Acta Crystallographica Section F
Structural Biology and Crystallization Communications
ISSN 1744-3091

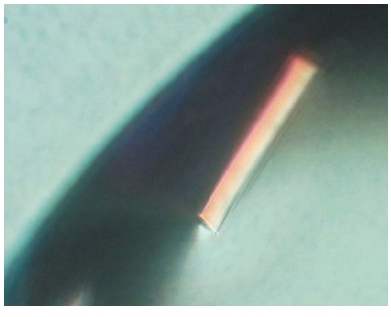
Purification, crystallization and preliminary X-ray diffraction of the C-terminal bromodomain from human BRD2

Takashi Umehara,[a] Masatoshi Wakamori,[a] Akiko Tanaka,[a] Balasundaram Padmanabhan[a]* and Shigeyuki Yokoyama[a,b]*

[a]Genomic Sciences Center, Yokohama Institute, RIKEN, 1-7-22 Suehiro-cho, Tsurumi, Yokohama 230-0045, Japan, and [b]Department of Biophysics and Biochemistry, Graduate School of Science, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan

Correspondence e-mail: paddy@gsc.riken.jp, yokoyama@biochem.s.u-tokyo.ac.jp

Received 23 April 2007
Accepted 11 June 2007



BRD2 is a bromodomain-containing BET-family protein that associates with acetylated histones throughout the cell cycle. Although the tertiary structures of the bromodomains involved in histone acetyl transfer are already known, the structures of the BET-type bromodomains, which are required for tight association with acetylated chromatin, are poorly understood. Here, the expression, purification and crystallization of the C-terminal bromodomain of human BRD2 are reported. The protein was crystallized by the sitting-drop vapour-diffusion method in the orthorhombic space group $P2_12_12$, with unit-cell parameters $a = 71.78$, $b = 52.60$, $c = 32.06$ Å and one molecule per asymmetric unit. The crystal diffracted beyond 1.80 Å resolution using synchrotron radiation.

1. Introduction

Eukaryotic genomic DNA forms a nucleoprotein complex with core histone particles, composed of H2A, H2B, H3 and H4 (Kornberg & Lorch, 1999). Each core histone has N- and C-terminal flanking tail regions and a central core domain. Several amino acids in the histone-tail regions are covalently modified by various enzymes (Strahl & Allis, 2000). The combinations of the histone-tail modifications are the key to determining the active or inactive state of the nucleosome and the particular DNA-mediated reaction(s) (Strahl & Allis, 2000; Roth *et al.*, 2001; Fischle *et al.*, 2003). Acetylation of the histone tails is one of the important hallmarks for the activation of the nucleosomal state (Roth *et al.*, 2001). The acetylated N-terminal tails of the histones are specifically recognized by the bromodomain, which is present in different histone-associated factors, including acetyl-transferases, chromatin-remodelling factors and BET-family proteins (Haynes *et al.*, 1992; Zeng & Zhou, 2002).

The BET family of nuclear proteins contain two tandem bromodomains and an additional ET domain (Dey *et al.*, 2000). BRD2, a BET-family member, selectively interacts with the acetylated lysine 12 of histone H4 through its bromodomains and leads to transcription activation *in vivo* (Kanno *et al.*, 2004). Notably, BRD2 strongly associates with acetylated chromatin during mitosis, in contrast to the observation that other non-BET bromodomain proteins dissociate from the chromatin during mitosis (Kanno *et al.*, 2004). Although this retention on chromosomes during mitosis is supposed to be a unique feature of the BET-family proteins (Dey *et al.*, 2000, 2003; Kanno *et al.*, 2004), it is still unclear how the BET-type bromodomains recognize the acetylated histone. We recently showed that the N-terminal bromodomain of BRD2 forms a dimer in the crystal as well as in solution (Nakamura *et al.*, 2007). To understand the molecular mechanism of the association of BRD2 with mitotic chromosomes, we have studied the C-terminal bromodomain structure of human BRD2 (amino-acid residues 348–455; hereafter, we refer to this bromodomain as BD2). In this report, we describe the expression, purification, crystallization and preliminary crystallographic studies of the BRD2 bromodomain BD2.

