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Purification, crystallization and preliminary X-ray crystallographic analysis of human CCG1-interacting factor B

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A novel human factor CIB (CCG1-interacting factor B) has been isolated using the yeast two-hybrid system. The 22 kDa CIB protein has been expressed in *Escherichia coli*, purified to homogeneity and crystallized in a form suitable for crystallographic studies. The protein was crystallized in the orthogonal space group $P2_12_12_1$, with unit-cell parameters a = 43.60 (2), b = 44.45 (1), c = 110.70 (5) Å and one molecule in the asymmetric unit. The crystal diffracted beyond 2.2 Å resolution using synchrotron radiation.

1. Introduction

Spatial and temporal regulation of gene expression from a genome is responsible for a variety of biological functions. Biochemical and genetic studies have demonstrated that one of the key players in the regulation of RNA polymerase II dependent transcription from naked DNA (Hai *et al.*, 1988; Horikoshi, Carey *et al.*, 1988; Horishoki, Hai *et al.*, 1988) and chromatin DNA (Workman & Roeder, 1987; Workman *et al.*, 1988) is transcription initiation factor IID (TFIID). TFIID is a multisubunit complex consisting of TATA box-binding protein (TBP) and TBP-associated factors (TAFs) (reviewed in Burley & Roeder, 1996).

The cDNA encoding the largest subunit of TFIID (Hisatake *et al.*, 1993; Ruppert *et al.*, 1993) is identical to CCG1 (cell cycle gene 1), a gene able to complement the late G1 arrest of the temperature-sensitive (*ts*) mutant hamster cell lines (Sekiguchi *et al.*, 1988). CCG1 is able to acetylate lysine residues in the N-terminal tails of histones H3 and H4 *in vitro* (Mizzen *et al.*, 1996). Acetylation of histones induces changes in chromatin structure and a strong correlation has been shown between the level of histone acetylation and the activity of transcription from chromatin DNA (reviewed in Workman & Kingston, 1998). Thus, the histone acetyltransferase (HAT) activity of CCG1 may provide a mechanism for TFIID to access transcriptionally repressed chromatin (which is reviewed in Kornberg & Lorch, 1999). Deletion analysis of human CCG1 indicates that the HAT domain of CCG1 maps between amino acids 517 and 976 (Mizzen *et al.*, 1996). The *ts* mutation, which corresponds to amino acid 716 in the human CCG1, resides in the HAT domain of CCG1, suggesting the importance of this domain in transcriptional regulation of the cell cycle *in vivo* (Dunphy *et al.*, 2000).

To investigate the functional regulation of CCG1 HAT domain, we screened interacting factors with this domain using a yeast two-hybrid system. As a result of our search, we have isolated the cDNA of a novel factor CIB (CCG1-interacting factor B). The deduced amino-acid sequence of CIB reveals that it is a 22 kDa protein with similarity to bacterial hydrolase (20% identity, 43% similarity), not to any transcription factors reported to date. This protein is found to be localized in both the nucleus and cytosol, suggesting the regulation of its traffic and the ability of functional interaction between CCG1 and CIB *in vivo* (Kuzuhara & Horikoshi, unpublished results). To elucidate the functional and structural roles of CIB at the atomic level, we have initiated crystallographic, biochemical and genetic studies. The three-dimensional structure of CIB will reveal the unique molecular action of CIB protein taking place in the cell. Here, we report the purification, crystallization and preliminary crystallographic study of the CIB protein in order to solve its three-dimensional structure.

2. Materials and methods

2.1. Protein expression and purification

The open reading frame of the gene encoding CIB was amplified using PCR with primers including a 5′ *Nde*I site and 3′ *Bam*HI site to facilitate cloning into the expression vector 6His-pET11d (Hoffmann & Roeder, 1991). In order to overexpress CIB protein, *E. coli* BL21(DE3) cells were transformed with the 6His-pET11d-CIB recombinant plasmid and grown at 300 K in TBGM9 medium (Horikoshi *et al.*, 1990) containing 50 µg ml^{-1} ampicillin until OD_{600} reached 0.6–0.8. Overexpression of CIB was induced by the addition of 0.4 m*M* IPTG. After 3 h of culture at 300 K,

Table 1
Data-collection statistics.

Resolution (Å)	30.0–2.2
No. of observed reflections	47199
No. of unique reflections	10809
R_{merge}† (%)	3.9 (10.5‡)
Completeness (%)	94.0 (88.6‡)
Temperature	Room temperature

† $R_{merge} = \sum|I(h) - \langle I(h)\rangle|/\sum I(h)$, where $I(h)$ is the observed intensity and $\langle I(h)\rangle$ is the mean intensity of reflection h over all measurements of $I(h)$. ‡ Last shell, 2.28–2.20 Å.

the cells were harvested by centrifugation (3000 rev min^{-1}, 10 min, 277 K), resuspended in a buffer containing 10 m*M* Tris–HCl pH 7.9 (277 K), 500 m*M* NaCl, 10 m*M* β-mercaptoethanol (β-ME), 0.5 m*M* phenylmethylsulfonyl fluoride and stored at 193 K until further use. The cell suspension was thawed and lysated by sonication. The cell lysate was centrifuged at 18 500 rev min^{-1} for 30 min at 277 K. The resulting supernatant was applied to Ni^{2+}-agarose (Invitrogen) and eluted with a buffer containing 0.2 *M* imidazole. For further purification, the eluted protein was fractionated by gel filtration (Sephacryl S-200) and analysed by SDS–PAGE, resulting in 98% homogeneity of CIB protein. The histidine tag was cleaved by thrombin and the digested protein was fractionated again by gel filtration to remove the tag and stored at 193 K.

