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Purification, crystallization and preliminary X-ray diffraction analysis of human oncoprotein SET/TAF-1β

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The human oncoprotein SET/TAF-1β has been crystallized by the sitting-drop vapour-diffusion method using ammonium sulfate as a precipitant. The crystal belongs to space group $C2$, with unit-cell parameters $a = 119.6$, $b = 62.8$, $c = 61.0$ Å, $\beta = 89.7^\circ$, and contains two molecules in the asymmetric unit. A complete data set was collected to 2.8 Å resolution using synchrotron radiation.

1. Introduction

SET/TAF-1β is a multifunctional molecule which is involved in many biological phenomena. The *set* gene was originally identified as a fusion gene with *can* in acute undifferentiated leukaemia (AUL). The *set–can* fusion gene is the product of the translocation (6;9)(p23q34) that is a hallmark of acute myeloid leukaemia. This translocation usually results in the formation of a *dek–can* fusion gene on chromosome 6p−. In the case of AUL, one of the classes of acute myeloid leukaemia, *set* is fused to *can* instead of *dek* (Von Lindern *et al.*, 1992; Adachi *et al.*, 1994). The *set* gene is suggested to play a key role in the leukaemogenesis of AUL.

TAF-1β was independently characterized as a host factor that stimulates adenovirus core DNA replication (Matsumoto *et al.*, 1993). Cloning of TAF-1β revealed that it is encoded by the *set* gene (Nagata *et al.*, 1995). Further studies showed that SET/TAF-1β is a multifunctional factor which is involved in transcription (Matsumoto *et al.*, 1995), silencing (Cervoni *et al.*, 2002) and apoptosis (Fan *et al.*, 2003). SET/TAF-1β was also characterized as a histone chaperone that has been suggested to be involved in alteration of chromatin structure (Kawase *et al.*, 1996). Since SET/TAF-1β inhibits the acetylation of histones (Seo *et al.*, 2001) and DNA-binding transcription factors (Miyamoto *et al.*, 2003) as well as the methylation of DNA (Cervoni *et al.*, 2002), SET/TAF-1β may therefore function as a regulator of transcription and replication by affecting the chemical modifications of nucleosome and transcription factors.

We recently isolated SET/TAF-1β as an interacting factor of the DNA-binding domain of transcription factors Sp1 and KLF5 (Suzuki *et al.*, 2003; Miyamoto *et al.*, 2003). SET/TAF-1β inhibits the DNA binding of Sp1 and KLF5, consequently down-regulating their transcriptional activities, contrary to the case of the coactivator/acetyltransferase p300 (Suzuki *et al.*, 2000). Furthermore, SET/TAF-1β inhibits the acetylation of KLF5 by p300. Therefore, SET/TAF-1β is suggested to function as a multifunctional factor by interacting with DNA-binding proteins.

To analyze the molecular action of SET/TAF-1β at the atomic level, we have initiated structural studies of SET/TAF-1β. We utilized the protein without its acidic stretch (amino acids 1–225) because the acidic stretch is difficult to crystallize; importantly, however, the protein is still functional and shows negative effects on DNA binding, acetylation and transcriptional activity of KLF5 (Miyamoto *et al.*, 2003). Here, we report the purification, crystallization and preliminary crystallographic analysis of human SET/TAF-1β.

2. Materials and methods

2.1. Protein expression and purification

To overexpress SET/TAF-1β, *Escherichia coli* BL21 (DE3) pLysS (Stratagene) cells were transformed with the pET14b-SET/TAF-1β (amino acids 1–225) recombinant plasmid (Nagata *et al.*, 1995). Transformed cells were grown at 300 K in TBG-M9 medium containing 100 μg ml^{-1} ampicillin and 34 μg ml^{-1} chloramphenicol until OD_{595} reached 0.6. Overexpression of SET/TAF-1β was induced by the addition of 0.4 m*M* IPTG. After a 3 h culture at 300 K, the cells were harvested by centrifugation (3000 rev min^{-1}, 10 min, 277 K), resuspended in buffer *A* containing 20 m*M* Tris–HCl (pH 7.9 at 277 K), 10% glycerol, 500 m*M* NaCl, 50 m*M* 2-mercaptoethanol, 0.5 m*M* PMSF, 10 μg ml^{-1} leupeptin and 10 μg ml^{-1} pepstatin A and then lysed by EmulsiFlex-C5 (Avestin). The cell lysate was centrifuged at 24 000 rev min^{-1} for 30 min at 277 K. The supernatant was then applied to ProBond resin (Invitrogen); after washing the resin, the protein was eluted with buffer *A* containing 0.2 *M* imidazole. For further purification, the eluted protein was concentrated by

Centriprep YM-30 (Millipore) and then fractionated by gel filtration on a HiLoad 26/60 Superdex 200 column (Amersham Pharmacia). The pooled SET/TAF-1β protein was concentrated to 70 mg ml^{-1} by Centriprep YM-30 (Millipore). The purity of SET/TAF-1β was examined by SDS–PAGE (Fig. 1). Although the molecular weight of SET/TAF-1β is calculated to be 28 kDa from the sequence, the SET/TAF-1β protein was detected as a molecule of around 38 kDa by SDS–PAGE.

