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

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, $\beta = 115.93^\circ$ 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'-GGGATGCGGGGGATCCTCATTAAATTATC-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 $\mu\text{g ml}^{-1}$ ampicillin until the OD₆₀₀ reached 0.4–0.5. Overexpression of His₆-tagged *cia1*(1–161) was induced by the addition of 1.0 mM IPTG. After 3 h of culture at 303 K, the cells were harvested by centrifugation, resuspended in a buffer containing

20 mM Tris-HCl pH 7.5, 20 mM imidazole and 150 mM NaCl and lysed by sonication. The cell lysate was centrifuged at 16 000g 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 His₆-tagged *cia1*(1–161) protein was eluted with a buffer containing 20 mM Tris-HCl pH 7.5, 500 mM imidazole and 150 mM NaCl and the His₆ 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 mM Tris-HCl pH 7.5, 100 mM NaCl and 2 mM 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. 1a). 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. 1b).

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	C2
Unit-cell parameters (Å, °)	$a = 79.16$, $b = 40.53$, $c = 69.79$, $\alpha = 90.00$, $\beta = 115.93$, $\gamma = 90.00$
Resolution range (Å)	50.0–2.10
Wavelength (Å)	1.000
No. of measured reflections	41832
No. of unique reflections	11430
$R_{\text{merge}}^{\dagger}$ (%)	5.6 (22.0)
Completeness (%)	98 (95)
Redundancy	3.7 (3.5)

$\dagger R_{\text{merge}} = \sum |I(h) - \langle I(h) \rangle| / \sum I(h)$, 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 HKL2000 suite (Otwinowski & Minor, 1997).

3. Results

The cial1–161 protein from *S. pombe* was purified to near-homogeneity (Fig. 1a) and crystallized (Fig. 1b). After optimizing the crystallization conditions, good crystals with dimensions of approximately $0.05 \times 0.025 \times 1.2$ 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 C2, with unit-cell parameters $a = 79.16$, $b = 40.53$, $c = 69.79$ Å, $\beta = 115.93^\circ$. 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_{merge} of 5.6%. The merged data set is 98% complete to 2.10 Å resolution. A value for the Matthews coefficient of $3.0 \text{ Å}^3 \text{ Da}^{-1}$ (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 cial1 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 cial1 from *S. pombe* by the molecular-replacement method.

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