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Crystallization and preliminary crystallographic analysis of a PHD domain of human JARID1B

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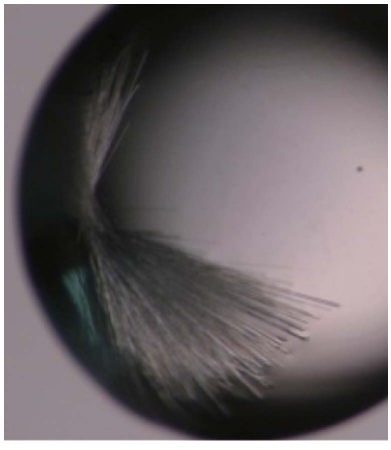
Histone lysine methylation can be removed by proteins containing JmjC domains in a sequence- and methylation state-specific manner. JARID1B, a protein containing PHD and JmjC domains, is a histone demethylase specific for H3K4me2 and H3K4me3 which requires Fe(II) and α-ketoglutarate (α-KG) as cofactors to remove the methyl group. JARID1B has also been shown to play a critical role in the development of breast cancer. JARID1B contains JmjN, Arid and JmjC domains, a C5HC2 zinc-finger domain and three PHD domains. The first PHD domain ($PHD1_{JARID1B}$; residues 306–360) is located at the N-terminus and is important for both histone demethylase activity and histone-tail recognition of JARID1B. Here, the expression, purification and crystallization of $PHD1_{JARID1B}$ is reported. A $PHD1_{JARID1B}$ crystal was grown by the hanging-drop vapour-diffusion method in reservoir solution consisting of 0.1 *M* HEPES pH 7.0, 2.2 *M* ammonium sulfate at 277 K. A zinc SAD data set was collected from a $PHD1_{JARID1B}$ crystal. The diffraction pattern of the $PHD1_{JARID1B}$ crystal extended to 1.65 Å resolution using synchrotron radiation. The crystal belonged to space group $P4_3$, with unit-cell parameters a = 51.7, b = 51.7, c = 36.2 Å.

1. Introduction

Histone methylation is recognized as an important modification that is linked to chromatin and transcription regulation as well as DNA-damage response. Five lysine (K) residues of histone H3 (H3K4, H3K9, H3K27, H3K36 and H3K79) and Lys20 of histone H4 (H4K20) have been identified to be sites for this modification (Margueron *et al.*, 2005; Zhang & Reinberg, 2001). In general, methylation at H3K9, H3K27 and H4K20 is associated with transcription repression, and methylation at H3K4, H3K36 and H3K79 is associated with transcription activation (Zhang & Reinberg, 2001; Martin & Zhang, 2005). The first histone demethylase, LSD1 (lysine-specific demethylase 1), was identified in 2004 (Shi *et al.*, 2004). To date, two distinct classes of demethylases have been characterized. The first class, represented by LSD1, use FAD as a cofactor through an amine-oxidation reaction to remove the lysine methyl group. The second class are the family of JmjC-domain-containing demethylases, which require Fe^{II} and α-ketoglutarate (α-KG) as cofactors in the demethylation process (Shi & Whetstine, 2007; Whetstine *et al.*, 2006; Tsukada *et al.*, 2006; Cloos *et al.*, 2006; Chen *et al.*, 2006).

Over 30 human JmjC-domain-containing proteins have been found through analysis of public protein-domain databases (Klose, Kallin *et al.*, 2006; Shin & Janknecht, 2007; Xiang, Zhu, Han, Ye *et al.*, 2007). Many of these proteins have been identified to be histone lysine demethylases (Lee, Villa *et al.*, 2007; Lee, Norman *et al.*, 2007; Xiang, Zhu, Han, Lin *et al.*, 2007; Lan *et al.*, 2007; Christensen *et al.*, 2007; Cloos *et al.*, 2006; Iwase *et al.*, 2007; Klose, Yamane *et al.*, 2006; Klose *et al.*, 2007; Whetstine *et al.*, 2006; Yamane *et al.*, 2007; Agger *et al.*, 2007). The structures of several representative JmjC-domain-containing histone lysine demethylase family members, including JMJD2A, PHF8, Kiaa1718 and JHDM1A, have been determined in



the apo form or in complex with the substrate peptide and α-KG (Horton *et al.*, 2010; Chen *et al.*, 2006, 2007; Couture *et al.*, 2007; Ng *et al.*, 2007; Yang *et al.*, 2010; Han *et al.*, 2007). However, no structure has been reported for a member of the JARID1 subgroup which can specifically remove trimethylated histone H3K4.

