HHS Public Access

Author manuscript

Proteins. Author manuscript; available in PMC 2018 May 01.

Published in final edited form as:

Proteins. 2017 May ; 85(5): 957–962. doi:10.1002/prot.25259.

Solution NMR structure of zinc finger 4 and 5 from human INSM1, an essential regulator of neuroendocrine differentiation

Jiang Zhu[1], **Huapu Wang**[1,2], **Theresa A. Ramelot**[3], **Michael A. Kennedy**[3], **Rui Hu**[1], **Xiali Yue**[2], **Maili Liu**[1,*], and **Yunhuang Yang**[1,*]

[1]State Key Laboratory of Magnetic Resonance and Atomic Molecular Physics, Wuhan Center for Magnetic Resonance, Wuhan Institute of Physics and Mathematics, Chinese Academy of Sciences, Wuhan 430071, China

[2]College of Science, Huazhong Agricultural University, Wuhan 430070, China

[3]Department of Chemistry and Biochemistry, and the Northeast Structural Genomics Consortium, Miami University, Oxford, Ohio 45056, USA

Abstract

Human INSM1 containing five C-terminal C2H2-type zinc fingers (ZFs), is a key regulator of neuroendocrine development. Previous research reported that full-length INSM1 containing all five ZFs recognized a consensus DNA sequence. Structure elucidation of human INSM1 ZFs is currently insufficient to understand the DNA binding mechanism. Herein, we present the solution NMR structure of ZF4-5, in which the two ZFs adopt a head-to-tail arrangement and each ZF features a canonical ββα fold. NMR titrations and isothermal titration calorimetry experiments showed that ZF4-5 binds weakly to the consensus DNA sequence.

Keywords

INSM1; ZF4-5; Consensus DNA; Solution structure; Mechanism

INTRODUCTION

The human *insulinoma-associated 1* (*INSM1*, formerly named *IA-1*; Uniprot ID: Q01101) gene widely expressed in neuroendocrine tumors, encodes a five C2H2-type zinc finger (ZF) containing transcriptional repressor that plays an essential role in regulating the differentiation of endocrine cells and the development of neurogenic progenitor cells.[1] The C2H2-type ZF is the most common type of zinc finger domain, and is a well-studied DNA-binding domain utilized by numerous transcription factors. Transcription factors often employ multiple ZFs to achieve specific DNA recognition and binding.[2] C2H2-type zinc fingers are short protein motifs containing 20–30 residues, with structures consisting of a short antiparallel β-sheet formed by two β-strands at the N-terminus and one α-helix at the

*Correspondence to: Maili Liu and Yunhuang Yang, State Key Laboratory of Magnetic Resonance and Atomic Molecular Physics, Wuhan Center for Magnetic Resonance, Wuhan Institute of Physics and Mathematics, Chinese Academy of Sciences, Wuhan 430071, China; Phone: +86-27-87199541, Fax: +86-27-87199543; ml.liu@wipm.ac.cn; yang_yh@wipm.ac.cn.

C-terminus. Two cysteine and two histidine residues at discrete positions contribute to the binding of the zinc ion with coordination bonds.[3] The C2H2-type ZF typically recognizes sequence-specific DNA with the amino acids at positions -1, 2, 3, and 6 of the α-helix where it contacts specific nucleotides within the major groove of DNA.[4] Also, it is worth pointing out that progressively more evidence has shown that ZFs could mediate protein-RNA or protein-protein interactions.[5]

Full length human protein INSM1 has 510 amino acids, and is made up of one Snail/Ggi-1 (SNAG) motif and two proline-rich regions in the N-terminus, as well as five tandem C2H2-type zinc fingers in the C-terminus.[1, 6] INSM1 represses gene transcription through directly binding to the promoters containing the consensus sequence 5′-$T^{G}/_{T}{}^{C}/_{T}{}^{C}/_{T}{}^{T}/_{A}GGGG^{G}/_{T}C^{G}/_{A}$-3′, and the binding is mediated by the C-terminal ZFs.[6] The five C-terminal ZFs in the human INSM1 are symmetrically spaced with ZF3 located in the middle, while ZF1-2 and ZF4-5 are separated by 45 and 46 amino acids from ZF3, respectively. Among these ZFs, a reported study on INSM1 transcriptional activity revealed that ZF2-3 was sufficient for regulating transcription of one particular INSM1 target, while ZF4-5 was dispensable for transcriptional activity of this target in cells.[6] However, much work remains to characterize the roles of the 5 ZFs for specificity of binding to different consensus sequence targets, and there are no structures of these ZFs available, to date, to elucidate the transcriptional regulation mechanism of INSM1.

