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Crystallographic Study of lipid-raft Protein Stomatin

Hideshi Yokoyama[1,*]
[1]Faculty of Pharmaceutical Sciences, Tokyo University of Science, 2641 Yamazaki, Noda, Chiba, 278-8510, Japan

1 Introduction

Stomatin, prohibitin, flotillin, and HflK/C (SPFH) domain proteins are found in the lipid raft microdomains of various cellular membranes. 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. Stomatin is also widely expressed in various tissues and cell lines, and localized in detergent-resistant membrane domains. It is organized into high order homo-oligomeric complexes of about 300 kDa, comprised of 9- to 12-mers. Stomatin and STOPP (stomatin operon partner protein) genes form an operon in more than 350 archaeal and bacterial genomes, and their protein products may be involved in the quality control of membrane proteins. Two sets of STOPP/stomatin gene pairs, PH1510 (STOPP)/PH1511 (stomatin) and PH0471 (STOPP)/PH0470 (stomatin), have been identified in the hyperthermophilic archaeon *Pyrococcus horikoshii*.

The N-terminal region of PH1510 (residues 16-236, 1510-N) from *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 PH1511 [1]. We previously determined the first crystal structure of the core domain of stomatin PH1511 from *Pyrococcus horikoshii*. In this structure, the SPFH domain was found to form a stable trimer, while three α-helical domains extended from the apexes of the triangle [2]. PH1511 has two membrane-spanning regions at the N-terminus. The structure of full-length stomatin containing membrane-spanning regions and C-terminal hydrophobic region remains to be elucidated. In order to understand the function of oligomeric protein stomatin, we tried to elucidate the structure of full-length stomatin.

2 Experiment

Stomatin PH1511 was mostly prepared as described previously [1, 2]. Briefly, *E. coli* BL21 (DE3) Codon-Plus RIL (Stratagene) cells were transformed with the prepared expression vector. The protein was solubilized in the presence of 1.0% (w/v) dodecyl-β-D-maltoside (DDM, Anatrace), and purified in 0.05% (w/v) DDM and other buffer solution. The resultant protein contains whole residues 1-266 of PH1511, and additionally contains an initial methionine at its N terminus and LEHHHHHH at its C terminus.

Crystallization drops were prepared by mixing equal volumes of the protein and reservoir solutions. The protein solution was approximately 1 to 5 mg/mL stomatin PH1511 in a buffer containing 50 mM Tris-HCl (pH 7.5), 50 mM NaCl, and 0.05% (w/v) DDM. Crystallization was performed at 20 °C by the hanging-drop vapor diffusion method.

3 Results and Discussion

For the wild-type stomatin PH1511, hexagonal plate-shaped crystals were grown to an approximate size of 0.1 mm on a side using the reservoir solution containing 10% (w/v) PEG400, 0.1 M HEPES-NaOH (pH 7.5), and 0.2 M $MgCl_2$. Crystals were transferred into the cryoprotectant solution containing 40% (w/v) PEG400, 0.1 M HEPES-NaOH (pH 7.5), 0.2 M $MgCl_2$, 50 mM NaCl, and 0.05% (w/v) DDM, and then flash-frozen at 95 K. X-ray diffraction images were collected to check the crystal quality. In almost all the diffraction images, concentric ring patterns around 15 Å resolution were detected. They were not suitable for the collection of X-ray data.

The C-terminal hydrophobic region of the human stomatin is reported to be crucial for oligomerization. Based on the sequence alignment, the residues 234-242 of PH1511 corresponds to the region. For the purpose of improving the crystal quality, the stomatin PH1511 mutant M239A was also prepared in the same procedure as described. Crystallization of the mutant M239A was tried at 20 °C by the hanging-drop vapor diffusion method, and then hexagonal plate-shaped or round crystals appeared using the reservoir solution containing 16% (w/v) PEG400, 0.1 M Tris-HCl (pH 7.5), and 0.1 M $MgCl_2$. Under the same protocol as the wild-type crystals, X-ray diffraction images were collected. As a result, concentric ring patterns around 15 Å resolution were observed.

Now, we try to prepare and crystallize the stomatin PH1511 in the buffer containing several types of detergents.

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References

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* yokoyama@rs.tus.ac.jp