

1H, 13C and 15N resonance assignments of TbBDF5-bromo1 domain from Trypanosoma brucei

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Research Article

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Abstract

^1H , ^{13}C and ^{15}N resonance assignments are presented for the first bromo domain of TbBDF5 (TbBDF5-bromo1) from *Trypanosoma brucei*. TbBDF5 is localized in the nucleus and plays a potential role in transcription regulation. Bromo domain can recognize acetylated histone through a conserved binding pocket. Here we report the NMR resonance assignments of TbBDF5-bromo1 domain for further studies of the relationship between its structure and function.

Biological Context

Bromo domain has been identified to play an essential role in gene expression in all kinds of eukaryotes including protozoan parasites. Bromodomain-containing proteins, which can exist alone or as part of a larger protein complex, have a variety of physiological functions. These proteins participate in histone modifications, act as components of chromatin remodeling complexes and serve as scaffolds that control the recruitment of other transcriptional regulators to chromatin (Fujisawa and Filippakopoulos 2017). Bromo domain is a kind of motif recognizing acetylated lysine of histones that occur in the N-terminal regions of proteins (Zeng and Zhou 2002). It is well known that histone lysine acetylation can neutralize the positive charge of histone, increase hydrophobicity and bind to the conserved amino acid residues in the hydrophobic pocket of bromo domain. The bromo domain is evolutionarily conserved, consists of about 110 amino acids and shares a globular fold that comprises a left-handed bundle of four α -helices linked by two variable-loop regions, which is conserved across all bromo domains (Filippakopoulos et al. 2012). A number of evidences have demonstrated that bromodomain-containing protein can be used as targets for the treatment of cancer and other diseases (Alonso et al. 2019; Schulz et al. 2015).

Trypanosoma brucei (*T. brucei*) is a single-cell eukaryote, which is different from other eukaryotic cells and has unique characteristics in gene transcription. There are seven bromodomain-containing proteins in the *T. brucei* (Fleck, Nitz, and Jeffers 2021), but only TbBDF5 contains a tandem bromo domain, which happens mostly in the BET (bromodomain and extra-terminal) protein family. TbBDF1-6 are enriched at transcription start sites to promote RNAPII-mediated transcription and TbBDF7 is enriched at the transcription termination (Staneva et al. 2021). TbBDF5 interacts with other uncharacterized proteins to form a complex at transcription initiation sites. The bromo domain of TbBDF5 may guide HAT2 (acetyltransferase) to acetylation modification at the transcription initiation site to maintain the acetylation state required for effective transcription.

To gain critical insights into the structure and function of TbBDF5-bromo1 domain, we analyzed the NMR data of TbBDF5-bromo1 domain to establish its structural information. In this article, the backbone and side-chain ^1H , ^{13}C and ^{15}N resonance assignments of TbBDF5-bromo1 domain from *T. brucei* are determined.

Methods And Experiments

The DNA fragment encoding the first bromo domain (residue 1-122) of TbBDF5 (Tb927.11.13400) from *T. brucei* was amplified by polymerase chain reaction (PCR) from *T. brucei* genomic DNA. The forward and reverse primers were 5'-GGAGATATACATATGAGTCAAACCGGCAG-3' and 5'-GTGGTGGTGTCTCGAGGCCACCCACTTTCTG-3', respectively. PCR products were then cloned into the vector pET-22(b) (Novagen) cleaved with NdeI/XhoI by infusion method. The recombinant vectors were transformed into *Escherichia coli* strain BL21 (DE3). The recombinant TbBDF5-bromo1 domain possesses 130 amino acids with a His-tag in the C-terminus (LEHHHHHH). To obtain isotopically labeled TbBDF5-bromo1 domain, the domain was overproduced in *Escherichia coli* BL21 (DE3) using minimal medium containing 2.5 g/L 99% ^{13}C -glucose and 0.5 g/L 99% $^{15}\text{NH}_4\text{Cl}$ as the sole carbon and nitrogen source, respectively. Cells were cultured at 37°C until $\text{OD}_{600} = 0.8$, then induced with 0.5 mM IPTG at 16°C for 20 hours. The induced cells were harvested and resuspended in 35 mL lysis buffer (20 mM Tris, 500 mM NaCl, pH 7.8), then lysed by sonication on ice. The lysate was centrifuged at 11000 \times g and 4°C for 30 min to remove precipitate. The protein was purified by Ni^{2+} -NTA column. The labeled protein was eluted with 15 ml Tris NaCl buffer containing 500 mM imidazole. The eluted protein was further purified using a Superdex 75 column on an AKTA purification system. The purified proteins were dialyzed with buffer containing 25 mM NaH_2PO_4 , 100 mM NaCl, 1 mM EDTA and 1 mM DL-Dithiothreitol (DTT) (pH 6.8) for 4 times and then concentrated to 500 μL with a concentration of 0.8 mM. The final NMR buffer contains 25 mM phosphate (pH 6.8), 100 mM NaCl, 1 mM EDTA, 1 mM DTT in 10:90% D_2O : H_2O .

