BAZ2B is a regulatory subunit of the ISWI (Imitation Switch) remodeling complex and engages in nucleosome remodeling. Loss-of-function and haploinsufficiency of BAZ2B are associated with different diseases. BAZ2B is a large multidomain protein. In addition to the epigenetic reader domains plant homeodomain (PHD) and bromodomain (BRD), BAZ2B also has a Tip5/ARBP/MBD (TAM) domain. Sequence alignment revealed that the TAM domains of BAZ2A and BAZ2B share 53% sequence identity. How the BAZ2A TAM domain bound with DNA has been characterized recently, however, the DNA binding ability and methylation preference, as well as the structural basis of the BAZ2B TAM domain are not studied yet. In this study, we measured the DNA binding affinity of the TAM domain of BAZ2B, and also determined its apo crystal structure. We found that the TAM domains of BAZ2A and BAZ2B adopt almost the same fold, and like BAZ2A, the BAZ2B TAM domain also binds to dsDNA without methyl-cytosine preference, implying that the BAZ2B TAM domain might recognize DNA in a similar binding mode to that of the BAZ2A TAM domain. These results provide clues for the biological function study of BAZ2B in the future.
Here, we carried out fluorescence polarization (FP) and isothermal titration calorimetry (ITC) assays to measure the DNA binding ability of the BAZ2B TAM domain, and further solved the structure of the BAZ2B TAM domain in its apo-state. We found that the BAZ2B TAM domain adopts a fold similar to the BAZ2A TAM domain, and also recognizes dsDNA independent of methyl-cytosine, implying that the BAZ2B TAM domain might recognize DNA in a similar binding mode to that of the BAZ2A TAM domain.

2. Materials and methods

2.1. Protein expression and purification

We cloned the human BAZ2B (aa.736-846) fragment into the pET28-MHL vector and further expressed the protein using Escherichia coli BL21 (DE3) by inducing with 0.5 mM IPTG at 14 °C. The cell was collected and resuspended in lysis buffer (500 mM NaCl, 20 mM Tris-HCl pH 7.5, and 5% glycerol). After sonication and centrifugation, the collected supernatant of the target protein was applied to Ni-NTA resin (Qiagen). After treatment with Tobacco etch virus, the target protein was further purified using Ni-NTA column and gel filtration column chromatography (GE Healthcare). The BAZ2B C827S mutant was obtained using the DNA sequence encoding BAZ2B (aa.736-846) as a template, and the protein was purified as mentioned for BAZ2B WT protein. Finally, the WT and C827S mutant protein samples were stored in 150 mM NaCl, 20 mM Tris-HCl pH 7.5 at 10 mg/mL with or without 1 mM DTT.

2.2. Fluorescence polarization (FP) binding assay

The single-strand DNA oligos were synthesized by General Biosystems (Anhui) Co. Ltd. with the 5’ FAM-labeled. The DNA oligos were dissolved in a buffer that the protein is in but without DTT. After pH adjustment, the ssDNA was annealed to DNA duplex by PCR instrument. Fluorescence polarization experiment was performed with a constant 5’ FAM-labeled dsDNA concentration of 40 nM and BAZ2B (aa. 736–846) protein at a concentration ranging from low to high micromolar in a 10 μL with a buffer containing 150 mM NaCl, 20 mM Tris-HCl pH 7.5, 5% glycerol, 2 mM MgCl2, 1 mM DTT, and 0.01% Triton X-100. Fluorescence polarization signal was analyzed with an excitation wavelength and emission wavelength of 485 nm and 528 nm, respectively, by Synergy H1 multi-mode reader (BioTek). For the Kd values, the data were fitted to a hyperbolic function using Origin 6.1.

