Published in final edited form as:

Biomol NMR Assign. 2011 October ; 5(2): 215–219. doi:10.1007/s12104-011-9303-8.

Backbone and Ile- $\delta 1$, Leu, Val Methyl ^1H , ^{13}C and ^{15}N NMR chemical shift assignments for human interferon-stimulated gene 15 protein

Cuifeng Yin,

Department of Molecular Biology and Biochemistry, Center for Advanced Biotechnology and Medicine, Northeast Structural Genomics Consortium, Rutgers, The State University of New Jersey, Piscataway, NJ 08854, USA

James M. Aramini,

Department of Molecular Biology and Biochemistry, Center for Advanced Biotechnology and Medicine, Northeast Structural Genomics Consortium, Rutgers, The State University of New Jersey, Piscataway, NJ 08854, USA

Li-Chung Ma,

Department of Molecular Biology and Biochemistry, Center for Advanced Biotechnology and Medicine, Northeast Structural Genomics Consortium, Rutgers, The State University of New Jersey, Piscataway, NJ 08854, USA

John R. Cort,

Biological Sciences Division, Pacific Northwest National Laboratory, Northeast Structural Genomics Consortium, Richland, WA 99352, USA

G. V. T. Swapna,

Department of Molecular Biology and Biochemistry, Center for Advanced Biotechnology and Medicine, Northeast Structural Genomics Consortium, Rutgers, The State University of New Jersey, Piscataway, NJ 08854, USA

Robert M. Krug, and

Section of Molecular Genetics and Microbiology, Institute for Cellular and Molecular Biology, University of Texas, Austin, TX 78712, USA

Gaetano T. Montelione

Department of Molecular Biology and Biochemistry, Center for Advanced Biotechnology and Medicine, Northeast Structural Genomics Consortium, Rutgers, The State University of New Jersey, Piscataway, NJ 08854, USA

Department of Biochemistry, Robert Wood Johnson Medical School, University of Medicine and Dentistry of New Jersey, Piscataway, NJ 08854, USA

James M. Aramini: jma@cabm.rutgers.edu; Gaetano T. Montelione: guy@cabm.rutgers.edu

Abstract

Human interferon-stimulated gene 15 protein (ISG15), also called ubiquitin cross-reactive protein (UCRP), is the first identified ubiquitin-like protein containing two ubiquitin-like domains fused in tandem. The active form of ISG15 is conjugated to target proteins via the C-terminal glycine residue through an isopeptide bond in a manner similar to ubiquitin. The biological role of ISG15

is strongly associated with the modulation of cell immune function, and there is mounting evidence suggesting that many viral pathogens evade the host innate immune response by interfering with ISG15 conjugation to both host and viral proteins in a variety of ways. Here we report nearly complete backbone $^1\text{H}^{\text{N}}$, ^{15}N , $^{13}\text{C}'$, and $^{13}\text{C}^{\alpha}$, as well as side chain $^{13}\text{C}^{\beta}$, methyl (Ile- $\delta 1$, Leu, Val), amide (Asn, Gln), and indole N-H (Trp) NMR resonance assignments for the 157-residue human ISG15 protein. These resonance assignments provide the basis for future structural and functional solution NMR studies of the biologically important human ISG15 protein.

Keywords

Backbone NMR resonance assignment; Human ISG15; Innate immune response; Ubiquitin-like protein

