6A/2007G052

# Structural study of a membrane-bound protease specific for stomatin

Hideshi YOKOYAMA*[1], Shiho HAMAMATSU[1], Satoshi FUJII[1], Ikuo MATSUI[2]
[1]School of Pharmaceutical Sciences, University of Shizuoka, Shizuoka 422-8526, Japan
[2]BIRC, Natl. Inst. Adv. Indust. Sci. & Tech. (AIST), Tsukuba, Ibaraki 305-8566, Japan

## Introduction

Membrane-bound proteases play several important roles in protein quality control and regulation. 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. However, the function of stomatin is not fully understood. The N-terminal region of PH1510 (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), PH1511 [1]. According to the crystal structure of the wild-type 1510-N in dimeric form, the active site around Ser-97 is in hydrophobic environment suitable for the hydrophobic substrates [2].

It is not known how 1510-N recognizes and degrades the p-stomatin PH1511. Therefore we tried to determine the crystal structure of the K138A mutant of 1510-N with the substrate peptide.

## Materials and Methods

For the structure determination of 1510-N in complex with its substrates, crystallization attempts were carried out using the 1510-N K138A mixed with a synthetic peptide containing the cleavable sequence of PH1511 by 1510-N protease. The protein solution contains 5 mg/mL K138A and 5 mg/mL peptide in 50 mM Tris-HCl (pH8.5) containing 0.1 M NaCl, 0.05 % (w/v) *N,N*-dimethyldodecylamine *N*-oxide, and 10 % (v/v) dimethyl sulfoxide. Crystallization trials were carried out with the hanging-drop vapor-diffusion method by mixing equal volumes of the protein and reservoir solutions. Crystals were grown at 20 °C using a reservoir solution containing 18 % (w/v) PEG4000, 0.1 M $MgCl_2$, and 0.1 M sodium acetate (pH4.5). Cubic crystals grew to an approximate size of 0.2 mm on a side.

X-ray diffraction data from a flash-frozen crystal were collected at beamline BL6A up to 2.3 Å resolution. The cryoprotectant solution used was 20 % (w/v) PEG4000, 25 % (w/v) sucrose, 0.1 M $MgCl_2$, and 0.1 M sodium acetate (pH 4.5), with Tris-HCl (pH 7.5) added to a final concentration of 0.1 M. Data were processed and scaled with HKL2000. The molecular replacement and structure refinement were performed with CNS and REFMAC, respectively.

## Results and Discussion

Although diffraction data were collected using the co-crystal of the 1510-N K138A and the substrate peptide, no electron densities corresponding to the peptide were observed around the active sites. The determined structure of the 1510-N K138A mutant [3] contains one molecule per asymmetric unit, but 1510-N is active in dimer. By calculating the buried surface area between one monomer and each symmetry-related molecule, two possible sets of dimer were found. One dimer is almost the same as the wild-type 1510-N (Fig. 1). Another dimer uses the hydrophobic active site to form the dimeric interface, and thus it is probably in an inactive form. The L2 loop, which is disordered in the wild-type structure, is enormously kinked at around A-138 in the K138A mutant. And thus Lys-138 probably has an important role on the conformation of L2.

Further crystallization attempts are now carried out using the complex of 1510-N and its substrate PH1511 for elucidating how 1510-N recognizes its substrate.

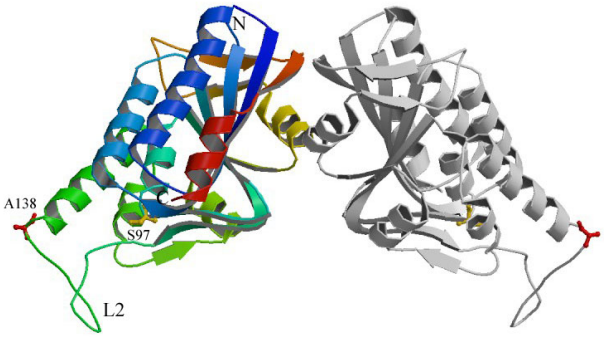


Figure 1. Ribbon representation of 1510-N K138A. A mononer is colored in a rainbow ramp. One symmetry-related molecule is also shown in grey. The catalytic residue Ser-97 is drawn in yellow sticks and Ala-138 is drawn in red sticks. L2 corresponds to the disordered loop in the 1510-N wild-type structure.

## References

[1] H. Yokoyama and I. Matsui, J. Biol. Chem. 280, 6588 (2005).
[2] H. Yokoyama et al., J. Mol. Biol. 358, 1152 (2006).
[3] H. Yokoyama et al., J. Synchrotron Rad. 15, 254 (2008).

* h-yokoya@u-shizuoka-ken.ac.jp