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Letter to the Editor: ^1H , ^{15}N and ^{13}C chemical shift assignments of the Resuscitation Promoting Factor domain of Rv1009 from *Mycobacterium tuberculosis*.

Martin Cohen-Gonsaud^a, Philippe Barthe^b, Richard Harris^c, Paul C. Driscoll^c, Nicholas H. Keep^a and Christian Roumestand^{b*}.

^a School of Crystallography and Bloomsbury Centre for Structural Biology, Birbeck College, University of London, Malet Street, London, WC1E 7HX, UK

^b Centre de Biochimie Structurale, UMR 5048 CNRS/UM1 - UMR 554 INSERM/UM1, Faculté de Pharmacie, BP 14491, 15 Avenue Charles Flahault, 34093 Montpellier Cedex 5, France

^c Department of Biochemistry and Molecular Biology and Bloomsbury Centre for Structural Biology, University College London, Gower Street, London WC1E 6BT, UK

* *To whom correspondence should be addressed.*

Email address: christian.roumestand@cbs.cnrs.fr

Key words:

Rpf, NMR assignments, Resuscitation promoting factors, *Mycobacterium tuberculosis*.

Biological context

Studies of starved *Micrococcus luteus* have led to the identification of a secreted protein which resuscitates dormant cells allowing them to enter the cell cycle. This factor was named resuscitation promoting factor (Rpf) and has been classified as the first bacterial cytokine (Mukamolova, Kaprelyants et al. 1998). Homologues of the *rpf* genes are widely distributed among the high G+C cohort of Gram-positive bacteria including the mycobacterial pathogens *M. tuberculosis* and *M. leprae* (Mukamolova, Turapov et al. 2002). *M. tuberculosis* can spend many years dormant in human tissue so the mechanism of revival of dormant *M. tuberculosis* is of major medical interest. Genetic ablation of the unique *rpf* gene in *M. luteus* has been shown to be lethal (Mukamolova, Turapov et al. 2002), while in *M. tuberculosis*, which has five *rpf* genes, none of these genes was individually found to be essential suggesting functional redundancy (Tufariello, Jacobs et al. 2004). However, in a genome wide transposon mutagenesis study, the *rpfB* knockout exhibited a slow growth phenotype (Sasseti, Boyd et al. 2003). All five *rpf* homologues are expressed in *M. tuberculosis* in extended-stationary-phase cultures indicating a potential for Rpf to play a role in the reactivation of quiescent bacilli. Recently, using sequence analysis and homology modelling, we predicted that the structure of the common sequence region of about 100 amino acids in the Rpf proteins possesses a lysozyme-like domain (Cohen-Gonsaud, Keep et al. 2004). Based on this analysis, we sub-cloned the core domain of *rpfB* (Rv1009). Here we report the expression, purification and the ^1H , ^{15}N and ^{13}C resonance assignment of the corresponding protein called RpfBc. This work is the preliminary step toward obtaining the first atomic structure of a protein of the Rpf family, and an understanding of how the resuscitation promoting factors work particularly in *M. tuberculosis*.

Methods and experiments

Protein expression and Purification: The cDNA encoding for the 108 residues of RpfBc domain from *M. tuberculosis* was sub-cloned into a NdeI/BamHI site of an in-house engineered variant of pET15b (Novagen) that includes the replacement of the thrombin site coding sequence with a tobacco etch virus (TEV) protease site. The construct was transformed into *Escherichia coli* BL21-Rosetta (DE3) pLysS (Novagen) containing the pRARE plasmid (Novagen) to supply rare tRNA. Uniform ^{15}N and $^{15}\text{N}/^{13}\text{C}$ labelling was obtained by growing cells (30 °C) in ECPM1 medium containing $^{15}\text{NH}_4\text{Cl}$ and $^{13}\text{C}_6$ glucose as the sole nitrogen and carbon sources respectively. Protein expression was induced for 3 hours by addition of 0.5 mM IPTG. The cells were then harvested by centrifugation, and the pellet was sonicated in a lysis buffer (100 mM Tris/HCl pH 8.5, 5 mM β -mercaptoethanol). The supernatant was applied to a Ni-NTA column (Amersham Biosciences). After elution with imidazole and desalting into the TEV protease buffer (50 mM Tris-HCl pH 8.0, 0.5 mM EDTA, 10 mM DTT), the His₆-fusion protein was cleaved overnight at 14 °C by addition of TEV protease. The cleaved protein was further purified using size exclusion chromatography with a Sephadex-HR75 column (Amersham Biosciences) equilibrated with the final sample buffer (25 mM Na-acetate pH 4.6, 2 mM β -mercaptoethanol) and finally concentrated to 0.5 mM.

NMR Spectroscopy: All NMR experiments were performed at 20 °C on a Bruker AVANCE 500 MHz spectrometer equipped with a 5 mm Z-gradient ^1H - ^{13}C - ^{15}N cryogenic probe. ^1H chemical shifts were directly referenced to the methyl resonance of DSS, while ^{13}C and ^{15}N chemical shifts were indirectly referenced. The following spectra were used for the ^1H , ^{15}N , $^{13}\text{C}\alpha$ and $^{13}\text{C}\beta$ and ^{13}CO resonance assignments: ^1H - ^{15}N -HSQC, HNCA, HN(CO)CA, HNCO, HN(CA)CO, HNCACB, CBCA(CO)NH essentially for sequential assignments, and ^{15}N -

edited HSQC-NOESY and HSQC-TOCSY experiments essentially for side chain assignments. All NMR experiments were processed with GIFA (Pons et al., 1996).

Extent of assignments and data deposition

The ^1H - ^{15}N HSQC spectrum of RpfBc domain from *M. tuberculosis* is shown in Figure 1a. By combining the information from the heteronuclear experiments, we were able to assign 98.5 % of the expected backbone resonances and 94.6 % of the side chain resonances. Residues from the C-terminal segment (Gly¹⁰⁶-Arg¹⁰⁸) and from the Pro¹⁸-Pro²³ segment exhibit evidence for the presence of major and minor components. Potential sources for the minor peaks presumably involve local conformational exchange for the C-terminal segment, and proline isomerization for the Pro¹⁸-Pro²³ segment. A ^1H - ^{15}N heteronuclear NOE experiment was important for identifying the structured regions of the RpfBc construct. The ^1H - ^{15}N NOEs, shown in Figure 1b, indicate that the first 20 residues are very mobile. On the other hand, preliminary structures revealed that, consistent with previous proposals (Cohen-Gonsaud, Keep et al. 2004), the core structure of RpfBc adopts roughly a lysozyme-like fold. The chemical shifts of the RpfBc domain of *M. tuberculosis* (major conformation) have been deposited in the BioMagResBank under the accession number BMRB-6221. A full 3D structure determination is in progress.

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Figure legend

Figure 1: (a) ^1H - ^{15}N HSQC spectrum of RpfBc domain from *M. tuberculosis* recorded at 20 °C. Negative peaks are shown in grey. The assignment of peaks is indicated with their one-letter amino acid and number. Peaks belonging to a minor conformational component are indicated with an asterisk. (b) ^1H - ^{15}N heteronuclear NOE ratios for RpfBc.

