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Crystal structure of the yeast condensin hinge with long coiled coils

Young-Min Soh[1], Byung-Ha Oh[1]
[1]Department of Biological Sciences, KAIST Institute for the Biocentury, Korea Advanced Institute of Science and Technology, Daejeon 305-701, Korea

1 Introduction

During cell division, the cell is given the daunting task of packaging enormous amount of DNA into a limited amount of space as well as proper segregation of replicated DNA into daughter cells. The condensin complex is essential for both processes. The eukaryotic condensin complex consists of Smc2, Smc4, and three other subunits [2]. The Smc subunits are part of a large family of proteins called the Structural Maintenance of Chromosomes. The N-terminal and C-terminal fold to form an ABC ATPase on one end while the opposite end consists of the hinge domain responsible for hetero-dimerization. The two globular domains are separated by a 50 nm long coiled coil domain [1]. Here we solved the crystal structure of the yeast Smc2 and Smc4 hinge heterodimer with long stretches of coiled coils. Contrary to expectation, the crystal structure revealed that the coiled coils are juxtaposed onto each other forming a closed structure rather than open structure. This was also observed in solution through SAXS analysis.

2 Experiment

The DNA fragments encoding Smc2 residues 396-792 (Smc2H-LCC) and Smc4 residues 555-951 (Smc4H-LCC) were inserted into pProExHTa (Invitrogen) and pET30a (Novagen) by standard PCR-based cloning methods. SelMet-substituted Smc4H-LCC was expressed in *E.coli* B834(DE3) RIL (Novagen), and native Smc2H-LCC was expressed in *E. coli* BL21(DE3) RIPL strain. The cells obtained from the two different cultures were co-sonicated and purified using affinity chromatography, ion exchange chromatography, and size exclusion chromatography. The final buffer contained 100 mM NaCl, 20 mM Tris-HCl pH 7.5, and 2 mM DTT. The Smc2H-LCC–Smc4H-LCC complex (20 mg/ml) was crystallized using the hanging-drop vapor diffusion technique at 20 °C in a precipitant solution containing 16% PEG 300, 0.1 M Na/K phosphate pH 6.0, 8% glycerol and 10 mM dithiothreitol (DTT). X-ray diffraction data were collected at the beamline BL17A at the Photon Factory, Japan. A single-wavelength anomalous dispersion (SAD) data set was collected with a SelMet-substituted Smc2H-LCC–Smc4H-LCC crystal at the Se absorption peak. Diffraction data were processed with HKL2000. The Smc2H-LCC–Smc4H-LCC structure was solved by the single isormorphous replacement with anomalous scattering (SIR-AS) method. The model building and structure refinement were carried out using the programs *COOT* and *CNS*. The final model of Smc2H-LCC–Smc4H-LCC does not include residues 671-673 of Smc2 and 836-841 of Smc4, which are disordered in the crystals. The structure factor and coordinates of the structure are deposited in the Protein Data Bank (www.rcsb.org, PDB ID:4RSI).

3 Results and Discussion

The overall structure of yeast Smc2/4 hinge heterodimer is depicted in Figure 1. It contains about 50 residues of coiled coil stretches for the Smc2 hinge and more than 100 residues for the Smc4 hinge.

Similar to previous Smc hinge domain structures, the yeast Smc2/4 hinge forms a toroid structure which contains a central hole. However, because the coiled coils are oriented differently in Smc2 and Smc4, upon heterodimerization, the coiled coils form closed structures unlike the open structures depicted in previous bacterial Smc hinge structures.

In order to know whether or not the coiled coils form closed structures in solution, the same construct was analyzed using SAXS. The distance distribution function *P*(r) indicated a maximum dimension of 200 Å, which is close to the measured length (190 Å) of the complex in the crystal structure. The molecular envelope derived from SAXS analysis fits well with the crystal structure (Fig. 2). Hence, the coiled coils of yeast Smc2/4 hinge form closed structures also in solution.

In summary, these results provide new insight into the conformation of the coiled coils of Smc proteins.

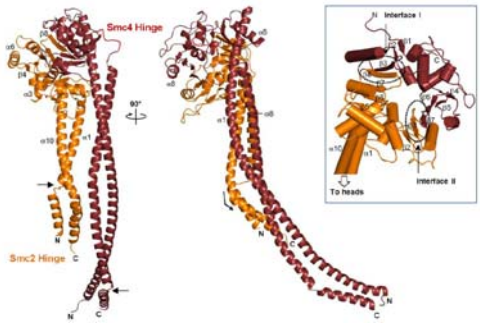

Fig. 1: Overall structure of yeast Smc2/4 hinge with long coiled coil stretches. In depth view of dimerization interface is detailed in the box.

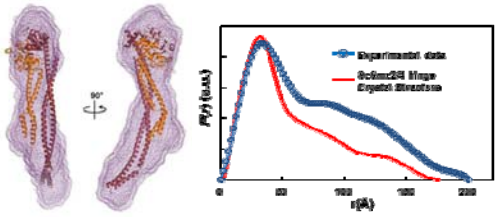

Fig. 2: crystal structure superimposed to SAXS envelope. The distance distribution function *P*(r) is boxed.

References
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*youngminsoh@gmail.com