In this short note, we present the solution NMR structure of ZF4-5, a truncated INSM1 fragment (G424-R497) containing ZF4 and ZF5 connected by a short ‘AAQV’ linker. The NMR structure reveals that each ZF consists of one short antiparallel β-sheet at the N-terminus and one α-helix at the C-terminus, and adopts a canonical ββα fold. The interaction between ZF4-5 and the consensus sequence-containing DNA was investigated using NMR titrations and isothermal titration calorimetry (ITC) experiments. NMR titrations exhibited small chemical shift perturbations for the ^{1}H-^{15}N resonances from five residues in ZF4-5 after additions of the dsDNA. ITC experiments revealed that ZF4-5 interacted weakly with the dsDNA ($K_d \approx 20$ μM). Our results suggested that although ZF4-5 may not play a major role in recognition of the sequence-specific DNA, it could still contribute to the interaction in a non-canonical manner. Further exploration of the rest of the INSM1 ZFs structures and DNA binding properties is needed to fully understand the DNA binding mechanism of INSM1.

MATERIALS AND METHODS

(1) Protein expression, purification and uniformly [^{13}C, ^{15}N] labeling

The DNA fragment encoding human INSM1 ZF4-5 was cloned into a pET15 expression vector derivative with an additional N-terminal His_6-tag (MGHHHHHHSH). The *U*-[^{13}C,^{15}N]-labeled (NC) and *U*-^{15}N, 5% biosynthetically-directed ^{13}C-labeled (NC5) samples of ZF4-5 were prepared. Briefly, BL21 (DE3) *E. coli* cells containing above plasmid construct were grown in MJ minimal medium supplemented with 0.25 mM $ZnCl_2$ at 37 °C until the OD_{600} was 0.6–0.8. At this point, 0.1 mM isopropyl-β-D-thiogalactopyranoside (IPTG) was added and the incubation temperature was shifted to 17 °C for protein expression. Overnight culture was harvested by centrifugation and lysed by sonication. The

clarified supernatant containing INSM1 ZF4-5 protein was purified using an ÄKTAxpress™ (GE Healthcare) with a Ni-affinity column (HisTrap IMAC HP™ column) followed by a gel filtration column (HiLoad 26/60 Superdex 75). The purified protein was concentrated to 0.69 mM in the NMR buffer containing 10% D_2O (*v*/*v*), 20 mM MES, 100 mM NaCl, 10 mM DTT, 5 mM $CaCl_2$, and 0.02 % NaN_3 at pH 6.5.

(2) Rotational correlation time (τ_c) estimate

Average ^{15}N relaxation times were determined from 1D ^{15}N-edited T_1 and T_2 (CPMG) experiments on *U*-[^{13}C, ^{15}N]-labeled ZF4-5 recorded at a Varian Inova 600 MHz at 298 K. Longitudinal T_1 relaxation delays were 50, 100, 200, 300, 400, 600, 800, 1000, 1500, and 2000 ms; transverse T_2 relaxation CPMG delays were 10, 20, 30, 50, 70, 100, 130, 170, 210, and 250 ms; both experiments had 1.5 s recycle delays. T_1 and T_2 relaxation times were obtained by integration from 8.5 to 10.5 ppm, and τ_c was approximated following the literature equation.[7] The isotropic overall rotational correlation time of *U*-[^{13}C, ^{15}N]-labeled ZF4-5 was 7.3 ns based on the backbone ^{15}N T_1 and T_2 relaxation time measurements. From the linear fit of protein molecular weight (M.W.) versus correlation time for a series of standard proteins,[8] ZF4-5 was estimated to have a M.W. of 9.6 kDa (Supporting Information Fig. S1). This result indicated that INSM1 ZF4-5 under the NMR study conditions existed predominantly as a monomer (unlabeled ZF4-5 M.W. of 9.2 kDa).

