High resolution crystal structure of DPF3b in complex with an acetylated histone peptide

Weiguo Li^{a, b#}, Anthony Zhao^{b#}, Wolfram Tempel^b, Peter Loppnau^b and Yanli Liu^{b, c*}

^a Key Laboratory of Pesticide & Chemical Biology, College of Chemistry, Central China Normal University, Wuhan 430079, P.R. China.

^b Structural Genomics Consortium, University of Toronto, 101 College Street, Toronto, Ontario M5G 1L7, Canada.

^c Hubei Key Laboratory of Genetic Regulation and Integrative Biology, College of Life Science, Central China Normal University, Wuhan 430079, PR China.

[#] These authors contributed equally to the work.

* To whom correspondence should be addressed. Tel: +86-27-67862703 Email: yanliliu@mail.ccnu.edu.cn

Abstract

Histone acetylation plays an important role in chromatin dynamics and is associated with active gene transcription. This modification is written by acetyltransferases, erased by histone deacetylases and read out by bromodomain containing proteins, and others such as tandem PHD fingers of DPF3b. Here we report the high resolution crystal structure of the tandem PHD fingers of DPF3b in complex with an H3K14ac peptide. In the complex structure, the histone peptide adopts an α -helical conformation, unlike previously observed by NMR, but similar to a previously reported MOZ-H3K14ac complex structure. Our crystal structure adds to existing evidence that points to the α -helix as a natural conformation of histone tails as they interact with histone-associated proteins.

Keywords: DPF3b; tandem PHD fingers; H3K14ac; α -helical conformation

1. Introduction

Histone N-terminal tails are subject to various post-translational modifications (PTMs), which dynamically regulate chromatin structure and affect many processes that involve DNA, including replication, transcription, repair and recombination (Berger, 2007; Downs et al., 2007; Groth et al., 2007; Kouzarides, 2007; Strahl and Allis, 2000; Vaquero et al., 2003). One such modification, histone acetylation, which occurs at the ϵ -amino group of lysine in the N-terminus of H3 and H4, has been associated with activation of transcription (Liu et al., 2005; Pokholok et al., 2005; Wang et al., 2008). Acetylation of lysines is catalyzed by histone acetyltransferases (HATs), removed by histone deacetylases (HDACs) and read out by readers in a sequence specific manner (Kouzarides, 2007). For a long time, the bromodomain was the only known acetyllysines recognition module (Filippakopoulos et al., 2012; Owen et al., 2000). Recently, a number of additional modules have been identified as acetylated histone readers, including tandem plant homeodomain (PHD) fingers of DPF3b (Zeng et al., 2010), MOZ (Dreveny et al., 2014; Qiu et al., 2012) and MORF (Ali et al., 2012), tandem plekstrin-homology (PH) domains of yeast histone chaperone Rtt106 (Su et al., 2012) and the Yaf9, ENL, AF9, Taf14 and Sas5 (YEATS) domain of AF9 (Li et al., 2014).

DPF3, also known as BAF45C, BRG1-associated factor 45C or Zinc finger protein cer-d4, is a member of the d4 protein family and composed of an N-terminal 2/3 domain, a C2H2-Krüppel-like zinc finger and C-terminal tandem PHD zinc fingers (Fig. 1A) (Ninkina et al., 2001). There are two splicing variants of *DPF3* in the human genome, namely *DPF3a* and *DPF3b* (Lange et al., 2008). DPF3 is associated with the BAF complex and involved in heart and skeletal muscle development. A pull-down assay has revealed that DPF3b interacts with histone H3 and H4 in a lysine acetylation-dependent manner (Lange et al., 2008). The three-dimensional solution structure indicates that the tandem PHD fingers of DPF3b bind the N-terminal tail of histone H3 and that acetylation at H3 lysine 14 site (H3K14ac) enhances the interaction, whereas methylation at the lysine 4 site (H3K4me) precludes the binding (Zeng et al., 2010).

Here we report the high resolution crystal structure of the tandem PHD fingers of DPF3b in complex with an H3K14ac peptide, and compare our findings with known structures of this (Zeng et al., 2010) and other acetyllysine reader modules (Dreveny et al., 2014; Li et al., 2014; Owen et al., 2000; Qiu et al., 2012; Su et al., 2012).

