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Purification, crystallization and preliminary X-ray diffraction analysis of the histone chaperone cia1 from fission yeast

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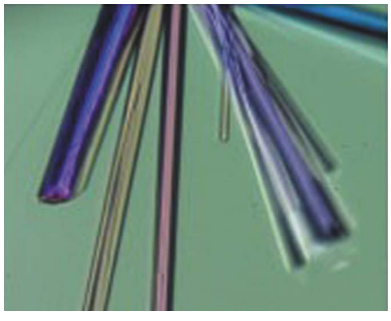

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In fission yeast, *cia1*$^+$ is an essential gene that encodes a histone chaperone, a homologue of human CIA (CCG1-interacting factor A) and budding yeast Asf1p (anti-silencing function-1), which both facilitate nucleosome assembly by interacting with the core histones H3/H4. The conserved domain (residues 1–161) of the *cia1*$^+$-encoded protein was expressed in *Escherichia coli*, purified to near-homogeneity and crystallized by the sitting-drop vapour-diffusion method. The protein was crystallized in the monoclinic space group *C*2, with unit-cell parameters a = 79.16, b = 40.53, c = 69.79 Å, β = 115.93° and one molecule per asymmetric unit. The crystal diffracted to beyond 2.10 Å resolution using synchrotron radiation.

1. Introduction

In eukaryotes, genomic DNA forms a nucleoprotein complex with the core histone particles H2A, H2B, H3 and H4, which is the basic unit of genomic compaction. As a consequence of this nucleosome structure, the core histones in general negatively regulate DNA-mediated reactions such as transcription, DNA replication and DNA repair (for a review, see Kornberg & Lorch, 1999). Histone chaperones are factors that mediate nucleosome assembly in a synchronized manner with DNA replication, repair and transcription (for a review, see Loyola & Almouzni, 2004). They bind to the core histones and facilitate their deposition onto the nucleosome in an ATP-independent manner. CCG1-interacting factor (CIA) is one such mammalian histone chaperone and was originally isolated by its interaction with the human TFIID subunit TAF1/CCG1 (Munakata *et al.*, 2000). The CIA-related mammalian protein, designated CIA-II, also has histone chaperone activity (Umehara & Horikoshi, 2003). Importantly, its homologue Asf1p from the budding yeast *Saccharomyces cerevisiae*, which was identified by its involvement in gene silencing (Le *et al.*, 1997), also interacts with core histones and possesses histone chaperone activity (Umehara *et al.*, 2002). Moreover, Asf1p interacts with various nuclear proteins, such as the bromodomain-containing factor Bdf1p (Chimura *et al.*, 2002), the DNA-repair coupled factor Rad53p (Emili *et al.*, 2001; Hu *et al.*, 2001), the transcriptional co-repressor Hir proteins (Sharp *et al.*, 2001; Sutton *et al.*, 2001) and the chromatin-assembly factor CAF-I (Tyler *et al.*, 2001; Mello *et al.*, 2002). In addition, *ASF1* is involved in transcriptional regulation (Chimura *et al.*, 2002; Adkins *et al.*, 2004), gene silencing (Le *et al.*, 1997; Singer *et al.*, 1998; Osada *et al.*, 2001; Umehara *et al.*, 2002), DNA replication (Tyler *et al.*, 1999), DNA repair (Mello *et al.*, 2002), DNA recombination (Prado *et al.*, 2004), the cell cycle (Le *et al.*, 1997; Singer *et al.*, 1998) and cell death (Yamaki *et al.*, 2001). These results suggest that Asf1p and presumably also its homologues contribute to the fundamental regulation of DNA-mediated reactions. Thus, structural analyses of Asf1p and its homologues are essential to understand the mechanism of the nucleosome-assembly process and the resultant regulation of DNA-mediated reactions. Recently, the crystal structure of the functional domain (residues 1–155) of yeast Asf1p revealed the presence of an immunoglobulin-like β-sandwich fold (Daganzo *et al.*, 2003). We have also observed that the tertiary structure of yeast Asf1p, corresponding to the region of amino acids 1–169, is indeed similar to the published structure; however,

substantial structural deviations existed in the β-sheet-intervening regions (Padmanabhan *et al.*, 2002; unpublished results).

We have focused on structural and functional analyses of *S. pombe* cia1, the fission yeast orthologue of CIA/Asf1p, to elucidate the common as well as the diverse features of CIA homologues through comparative studies between these two evolutionarily distant yeast species. *S. pombe* cia1 and *S. cerevisiae* Asf1p share 66% sequence identity in their functional N-terminal domains (residues 1–159 of cia1). The cia1 and Asf1p proteins each have a polyanionic stretch in their C-termini which is specific to yeast but dispensable *in vivo*. The *cia1*$^+$ and *ASF1* genes are unique in their respective genomes; however, *cia1*$^+$ is indispensable for cell viability, whereas *ASF1* is dispensable, suggesting structural and functional differences *in vivo* (Le *et al.*, 1997; Umehara *et al.*, 2002). In this study, we report the purification, crystallization and preliminary crystallographic studies of the conserved functional domain of *S. pombe* cia1.

