Chem. Pharm. Bull. 28(4)1342—1344(1980)

The Molecular Orbital Study on the Role of Hydrogen Bonding System in the Active Site of Serine Proteases

The active sites of serine proteases include the hydrogen bonds structure composed of serine, histidine, and aspartate. The two reaction mechanisms for the hydrogen bonds structure have been proposed. One is the charge relay system proposed by Blow *et al.* The other mechanism is the hydrogen bonding system proposed by Wang, and Polgar and Bender. In this system the form of Ser(anion)-His(cation)-Asp(anion) is stabilized with the proton transfer from Ser to His. In order to decide between two mechanisms, LCAO MO SCF *ab initio* calculations by using a 4-31G basis set were carried out. In the active site of the trypsin with pancreatic trypsin inhibitor (PTI), the potential energy surface of the two proton transfers from Ser to His and from His to Asp is described. The form of Ser(neutral)-His(neutral)-Asp(anion) is most stable. The form of Ser(anion)-His(cation)-Asp(anion) is much more stable than that of Ser(anion)-His(neutral)-Asp-(neutral). Accordingly, the hydrogen bonding system is supported as the catalytic mechanism of serine proteases.

Keywords——*ab initio*, molecular orbital; trypsin; serine proteases; molecular structure; charge relay structure; enzymatic reaction; mechanism; enzyme

The reaction mechanism of serine proteases has been studied by many researchers in connection with a hydrogen-bonds structure composed of hydroxyl, imidazole and carboxyl groups.[1–5] The two reaction mechanisms for the hydrogen-bonds structure have been proposed. One is the charge relay system, in which the proton transfer from His to Asp plays a significant role in lowering the barrier of the proton transfer from Ser to His.[1] The other mechanism is the hydrogen bonding system proposed by Wang[6] and Polgar and Bender;[7] the form of Ser(anion)-His (cation)-Asp(anion) is stabilized with the proton transfer from Ser to His, and only one proton transfer occurs in the enzymatic reaction. In this paper, it is shown that the latter system is preferable to the former one from a quantum chemical point of view.

Ab initio MO calculations have been carried out for the charge relay system by Sheiner *et al.*[8] However, in their calculations, the potential curves of two proton transfers from His to Asp and from Ser to His were not described. The energy surface of the proton transfers will help to decide between two mechanisms mentioned above. Since the results using a double zeta basis set were in good agreement with the experiments,[8] a 4-31G basis set was used in this paper.[9] All the calculations were performed within the framework of the closed shell single determinant LCAO-SCF-MO theory. The GAUSSIAN 70 program was used as a nonempirical method.[10] Calculations were carried out using M-180 computer at the Institute for Molecular Science.

1) D.M. Blow, J.J. Birktoft, and B.S. Hartley, *Nature* (London), **221**, 337 (1969).
2) W. Bode and P. Schwager, *J. Mol. Biol.*, **98**, 693 (1975).
3) L. Sawyer, D.M. Shotton, and H.C. Watson, *Biochem. Biophys. Res. Commun.*, **53**, 944 (1973).
4) R.A. Alden, J.J. Birktoft, J. Kraut, J.D. Robertus, and C.S. Wright, *Biochem. Biophys. Res. Commun.*, **45**, 337 (1971).
5) R. Henderson, *J. Mol. Biol.*, **54**, 341 (1970).
6) J.H. Wang, *Science*, **161**, 328 (1968).
7) L. Polgar and M.L. Bender, *Proc. Natl. Acad. Sci. U.S.A.*, **64**, 1335 (1969).
8) *a*) S. Scheiner, D.A. Kleier, and W.N. Lipscomb, *Proc. Natl. Acad. Sci. U.S.A.*, **72**, 2606 (1975); *b*) S. Scheiner and W.N. Lipscomb, *ibid.*, **73**, 432 (1976).
9) R. Ditchfield, W.J. Hehre, and J.A. Pople, *J. Chem. Phys.*, **54**, 724 (1971).
10) W.J. Hehre, W.A. Lathan, R. Ditchfield, M.D. Newton, and J.A. Pople, GAUSSIAN 70, Program 236, Quantum Chemistry Program Exchange, Indiana University, 1973.

