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Structure of a thermostable membrane-bound stomatin-specific protease

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1 Introduction

Membrane-bound proteases are involved in various regulatory functions. 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 stomatin PH1511p [1]. Stomatin is one of the major integral membrane proteins of human erythrocytes. In a form of human hemolytic anemia known as hereditary stomatocytosis, the stomatin protein is deficient in the erythrocyte membrane due to mis-trafficking. It is not known how 1510-N recognizes and degrades the stomatin PH1511p. We determined the crystal structure of the catalytically inactive mutant K138A of 1510-N in complex with a 10-amino-acid peptide of the stomatin PH1511p [2]. In order to understand the catalytic mechanism of 1510-N in more detail, we determined the crystal structure of the heat-treated protein-peptide complex of 1510-N K138A with a substrate peptide [3].

2 Experiment

1510-N K138A was prepared mostly as described previously [1, 2]. The protein was mixed with a substrate peptide at a molar ratio of 1:10. The protease-peptide complex was heated at 80 °C for 10 min, kept on ice for 10 min, and then crystallization trials were performed. The protein-peptide solution contained 5.2 mg/mL of 1510-N K138A and 2.4 mg/mL of the 234P10 peptide (its sequence is ^{234}NVIVLMLPME^{243}, in which superscripts show residue numbers of stomatin PH1511p) in a buffer containing 40 mM Tris-HCl (pH8.5), 0.15 M NaCl, and 4.8% (v/v) dimethylsulfoxide. Crystallization drops were prepared by mixing equal volumes of the protease-peptide and reservoir solutions. Crystals obtained by heat-treatment were grown at 20 °C with the hanging-drop vapor diffusion method, using a reservoir solution containing 0.8 M imidazole (pH7.5). Cubic crystals grew to an approximate size of 0.15 mm per side.

The crystal was cryoprotected in a solution containing 1.0 M imidazole (pH7.5), 30% (v/v) glycerol, and flash-frozen at 100 K. X-ray diffraction data were collected, processed, and scaled with HKL2000.

The structure was determined by the TLS-restrained crystallographic refinement with REFMAC5 in the CCP4 suite using the structure of 1510-N K138A in complex with the 234P10 peptide (PDB code, 3VIV) as the initial model followed by slight model fitting with COOT.

3 Results and Discussion

In the previously determined structure of 1510-N K138A in complex with the 234P10 peptide, a 1510-N dimer binds to one peptide. In the structure, the catalytic Ser97 Oγ of chain A is hydrogen-bonded to the Asn234 O of the peptide, and the catalytic Ser97 Oγ of chain B is hydrogen-bonded to the Leu240 O. The structure shows the first substrate-binding step of 1510-N. 1510-N degrades the substrate stomatin PH1511p between Leu238 and Met239 at only one point [1]. Thus, Ser97 of chain A should move along the substrate 4-residue C-terminally, or Ser97 of chain B should move along the substrate 2-residue N-terminally.

1510-N is a thermostable protease, and the elevated activity was observed with temperatures from 50 to 98 °C [1]. Thus, we presume that heat-treatment is a good candidate for approaching the second catalytic step of 1510-N. The structure with heat-treatment determined here is fitted to the structure with no heat-treatment determined previously [2], resulting in a low root-mean-square (rms) difference of 0.15 Å for Cα atoms of the 1510-N dimer, and 0.40 Å for all atoms of the peptide. The result indicates that the structure with heat-treatment is almost identical to that with no heat-treatment. The N-terminal half of the peptide (^{234}NVIVL^{238}) shows clear electron densities, whereas the C-terminal half of the peptide (^{239}MLP^{241}) shows weak densities. Almost all the main-chain nitrogen and carboxyl oxygen atoms of ^{234}NVIVL^{238} are hydrogen-bonded to the protease. The main-chain atoms of ^{239}MLP^{241} have fewer hydrogen bonds than ^{234}NVIVL^{238}. According to the superposition between the structures with heat-treatment and no heat-treatment, the ^{234}NVIVL^{238} peptide is superposed well, whereas the ^{239}MLP^{241} peptide is slightly deviated. The distance between both Pro241 Cα atoms of two structures is 0.74 Å. According to the results of hydrogen bonds and superposition, the N-terminal half of the peptide binds to 1510-N more tightly than the C-terminal half of the peptide.

References

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