2. Materials and methods

2.1. Construction of the cia1(1–161) expression vector

The cDNA encoding the region of amino acids 1–161 of *S. pombe* cia1 was amplified by PCR using the plasmid carrying the *cia1*$^+$ gene (Umehara *et al.*, 2002) as a template and the following primers: forward, 5′-GGGATGCGGGGGATCCGATGTCAATCGTGAA-TAT-3′; reverse, 5′-GGGATGCGGGGGATCCTCATTAATTATC-CCACTGAATGTT-3′. This cDNA fragment was digested with *Bam*HI and ligated into the *Bam*HI-digested pET15b vector (Novagen). The orientation and the coding sequence of this subclone, designated as pET15b-cia1(1–161), were confirmed by DNA sequencing.

2.2. Expression and purification

Escherichia coli strain BL21 Star (DE3) (Invitrogen) was transformed with pET15b-cia1(1–161) and grown at 303 K in LB medium containing 50 µg ml^{-1} ampicillin until the OD_{600} reached 0.4–0.5. Overexpression of His_6-tagged cia1(1–161) was induced by the addition of 1.0 m*M* IPTG. After 3 h of culture at 303 K, the cells were harvested by centrifugation, resuspended in a buffer containing 20 m*M* Tris–HCl pH 7.5, 20 m*M* imidazole and 150 m*M* NaCl and lysed by sonication. The cell lysate was centrifuged at 16 000*g* for 20 min at 277 K. The resulting supernatant was filtered through a MILLEX-HV PVDF 0.45 µm membrane (Millipore) and applied onto HisTrap resin (Amersham Biosciences). The His_6-tagged cia1(1–161) protein was eluted with a buffer containing 20 m*M* Tris–HCl pH 7.5, 500 m*M* imidazole and 150 m*M* NaCl and the His_6 tag was then cleaved using thrombin (10 units per milligram of protein) at 277 K for 16 h. This reaction mixture was loaded onto a HiPrep desalting column (Amersham Biosciences) and the fraction containing cia1(1–161) was eluted with a buffer containing 20 m*M* Tris–HCl pH 7.5, 100 m*M* NaCl and 2 m*M* DTT. The thrombin was removed using a Benzamidine-Sepharose 6B column (Amersham Biosciences). The cia1(1–161) fraction was collected and loaded onto a 1 ml HiTrap column (Amersham Biosciences), which was eluted with a stepwise gradient from 0 and 0.5 *M* imidazole to remove the cleaved tag. The cia1(1–161) fraction was pooled and loaded onto a 5 ml HiTrap Q column (Amersham Biosciences), which was eluted with a linear gradient from 0.1 to 1.0 *M* NaCl. The eluted protein was finally fractionated by gel filtration on a HiLoad 16/60 Superdex 75 column (Amersham Biosciences). The cia1(1–161) protein was eluted as a single peak and was analyzed by SDS–PAGE (Fig. 1*a*). The purified protein solution was concentrated to 8.2 mg ml^{-1} with an Amicon Ultra (5000 MWCO; Millipore).

2.3. Crystallization

Crystallization trials were performed by the sitting-drop vapour-diffusion method at 293 K. The drops were prepared by mixing 1.5 µl protein solution (8.2 mg ml^{-1}) with 1.5 µl reservoir solution on siliconized cover slides and were equilibrated against 500 µl reservoir solution. Single crystals appeared within 2–4 d in a precipitant solution containing 32% PEG 6000, 0.18 *M* ammonium sulfate and 0.1 *M* Tris–HCl pH 8.0 (Hampton Research) (Fig. 1*b*).

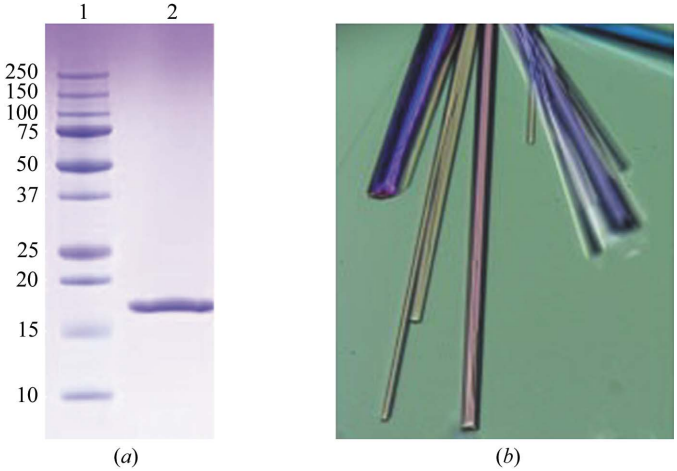

Figure 1
Purification and crystallization of *S. pombe* cia1(1–161). (*a*) SDS–PAGE profile of the *S. pombe* cia1(1–161) fraction. Lane 1, molecular-weight markers (kDa). The molecular weights of each band are shown on the left. Lane 2, purified cia1(1–161) fraction. Electrophoresis was performed with a 17.5% SDS–PAGE gel. (*b*) Single crystal of *S. pombe* cia1(1–161).

