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ARTICLE

^{1}H, ^{15}N and ^{13}C backbone resonance assignment of Rv1567c, an integral membrane protein from *Mycobacterium tuberculosis*

Hau B. Nguyen · Timothy A. Cross

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Abstract We report here the backbone assignment of Rv1567c, an integral membrane protein from *Mycobacterium tuberculosis*. The backbone resonance assignments were determined based on triple-resonance experiments with uniformly [^{13}C,^{15}N]-labeled protein in LMPG detergent micelles.

Keywords Integral membrane protein · Solution NMR · *Mycobacterium tuberculosis* · Backbone assignment · Structural genomics

Biological context

Mycobacterium tuberculosis is the causative agent of tuberculosis, the leading cause of infectious disease mortality in the world infecting over one third of the world's population (WHO 1999). Nearly 10% of the people infected with this bacterium will develop the tuberculosis disease (WHO 1999). Although several drugs and the Bacille-Guerin vaccine (BCG) are available and a short-course chemotherapy (DOTS) are utilized, the tubercle bacillus still claims more lives than any single infectious agent (Snider et al. 1994, WHO 2004). This situation is even worse in developing countries where drug-resistant strains of *M. tuberculosis* and human immunodeficiency virus are wide spread. Recently, a new drug has been reported, diarylquinoline, R207910 that potently inhibits both drug-sensitive and drug-resistant *Mycobacterium tuberculosis* in vitro by inhibiting ATP synthase function. However, its clinical potential has not been verified. Although *M. tuberculosis* is an enormously devastating agent, little is known about its molecular mechanism of pathogenesis. Effective treatments of tuberculosis thus require the discovery of new drugs, the characterization of drug targets and drug-resistance factors.

Since its first isolation in 1905, the H37Rv strain of *M. tuberculosis* has been used widely in biomedical research because it retains full virulence in animal models of tuberculosis and it is susceptible to drugs. The genome for the H37Rv strain was found to encode for almost 4,000 proteins (Cole et al. 1998) and 30% of these are believed to be membrane or membrane-associated proteins (Korepanova et al. 2005). A detailed structural understanding of the membrane proteins and cell envelope of this strain will help unravel the mechanism through which *M. tuberculosis* infects and survives within human macrophages, and thus may help to facilitate the development of new treatments for tuberculosis. In fact, it is anticipated that more than 50% of all drug targets are membrane proteins. However, there is very little structural information on these proteins. A structural investigation for Rv1567c by solution NMR spectroscopy represents an initial effort towards NMR characterization of these targets. Rv1567c is an integral membrane protein containing 94 residues. It is predicted to contain 2 transmembrane α helices and to participate in cell wall and cell processes, however its specific biological function is unknown (Cole

H. B. Nguyen · T. A. Cross (✉)
National High Magnetic Field Laboratory,
1800 E. Paul Dirac Dr, Tallahassee, FL 32310, USA
e-mail: cross@magnet.fsu.edu

H. B. Nguyen · T. A. Cross
Institute of Molecular Biophysics, Florida State University,
Tallahassee, FL 32306, USA

T. A. Cross
Department of Chemistry and Biochemistry, Florida State
University, Tallahassee, FL 32306, USA

et al. 1998; http://genolist.pasteur.fr/TubercuList/, Krogh et al. 2001; http://www.cbs.dtu.dk/services/TMHMM/).

Methods and experiments

The nucleotide sequence of Rv1567c was obtained from the Tuberculosis Research Materials and Vaccine Testing Contract at Colorado State University. It was cloned into pET15b (Novagen) by PCR. Rv1567c was expressed with the N-terminal Histidine tag followed by the Thrombin protease cleavage site.

The DNA plasmid containing the Rv1567c gene was transformed into an *E. coli* competent cell strain BL-21 (DE3) codon Plus-RP strain (Stratagene) for protein over-expression. This strain is designed to compensate for codon usage differences between *M. tuberculosis* and *E. coli* and was successfully used in our previous expression of membrane proteins (Korepanova et al. 2005, 2007).

The expression level in M9 minimal media for protein isotope labeling was enhanced by increasing cell densities using a 4:1 cell concentrating method (Marley et al. 2001). For ^{15}N uniform labeling and ^{13}C/^{15}N double labeling, cells from 4 l LB cultures were grown until an OD_{600} of 0.6–0.7 was reached, cells were then harvested and resuspended in 1 l of M9 minimal media containing 1g $^{15}NH_4Cl$. In ^{13}C/^{15}N labeling, 2g ^{13}C-glucose was added after 20 min of shaking at 37°C to assure that all left-over ^{12}C from LB media was completely utilized. Protein expression was induced with 0.4 mM IPTG after 30 min of shaking at 37°C and cells were harvested after 5 h of induction by centrifugation at 5,000 rpm.

