Journal of Structural Biology 180 (2012) 165–173

Contents lists available at SciVerse ScienceDirect

Journal of Structural Biology

journal homepage: www.elsevier.com/locate/yjsbi

Solution structure of an atypical PHD finger in BRPF2 and its interaction with DNA

Lei Liu, Su Qin, Jiahai Zhang, Peng Ji, Yunyu Shi *, Jihui Wu *

Hefei National Laboratory for Physical Sciences at Microscale and School of Life Sciences, University of Science and Technology of China, Hefei, Anhui 230026, PR China

ARTICLE INFO

Article history:
Received 6 March 2012
Received in revised form 19 June 2012
Accepted 26 June 2012
Available online 20 July 2012

Keywords:
PHD finger
PZPM
BRPF2
NMR structure
DNA

ABSTRACT

Plant homeodomain (PHD) finger is found to be a versatile reader that functions in recruiting transcription factors and chromatin modification complexes. Bromodomain- and PHD finger-containing (BRPF) proteins are identified as scaffold component in a couple of histone acetyltransferase (HATs) complexes but the biological function of PHD fingers, composing the motif called PZPM (PHD/Zn-knuckle/PHD Motif), in BRPF proteins is far from being well understood. Here we report the three-dimensional solution structure of the second PHD finger of PZPM in human BRPF2. According to the structure, BRPF2 PHD2 possesses a two-strand β sheet which is different from any other PHD fingers. Functionally, this PHD finger can potentially bind DNA non-specifically with an evolutionarily conserved and positively charged surface. We provide the structural and interaction information of this atypical PHD finger and categorize this BRPF2 PHD2 into a new subset of PHD finger. Moreover our work also shed light on the functional aspect of the PZPM.

1. Introduction

PHD (plant homeodomain) finger was first identified in HAT3.1 of Arabidopsis in 1993 (Schindler et al., 1993). It is characterized by chelating two zinc ions with a conserved Cys4-His-Cys3 motif. This ~60-residue module exhibits a two-strand β-sheet flanked by two short alpha helices (Yap and Zhou, 2010). PHD fingers are categorized according to their different binding properties to histone H3 with different modifications. The first type PHD finger specifically recognizes tri-methylated K4 on histone H3 tails (H3K4me3) (Wysocka et al., 2006) by embracing the trimethylammonium group with a hydrophobic pocket formed by side chains of several aromatic residues (Li et al., 2006; Palacios et al., 2006; Pena et al., 2006). The second type PHD finger, which does not possess the hydrophobic pocket, recognizes non-modified histone tails (Lan et al., 2007). The histone H3 tail forms an anti-parallel β-sheet with the PHD finger upon binding and the substrate specificity is determined cooperatively by amino terminus, Lys4 and Arg8 on histone H3 tail (Lan et al., 2007). In addition, two more PHD subsets were identified recently. One binds H3K9me3 (Mansfield et al., 2011; Musselman et al., 2009) the other recognizes acetylated lysine on histone H3 or H4 (Lange et al., 2008; Matsuyama et al., 2010; Zeng et al., 2010). These properties make PHD finger a versatile reader that functions in recruiting transcription factors and chromatin modification complexes (Slama and Geman, 2011).

BRPF (Bromodomain-and PHD finger-containing proteins) is identified as a component of MOZ (monocytic leukemia zinc finger protein)/MORF (MOZ-related factor) histone acetyltransferase (HATs) complex (Brown et al., 2000; Li et al., 2007). It has three paralogs, BRPF1, 2, and 3. BRPF1 and 2 are also referred to as BRD140 (bromodomain protein of 140 kDa) and BRD1 (bromodomain protein 1), respectively. In the HAT complex, MOZ/MORF, the catalytic subunit (Doyon et al., 2006), is bridged with another two components, EAF6 (homolog of yeast Esa1-associated factor6) and ING5 (inhibitor of growth protein 5), by the scaffold protein BRPF (Pelletier et al., 2002; Ullah et al., 2008). Members of BRPF family contain a C-terminal PWWP domain which is a H3K36me3 binding module (Vezzoli et al., 2010), a bromodomain which associates with acetyllysine residues on proteins (Sanchez and Zhou, 2009), and a unique N-terminal PZPM (PHD/Zn-knuckle/PHD Motif) which consists of two PHD fingers linked by a mononuclear zinc knuckle. The PZPM defines the subfamilies III and IV of Epc-N (Enhancer of the Polycomb-N-terminus) domain, a protein–protein interaction module found in chromatin associated proteins, and all proteins studied to date that contain PZPM have been implicated in histone methylation and/or gene silencing (Perry, 2006). The first PHD finger of PZPM in BRPF proteins is highly conserved in yeast-NuA3-like HAT complexes and the one in BRPF2, which belongs to the second

