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Expression, crystallization and preliminary X-ray crystallographic studies of SCP3 coiled-coil domain

The synaptonemal complex protein SCP3 is one of the components of the lateral element of the synaptonemal complex, which is a meiosis-specific complex structure formed at the synapse of homologous chromosomes. In this study, a C-terminal coiled-coil domain, SCP3, was overexpressed in *Escherichia coli* with an engineered C-terminal His tag. The coiled-coil domain of SCP3 was then purified to homogeneity and crystallized at 293 K. X-ray diffraction data were collected to a resolution of 3.2 Å from a crystal belonging to space group C2, with unit-cell parameters $a = 121.29$, $b = 43.08$, $c = 57.42$ Å, $\beta = 100.71^\circ$. The asymmetric unit was estimated to contain three molecules.

1. Introduction

Meiosis is critical for the production of healthy gametes in diploid organisms (Handel & Schimenti, 2010). During meiosis, homologous chromosomes are aligned next to each other to undergo crossing-over and the formation of chiasmata (Kleckner, 2006; Qiao *et al.*, 2012). Pairing of homologous chromosomes during this process is mediated by a complicated molecular complex structure known as the synaptonemal complex, which is a ladder-like structure composed of meiosis-specific proteins (Page & Hawley, 2004; Fraune *et al.*, 2012).

Assembly of the synaptonemal complex is initiated in the early stage of meiotic prophase I, which is commonly divided into five substages: the leptotene, zygotene, pachytene, diplotene and diakinesis stages (Zickler, 2006). For proper assembly of the synaptonemal complex followed by proper pairing of the homologous chromosomes, axial elements (AEs) should be formed along each chromosome at the initial stage during the leptotene stage. Later, in the zygotene stage, the two AEs from a given pair of homologous chromosomes closely align and connect through the central element (CE) and are known as lateral elements (LEs). The central region (CR) is composed of transverse filaments (TFs) and the central element. The synaptonemal complex is fully assembled in the pachytene and disassembly is started in the diplotene (Zickler, 2006; Fraune *et al.*, 2012; Bisig *et al.*, 2012). The failure of proper assembly of the synaptonemal complex leads to infertility or aneuploidy in mammals (Hassold & Hunt, 2001; Roos *et al.*, 2013). Despite the importance of this complex, there is no structural information regarding the synaptonemal complex and the structure of each component is totally unknown. Synaptonemal complex protein 3 (SCP3) is the main structural constituent of LEs and is known to form multi-stranded, cross-striated fibres *in vivo* (Yuan *et al.*, 1998). SCP3 is also a well known meiosis marker that has recently been found to be a novel prognostic marker in early-stage non-small-cell lung cancer (Chung *et al.*, 2013). Human SCP3 is a 236 amino-acid protein with two C-terminal coiled-coil domains.

In the present study, we overexpressed, purified and crystallized the coiled-coil domain of SCP3 (SCP3 CC), which forms the core of the lateral elements of the synaptonemal complex and functions as a molecular scaffold onto which other synaptonemal proteins attach, as a first step towards elucidation of the molecular structure of the synaptonemal complex. Although SCP3 usually forms cross-striated fibres *in vitro* via its C-terminal coiled-coil domain, we were able to obtain and overexpress a construct by designing, constructing and testing many plasmid constructs. Structural studies of functional domains occasionally provide valuable information (Bae & Park,

2011; Park, 2011). Overexpressed human SCP3 CC was purified by affinity chromatography followed by gel-filtration chromatography. SCP3 CC crystals were obtained in a solution consisting of 0.65 M ammonium citrate dibasic, 0.1 M sodium acetate pH 4.2. The crystals belonged to space group C2, with unit-cell parameters $a = 121.29$, $b = 43.08$, $c = 57.42$ Å, $\beta = 100.71^\circ$. The crystals were obtained at 293 K and diffracted to a resolution of 3.2 Å. Details regarding the atomic structure of SCP3 CC should enable us to understand the assembly mechanism of the synaptonemal complex and the role of SCP3 in this complicated but important molecular complex.

