BL-1A, 17A/2014G517, 2012G584

Structure of the stomatin operon partner protein

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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 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 C-terminal region of STOPP PH1510 (residues 371-441, 1510-C) was found to be homologous to the soluble core domain of another STOPP PH0471 (residues 72-143, 471-C). In cross-linking and blue native polyacrylamide gel electrophoresis (BN-PAGE) experiments on 1510-C, the 1510-C domain formed 12- to 24-mer oligomers [1]. However, the multimeric structure of STOPP has not yet been examined. To understand the multimeric properties and possible molecular function of 1510-C in more detail, we herein described the crystal structure of 1510-C, which indicated the formation of a multimeric assembly [2].

2 Experiment

1510-C was mostly prepared as described previously [1, 3]. Crystallization drops were prepared by mixing equal volumes of the protein and reservoir solutions. The protein solution contained 5.7 mg/mL 1510-C in a buffer containing 50 mM Tris-HCl (pH 7.5) and 50 mM NaCl. The reservoir solution was the Crystal Screen II (Hampton Research) #31 condition that contained 20% (v/v) Jeffamine M-600 and 0.1 M HEPES-NaOH (pH 7.5). Crystals were grown at 20 °C by the sitting-drop vapor diffusion method. Rod-shaped crystals grew to an approximate size of 0.20 × 0.02 × 0.02 mm.

A crystal was transferred into a solution of 20% (v/v) glycerol in the reservoir solution, and flash-frozen at 95 K. X-ray diffraction data were collected, and were then integrated and scaled with XDS and SCALA.

The structure was determined by the molecular replacement method with the program PHENIX. The solution structure of 471-C was used as an initial model (PDB code, 2EXD), although the sequence identity between 1510-C and 471-C was relatively low, 33% [1]. The model was subjected to several cycles of refinement with REFMAC5 in the CCP4 suite, followed by manual model fitting with COOT.

3 Results and Discussion

The crystal structure of 1510-C was determined at 2.4 Å resolution [2]. The structure contained one 1510-C molecule in an asymmetric unit. 1510-C was organized into a compact five-stranded β-barrel fold with a 1-2-3-5-4-1 topology, which is known as an oligosaccharide/oligonucleotide-binding fold (OB-fold) domain. Based on the superposition between 1510-C and 471-C, a root-mean-square deviation (r.m.s.d.) was 1.69 Å for 57 Cα atoms. The five β strands were superposed well, whereas the N-terminus and loop region between the two β strands of 1510-C differed from those of 471-C.

The structure contained one 1510-C molecule in an asymmetric unit. However, according to our previous BN-PAGE results, 1510-C (monomeric Mw = 9.2 kDa) showed a broad band of 110-220 kDa, indicating that 1510-C forms 12- to 24-mer oligomers. By calculating the interface areas between 1510-C and its symmetry-related molecules, five pairs of 1510-C and its symmetry-related molecules showing large interface areas were observed, and all these pairs were related by crystallographic two-fold axes. The largest interface area of 1510-C (interface 1) was 698 $Å^2$, which was 16.3% of the total surface area (4,280 $Å^2$). Perfectly conserved residues Gly381, Val421, and Val431 were included in interface 1.

According to crystal packing, 1510-C could assemble into multimers based on the dimer (interface 1) as a basic unit. 1510-C also forms a large cylinder-like structure composed of 24 subunits, or a large triangular prism-like structure composed of 12 subunits. The multimeric assembly formed by the crystal packing appears to conform to the cross-linking and BN-PAGE results. Stomatin has also been shown to form high order homo-oligomeric complexes comprising 9- to 12-mers. 1510-C may function as a scaffold protein to form a multimeric assembly of STOPP and stomatin.

References

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