2. Materials and methods

2.1 Protein expression and purification

The tandem PHD fingers of DPF3b (residues 255-368) were subcloned into a modified pET28-MHL vector. The encoded N-terminal His-tagged fusion protein was overexpressed in *Escherichia coli* BL21 (DE3) Codon plus RIL (Stratagene) cells at 15 °C and purified by affinity chromatography on Ni-nitrilotriacetate resin (Qiagen) in a buffer containing 20 mM Tris, pH 7.5, 500 mM NaCl, 5 mM β -mercaptoethanol (β -ME) and 5% glycerol, followed by TEV protease treatment to remove the tag by dialysis in a buffer containing 20 mM Tris, pH 7.5, 150 mM NaCl, 50 μ M ZnCl₂ and 1 mM DTT. Protein was further purified by Superdex75 gel-filtration in the same buffer as dialysis (GE Healthcare, Piscataway, NJ). For crystallization experiments, purified protein was concentrated to 5 mg/mL in the same buffer as dialysis. The molecular weight of the purified protein was determined by mass spectrometry, which showed the expected molecular weight (15005.9 Da after tag removing).

2.2 Crystallization

Purified protein was mixed with H3K14ac peptide (residues 1-20) at a 1:2 stoichiometric ratio and crystallized using the sitting drop vapor diffusion method at 20 °C by mixing 0.5 μ L of the protein with 0.5 μ L of the reservoir solution, which contained 1.4 M sodium citrate, 0.1 M Hepes, pH 7.5, 5% MPD. Before flash-freezing in liquid nitrogen, crystals were soaked in a cryoprotectant consisting of 85% reservoir solution and 15% ethylene glycol.

2.3 Data collection and structure determination

Diffraction data were collected under cooling to 100 K at beam line 19ID of the Advanced Photon Source (Argonne, IL) and reduced with XDS (Kabsch, 2010) and AIMLESS (Evans and Murshudov, 2013) software. The structure was solved by molecular replacement with the program PHASER (McCoy et al., 2007). DPF3b coordinates from an unpublished crystal structure, ultimately based on molecular replacement with KAT6A coordinates of PDB entry 3V43 (Qiu et al., 2012), were used as a search model. The present model was obtained through iterative manual re-building with COOT (Emsley et al., 2010), restrained refinement with REFMAC (Murshudov et al., 2011) and model validation with MOLPROBITY (Chen et al., 2010). Some terminal DPF3b and histone amino acid residues are absent from the model entirely, and some individual atoms were not modeled when poor electron density did not allow their confident placement in favorable conformations and without clashes. Glu326 in one of the DPF3b chains is located near a special position and we could not discern a plausible side chain conformer given its symmetry environment. In addition to DPF3b and histone, zinc ion and water coordinates, the current model contains an ethylene glycol molecule, a sodium ion and several dummy atoms that point to unidentified features in the density maps. CCP4 (Winn et al., 2011), PHENIX (Adams et al., 2010), PDB_EXTRACT (Yang et al., 2004) programs and the IOTBX library (Gildea et al., 2011) were used in preparing model summaries (Table 1) and Protein Data Bank deposition. Additional information on

crystallographic experiments and models is listed in Table 1.