2.3. Isothermal titration calorimetry (ITC) assays

The single-strand DNA oligos were synthesized by General Biosystems (Anhui) Co. Ltd. and annealed to DNA duplex as mentioned for the FP assay. The concentrations of BAZ2B (aa. 736–846) protein and DNA oligos are 30 μM and 0.8 mM, respectively, which were determined by TGem Plus Spectrophotometer (TIANGEN). The ITC titration was carried out with MicroCal ITC200 (Malven) at 25 °C, and the ITC curve was processed by Origin 7.0 (MicroCal Inc.) with the one-site fitting model.
2.4. Size-exclusion chromatography (SEC) analysis

The size-exclusion chromatogram analysis was carried out to assess the BAZ2B TAM domain protein aggregation. The BAZ2B TAM domain WT and C827S mutant samples, as well as the protein standard, were analyzed using Superdex 75 10/300 GL (GE Healthcare) gel filtration column with a buffer of 20 mM Tris-HCl pH 7.5 and 150 mM NaCl.

2.5. Crystallization

For crystallization, the BAZ2B TAM domain WT and C827S mutant proteins (10 mg/mL) were incubated with different dsDNA at a molar ratio of 1:1.2 for approximately 20 min, respectively. The samples and reservoir solution were then mixed with equal volumes (0.5 μL) by the sitting-drop vapor diffusion method under 18 °C. The crystal of the BAZ2B TAM domain protein appeared in a condition with 30% w/v PEG 400, 0.2 M Sodium citrate tribasic dihydrate, and 0.1 M Tris pH 8.5.

2.6. Data collection and structure determination

Before data collection, the BAZ2B crystal was briefly soaked in the original crystallization solution supplemented with additional 15% glycerol, followed by liquid nitrogen freezing. Diffraction data were collected using home X-ray sources at 100 K (HighFlux HomeLab, Rigaku), and then processed with the HKL2000 software package [27]. The BAZ2B (aa 736–846) structure was solved by molecular replacement with Molrep using coordinates from the BAZ2A TAM domain (PDB: 7MWL) [28, 29]. The crystal structure was refined using REFMAC [30] with the iterative refinement statistics are presented in Table 1.

3. Results and discussion

3.1. TAM domain of BAZ2B binds to dsDNA independent of methylcytosine

Different from the binding specificity of canonical MBDs, the BAZ2A TAM domain recognizes dsDNA without sequence and methylation state selectivity [24]. To investigate if the BAZ2B TAM domain prefers DNA methylation or not, we performed FP binding experiments using methylated and unmethylated dsDNA. Our FP results revealed that the TAM domain of BAZ2B bound to methylated and unmethylated dsDNA with almost the same affinity (Figure 2A). We also confirmed the DNA binding affinity of the BAZ2B TAM domain using ITC assays, and found that the TAM domain of BAZ2B bound to methylated dsDNA in a binding affinity comparable to that of the BAZ2A TAM domain (Figure 2B) [24]. Thus, our binding assays revealed that, similar to that of BAZ2A, the BAZ2B TAM domain binding to the dsDNA has no preference for DNA methylation.

3.2. Overall structure of BAZ2B TAM domain

To elucidate the structural basis for the BAZ2B TAM domain binding to DNA, we tried co-crystallization using a mixture of the BAZ2B TAM domain protein with dsDNA. Unfortunately, we only obtained the apo-state crystal of the BAZ2B TAM domain and determined its structure (Table 1). In the structure, two TAM molecules form an antiparallel dimer, which is fixed by a disulfide bond formed by residue C827 of each TAM molecule (Figure 2C). We believe that this disulfide bond is introduced during crystallization, because during protein purification, our gel filtration chromatography showed that the WT and C827S mutant proteins behaved as a monomer (Figure 2D). As expected, the two TAM molecule structures are essentially isomorphous, and each BAZ2B TAM molecule consists of an MBD-like moiety containing β1, β2, β3, α1, α2 and α1, as well as a C-terminal BAZ-specific moiety composed of β4, β5 and α3 (Figures 1B and 2C).

