PROTEIN STRUCTURE REPORT

The solution structure of ZNF593 from *Homo sapiens* reveals a zinc finger in a predominately unstructured protein

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Abstract

Here, we report the solution structure of ZNF593, a protein identified in a functional study as a negative modulator of the DNA-binding activity of the Oct-2 transcription factor. ZNF593 contains a classic C_2H_2 zinc finger domain flanked by about 40 disordered residues on each terminus. Although the protein contains a high degree of intrinsic disorder, the structure of the zinc finger domain was resolved by NMR spectroscopy without a need for N- or C-terminal truncations. The tertiary structure of the zinc finger domain is composed of a β-hairpin that positions the cysteine side chains for zinc coordination, followed by an atypical kinked α-helix containing the two histidine side chain ligands. The structural topology of ZNF593 is similar to a fragment of the double-stranded RNA-binding protein Zfa and the C-terminal zinc finger of MBP-1, a human enhancer binding protein. The structure presented here will provide a guide for future functional studies of how ZNF593 negatively modulates the DNA-binding activity of Oct-2, a POU domain-containing transcription factor. Our work illustrates the unique capacity of NMR spectroscopy for structural analysis of folded domains in a predominantly disordered protein.

Keywords: structural genomics; NMR; C_2H_2 zinc finger; ZNF593; Oct-2

Zinc-binding domains, typified by the zinc finger motif, are found in a variety of proteins and play crucial roles in protein–protein and protein–nucleic acid interactions (DNA and/or RNA). The assembly of multiple zinc fingers within a single protein increases ligand binding specificity or allows for interactions with multiple partners (Iuchi 2001). Zinc finger-containing transcription factors represent one of the largest and most complex eukaryotic gene superfamilies and regulate gene expression in an array of cellular activities including differentiation, development, and tumor suppression (Nyborg and Peersen 2004). Zinc finger domains are formed by short peptide sequences (about 40 residues) that exhibit a secondary structure stabilized by a zinc ion that is coordinated by cysteine and histidine side chains. The several common zinc-binding motifs, C_2H_2, C_2HC, C_2C_2, $C_2HCC_2C_2$, and $C_2C_2C_2C_2$, exhibit diversity in the composition and spacing of the four zinc-coordinating residues (Iuchi 2001).

The canonical C_2H_2 zinc finger motif is common in nucleic acid binding proteins and shows the typical $CX_{2-4}CX_{12}HX_{2-6}H$ amino acid pattern of the zinc-coordinating residues (Iuchi 2001). This motif folds into a compact structure that is comprised of a two- or three-stranded N-terminal β-sheet containing the two conserved cysteine residues and a C-terminal α-helix containing the two

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conserved histidine residues. The sulfur and nitrogen ligands coordinate the zinc ion in a tetrahedral arrangement (Lachenmann et al. 2004). Here, we report the structure of human ZNF593, a protein that contains a core C_2H_2 zinc finger bracketed by about 40 disordered residues on each terminus, solved by NMR spectroscopy at the Center for Eukaryotic Structural Genomics (CESG, http://www.uwstructuralgenomics.org). The ZNF593 gene was first isolated in 1997 as *BC019267.1* from a functional study of the Oct-2 transcription factor (Terunuma et al. 1997). This study showed that ZNF593 displayed a dominant negative effect on the DNA-binding activity of Oct-2, which is a Pit-1, Oct-1, Oct-2, and Unc-86 (POU) domain-containing protein that targets the specific octamer site ATGCAAAT found upstream of the TATA box (Kemler et al. 1991). Structural characterization of ZNF593 serves as an initial step toward understanding how the protein regulates the DNA-binding activity of Oct-2.

Results and Discussion

Structure of ZNF593

ZNF593 consists of 115 residues with a molecular weight of 13.2 kDa. The two-dimensional ^{15}N–^{1}H HSQC spectrum showed two distinct sets of resonances with nonuniform peak intensities (Fig. 1A). One set of resonances displayed a dispersed pattern with uniform intensity that is consistent with a stable folded domain. The second set of resonances displayed a narrow chemical shift distribution that suggested a segment of the protein was unstructured. Analysis of the ZNF593 amino acid sequence using the Pfam database (Finn et al. 2006) identified a potential C_2H_2 zinc finger domain ($C–X_2–C–X_{12}–H–X_5–H$) that spanned residues 24–92, consistent with the previous report of a TFIIIA-type zinc finger centrally located within the protein (Terunuma et al. 1997). Addition of 2 mM

