Crystal Structure of the Human BRD2 Bromodomain

INSIGHTS INTO DIMERIZATION AND RECOGNITION OF ACETYLATED HISTONE H4

The BET (bromodomains and extra terminal domain) family proteins recognize acetylated chromatin through their bromodomain and act as transcriptional activators. One of the BET proteins, BRD2, associates with the transcription factor E2F, the mediator components CDK8 and TRAP220, and RNA polymerase II, as well as with acetylated chromatin during mitosis. BRD2 contains two bromodomains (BD1 and BD2), which are considered to be responsible for binding to acetylated chromatin. The BRD2 protein specifically recognizes the histone H4 tail acetylated at Lys12. Here, we report the crystal structure of the N-terminal bromodomain (BD1, residues 74–194) of human BRD2. Strikingly, the BRD2 BD1 protein forms an intact dimer in the crystal. This is the first observation of a homodimer among the bromodomain-containing proteins, BRD2, associates with the transcription factor E2F, the mediator components CDK8 and TRAP220, and RNA polymerase II, as well as with acetylated chromatin during mitosis. BRD2 contains two bromodomains (BD1 and BD2), which are considered to be responsible for binding to acetylated chromatin. The BRD2 protein specifically recognizes the histone H4 tail acetylated at Lys12. Here, we report the crystal structure of the N-terminal bromodomain (BD1, residues 74–194) of human BRD2. Strikingly, the BRD2 BD1 protein forms an intact dimer in the crystal. This is the first observation of a homodimer among the known bromodomain structures, through the buried hydrophobic core region at the interface. Biochemical studies also demonstrated BRD2 BD1 dimer formation in solution. The two acetyllysine-binding pockets and a negatively charged secondary binding pocket, produced at the dimer interface in BRD2 BD1, may be the unique features that allow BRD2 BD1 to selectively bind to the acetylated H4 tail.

The eukaryotic genome forms a polymeric structure consisting of repetitive units of nucleosomes (1). In each nucleosome, the DNA is wrapped around an octamer of core histones: two H2A/H2B dimers and an H3/H4 tetramer (2). All of the core histones are basic proteins comprising N- and C-terminal tail regions and a central core domain. The N-terminal tail regions are rich in lysine and, to a lesser extent, arginine and serine. The tail regions are assumed to be flexible in the nucleosome, thus allowing access to enzymes such as acetyltransferases, methyltransferases, and kinases for covalent modifications (3, 4). The histone code, which is defined by covalent modifications such as acetylation, methylation, and phosphorylation of the histone tails, represents a fundamental regulatory mechanism of gene expression/repression (5). Various hypotheses have suggested that a single modification or specific combinations of histone modifications may affect distinct downstream events by altering the chromatin structure and/or generating a binding platform for protein effectors that specifically recognize the modification(s) and initiate gene transcription or repression (5, 6).

Acetylation of the histone tails is one of the most thoroughly characterized features of chromatin remodeling and transcriptional activation (4, 7). The acetylated N-terminal tails of the histones are recognized by the bromodomain, a conserved sequence motif composed of ~110 amino acids (8, 9) that was originally identified in the Drosophila protein brahma (8, 10). The specific recognition of acetylated lysine on the histone N-terminal tails by the bromodomain is considered to be a major characteristic of chromatin activation. The bromodomain is present in many chromatin-associated factors such as nuclear histone acetyltransferases, ATP-dependent chromatin-remodeling factors, and the BET family of nuclear proteins. Recent evidence has shown that the development of human cancers by transcriptional dysfunction is caused by genetic alterations of these bromodomain genes (11–15).