Table 1. Data collection and refinement statistics.

| Structure          | BAZ2B (aa 736–846) |
|--------------------|--------------------|
| PDB ID             | 7WMN               |
| Data Collection    |                    |
| Space group        | P2₁                |
| Cell dimensions    |                    |
| a,b,c [Å]          | 41.34,53.86,44.51   |
| α, β, γ [°]        | 90.00,96.78,90.00   |
| Resolution [Å]     | 50.00–1.95 (1.98–1.95) |
| Completeness [%]   | 97.1 (72.5)        |
| Rmerge             | 0.050 (0.281)      |
| Rfree              | 24.39 (3.02)       |
| CC1/2              | 0.998 (0.883)      |
| Redundancy         | 3.1 (2.3)          |
| Refinement         |                    |
| Resolution [Å]     | 34.19–1.95         |
| Reflections used   | 13207/666          |
| No. atoms/B-factor [Å²] | 1739/33.54    |
| Protein            | 1694/33.62         |
| Water              | 45/31.07           |
| Rmerge/Rfree       | 0.200/0.246        |
| R.m.s deviations   |                    |
| Bond lengths [Å]   | 0.008              |
| Bond angles [°]    | 1.60               |
| Ramachandran Plot % residues |
| Favoured           | 99.0               |
| Allowed            | 1.0                |
| Outliers           | 0.0                |

Values in parentheses are for the highest-resolution shell.

3.3. Structural comparison of BAZ2B TAM domain with that of BAZ2A TAM domain

Superposition of the structures of the BAZ2B and BAZ2A TAM domains revealed that, except for the β4 and β5 strands, the rest structure regions of the BAZ2B TAM domain overlapped well with that of the BAZ2A TAM domain. In addition, the DNA interacting residues, including K585, R586, K588, K595 and R599 of BAZ2A, were positioned at almost identical positions in the BAZ2B TAM domain (Figures 1B and 3A). Electrostatic potential surface analysis further showed that the BAZ2B TAM domain contained a similar positively charged DNA-binding surface to the BAZ2A TAM domain (Figures 3B and 3C). Thus, our binding assays and crystal structure indicated that the BAZ2B TAM domain might recognize dsDNA using a similar mode to the BAZ2A TAM-dsDNA complex.

A continuous positively charged surface formed by two TAM molecules contributes to the electrostatic and hydrogen bond interactions with the dsDNA in the BAZ2A-dsDNA complex [24]. However, this continuous surface is interrupted due to the antiparallel dimer mediated by the disulfide bond of the BAZ2A TAM domain (Figures 3D and 3E). We made a dimer model for the BAZ2B TAM domain based on the structure of the BAZ2A TAM domain. The structural model showed that the loop linking β4 and β5 strands forms a clash with the other TAM molecule when two BAZ2B TAM molecules pack in the same manner as that of the BAZ2A TAM domain (Figure 3F). Thus, these findings might explain why we did not obtain the BAZ2B TAM-DNA complex crystal.

3.4. Structural comparison of BAZ2B TAM domain with that of the canonical MBDs

The canonical MBDs bind to mCG and TG DNA by the conserved base-specific interactions between arginine residues and guanine base of mCG/TG dinucleotide, as well as electrostatic interactions with the
negatively charged backbone phosphate of dsDNA [18, 19, 20, 21, 22, 23, 32, 33]. Notwithstanding, sequence alignment revealed that R760 and R781, corresponding R166 and R188 of human MBD2, are conserved in the TAM domain of BAZ2B (Figure 1B). However, structural comparison of the BAZ2B TAM domain with the MBD2 MBD-dsDNA complex showed that, different from the positively binding surface of the MBD2 MBD, the corresponding mCG dinucleotide binding face is negatively and/or neutrally charged in the BAZ2B TAM domain (Figures 4A and 4B), implying that the BAZ2B TAM domain might adopt a different DNA binding mode from that of the canonical MBD-DNA complex.

Moreover, the β1 and β2 strands of the BAZ2B TAM domain are longer than that of MBD2, and the R760 residue sits on the β1 strand (Figure 4C). Similar to the BAZ2A TAM domain, the three β strands (β1, β2 and β3) of MBD-like moiety integrating with β4 and β5 strands form a β-sheet in the BAZ2B TAM domain. Thus, we speculate that these structural features might restrain R760 to point to the mCG dinucleotide, and also hamper the R760 to specifically bind to mCG dinucleotide as the R166 of MBD2 does (Figure 4C). Besides the arginine fingers, the loop connecting β1 and β2, and the α1 of MBD2 make contact with the sugar-phosphate backbone, which recently has been shown to be crucial for DNA binding [23]. However, there is a short loop between the β1 and β2 of the BAZ2B TAM domain, and it fails to form interaction with the DNA backbone (Figures 1B and 4D). In addition, the S189 in MBD2 is substituted by glutamine in BAZ2B, which also disrupts the DNA backbone phosphates binding of