JARID1-subgroup members are highly conserved from yeast to humans and contain a similar domain architecture comprising JmjN, Arid and JmjC domains, a C5HC2 zinc-finger domain and two or three PHD domains (designated PHD1, PHD2 and PHD3 from the N-terminus to the C-terminus). There are four JARID1-group members in mammals, JARID1A (RBP2), JARID1B (PLU-1), JARID1C (SMCX) and JARID1D (SMCY), which have all been identified to be demethylases for trimethylated and dimethylated H3K4 (Iwase *et al.*, 2007; Christensen *et al.*, 2007; Klose *et al.*, 2007; Tahiliani *et al.*, 2007; Lee, Norman *et al.*, 2007; Yamane *et al.*, 2007). The four proteins are all important transcriptional corepressors since they remove the transcription-activating mark trimethylated H3K4. Studies of human malignancies show that JARID1B plays a critical role in the development of breast cancer (Lu *et al.*, 1999; Barrett *et al.*, 2002; Yamane *et al.*, 2007).

Among the recognizable domains in JARID1B, PHD domains are predicted to be involved in histone-tail recognition. JARID1B contains three PHD domains: PHD1 is the first PHD domain, located between the Arid and JmjC domains, while the PHD2 and PHD3 domains are located at the C-terminus. Recent studies of Lid, a homologue of JARID1B in *Drosophila*, found that PHD1 is required for demethylase activity towards H3K4me3, while PHD2 and PHD3 are not (Li *et al.*, 2010). Interestingly, unbiased peptide screening for PHD1 interaction indicates that PHD1 can specifically bind to histone H3, either unmodified or with K9 methylation, suggesting that PHD1 plays an important role in the nucleosome-localization and demethylase activity of JARID1B (Li *et al.*, 2010). Given the highly conserved sequence homology of PHD1 of Lid and JARID1B (58% identity, 80% homology), $PHD1_{JARID1B}$ may also be important for the function of JARID1B. We have found that $PHD1_{JARID1B}$ indeed plays an important role in histone-tail recognition and histone demethylase activity (unpublished data). Here, we report the expression, purification and crystallization of $PHD1_{JARID1B}$. The structure will reveal the mechanism of histone-tail recognition by $PHD1_{JARID1B}$.

2. Materials and methods

2.1. Protein expression and purification

The ORF of $PHD1_{JARID1B}$ (residues 306–360 of JARID1B; NP_006609.3; *Homo sapiens*) was engineered into modified pGEX-6P-1 vector (GE Healthcare) using *Bam*HI and *Xho*I restriction sites. The construct was verified by DNA sequencing and the plasmid was transformed into *Escherichia coli* strain BL21 (DE3) competent cells. The transformants were grown at 310 K to an OD_{600} of 1.0 in Luria broth medium containing 100 µg ml^{-1} ampicillin and were induced by the addition of 0.2 m*M* isopropyl β-D-1-thio-galactopyranoside. $PHD1_{JARID1B}$ was overexpressed as a fusion protein with a GST tag at the N-terminus. After a further 12 h incubation at 288 K, the cells were pelleted and resuspended in lysis buffer consisting of 25 m*M* Tris pH 8.0, 150 m*M* NaCl supplemented with DNAse and protease inhibitors. Cells were lysed on ice using a French press and the solution was clarified by centrifugation at 15 000 rev min^{-1} for 30 min at 277 K. The supernatant was applied onto a Glutathione Sepharose 4B column (1 ml resin per column; GE Healthcare) equilibrated with lysis buffer. After washing with buffer containing 25 m*M* Tris pH 8.0, 150 m*M* NaCl, the fusion protein was digested on the column with PreScission (3C) protease overnight at 277 K. The molecular weight of $PHD1_{JARID1B}$ is 6.1 kDa including the additional Gly-Pro-Leu-Gly-Ser sequence from 3C cleavage. The eluted protein was then loaded onto an Source 15Q anion-exchange column (GE Healthcare) and eluted with a linear gradient of 0–0.5 *M* NaCl at a flow rate of 10 ml min^{-1}. The peak fractions were collected and further purified by gel-filtration chromatography on a Superdex 200 10/300 GL column (GE Healthcare) with buffer consisting of 10 m*M* Tris pH 8.0, 50 m*M* NaCl and 3 m*M* DTT. $PHD1_{JARID1B}$-containing fractions were concentrated to 40 mg ml^{-1} using an ultracentrifugal filter tube (Millipore) and used for crystallization.