(3) Chemical shift assignments and structure calculation

A Varian Inova 600 MHz spectrometer and a Bruker Avance III 850 MHz spectrometer were used to record the NMR data at 298 K. NMR data collected for chemical shift assignments and structural calculation were as follows: 2D ^1H-^{15}N HSQC and ^1H-^{13}C HSQC, 3D HNCO, HNCA, HN(CO)CA, HNCACB, CBCA(CO)NH, HNHA, H(C)CH-TOCSY, two ^{13}C-edited NOESY-HSQC (τ_m = 70 ms) optimized for either aliphatic or aromatic carbons, and ^{15}N-edited NOESY-HSQC (τ_m = 70 ms) on the NC sample, 3D (H)CCH-TOCSY and 4D ^{13}C-^{13}C-HMQC-NOESY-HMQC (τ_m = 70 ms) on the NC sample in D_2O, 2D constant time ^1H-^{13}C HSQC (CT-HSQC) on the NC5 sample. The backbone and side chain resonances were automatically assigned using the PINE server from NMRFAM,[9] followed by manual validation and correction. Stereospecific assignments of isopropyl methyl groups of Leu and Val residues were determined from 2D ^1H-^{13}C CT-HSQC spectrum on the NC5 sample.[10] Overall, non-proline backbone cross peaks of amide proton and nitrogen were completely assigned with total 92.4% complete assignments of ^1H, ^{13}C and ^{15}N chemical shifts (Supporting Information Fig. S2). These ^1H, ^{13}C and ^{15}N assignments have been deposited to the BioMagResDB (BMRB accession number, 18551).

NOE-based inter-proton distance restraints were determined automatically for INSM1 ZF4-5 using CYANA 3.0. Input for CYANA consisted of chemical shift assignments, NOESY peak lists from four NOESY spectra with peak intensities, the restraints for backbone phi (ϕ) and psi (ψ) torsion angle derived from chemical shifts of backbone atoms using the TALOS+ software program.[11] Distance restraints for two zinc ions coordinated to the corresponding histidines or cysteines were introduced as pseudo-NOEs as previously described.[12] Manual and iterative refinements of NOESY peak picking lists were guided using NMR RPF quality to assess “goodness of fit” between calculated structures and NOESY peak lists.[13] The 20

lowest energy structures calculated by CYANA 3.0 were further refined using restrained molecular dynamics in explicit water CNS 1.2[14] and the PARAM19 force field, using the final NOE-derived distance restraints and TALOS-derived dihedral angle restraints. The final NMR ensemble of 20 structures has been deposited to the Protein Data Bank (PDB ID 2LV2). Structural statistics and global structure quality factors were computed using PSVS version 1.4 (Table 1).[15]

(4) NMR titration with the consensus DNA

Single-stranded DNA of 5′-TGTCAGGGGGCA-3′ and 5′-TGCCCCCTGACA-3′ (Sangon Biotech Co.), were re-suspended with Milli-Q water and mixed at equimolar concentrations to obtain the dsDNA. Mixtures containing 0.5 mM ZF4-5 and dsDNA with a series of concentrations (0, 0.25, 0.5, and 0.75 mM) were pre-mixed and allowed to equilibrate for one hour. ^{1}H-^{15}N HSQC spectra were collected on a Bruker Avance III 600 MHz instrument at 298 K.

(5) Isothermal titration calorimetry

The isothermal titration calorimetry (ITC) experiments were carried out on a VP-ITC instrument (MicroCal Inc., Northampton, MA, USA) at 298 K. All samples were prepared in the same buffer as that was used in the NMR experiments to avoid any heat changes resulted from mixing buffers. A 289 μM protein solution of INSM1 ZF4-5 was titrated into the reservoir containing 33 μM dsDNA, which was identical to that used for the NMR titrations with DNA. The experiments consisted of a preliminary injection of 4 μl followed by 17 injections of 15 μl protein solution. After converting the raw data into heat per injection, the curve was fitted using MicroCal Origin to obtain the binding constant (K_d), stoichiometry, and binding enthalpy. The first data point was excluded when fitting the titration curve. The heat of dilution was determined by titrating protein solution into buffer alone and subtracted from the titration data prior to curve fitting.