The proteins belonging to the BET family have a unique feature in that they contain two tandem bromodomains and an additional conserved ET domain (16). In vertebrates, four members of the BET family have been identified: BRD2/RING3/FSRG1 (16, 17), BRD3/ORFX/FSRG2 (18), BRD4/HUNK1/MCAP (mitotic chromosome-associated protein) (19), and BRD5/BRDT (20). BRD2/RING3/FSRG1 was initially identified as a novel gene involved in the regulation of the human major histocompatibility complex class II region and was initially named RING3 (really interesting new gene-3) (17, 21), but was subsequently renamed BRD2. BRD2 contains a...
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kinase-like domain between the two bromodomains, consistent with the fact that it has mitogen-activated nuclear kinase activity and is involved in signal transduction (22). Through its bromodomains, BRD2 selectively interacts with the acetylated Lys12 of histone H4 in the intact nuclei of living cells and activates transcription from the cyclin E promoter (23). Furthermore, BRD2 associates with acetylated chromatin during mitosis (19, 23, 24), whereas the other bromodomain family proteins are known to be displaced from the chromosomes during mitosis (25–27). In addition, BRD4/MCAP, another member of the BET family, also associates with acetylated chromatin throughout the cell cycle (19, 24) and helps viral DNA to remain attached to the host’s mitotic chromosomes (28). Hence, the retention on chromosomes during mitosis is likely to be a distinctive feature of the BET family. The bromodomains within the BET family members probably play an important role in the epigenetic memory of transcription and viral inheritance across cell division. Nevertheless, it is still not clear 1) how BRD2 recognizes the specific acetylated histone code and 2) how BRD2 can associate with the acetylated chromatin for a prolonged time during cell division. To understand the molecular mechanism of the BRD2 bromodomain in more detail, we determined the crystal structure of the N-terminal bromodomain I (BD1; residues 74–194) of human BRD2. Intriguingly, BRD2 BD1 forms an intact homodimer in both crystals and solution. In addition to the two acetyllysine-binding pockets, the dimer interface makes a potential acidic cleft, suggesting that this secondary binding pocket, together with the acetyllysine-binding pockets, may play a crucial role in BRD2 BD1 recognition of a specific acetylated histone H4 tail.

EXPERIMENTAL PROCEDURES

Construction of BRD2 BD1 Expression Vectors—The cDNA encoding BD1 (residues 74–194) of human BRD2 (29) was amplified by PCR using the following primers 5′-CCAGGGCTCTCTCCGGGGAGTTACCAAAGCGTG-3′ (forward) and 5′-GGCCGGATCAATCATTAGTTCTTAGG-GATGGTCAC-3′ (reverse). This cDNA fragment was further amplified by PCR to add the T7 promoter and a histidine affinity tag-encoding sequence within the 5′-region and the T7 terminator sequence within the 3′-region (30). This subclone was ligated into the pCR2.1-TOPO vector (Invitrogen) for cell-free protein expression.

Expression and Purification of Human BRD2 BD1—BD1 of human BRD2 was produced as a 161-amino acid recombinant protein with an N-terminal histidine affinity tag and a TEV cleavage site. The selenomethionine-labeled protein was produced by the Escherichia coli cell-free synthesis system (30). The protein was first adsorbed to a HiTrap HP column (Amersham Biosciences) in 20 mM Tris-HCl buffer (pH 8.0) containing 1 mM NaCl and 15 mM imidazole and eluted with 20 mM Tris-HCl buffer (pH 8.0) containing 500 mM NaCl and 500 mM imidazole. The histidine affinity tag was then removed by incubation with the TEV protease at 30 °C for 1 h. To remove the histidine tag and the TEV protease from the reaction mixture, the solution was applied to a HiTrap HP column. The flow-through fraction was desalted and loaded onto a HiTrap SP column (Amersham Biosciences) previously equilibrated with 20 mM MES (pH 5.5) containing 2 mM dithiothreitol (DTT). The protein was eluted by a linear gradient of 0–1 M NaCl. The BD1-containing fractions were collected, concentrated, and applied to a HiLoad 16/60 Superdex 75 gel filtration column, (Amersham Biosciences) equilibrated with 20 mM Tris-HCl buffer (pH 8.0) containing 150 mM NaCl and 2 mM DTT. The purified protein was concentrated to 10–17 mg/ml.