Biological context

Human interferon-stimulated gene 15 protein product (ISG15) is a key ubiquitin-like protein (UBL) involved in cell innate immunity and antiviral defense (for recent reviews, see: Jeon et al. 2010, Skaug and Chen 2010). Mature human ISG15, synthesized initially within the cell as a transient inactive precursor (Potter et al. 1999), is a 157-residue protein comprised of two UBL domains, sharing 29% and 37% sequence identity with human ubiquitin, respectively, tethered by a short polypeptide linker (Narasimhan et al. 2005). The active form of ISG15 features a conserved C-terminal LRLRGG sequence. ISG15 conjugation (ISGylation) to target proteins proceeds through this C-terminal glycine via an isopeptide bond in a manner analogous to ubiquitylation. Both ISG15 and its conjugates can be induced in many cell types, and are among the earliest proteins produced by pathogen-infected cells in response to interferons (IFNs) α and β (Haas et al. 1987). The functions of ISG15 and its conjugates also include inducing the production of IFN- γ from T cells (D'Cunha et al. 1996), and the regulation of signal transduction pathways and antiviral responses (Jeon et al. 2010). Indeed, it was recently demonstrated that ISGylation of non-structural protein 1 from influenza A virus (NS1A) attenuates viral replication (Zhao et al. 2010). However, some viruses have evolved means of counteracting the immune responses of ISG15. For example, non-structural protein 1 from influenza B virus (NS1B) can bind directly to ISG15 and inhibit ISGylation (Yuan and Krug 2001; Chang et al. 2008). Here we report nearly complete backbone $^1\text{H}^{\text{N}}$, ^{15}N , $^{13}\text{C}'$, $^{13}\text{C}^{\alpha}$, and $^{13}\text{C}^{\beta}$ NMR resonance assignments, as well as side chain ^{13}C - ^1H and ^{15}N - ^1H assignments for Ile ($\delta 1$), Leu, and Val methyls and amide and indole N-H moieties of Asn, Gln, and Trp, respectively, for the active wild type form of human ISG15. These resonance assignments provide the basis for future structural and functional solution studies of human ISG15, and its interactions with target proteins.

Methods and experiments

Using the Gateway[®] site-specific recombination cloning system (Invitrogen Corp.), the human *isg15* gene was subcloned from plasmid pGEM1/ISG15M into expression vector pKM596 (Nallamsetty et al. 2005). The resulting plasmid, pMBP-ISG15, encodes a maltose-binding protein (MBP), TEV protease site and 7-residue SerHis₆ purification tag fusion in frame with the N-terminus of *isg15*. pMBP-ISG15 was then transformed into *Escherichia coli* BL21PRO cells, which carry the plasmid for TEV protease, providing proteolytic cleavage of MBP protein from ISG15 within the cell. Two isotopically-enriched protein samples of ISG15 were prepared. The first was a uniformly triple-labeled protein sample, [U - ^2H , ^{13}C , ^{15}N]-ISG15; the second was triple-labeled with the methyl groups of Ile ($\delta 1$), Leu, and Val selectively protonated, [U - ^2H , ^{13}C , ^{15}N ; ^1H -Ile- $\delta 1$, Leu- δ , Val- γ]-ISG15.

Transformed cell cultures were grown at 37°C in MJ9 minimal media (Jansson et al. 1996) in 100% $^2\text{H}_2\text{O}$ containing either 0.1% (w/v) $(^{15}\text{NH}_4)_2\text{SO}_4$ and 0.4% (w/v) $U\text{-}^2\text{H}, ^{13}\text{C}$ -glucose as sole nitrogen and carbon sources (for $[U\text{-}^2\text{H}, ^{13}\text{C}, ^{15}\text{N}]\text{-ISG15}$), or 0.1% (w/v) $(^{15}\text{NH}_4)_2\text{SO}_4$ and 0.3% (w/v) $U\text{-}^2\text{H}, ^{13}\text{C}$ -glucose supplemented with $[U\text{-}^{13}\text{C}_4, 3,3\text{-}^2\text{H}_2]\text{-}\alpha$ -ketobutyrate (50 mg/L), $[U\text{-}^{13}\text{C}_5, 3\text{-}^2\text{H}]\text{-}\alpha$ -ketoisovalerate (CIL Inc.) (100 mg/L) (for $[U\text{-}^2\text{H}, ^{13}\text{C}, ^{15}\text{N}; ^1\text{H}\text{-Ile-}\delta 1, \text{Leu-}\delta, \text{Val-}\gamma]\text{-ISG15}$) (Goto et al. 1999). Protein expression was induced at mid-log phase ($\text{OD}_{600} \sim 0.5$) by addition of IPTG to a final concentration of 1 mM, and cultures were transferred to room temperature. After 3 h, tetracycline was added to a final concentration 0.1 $\mu\text{g}/\text{ml}$ to induce the expression of TEV protease, and cells were harvested after another 3 h. Expressed isotopically enriched samples of human ISG15 were purified using a two-step protocol consisting of nickel-affinity chromatography followed directly by HiLoad 16/60 Superdex 75 gel filtration chromatography (GE Healthcare). The final deuteration level for $[U\text{-}^2\text{H}, ^{13}\text{C}, ^{15}\text{N}]\text{-ISG15}$ was ca. 83%, according to the molecular mass measured by MALDI-TOF. For both samples, sample purity (>97%) was verified by SDS-PAGE. Isotopically enriched ISG15 samples for NMR spectroscopy were concentrated to ca. 0.7 mM concentration in H_2O solution containing 5% $^2\text{H}_2\text{O}$, 50 mM ammonium citrate, 10 mM DTT, and 5 mM CaCl_2 at $\text{pH } 6.5 \pm 0.02$, and transferred to 5-mm Shigemi susceptibility-matched NMR tubes for data collection.