RESULTS AND DISCUSSION

ZF4-5 was successfully expressed after addition of 0.25 mM $ZnCl_2$ to the MJ minimal medium. ICP-MS data confirmed that ZF4-5 contains two zinc ions in the binding state (data not shown). Chemical shift dispersions in both dimensions became much narrower in the presence of excess EDTA as shown in the 2D ^{1}H-^{15}N HSQC spectrum indicated that zinc ion is indispensible for ZF structural stability (Supporting Information Fig. S3). The good signal-to-noise ratio and chemical shift dispersions suggested that ZF4-5 was well folded and suitable for structure determination using NMR spectroscopy (Supporting Information Fig. S3).

Sequence similarity analysis shows that INSM1 ZF4-5 is highly conserved. As shown in Figure 1(A), the amino acid sequence of human INSM1 ZF4-5 (424-497) is 100% identical to its counterpart in the *Pan troglodytes*, and sequence identities were 93%, 66%, 66% against *Mus musculus*, *Xenopus laevis*, and *Danio Rerio*, respectively. Fig. 1(B) shows the superposition of Cα trace of the lowest energy conformers of INSM1 ZF4-5. The RMSD of backbone atoms is 0.9 Å, and is 1.3 Å for all heavy atoms in the ordered regions. Not

surprisingly, each ZF features the canonical zinc finger structure with the ββα motif containing two β strands in a short antiparallel β-sheet followed by one α-helix. As shown in Figure 1(C), all secondary structural elements in the ZF4-5 were well defined as follows: β1 (441-442), β2 (449-450), α1 (453-461) in the ZF4, β3 (469-470), β4 (477-478), and α2 (481-489) in the ZF5. In addition, one zinc ion is tetra-coordinated by C443, C446, H459 and H464 in ZF4, whereas a second zinc ion is tetra-coordinated by C471, C474, H487 and H492 in the ZF5. The two ZF cores are formed mainly by the van der Waals contacts from hydrophobic residues such as V445, F450, L460 in the ZF4, and F469, Y473, F478, L484 and I488 in the ZF5. The two ZFs form a head-to-tail arrangement connected by a short linker, "AAQV". The orientation of the two domains is mediated through a few long-range hydrophobic interactions between the linker and each separate ZF. In addition, all histidines were coordinated to the metal ion through the $N^{\delta 1}$ (in the $N^{\epsilon 2}$-H-tautomeric form), as determined from the cross peak patterns of ^{1}H-^{15}N (via $^{2}J_{HN}$ or $^{3}J_{HN}$) in a 2D ^{1}H-^{15}N HMQC spectrum (Supporting Information Fig. S4).[16]