Crystallization—All crystallization trials were carried out using the hanging drop vapor diffusion method by mixing 1 μl of protein solution (5 mg/ml in 20 mM Tris-HCl buffer (pH 8.0) containing 150 mM NaCl and 2 mM DTT) with 1 μl of various reservoir solutions and equilibrating the mixtures against 500 μl of reservoir solution at 20 °C. After the initial crystallization screening and optimization, good single crystals for the selenomethionine-labeled native protein were obtained within 1 week in a drop containing 25–30% methoxy polyethylene glycol 5000, 0.2 M ammonium sulfate, and 0.1 M HEPES (pH 7.7). The typical size of the native crystals used for data collection was −0.5 × 0.3 × 0.03 mm3. The crystals were soaked briefly in a cryoprotectant containing 10% (v/v) glycerol and flash-frozen in liquid nitrogen prior to the start of data collection.

Data Collection—A three-wavelength anomalous dispersion data set for the selenomethionine crystal was collected at beamline AR-NW12 at the Photon Factory (Tsukuba, Japan) using an ADSC Quantum-210 CCD detector. The crystal-to-detector distance was set to 190 mm. The oscillation range per image was 1.0°, with no overlap between two contiguous images. The data were processed and scaled with the HKL2000 program suite (31). The crystal belongs to the primitive monoclinic space group C2, with unit cell parameters of a = 113.92, b = 55.14, and c = 67.30 Å and β = 94.06°. Assuming the presence of three molecules in the asymmetric unit, the calculated Matthews coefficient and solvent content values are 2.3 Å3/Da and 47%, respectively. Data statistics are summarized in Table 1.

Structure Determination and Refinement—The structure determination was attempted with both the multiwavelength and single-wavelength anomalous dispersion methods using the program SOLVE (32). However, the single-wavelength anomalous dispersion phasing produced better experimental phases compared with the multiwavelength anomalous dispersion results. Of the 21 selenium atoms in the asymmetric unit, 14 were identified, giving an experimental figure of merit of 0.66 for all data up to 2.6 Å resolution. The initial experimental phases were further improved by solvent flattening with non-crystallographic symmetry (NCS) averaging and phase extension up to 2.0 Å resolution using the program RESOLVE (33). The modified experimental map obtained by RESOLVE was readily interpretable, and ~70% of the protein chains were traced automatically. The rest of the protein chains were fitted manually using the graphics program O (34). An initial round of rigid body refinement, followed by torsion angle dynamics and simulated annealing with NCS restraints between the mono-
The crystallographic statistics for this structure are provided in Table 1.

### Table 1

| Data collection | Wavelength (Å) | 0.9791 |
|-----------------|----------------|--------|
| Resolution (Å)  | 20 to 2.2      |        |
| Redundancy*     | 3.6 (3.6)      |        |
| Unique reflections | 28,500     |        |
| Completeness (%) | 99.7 (99.6) |        |
| Rmerge (%)      | 6.2 (32.1)     |        |

### Refinement statistics

- Resolution (Å) 20 to 2.0
- σ cutoff 0
- Reflections 27,884
- No. protein residues 333
- No. water molecules 352
- Rcryst (%) 18.7
- Rfree (%) 22.9
- Average B factors 51.94
- r.m.s. deviations Bond length (Å) 0.008
- Bond angle 1.26°

*The numbers in parentheses are the values in the highest resolution shell.

