Biochemical and Biophysical Research Communications 413 (2011) 58–61

Contents lists available at SciVerse ScienceDirect

Biochemical and Biophysical Research Communications

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

Crystal structure of the Cys2His2-type zinc finger domain of human DPF2

Wei Zhang [a,1], Chao Xu [b,1], Chuanbing Bian [b], Wolfram Tempel [b], Lissete Crombet [b], Farrell MacKenzie [b], Jinrong Min [a,b], Zhonglai Liu [a,*], Chao Qi [a,*]

[a] Hubei Key Laboratory of Genetic Regulation and Integrative Biology, College of Life Science, Huazhong Normal University, Wuhan 430079, People's Republic of China
[b] Structural Genomics Consortium, University of Toronto, 101 College St., Toronto, Ontario, Canada M5G 1L7

ARTICLE INFO

Article history:
Received 3 August 2011
Available online 24 August 2011

Keywords:
DPF2
Cys2His2 type zinc finger
Crystal structure
d4-Protein family

ABSTRACT

DPF2 is an evolutionary highly conserved member of the d4-protein family characterized by an N-terminal 2/3 domain, a C2H2-type zinc finger (ZF), and a C-terminal tandem PHD zinc finger. DPF2 is identified as a transcription factor and may be related with some cancers in human. Here, we report the crystal structure of the C2H2-type zinc finger domain of human DPF2 with a canonical C2H2 fold, which contains two beta strands and one alpha helix. Several conserved residues, including Lys207, Lys216 and Arg217, constitute a positively charged surface in C2H2 domain, which implicates that it has the potential to bind DNA. The side chains of the residues Y209, C211, C214, K216, Y218, L224, H227 and H232 form the hydrophobic core of C2H2 domain, which indicates a potential-binding surface in the human DPF2.

1. Introduction

DPF2 (ubi-d4/requiem) is a member of the d4-protein family characterized of structural similarity and evolutionary conservation [1,2]. All proteins of this family contain a unique domain 2/3 in the N-terminal region, a Cys2His2 (C2H2) ZF or Krüppel-type ZF in the central part of the protein followed by a C-terminal tandem plant homeodomain (PHD). In d4-protein family, neuro-d4 (DPF1) and cer-d4 (DPF3) are expressed in the central and peripheral nervous system of vertebrate, whereas DPF2 is equally expressed in all other tissues and organs. It was assumed that proteins DPF1 and DPF3 are involved in the transcription regulation of neurospecific gene clusters, while DPF2 was identified as an transcription factor and may participate in developmentally programmed cell death [4–7]. The human DPF2 has previously been mapped to 11q13 [2], a region associated with several cancer forms. So, DPF2 may be involved in leukemia or other cancers with other genes connected with cancer.

In d4-protein family, the PHD fingers are hall marker of the proteins implicated in chromatin-mediated transcriptional control. The d4 proteins are involved in transcriptional regulation of gene via changing the condensed/decondensed state of chromatin in nucleus. It has been reported that the PHD domain of its homolog, DPF3, could specifically recognize unmodified or acetylated histone H3 [8,9] and the interaction is severely diminished by histone methylation [10]. And the three-dimensional solution structures of the double tandem PHD fingers of DPF3b showed that the tandem PHD12 fingers worked as one functional unit in recognition of histone [10], while the link between the C2H2 finger and the PHD12 fingers is obscure. The C2H2 finger belongs to a family involved in sequence-specific DNA binding [3], which implied that DPF2 could bind histone and DNA simultaneously via individual domains. However, the molecular mechanism between DPF2 and DNA, or the tandem PHD12 fingers and C2H2 finger of DPF2 remains unclear. To shed light on further biological function investigation of DPF2, we present the high-resolution crystal structure of DPF2 C2H2 zinc finger domain and identify a conserved and positively charged interface potential to bind DNA.