2.2. Protein crystallization

Initial crystallization trials were performed using Crystal Screen, Index, SaltRX and PEG/Ion kits from Hampton Research and Wizard I and II kits from Emerald BioSystems at 277 K. These initial screens were set up using the hanging-drop vapour-diffusion method by mixing 1 µl protein solution and 1 µl reservoir solution. The initial conditions yielded clusters of needle-shaped crystals of about 0.02 mm in width. The crystal conditons were further optimized by variation of

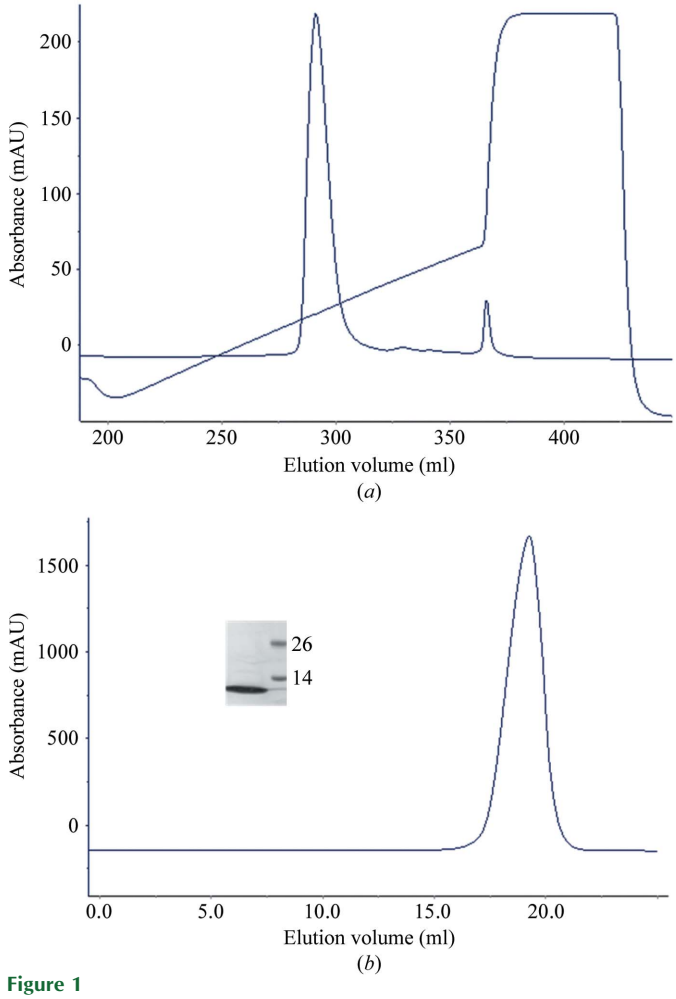


Figure 1
$PHD1_{JARID1B}$ protein purification. (*a*) Ion-exchange (Source 15Q; GE Healthcare) profile of $PHD1_{JARID1B}$ protein. (*b*) Gel-filtration (Superdex 200 10/300 GL; GE Healthcare) profile of $PHD1_{JARID1B}$ protein. Inset: one of the peak fractions was analyzed by SDS–PAGE with Coomassie Blue staining. The left lane contained the peak fraction and molecular weights are indicated in kDa in the right lane.

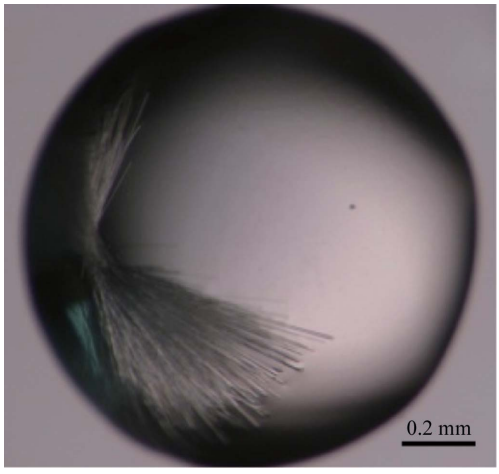


Figure 2
Crystals of PHD1$_{\text{JARID1B}}$ grown in 0.1 *M* HEPES pH 7.0, 2.2 *M* ammonium sulfate at 277 K.

the pH, protein concentration, precipitants and additives. Approximately 200 conditions were set up to optimize crystallization.

2.3. Data collection and processing

All crystals were mounted in nylon loops and flash-cooled in liquid nitrogen using the reservoir buffer as cryoprotectant. Data collection was carried out on BL17U at Shanghai Synchrotron Radiation Facility (SSRF, People's Republic of China) using a MAR CCD MX-225 detector. The wavelength of the radiation was 1.2823 Å and the crystal-to-detector distance was 90 mm. The exposure time for each frame was 1 s with 1° oscillation and 180 frames were collected. The data were indexed, integrated and scaled using *HKL*-2000 (Otwinowski & Minor, 1997).

Table 1
Diffraction data statistics for PHD1$_{\text{JARID1B}}$.

Values in parentheses are for the highest resolution shell.