All NMR data were collected at 293 K on a Bruker DMX 600 spectrometer. The following spectra were recorded to obtain backbone and side chain resonance assignments: ^1H - ^{15}N HSQC, CBCA(CO)NH, CBCANH, HC(CO)NH, HBHA(CO)NH, H(CCO)NH, HNCO, 3D ^{15}N -edited and ^{13}C -edited NOESY. NMR data was processed using NMRpipe and NMRDraw software (Delaglio et al. 1995), and then analyzed with Sparky 3 (Goddard and Kneller 1993).

Assignment And Data Deposition

The ^1H - ^{15}N -HSQC spectrum of TbBDF5-bromo1 is shown in Fig. 1. Except three non-proline residues (Ser2, Gly68 and Thr75), ^1H and ^{15}N backbone chemical shifts were assigned for almost all residues (97%). In addition, 95% of NH and ^{15}N , 97% of ^{13}C , and 90% of ^1H chemical shifts were assigned. 90% of side-chain resonance assignments were completed. The data sets from spectra NHCBCA, CBCA(CO)NH and HC(CO)NH contained the ^{13}C signals for the proline residues.

The consensus chemical shift index (CSI) derived from $^1\text{H}\alpha$, $^{13}\text{C}\alpha$ and $^{13}\text{C}\beta$ chemical shifts (Wishart and Sykes 1994) is shown in Fig. 2. This data indicates the TbBDF5-bromo1 domain contains five α -helices without β -sheet.

The chemical shift data have been deposited in the BioMagResBank (<http://www.bmrb.wisc.edu>) with accession number 51357.

Declarations

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Conflict of interest

The authors declare that they have no conflict of interest.