3. Results and discussion

3.1 DPF3b tandem PHD fingers interact with α -helical H3 tail

The three-dimensional structure of the DPF3b complex with an H3K14ac peptide was determined by NMR methods in 2010 (PDB code: 2KWJ) (Zeng et al., 2010). Analysis of coordinate variations (Sikic and Carugo, 2009) across the ensemble's models reveals an RMSD spike between H3A1 and H3K14ac. These differences may, with reservations, be considered a measure of uncertainty in the atomic coordinates' precision (Snyder et al., 2005). Intriguingly, an H3K14ac peptide bound to the tandem PHD fingers of MOZ, whose sequence is similar to that of DPF3b, forms a helix (Dreveny et al., 2014). How do the very similar DPF3b and MOZ readers affect the structure of bound histone? Here, we solved the crystal structure of DPF3b tandem PHD fingers (residues 255-368) in complex with the H3K14ac peptide at 1.85 Å resolution (Table 1 and Fig. 1 and 2). The H3K14ac peptide binds to one of two DPF3b molecules that compose the asymmetric unit of our crystal structure (Fig. 1B). The hypothetical binding of H3K14ac to the second DPF3b chain in the same mode as observed for the first chain would be incompatible with the current crystal packing. The backbones of the H3 peptide-free and H3 peptide-bound DPF3b monomers superimpose well, with a root mean square deviations around 0.6 Å, indicating that binding of the peptide did not induce significant conformational changes in the protein's backbone (Fig. 1C) (Krissinel and Henrick, 2004). In solution, the protein behaved as a monomer (data not shown). Therefore, the dimer was formed due to artificial crystal packing. In the crystal structure's dimer, each PHD finger adopts the canonical fold including a β -hairpin and two zinc ions coordinated by C4-H-C3 motifs (Fig. 1B) (Li et al., 2006; Pena et al., 2006). The C-terminus of PHD1 and the hairpin's turn in PHD2 engage in polar interactions (Fig. 1D). Significantly, peptide residues H3T4-T11 adopt an α -helical conformation (Fig. 1B). As a consequence, the ensuing description and discussion recapitulates many points raised by Dreveny et al. (Dreveny et al., 2014). Consistent with previous studies, the positively charged N-terminus of the H3K14ac peptide is attracted by negative electrostatic potential on the surface of the PHD2 finger. Toward the C-terminus of the peptide, the K14 acetyl moiety is embraced by a narrow, mainly hydrophobic and weakly positively charged pocket on the surface of the PHD1 finger (Fig. 1E).

The H3A1, H3R2 and H3K4 residues of the peptide are embedded in three negatively charged pockets formed by PHD2 (Fig. 2A). The amino group of H3A1 is anchored by the main chain carbonyl oxygen of Gly356 (Fig. 2A). The side chain of H3R2 is buried in a negatively charged pocket formed by residues Asp335 and Asp338. This may explain why the D335A mutation in DPF3b (Zeng et al., 2010) and the corresponding D282A mutation in MOZ weaken the binding significantly (Fig. 2A and 2C) (Qiu et al., 2012). The side chain of H3R2 additionally forms a hydrogen bond with the carbonyl moiety of Cys334 (Fig. 2A). The significance of H3R2 for the interaction between tandem PHD fingers and H3 tail is also supported by the prior observation that H3R2 methylation or H3R2A mutation disrupts the interaction with MOZ (Qiu et al., 2012). Weaker electron density for side chains of H3 residues K4 and R8 only approximately resolves their atomic coordinates, and places the former

into a narrow pocket, which is lined by back bone carbonyl oxygens of DPF3b residues Ile314 and Arg315, as well as the side chain of Asp328. Possible steric exclusion from his pocket might explain the previously reported (Zeng et al., 2010) rejection of H3K4me3 (Fig. 2A). Our model includes a stub of the H3R8 side chain that also points toward the H3K4 pocket, with the possibility of a salt bridge to Glu315 (Fig. 2A). This interaction was previously shown as significant to the recognition of H3K14ac by tandem PHD fingers, as mutation E315A (Zeng et al., 2010), like the corresponding E261A in MOZ (Fig. 2A and 2C) (Qiu et al., 2012), weakens the binding significantly. The unresolved side chain of H3K9 probably protrudes toward the solvent, in accordance with modification of H3K9 showing little effect on the interaction (Ali et al., 2012; Dreveny et al., 2014; Qiu et al., 2012; Zeng et al., 2010).

The side chain of K14 inserts into a pocket formed by residues Asp263, Phe264, Arg289, Leu296, Trp311, Cys313, Ile314 and Glu315 in PHD1 (Fig. 2B). The ϵ -amino group of H3K14ac forms a hydrogen bond with the carboxylate moiety of Asp263 (Fig. 2B). In addition, the amino group and carbonyl oxygen of H3A15 form hydrogen-bonds with the main chain carbonyl oxygen of Asp263 and side chain of Arg289, respectively (Fig. 2B). The importance of these two residues were confirmed by previous studies, in which the mutations of D263A (Zeng et al., 2010) and corresponding S210A and N235A (Fig. 2B and 2C) (Qiu et al., 2012) weaken the interaction significantly. Taken together, the tandem PHD fingers of DPF3b cooperatively “read out” the H3K14ac peptide by interaction of the N-terminal peptide residues with PHD2 and recognition of K14ac by PHD1.