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The tertiary structure of BD1 of BRD2 (residues 74–194) was solved by the single-wavelength anomalous dispersion method at 2.5 Å resolution and refined to 2.0 Å resolution (supplemental Fig. 1) with excellent overall stereochemistry. The structure contains a left-handed α-helical bundle formed by four α-helices (αz2, αA, αy, and αC) (Fig. 1B). The overall structure of BD1 of BRD2 is similar to those of the bromodomains of p300/CREB-binding protein-associated factor (PCAF) (37), human GCN5 (38), yeast Gcn5p (39), PCAF (41), and CBP (42) and the double-bromodomain module of TAF1 (40). The binding site within BD1 was directly introduced into the plasmid by PCRs using the QuikChange II XL site-directed mutagenesis Kit (Stratagene). Wild-type BRD2 BD1 and mutants Q78A, I154A, and Q182A were expressed in *E. coli* Rosetta (DE3) and purified by chromatography on a HiTrap HP column and a HiLoad 16/60 Superdex 75 gel filtration column. The purity of the proteins was confirmed by SDS-PAGE.

Biotinylated histone H4 peptide acetylated at Lys12 (H4K12Ac; H-SGRGKGGKGLGK(Ac)GGA-GSGSK-biotin) was immobilized on the surface of a Sensor Chip SA (Biacore), and the binding affinities of the wild-type and mutant BD2 BD1 proteins were tested by surface plasmon resonance (SPR) analysis on a Biacore 3000 system (Biacore) with phosphate-buffered saline (pH 7.4) as the running buffer. The assay was conducted in duplicate for each BD2 BD1 sample and analyzed using BIAevaluation Version 4.1 software (Biacore). The binding affinity (in response units) was calculated as (the value from the flow cell immobilized with H4 peptide) − (the value from the blank flow cell).

### Results

Crystal Structure of BD2 BD1—The tertiary structure of BD1 of BRD2 (residues 74–194) (Fig. 1A) was solved by the single-wavelength anomalous dispersion method at 2.5 Å resolution and refined to 2.0 Å resolution (supplemental Fig. 1) with excellent overall stereochemistry. The structure contains a left-handed α-helical bundle formed by four α-helices (αz2, αA, αy, and αC) (Fig. 1B). The overall structure of BD1 of BRD2 is similar to those of the bromodomains of p300/CREB-binding protein-associated factor (PCAF) (37), human GCN5 (38), yeast Gcn5p (39), PCAF (41), and CBP (42) and the double-bromodomain module of TAF1 (40). The binding site within BD1 is defined by an extended long loop (loop ZA) that connects helices αz2 and αA and by another loop (loop BC) that connects helices αy and αC. These two loops form a deep cleft, with a cavity in the middle of the pocket. The hydrophobic core of the domain is stabilized mainly by the conserved hydrophobic residues and additionally by some conserved hydrophilic residues. The BD1 sequence corresponding to the solved structure comprises residues 74–194 of BD2, with extended tail segments at the N- and C-terminal regions (Fig. 1B).
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**Dimeric Structure of BRD2 BD1**—The crystal contains three molecules in the asymmetric unit (Fig. 1C). The overall structures of the three NCS molecules (molecules A–C) are very similar in the helical bundle regions as judged from the root mean square deviation of 0.383 Å obtained by superposition of the main chain atoms. Intriguingly, the arrangement of the three NCS molecules is quite unique compared with those found in other protein structures. Molecule A is transformed into molecule B by 2-fold symmetry, whereas molecule C is related by a rotation of 110° to molecule B. Molecule C forms a dimer with a molecule generated by crystallographic symmetry in a manner similar to the association of molecules A and B (Fig. 1C). Between molecules A and B, the dimeric interaction is concentrated in the regions of the BC loop and helices αB and αC. In the other case of NCS formation between molecules B and C, the N- and C-terminal extended tail regions of molecule B have NCS contacts with the residues within the cleft region of molecule C.