All NMR data were collected at 18°C on four-channel Varian INOVA 600 and 750 MHz NMR spectrometers and referenced to external DSS. For backbone assignments of ISG15, the following experiments were collected on $[U\text{-}^2\text{H}, ^{13}\text{C}, ^{15}\text{N}]\text{-ISG15}$: 2D $^1\text{H}\text{-}^{15}\text{N}$ HSQC and 3D ^2H -decoupled HNCOC, HNCA, HN(CO)CA, HNCACB, HN(CO)CACB, HN(CA)CO spectra. In addition, 2D $^1\text{H}\text{-}^{13}\text{C}$ HSQC, 3D ^{15}N -edited NOESY (150 ms), and 3D ^2H -decoupled H(CCCO)NH- and (H)C(CCO)NH-TOCSY spectra were collected on $[U\text{-}^2\text{H}, ^{13}\text{C}, ^{15}\text{N}; ^1\text{H}\text{-Ile-}\delta 1, \text{Leu-}\delta, \text{Val-}\gamma]\text{-ISG15}$ for side chain resonance assignments of Ile ($\delta 1$), Leu, and Val methyls and N-H moieties of Asn, Gln, and Trp. The programs VNMR (Varian, Inc.), FELIX (Accelrys, Inc.), NMRPipe (Delaglio et al. 1995), and SPARKY (Goddard and Kneller, University of California, San Francisco) were used for data processing, peak picking, and spectral analysis. The majority of backbone assignments were done automatically using AutoAssign (Zimmerman et al. 1997; Moseley et al. 2001), and confirmed with the ^{15}N -edited 3D NOESY spectrum and spin system typing assignment information from the TOCSY spectra (Zheng et al. 2003). Side chain resonance assignments of Ile ($\delta 1$), Leu, and Val methyl, Trp $\text{N}^\epsilon\text{H}^\epsilon$, Asn $\text{N}^\delta\text{H}_2$, and Gln $\text{N}^\epsilon\text{H}_2$ groups were done manually. All NMR assignments were further validated by the Assignment Validation Suite (AVS) software package (Moseley et al. 2004).

Assignments and data deposition

A total of 152 out of 157 spin systems (excluding the N-terminal hexa-His tag) were assigned automatically using AutoAssign, and three more assignments (Ser²⁸, Ser²⁹ and Met³⁰) were made manually. Overall, >99% of the assignable backbone ($^1\text{H}^N$, ^{15}N , $^{13}\text{C}^\alpha$, and $^{13}\text{C}^\beta$) resonances were assigned in ISG15 (Table 1). The assigned 2D $^1\text{H}\text{-}^{15}\text{N}$ HSQC spectrum of ISG15 is shown in Fig. 1, and a summary of the resulting connectivity map for ISG15 is shown in Fig. 2. Chemical shift index (CSI) analysis (Wishart and Sykes 1994) and sequential backbone $\text{H}^N\text{-H}^N$ NOEs are consistent with the presence of two ubiquitin-like domains like those revealed in the crystal structure of mutant ISG15 (Narasimhan et al. 2005) (Fig. 2), and in the crystal structure of the complex between human ISG15 and a viral tumor domain protease (James et al. 2011). In addition, 3D H(CCCO)NH-TOCSY, (H)C(CCO)NH-TOCSY, and ^{15}N -edited NOESY spectra afforded $\sim 86\%$ side chain resonance assignments for Ile ($\delta 1$), Leu, and Val methyls, as well as the side chain NH or NH_2 groups of Trp, Asn, and Gln (Table 1). The chemical shift assignments for human ISG15 were deposited in the BioMagResBank (accession number 5658).