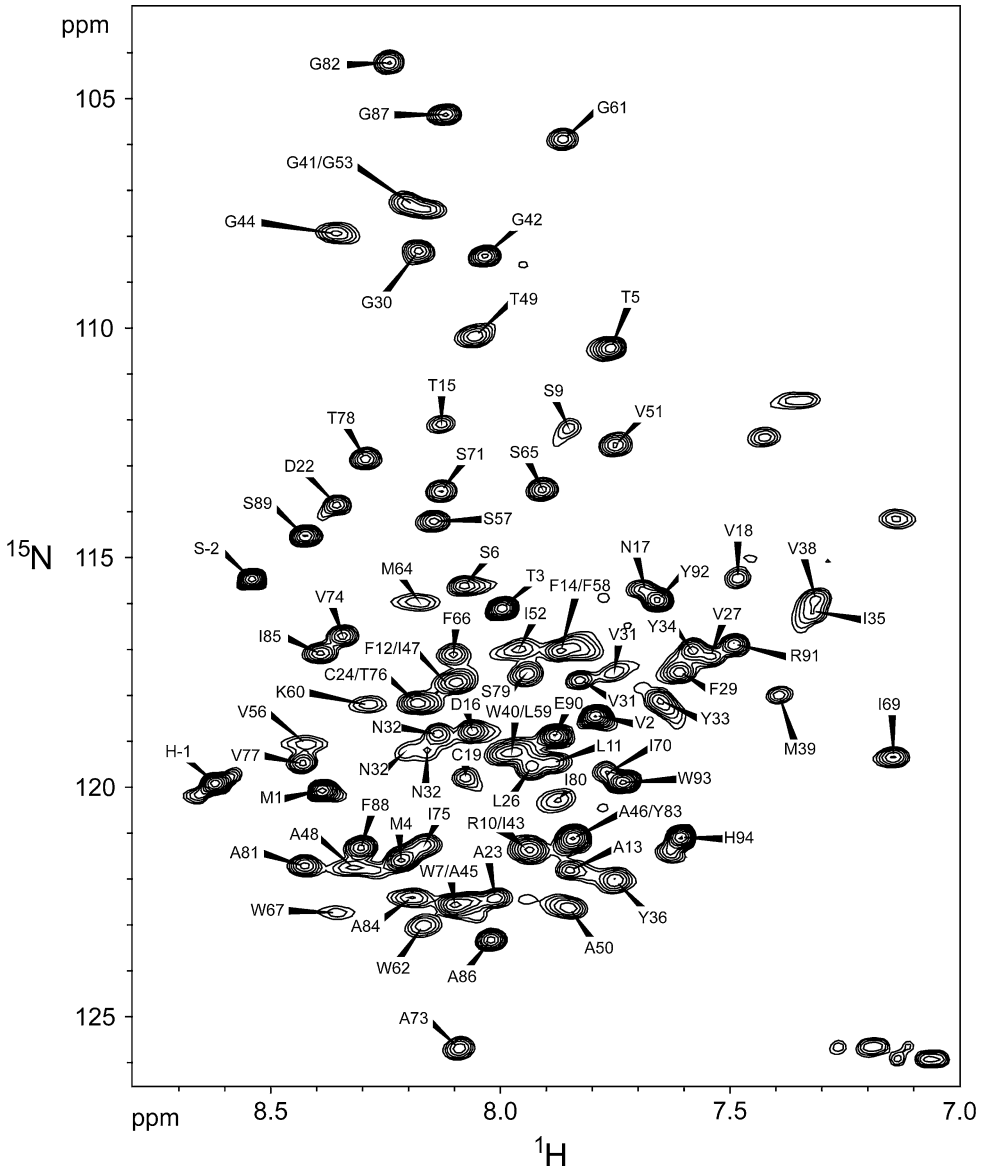

Fig. 1 2D [^{1}H-^{15}N]-HSQC spectrum of uniformly ^{15}N-labeled Rv1567c in 150 mM LMPG with 10 mM sodium acetate buffer at pH 4. The spectrum was recorded at 600 MHz at 323 K. Assignment of backbone amides are as indicated

The harvested cells were suspended in buffer (40 mM Tris-HCl pH 8, 500 mM NaCl, DNAase, lysozyme) and lysed by sonication. The His-tag fusion protein was solubilized in empigen and purified using nickel affinity (Qiagen). In the last step of purification, His-tagged protein was exchanged into an elution buffer containing LMPG ((1-myristoyl-2-hydroxy-sn-glycero-3-[phospho-RAC-(1-glycerol) detergent micelles. The His tag was then removed with Thrombin and the protein purified using LMPG-containing buffers and another nickel column. The NMR samples contain 0.8–1 mM Rv1567c in 150 mM LMPG, 10 mM Sodium acetate buffer and 10% D_2O at pH 4.