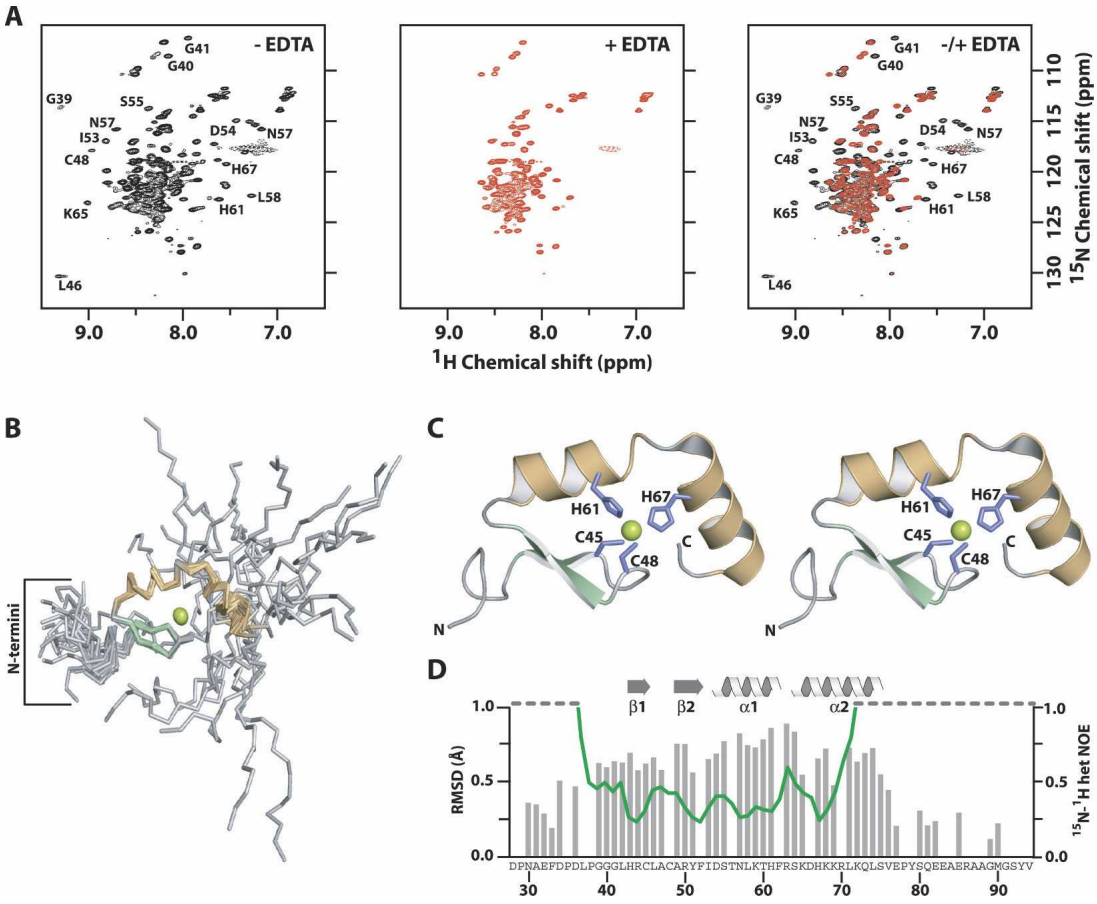

Figure 1. (*A*) Two-dimensional ^{15}N–^{1}H HSQC spectrum of ZNF593 before (black contours) and after (red contours) the addition of 2 mM EDTA. Resonance assignments are indicated by a one-letter amino acid code followed by the specified residue number. (*B*) Ensemble of 20 conformers of ZNF593. (Gray) The N- and C-terminal unstructured regions (residues 28–42 and 76–94), (green) β-sheet, (orange) α-helices, (green sphere) the zinc ion. (*C*) Cross-eyed stereoview of ZNF593, with unstructured residues 1–35 and 78–116 omitted for clarity. (*D*) Backbone r.m.s.d. and ^{15}N–^{1}H heteronuclear NOEs values displayed as a function of amino acid sequence. Locations of secondary structure elements are indicated.

EDTA to ZNF593 collapsed the ^{15}N–^{1}H HSQC spectrum to a narrow chemical shift range consistent with an unfolded protein, confirming that the secondary structure is stabilized by the coordination of a metal ion. Taken together, these data suggest that ZNF593 contains a C_2H_2 zinc finger domain.