INSM ZF4-5 was titrated with the consensus sequence-containing DNA (5′-TGTCAGGGGGCA-3′) to different protein to DNA ratios (1:0.5, 1:1, 1:1.5). By comparison to the 2D ^{1}H-^{15}N HSQC of apo- ZF4-5 (Fig. 2A), chemical shifts for ^{1}H-^{15}N cross peaks from five residues (A466, K472, Y473, T477 and F478) changed stepwise upon addition of dsDNA. The resulting 2D ^{1}H-^{15}N HSQC spectra at the protein to DNA ratios of 1:0.5 (cyan) and 1:1 (blue) are overlaid on the apo spectrum in Figure 2B. When ZF4-5 was titrated further with DNA to a final protein to DNA ratio of 1:1.5, there were no further changes (Supporting Information Fig. S5), indicating that the stoichiometry of DNA binding to ZF4-5 is 1:1. The shifts from these residues changed in a stepwise manner, as highlighted for F478 (enlarged in the inset, Fig. 2B), demonstrating that the exchange processes between free and DNA-bound ZF4-5 were fast on the NMR time scale. Upon mapping of all five shifted residues onto the solution structure of ZF4-5 (Fig. 2C), it can be clearly seen that most residues with shifted peaks are located in ZF5, with the exception of A466 that is located in the loop between ZF4 and ZF5. Residues K472 and Y473 are located in the loop between β3 and β4, and T477 and F478 are located in β3 and β4, respectively. Surprisingly, none of them are located in the α-helix, which would be expected in the typical DNA binding mode of ZFs that use the residues in positions −1, 2, 3, 6 of the α-helix to recognize a trinucleotide DNA sequence.[4] Additionally, ITC was carried out in order to determine the affinity of DNA binding. The heat changes showed that the interaction between ZF4-5 and the DNA was exothermic (top, Fig. 2D). The fitting curve for the injection heat versus ZF4-5/dsDNA ratio suggested the DNA binding stoichiometry was 1:1, a result that was consistent with the NMR titration result. The dissociation constant (K_d) derived from the ITC fitting was 21.8 μM, indicating a relatively low binding affinity of INSM1 ZF4-5 with INSM1-targeted DNA. For example, this was more than 40-fold lower than that reported for the zinc finger transcription factor YY1 (K_d, 0.56 μM, 298 K) binding to its target DNA as determined from ITC experiments,[17] Previous *In vitro* Electrophoric Gel Mobility Shift Assay (EMSA) results confirmed that the C-terminus of INSM1 containing all five ZFs could achieve sequence-specific DNA-binding, and ZF2-3 was further proven to play a major role in INSM1-mediated transcriptional repression in cells.[6] Our NMR titration and ITC assay showed that ZF4-5 bound to the same dsDNA at a low affinity using residues that

are not in the recognition α-helix, a result which implies that these two ZFs can still contribute to the binding of this DNA sequence, albeit in a noncanonical way. The presence of multiple ZFs in transcription factors can allow for variability in target DNA recognition, and the specificity of binding by the tandem ZFs of INSM1 may be modified by their neighboring ZF domains.[2] In order to understand these interactions, further structural and functional work remains to be done with the five ZFs of INSM1 and various target DNA sequences.

In summary, solution NMR structure of human INSM1 ZF4-5 consists of two C2H2-type ZFs, each exhibiting a typical ZF structural feature. Our NMR titration and ITC experiments showed that ZF4-5 interacts weakly with the previously reported consensus dsDNA sequence.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by funds from the National Institute of General Medical Sciences of USA (Grant Numbers: U54-GM074958 and U54-GM094597), the Hundred Talent Program by Chinese Academy of Sciences, the Ministry of Science and Technology (Grant number 2016YFA051201) and the National Natural Sciences Foundation of China (Grant Number: 21575155).

References

1. Lan MS, Breslin MB. Structure, expression, and biological function of INSM1 transcription factor in neuroendocrine differentiation. FASEB J. 2009; 23(7):2024–2033. [PubMed: 19246490]
2. Razin SV, Borunova VV, Maksimenko OG, Kantidze OL. Cys2His2 zinc finger protein family: classification, functions, and major members. Biochemistry (Mosc). 2012; 77(3):217–226. [PubMed: 22803940]
3. Laity JH, Lee BM, Wright PE. Zinc finger proteins: new insights into structural and functional diversity. Curr Opin Struct Biol. 2001; 11(1):39–46. [PubMed: 11179890]
4. Wolfe SA, Nekludova L, Pabo CO. DNA recognition by Cys2His2 zinc finger proteins. Annu Rev Biophys Biomol Struct. 2000; 29:183–212. [PubMed: 10940247]
5. Brayer KJ, Segal DJ. Keep your fingers off my DNA: protein-protein interactions mediated by C2H2 zinc finger domains. Cell Biochem Biophys. 2008; 50(3):111–131. [PubMed: 18253864]
6. Breslin MB, Zhu M, Notkins AL, Lan MS. Neuroendocrine differentiation factor, IA-1, is a transcriptional repressor and contains a specific DNA-binding domain: identification of consensus IA-1 binding sequence. Nucleic Acids Res. 2002; 30(4):1038–1045. [PubMed: 11842116]
7. Kay LE, Torchia DA, Bax A. Backbone dynamics of proteins as studied by 15N inverse detected heteronuclear NMR spectroscopy: application to staphylococcal nuclease. Biochemistry. 1989; 28(23):8972–8979. [PubMed: 2690953]
8. Rossi P, Swapna GV, Huang YJ, Aramini JM, Anklin C, Conover K, Hamilton K, Xiao R, Acton TB, Ertekin A, Everett JK, Montelione GT. A microscale protein NMR sample screening pipeline. J Biomol NMR. 2010; 46(1):11–22. [PubMed: 19915800]
9. Bahrami A, Assadi AH, Markley JL, Eghbalnia HR. Probabilistic interaction network of evidence algorithm and its application to complete labeling of peak lists from protein NMR spectroscopy. PLoS Comput Biol. 2009; 5(3):e1000307. [PubMed: 19282963]
10. Neri D, Szyperski T, Otting G, Senn H, Wuthrich K. Stereospecific nuclear magnetic resonance assignments of the methyl groups of valine and leucine in the DNA-binding domain of the 434

