Journal of Biomolecular NMR, **20:** 185–186, 2001.
KLUWER/ESCOM

Letter to the Editor: Sequence-specific assignment and determination of the secondary structure of the 163-residue *M. tuberculosis* and *M. bovis* antigenic protein mpb70

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Received 31 January 2001; Accepted 5 March 2001

Key words: mpb70, NMR resonance assignments, secondary structure, tuberculosis

Biological context

Tuberculosis is one of the most significant bacterial diseases of humans, with about one third of the world's population infected (Kochi, 1994). The complete sequence of the *M. tuberculosis* genome has recently been determined and codes for 3924 proteins (Cole et al., 1998), and the *M. bovis* genome will shortly be available. However, we still have very little information about which proteins are essential for pathogenesis and even less knowledge of their structures, functions and mechanisms of action.

The mature *M. bovis* protein mpb70 (163 residues) is an immunodominant antigen, which is secreted from *M. bovis* cells following cleavage of a 30-residue signal peptide (Hewinson and Russell, 1993). The *M. tuberculosis* homologue of mpb70 is mpt70 and the sequences of the two proteins are identical (Cole et al., 1998). Virulent *M. bovis* expresses high levels of mpb70 and although the expression of mpt70 is low in *M. tuberculosis* cells grown in vitro, both mycobacteria stimulate a strong immune response to mpb70/mpt70 on infection (Hewinson and Russell, 1993; Hewinson et al., 1996). In addition, treatment of *M. tuberculosis* infected mice with a DNA vaccine encoding mpb70 has a pronounced therapeutic action (Lowrie et al., 1999). Together, this suggests that mpb70 may be required for the survival of *M. tuberculosis* and *M. bovis* in vivo.

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To date, the function of mpb70 and its role in tuberculosis pathogenesis remains unknown. In this letter we report the determination of sequence-specific backbone resonance assignments for mpb70 and identification of the secondary structure of the protein. The continuation of this work will lead to the determination of a high resolution structure for mpb70, which will assist in determining its function and role in tuberculosis pathogenesis.

Methods and experiments

The NMR studies were carried out on the mature form of mpb70 secreted by *E. coli* DH5α cells transformed with pBluescript KS^+ containing the full coding sequence for the protein (pVW500, Hewinson and Russell, 1993). Uniformly ^{15}N and ^{13}C labelled mpb70 was prepared from cells grown at 37 °C in minimal medium containing 2 g/l ^{15}N ammonium sulphate and ^{13}C glucose. The mbp70 was purified from 40× concentrated culture supernatant using anion exchange and hydrophobic interaction chromatography. The concentrate was initially dialysed against 20 mM Tris-HCl buffer, pH 8.0, loaded onto a 20 ml Q-sepharose column (Pharmacia) and eluted with steps of increasing sodium chloride concentration. The mbp70-containing fraction was concentrated sixfold, dialysed against 20 mM sodium phosphate buffer, pH 7.2, and ammonium sulphate was added to a final concentration of 1 M. This sample was then loaded onto a 1 ml Resource-Phe column (Pharmacia) and the

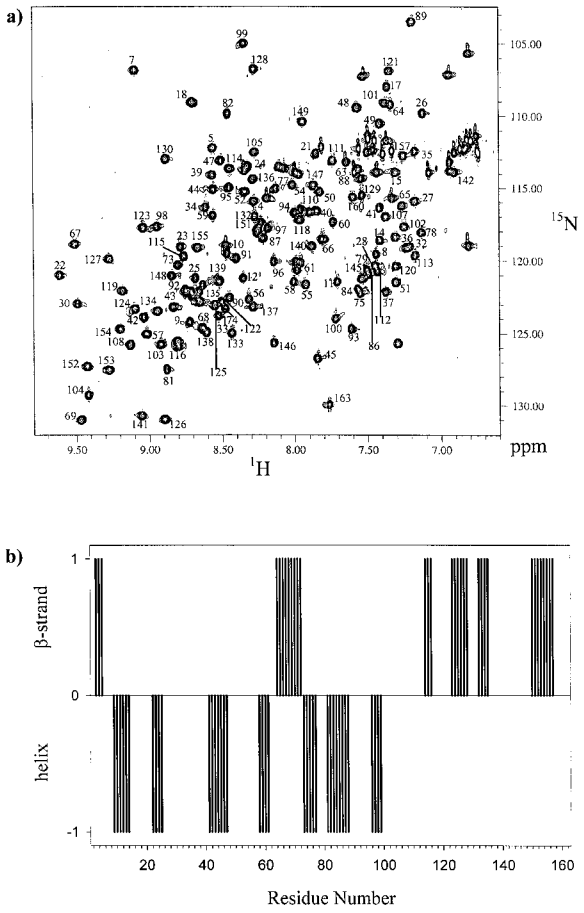

Figure 1. (a) A $^{15}N/^{1}H$ HSQC spectrum of uniformly ^{15}N labelled mpb70 at 30 °C. (b) A histogram summarising the results of chemical shift index-based (C_α, C_β and H_α) identification of the secondary structure of mpb70.

mpb70 was eluted by a linear gradient of ammonium sulphate from 1 to 0 M. Fractions containing pure mpb70 were pooled, dialysed against 20 mM sodium phosphate buffer, pH 6.0, containing 100 mM sodium chloride, and then concentrated for NMR analysis.

The NMR experiments were carried out on samples of 0.7 mM uniformly ^{15}N and ^{13}C labelled mpb70. All the NMR data were acquired at 30 °C on a 600 MHz Varian Inova spectrometer. The 3D HNCACB (Wittekind and Mueller, 1993) and CBCA(CO)NH (Grzesiek and Bax, 1993) spectra were recorded with acquisition times of 5.3 ms in F_1 (^{13}C), 12.8 ms in F_2 (^{15}N) and 51 ms in F_3 (^{1}H), whilst the HBHA(CBCACO)NH (Grzesiek and Bax, 1993) spectrum was acquired with acquisition times of 6.4 ms in F_1 (^{1}H), 12.8 ms in F_2 (^{15}N) and 51 ms in F_3 (^{1}H). The 3D NMR data were processed using NMRPipe (Delaglio et al., 1995), and analysed using the program XEASY (Bartels et al., 1995).

Mpb70 gives well-resolved NMR spectra at pH 6.0 and 30 °C, as illustrated by the $^{15}N/^{1}H$ HSQC spectrum shown in Figure 1a. The good dispersion and sensitivity observed in the 3D spectra allowed the determination of nearly complete sequence-specific backbone resonance assignments for the protein, which were used to map the secondary structure of mpb70 (Figure 1b, Wishart et al., 1992). This analysis clearly shows that the protein contains both helical and β-sheet structure in roughly equal quantities.

Extent of assignments and data deposition

Mpb70 contains 152 non-proline residues and backbone amide signals were observed and assigned for all residues except Gly 1 and Asp 2 (99.3%, Figure 1a); C_α resonances were identified for all residues apart from Gly 1 and Pro 161 (98.8%) and only Thr 42, Thr 44, Thr 95, Gln 117 and Pro 161 have unassigned C_β signals (96.6%). In the case of H_α and H_β resonances, sequence-specific assignments were obtained for 90.2% and 76.4% of the residues in mpb70. The ^{15}N, ^{13}C and ^{1}H resonance assignments for mpb70 have been deposited in the BioMagResBank database (accession number 3716).

Acknowledgements

This work was supported by the Wellcome Trust, the Arthritis Research Campaign and the Ministry of Agriculture, Fisheries and Food.

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