The present crystal and previous NMR models of DPF3b tandem PHD fingers in complex with H3K14ac generally superimpose well, but distinct main chain conformations correlate with differences in the peptide’s side chains (Fig. 2D), like previously noted (Dreveny et al., 2014). In our crystallographic model, the incompletely resolved side chain of H3R8 points toward the H3K4 binding pocket and presumably may form a salt bridge to Glu315, while the H3K9 side chain appears disordered, pointing away from the H3K4 pocket (Fig. 2D). Within the crystal context, the side of H3 pointing away from the DPF3b receptor faces a symmetry-related copy of H3 at some distance. In our crystallographic model, the closest contact occurs between the truncated side chains of symmetry related H3R8 residues. Crystal packing, therefore, does not appear to significantly restrain the peptide. This could explain higher refined B-factors of the H3 model atoms, compared to the DPF3b receptor (see Table 1). Another explanation, compatible with the apparent lack of strong crystal contacts and possibly arising from correlation between occupancies and B-factors, would be incomplete occupancy of the H3 ligand inside the crystal lattice. When comparing structural models that are based on different structure determination methods, method-dependent artifacts must be considered. The crystalline state may impose structural perturbations and restrictions that could confound inference on function. In addition, crystallographic models typically are averages over the diffraction experiment’s time scale and over the multitude of a crystal’s asymmetric units. The underlying assumptions lead to biased crystallographic models with

reduced information on molecular motions. NMR data, on the other hand, tend to encode short-range distances (Clore and Gronenborn, 1989). But even small uncertainty in relative atomic positions at short range may give rise to significant long-range distance variances. Aware of these caveats, we proceed to a discussion of possible functional consequences of helical histone tail conformation.

3.2 Possible functional implications of α -helical H3 tails

Structural studies on complexes between histone readers and histone tail peptides have indicated that histone tails generally adopt an extended β -strand-like conformation (Arents et al., 1991; Bian et al., 2011; Krissinel and Henrick, 2005; Min et al., 2003; Nielsen et al., 2002). However, other studies show that histone tails can assume α -helical conformations (Arents et al., 1991; Banères et al., 1997; Hansen et al., 1998). In our crystallographic complex model of DPF3b, residues 4 through 11 of the H3K14ac peptide adopt an α -helical conformation, H3K4 inserts into a narrow pocket and the H3K9 side chain points away from the PHD fingers. This conformation could explain why modification of H3K4, such as tri-methylation, abrogates binding, but H3K9 modification is tolerated (Zeng et al., 2010). In the previous studies, helical histone conformations have been reported to facilitate protein function (Arita et al., 2012; Chen et al., 2015; Dreveny et al., 2014; Forneris et al., 2007). In the MOZ-H3K14ac complex, residues H3K4 through H3T11 compose a helical secondary structure bound to tandem PHD fingers of MOZ, and this helix is consistent with selectivity over histone tail PTMs and with MOZ's acetyltransferase activity on H3 tails (Fig. 3A) (Dreveny et al., 2014). In the complex of UHRF1-H3R2me0K9me3, an α -helix, spanning residues H3K4 through H3R8, facilitates bivalent recognition of H3R2me0 and H3K9me3 by tandem tudor domains and a PHD finger (Fig. 3B) (Arita et al., 2012). In the LSD1-H3K4M complex, residues 1 through 4 of the histone H3 tail-like peptide H3(1-16)K4M, which bears a methionine replacement of lysine at position 4, adopt a helical structure in complex with histone demethylase LSD1 (Fig. 3C) (Forneris et al., 2007). The helical terminus allows the peptide to fit in the narrow pocket of LSD1 and the side chain of K4M to point toward the FAD cofactor (Forneris et al., 2007). In the recently solved AF10 complex structure, H3K27 peptide residues H3T22-K27 also form a helix bound to the PZP (PHD finger-Zn knuckle-PHD finger) domain of AF10 (Fig. 3D) (Chen et al., 2015). Together, these studies indicate that histone tails may adopt specific secondary structures to complement the shapes of their partners, although they are an unstructured coil in solution (Böhm and Crane-Robinson, 1984; Hansen, 1997).