2. Materials and methods

2.1. Expression and purification

To express C-terminally histidine-tagged SCP3 CC, the coiled-coil region of SCP3, which corresponds to residues Met140–Phe236, was cloned into pET-24a. The plasmid was transformed into *Escherichia coli* BL21 competent cells and its expression in TB medium was induced by treatment with 0.5 mM isopropyl β -D-1-thiogalactopyranoside (IPTG) overnight at 293 K when the OD₆₀₀ reached 0.73. Cells expressing the SCP3 CC were pelleted by centrifugation, resuspended and lysed by sonication in 50 ml lysis buffer (20 mM Tris pH 7.9, 500 mM NaCl, 25 mM imidazole). The lysate was then centrifuged at 16 000 rev min⁻¹ for 30 min at 277 K, after which the supernatant fractions were applied onto a gravity-flow column (Bio-Rad) packed with Ni-NTA affinity resin (Qiagen). Next, the unbound bacterial proteins were removed from the column using 100 ml washing buffer (20 mM Tris pH 7.9, 500 mM NaCl, 60 mM imidazole). The C-terminal histidine-tagged SCP3 CC was eluted from the column using elution buffer (20 mM Tris buffer pH 7.9, 500 mM NaCl, 250 mM imidazole). 0.7 ml fractions were then collected yielding a total volume of 5.6 ml. The collected SCP3 CC was pooled, concentrated and applied onto a Superdex 200 gel-filtration column (GE Healthcare) that had been pre-equilibrated with 20 mM Tris-HCl pH 8.0, 150 mM NaCl. The SCP3 CC (molecular mass 11 250 Da) eluted as a broad peak between 13 and 16 ml and was collected and concentrated to 3–4 mg ml⁻¹. A total of 8 mg was obtained from a 1 l culture of bacteria. The protein concentration was

measured using a protein-assay kit (Bio-Rad) and then determined by the method of Bradford (1976). Purified SCP3 CC contained the additional residues LEHHHHHH at the C-terminus. The additional residues including the hexa-His tag at the C-terminus were not removed.

2.2. Crystallization

The crystallization conditions were initially screened at 293 K by the hanging-drop vapour-diffusion method and the sitting-drop vapour-diffusion method using screening kits from Hampton Research (Crystal Screen, Crystal Screen 2, SaltRx and Matrix). Initial crystals were grown in hanging-drop plates by equilibrating a mixture consisting of 1 μ l protein solution (3–4 mg ml⁻¹ protein in 20 mM Tris-HCl pH 8.0, 150 mM NaCl) and 1 μ l reservoir solution No. 15 from SaltRx (Hampton Research; 1.0 M ammonium citrate dibasic, 0.1 M sodium acetate pH 4.6) against 0.4 ml of reservoir solution. Crystals appeared within 3 d and grew to maximum dimensions of 0.1 $\times$ 0.1 $\times$ 0.02 mm, with the best quality occurring in the presence of 0.65 M ammonium citrate dibasic, 0.1 M sodium acetate pH 4.2. The crystals diffracted to a resolution of 3.2 Å.

2.3. Crystallographic data collection

For data collection, the crystals were transiently soaked in a solution corresponding to the reservoir solution supplemented with 30% (v/v) glycerol. The soaked crystals were then flash-cooled at 110 K using liquid nitrogen. A 3.2 Å resolution native diffraction data set was collected from a single crystal on a MAR CCD detector (crystal-to-detector distance of 300 mm, 1° oscillation per image, total rotation angle of 180°) at beamline 5C (SB II) at the Pohang Accelerator Laboratory (PAL), Republic of Korea. The data sets were indexed and processed using HKL-2000 (Otwinowski & Minor, 1997).

3. Results and discussion

The process of synaptonemal complex formation during meiosis is totally unknown. To obtain a better understanding of this process, we overexpressed, purified and crystallized SCP3 CC, which forms the core of the lateral elements of the synaptonemal complex.

Nickel-affinity chromatography followed by gel-filtration chromatography produced 95% pure SCP3 CC and no contaminating bands

Figure 1
Gel-filtration chromatography and SDS-PAGE of the SCP3 coiled-coil domain. Peak fractions that were loaded onto SDS-PAGE are indicated by a black bar.