Abbreviations

CSI	Chemical shift index
DTT	Dithiothreitol
DSS	2,2-Dimethyl-2-silapentane-5-sulfonic acid
HSQC	Heteronuclear single quantum coherence
IFN	Interferon
ISG15	Interferon-stimulated gene 15
IPTG	Isopropyl β -D-1-thiogalactopyranoside
UBL	Ubiquitin-like

Acknowledgments

This work was supported by National Institute of General Medical Sciences Protein Structure Initiative Grants U54-GM074958 and U54-GM094597 (to G.T.M.) and National Institutes of Allergy and Infectious Disease Grant AI11772 (to R.M.K.). The authors wish to thank Dr. David S. Waugh for kindly providing plasmid pKM596 and the BL21PRO cells. NMR spectra at 750 MHz were acquired in the Environmental Molecular Sciences Laboratory (a national scientific user facility sponsored by the U.S. Department of Energy Office of Biological and Environmental Research) located at Pacific Northwest National Laboratory and operated for DOE by Battelle (contract KP130103).

References

- Chang YG, Yan XZ, Xie YY, Gao XC, Song AX, Zhang DE, Hu HY. Different roles for two ubiquitin-like domains of ISG15 in protein modification. *J Biol Chem*. 2008; 283:13370–13377. [PubMed: 18356159]
- D’Cunha J, Knight E Jr, Haas AL, Truitt RL, Borden EC. Immunoregulatory properties of ISG15, an interferon-induced cytokine. *Proc Natl Acad Sci USA*. 1996; 93:211–215. [PubMed: 8552607]
- Delaglio F, Grzesiek S, Vuister GW, Zhu G, Pfeifer J, Bax A. NMRPipe: a multidimensional spectral processing system based on UNIX pipes. *J Biomol NMR*. 1995; 6:277–293. [PubMed: 8520220]
- Goto NK, Gardner KH, Mueller GA, Willis RC, Kay LE. A robust and cost-effective method for the production of Val, Leu, Ile (δ 1) methyl-protonated ^{15}N -, ^{13}C -, ^2H -labeled proteins. *J Biomol NMR*. 1999; 13:369–374. [PubMed: 10383198]
- Haas AL, Ahrens P, Bright PM, Ankel H. Interferon induces a 15-kilodalton protein exhibiting marked homology to ubiquitin. *J Biol Chem*. 1987; 262:11315–11323. [PubMed: 2440890]
- James TW, Frias-Staheli N, Bacik JP, Livingston Macleod JM, Khajepour M, Garcia-Sastre A, Mark BL. Structural basis for the removal of ubiquitin and interferon-stimulated gene 15 by a viral ovarian tumor domain-containing protease. *Proc Natl Acad Sci USA*. 2011; 108:2222–2227. [PubMed: 21245344]
- Jansson M, Li YC, Jendeborg L, Anderson S, Montelione GT, Nilsson B. High-level production of uniformly ^{15}N - and ^{13}C -enriched fusion proteins in *Escherichia coli*. *J Biomol NMR*. 1996; 7:131–141. [PubMed: 8616269]
- Jeon YJ, Yoo HM, Chung CH. ISG15 and immune diseases. *Biochim Biophys Acta*. 2010; 1802:485–496. [PubMed: 20153823]
- Moseley HN, Monleon D, Montelione GT. Automatic determination of protein backbone resonance assignments from triple resonance nuclear magnetic resonance data. *Methods Enzymol*. 2001; 339:91–108. [PubMed: 11462827]
- Moseley HN, Sahota G, Montelione GT. Assignment validation software suite for the evaluation and presentation of protein resonance assignment data. *J Biomol NMR*. 2004; 28:341–355. [PubMed: 14872126]