All ^{1}H, ^{15}N, and ^{13}C chemical shift assignments were completed by standard NMR methods (Markley et al. 2003), and the three-dimensional structure was solved using an automated procedure for iterative NOE assignment (Herrmann et al. 2002). The final ensemble of 20 conformers (PDB code: 1ZR9) is shown in Figure 1B, and the statistical analysis describing the agreement with experimental constraints, coordinate precision, and stereochemical quality are summarized in Table 1. The ensemble was generated from 46 ϕ and ψ dihedral angle constraints, 820 nonredundant NOE-derived distance restraints, and four fixed distance constraints to the zinc ion where the zinc–sulfur and zinc–nitrogen bond lengths were set to 2.3 Å and 2.0 Å, respectively (Summers et al. 1992; Wang et al. 2003). The tertiary structure of the zinc finger domain reveals a short β-hairpin that positions two cysteine side chain thiolates for zinc binding, followed by a kinked α-helix that orients two histidine side chains as ligands for zinc coordination (Fig. 1C). The $^{13}C^{\alpha}$ and $^{13}C^{\beta}$ chemical shift values for cysteines 45 (C^{α} 58.63 ppm and C^{β} 31.51 ppm) and 48 (C^{α} 60.51 ppm and C^{β} 31.08 ppm) are consistent with those reported when the sulfur groups are coordinated to a zinc ion (Lee et al. 1992; Kornhaber et al. 2006). Low backbone r.m.s.d. values and high ^{15}N–^{1}H heteronuclear NOE values for residues 36–71 (Fig. 1D) confirm that the zinc finger core of ZNF593 is well ordered. In contrast, the rapid increase in backbone r.m.s.d. values in concert with the rapid decrease in ^{15}N–^{1}H heteronuclear NOE values indicates that the first 35 and last 40 residues of the ZNF593 do not adopt stable secondary or tertiary structures and are highly mobile. Although ~65% ZNF593 is intrinsically disordered, the structure of the zinc finger domain was resolved by NMR spectroscopy without the need for N- or C-terminal truncations. In contrast, some proteins containing <25% disorder require N- or C-terminal truncations to make them amenable to NMR structure determination (Waltner et al. 2005). The case of ZNF593 highlights a valuable role for NMR in structural genomics, as proteins that contain high levels of disorder may be difficult to crystallize.

Table 1. *Statistics for the 20 ZNF593 conformers*

Experimental constraints	
Distance constraints	
Long	144
Medium [$1 < (i - j) \leq 5$]	186
Sequential [$(i - j) = 1$]	238
Intraresidue [$i = j$]	252
Total	820
Dihedral angle constraints (ϕ and ψ)	46
Average atomic r.m.s.d. to the mean structure (Å)	
Residues 36–71	
Backbone (C^{α}, C′, N)	0.52 ± 0.13
Heavy atoms	1.07 ± 0.11
Deviations from idealized covalent geometry	
Bond lengths RMSD (Å)	0.017
Bond angles RMSD (°)	1.4
Constraint violations[a]	
NOE distance number >0.5 Å	0 ± 0
NOE distance RMSD (Å)	0.035 ± 0.001
Torsion angle violations number >5°	0 ± 0
Torsion angle violations RMSD (°)	0.485 ± 0.097
Ramachandran statistics (% of residues 28–94)	
Most favored	77.6 ± 3.55
Additionally allowed	18.5 ± 4.2
Generously allowed	2.8 ± 2.5
Disallowed	1.2 ± 1.5
Lennard-Jones energy[b] (kJ mol^{-1})	−3,554 ± 124

[a]The largest NOE violation in the ensemble of structures was 0.42 Å. The largest torsion-angle violation in the ensemble of structures was 4.0°.
[b]Nonbonded energy was calculated in XPLOR-NIH.

Structural homologs of ZNF593

ZNF593 was identified in 1997 in a screen for proteins that could derepress a reporter gene under the control of Oct-2, a POU domain-containing transcription factor that targets the regulatory element, ATGCAAAT (Phillips Jr. and Luisi 2000; Shore et al. 2002). Thus, ZNF593 negatively regulates the DNA-binding activity of Oct-2 and its transcriptional regulatory activity (Terunuma et al. 1997). The inhibitory pathway of ZNF593 remains unknown; however, it has been speculated that ZNF593 inactivates Oct-2 by binding directly to the octamer sequence or by direct interaction with Oct-2 (Phillips et al. 2007).