The electrostatic surface potential analysis revealed that the dimer interface of molecules A and B is partially hydrophobic (supplemental Fig. 2). The main positive electrostatic potential area is created by a cluster of positively charged residues on the central parts of helices α2, αA, and αC (supplemental Fig. 2B). Calculation of the solvent-accessible area using the AREAIMOL program (CCP4 Program Suite) revealed that the buried surface area of a monomer in the AB dimer is 2183 Å², which corresponds to ~16% of the surface area of a monomer. Similarly, the buried surface area in the crystallographic CC dimer is 2125 Å². In contrast, for the BC dimer, the value is 1069 Å², corresponding to 8% of the monomer surface. These values indicate that BRD2 BD1 in the crystal is indeed consistent with the results obtained from *in vitro* and *in vivo* analyses (described below). The overall structures of the three NCS molecules are very similar in the helical bundle regions as judged from the root mean square deviation of 0.383 Å obtained by superposition of the main chain atoms.

**Homophilic Interaction of BD1 in Vitro and in Vivo**—The oligomeric structure of BD1 in solution was independently assessed by two different techniques. First, a monomodal fit of the data from the dynamic light scattering experiment predicted a molecular mass of 33 kDa, indicating that BRD2 BD1 (calculated molecular mass of 15 kDa) indeed exists as a dimer in solution (see “Experimental Procedures”). Second, cross-linking of BRD2 BD1 with ethylene glycol bis(sulfosuccinimidyl succinate) generated a result consistent with the dimerization of BD1 (supplemental Fig. 3). These results indicate that BRD2 BD1 exists as a dimeric form in *vitro*.

To ascertain whether the BRD2 protein interacts with another BRD2 molecule *in vivo*, we carried out a co-immunoprecipitation analysis with nuclear extracts from HeLa cells expressing GFP-BRD2 and FLAG-BRD2 (Fig. 2A). Notably, GFP-BRD2 coprecipitated with FLAG-BRD2 in cells expressing both proteins, but not in cells expressing the tag alone or a single protein, clearly indicating that the full-length BRD2 protein indeed interacts with another BRD2 molecule *in vivo* (Fig. 2A). To further substantiate the specificity of the BRD2-BRD2 interaction, we performed a competition experiment with the full-length BRD2 protein (Fig. 2B). These results provide strong evidence for the biological relevance of the BRD2-BRD2 interaction.

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**FIGURE 1.** Overall tertiary structure of BD1 of BRD2. A, schematic diagram of human (h) BRD2 aligned with other human BET proteins: human BRD2 (NCBI accession number AAH63840.1), BRD3 (accession number AAH32124.1), BRD4 (accession number AAL26987.1), and BRDT (accession number AAQ16198.1). The bromodomains (BD1 and BD2) and the extra terminal domain (ET) are indicated by blue and green boxes, respectively. Two other conserved regions are shown as black and gray boxes. The amino acid length of each protein is shown on the right. B, stereo view of ribbon diagram of the tertiary structure of BD1. Structural figures were generated with PyMOL (www.pymol.org). C, NCS arrangement of the BD1 molecules (molecules A–C) in the asymmetric unit. Molecules A and B form an intact dimer. Molecule C forms a similar dimer with the crystal symmetry molecule, C’ (slate).
in vivo interaction, we performed co-immunoprecipitation experiments using FLAG-BRD2 BD1. As shown in Fig. 2B, FLAG-BRD2 BD1 coprecipitated full-length BRD2 (GFP-BRD2) in vivo. To identify the residues responsible for the homophilic interaction of BRD2, we prepared BRD2 BD1 proteins with point mutations of the residues contributing to the dimeric interactions (Fig. 2C) and tested their abilities to interact with BRD2. Remarkably, six BRD2 BD1 point mutants, including Q78A, Y153K, E170A, L174E, V177E, and the double mutant M142A/Q143A, were completely unable to interact with BRD2 in vivo. The point mutants I154A and Q182A showed weak interactions with BRD2 (Fig. 2B). These results suggest that the aforementioned residues within the BRD2 BD1 dimer interface, which were identified by the structural analysis, are significantly involved in the homophilic interaction of BRD2 in vivo.

