows that α proton transfer is more favorable in the complex trypsin than in the native trypsin.

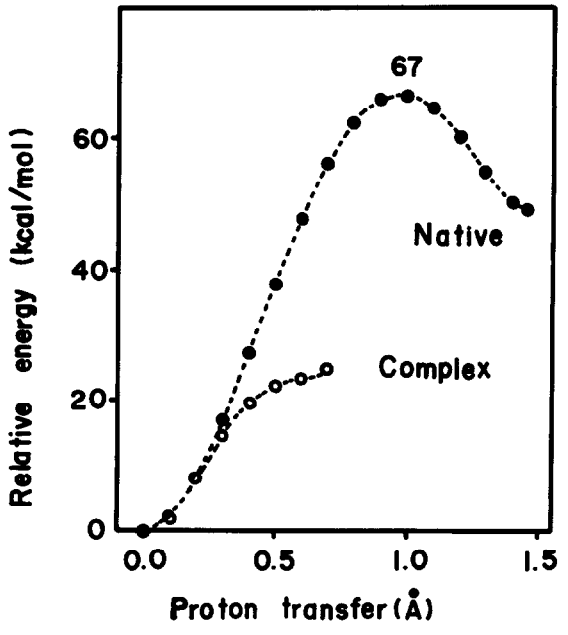

Fig.3. Potential energy curves for α proton transfer in native trypsin (•———•) and complex trypsin (o———o).

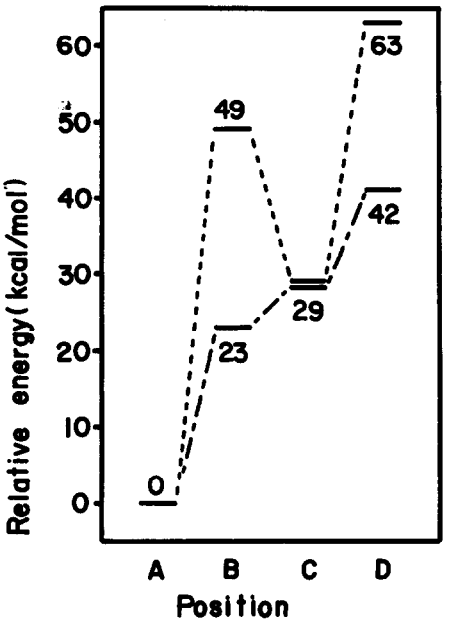

Fig.4. Energy levels in kcal/mol at the B, C and D positions relative to that at the A position: (———) native trypsin (model I); (– – – -) complex trypsin (model II).

The potential energy changes for α and β proton transfers in models I and II are shown in fig.4. The 4 states of A, B, C and D involving 2 proton transfers are defined as follows: (A) both α and β protons do not transfer; (B) only the α proton is at the transfer distance of 1.46 Å in model I and at the transfer distance of 0.60 Å in model II; (C) only the β proton is at the transfer distance of 0.68 Å in model I and at the transfer distance of 0.90 Å in model II; (D) both α and β protons are at the transfer distances of 1.46 and 0.68 Å, respectively, in model I and at the transfer distances of 0.60 and 0.90 Å, respectively, in model II. In the native trypsin the energies of states B–D in model I relative to the initial states were calculated to be 49 kcal/mol, 29 kcal/mol and 63 kcal/mol, respectively (fig.4). In the complex trypsin, the relative energies of states B–D in model II were calculated to be 23 kcal/mol, 29 kcal/mol and 42 kcal/mol, respectively. Position A was found

to be minimum energy. The form Asp 102$^-$–His 57^n–Ser 195^n at the A position is thought to be the initial-state structure of the catalytic site in both native trypsin and complex trypsin.

Since the α proton must transfer from Ser 195 to His 57 during the catalysis, the comparison between the relative energies of B and D is significant. In both native trypsin and complex trypsin, the potential energy change from A to B is significantly lower than that from A to D. Therefore, the attack of Ser 195 O^γ to the carbonyl carbon of the substrate is accompanied with only α proton transfer. The potential energy change from A to B is greatly lower in the complex trypsin than in the native trypsin (lower by 26 kcal/mol). This result shows that the formation of the Asp 102$^-$–His 57$^+$–Ser 195$^-$ state in the enzymatic reaction is easier in the complex trypsin than in the native trypsin.