Abbreviations: PHD, plant homeodomain; BRPF, bromodomain- and PHD finger-containing protein; HAT, histone acetyltransferase; PZPM, PHD/Zn-knuckle/PHD Motif; MOZ, monocytic leukemia zinc finger protein; MORF, MOZ-related factor; HBO1, human acetylase binding to ORC1; ING, inhibitor of growth protein; IR-EMSA, infrared electrophoretic mobility shift assays; FPA, fluorescence polarization assays; CD, circular dichroism.

* Corresponding authors. Present address: School of Life Sciences, University of Science and Technology of China, Hefei, Anhui 230026, PR China. Fax: +86 551 3600374 (Y. Yu), (J. Wu).

E-mail addresses: yyshi@ustc.edu.cn (Y. Shi), wujihui@ustc.edu.cn (J. Wu).

1047-8477/$ - see front matter © 2012 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.jsb.2012.06.014

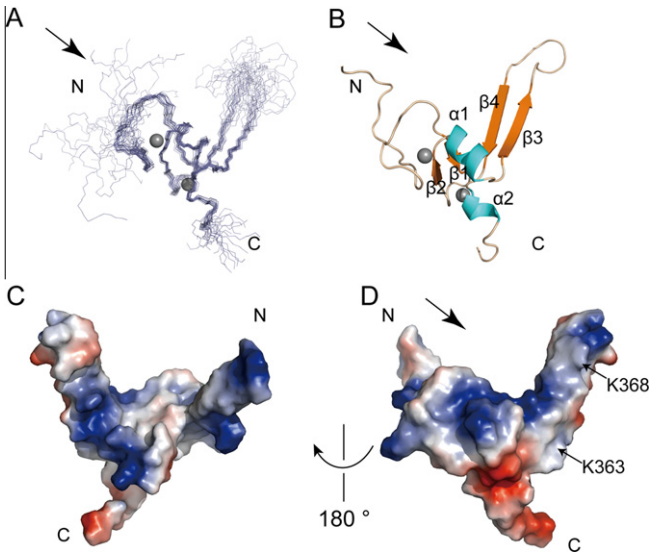

Fig.1. Structure of BRPF2 PHD2. (A) Ensemble of 20 lowest-energy structures for residues 317–394 of the BRPF2 PHD2 domain. (B) Ribbon show of BRPF2 PHD2 domain. (C and D) Stereoscopic show of the electronic potential surface of PHD2 in which (D) is oriented the same as (A and B).

type of PHD finger, was found to bind unmodified histone H3 tails and this interaction contributes to the localization of BRPF2 onto HOXA9 locus. (Qin et al., 2011; Slama and Geman, 2011). Despite much knowledge has been acquired, the role of the PZPM, especially its second PHD finger, in BRPF proteins remains unclear. Even more, the biological function of BRPF proteins in MOZ/MORF or related HAT complex is far from being understood and the chromatin-targeting and histone-modifying mechanism of MOZ/MORF HAT complex are yet to be revealed. Here we determined the solution structure of the second PHD finger (PHD2) of BRPF2 by NMR, which is the first structure of PHD2 in PZPMs and represents an atypical PHD finger in structural aspect. Meanwhile we revealed the DNA binding activities of the PHD finger by fluorescence polarization assay (PFA) and identified a positively charged area responsible for this DNA binding by NMR titration and mutagenesis experiments. Based on our results, we categorize the PHD2 of BRPF2 into a new subset of PHD finger that have the potential capacity to bind DNA non-specifically with an evolutionarily conserved and positively charged surface. Although the detailed mechanism of BRPF2 PHD2-DNA interaction remains elusive, the structural and interaction information of this atypical PHD finger provided by this study might suggest a novel regulatory role of BRPF proteins in the process of histone acetylation.

