STRUCTURAL BIOLOGY COMMUNICATIONS

ISSN 2053-230X

X-ray crystallographic studies of the middle part of the human synaptonemal complex protein 1 coiled-coil domain

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Received 3 June 2015
Accepted 1 July 2015

Edited by T. Bergfors, Uppsala University, Sweden

Keywords: meiosis; syneptonemal complex; SYCP1; coiled-coil domain; crystallization.

The synaptonemal complex is a meiosis-specific complex structure formed at the synapse of homologous chromosomes to hold them together during meiosis. Synaptonemal complex protein 1 (SYCP1) is one of the components of the syneptonemal complex. In this study, the short form of the coiled-coil domain of SYCP1 was overexpressed in *Escherichia coli* with an engineered C-terminal His tag. The short form of the coiled-coil domain of SYCP1 was then purified to homogeneity and crystallized at 293 K. X-ray diffraction data were collected to a resolution of 3.0 Å from a crystal belonging to space group *I*4, with unit-cell parameters $a = 41.95$, $b = 41.95$, $c = 318.78$ Å. The asymmetric unit was estimated to contain two molecules.

1. Introduction

To ensure proper recombination events during meiosis, homologous chromosomes need to be aligned next to each other to undergo recombination events (Kleckner, 2006; Qiao *et al.*, 2012). Pairing of homologous chromosomes is mediated by a complicated, well organized protein complex structure known as the syneptonemal complex, which is a large structure composed of many meiosis-specific proteins such as synemtonemal complex proteins 1, 2 and 3 (SYCP1, SYCP2 and SYCP3; Page & Hawley, 2004; Fraune *et al.*, 2012).

Syneptonemal complex formation is initiated in the early stage of meiotic prophase I (Zickler, 2006). The syneptonemal complex is composed of three distinct structures: axial elements (AEs), lateral elements (LEs) and central elements (CEs). For proper pairing of the homologous chromosomes during meiotic prophase I, axial elements (AEs), which are composed of SYCP2 and SYCP3, are formed along each chromosome. Subsequently, the two AEs associate with linker parts called lateral elements (LEs), which contain SYCP1 as the main protein component. Finally, the central element (CE), which is composed of SYCE1 and SYCE2, connects to the AE *via* the LE to form the full syneptonemal structure (Zickler, 2006; Fraune *et al.*, 2012; Bisig *et al.*, 2012). Failure of proper assembly of the syneptonemal complex leads to infertility or aneuploidy in mammals (Hassold & Hunt, 2001; Roos *et al.*, 2013). Despite the importance of this complex, structural information is still limited. SYCP1 is the main structural constituent of LEs and is known to form multi-stranded, cross-striated fibres *in vivo* (Yuan *et al.*, 1998). SYCP1 is a well known meiosis marker that is also known to be prognostic in early-stage cancers, including breast, glioma and ovarian cancers (Türeci *et al.*, 1998). Human SYCP1 is a 993-amino-acid protein with several coiled-coil domains.

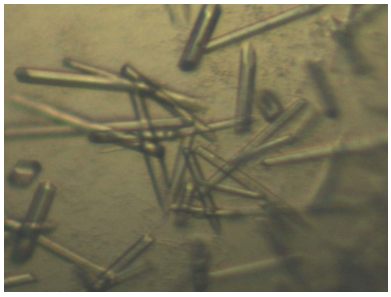

Table 1
Macromolecule-production information.

Source organism	Human
DNA source	GenBank NM_003176
Forward primer	5′-GGGCATATGGACACCTATCAAAAAGAA-3′
Reverse primer	5′-GGGCTCGAGGAACTCAGCGCGAGGCTT-3′
Cloning vector	pET-24a
Expression vector	pET-24a
Expression host	*E. coli*
Complete amino-acid sequence of the construct produced	DTYQKEIEDKKISEENLLEEVEKAKVIADEAVKL-QKEIDKRCQHKIAEMVALMEKHKHQYDKIIEE-RDSELGLYKSKEQEQSSLRASLELEHHHHHH

Table 2
Crystallization.