- Nallamsetty S, Austin BP, Penrose KJ, Waugh DS. Gateway vectors for the production of combinatorially-tagged His₆-MBP fusion proteins in the cytoplasm and periplasm of *Escherichia coli*. *Protein Sci.* 2005; 14:2964–2971. [PubMed: 16322578]
- Narasimhan J, Wang M, Fu Z, Klein JM, Haas AL, Kim JJ. Crystal structure of the interferon-induced ubiquitin-like protein ISG15. *J Biol Chem.* 2005; 280:27356–27365. [PubMed: 15917233]
- Potter JL, Narasimhan J, Mende-Mueller L, Haas AL. Precursor processing of pro-ISG15/UCRP, an interferon- β -induced ubiquitin-like protein. *J Biol Chem.* 1999; 274:25061–25068. [PubMed: 10455185]
- Skaug B, Chen ZJ. Emerging role of ISG15 in antiviral immunity. *Cell.* 2010; 143:187–190. [PubMed: 20946978]
- Wishart DS, Sykes BD. The ¹³C Chemical-Shift Index: a simple method for the identification of protein secondary structure using ¹³C chemical-shift data. *J Biomol NMR.* 1994; 4:171–180. [PubMed: 8019132]
- Yuan W, Krug RM. Influenza B virus NS1 protein inhibits conjugation of the interferon (IFN)-induced ubiquitin-like ISG15 protein. *EMBO J.* 2001; 20:362–371. [PubMed: 11157743]
- Zhao C, Hsiang TY, Kuo RL, Krug RM. ISG15 conjugation system targets the viral NS1 protein in influenza A virus-infected cells. *Proc Natl Acad Sci USA.* 2010; 107:2253–2258. [PubMed: 20133869]
- Zheng D, Cort JR, Chiang Y, Acton T, Kennedy MA, Montelione GT. ¹H, ¹³C and ¹⁵N resonance assignments for methionine sulfoxide reductase B from *Bacillus subtilis*. *J Biomol NMR.* 2003; 27:183–184. [PubMed: 12913416]
- Zimmerman DE, Kulikowski CA, Huang Y, Feng W, Tashiro M, Shimotakahara S, Chien C, Powers R, Montelione GT. Automated analysis of protein NMR assignments using methods from artificial intelligence. *J Mol Biol.* 1997; 269:592–610. [PubMed: 9217263]

Fig. 1. Two dimensional ^1H - ^{15}N HSQC spectrum of 0.7 mM human [U - ^2H , ^{13}C , ^{15}N]-ISG15 at 18°C, pH 6.5 in 95% H_2O /5% $^2\text{H}_2\text{O}$ containing 50 mM ammonium citrate, 10 mM DTT, 5 mM CaCl_2 . Backbone resonance assignments are labeled with one-letter amino acid codes followed by their sequence numbers. Side chain NH resonances of Trp and side chain NH_2 resonances of Asn and Gln are also labeled; folded side chain NH resonances of Arg are all labeled Arge since their side chain assignments are beyond the scope of the methods used here. Note that our residue numbering starts from the N-terminal tag, meaning that it is shifted +7 with respect to wild type human ISG15

Fig. 2.

AutoAssign (Moseley et al. 2001) connectivity map showing sequential (yellow, s) and intra-residue (red, i) connectivities used to establish backbone resonance assignments for human ISG15. The 7-residue N-terminal purification tag has been omitted; again, residue numbering is shifted +7 with respect to wild type human ISG15. Secondary structural elements found in the crystal structure of human [C78S]-ISG15 (Narasimhan et al. 2005) are depicted above the sequence. CSI (Wishart and Sykes 1994) data for C^α are represented by a blue bar graph. Interresidue H^N-H^N NOE connectivities are shown as thin, medium, and thick black lines, corresponding to weak, medium, and strong NOE interactions. In general, the secondary structural elements in human ISG15 agree well with the CSI and ¹⁵N-edited NOESY data

Table 1

Summary of NMR assignment statistics for human ISG15

<i>Backbone</i>	
$^1\text{H}^{\text{N}}$	148/150
^{15}N	148/150
$^{13}\text{C}'$	156/157
$^{13}\text{C}^{\alpha}$	157/157
<i>Side chain</i>	
$^{13}\text{C}^{\beta}$	142/142
Ile ($\delta 1$)	3/3
Leu (δ)	42/48
Val (γ)	30/32
Trp ($\epsilon 1$)	2/2
Asn/Gln (NH_2)	24/32

Computed using AVS software (Moseley et al. 2004) excluding the N-terminal SerHis₆ purification tag