Figure 2. Structural architecture of the BAZ2B TAM domain. (A) The binding curves of fluorescence polarization assays of methylated and unmethylated dsDNA to the BAZ2B TAM domain. (B) ITC fitting curve of the TAM domain of BAZ2B with methylated dsDNA. (C) Crystal structure of the BAZ2B TAM domain in its apo-state. Two BAZ2B TAM molecules were colored blue and green, respectively. (D) Size-exclusion chromatogram analysis of the WT and C827S mutant proteins for the BAZ2B TAM domain.
Figure 3. Structural comparison of BAZ2B TAM domain with that of BAZ2A TAM domain. (A) Structural superposition of the TAM domains of BAZ2B (blue) and BAZ2A (pink, PDB code 7MWI). (B) The electrostatic potential surface of one BAZ2A TAM molecule bound to mCA dsDNA (PDB code 7FHJ). (C) A model of the BAZ2B TAM domain bound to mCA dsDNA. The model was built using PyMOL according to the BAZ2A-DNA structure (PDB code 7FHJ). (D) The electrostatic potential surface of two BAZ2A TAM molecules bound to one mCA dsDNA (PDB code 7FHJ). (E) A model of two BAZ2B TAM molecules in complex with one mCA dsDNA. (F) The model of two BAZ2B TAM molecules (blue) packing in the same manner as that of the BAZ2A TAM domain (pink). The model was generated and presented using PyMOL.
BAZ2B MBD-like moiety (Figures 1B and 4D). In addition, we found the truncated protein that only contains the MBD-like moiety of the BAZ2B TAM domain could barely bind to DNA (data not shown). Thus, these findings might also explain why the BAZ2B TAM domain binds to DNA without preference for methyl-cytosine as the canonical MBD protein does.

Unlike catalytic subunit SMARCA1 or SMARCA5, BAZ2B functions as a regulatory subunit of the ISWI remodeling complex [1], how BAZ2B functions within the ISWI complex to involve the chromatin remodeling is poorly understood. It was proposed that the transient binding to chromatin of the ISWI complex is crucial for its efficient target recognition [34], in which the regulatory subunits of the ISWI complex play important roles [35]. For example, ACF1 (also known as BAZ1A) functions as a regulator to ensure efficient nucleosome sliding and the SMARCA5 loading onto its targets [36, 37]. Williams syndrome transcription factor (WSTF, also named BAZ1B) can recruit SMARCA5 to replication foci by interacting with the sliding clamp PCNA [38]. ACF1 and WSTF belong BAZ family together with BAZ2A and BAZ2B [39]. The BAZ2B-containing ISWI remodeling complex can control DNA accessibility by regulating the sliding of the histone octamer on the DNA template [1]. Thus, we hypothesized that the DNA binding ability of the BAZ2B TAM domain reported here might involve mediating the transient binding and nucleosome sliding of the ISWI complex or other functional proteins, which needs to be further investigated.

Figure 4. Structural comparison of the BAZ2B TAM domain with that of the canonical MBDS. (A) The electrostatic potential surface of the MBD2 MBD bound to mCG DNA (pink, PDB code 6CNQ). (B) A model of the BAZ2B TAM domain bound to mCG DNA. The model was built using PyMOL according to the MBD2-mCG structure. (C) Superposition of the structures of BAZ2B TAM domain apo-state (blue) with the MBD2 MBD-mCG complex (green, PDB code 6CNQ). (D) A different view of Figure 4C emphasizes the interactions between the β1-β2 loop and the α1 in MBD2 with DNA, which is lacking in the BAZ2B TAM domain.
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Declarations

Author contribution statement

Yingying Feng, Sizhuo Chen, Mengqi Zhou, Jin Zhang: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.
Jinrong Min, Ke Liu: Conceived and designed the experiments; Analyzed and interpreted the data; Wrote the paper.

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Data availability statement

Data associated with this study has been deposited at Protein Data Base under the accession number 7WIN.

Declaration of interests statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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