Method	Hanging-drop vapour diffusion
Plate type	24-well plates, Hampton Research
Temperature (K)	293
Protein concentration (mg ml^{-1})	8–9
Composition of protein buffer solution	20 m*M* sodium citrate pH 5.0, 150 m*M* NaCl
Composition of reservoir solution	1.9 *M* ammonium phosphate, 16%(*v*/*v*) glycerol, 0.08 *M* Tris–HCl pH 8.0
Volume and ratio of drop	2 µl, 1:1
Volume of reservoir (µl)	400

In the present study, we overexpressed, purified and crystallized the middle part of the SYCP1 coiled-coil domain (SYCP1$^{662–750}$) as a first step towards the elucidation of the molecular structure of the synaptonemal complex. Although SYCP1 usually forms fibres *in vitro*, we were able to obtain the overexpressed construct by designing, constructing and testing many plasmid constructs. SYCP1$^{662–750}$ was the most well behaved and well expressed construct among these. Overexpressed human SYCP1$^{662–750}$ was purified by affinity chromatography followed by gel-filtration chromatography and diffracting SYCP1$^{662–750}$ crystals were obtained. X-ray diffraction data were collected to a resolution of 3.0 Å from a crystal belonging to space group *I*4, with unit-cell parameters a = 41.95, b = 41.95, c = 318.78 Å. The asymmetric unit was estimated to contain two molecules.

2. Materials and methods

2.1. Macromolecule production

To express C-terminally histidine-tagged SYCP1$^{662–750}$, the coiled-coil domain including the middle region of SCP1, which corresponds to residues Asp662–Glu750, was cloned into pET-24a. The plasmid was then transformed into *Escherichia coli* BL21 competent cells, after which its expression in LB medium was induced by treatment with 0.5 m*M* isopropyl β-D-1-thiogalactopyranoside (IPTG) overnight at 293 K when the OD_{600} reached 0.8. Cells expressing SYCP1$^{662–750}$ were pelleted by centrifugation, resuspended and lysed by sonication in 25 ml lysis buffer (20 m*M* Tris–HCl pH 7.9, 500 m*M* NaCl, 25 m*M* imidazole). The lysate was then centrifuged at 16 000*g* 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). The unbound bacterial proteins were then removed from the column using 150 ml washing buffer (20 m*M* Tris–HCl pH 7.9, 500 m*M* NaCl, 60 m*M* imidazole). The C-terminally histidine-tagged SYCP1$^{662–750}$ was then eluted from the column using elution buffer (20 m*M* Tris–HCl buffer pH 7.9, 500 m*M* NaCl, 250 m*M* imidazole). The elution fractions were subsequently collected in 0.5 ml volumes to give a total volume of 5.0 ml. The collected SYCP1$^{662–750}$ was pooled, concentrated and applied onto a Superdex 200 gel-filtration column (GE Healthcare) that had been pre-equilibrated with a solution consisting of 20 m*M* sodium citrate pH 5.0, 150 m*M* NaCl. SYCP1$^{662–750}$ (molecular mass 11 800 Da) eluted at around 14–15 ml and was collected and concentrated to 8–9 mg ml^{-1}. The protein concentration was measured using a protein-assay kit (Bio-Rad) and was determined by the Bradford method (Bradford, 1976). Purified SYCP1$^{662–750}$ contained the additional residues LEHHHHHH at the C-terminus. The additional residues, including the hexahistidine tag, at the C-terminus were not removed. The purified protein was used directly in crystallization trials. The purification procedures are summarized in Table 1.

2.2. Crystallization

The crystallization conditions were initially screened at 293 K by the hanging-drop vapour-diffusion method using screening kits from Hampton Research (Crystal Screen, Crystal Screen 2, Crystal Screen Cryo, SaltRx and Natrix) and Rigaku Reagents (Wizard I, II, III and IV). The initial crystals were grown on plates by equilibrating a mixture consisting of 1 µl protein solution (8.5 mg ml^{-1} protein in 20 m*M* sodium citrate pH 5.0, 150 m*M* NaCl) and 1 µl solution No. 48 from Crystal Screen Cryo [Hampton Research; 1.6 *M* ammonium phosphate monobasic, 20%(*v*/*v*) glycerol, 0.08 *M* Tris–HCl pH 8.5] against 0.4 ml reservoir solution. After optimization, crystals appeared within 4 d and grew to maximum dimensions of 0.05 × 0.05 × 0.4 mm, with the best quality occurring in the presence of 1.9 *M* ammonium phosphate, 16%(*v*/*v*) glycerol, 0.08 *M* Tris–HCl pH 8.0. The crystals diffracted to a resolution of 3.0 Å. A summary of the crystallization is provided in Table 2.

