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Structure of a membrane-bound stomatin-specific protease in complex with a substrate peptide

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1 Introduction

Membrane-bound proteases play several important roles in protein quality control and regulation. The N-terminal region of PH1510p (residues 16-236, 1510-N) from the hyperthermophilic archaeon *Pyrococcus horikoshii* is a thermostable serine protease with a catalytic Ser-Lys dyad (Ser-97 and Lys-138), and specifically cleaves the C-terminal hydrophobic region of the prokaryotic stomatin (p-stomatin), PH1511p [1]. Stomatin is one of the major integral membrane proteins of human erythrocytes, the absence of which is associated with a form of hemolytic anemia known as hereditary stomatocytosis. It is not known how 1510-N recognizes and degrades the p-stomatin PH1511p. Therefore, we determined the crystal structure of the catalytically inactive mutant K138A of 1510-N with the substrate peptide [2].

2 Experiment

The K138A mutant of 1510-N was prepared mostly as described previously [1, 3]. The 1510-N protease specifically cleaves the middle of the C-terminal hydrophobic region of the p-stomatin PH1511p between residues 238 and 239 (^{234}NVIVL↓MLPME^{243}, the arrow indicates the cleaved point). Therefore, 1510-N K138A was mixed with a 10-aa synthetic peptide containing the sequence of PH1511p (^{234}NVIVLMLPME^{243}) at a molar ratio of 1:10. The protein-peptide solution contained 9.1 mg/mL of 1510-N K138A and 4.2 mg/mL of the peptide in a buffer containing 30 mM Tris-HCl (pH8.5), 0.1 M NaCl, and 8.4% (v/v) dimethylsulfoxide. Crystallization drops were prepared by mixing equal volumes of the protein-peptide and reservoir solutions. Crystals were grown at 20 °C with the hanging-drop vapor diffusion method, using a reservoir solution containing 1.0 M imidazole (pH7.5). Cubic crystals grew to an approximate size of 0.20 mm per side.

A crystal was cryoprotected in a solution containing 1.5 M imidazole (pH7.5), 30% (v/v) glycerol, 25 mM Tris-HCl (pH8.5), and 0.15 M NaCl, and flash-frozen at 95 K. X-ray diffraction data were collected at beamline NW12A, and processed and scaled with HKL2000.

The structure was determined at 2.25 Å resolution by the molecular replacement method with the program MOLREP in the CCP4 suite. After rigid-body refinement, the model was subjected to several cycles of crystallographic refinement with REFMAC5, followed by manual model building and fitting with COOT.

3 Results and Discussion

In the structure, a 1510-N dimer binds to one peptide. The catalytic Ser97 Oγ of chain A is hydrogen-bonded to the Asn234 O of the peptide, and another Ser97 Oγ of chain B is hydrogen-bonded to the Leu240 O. As both catalytic Ser97 residues are located around the exit of the active tunnel, the peptide residues bound to the hydrophobic active tunnel are the central 6 residues ^{235}VIVLML^{240}. The pseudo two-fold axis running between chains A and B of 1510-N K138A also runs through the peptide bond between Val237 and Leu238 of the peptide. The central 6 residues ^{235}VIVLML^{240} of the peptide are hydrophobic and in a pseudo-palindromic structure, and therefore favorably fit into the hydrophobic active tunnel of the 1510-N dimer.

By binding the peptide, the flexible L2 loop of one protomer forms β-strands, whereas that of the other protomer remains in a loop form, indicating that one protomer binds to the peptide more tightly than the other protomer. Therefore, the two protomers of 1510-N may have different roles (catalytic and substrate binding).

A comparison with unliganded 1510-N K138A [3] revealed the binding of the substrate to cause a large rotational and translational displacement between protomers, and produce a tunnel suitable for binding the peptide. If the peptide approaches from the open space beneath the peptide-binding site of 1510-N, the conformation of the dimer is changed, and salt bridges of Arg66 and Asp68 of chains A and B are formed, and then the peptide-binding tunnel is produced.

In both protomers, the catalytic Ser97 residues are relatively far away from the other catalytic Ala138 residues that replaced Lys. The Ala138 residues of the two protomers are located very close together (the distance between two Cβ atoms, 3.6 Å). Thus in the wild-type 1510-N, the close positioning of the catalytic Ser97 and Lys138 may be induced by the conformational changes caused by electrostatic repulsion of the two Lys138 side chains of the protomers.

References

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