2. Materials and methods

2.1. Protein expression and purification

The fragment of human DPF2 (residues D206-H232) coving C2H2 ZF domain was subcloned into a pET-28a-MHC vector via ligase-independent cloning. The recombinant protein was over expressed in *Escherichia coli* BL21 (DE3) with the pRARE plasmid for codon-biased expression. Cells were grown in minimal media at 37 °C to an optical density of approximately OD_{600nm} = 2.5. Protein expression was induced with 100 μM isopropyl-1-thio-D-galactopuranoside (IPTG) and the cell cultures were grown for approximately 16 h at 15 °C after induction. The protein was purified by affinity chromatography on Ni–NTA resin (Qiagen Mississauga, ON) and size exclusion chromatography using a Superdex 75 col-

* Corresponding authors.
E-mail addresses: hslzl2007@163.com (Z. Liu), qichao@mail.ccnu.edu.cn (C. Qi).
[1] These authors contributed equally to this work.

0006-291X/$ - see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2011.08.043

umn (GE Healthcare, Tyrone, PA) in 20 mM Tris–HCl pH 7.5, 200 mM NaCl, 1 mM DTT, 20 μM $ZnCl_2$. The His-tag was cleaved from C2H2 ZF by the addition of 0.05 mg of TEV protease per milligram of C2H2 ZF protein, followed by incubation in ice for 12 h. The sample was then passed through a Ni–NTA column and the flow-through was collected and concentrated to 5 mg/mL for crystallization.

2.2. Crystallization, X-ray data collection and structure determination

The C2H2 ZF domain of human DPF2 was crystallized by the sitting-drop vapor diffusion method at 291 K with mixing the equal volumes of the protein solution 5 mg/mL and the reservoir solution, which consists of 0.1 M CHES pH 9.5 and 30% PEG 3000. Diffraction experiments were performed at 100 K. Diffraction data from two isomorphous crystals were collected at wavelengths of 1.2651 Å and 1.2834 Å, respectively at beamline 19ID of the Advanced Photon Source (Argonne, Illinois, USA) and reduced with the HKL software suite [21]. Further experimental details are listed in Table 1. The structure was solved by the multiple wavelength anomalous diffraction [22] method using aforementioned high energy remote and peak wavelength data sets SHELX [23] software. The protein model was traced automatically with ARP/WARP [24]. Further refinement was performed with the programs COOT [25] PHENIX [26], and REFMAC [27]. The MOLPROBITY server was used periodically for validation of the model's geometry [28].

2.3. Accession numbers

The structure factors and coordinates have been deposited in the protein Data Bank under accession codes 3IUF.

3. Results and discussion

3.1. Overall structure

The crystal structure of the C2H2 zinc finger of DPF2 (residues D206-H232) is well defined. The overall structure exhibits a canonical C2H2 fold, which contains a short antiparallel β-sheet, where β1 (209–211) and β2 (215–218) strands are connected by β-turn ($C_{211}DIC_{214}$) and a α helix (221–230). The ββα fold is stabilized by tetrahedral coordination to a zinc ion involved in the thiol groups of cys211, cys214 and the $N^{\varepsilon 2}$ atom of His227 and His232 (Fig. 2A). In d4 protein family, all d4 proteins have PHD zinc finger, while other domains may be absent. In *Drosophila melanogaster*, the DPF2 does not have a C2H2 ZF, instead of the stretches of the positively charged amino acids that could serve as the nuclear localization signals [11]. From the crystal structure of DPF2 ZF domain, we found a novel interface distributed with the positive charge, which consists of Lys_{207}, Lys_{216} and Arg_{217}. The positively charged interface is found to be conserved among its orthologs, which implicates that its potential to bind DNA is well conserved, and it likes other members in C2H2 ZF family (Fig. 2B1 and B2).

DPF2 was found to bind to several SWI/SNF complex subunits and also to the p52 NF-κ B subunit through its nuclear localization signal containing the N-terminal region [5].The data in this paper suggest that the C2H2 ZF domain of DPF2 plays an important role in regulation of gene transcription via the interaction with DNA. On the other hand, we speculate that the different domains of DPF2 may function in a cooperative manner in some intracellular processes, which could bind histone and DNA via tandem PHD domain and C2H2 ZF domain, respectively in the context of nucleosome.

Table 1
Data collection and refinement statistics.