Figure 2
Crystal of the SCP3 coiled-coil domain. Crystals were grown in 3 d in the presence of 0.65 M ammonium citrate dibasic, 0.1 M sodium acetate pH 4.2. The approximate dimensions of the crystals were 0.1 $\times$ 0.1 $\times$ 0.02 mm.

Table 1

Diffraction data describing the SCP3 coiled-coil domain crystals.

Values in parentheses are for the outermost resolution shell.

X-ray source	5C (SB II), PAL
Wavelength (Å)	0.97182
Space group	C2
Unit-cell parameters (Å, °)	$a = 121.29$, $b = 43.08$, $c = 57.42$, $\beta = 100.71$
Resolution range (Å)	50–3.2 (3.32–3.20)
No. of observations	26066
No. of unique reflections	4897
Mean $I/\sigma(I)$	29.5 (3.5)
Completeness (%)	99.4 (95.8)
$R_{\text{merge}}^{\dagger}$ (%)	9.9 (63.9)

$\dagger R_{\text{merge}} = \sum_{hkl} \sum_i |I_i(hkl) - \langle I(hkl) \rangle| / \sum_{hkl} \sum_i I_i(hkl)$, where $I_i(hkl)$ is the i th observation of reflection hkl and $\langle I(hkl) \rangle$ is the weighted average intensity for all observations i of reflection hkl .

were observed upon SDS–PAGE analysis (Fig. 1). SCP3 CC eluted at around 14–16 ml in the gel-filtration chromatography, indicating that SCP3 CC exists in an oligomeric form in solution. Highly oligomeric or an aggregated state of SCP3 CC, which eluted at around 8–9 ml, was also detected in the profile of gel-filtration chromatography. Because aggregated protein cannot be used for crystallization trials, we collected the SCP3 CC sample that eluted at around 14–16 ml. The calculated monomeric molecular mass of SCP3 CC, including the additional residues at the C-terminus, was 11 250 Da and its size-exclusion chromatography elution profile suggests that it exists as a trimer or a tetramer in solution (Fig. 1). A gel-filtration standard (Bio-Rad) containing a mixture of molecular-mass markers (thyroglobulin, 670 000 Da; globulin, 158 000 Da; ovalbumin, 44 000 Da; myoglobulin, 17 000 Da; vitamin B₁₂, 1350 Da), was used for size calibration.

An initial crystal was obtained using condition No. 15 of the SaltRx kit (Hampton Research; 1.0 M ammonium citrate dibasic, 0.1 M sodium acetate pH 4.6). Optimization of the crystallization conditions using a range of concentrations of protein and precipitant and various pH values led to better crystals (Fig. 2). The optimized crystals grew to dimensions of $0.1 \times 0.1 \times 0.02$ mm in 3 d and diffracted to 3.2 Å resolution (Fig. 3). The crystals belonged to space group C2, with unit-cell parameters $a = 121.29$, $b = 43.08$, $c = 57.42$ Å, $\beta = 100.71^\circ$. Diffraction data statistics are shown in Table 1.

Assuming the presence of one trimeric molecule in the crystallographic asymmetric unit, the Matthews coefficient (V_M) was calculated to be $2.21 \text{ Å}^3 \text{ Da}^{-1}$, which corresponds to a solvent content of 44.4% (Matthews, 1968). There was no clear peak indicating noncrystallographic symmetry on analyzing the self-rotation function. The molecular-replacement phasing method (MR) was conducted using *Phaser* (McCoy, 2007). Several coiled-coil domains including fibrinogen (PDB entry 1ei3; Yang *et al.*, 2000), which shares 22% sequence identity, and the HAMP domain (PDB entry 3zrv; Ferris *et al.*, 2012), which shares 18% sequence identity, were used as search models. No promising MR solutions were found in the initial MR attempt. We also produced a selenomethionine-substituted protein for single-wavelength anomalous diffraction (SAD) phasing. Efforts to determine the structure of SCP3 CC are under way.

Figure 3

A diffraction image (1° oscillation) of the SCP3 coiled-coil domain crystal at a 3.2 Å resolution limit.

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