Analysis of the ZNF593 protein sequence using the VAST (http://www.ncbi.nlm.nih.gov/Structure/VAST/vastsearch.html) (Gibrat et al. 1996), FATCAT (http://fatcat.burnham.org) (Ye and Godzik 2003), and FFAS03 servers (http://ffas.burnham.org) (Jaroszewski et al. 2005) failed to return a consensus set of closely related proteins. Inspection of the results from all three servers identified only three significant matches that included the second zinc finger of the double-stranded RNA-binding protein Zfa (dsRBP-Zfa), the human enhancer binding protein MBP-1, and ZFY-swap (Fig. 2A,B). The second zinc finger of dsRBP-Zfa (Moller et al. 2005) (PDB code: 1ZU1) showed the greatest structural similarity (3.23 Å r.m.s.d. over 38 aligned C^{α} atoms) even though it shares only 21% sequence identity with ZNF593. Double-stranded RNA has recently received much attention because of its functional roles, which include defense against viruses, processing of cellular RNA, and involvement in gene silencing and RNA interference as a mechanism of post-transcriptional gene regulation (Bernstein et al. 2001; Saunders and Barber

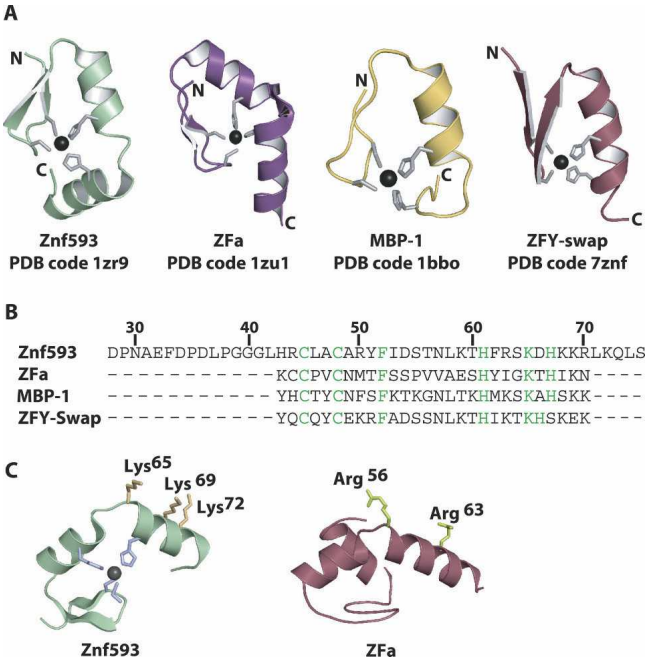

Figure 2. (*A*) Ribbon diagrams of ZNF593 (PDB code: 1zr9), the second zinc finger of dsRNA-ZFa (residues 94–128) (PDB code: 1zu1), the C-terminal zinc finger of MBP-1 (residues 30–57) (PDB code: 1bbo), and ZFY-swap (PDB code: 7znf). (*B*) Multiple sequence alignments demonstrating residue conservation between similar zinc fingers. (Green residues) Conserved amino acids. (*C*) Positively charged residues located on the solvent-exposed side of the α-helix are compared between ZNF593 and double-stranded RNA-binding protein ZFa.

2003). Two of the seven zinc fingers found in dsRBP-Zfa, a protein originally identified in *Xenopus laevis*, and the single zinc finger of ZNF593 share a common cluster of basic residues (Fig. 2C) and an atypical kinked α-helix motif (Moller et al. 2005). Studies showed that dsRBP-Zfa bound preferentially to double-stranded RNA, and it was speculated that recognition of dsRNA by dsRNA-Zfa will most likely prove to be a shape-dependent interaction (Batey et al. 1999; Hermann and Patel 1999; Carlson et al. 2003).

The human enhancer binding protein MBP-1 (PDB code: 1BB0) contains two zinc fingers in a tandem arrangement, with the C-terminal zinc finger having 27% sequence identity with ZNF593 and an r.m.s.d of 3.42 Å over 37 aligned C^{α} atoms with ZNF593. Both zinc fingers contain a kinked α-helix that is not commonly found in the classical C_2H_2 zinc finger motif (Omichinski et al. 1990). MBP-1 has been reported to bind specifically to the promoter of the MHC class 1H-2Kb gene (Singh et al. 1988). MBP-1 also includes a large number of positively charged and polar side chains located on the solvent-exposed surface of the helix, which is speculated to be the area involved in DNA recognition (Omichinski et al. 1990).