Huber's x-ray structure of the complex of bovine trypsin with pancreatic trypsin inhibitor-(PTI)[11] was used for the calculations. Ser 195, His 57 and Asp 102 of the active site residues were modeled by methanol, imidazole and formic acid, respectively.[12] The peptide chain between Lys 15(I) and Ala 16(I) of the inhibitor was modeled by formamide.[8] The coordinates of heavy atoms were obtained from the work of Huber.[11] The coordinates of hydrogens were determined using the optimized geometries of methanol,[13] imidazole,[14] formic acid[14] and formamide.[15] Hydrogens in the hydrogen bonds were made orient to preferable direction from the results of calculations. $\angle HO^{\gamma} C^{\beta}$ in Ser 195 is 110.42°. $\angle HN^{\varepsilon 2} C^{\varepsilon 1}$ and $\angle HN^{\delta 1} C^{\varepsilon 1}$ in His 57 are 119.68° and 120.69°, respectively. $\angle HO^{\delta 2} C^{\gamma}$ in Asp 102 is 111.66°. A proton, α, transfers from the position covalently-bonded to O^{γ} of Ser 195 to that covalently-bonded to $N^{\varepsilon 2}$ of His 57 by 0.771 Å. A proton, β, transfers from the position covalently-bonded to $N^{\delta 1}$ of His 57 to that covalently-bonded to $O^{\delta 2}$ of Asp 102 by 0.945 Å.

Figure 1 shows the potential energy surface for the transfers of α and β in the hydrogen-bonds structure without inhibitor part (formamide). The most stable state is near the original position in the map. On the other hand, the state where the transfers of both α and β have finished is most unstable. The state after the transfer of α or β is less stable than the original position.

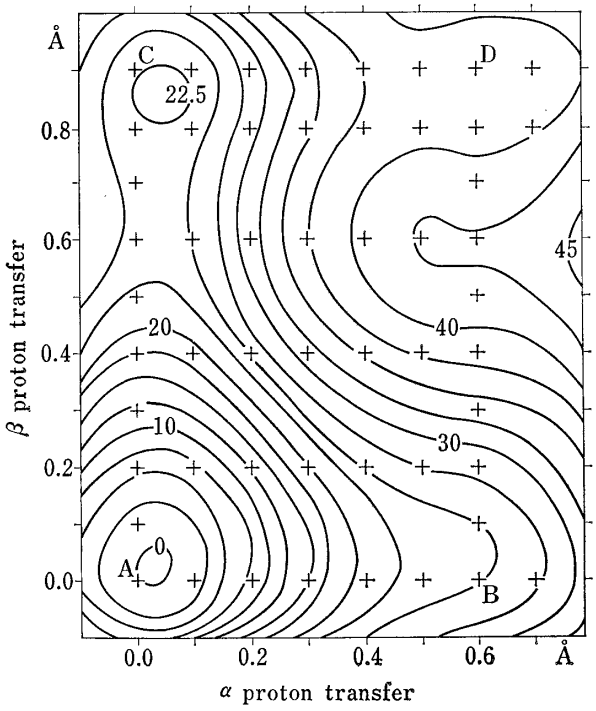

Fig. 1. The Potential Energy Surface in kcal/mol for the Transfers of α and β Protons in the Hydrogen-bonds Structure

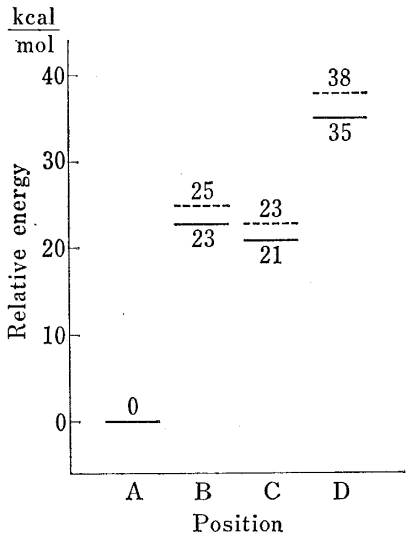

Fig. 2. The Energies in kcal/mol of the Hydrogen Bonds Structure (·····) and the Complex Structure (——) at the Positions of B, C, and D Relative to the Energy at A

