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Purification, crystallization and preliminary X-ray diffraction analysis of yeast nucleosome-assembly factor Cia1p

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Yeast Cia1p is a homologue of human CIA (CCG1-interacting factor A), which possesses nucleosome-assembly activity and interacts with the human TFIID subunit CCG1 and the C-terminal domain of histone H3. The yeast Cia1p without the C-terminal polyanionic stretch has been expressed in *Escherichia coli*, purified to homogeneity and crystallized by the hanging-drop vapour-diffusion method using PEG 8000 as precipitant. The protein was crystallized in orthorhombic space group $P2_12_12_1$, with unit-cell parameters $a = 106.70$, $b = 46.92$, $c = 40.60$ Å and one molecule in the asymmetric unit. The crystal diffracted beyond 2.95 Å resolution using synchrotron radiation.

1. Introduction

Eukaryotic genomic DNA forms the nucleosome structure with histones through which the DNA-mediated reactions such as gene expression are controlled (Kornberg, 1974). Although formation and disruption of nucleosome structure are important for control of the function of the genome (for a review, see Kornberg & Lorch, 1999), the mechanism is still unclear. Since the structural modification of the nucleosome is modulated by various factors, such as (i) nucleosome-assembly factor, (ii) nucleosome-remodelling enzyme and (iii) histone-modification enzyme (for a review, see Travers, 1999), elucidation of the mechanism requires not only conventional biochemical analysis, but also structural analysis.

CIA was identified as a human CCG1-interacting factor and is highly conserved from yeast to human (Munakata *et al.*, 2000). On the other hand, the yeast homologue was previously isolated genetically and identified as anti-silencing function 1 (Asf1p; Le *et al.*, 1997). We have found recently by micrococcal nuclease digestion and plasmid supercoiling assays that yeast Cia1p as well as human CIA possess nucleosome-assembly activity (Munakata *et al.*, 2000; Umehara *et al.*, 2002). Previous studies have shown that the H3 region responsible for CIA binding is thought to be (i) required for formation of the $(H3/H4)_2$ tetramer (Freeman *et al.*, 1996) and (ii) contain the start and terminal points of interaction between histones and DNA in a nucleosome core particle (Luger *et al.*, 1997). These results indicate that Cia1p plays a fundamental and central role in the alteration of the nucleosome structure through the modulation of histone–histone and/or histone–DNA interactions (Munakata *et al.*, 2000). Because nucleosome assembly/disassembly processes are the molecular foundation for chromatin-related reactions, Cia1p is expected to participate in various chromatin functions. In fact, Cia1p participates in several reactions such as gene silencing (Osada *et al.*, 2001; Umehara *et al.*, 2002), DNA replication (Tyler *et al.*, 1999), DNA repair (Mello *et al.*, 2002), the cell cycle (Le *et al.*, 1997; Singer *et al.*, 1998) and cell death (Yamaki *et al.*, 2001). Moreover, Cia1p regulates transcriptional activity in association with the general transcription initiation factor TFIID (Chimura *et al.*, 2002). These findings indicate that elucidation of the mechanism of action of Cia1p is necessary to establish the mechanisms involved in chromatin structure and function.

Since the X-ray crystal structure of the nucleosome, which is the target of Cia1p, is known (Luger *et al.*, 1997), determination of the tertiary structure of Cia1p will provide further information on the molecular mechanism of nucleosome assembly. Hence, in order to better understand the mechanism of nucleosome assembly carried out by histone chaperone Cia1p, we have initiated the determination of the tertiary structure of Cia1p.

In a previous study, we constructed a series of Cia1p deletion fragments to localize the functional nucleosome-assembly domain of Cia1p. We have recently shown that Cia1p-ΔC2 (comprising residues 1–169 of Cia1p), which contains the evolutionally conserved region, has nucleosome-assembly activity and binds to H3/H4 *in vitro* and complements the *cia1*$^-$ growth defect *in vivo* (Umehara *et al.*, 2002). On the other hand, the polyanionic stretch-containing fragment Cia1p-ΔN3 (comprising residues 170–279 169 of Cia1p) does not complement the *cia1*$^-$ growth defect

in vivo and binds to core histones H2A/H2B/H3/H4, unlike the full-length Cia1p or Cia1p-ΔC2 (Umehara *et al.*, 2002). These findings suggest that Cia1p-ΔC2 is necessary for the functional activity and histone selectivity of Cia1p as a histone chaperone. Here, we report the purification, crystallization and preliminary crystallographic studies of Cia1p-ΔC2 (Umehara *et al.*, 2002), the polyanionic stretch-deleted form of Cia1p from *S. cerevisiae*.