Data collection	
Space group	$P2_12_12$
Cell dimensions	
a, b, c (Å)	24.71, 57.66, 22.58
α, β, γ (°)	90, 90, 90
Wavelength (Å)	1.2651
Resolution (Å)	40.0–1.80 (2.44–2.55)
R_{merge} (%)	11.8 (52.2)
I/σI	27.8 (3.4)
Completeness (%)	99.9 (98.7)
Redundancy	8.2 (6.0)
Refinement	
Resolution (Å)	22.7–1.8
No. reflections	3240
R_{work}/R_{free}	19.3/23.7
No. atoms	
Protein	263
Water	18
Average B-factors (Å^2)	
Protein	22.1
Water	29.6
R.m.s. deviations	
Bond lengths (Å)	0.016
Bond angles (°)	1.49

Values in parentheses correspond to the highest resolution shells.

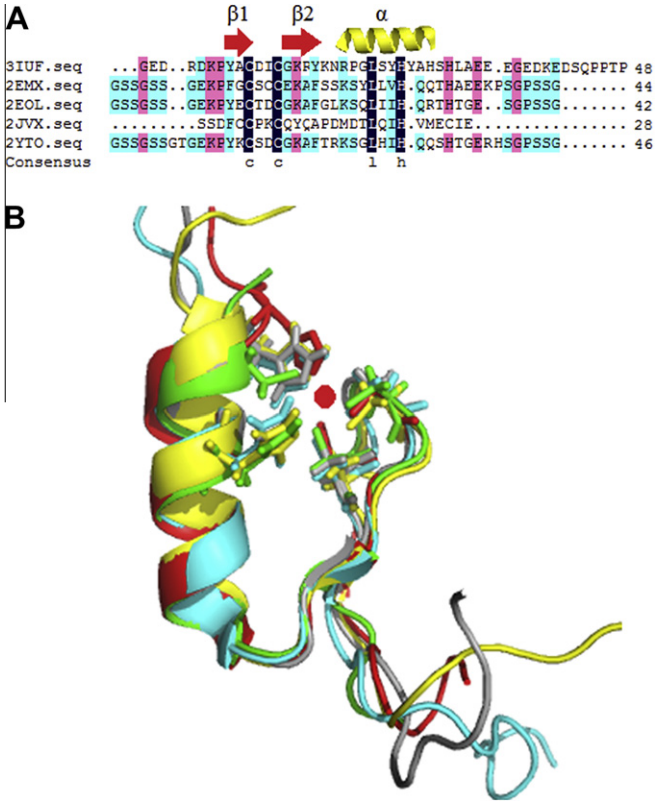

Fig. 1. Structural and sequence comparison of zinc finger (ZF) domains. (A) Structure-guided sequence alignment of the members of the ZF domains. The secondary structure of C2H2 zinc finger domain of DPF2 is indicated above the sequence. The dashed lines indicate gaps introduced to optimize alignments. The PDB codes of the protein are the same as used in the Fig. 1B. (B) Structural comparison of ZF domains. Superimposition of NEMO ZF (PDB code 2JVX, green), Zif268 ZF1 (PDB code 2EMX, yellow), Zif268 ZF2 (PDB code 2EOL, gray) and Zif484 (PDB code 2YTO, cyan) onto DPF2 C2H2 ZF (PDB code 3IUF, red). The side chains are depicted for the zinc-chelating residues (ball and sticks), the zinc atoms are highlighted as the red spheres. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