Wavelength (Å)	1.2823
Resolution (Å)	50.00–1.65 (1.71–1.65)
Space group	$P4_3$
Unit-cell parameters (Å)	a = 51.7, b = 51.7, c = 36.2
Completeness (%)	96.7 (80.5)
R_{merge}† (%)	7.8 (37.4)
$\langle I/\sigma(I)\rangle$	46.9 (3.1)
Multiplicity	6.8 (4.7)
Total No. of reflections	77112 (4441)
No. of unique reflections	11340 (945)

† $R_{\text{merge}} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$, where $I_i(hkl)$ is the ith measurement of the intensity of reflection hkl and $\langle I(hkl)\rangle$ is the mean intensity of reflection hkl.

3. Results and discussion

Recombinant PHD1$_{\text{JARID1B}}$ protein was expressed, purified to homogeneity (Fig. 1) and used for crystallization. The purified PHD1$_{\text{JARID1B}}$ protein was estimated to have a purity of >95% (inset in Fig. 1*b*). Initial screening was performed at 277 K and crystals appeared in several conditions as clusters of needle-shaped crystals of about 0.02 mm in width. Most of the initial crystallization conditions contained ammonium sulfate, which was chosen to be the essential component for further optimization. After a further optimization screen of solution buffer pH, precipitants and additives, we obtained crystals that were suitable for diffraction. The crystals (Fig. 2) used for data collection were obtained by mixing 1 µl protein solution (40 mg ml^{-1}) with 1 µl reservoir solution consisting of 0.1 *M* HEPES pH 7.0, 2.2 *M* ammonium sulfate at 277 K. The crystals appeared after four weeks.

All PHD domains contain two or three Zn atoms according to their primary sequences. In order to solve the phase problem, anomalous data at a wavelength of 1.2823 Å were collected for zinc SAD (Fig. 3). The diffraction extended to 1.65 Å resolution. The crystals belonged to space group $P4_3$, with unit-cell parameters a = 51.7, b = 51.7,

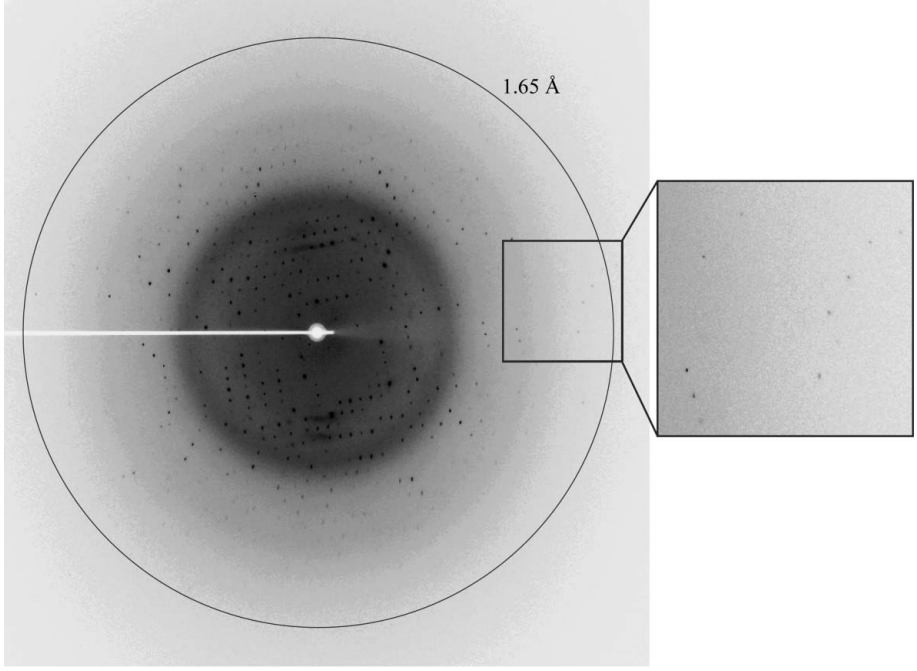


Figure 3
X-ray diffraction pattern of the PHD1$_{\text{JARID1B}}$ crystal. The ring indicates a resolution of 1.65 Å. An enlargement of the region in the box is shown on the right.

c = 36.2 Å. After initial data processing, two molecules were predicted to be present in the asymmetric unit. The estimated solvent content was 38% and the Matthews coefficient was 1.99 Å^3 Da^{-1}. Based on sequence analysis, PHD1$_{\rm JARID1B}$ contains two zinc ions in each monomer. Data-collection statistics are summarized in Table 1. The PHD domain of SmcY protein (PDB entry 2e6r; S. Kadirvel, F. He, Y. Muto, M. Inoue, T. Kigawa, M. Shirouzu, T. Terada & S. Yokoyama, unpublished work) shows 81% sequence similarity and 57% identity to PHD1$_{\rm JARID1B}$. Therefore, it should be possible to solve the PHD1$_{\rm JARID1B}$ structure by molecular replacement.

The crystal structure of PHD1$_{\rm JARID1B}$ will deepen our understanding of the molecular mechanism of histone-tail recognition by JARID1B.

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