2.3. Data collection and processing

For data collection, the crystals were transiently soaked in cryoprotectant solution and directly cooled in liquid nitrogen. During this process, providing an additional 30%(*v*/*v*) glycerol [1.5 µl 60%(*v*/*v*) glycerol was added to 1.5 µl mother liquor] in the crystal drop was sufficient as a cryoprotectant. A 3.0 Å resolution native diffraction data set was collected on beamline 5C (SB II) of the Pohang Accelerator Laboratory (PAL), Republic of Korea. The data set was indexed and processed using *HKL*-2000 (Otwinowski & Minor, 1997). Diffraction data statistics are given in Table 3.

3. Results and discussion

Formation of the synaptonemal complex between homologous chromosomes during meiosis is critical for homologous

recombination and proper chromosome segregation. Although several electron-microscopic (EM) analyses have shown the overall structure of the syneptonemal complex (Gillies, 1975; Ollinger *et al.*, 2005; Comings & Okada, 1971), the atomic structure of this complicated molecule is completely unknown. To obtain a better understanding of this process, we overexpressed, purified and crystallized the middle part of SYCP1 (SYCP1$^{662–750}$). SYCP1 is the main component of the lateral element of the syneptonemal complex. We made 32 expression constructs, including full-length SYCP1. Among them, SYCP1$^{662–750}$, which contains amino acids 662–750, and SYCP1$^{662–801}$, which contains amino acid 662–801, were well expressed and were used to produce the coiled-coil domain of SYCP1. Although our target, SYCP1$^{662–750}$, is only the middle part of the coiled-coil domain of SYCP1, the target domain used in this study will be representative of the overall structure of SYCP1 because the majority of the structure of SYCP1 is known to be composed of coiled-coil domain.

His-tag affinity chromatography followed by gel-filtration chromatography produced approximately 95% pure SYCP1$^{662–750}$, and no contaminating bands were observed upon SDS–PAGE analysis (Fig. 1). The calculated monomeric molecular weight of SYCP1$^{662–750}$, including the additional residues at the C-terminus, was 11 800 Da, and its elution peak from size-exclusion chromatography suggests that it exists as a dimer in solution. A gel-filtration standard (Bio-Rad) containing a mixture of molecular-weight markers (thyroglobulin, 670 000 Da; globulin, 158 000 Da; ovalbumin, 44 000 Da; myoglobulin, 17 000 Da; vitamin B_{12}, 1350 Da) was used for size calibration.

An initial crystal was obtained in solution No. 48 from Crystal Screen Cryo [Hampton Research; 1.6 *M* ammonium phosphate monobasic, 20%(*v*/*v*) glycerol, 0.08 *M* Tris–HCl pH 8.5]. Optimization of the crystallization conditions using a range of concentrations of protein and precipitant and various

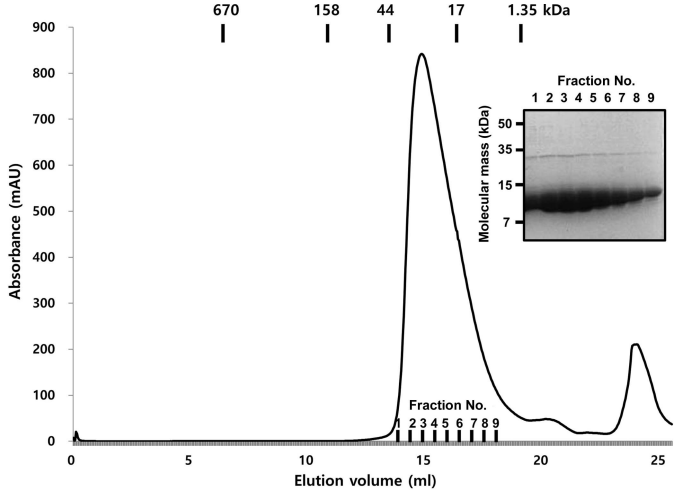

Figure 1
Purification of SYCP1$^{662–750}$. Size-exclusion chromatography and SDS–PAGE of SYCP1$^{662–750}$. The numbered fractions were loaded onto SDS–PAGE (inset).

Table 3
Data collection and processing.

Values in parentheses are for the outer shell.