2. Materials and methods

2.1. Construction of the expression vector

The cDNA encoding the C-terminal bromodomain of human BRD2 (BD2; residues 348–455) was amplified by PCR using a human BRD2 cDNA clone (OriGene Technologies, Inc.) with forward primer 5′-GGGATGCGGGCATATGCAGGATCCGGAACAGTTAAAACATTGC-3′ and reverse primer 5′-GGGATGCGGGCTCGAGTCATTAATCTGGCATCTTGGCATAAC-3′. The amplified cDNA fragment was digested with *Nde*I and *Xho*I and was ligated into the pET15b vector (Novagen). The orientation and the complete coding sequence of this subclone, pET15b-BRD2BD2, were confirmed by DNA sequencing.

2.2. Expression and purification

Escherichia coli strain BL21 Star (DE3) (Invitrogen) was transformed with pET15b-BRD2BD2 and grown at 303 K in LB medium containing 50 µg ml^{-1} ampicillin until the OD_{600} reached 0.7–0.8. Overexpression of the six-His-tagged BD2 protein was induced by the addition of 1.0 m*M* IPTG. After a 3 h culture at 303 K, the cells were harvested by centrifugation, resuspended in buffer containing 20 m*M* MES pH 5.5, 20 m*M* imidazole and 100 m*M* NaCl and lysed using a sonicator. The cell lysate was centrifuged at 16 000*g* for 20 min at 277 K. The supernatant was filtered using a MILLEX-HV PVDF 0.45 µm filter (Millipore) and was applied onto a column containing HisTrap resin (GE Healthcare). The six-histidine-tagged BD2 protein was eluted with buffer containing 20 m*M* MES pH 5.5, 500 m*M* imidazole and 100 m*M* NaCl and the tag was cleaved at 277 K for 16 h using 500 units of thrombin per approximately 20 mg of BD2 protein. The tag-cleaved BD2 protein has three non-native residues (Gly-Ser-His) at its N-terminus. This reaction mixture was loaded onto a HiPrep 26/10 desalting column (GE Healthcare) and the fraction containing the BD2 protein was eluted with a buffer containing 20 m*M* MES pH 5.5, 100 m*M* NaCl and 2 m*M* DTT. The thrombin was removed using a Benzamidine-Sepharose 6B column (GE Healthcare). The BD2 fraction was loaded onto a 1 ml column of HiTrap SP (GE Healthcare), which was eluted using a linear gradient of NaCl (from 0.1 to 1.0 *M*). The eluted BD2 fraction was further loaded onto a gel-filtration HiLoad 16/60 Superdex 75 column (GE Healthcare) and the BD2 protein was eluted as a single peak. The BD2 protein was analyzed by SDS–PAGE and Coomassie Brilliant Blue staining (Fig. 1).

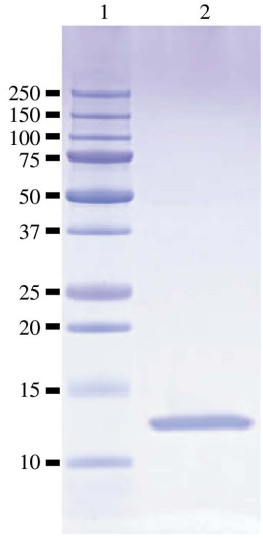


Figure 1
Purification of the C-terminal bromodomain of human BRD2: an SDS–PAGE profile of the purified BD2 bromodomain. Lane 1, molecular-weight markers. The size of each protein band is shown on the left in kDa. Lane 2, purified BD2 bromodomain fraction. Electrophoresis was performed with a 17.5% SDS polyacrylamide gel, which was stained with Coomassie Brilliant Blue.

Table 1
Data-collection and processing statistics.

Values in parentheses are for the last shell (1.86–1.80 Å).