4. Discussion

Our calculated results show that the barrier height of α proton transfer, which is connected with the nucleophilicity of Ser 195, is greatly affected by the induced-fit movement of Ser 195 upon complex formation between trypsin and the substrate. That is, the improvement of the hydrogen-bond distance from 3.1–2.7 Å in the trypsin complex with the substrate lowers the barrier height of α proton transfer, and stabilizes the Asp 102$^-$–His 57$^+$–Ser 195$^-$ in the reaction step. We show that, from the proton transfer energetics, no significant hydrogen bond exists between Ser 195 O^γ and His 57 $N^{\epsilon 2}$ in the native trypsin, and that the nucleophilicity of Ser 195 O^γ is enhanced by the induced-fit movement of Ser 195 due to the decrease of the barrier height of α proton transfer.

In [21] the position of the proton between Asp 102 and His 57 was determined in DIP–trypsin from a neutron diffraction study in agreement with the predicted position from the ab initio MO study [13]. In the native trypsin, the total energy at the A position was most stable. This result shows that the Asp 102$^-$–His 57^n–Ser 195^n form exists in the native trypsin. The position of the α proton covalently bonded to Ser 195 O^γ will be determined by a neutron diffraction study on native trypsin.

Acknowledgements

The authors are grateful to Professor I. Moriguchi of this university for his support. Numerical calculations were done with a HITAC M-200H computer at the Computer Center of Institute for Molecular Science.

References

[1] Laskowski, M. jr and Sealock, R. W. (1971) The Enzyme 3, 375–473.
[2] Luthy, J., Praissman, M., Finkenstadt, W. R. and Laskowski, M. jr (1973) J. Biol. Chem. 248, 1760–1771.
[3] Huber, R. and Bode, W. (1978) Acc. Chem. Res. 11, 114–122.
[4] Umeyama, H., Imamura, A., Nagata, C. and Hanano, M. (1973) J. Theor. Biol., 41, 485–502.
[5] Amidon, G. L. (1974) J. Theor. Biol., 46, 101–109.
[6] Scheiner, S., Kleier, D. A. and Lipscomb, W. N. (1975) Proc. Natl. Acad. Sci. USA 72, 2606–2610.
[7] Scheiner, S. and Lipscomb, W. N. (1976) Proc. Natl. Acad. Sci. USA 73, 432–436.
[8] Beppu, Y. and Yomosa, S. (1977) J. Phys. Soc. Jap. 42, 1694–1700.
[9] Blow, D. M., Birktoft, J. J. and Hartley, B. S. (1969) Nature 221, 337–340.
[10] Nakagawa, S., Umeyama, H. and Kudo, T. (1980) Chem. Pharm. Bull. 28, 1342–1344.
[11] Umeyama, H. and Nakagawa, S. (1980) Chem. Pharm. Bull. 28, 2292–2300.
[12] Kollman, P. A. and Hayes, D. M. (1981) J. Am. Chem. Soc. 103, 2955–2961.
[13] Umeyama, H., Nakagawa, S. and Kudo, T. (1981) J. Mol. Biol. 150, 409–421.
[14] Bode, W. and Schwanger, P. (1975) J. Mol. Biol. 98, 693–717.
[15] Huber, R., Kukla, D., Bode, W., Schwanger, P., Bartels, K., Deisenhofer, J. and Steigemann, W. (1974) J. Mol. Biol. 89, 73–101.
[16] Bernstein, F. C., Koetzle, T. F., Williams, G. J. B., Meyer, E. F. jr, Brice, M. D., Rodgers, J. R., Kennard, O., Shimanouchi, T. and Tasumi, M. (1977) J. Mol. Biol. 112, 535–542.
[17] Morokuma, K., Kato, S., Kitaura, K., Ohmine, I. and Sakai, S. (1979) 'An ab initio program package consisting of Gaussian 70 and many other additional routines', unpublished.
[18] Hehre, W. J., Lathan, W. A., Ditchfield, R., Newton, M. D. and Pople, J. A. (1974) Gaussian 70, Quantum Chemistry Program Exchange, Indiana University.
[19] Ditchfield, R., Hehre, W. J. and Pople, J. A. (1971) J. Chem. Phys. 54, 724–728.
[20] Hehre, W. J., Stewart, R. F. and Pople, J. A. (1969) J. Chem. Phys. 51, 2657–2664.
[21] Kossiakoff, A. A. and Spencer, S. A. (1980) Nature 288, 414–416.