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Comment on "Cleavage mechanism of the H5N1 hemagglutinin by trypsin and furin" [Amino Acids 2008, January 31, Doi: 10.1007/s00726-007-0611-3]

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Summary. Recently, Guo et al. have reported structural as well as the binding energy data of the particular interactions between the cleavage sites of hemagglutinin and serine proteases, trypsin and furin, using molecular docking approach. Due to a wrong assignment of protonation state on the histidine, one of the catalytic triad in the active site of both enzymes, their docking results are contradictory with the fundamental principle and previous theoretical studies of the known cleavage mechanism in serine proteases.

Keywords: Catalytic triad – Cleavage mechanism – Serine proteases – Trypsin – Furin

A recent paper in this journal uses molecular docking tools to study the specific recognition between the cleavage sites of hemagglutinin (HA) and two serine proteases, trypsin and furin (Guo et al., 2008). Based on the docking results, the authors report that "*...trypsin was able to recognize and cleave both the high pathogenic and low pathogenic hemagglutinin, while furin could only cleave the high pathogenic hemagglutinin...*". It seems that the results support this idea. However, there are critical issues concerning on the working model and structural inconsistency of the enzyme-substrate complexes.

The catalytic histidine at the active site of both proteases in the paper was not the correct model to represent the proton acceptor in proton transfer reaction. These are the charged histidine (His57) in trypsin and the neutral histidine with a hydrogen at the epsilon position (His194) in furin (Figs. 2b and 4a of the paper). In general, the catalytic histidine exists in the neutral form in which δ-nitrogen (Nδ) and ε-nitrogen (Nε) are protonated and non-protonated, respectively (Fersht, 1985; Perona and Craik, 1995; Hedstrom, 2002; Topf et al., 2002; Ishida and Kato, 2003). With this state, Nε on imidazole ring is then feasible to accept a proton from the hydroxyl group of serine to generate a nucleophile which directly attacks on the carbonyl carbon of the scissile peptide. The use of incorrect histidine type in the paper is a direct source of discrepancy in the following results: (i) This histidine can not play its important role as proton acceptor in proton transfer to initiate the nucleophile addition with a formation of tetrahedral intermediate. (ii) Differences of the atomic charge and molecular geometry due to the use of non-appropriate protonation state lead to an inaccurate electrostatic energy and, hence, the binding energy of the investigated systems. (iii) In the furin-HA complex, the docking geometry at the P1-residue of the high pathogenic hemagglutinin lies in a wrong conformation within the S1 binding pocket (Fig. 4a of the paper). The P1-carbonyl oxygen forms two hydrogen bonds with HNε of His194's imidazole ring and HO of Ser368's side chain. This is in contrast to the fact that this oxygen is stabilized by the oxyanion hole of furin which is structurally constructed by the carboxamide nitrogen of Asn295 and the backbone nitrogen of Ser368 (Henrish et al., 2003). Surprisingly, inconsistent with what appears in Fig. 4a, this fact was stated correctly in the text "*...The carbonyl oxygen also inserts into an oxyanion hole formed by the carboxamide nitrogens of Asn295 and Ser368...*".

In summary, the histidine model at the active site used in the paper does not correspond to the fundamental basis of known catalytic histidine involved in enzyme mechanism of serine proteases. The docking results presented by Guo et al. cannot be used for explanation on the specific recognition between the cleavage site of low/high pathogenic hemagglutinin and serine proteases, trypsin and furin. Therefore, the protonation states of amino acids with electrically charged side chains should be carefully

assigned, particularly in computational study of biological systems.

References

Fersht AR (1985) Enzyme structure and mechanism, 2nd edn. W. H. Freeman & Co

Guo XL, Li L, Wei DQ, Zhu YS, Chou KC (2008) Cleavage mechanism of the H5N1 hemagglutinin by trypsin and furin. Amino Acids 35: 375–382

Hedstrom L (2002) Serine protease mechanism and specificity. Chem Rev 102: 4501–4524

Henrich S, Cameron A, Bourenkov GP, Kiefersauer R, Huber R, Lindberg I, Bode W, Than ME (2003) The crystal structure of the proprotein processing proteinase furin explains its stringent specificity. Nature Struct Biol 10: 520–526

Ishida T, Kato S (2003) Theoretical perspectives on the reaction mechanism of serine proteases: the reaction free energy profiles of the acylation process. J Am Chem Soc 125: 12035–12048

Perona JJ, Craik CS (1995) Structural basis of substrate specificity in the serine proteases. Protein Sci 4: 337–360

Topf M, Várnai PV, Richards WG (2002) Ab initio QM/MM dynamics simulation of the tetrahedral intermediate of serine proteases: insights into the active site hydrogen-bonding network. J Am Chem Soc 124: 14780–14788

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