Significance of BRD2 BD1 Dimerization in the Histone H4 Peptide Interaction—As the BRD2 BD1 proteins form an intact homodimer, we carried out additional binding studies to determine whether the homodimerization affects the interaction of the histone H4 peptide acetylated at Lys12. SPR analysis was performed with an immobilized H4 peptide corresponding to the N-terminal region (residues 1–15) and acetylated at Lys12 (H4K12Ac) against BRD2 BD1. As shown in Fig. 3, wild-type BRD2 BD1 bound to the H4K12Ac peptide, consistent with our previous results (23). We next carried out SPR analyses with the BRD2 BD1 point mutants Q78A, I154A, and Q182A, which are located in the dimer interface (Fig. 2C). As expected, the results clearly show that the binding affinity of BRD2 BD1 for the H4K12Ac peptide was drastically reduced by the mutations (Fig. 3).

DISCUSSION

In this study, we determined the tertiary structure of the N-terminal bromodomain of BRD2, which belongs to the BET family. Our data provide further insight into the molecular mechanism of histone tail recognition by BD1 of BRD2 as a dimer. We reported recently that BRD2 specifically recognizes acetylated histone H4 through Lys12 (H4K12Ac) against BRD2 BD1. As shown in Fig. 3, wild-type BRD2 BD1 bound to the H4K12Ac peptide, consistent with our previous results (23). We next carried out SPR analyses with the BRD2 BD1 point mutants Q78A, I154A, and Q182A, which are located in the dimer interface (Fig. 2C). As expected, the results clearly show that the binding affinity of BRD2 BD1 for the H4K12Ac peptide was drastically reduced by the mutations (Fig. 3).
are highly homologous compared with the nonequivalent bromodomains, i.e. BD1 versus BD2 (Fig. 4A). The corresponding bromodomains share >75% sequence identity, whereas only ~44% sequence identity exists between BD1 and BD2. Sequence comparisons among other BET proteins also showed similar results (data not shown). The properties of the BRD2 BD1 residues that contribute to the dimer interface are highly conserved between BRD2 BD1 and BRD4 BD1 (Fig. 4A). This suggests that BD1 of BRD4 may also possess the ability to form a similar dimer, as observed in the BRD2 BD1 structure.

In the case of BD2, the residues within the dimer interface are not highly conserved compared with their equivalent residues in BD1 (Fig. 4A). Because of the partial conservation of the dimeric residues, along with the low sequence similarity to BD1, it is likely that the arrangement of the dimeric form might be different in the BD2 regions of BET proteins compared with...
that in BD1. However, additional structural analyses of BD2 will be necessary to clarify this hypothesis.

A comparison of the structure of BRD2 BD1 with the known histone acetyltransferase-type bromodomain structures of human GCN5 (38), yeast Gcn5p (39), human PCAF (41), and human CBP (42) and the double-bromodomain module of TAF1 (40) revealed that the left-handed four-helix bundle of BRD2 BD1 has been conserved among the bromodomain family proteins (37). Although the overall structures of these bromodomains are similar, major structural variations exist in the ZA and BC loop regions (Fig. 4). An overall sequence similarity of ~32% is observed among these bromodomains. The complex structure of the Gcn5p bromodomain with the histone H4 peptide acetylated at Lys16 revealed that this bromodomain preferentially binds to peptides containing an N-acetyllysine residue at position 16 (39). Although a 15-amino acid-long peptide (positions 15–29) was used, just four residues (position 16–19) actually bound to the protein, and only a limited number of contacts were formed with the protein. Considering the large structural and sequence variations in the ZA and BC loop regions between the Gcn5p bromodomain and BRD2 BD1, we speculate that BRD2 BD1 may recognize the H4 tail in a different manner. However, additional structural data of the BRD2 BD1 complex with the H4K12Ac peptide are required to understand how the BRD2 BD1 protein recognizes the acetylated H4 tail.