3.3 PHD zinc fingers in comparison with structures of other acetyllysine readers.

To date, four types of domain have been identified as acetyllysine readers. For three, namely for bromodomains (Filippakopoulos et al., 2012; Owen et al., 2000), YEATS (Li et al., 2014) domain and tandem PHD fingers (Ali et al., 2012; Dreveny et al., 2014; Qiu et al., 2012; Zeng et al., 2010), structures in complex with acetyllysine peptides have been determined. Although the sequences and structures of these domains are diverse, they bind acetyllysine side chains in deep pockets through a

combination of hydrophobic contacts and direct or solvent-mediated hydrogen bonds (Fig. 4A-C). However, these similar binding modes are supported by diverse reader protein folds. The individual PHD subdomains of tandem PHD fingers pack compactly into a single functional unit with a cleft running across both the domains' surfaces to accommodate the acetylated peptide (Fig. 1E and 4A) (Dreveny et al., 2014; Zeng et al., 2010). In contrast, the bromodomain recognizes acetyllysine with conserved loops at one end of its helix bundle (Fig. 4B) (Filippakopoulos et al., 2012; Owen et al., 2000). Loops between β -strands shape the acetyllysine binding site of YEATS domains (Fig. 4C) (Li et al., 2014). A previous study on a fourth type of reader, tandem PH domain, proposes a set of Rtt106 residues that may form the binding cleft for H3K56ac peptides (Fig. 4D) (Su et al., 2012). This selection of Rtt106 residues could hypothetically support, again, both lipophilic interactions and hydrogen bonds. Taken together, the diversity of folds that support acetyllysine read-out, in turn enables a multitude of functions of this common PTM in highly diverse contexts.

4. Conclusion

The high resolution crystal structure of tandem PHD fingers of DPF3b in complex with H3K14ac peptide reveals a helix encompassing residues 4 through 11 of the peptide, similar to a peptide complex of MOZ (Dreveny et al., 2014), but in contrast to the previously reported NMR structure of the DPF3b complex (Zeng et al., 2010). The present study adds support to the hypothesis that secondary structure plasticity in histone tails enables histone modifications and their readout in a multitude of contexts.

Accession number

Coordinates and structure factor amplitudes of DPF3b tandem PHD fingers-H3K14ac were deposited in the PDB with accession code 5I3L.

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Author contributions

Weiguo Li and Anthony Zhao purified and crystallized the protein; Wolfram Tempel determined the crystal structure; Peter Loppnau cloned the constructs; Yanli Liu conceived, designed the study and wrote the paper. All authors analyzed results and approved the final version of the manuscript.

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Figure legends

Fig. 1. DPF3b tandem PHD fingers bind α -helical histone H3 tail.

(A) DPF3b consists of an N-terminal 2/3 domain, a C2H2-Krüppel-like zinc finger and tandem PHD fingers (PHD1 and PHD2). (B) Model of the crystal asymmetric unit of the tandem PHD fingers and H3K14ac peptide complex of DPF3b. (C) Superimposition of the structures of the tandem PHD fingers of the H3 peptide-free and H3 peptide-bound DPF3b monomers. (D) The PHD1 and PHD2 fingers together form a functional unit through intra-molecular, inter-domain hydrogen bonds. Interacting residues are shown in stick mode and intra-molecular hydrogen bonds are depicted as yellow dashed lines. (E) Electrostatic potential surface representation of tandem PHD fingers in complex with H3K14ac peptide. Molecular representations were generated with PyMOL (<http://pymol.sourceforge.net>). DPF3B coordinates were processed with PDB2PQR (Dolinsky et al., 2007) with a pH setting of 7. The potential surface was calculated with APBS (Baker et al., 2001). The APBS surface is colored in a red-blue spectrum in the ± 10 kT/e range. A Ca trace of the histone peptide is shown in yellow.

Fig. 2. Structural basis of H3K14ac recognition by the tandem PHD fingers of DPF3b.