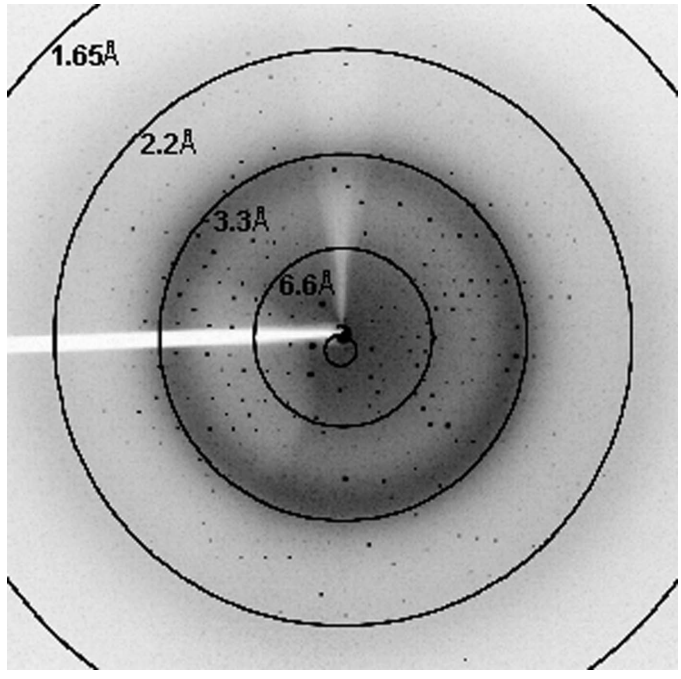

Figure 2
An X-ray diffraction pattern of the *S. pombe* cia1(1–161) crystal. The crystal-to-detector distance was 180 mm, the oscillation angle was 1.0° and the exposure time was 10 s.

Table 1
Data-collection and processing statistics.

Values in parentheses refer to the last shell (2.18–2.10 Å).

Space group	*C*2
Unit-cell parameters (Å, °)	a = 79.16, b = 40.53, c = 69.79, α = 90.00, β = 115.93, γ = 90.00
Resolution range (Å)	50.0–2.10
Wavelength (Å)	1.000
No. of measured reflections	41832
No. of unique reflections	11430
$R_{\rm merge}$† (%)	5.6 (22.0)
Completeness (%)	98 (95)
Redundancy	3.7 (3.5)

† $R_{\rm merge} = \sum |I(h) - \langle I(h)\rangle| / \sum I(h)$, where $I(h)$ is the observed intensity and $\langle I(h)\rangle$ is the mean intensity of reflection h over all measurements of $I(h)$.

2.4. Data collection

The crystal was briefly soaked in a cryoprotectant containing 30% PEG 6000, 0.18 *M* ammonium sulfate, 0.1 *M* Tris–HCl pH 8.0 and 10% glycerol (Hampton Research). Diffraction data were collected from a single crystal on beamline BL26B1 using a Jupiter CCD detector at SPring-8, Harima, Japan. The wavelength used was 1.0 Å and the incident beam was collimated to a 0.1 mm diameter. The crystal-to-detector distance was set to 180 mm and the oscillation range was 1° with an exposure time of 10 s. A complete data set was collected at 100 K under a liquid-nitrogen stream to a maximum resolution of 2.10 Å (Fig. 2). All data were processed and scaled using the *HKL*2000 suite (Otwinowski & Minor, 1997).

3. Results

The cia1(1–161) protein from *S. pombe* was purified to near-homogeneity (Fig. 1*a*) and crystallized (Fig. 1*b*). After optimizing the crystallization conditions, good crystals with dimensions of approximately 0.05 × 0.025 × 1.2 mm were obtained. The crystals diffracted to beyond 2.10 Å resolution (Fig. 2) using synchrotron radiation. The crystals belong to the monoclinic space group *C*2, with unit-cell parameters a = 79.16, b = 40.53, c = 69.79 Å, β = 115.93°. Data-collection statistics of the processed data are summarized in Table 1. A total of 41 832 measured reflections were merged into 11 430 unique reflections with an $R_{\rm merge}$ of 5.6%. The merged data set is 98% complete to 2.10 Å resolution. A value for the Matthews coefficient of 3.0 Å^3 Da^{-1} (Matthews, 1968) with a solvent content of 58% was obtained assuming the presence of one molecule in the asymmetric unit and a molecular weight of 19 201 Da. The sequence of cia1 from fission yeast (Umehara *et al.*, 2002) shares 66% sequence identity with that of the *S. cerevisiae* Asf1p protein. The Asf1p structure, which was recently determined (Daganzo *et al.*, 2003), will be used as a model to solve the structure of cia1 from *S. pombe* by the molecular-replacement method.

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