The TFIIID complex reportedly dimerizes when not bound to DNA (44, 45), and the dimerization is regulated by transcription factor IIA (46). Therefore, the largest subunit of transcription factor IID, TAF1, which contains the double bromodomain, might form a complex with four bromodomains. The structure of the TAF1 double bromodomain (40) is quite different from that of the BRD2 BD1 homodimer (Fig. 5). In the case of the TAF1 double-bromodomain structure, the dimeric form is maintained by interactions between helix $\alpha_A$ and the ZA loop region of BD1 and between helix $\alpha_B$ and the ZA loop region of BD2, and the dimeric form of TAF1 lacks a 2-fold symmetry axis (40). On the other hand, in the case of the BRD2 BD1 homodimer, the dimeric interaction between molecules A and B of BRD2 BD1 is concentrated in the regions of helices $\alpha_A$ and $\alpha_C$ and the BC loop, and the dimerized structure has a 2-fold non-crystallographic symmetry axis (Figs. 1C and 5). Therefore, we searched for a correlation between the dimeric structures mentioned above and the histone tail recognition. TAF1 binds to the N-terminal tail of histone H4 monoacetylated at Lys8, Lys12, or Lys16. It also binds to the N-terminal tail of histone H3 acetylated at Lys14. On the other hand, the BET family proteins BRD2 and BRD4 specifically recognize the histone H4 N-terminal tail acetylated at Lys12, as confirmed by our previous biochemical studies (23, 24). These observations suggest that BRD2 and BRD4 have a more specific recognition mechanism for the acetylated histone tail compared with TAF1.

Indeed, we speculated that the specific recognition of a certain acetylated status of the histone H4 tail by BRD2 BD1 would be mediated by the electrostatic surface potential of the BRD2 BD1 homodimeric structure. In the present SPR analysis, we demonstrated that mutations of the residues within the BRD2 BD1 dimer interface significantly reduced the binding affinity of this protein for the H4K12Ac peptide. We also confirmed that mutants Q78A, I154A, and Q182A (used in the binding assay) were unable to form stable dimeric interactions compared with wild-type BRD2 BD1 (Fig. 2B). These results suggest that the dimer formation is critical for the effective interaction of BRD2 BD1 with the H4K12Ac tail. Intriguingly, the electrostatic surface potential analysis of the BRD2 BD1 homodimer clearly revealed a negatively charged cavity, which is absent in the TAF1 double bromodomain (data not shown), in addition to the two hydrophobic pockets that recognize acetylated lysines (Fig. 6). The acidic cavity, which exists at the dimer interface, is formed by the main chain carbonyl groups of $\text{Tyr}^{153}$, $\text{Tyr}^{153'}$, $\text{Ile}^{154}$, $\text{Ile}^{154'}$, $\text{Tyr}^{155}$, $\text{Tyr}^{155'}$, $\text{Asn}^{156}$, and $\text{Asn}^{156'}$. We thus speculate that this negatively charged cleft might be a potential secondary binding site for interacting with a basic residue of the acetylated histone H4 tail. As the histone H4 N-terminal tail is quite basic and as Lys8, Lys16, and Arg17 are near the acetylated Lys12 residue, it is possible that Lys8, Lys16, or Arg17 interacts with BRD2 BD1 through this putative secondary binding pocket. Moreover, as this and previous (23, 24) studies also confirmed the BRD2 BD1 recognition of the monoacetylated Lys12 residue of the H4 tail, the present structure suggests that the BRD2 BD1 homodimer may recognize two acetylated H4 tails simultaneously. However, additional structural analyses of BRD2 BD1 complexed with the acetylated H4 tail peptides are necessary to understand the molecular mechanism of BRD2 BD1 recognition of the acetylated H4 tail.

In summary, BRD2 BD1 forms a homodimer in the crystal as well as in solution. The acidic cleft at the dimer interface and
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FIGURE 6. Features of the molecular surface of the BRD2 BD1 homodimer. A, electrostatic surface potential of the BD1 homodimer; B, close-up view of the dimer interface showing the two acetyllysine (Ack)-binding pockets and the proposed secondary binding acidic cleft formed by the dimer interface.

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