2. Materials and methods

2.1. Protein expression and purification

To overexpress Cia1p-ΔC2, *Escherichia coli* BL21 (DE3) pLys-RIL (Stratagene) cells were transformed with the pGEX5x-2-*CIA1*-ΔC2 recombinant plasmid and grown at 300 K in TBG-M9 medium (Munakata *et al.*, 2000) containing 50 μg ml^{-1} ampicillin and 30 μg ml^{-1} chloramphenicol until OD_{650} reached 0.8–1.0. Overexpression of GST-Cia1p-ΔC2 was induced by addition of 0.4 m*M* IPTG. After 3 h of culture at 300 K, the cells were harvested by centrifugation (3000 rev min^{-1}, 10 min, 277 K), resuspended in a buffer containing 20 m*M* Tris–HCl (pH 7.9 at 277 K), 10% glycerol, 500 m*M* KCl, 50 m*M* 2-mercaptoethanol, 1 m*M* PMSF, 20 μg ml^{-1} leupeptin and 20 μg ml^{-1} pepstatin A and lysed by EmulsiFlex-C5 (Avestin). The cell lysate was centrifuged at 24 000 rev min^{-1} for 30 min at 277 K. The resulting supernatant was applied to glutathione-Sepharose resin (Amersham Biosciences) and after washing the resin, GST-Cia1p-ΔC2 was cleaved by factor Xa (Amersham Biosciences) at 295 K for 16 h. Benzamidine-Sepharose (Amersham Biosciences) was added to the mixture to remove factor Xa. The reaction buffer containing Cia1p-ΔC2 was collected and loaded onto a Poros QE column (Applied Biosystems) and eluted with a linear gradient from 0.2 to 1.5 *M* NaCl. Cia1p-ΔC2 was eluted from about 0.3–0.5 *M* NaCl. The eluted protein was fractionated by gel-filtration HiLoad 26/60 Superdex 200 prep-grade (Amersham Biosciences). Cia1p-ΔC2 was eluted as a single peak and fractions containing Cia1p-ΔC2 were analysed by SDS–PAGE to check the purity of the protein sample. Purified protein solution was concentrated to 4 mg ml^{-1} by Centriprep YM-10 (Millipore) for the crystallization experiment.

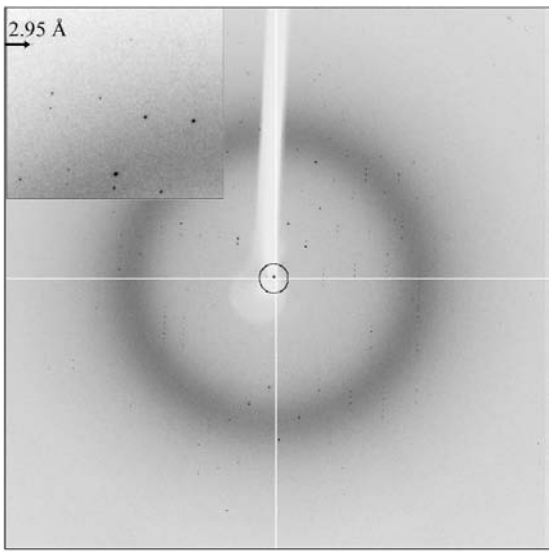

Figure 1
An X-ray diffraction pattern of *S. cerevisiae* Cia1p-ΔC2 crystal (oscillation range 2.0°). A magnified view of the upper left area is shown in the upper left corner of the main figure. The arrow indicates a resolution of 2.95 Å.

2.2. Crystallization

Crystallization trials were initially performed by the sitting-drop vapour-diffusion method at 278 and 293 K. The drops were prepared by mixing 2 μl of protein solution (4 mg ml^{-1}) with 2 μl of reservoir solution in siliconized cover slides. Microcrystals initially appeared within 2–3 d in a precipitant solution containing 0.1 *M* Tris–HCl (pH 7.5 at 293 K), 35% PEG 8000 and 0.2 *M* ammonium sulfate (Hampton Research). Systematic screening of buffer pH, PEGs (4K, 6K and 8K) and different salts did not improve crystal size or morphology. Hence, the streak-seeding method (Stura & Wilson, 1999) was employed to improve the growth of these microcrystals. Clusters of thin plate-shaped crystals (0.1–0.15 mm) appeared in 7–10 d with 30% PEG 8000, 0.18 *M* ammonium sulfate and 0.1 *M* MES pH 6.5.

2.3. Data collection

Diffraction data were collected from a single crystal on beamline BL18B using the ADSC Quantum-4 CCD detector at the Photon Factory, Tsukuba, Japan. The wavelength used was 1.00 Å and the incident beam was collimated to 0.1 mm in diameter. The crystal-to-detector distance was set to 200 mm. A complete data set was collected at room temperature to a maximum resolution of 2.95 Å (Fig. 1). All data were processed and scaled using the programs *DPS*/*MOSFLM* (Rossmann & van Beek, 1999) and *SCALA* from the *CCP*4 package (Collaborative Computational Project, Number 4, 1994).

Table 1
Data-collection and processing statistics.

Values in parentheses refer to the last shell, 3.13–2.95 Å.

Space group	$P2_12_12_1$
Unit-cell parameters (Å)	a = 106.70, b = 46.92, c = 40.60
Resolution range (Å)	30.0–2.95
No. of measured reflections	17216
No. of unique reflections	4606
R_{merge}† (%)	8.5 (28.7)
Completeness (%)	99.9 (99.9)
Average $I/\sigma(I)$	5.3 (2.5)

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

3. Results

Cia1p-ΔC2 from *S. cerevisiae* was crystallized and preliminary crystallographic studies were carried out. Crystals of improved quality with dimensions of approximately 0.15 × 0.1 × 0.03 mm were obtained by the streak-seeding method. Crystals of yeast Cia1p-ΔC2 diffracted to beyond 2.95 Å resolution. They belong to the orthorhombic space group $P2_12_12_1$, with unit-cell parameters a = 106.70, b = 46.92, c = 40.60 Å. Data-collection statistics are summarized in Table 1. A total of 17 216 measured reflections were merged into 4606 unique reflections with an R_{merge} of 8.5%. The merged data set is 99.9% complete to 2.95 Å resolution. A value for the Matthews coefficient of 2.54 Å^3 Da^{-1} (Matthews, 1968) with a solvent content of 51% was obtained assuming one molecule in the asymmetric unit and a molecular weight of 19 125 Da. A search for heavy-atom derivatives for phase determination is in progress.

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