Diffraction source	5C (SB II), PAL
Wavelength (Å)	0.9776
Temperature (K)	110
Detector	ADSC Quantum 315r
Crystal-to-detector distance (mm)	300
Rotation range per image (°)	1
Total rotation range (°)	180
Exposure time per image (s)	1
Space group	*I*4
a, *b*, *c* (Å)	41.95, 41.95, 318.78
α, β, γ (°)	90, 90, 90
Mosaicity (°)	0.6
Resolution range (Å)	50–3.0
Total No. of reflections	40271
No. of unique reflections	5490
Completeness (%)	99.9 (99.6)
Multiplicity	7.3 (6.8)
$\langle I/\sigma(I)\rangle$	22.3 (3.2)
$R_{\rm merge}$ (%)	12.4 (60.8)

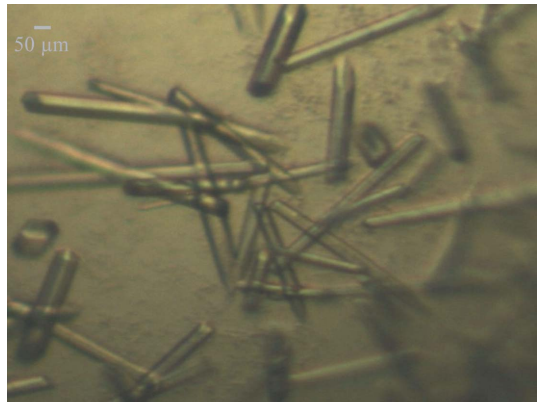

Figure 2
Crystals of SYCP1$^{662–750}$. Crystals grew in 4 d in the presence of 1.9 *M* ammonium phosphate, 16%(*v*/*v*) glycerol, 0.08 *M* Tris–HCl pH 8.0. The approximate dimensions of the crystals were 0.05 × 0.05 × 0.4 mm.

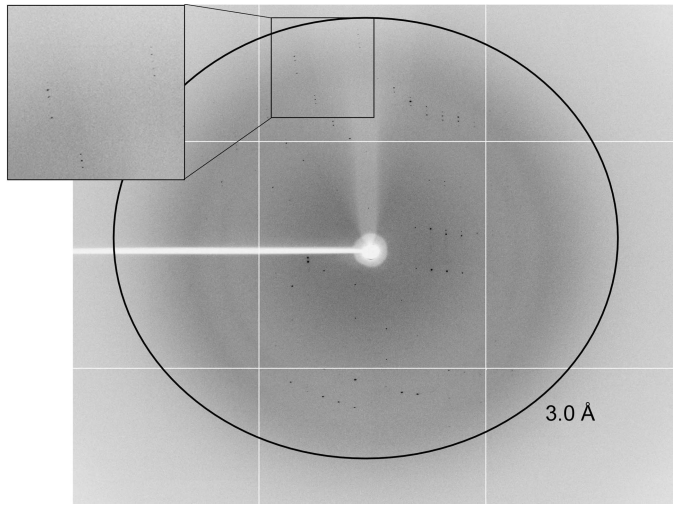

Figure 3
A diffraction image (1° oscillation) from an SYCP1$^{662–750}$ crystal. The resolution limit of 3.0 Å is shown as a circle.

pH values led to better crystals that were suitable for diffraction in the presence of 1.9 *M* ammonium phosphate, 16%(*v*/*v*) glycerol, 0.08 *M* Tris–HCl pH 8.0 (Fig. 2). The optimized crystals grew to dimensions of 0.05 × 0.05 × 0.4 mm in 4 d and diffracted to 3.0 Å resolution (Fig. 3). The crystals belonged to space group *I*4, with unit-cell parameters $a = 41.95$, $b = 41.95$, $c = 318.78$ Å.

Assuming the presence of two monomers or one dimer in the crystallographic asymmetric unit, the Matthews coefficient (V_M) was calculated to be 2.78 Å^3 Da^{-1}, which corresponds to a solvent content of 55.82% (Matthews, 1968). The molecular-replacement phasing method was conducted using *Phaser* (McCoy, 2007); several coiled-coil domains, including fibrinogen (PDB entry 1ei3; Yang *et al.*, 2000), which has 22% sequence identity to SYCP1$^{662–750}$, and HAMP (PDB entry 3zrv; Ferris *et al.*, 2012), which has 15% sequence identity to SYCP1$^{662–750}$, were used as search models. Because of poor models, an MR solution has not yet been obtained. To overcome the phasing problem, we also produced selenium-substituted crystals using similar conditions and are currently conducting SAD phasing using *SOLVE*/*RESOLVE* (Terwilliger, 2004).

Acknowledgements

This research was supported by the Korea Healthcare Technology R&D project, Ministry of Health & Welfare, Republic of Korea (HI13C1449).

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