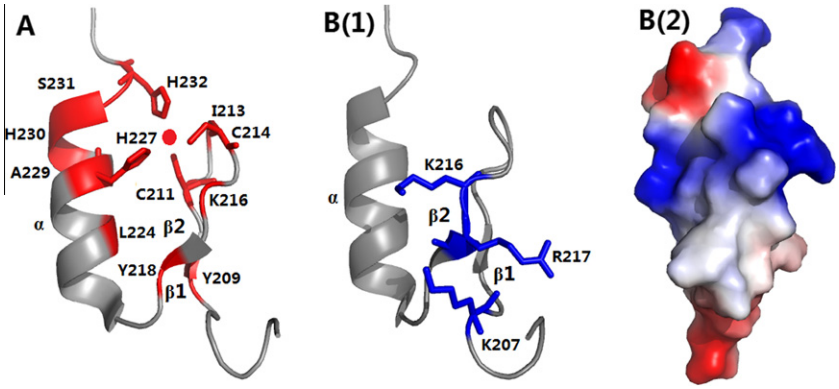

Fig. 2. Stereo diagram overall structure of DPF2 C2H2 ZF. (A) Overall structure of DPF2 C2H2 ZF. The side chains of chelating the zinc atom (C211, C214, H227, H232) are shown in sticks and colored in red, the zinc atom is highlighted as a red sphere. The side chains of the residues Y209, C211, C214, K216, Y218, L224, H227, and H232 form the hydrophobic core of the finger, which are colored red and shown in sticks. (B) (1) The distribution of the positive residues. The conserved residues, including k207, K216 and R217, constitute a positively charged surface of the protein. The positive radiuses are shown in sticks and colored in blue. (2) Orthogonal view of the surface with the charge distribution of the protein (blue for the positive charge, and red for the negative charge). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.2. Structural comparisons

In eukaryote, C2H2 zinc finger proteins comprise a large class of transcription factors, which are observed to be involved in DNA binding. But there are some examples of zinc fingers, that support both DNA and protein binding, which can be found in Sp1, human ZF protein 268(Zif268), YY1, NF-κB essential modulator (NEMO) and so on [12–15]. To better understand the potential roles of the C2H2 ZF domain of DPF2, the structural similarity search of the protein data bank with the pairwise structural comparison serve DALI [16] was applied. The multiple sequence and crystal structure alignments of these C2H2 ZF domains show that there is the high structural similarity among them (Fig. 1A and B). The high degree of the structural similarity among the C2H2 ZF domain family allowed us to create a model of this family. By using the coordinates of NEMO ZF and Zif268 (regions 659–691), our modeling suggest that the side chains of the residues Y209, C211, C214, K216, Y218, L224, H227 and H232 form the hydrophobic core of the finger of DPF2 (Fig. 2A). The residues Y209, L224, H227 and Y218 stabilized the hydrophobic core. And the aromatic residue Y218 is involved in edge-to-face packing to the zinc-chelating His227 (Fig. 2A).

DPF2 is a hypothesized transcriptional factor, which its nuclear localization and its conserved PHD zinc finger support the hypothesis that DPF2 encodes for a potential transcription factor [6]. And the solution structure of the PHD finger of DPF2's homolog, DPF3b, has shown the molecular basis of the integrated tandem PHD finger in these cellular processes [10], while the exact role of its C2H2 domain is obscure. Recent studies have shown that some zinc fingers do not participate in DNA recognition, instead they may serve in protein-binding or RNA-binding in the protein with multiple ZF domains [17–20]. The data obtained in this paper suggests C2H2 ZF domain's potential roles in transcriptional regulation via both DNA-binding and protein-binding, respectively in a cooperative way. The hydrophobic core of C2H2 ZF domain indicates a potential-binding surface in the human DPF2. To look for exact targets of DPF2 C2H2 ZF and the link with other domains of DPF2 requires further biological functional studies.

Acknowledgments

This research was supported by the Structural Genomics Consortium, a registered charity (Number 1097737) that receives funds from the Canadian Institutes for Health Research, the Canadian Foundation for Innovation, Genome Canada through the Ontario Genomics Institute, GlaxoSmithKline, Karolinska Institutet, the Knut and Alice Wallenberg Foundation, the Ontario Innovation Trust, the Ontario Ministry for Research and Innovation, Merck & Co., Inc., the Novartis Research Foundation, the Swedish Agency for Innovation Systems, the Swedish Foundation for Strategic Research and the Wellcome Trust. Additional support was provided by the National Science Foundation of China (No. 30670429 to J.M. and C.Q.) and Natural Science Foundation of Hubei Province of China (No. 2010CDB01206). Results shown in this report are derived from work performed at Argonne National Laboratory, Structural Biology Center at the Advanced Photon Source. Argonne is operated by UChicago Argonne, LLC, for the U.S. Department of Energy, Office of Biological and Environmental Research under Contract DE-AC02-06CH11357.