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Figures

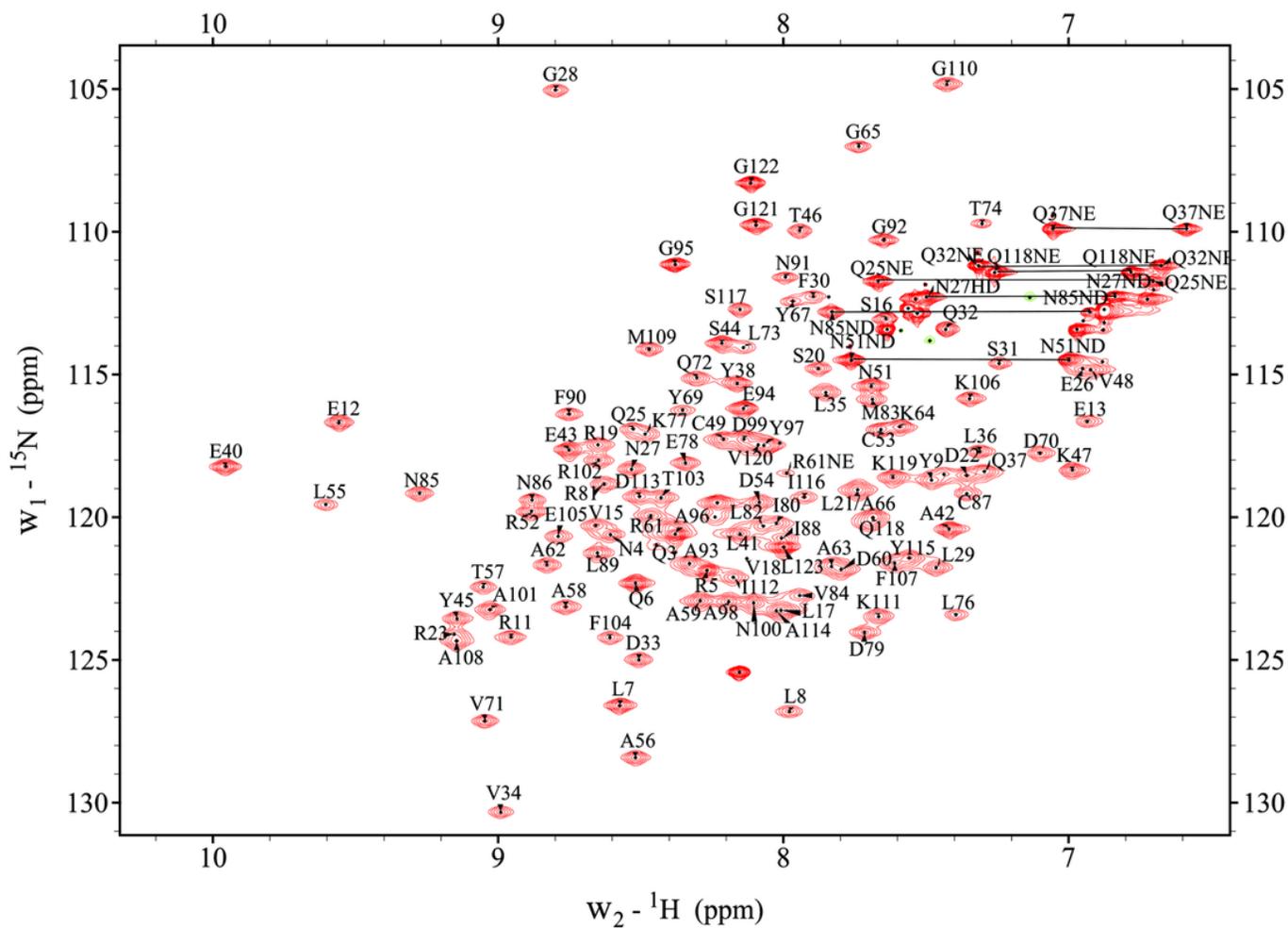


Figure 1

600 MHz ${}^1\text{H}$ - ${}^{15}\text{N}$ HSQC spectrum of TbBDF5-bromo1 domain from *T. brucei* at 293 K and pH 6.8. The amide side chains resonances are indicated using horizontal lines

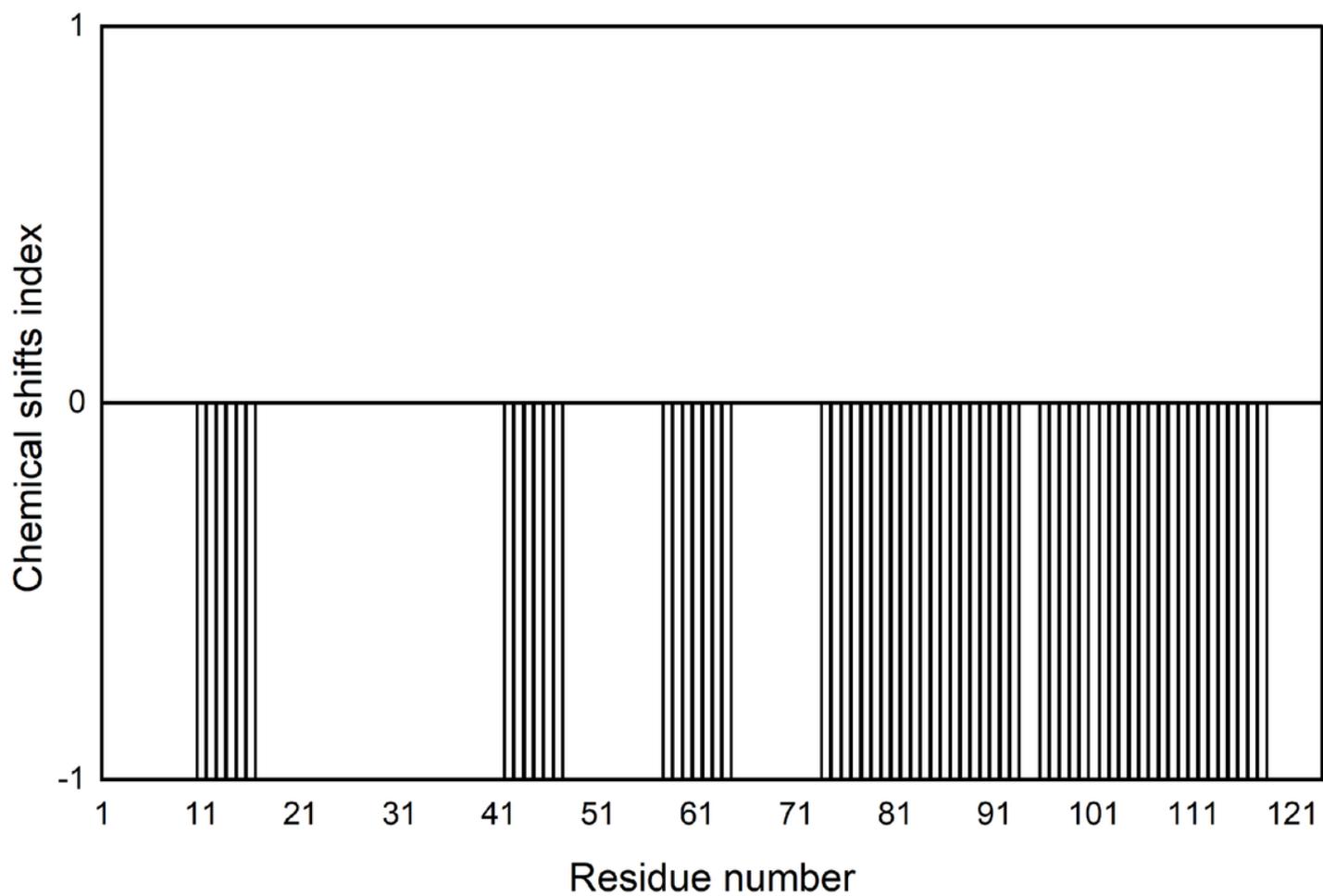


Figure 2

The consensus chemical shift index (CSI), obtained from $^1\text{H}\alpha$, $^{13}\text{C}\alpha$ and $^{13}\text{C}\beta$ chemical shifts (values of 1, 0, -1 indicate β -sheet, random coil and α -helical structure, respectively)