Structural Determinants of Furin Inhibitors Derived from Influenza Hemagglutinin

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Introduction

Furin belongs to a small family of the proprotein convertases (PCs) and is the best-characterized member with a ubiquitous tissue distribution. Furin is responsible for posttranslational transformation and activation of proproteins into biologically active proteins and regulation of many physiological processes in living organisms [1]. In addition to their normal physiological role, furin and related convertases contribute to the maturation of many diseases-related proteins and are involved in tumorigenesis, neurodegenerative disorders, diabetes and atherosclerosis [2]. Moreover, furin is also required for the activation of many bacterial and viral toxin precursors (including *Pseudomonas aeruginosa*, pathogenic Ebola strains, Marburg, HIV gp160, the avian influenza virus hemagglutinin) [3]. Studies have shown that the hemagglutinin of avian influenza virus A contains a TPRERRRKKRTGL sequence, which is a furin cleavage site. The optimization of TPRERRRKKRTGL peptide led to the discovery of a potent inhibitor with the following sequence: Ac-RARRRKKRT-NH₂ [4,5]. In the present work, the structural determinants for furin inhibition were investigated using positional-scanning approach. We synthesized peptide libraries substituted by each natural amino acid residue (with the exception of the Cys) in the P5-P8 positions, while maintaining the furin recognition motif (the P4-P1 positions) and we determined inhibitor activity of the resulting peptides towards recombinant furin.

Results and Discussion

The peptides were prepared by solid-phase synthesis and inhibition constants (K_i) were determined *via* competitive kinetic assays using recombinant human furin. The results of kinetic studies (Figure 1) contain four series of analogs, which were modified at the P5 (A), P6 (B), P7 (C) and P8 (D) position. The most potent peptides are indicated in dark blue and our initial peptide (control) is indicated in green. The most potent inhibitors derived from this study include analogs modified at the P5 position with small hydrophobic residues. Moreover, almost all of the peptides substituted at the P8 position possessed improved inhibitory activity towards furin with the exception of compound containing Trp, Phe and Tyr.

The results of the kinetic studies showed that the incorporation of the small, hydrophobic residues like Leu, Ile and Val at the P5 position led to the most potent furin inhibitors. Therefore, in order to improve the stability of our analogs we decided to substitute this position with the unnatural amino acid residues with the similar character (Figure 2).

Screening the P5 to P8 libraries with recombinant furin revealed that only the modification of the P5 or P8 position might have a beneficial effect on the inhibitory activity of the resulting analogs. We demonstrated that the incorporation of small, hydrophobic residues at the P5 position led to analogs with improved potency towards furin, whereas at the P8 position almost all of the residues used enhanced inhibitory activity, with the exception of the aromatic amino acids residues. Keeping in mind that polybasic peptides like our initial inhibitor suffer from poor metabolic stability, we designed an additional series of compounds having the unnatural substitutions at the P5 position. From this group, the peptide modified with the Abu residue displayed improved inhibitory activity. The next step of this project will be to combine the most efficient modifications at the P5 and P8 position in order to obtain even more potent inhibitor. Then, the plasma stability studies of the leading compounds will be assessed. We believe that our results could be a solid basis for further structure optimization aiming at improving potency and stability of furin inhibitors and might lead to a new drug candidate to treat infectious disease.

Fig. 1. Screening the P5 to P8 libraries with recombinant furin presented in comparison to our initial inhibitor (Ac-RARRRKKRT-NH_2).

Fig. 2. Inhibition of furin by analogs modified at the P5 position by unnatural amino acids residues. The most potent inhibitor was the peptide containing L-2-aminobutyric acid that is marked by dark blue color.

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References

1. Couture, F., et al. *Biomol. Concepts* **2**, 421-438 (2011), <http://dx.doi.org/10.1515/BMC.2011.034>
2. Thomas, G. *Nat. Rev. Mol. Cell Biol.* **3**, 753-766 (2002), <http://dx.doi.org/10.1038/nrm934>
3. Artenstein, A.W., Opal S.M. *N. Eng. J. Med.* **365**, 2507-25183 (2011), <http://dx.doi.org/10.1056/NEJMr1106700>
4. Remacle, A.G., et al. *Int. J. Biochem. Cell Biol.* **42**, 987-995 (2010), <http://dx.doi.org/10.1016/j.biocel.2010.02.013>
5. Gagnon, H., et al. *J. Med. Chem.* **57**, 29-41 (2014), <http://dx.doi.org/10.1021/jm400633d>