repressor by biosynthetically directed fractional 13C labeling. Biochemistry. 1989; 28(19):7510–7516. [PubMed: 2692701]

11. Shen Y, Delaglio F, Cornilescu G, Bax A. TALOS+: a hybrid method for predicting protein backbone torsion angles from NMR chemical shifts. J Biomol NMR. 2009; 44(4):213–223. [PubMed: 19548092]
12. Wu B, Lukin J, Yee A, Lemak A, Semesi A, Ramelot TA, Kennedy MA, Arrowsmith CH. Solution structure of ribosomal protein L40E, a unique C4 zinc finger protein encoded by archaeon Sulfolobus solfataricus. Protein Sci. 2008; 17(3):589–596. [PubMed: 18218710]
13. Huang YJ, Powers R, Montelione GT. Protein NMR recall, precision, and F-measure scores (RPF scores): structure quality assessment measures based on information retrieval statistics. J Am Chem Soc. 2005; 127(6):1665–1674. [PubMed: 15701001]
14. Linge JP, Williams MA, Spronk CA, Bonvin AM, Nilges M. Refinement of protein structures in explicit solvent. Proteins. 2003; 50(3):496–506. [PubMed: 12557191]
15. Bhattacharya A, Tejero R, Montelione GT. Evaluating protein structures determined by structural genomics consortia. Proteins. 2007; 66(4):778–795. [PubMed: 17186527]
16. Pelton JG, Torchia DA, Meadow ND, Roseman S. Tautomeric states of the active-site histidines of phosphorylated and unphosphorylated IIIGlc, a signal-transducing protein from Escherichia coli, using two-dimensional heteronuclear NMR techniques. Protein Sci. 1993; 2(4):543–558. [PubMed: 8518729]
17. Houbaviy HB, Burley SK. Thermodynamic analysis of the interaction between YY1 and the AAV P5 promoter initiator element. Chem Biol. 2001; 8(2):179–187. [PubMed: 11251291]
18. Robert X, Gouet P. Deciphering key features in protein structures with the new ENDscript server. Nucleic Acids Res. 2014; 42(Web Server issue):W320–324. [PubMed: 24753421]

