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Ser-His dipeptide : a potential candidate of the prototype for serine protease

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Introduction: Ser-His is a magical dipeptide which was found to be the smallest functional dipeptide with protease activity[1-3]. In order to explore its protein cleavage spectrum, four differently folded proteins, namely GFP, CyPA, BSA and myoglobin, were treated with Ser-His recently. The resulting digestion products were evaluated with high-resolution mass spectrometry.The cleavage efficiency and cleavage propensity of Ser-His against these protein substrates were calculated at both the primary and secondary sequence levels. The above experiments show that Ser-His cleaves a broad spectrum of substrate proteins of varying secondary structures. Moreover, Ser-His could cleave at all 20 amino acids with different efficiencies according to the substrateprotein, which means that Ser-His has the original digestion function of serine proteases.

We also collected and compared the catalytic sites and cleavage sites of 340 extant serine proteases derived from 17 representative organisms. A consensus motif Ser-[X]-His was identified as the major pattern at the catalytic sites of serine proteases from all of the organismsrepresented except *D. rerio*, which uses Ser-Lys instead. This finding indicates that Ser-His is the core component of the serine protease catalytic site. Moreover, our analysis revealed that the cleavage sites of modern serine proteases have become more specific over the evolutionary history of this family. Excitingly, Prof. Szostak and his co-workers used Ser-His to catalyze the formation of peptide bonds[4]. Thus, Ser-His exhibits microscopic reversibility, similar to modern serine proteases[5].

Another question should be answered is if Ser-His is easy to obtained in prebiotic conditions. At present in our lab, we found that Ser with the activation of phosphorus at N-terminal is more liable to form amide bond with histidine than other ancient amino acids[6], such as Ser, Ala, Pro, Asp. To some extent, it implied Ser and His are perfect couple to form dipeptide among other amino acids.

In summary, to some extent, all above findings indicates that Ser-His is a potentialcandidate of the prototype for serine protease.

References:

[1]Chen J, Wan R, Liu H, et al. (2000). Cleavage of BSA by a dipeptide seryl-histidine. *Letters in Peptide Science* 7(6): 325-329.[2] Chen J, Wan R, Liu H, et al. (2001). Studies on the cleavage of bovine serum albumin by Ser-His. *Chemical Journal of Chinese Universities-Chinese* 22(8): 1349-1351.[3] Du HL, Wang YT, Yang LF, et al. (2002). Appraisal of green fluorescent protein as a model substrate for seryl-histidine dipeptide cleaving agent. Letters in Peptide Science 9(1): 5-10. [4]Gorlero M, Wieczorek R, Adamala K, et al. (2009). Ser-His catalyses the formation of peptides and PNAs. *Febs Letters* 583(1): 153-156. [5]Adamala K and Szostak JW (2013). Competition between model protocells driven by an encapsulated catalyst. *Nature Chemistry* 5(7): 495-501. [6] Trifonov EN, (2000). Consensus temporal order of amino acids and evolution of the triplet code. *Gene*.261: 139-151.

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