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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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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 (Sassetti, 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 ^{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.*

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 $^{15}N/^{13}C$ labelling was obtained by growing cells (30 ^{o}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 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 His_6-fusion protein was cleaved overnight at 14 ^{o}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, $^{13}C\alpha$ and $^{13}C\beta$ 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. All NMR experiments were processed with GIFA (Pons et al., 1996).

Extent of assignments and data deposition

The ^{1}H-^{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 (Gly^{106}-Arg^{108}) and from the Pro^{18}-Pro^{23} 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 Pro^{18}-Pro^{23} segment. A ^{1}H-^{15}N heteronuclear NOE experiment was important for identifying the structured regions of the RpfBc construct. The ^{1}H-^{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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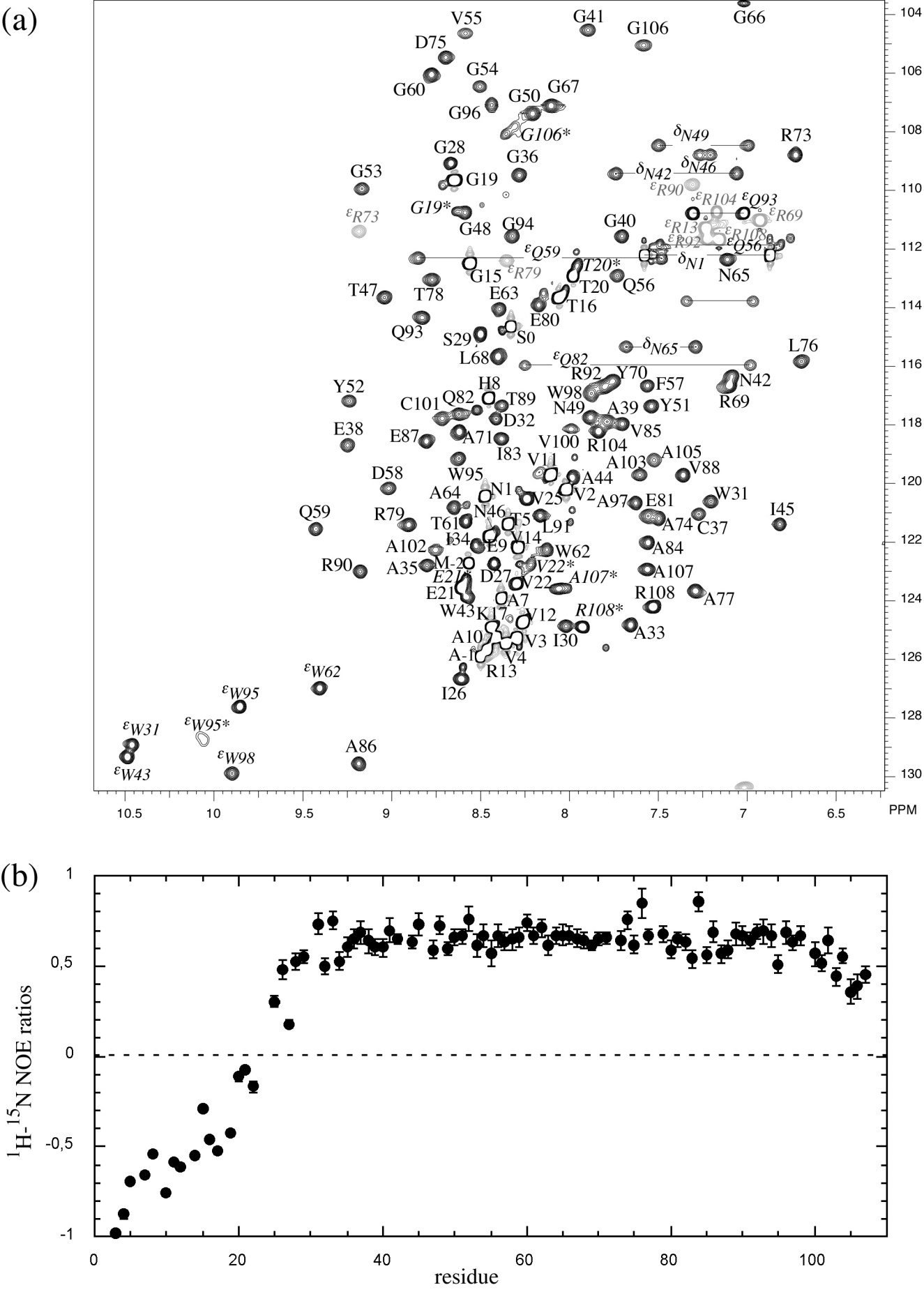
Figure legend

Figure 1: (a) ^{1}H-^{15}N HSQC spectrum of RpfBc domain from *M. tuberculosis* recorded at 20 ^{o}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) ^{1}H-^{15}N heteronuclear NOE ratios for RpfBc.


(a)
V55
G41
G106
G66
D75
G54
G60
G50
G67
G96
G106*
δN49
R73
G28
G36
δN46
G53
G19
δN42
εR90
εR104
εQ93
εR73
G19*
G48
G94
G40
εR69
εR13
εR108
εR92
εQ56
εQ59
δN1
T20*
G15
εR79
N65
T47
T78
E63
T20
Q56
T16
E80
Q93
S29
S0
δN65
L76
L68
εQ82
R92
Y70
F57
N42
Y52
H8
W98
R69
C101
Q82
T89
N49
A39
Y51
D32
E38
E87
A71
V85
V100
R104
I83
V11
A105
A103
V88
D58
W95
A44
A64
N1
V25
V2
A97
E81
W31
Q59
R79
N46
T5
L9
A74
C37
I45
T61
I34
V14
A102
E9
W62
A84
R90
A35
M-2
V22*
E21*
D27
V22
A107*
A107
E21
R108
A77
A7
W43
K17
V12
R108*
A10
V3
I30
A33
A-1
V4
εW62
R13
εW95
I26
εW31
εW95*
A86
εW98
εW43
10.5
10
9.5
9
8.5
8
7.5
7
6.5
PPM
104
106
108
110
112
114
116
118
120
122
124
126
128
130
(b)
1
0,5
0
-0,5
-1
^{1}H-^{15}N NOE ratios
0
20
40
60
80
100
residue