References

[1] A.V. Chestkov, I.D. Baka, M.V. Kost, G.P. Georgiev, V.L. Buchman, The d4 gene family in the human genome, Genomics 36 (1996) 174–177.
[2] T.G. Gabig, C.D. Crean, A. Klenk, H. Long, N.G. Copeland, D.J. Gilbert, N.A. Jenkins, D. Quincey, F. Parente, F. Lespinasse, G.F. Carle, P. Gaudray, C.X. Zhang, A. Calender, J. Hoeppener, K. Kas, R.V. Thakker, F. Farnebo, B.T. Teh, C. Larsson, F. Piehl, J. Lagercrantz, S. Khodaei, E. Carson, G. Weber, Expression and chromosomal localization of the Requiem gene, Mamm. Genome 9 (1998) 660–665.
[3] I.B. Mertsalov, N.N. Ninkina, J.S. Wanless, V.L. Buchman, L.I. Korochkin, D.A. Kulikova, Generation of mutant mice with targeted disruption of two members of the d4 gene family: neuro-d4 and cer-d4, Dokl Biochem. Biophys. 419 (2008) 65–68.
[4] T.G. Gabig, P.L. Mantel, R. Rosli, C.D. Crean, Requiem: a novel zinc finger gene essential for apoptosis in myeloid cells, J. Biol. Chem. 269 (1994) 29515–29519.
[5] T. Tando, A. Ishizaka, H. Watanabe, T. Ito, S. Iida, T. Haraguchi, T. Mizutani, T. Izumi, T. Isobe, T. Akiyama, J. Inoue, H. Iba, Requiem protein links RelB/p52 and the Brm-type SWI/SNF complex in a noncanonical NF-kappaB pathway, J. Biol. Chem. 285 (2010) 21951–21960.
[6] Y. Lim, A. Mantalaris, M.G. Yap, D.C. Wong, Simultaneous targeting of Requiem & Alg-2 in Chinese hamster ovary cells for improved recombinant protein production, Mol. Biotechnol. 46 (2010) 301–307.
[7] Y. Lim, V.X. Seah, A. Mantalaris, M.G. Yap, D.C. Wong, Elucidating the role of requiem in the growth and death of Chinese hamster ovary cells, Apoptosis 15 (2010) 450–462.
[8] R. Matsuyama, I. Takada, A. Yokoyama, S. Fujiyma-Nakamura, N. Tsuji, H. Kitagawa, R. Fujiki, M. Kim, M. Kouzu-Fujita, T. Yano, S. Kato, Double PHD fingers protein DPF2 recognizes acetylated histones and suppresses the function of estrogen-related receptor alpha through histone deacetylase 1, J. Biol. Chem. 285 (2010) 18166–18176.
[9] M. Lange, B. Kaynak, U.B. Forster, M. Tönjes, J.J. Fischer, C. Grimm, J. Schlesinger, S. Just, I. Dunkel, T. Krueger, S. Mebus, H. Lehrach, R. Lurz, J. Gobom, W. Rottbauer, S. Abdelilah-Seyfried, S. Sperling, Regulation of muscle

