Structural basis for FXIIa inhibition by a peptide foldamer containing cyclic β-amino acids

Foldamers are oligomers that form a specific conformation and attract attention as a novel modality for functional molecules [1]. Cyclic amino acids are known to form peptide foldamers by inducing secondary structures such as helices and sheets.

Suga and colleagues have developed a method of obtaining macrocyclic peptides with various unnatural amino acids that strongly bind to target molecules (RaPID system) by combining genetic code reprogramming and in vitro selection methods [2]. Recently, Suga and Kato modified the RaPID system to efficiently incorporate cyclic β-amino acids (cβAAs) such as (1R,2R)-2-aminocyclohexane carboxylic acid (ACHC, Fig. 1(a)) into peptides [3]. Using this modified RaPID system, they discovered macrocyclic peptides with cβAAs that strongly inhibit FXIIa (a serine protease involved in blood coagulation). Here, the crystal structure of FXIIa bound with one such peptide, F3 [3] (Fig. 1(b)). F3 consists of 18 residues and inhibits FXIIa with a Ki value of about 1.5 nM. Note that we have use the term "cyclic" in two different contexts. F3 has a macrocyclic structure due to the formation of a thioether bond while containing ACHC, an unnatural amino acid with a cyclic side-chain structure.

The FXIIa-F3 complex yielded polycrystals consisting of stacked thin plates. Diffraction experiments using ordinary synchrotron radiation beams gave smeared diffraction spots with a resolution of about 7.5 Å, but we could not process the obtained images for analysis. Using a microbeams at SPring-8 **BL32XU**, diffraction images were collected from eight crystals with the helical scheme, then processed with the program KAMO [4] to obtain a final data set at a resolution of about 3.0 Å. This is an excellent example of how advances in beamlines and fully automated measurement methods have made it possible to solve previously difficult structural problems.

In the overall structure of the FXIIa-F3 complex (Fig. 1(c)), F3 forms a β-hairpin structure. ACHC is known to induce turns, and one of the two ACHC residues of F3 (ACHC8) is located at the tip of the β-hairpin, possibly stabilizing the overall β-hairpin structure of F3 by inducing a turn. In addition, the side chain of ACHC8 forms hydrophobic interactions with Tyr515 and His507 of FXIIa. The other ACHC residue (ACHC13) also stabilizes the F3 structure by forming intramolecular hydrogen bonds via its main-chain oxygen and nitrogen and hydrophobic interactions with Ala3 and Tyr16 via its side chain. ACHC13 also induces intermolecular hydrophobic interactions with Trp586 of FXIIa.

The unique chemical structure of ACHC enables the intra- and intermolecular interactions described above. As a cβAA, ACHC has a large hydrophobic side chain capable of forming hydrophobic interactions while limiting the structural flexibility of peptide main-chain atoms. It also contains the main-chain nitrogen that can accept a hydrogen bond. The unique chemical structure of ACHC allows F3 to have a stable folded structure (peptide foldamer) and bind strongly to FXIIa.

The details of the interactions between F3 and the active center of FXIIa are shown in Fig. 2. Arg6 of F3 fits into the S1 site of the substrate recognition pocket of FXIIa and forms an intermolecular salt bridge with Asp557 of FXIIa. The peptide bond between Arg6 and Arg7 of F3 is in the vicinity of Ser563, the catalytic residue of FXIIa, and the N-terminal residue of Arg6 forms an intermolecular sheet with FXIIa. These features show that F3 acts as a "standard mechanism inhibitor" that interacts with FXIIa in a manner similar to its substrates.

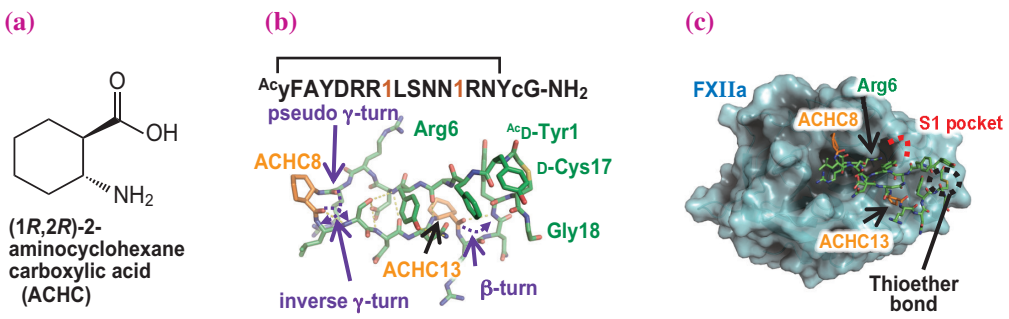

Fig. 1. Structure of F3-FXIIa complex. **(a)** Chemical structure of ACHC. **(b)** Structure of F3. 1, Acy, and c represent ACHC, acetyl-D-tyrosine, and D-cysteine, respectively. **(c)** Crystal structure of FXIIa-F3.

