Journal of Biomolecular NMR 30: 373–374, 2004.
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Letter to the Editor: ^{1}H, ^{15}N, and ^{13}C chemical shift assignments of the resuscitation promoting factor domain of Rv1009 from *Mycobacterium tuberculosis*

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Received 10 June 2004; Accepted 2 September 2004

Key words: *Mycobacterium tuberculosis*, NMR assignments, Rpf, resuscitation promoting factors

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 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 *Mycobacterium tuberculosis* and *Mycobacterium leprae* (Mukamolova 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. 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 et al., 2004). Based on this analysis, we sub-cloned the core domain of *rpfB* (Rv1009). Here we report the expression, purification and the ^{1}H, ^{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*.

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Methods and experiments

Protein expression and purification

The cDNA encoding for the 108 residues of RpfBc domain from *M. tuberculosis* was subcloned into a *Nde*I/*Bam*HI 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. Uniform ^{15}N and ^{15}N/^{13}C labelling was obtained by growing cells (30 °C) in ECPM1 medium containing $^{15}NH_4Cl$ and $^{13}C_6$ glucose as the sole nitrogen and carbon sources respectively. Protein expression was induced for 3 h 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,

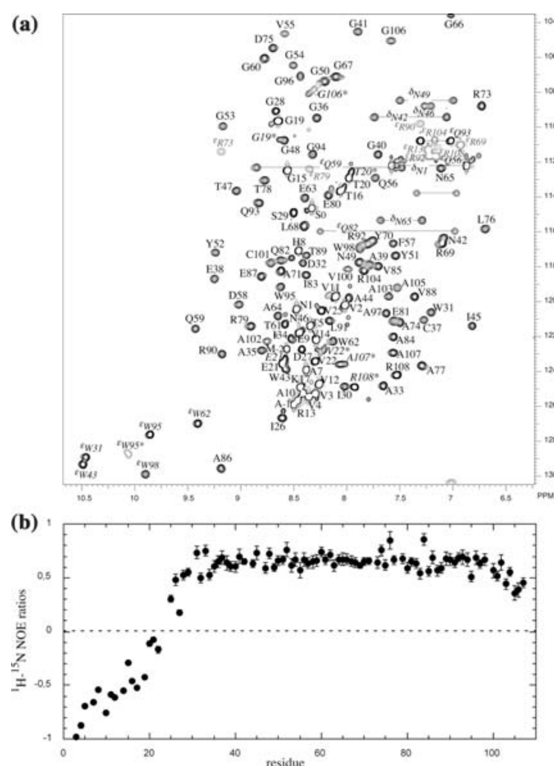

Figure 1: (a) ^{1}H–^{15}N HSQC spectrum of RpfBc domain from *M. tuberculosis* recorded at 20 °C. Negative peaks are shown in grey. Cross peak assignments are indicated using the one-letter amino acid. Signals originating from a minor conformational component are indicated with an asterisk. (b) ^{1}H–^{15}N heteronuclear NOE ratios for RpfBc plotted as a function of residue number.

10 mM DTT), the His$_6$-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 ^{1}H–^{13}C–^{15}N cryogenic probe. ^{1}H 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 ^{1}H, ^{15}N, ^{13}Cα and ^{13}Cβ and ^{13}CO resonance assignments: ^{1}H–^{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.

Extent of assignments and data deposition

The ^{1}H–^{15}N HSQC spectrum of the 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-terminus (Gly^{106}–Arg^{108}) and from the Pro^{18}–Pro^{23} segment exhibit evidence for the presence of major and minor components. A ^{1}H–^{15}N heteronuclear NOE experiment was important for identifying the structured regions of the RpfBc construct: the first approximately 20 residues of the construct are highly mobile (Figure 1b). Preliminary calculations for the 3D structures reveal that, consistent with previous proposals, the core region 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.

Acknowledgements

This work was supported by EU Grant QLK2–2001–02018 (M.C-G. and N.H.K.). NMR experiments were recorded and analysed using the facilities of the Structural Biology RIO platform (C.B.S., Montpellier, France).

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