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Crystallization and X-ray diffraction study of membrane-bound, oligomeric, and lipid-raft associated protein stomatin

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1 Introduction

Stomatin is one of the major integral membrane proteins of human erythrocytes. In a form of human hemolytic anemia which is known as hereditary stomatocytosis and is characterized by stomatocytic red blood cells with abnormal permeability to Na^+ and K^+, the stomatin protein is deficient in the erythrocyte membrane due to mis-trafficking during erythropoiesis. Stomatin is also widely expressed in various tissues and cell lines, and localized in detergent-resistant membrane domains, which are also termed lipid rafts. Stomatin is organized into high order homo-oligomeric complexes of about 300 kDa, comprising of 9- to 12-mers.

Stomatin, prohibitin, flotillin, and HflK/C (SPFH) domain proteins are found in the lipid raft microdomains of various cellular membranes. Stomatin-like proteins have been found in most species of eukaryotes, bacteria, and archaea. These SPFH domain proteins could function as a membrane-bound oligomeric scaffolding protein in lipid rafts.

We previously determined the first crystal structure of the core domain of stomatin PH1511 from hyperthermophilic archaeon *Pyrococcus horikoshii* [1]. In this structure, the SPFH domain was found to form a stable trimer, while three C-terminal α-helical domains extended from the apexes of the triangle. PH1511 has two membrane-spanning regions at the N-terminus. The crystal structures of the mouse stomatin-domain were shown to assemble into a banana-shaped dimer [2]. As an overall structure of full-length stomatin containing membrane-spanning regions and C-terminal hydrophobic region has not been determined, it remains to be elucidated how stomatin proteins oligomerize for themselves, and how they are associated with the membranes. 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]. The protein was solubilized in the presence of 1.5% (w/v) dodecyl-β-D-maltoside (DDM, Anatrace), and purified on a nickel affinity column and an anion-exchange Q Sepharose column 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.

Firstly, crystallization trials were performed at 20 °C by the hanging-drop vapor diffusion method. 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.

Next, lipidic cubic phase (LCP) methods were tried to crystallize membrane protein stomatin. Monoolein (MO, Nu Chek Prep) and the protein solution were mixed in mass ratio of 3:2 using two syringes connected with a coupler to produce cubic phase. Then, 0.2 μL of the protein-MO mixture was dispensed and 1 μL of a precipitant solution were added to it on a glass sandwich crystallization plate (Hampton Research), and were kept at 20 °C.

3 Results and Discussion

Using the hanging-drop vapor diffusion method, hexagonal plate-shaped crystals appeared, but did not appear reproducibly. On the other hand, using the LCP method, rectangular-shaped crystals were grown to an approximate size of 0.1 mm on a side using the precipitant solution containing 0.1 M sodium chloride, 0.1 M sodium citrate (pH 5.6), and 12% (w/v) 2-methyl-2,4-pentanediol in several days. According to the reference [3] about how to harvest the crystals in LCP, crystals were fished out from the cubic phase using a cryo loop, and then flash-frozen at 95 K. X-ray diffraction images were collected to check the crystal quality. In almost the diffraction images, concentric ring patterns around 20 Å resolution or no patterns were detected.

Now, we try to improve methods to harvest the crystals in LCP, and try to prepare and crystallize the stomatin PH1511 in the buffer containing several types of detergents. We will next try to prepare and crystallize the human stomatin. Given the structure of stomatin is determined, it will provide useful information about how stomatin oligomerizes, how it is associated with membranes.

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References

[1] H. Yokoyama *et al.*, *J. Mol. Biol.* **376**, 868 (2008).
[2] J. Brand *et al.*, *EMBO J.* **31**, 3635 (2012).
[3] D. Li *et al.*, *J. Vis. Exp.* **67**, e4001 (2012).

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