(A) PHD2 finger of DPF3b recognizes the N-terminal residues of the H3K14ac peptide, which includes an α -helix. Detailed interactions of the N-terminal residues of H3K14ac peptide, with PHD2 finger shown in cartoon (left) or electrostatic potential surface (right) representation. H3K14ac peptide is shown in stick mode and intermolecular hydrogen bonds are depicted as yellow dashed lines. (B) PHD1 finger of DPF3b recognizes the acetylated K14 residue of H3K14ac peptide. Detailed interactions of the C-terminal residues of H3K14ac peptide, with PHD1 finger shown as cartoon (left) or electrostatic potential surface (right) representation. H3K14ac peptide is shown in stick mode and hydrogen bonds are colored yellow. (C) Superposition of the DPF3b-H3K14ac complex (PDB: 5I3L, this work, cyan) on MOZ-H3K14ac complex (PDB code: 4LLB, salmon), with key interacting residues shown in stick mode and labeled. (D) Superposition of the crystal structure (PDB: 5I3L, this work, cyan) on model 1, “best conformer”, of the NMR structure (PDB code: 2KWJ, blue) of the DPF3b-H3K14ac complex. DPF3b proteins and H3K14ac peptides are shown in ribbon and cartoon mode, respectively. The residues of H3K4, H3R8, H3K9 and H3K14ac of H3K14ac peptides and peptide interaction associated residues of DPF3b are shown in stick mode. The peptide residues of crystal and NMR structure are colored in red and orange, respectively, and protein residues are colored in cyan.

Fig. 3. Structures of published complexes with α -helical H3 tail. (A) Structure of tandem PHD fingers of MOZ in complex with H3K14ac peptide (PDB code: 4LLB). (B) Structure of tandem tudor and PHD domains of UHRF1 in complex with H3R2me0K9me3 peptide (PDB code: 3ASK). (C) Structure of LSD1 in complex with H3K4M peptide (PDB code: 2V1D). (D) Structure of PZP domain of AF10 in complex with H3K27 peptide (PDB code: 5DAH). Structures are shown as cartoons

and colored grey and yellow for proteins and peptides, respectively.

Fig. 4. PHD zinc fingers in comparison with structures of other acetyllysine readers. **(A, B, C)** Acetyllysine binding modes of DPF3b tandem PHD fingers (this work, A), Gcn5p bromodomain (PDB code: 1E6I, B) and AF9 YEATS domain (PDB code: 4TMP, C). The domains are shown in cartoon representation and acetyllysine recognition residues are shown in stick mode. **(D)** Crystal structure of Rtt106 tandem PH domain (PDB code: 3TW1). Possible of H3K56ac binding cleft-forming residues are shown in stick mode.

Table 1 Data collection and refinement statistics*Data collection and reduction #*

Radiation wavelength [Å]	1.28
Space group	C222 ₁
Cell dimensions a, b, c [Å]	99.82, 121.64, 56.45
Resolution limits [Å]	45.56-1.85 (1.89-1.85)+
Unique HKLs	29753 (1817)
Completeness [%]	100.0 (100.0)
R _{merge}	0.102 (1.125)
R _{p.i.m.}	0.041 (0.453)
CC _{1/2}	0.998 (0.717)
Mean(I/σ)	17.7 (2.0)
Multiplicity	7.1 (7.1)

Model refinement

Refinement resolution [Å]	45.56-1.85
Reflections used/free	28115/1624
Number of atoms/average B-factor [Å ²]	2010/35.3
DPF3b	1750/34.8
Histone peptide	124/44.0
Water	91/35.4
Other molecules, ions or atoms	45/32.2
R _{work} /R _{free}	0.182/0.212
RMSD bonds [Å]/angles [°]	0.015/1.7
Molprobit Ramachandran favored/outliers [%]	98.76/0.00

Statistics were calculated by AIMLESS (Evans and Murshudov, 2013).

+ Values in parentheses are for the highest resolution shell.

Figure 1

Figure 2

Figure 3

Figure 4

A

DPF3-H3K14ac

B

Gcn5p-H4K16ac (1E6I)

C

AF9-H3K9ac (4TMP)

D

Rtt106 (3TW1)