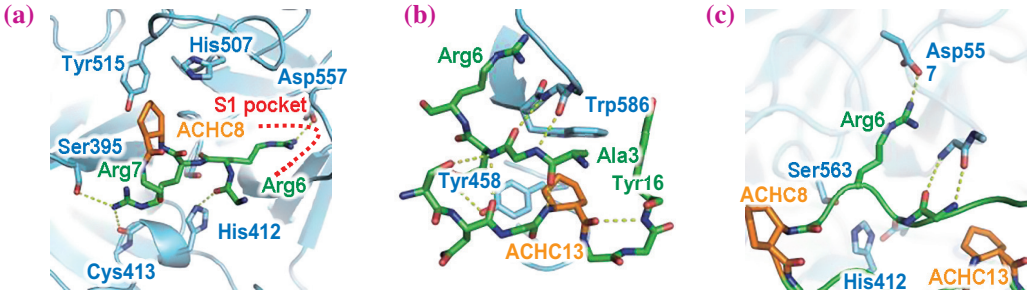

Fig. 2. Details of interaction. **(a)** Interactions near Arg6 and ACHC8. **(b)** Interactions near HCHC13. **(c)** Interactions involving Arg6 and its N-terminal region.

Protease activity in vivo must be tightly controlled, and various proteinaceous serine protease inhibitors exist naturally [5]. When we compared the inhibition mechanisms of F3 with those of the proteinaceous inhibitors, we found interesting similarities and differences (Fig. 3). They share a key basic residue that binds to the S1 site of the protease, and its N-terminal region commonly forms an intermolecular β-sheet with the protease. The interaction area of F3 with FXIIa is 703 Å^2, which is comparable to that of proteinaceous inhibitors (700–900 Å^2). ACHC8 and ACHC13 of F3 contribute to the stabilization of the compact structure, while the proteinaceous inhibitors often form disulfide bonds at the position of ACHC13 to stabilize the conformation of the basic residue. They also contain relatively large scaffold domains with various sizes and folds. The various proteinaceous inhibitors have likely developed a common inhibitory mechanism (binding of basic residues to the S1 site and intermolecular β-sheet formation) by convergent evolution to inhibit the target protease efficiently. On the other hand, F3, obtained by only a few cycles of in vitro selection, uses the same inhibitory mechanism as natural proteinaceous inhibitors, with a much smaller molecular size. The *de novo* discovery of F3 thus demonstrates the potential of the RaPID system adopted for cβAAs to obtain functional peptide foldamers.

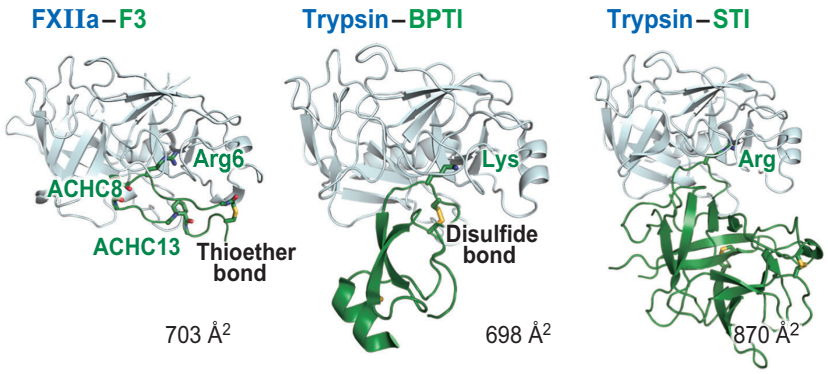

Fig. 3. Comparisons with protein-based protease inhibitors.

Toru Sengoku

Department of Biochemistry, School of Medicine, Yokohama City University

Email: tsengoku@yokohama-cu.ac.jp

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