Backbone assignments were obtained from the spectra of 2D [$^{15}N/^{1}H$]-HSQC, HNCA, HN(CO)CA, HNCO, CBCA(CO)NH and HNCACB (Sattler et al. 1999). In addition, three amino acid selectively labeled samples (Ala, Ile and Val) were used to help with the assignment process. All NMR experiments were recorded at 323 K on Varian 600 and 720 MHz spectrometers. All spectra were processed by nmrPipe (Delaglio et al. 1995). Data were analyzed by the Sparky software (T. D. Goddard and D. G. Kneller, University of California, San Francisco). Chemical shifts were referenced to 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) (Wishart et al. 1995).

Assignments and data deposition

Backbone assignments have been obtained for 97% of non-proline residues as shown in Fig. 1. The exceptions are M58, Y63 and I72. The ^{1}H, ^{15}N, and ^{13}C chemical shifts of the backbone resonances have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under the accession number 15638. The chemical shift indices obtained by plotting the differences between the observed ^{13}C chemical shifts and the standardized values (Schwarzinger et al. 2000) indicate that Rv1567c may contain 3 helices with two possible transmembrane helices from residue 43 to 65 and 70 to 90, respectively. The result for the second transmembrane helix is in good agreement with the predicted transmembrane helix from TMHMM: residues 66–88 (Krogh et al. 2001). However, TMHMM predicted the first transmembrane helix to be between residues 34 and 56, a shift of approximately 9 residues from that based on chemical shift indices. In addition, we have observed multiple resonances for residues V31 and N32 in the loop just before transmembrane helix 1. This result suggests that there may be a conformational switch in this region as a result of the observed heterogeneity.

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References

Cole ST, Brosch R, Parkhill J et al (1998) Deciphering the biology of *Mycobacterium tuberculosis* from the complete genome sequence. Nature 393:539–544

Delaglio F, Grzesiek S, Vuister GW, Zhu G, Pfeifer J, Bax A (1995) NMRPipe: a multidimensional spectral processing system based on UNIX pipes. J Biomol NMR 6:277–293

Korepanova A, Gao FP, Hua Y, Qin H, Nakamoto RK, Cross TA (2005) Cloning and expression of multiple integral membrane proteins from *Mycobacterium tuberculosis* in *Escherichia coli*. Protein Science 14:148–158

Korepanova A, Moore JD, Nguyen HB, Hua Y, Cross TA, Gao F (2007) Expression of membrane proteins from *Mycobacterium tuberculosis* in *Escherichia coli* as fusions with maltose binding protein. Protein Expr Purif 53:24–30

Krogh A, Larsson B, von Heijne G, Sonnhammer ELL (2001) Predicting transmembrane protein topology with a hidden Markov model: Application to complete genomes. J Mol Biol 305:567–580

Marley J, Lu M, Bracken C (2001) A method for efficient isotopic labeling of recombinant proteins. J Biomol NMR 20:71–75

Sattler M, Schleucher J, Griesinger C (1999) Heteronuclear multidimensional NMR experiments for the structure determination of proteins in solution employing pulsed field gradients. Prog NMR Spectrosc 34:93–158

Schwarzinger S, Kroon GJA, Foss TR, Wright PE, Dyson HJ (2000) Random coil chemical shifts in acidic 8 M urea: implementation of random coil shift data in NMRView. J Biomol NMR 18:43–48

Snider DE Jr, Raviglione M, Kochi A (1994) Tuberculosis: pathogenesis, protection, and control vol 2(11). American Society for Microbiology, Washington DC

Wishart DS, Bigam CG, Yao J, Abildgaard F, Dyson HJ, Oldfield E, Markley JL, Sykes BD (1995) ^{1}H, ^{13}C and ^{15}N Chemical-Shift Referencing in Biomolecular NMR. J Biomol NMR 6:135–140