11) *a*) R. Huber, D. Kukla, W. Bode, P. Schwager, K. Bartels, J. Deisenhofer, and W. Steigemann, *J. Mol. Biol.*, **89**, 73 (1974); *b*) F.C. Bernstein, T.F. Koetzle, G.J.B. Williams, E.F. Meyer, Jr., M.D. Brice, J.R. Rodgers, O. Kennard, T. Shimanouchi, and M. Tasumi, *J. Mol. Biol.*, **112**, 535 (1977).
12) H. Umeyama, A. Imamura, C. Nagata, and M. Hanano, *J. Theor. Biol.*, **41**, 485 (1973).
13) H. Umeyama and S. Nakagawa, *Chem. Pharm. Bull.*, **25**, 1671 (1977).
14) S. Nakagawa, H. Umeyama, K. Kitaura, and K. Morokuma, 3rd International Congress of Quantum Chemistry in Japan, 2-p-9 (1979).
15) Y. Sugawara, Y. Hamada, A.Y. Hirakawa, M. Tsuboi, S. Kato, and K. Morokuma, 3rd International Congress of Quantum Chemistry in Japan, 31-s-18 (1979).

Next we calculated the hydrogen-bonds structure with including formamide. The four positions (A, B, C, and D) which are near the local minima are selected from the map in Fig. 1. "A" is the original position. "B" is the position (0.6, 0.0) where the places of α and β are 0.6 and 0.0 Å, respectively, from the A position. "C" is (0.0, 0.9). "D" is (0.6, 0.9). The structure at A corresponds to the form of Ser(neutral)-His(neutral)-Asp(anion); the structure which is most stable. The form at B is Ser(anion)-His(cation)-Asp(anion); the structure in which only α transfers. That at C is Ser(neutral)-His(anion)-Asp(neutral); the structure in which only β transfers. That at D is Ser(anion)-His(neutral)-Asp(neutral); the structure in which both α and β transfer. The various interaction energies in the complex structure between the hydrogen-bonds structure and formamide are −3.4, −5.1, −4.8 and −6.6 kcal/mol at the positions at A, B, C and D, respectively. Figure 2 shows the energies at B, C and D relative to the total energy at A with or without formamide. Each energy level at B, C and D with formamide is smaller than that in the case without formamide. However, even though the inhibitor molecule is included in the calculations, the conclusion does not change.

If the α transfer from Ser to His in the enzymatic reaction occurs according to the charge relay system,[1] the form at D should be more stable than that at B; in this mechanism, the β proton must transfer simultaneously or step by step. In the hydrogen bonding system, on the other hand, the form at B should be more stable than that at D. In this mechanism, the β proton does not transfer from His to Asp. Fig. 2 shows that the form at B is more stable than that at D. Accordingly, our results support the hydrogen bonding system[6,7] in which only α transfer occurs in the hydrogen-bonds structure during a general base catalysis. Moreover the calculations including three amino acid residues of Ser 214, His 57 and Ala 56, which form three hydrogen bonds with Asp 102, in addition to three amino acid residues of Ser 195, His 57 and Asp 102 were performed. From the results, the conclusion mentioned above does not change. Even though the calculations including the substrate are performed, the conclusion will not change. Because the interaction energy between the substrate and the three amino acids in the hydrogen bonding system seems very similar to that in the charge relay system. Since the proton affinity is significant in order to decide between two mechanisms, the small rotation of the imidazole plane around the C^{β}–C^{γ} bond of His 57 will not change the conclusion.

Acknowledgement The authors are very grateful to Professor I. Moriguchi of this university for his support for this research.

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

Setsuko Nakagawa
Hideaki Umeyama[16]
Takako Kudo

Received February 9, 1980

16) The author to whom correspondence is to be addressed.