Space group	$P2_12_12$
Unit-cell parameters (Å)	a = 71.78, b = 52.60, c = 32.06
Resolution range (Å)	50–1.80
Wavelength (Å)	1.0
No. of measured reflections	184981
No. of unique reflections	11743
$R_{\rm merge}$† (%)	7.2 (24.7)
Completeness (%)	99.5 (95.2)
Redundancy	6.8 (6.0)

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

2.3. Crystallization

Crystallization trials were performed by the sitting-drop vapour-diffusion method at 293 K. The drops were prepared by mixing 1.0 µl BD2 protein solution (6–9 mg ml^{-1}) in 20 m*M* Tris–HCl buffer pH 8.0 containing 150 m*M* NaCl and 2 m*M* DTT with 1.0 µl reservoir solution on siliconized cover slides and were equilibrated against 500 µl reservoir solution. Typical single crystals appeared within 3–7 d in a precipitant solution containing 30–34% PEG MME 2000 and 0.1 *M* Tris–HCl pH 8.0 (Hampton Research) (Fig. 2).

2.4. Data collection

The crystal was briefly soaked in a cryoprotectant solution containing 30% PEG MME 2000, 0.1 *M* Tris–HCl pH 8.0 and 10% glycerol (Hampton Research). Diffraction data were collected from a single crystal using a Jupiter CCD detector on beamline BL26B1 at SPring-8, Harima, Japan. The wavelength used was 1.0 Å and the incident beam was collimated to 0.1 mm in diameter. The crystal-to-

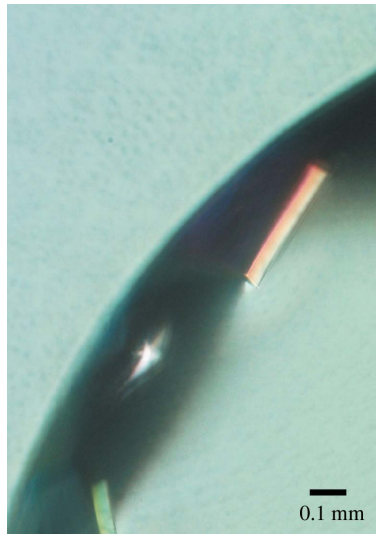


Figure 2
Single crystal of the human BRD2 bromodomain BD2.

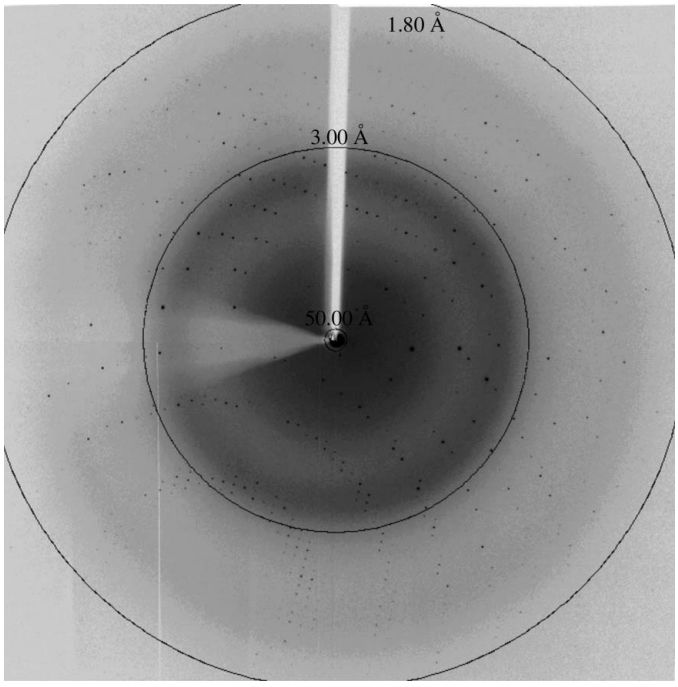


Figure 3
X-ray diffraction pattern of the BRD2 BD2 crystal collected at beamline BL26B1, SPring-8. The crystal-to-detector distance was 170 mm, the oscillation angle was 1.0° and the exposure time was 10 s.

detector distance was set to 170 mm and the oscillation range was 1.0° with an exposure time of 10 s. A complete data set was collected in a nitrogen-gas stream at 100 K to a maximum resolution of 1.80 Å. Diffraction images were processed and scaled using the *HKL*-2000 suite (Otwinowski & Minor, 1997).