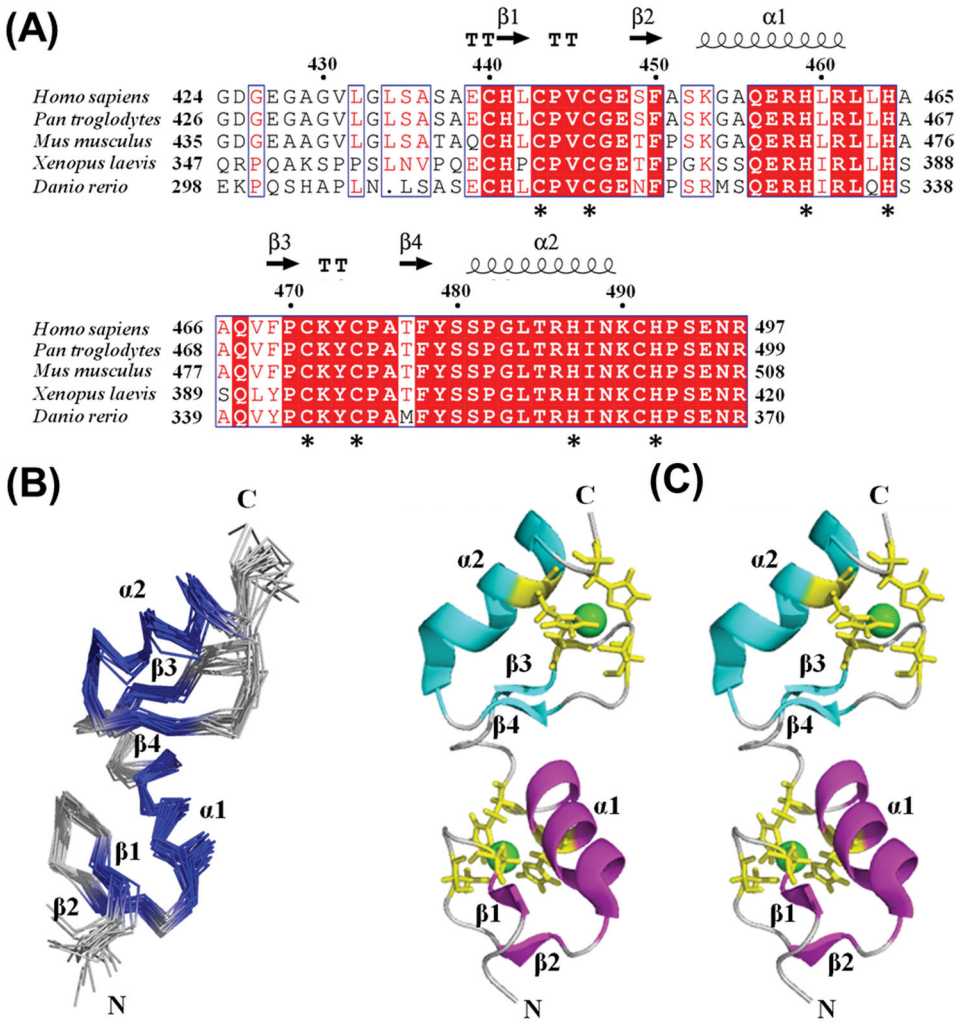

Figure 1.

Sequence Alignment and NMR structures of human ZF4-5. (A) Sequence alignment of ZF4-5 from INSM1 in *Homo sapiens* (424-497), *Pan troglodytes* (426-499), *Mus musculus* (435-508), *Xenopus laevis* (347-420), and *Danio Rerio* (INSM1a 288-370). Alignment was rendered using ESPript [18] with default setting for similarity calculations. Identical (red box with white letters) and similar (red letters) amino acids are denoted. All secondary structure elements are labeled on the top. Asterisk symbols mark the C2H2 residues coordinated to a zinc ion. (B) Superposition of Cα trace of 20 lowest energy conformers of INSM1 ZF4-5 determined by NMR spectroscopy, α-helix and β-strand colored in blue, and loop regions in gray. The disordered N-terminal His_6-tag is not shown for clarity. (C) Stereo-view of cartoon representation of the INSM1 ZF4-5 structure with the lowest overall energy. ZF4 and ZF5 are colored in violet and light blue, respectively, and all α-helices and β-strands are labeled. Sidechains of Zn^{2+}-chelating C2H2 residues are displayed, and zinc ions are represented by green spheres.