2. Material and method

2.1. Plasmids construction

For NMR and FPA experiments, the DNA fragments of BRPF2 PHD2 (corresponding to residue Gln317 to Thr394) were amplified from human brain cDNA library by PCR reactions. The DNA fragments were then cloned into a modified pET-28a (+) (Novagen) plasmid, expressing a fusion protein with a MGHHHHHHM tag at the N terminus. All BRPF2 PHD2 domain mutants were generated from the modified pET-28a (+) expression vector template encoding the wild-type protein by the QuickChange site-directed mutagenesis kit (Stratagene) and confirmed by DNA sequencing. For DNA binding EMSA experiment, the DNA fragments of BRPF2 PHD2 were cloned into pGEX4T-1 expressing a glutathione *S*-transferase (GST) fused protein.

2.2. Protein preparation

The recombinant plasmids were transformed into *Escherichia coli* BL21 (GOLD) competent cells. The cells were incubated in SV40 media with 100 μM $ZnSO_4$ added after transformation. Generally, the protein expression was induced at A600 = 1.0–1.5 by addition of IPTG to a final concentration of 0.1 mM, prolonged for 24 h at 16 °C. The expressed recombinant protein was purified using a Ni^{2+}-chelating column (Qiagen) followed by gel filtration chromatography using HiLoad 16/60 Superdex 75 column (GE Healthcare). The purified protein was then dialyzed against buffer A (20 mM Bis-Tris, 150 mM NaCl, pH6.5) for next study. Uniformly ^{15}N and $^{15}N/^{13}C$ labeled proteins were prepared by using $^{15}NH_4Cl$ (0.5 g L^{-1}) and $^{13}C_6$-glucose (2.5 g L^{-1}) as Nitrogen and Carbon sources.

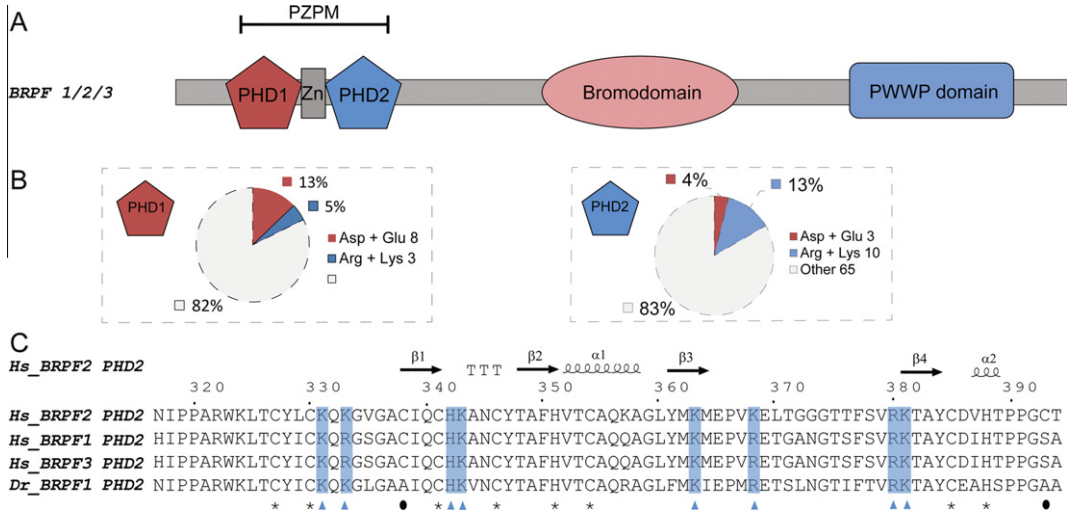

Fig.2. (A) Domain arrangement BPRF proteins (B) Pie charts of charged residues in PHD fingers of PZPM. (C) Sequence alignment of PHD2 of BRPF proteins. The sequences alignment Result were generated by Multalin (Corpet, 1988). The secondary structure and residue number of BPRF2 PHD2 are shown on the top. The residues chelating zinc ions are marked by asterisks. Conserved positively charged residues are colored blue and black circles indicate two non-chelating cysteine residues that are mutated to serine residues.