2.2. Crystallization

Crystallization trials were initially performed by the hanging-drop/sitting-drop vapour-diffusion method at both 278 and 293 K. The drops were prepared by mixing 2 µl of protein solution (5 mg ml^{-1}) with 2 µl of reservoir solution in siliconized cover slides. Hampton Crystal Screens I and II (Hampton Research) were used to determine initial crystallization conditions. Extremely thin plate-like crystals grown in cluster forms were initially obtained within 3–4 d when using a precipitant solution containing 2.2 *M* ammonium sulfate, 2–4% PEG 400, 0.1 *M* Tris–HCl pH 8.0 at 293 K. Further screenings were accomplished by varying the pH, precipitant concentration, PEGs (PEG 400, PEG 1K, PEG 4K and PEG 6K) and temperature. In this search, crystals growing in cluster forms were minimized. However, the thickness of crystals was not greatly improved. Therefore, screenings were improved in conjunction with the addition of different additives from the Hampton Research additive-screening kit. The best large crystals were obtained using 2 µl of 5 mg ml^{-1} protein solution (10 m*M* Tris–HCl, 150 m*M* NaCl, 10 m*M* β-ME), 4 µl of reservoir solution (1.55 *M* ammonium sulfate, 12 m*M* urea, 80 m*M* Tris–HCl pH 7.5) and 2 µl of 5% glycerol. At 278 K, the crystals grew to dimensions of 0.4 × 0.25 × 0.05 mm in one week (Fig. 1).

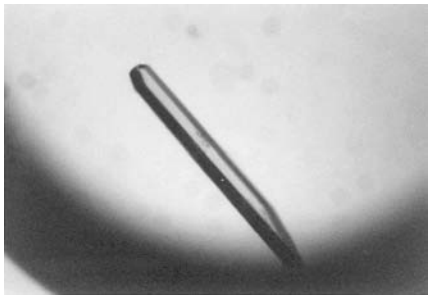

Figure 1
Photograph of human CIB crystal. Typical crystal dimensions are 0.4 × 0.25 × 0.05 mm.

2.3. Data collection and reduction

Initial inspection of the crystals was performed on an R-AXIS IV imaging-plate system mounted on a Rigaku rotating-anode generator equipped with a double-mirror focusing system, operated at 40 kV and 90 mA. The crystal diffracted to around 3.0 Å resolution, but suffered from severe radiation damage. In order to obtain a good data set, the data collection was carried out at room temperature using the Weissenberg camera for macromolecules (Sakabe, 1991) on beamline BL-18B at the synchrotron facility, 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 429.7 mm and the imaging-plate size was 200 × 400 mm. A complete data set was collected to a maximum resolution of 2.2 Å. All data were processed and scaled using the packages *DENZO* and *SCALEPACK* (Otwinowski & Minor, 1997).

3. Results

Thin plate-like crystals were grown using ammonium sulfate as the primary precipitant. As the CIB protein always precipitated in the presence of ammonium sulfate alone, an additional additive was essential along with ammonium sulfate to initiate the nucleation process. The rate of crystal growth of CIB protein was rapid and the crystals grew in cluster form. Though the rate of crystal growth and cluster formation could be controlled in the course of the first screening, the thickness of the crystals was not improved beyond 0.05 mm. After exhaustive screening, CIB protein crystals suitable for diffraction studies were obtained using 1.45–1.55 *M* ammonium sulfate, 12 m*M* urea and 80 m*M* Tris–HCl pH 7.5 at 278 K. The crystal belongs to the orthorhombic space group $P2_12_12_1$, with unit-cell parameters a = 43.60 (2), b = 44.45 (1), c = 110.70 (5) Å. A Matthews coefficient of 2.43 Å^3 Da^{-1} (Matthews, 1968) suggests one protein molecule in the asymmetric unit and a solvent content of 50%.

The crystal diffracted beyond 2.0 Å resolution using synchrotron radiation, but suffered substantial radiation damage during the course of X-ray exposure. The completeness of the data was 94.0% of the expected number of reflections to a resolution of 2.2 Å (Table 1).

We are currently searching for a cryo-protectant suitable for a cryo-experiment to overcome the crystal decay problems. We could not perform the molecular-replacement analysis of CIB protein because the sequence of human CIB protein does not show strong homology to any other known protein structures, except a weak similarity to bacterial hydrolase, whose three-dimensional structural data was not sufficient to determine the CIB structure. Thus, a search for suitable heavy-atom derivatives is now in progress in order to use the MIR method.

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