ZFY-swap (PDB code: 7ZNF), a male-associated zinc finger protein located on the human Y chromosome, shares 40% sequence identity (over 29 residues) with ZNF593. Although ZFY-swap differs structurally (2.22 Å r.m.s.d. over 29 aligned C^{α} atoms) because it lacks the kinked α-helix, it nevertheless maintains the ββα-helix topology common to the classical C_2H_2 zinc finger. ZFY is a putative transcription factor encoded by the sex-determining region of the Y chromosome and is believed to exhibit DNA-binding activity based on a conserved alteration in amino acid pattern observed in the odd- and even-numbered zinc finger domains that pair together to form a two-finger repeat (Page et al. 1987; Weiss et al. 1990; Kochoyan et al. 1991). ZFY-swap is a variant of ZFY zinc finger domain 6 in which the position of the conserved aromatic residue in the even-numbered domain was ''swapped'' with that of an odd-numbered domain, yielding a Y10K and S12F double mutant. Swapping of the aromatic residues altered the packing of the hydrophobic core and the respective DNA-binding surface but maintained the overall fold. This alternation of residues in two-finger repeats is not commonly found in zinc fingers but has been reported for a number of DNA-binding proteins and may have implications for the recognition of nucleic acids.

The present study shows that ZNF593 contains a classical C_2H_2 zinc finger domain that utilizes a canonical N-terminal β-hairpin and an atypical helix–kink–helix motif for the coordination of the zinc ion. Of the available three-dimensional structures, that of the second zinc finger from dsRNA-Zfa most resembles the ZNF593 structure, including a common electropositive patch on the helix surface. It remains to be seen whether this structural similarity will correlate with a common functionality, because the function of neither protein has been determined.

Materials and Methods

Protein expression and purification

The protein product of *Homo sapiens* gene *BC019267.1*, ZNF593, was expressed and purified in [*U*-^{13}C, ^{15}N]-labeled form using a wheat germ cell-free expression system as previously described (Vinarov et al. 2004). In short, the protein was expressed with an N-terminal His_6 fusion tag and purified by Ni-NTA affinity chromatography, followed by size-exclusion chromatography (Tyler et al. 2005).

NMR spectroscopy

All NMR data were obtained at 25°C on a Bruker 600-MHz spectrometer equipped with a CryoProbe and processed with NMRPipe software (Delaglio et al. 1995). Total acquisition time was ~289 h. The protein was shown to be folded throughout the data collection time as indicated by one-dimensional and two-dimensional spectra collected at the start and end of data compilation. The ^{1}H, ^{15}N, and ^{13}C resonance assignments were 89% complete for residues 28–94 and were obtained in an automated

manner using the program Garant (Bartels et al. 1996), with peak lists from 3D HNCO, HNCACO, HNCA, HNCOCA, HNCACB, and CCONH spectra that were generated automatically with SPSCAN. The assignments were manually inspected, edited, and completed by XEASY (Bartels et al. 1996). Side chain assignments were analyzed and completed from HCCONH, HBHACONH, and HCCH-TOCSY spectra using XEASY.

Structure determination

A total of 820 unique NOE distance constraints were collected from three-dimensional ^{15}N-edited NOESY-HSQC, ^{13}C-edited NOESY-HSQC, and ^{13}C-aromatic-edited NOESY-HSQC spectra ($\tau_{mix} = 80$ ms). The distance constraints included four fixed distances from the zinc ion to ZNF593 where the zinc–sulfur and zinc–nitrogen bond lengths were set to 2.3 Å and 2.0 Å, respectively (Summers et al. 1992; Wang et al. 2003). Backbone ϕ and ψ dihedral angle constraints were generated from secondary shifts of the $^{1}H^{\alpha}$, $^{13}C^{\alpha}$, $^{13}C^{\beta}$, $^{13}C'$, and ^{15}N nuclei shifts using the program TALOS (Cornilescu et al. 1999). Structures were generated in an automated manner using the torsion angle dynamics program CYANA (Herrmann et al. 2002). The automated NOEAssign module produced an ensemble with high precision and low residual constraint violations that required minimal manual refinement. The ensemble of 20 CYANA conformers with the lowest target function were subjected to a molecular dynamics protocol in explicit solvent (Linge et al. 2003) using XPLOR-NIH (Schwieters et al. 2003).

Data deposition

Coordinates and constraints have been placed in the Protein Data Bank, Research Collaboratory for Structural Bioinformatics, Rutgers University, New Brunswick, New Jersey (http://www.rcsb.org/pdb/home/home.do) under PDB code 1ZR9. All time-domain NMR data and chemical shift assignments have been deposited in BioMagResBank (http://www.bmrb.wisc.edu/) under BMRB Accession 6682.

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