development by DPF3, a novel histone acetylation and methylation reader of the BAF chromatin remodeling complex, Genes Dev. 22 (2008) 2370–2384.
[10] L. Zeng, Q. Zhang, S. Li, A.N. Plotnikov, M.J. Walsh, M.M. Zhou, Mechanism and regulation of acetylated histone binding by the tandem PHD finger of DPF3b, Nature 466 (2010) 258–262.
[11] E. Nabirochkina, O.B. Simonova, I.B. Mertsalov, D.A. Kulikova, N.G. Ladigina, L.I. Korochkin, V.L. Buchman, Expression pattern of *dd4*, a sole member of the d4 family of transcription factors in *Drosophila melanogaster*, Mech. Dev. 114 (2002) 119–123.
[12] S. Oka, Y. Shiraishi, T. Yoshida, T. Ohkubo, Y. Sugiura, Y. Kobayashi, NMR structure of transcription factor Sp1 DNA binding domain, Biochemistry 43 (2004) 16027–16035.
[13] E. Peisach, C.O. Pabo, Constraints for zinc finger linker design as inferred from X-ray crystal structure of tandem Zif268-DNA complexes, J. Mol. Biol. 330 (2003) 1–7.
[14] J.H. Viles, S.U. Patel, J.B. Mitchell, C.M. Moody, D.E. Justice, J. Uppenbrink, P.M. Doyle, C.J. Harris, P.J. Sadler, J.M. Thornton, Design, synthesis and structure of a zinc finger with an artificial beta-turn, J. Mol. Biol. 279 (1998) 973–986.
[15] F. Cordier, E. Vinolo, M. Véron, M. Delepierre, F. Agou, Solution structure of NEMO zinc finger and impact of an anhidrotic ectodermal dysplasia with immunodeficiency-related point mutation, J. Mol. Biol. 377 (2008) 1419–1432.
[16] L. Holm, S. Kääriäinen, P. Rosenström, A. Schenkel, Searching protein structure databases with DaliLite v. 3, Bioinformatics 24 (2008) 2780–2781.
[17] K.J. Brayer, D.J. Segal, Keep your fingers off my DNA: protein-protein interactions mediated by C2H2 zinc finger domains, Cell Biochem. Biophys. 50 (2008) 111–131.
[18] U. Schäfer, O. Rausch, T. Bouwmeester, T. Pieler, Sequence-specific recognition of a repetitive DNA element by a C2H2 zinc-finger protein in *Xenopus*, Eur. J. Biochem. 226 (1994) 567–576.
[19] R.T. Nolte, R.M. Conlin, S.C. Harrison, R.S. Brown, Differing roles for zinc fingers in DNA recognition: structure of a six-finger transcription factor IIIA complex, Proc. Natl. Acad. Sci. USA 95 (1998) 2938–2943.
[20] A.V. Persikov, M. Singh, An expanded binding model for Cys(2)His(2) zinc finger protein-DNA interfaces, Phys. Biol. 8 (2011) 035010.
[21] W. Minor, M. Cymborowski, Z. Otwinowski, M. Chruszcz, HKL-3000: the integration of data reduction and structure solution – from diffraction images to an initial model in minutes, Acta Crystallogr. D Biol. Crystallogr. 62 (2006) 859–866.
[22] J.M. Guss, E.A. Merritt, R.P. Phizackerley, B. Hedman, M. Murata, K.O. Hodgson, H.C. Freeman, Phase determination by multiple-wavelength X-ray diffraction: crystal structure of a basic "blue" copper protein from cucumbers, Science 4867 (1998) 806–811.
[23] T.R. Schneider, G.M. Sheldrick, Substructure solution with SHELXD, Acta Crystallogr. D Biol. Crystallogr. 58 (2002) 1772–1779.
[24] A. Perrakis, M. Harkiolaki, K.S. Wilson, V.S. Lamzin, ARP/wARP and molecular replacement, Acta Crystallogr. D Biol. Crystallogr. 57 (2001) 1445–1450.
[25] P. Emsley, B. Lohkamp, W.G. Scott, K. Cowtan, Features and development of Coot, Acta Crystallogr. D Biol. Crystallogr. 66 (2010) 486–501.
[26] P.D. Adams, R.W. Grosse-Kunstleve, L.W. Hung, T.R. Ioerger, A.J. McCoy, N.W. Moriarty, R.J. Read, J.C. Sacchettini, N.K. Sauter, T.C. Terwilliger, PHENIX: building new software for automated crystallographic structure determination, Acta Crystallogr. D Biol. Crystallogr. 58 (2002) 1948–1954.
[27] G.N. Murshudov, A.A. Vagin, E.J. Dodson, Refinement of macromolecular structures by the maximum-likelihood method, Acta Crystallogr. D Biol. Crystallogr. 53 (1997) 240–255.
[28] I.W. Davis, A. Leaver-Fay, V.B. Chen, J.N. Block, G.J. Kapral, X. Wang, L.W. Murray, 3rd Arendall WB, J. Snoeyink, J.S. Richardson, D.C. Richardson, MolProbity: all-atom contacts and structure validation for proteins and nucleic acids, Nucleic Acids Res. 35 (2007) 375–383.