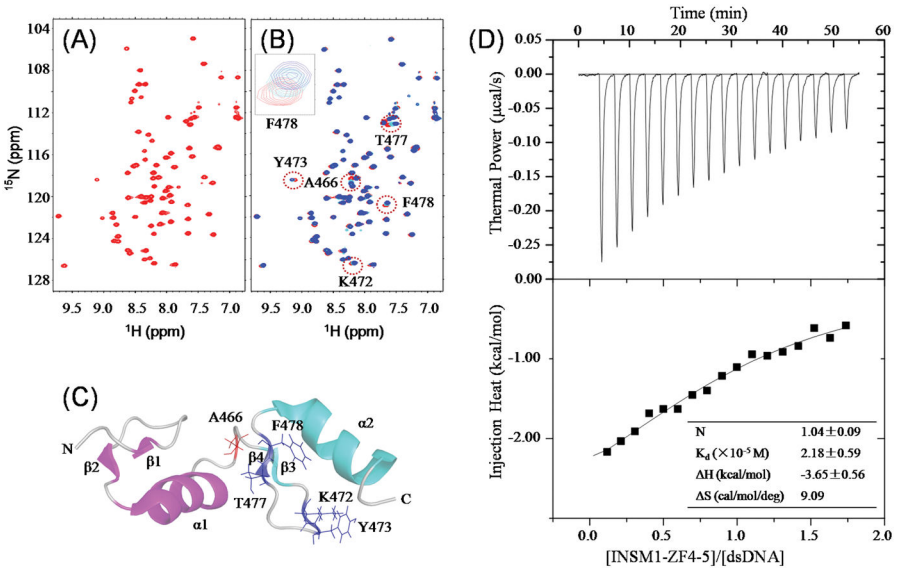

Figure 2.

Interactions between INSM1-ZF4-5 with the consensus dsDNA (5′-TGTCAGGGGGCA-3′) by NMR titration (A–C) and ITC (D) experiments. (A) 2D ^{1}H-^{15}N HSQC spectrum for apo-ZF4-5; (B) Overlay of 2D ^{1}H-^{15}N HSQC spectra for the apo- ZF4-5 (red), mixtures of ZF4-5 and dsDNA at the ratio of 1:0.5 (cyan), and 1:1 (blue). Five cross peaks with significant chemical shift perturbations were indicated. Chemical shift perturbation for the residue F478 ^{1}H-^{15}N cross peak is shown as an expanded inset. (C) DNA binding sites mapped onto the three-dimensional NMR structure of ZF4-5. Five residues with significant chemical shift perturbations are indicated. (D) ITC results for the interaction between ZF4-5 and dsDNA. (Top) Thermal power versus time. (Bottom) Injection heat versus ZF4-5/dsDNA ratio. The resulting parameters derived from the fitting are shown in the inset.

Table 1

Structural statistics for human INSM1 ZF4-5 domains [a]

Conformationally-restricting restraints [b]	
Distance restraints	
Total	782
Intra-residue (i=j)	150
Sequential (|i-j|=1)	249
Medium-range (1<|i–j|<5)	179
Long-range (|i–j≥5)	204
Dihedral angle restraints	60
Residue restraint violations [b]	
Average number of distance violations per structure	
0.1–0.2Å/0.2–0.5Å	3.65/0.6
>0.5Å	0
Average RMS distance violation/restraint (Å)	0.01
Maximum distance violation (Å)	0.31
Average number of dihedral angle violations per structure	
1–10°	7.75
>10°	0
Average RMS dihedral angle violation/restraint (degree)	1.04
Maximum dihedral angle violation (degree)	7.10
RMSD from average coordinates [b,c]	
Backbone/Heavy atoms (Å)	0.9/1.3
Molprobity Ramachandran plot statistics [b,c]	
Most favored/Allowed regions (%)	88.0/12.0
Disallowed regions (%)	0
Global quality scores (raw/Z-score) [b]	
Verify3D	0.17/−4.65
Prosall	−0.11/−3.14
Procheck (phi-psi) [c]	−0.54/−1.81
Procheck (all) [c]	−0.38/−2.25
Molprobity clash	11.44/−0.44
RPF Scores [d]	
Recall/Precison	0.98/0.88
F-measure/DP-score	0.93/0.75

[a] Structural statistics were computed for the ensemble of 20 deposited structures.

[b] Calculated using the PSVS 1.4 program. Residues (424-497) were analyzed.

[c] Ordered residues ranges (with sum of phi and psi > 1.8): 441-474, 477-492.

[d] RPF scores reflected the goodness-of-fit of the final ensemble of structures including disordered residues to the NMR data.