3. Results

The bromodomain BD2 of human BRD2 was purified to more than 95% homogeneity, as judged by the SDS–PAGE profile (Fig. 1), and crystallization screening was performed using the protein sample. Single crystals with dimensions of approximately 0.1 × 0.5 × 0.5 mm were obtained using PEG MME 2000 as the major precipitant (Fig. 2). The crystal diffracted to beyond 1.80 Å resolution using synchrotron radiation (Fig. 3). The crystals belong to the monoclinic space group $P2_12_12$, with unit-cell parameters $a = 71.78$, $b = 52.60$, $c = 32.06$ Å. The data-collection statistics of the processed data are summarized in Table 1. A total of 184 981 measured reflections were merged into 11 743 unique reflections with an $R_{\rm merge}$ of 7.2%. The merged data set is 99.5% complete to 1.80 Å resolution. A Matthews coefficient value of 2.5 Å^3 Da^{-1} (Matthews, 1968), with a solvent content of 51%, was obtained by assuming the presence of one molecule in the asymmetric unit and a molecular weight of about 12 000 Da. The molecular-replacement procedure, using the program *MOLREP* (Vagin & Teplyakov, 1997) from the *CCP*4 suite (Collaborative Computational Project, Number 4, 1994), was employed to determine the BD2 structure. The BD2 structure of BRD2 was solved using the N-terminal bromodomain structure of BRD2 (Nakamura *et al.*, 2007), which possesses about 44% sequence identity, as a model. Crystallographic refinement is now in progress.

We thank Drs M. Yamamoto and G. Ueno for technical support in the use of beamline BL26B1, SPring-8, Japan, Dr Y. Nakamura for help with data collection and Ms K. Tsuganezawa and Dr Y. Otta for technical assistance. We are grateful to Ms T. Nakayama, Ms K. Yajima and Ms A. Ishii for their clerical assistance. This work was supported in part by the RIKEN Structural Genomics/Proteomics Initiative (RSGI), the National Project on Protein Structural and Functional Analyses, Ministry of Education, Culture, Sports, Science and Technology of Japan.

References

Collaborative Computational Project, Number 4 (1994). *Acta Cryst.* D**50**, 760–763.
Dey, A., Chitsaz, F., Abbasi, A., Misteli, T. & Ozato, K. (2003). *Proc. Natl Acad. Sci. USA*, **100**, 8758–8763.
Dey, A., Ellenberg, J., Farina, A., Coleman, A. E., Maruyama, T., Sciortino, S., Lippincott-Schwartz, J. & Ozato, K. (2000). *Mol. Cell. Biol.* **20**, 6537–6549.
Fischle, W., Wang, Y. & Allis, C. D. (2003). *Curr. Opin. Cell Biol.* **15**, 172–183.
Haynes, S. R., Dollard, C., Winston, F., Beck, S., Trowsdale, J. & Dawid, I. B. (1992). *Nucleic Acids Res.* **20**, 2603.
Kanno, T., Kanno, Y., Siegel, R. M., Jang, M. K., Lenardo, M. J. & Ozato, K. (2004). *Mol. Cell*, **13**, 33–43.
Kornberg, R. D. & Lorch, Y. (1999). *Cell*, **98**, 285–294.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
Nakamura, Y. *et al.* (2007). *J. Biol. Chem.* **282**, 4193–4207.
Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.
Roth, S. Y., Denu, J. M. & Allis, C. D. (2001). *Annu. Rev. Biochem.* **70**, 81–120.
Strahl, B. D. & Allis, C. D. (2000). *Nature (London)*, **403**, 41–45.
Vagin, A. & Teplyakov, A. (1997). *J. Appl. Cryst.* **30**, 1022–1025.
Zeng, L. & Zhou, M. M. (2002). *FEBS Lett.* **513**, 124–128.