2.2. Crystallization

Crystallization trials were initially performed by the hanging-drop vapour-diffusion method at 293 K. Hampton Crystal Screen and Crystal Screen 2 kits (Hampton Research) were used to determine the initial crystallization conditions. The drop was prepared by mixing 1 µl of protein solution with 1 µl of reservoir solution. Twinned thin plate-like crystals were grown within one month using a solution containing 2.0 *M* ammonium sulfate, 0.1 *M* sodium citrate buffer pH 5.4 and 0.2 *M* potassium/sodium tartrate. Further screenings to obtain single crystals were accomplished by varying the pH, the concentration of both the precipitant and potassium/sodium tartrate, and by adding different additives. The best large crystals were obtained using 5 µl of 70 mg ml^{-1} protein solution (20 m*M* Tris–HCl, 100 m*M* NaCl, 10 m*M* 2-mercaptoethanol), 5 µl reservoir solution (2.75 *M* ammonium sulfate, 0.1 *M* sodium citrate buffer pH 5.4, 0.2 *M* potassium/sodium tartrate and 30 m*M* $MgCl_2$). At 293 K, crystals grew to approximate dimensions of 0.3 × 0.2 × 0.02 mm in one week (Fig. 2). A solution containing artificial mother liquor (2.75 *M* ammonium sulfate, 0.1 *M* sodium citrate buffer pH 5.4, 0.2 *M* potassium/sodium tartrate and 30 m*M* $MgCl_2$) and 30%(*w*/*v*) trehalose was used as a cryoprotectant. Instead of trehalose, 30% glycerol and 30% glucose were also tried, but yielded poor results.

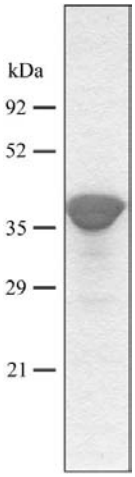

Figure 1
SDS–PAGE of SET/TAF-1β stained with Coomassie Brilliant Blue.

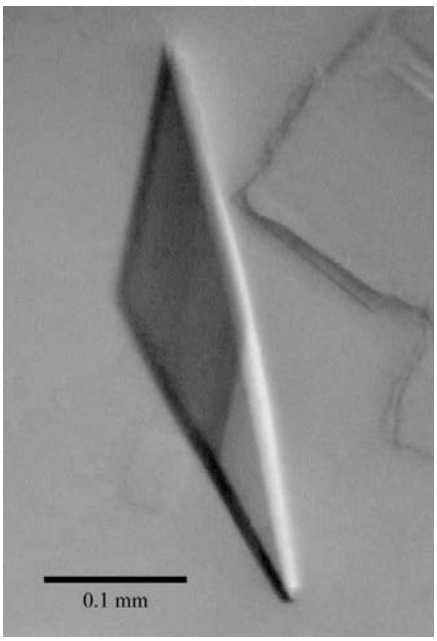

Figure 2
Crystal of human oncoprotein SET/TAF-1β.

2.3. Data collection

Initial inspection of the crystals was performed on an R-AXIS IV^{++} imaging-plate system mounted on a Rigaku rotating-anode X-ray generator (FR-D) operated at 50 kV and 60 mA. The crystal diffracted to around 3.5 Å resolution. In order to obtain a better data set, the data were collected from a single crystal on beamline 6A using an ADSC Quantum 4R CCD detector at the Photon Factory, Tsukuba, Japan. The wavelength used was 0.9780 Å and the incident beam was collimated to a diameter of 0.2 mm. The crystal-to-detector distance was set to 250 mm. A complete data set was collected to a maximum resolution of 2.8 Å (Fig. 3). All data were processed and scaled using the programs *MOSFLM* and *SCALA* from the *CCP*4 suite (Collaborative Computational Project, Number 4, 1994).

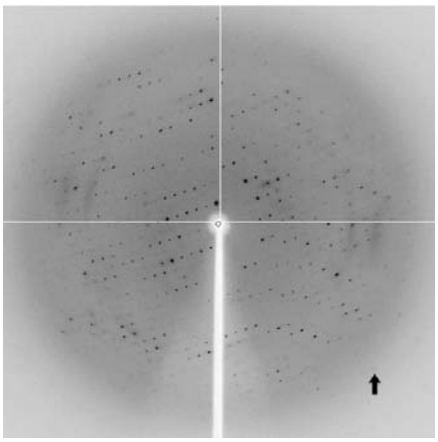

Figure 3
A 1° oscillation diffraction pattern from a crystal of SET/TAF-1β. The arrow indicates a resolution of 2.80 Å.

Table 1
Data-collection and processing statistics.

Values in parentheses are for the last shell, 2.95–2.80 Å.

Space group	*C*2
Unit-cell parameters (Å)	a = 119.6, b = 62.8, c = 61.0, β = 89.7°
Resolution range (Å)	42.64–2.80
No. measured reflections	67843
No. unique reflections	11065
$R_{\rm merge}$† (%)	6.1 (27.7)
Completeness (%)	98.4 (98.2)
Average $I/\sigma(I)$	7.2 (2.7)

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

3. Results

Examination of diffraction data from human SET/TAF-1β crystals revealed that the crystals diffracted to beyond 3 Å resolution and belonged to space group *C*2, with unit-cell parameters a = 119.6, b = 62.8, c = 61.0 Å, β = 89.7°. Data-collection statistics are summarized in Table 1. A total of 67 843 measured reflections were merged into 11 065 unique reflections with an $R_{\rm merge}$ of 6.1%. The merged data set is 98.4% complete to 2.8 Å resolution. A value for the Matthews coefficient of 2.3 Å^3 Da^{-1} and a solvent content of 46% were obtained assuming two molecules in the asymmetric unit and a molecular weight of 24 750 Da. An attempt to solve the structure using the MAD method is in progress.

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