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Letter to the Editor: ^{1}H, ^{13}C, ^{15}N backbone and sidechain resonance assignment of $Mip^{(77–213)}$ the PPIase domain of the *Legionella pneumophila* Mip protein

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Biological context

The Mip (macrophage infectivity potentiator) protein from the legionnaires, disease bacterium *Legionella pneumophila* belongs to the enzyme family of FK506 binding proteins (FKBP). This protein exhibits a peptidyl prolyl *cis*/*trans* isomerase activity (PPIase; EC5.2.1.8) and contributes to the intracellular infection of phagocytic host cells such as human alveolar macrophages and a variety of fresh water protozoa (Steinert et al., 2002).

Mip is a basic 24 kDa surface protein (pI 9.8). Cross-linking experiments demonstrated that Mip forms homodimers both in solution and on the outer membrane of *L. pneumophila* (Schmidt et al., 1994; Helbig et al., 2001). The crystal structure of Mip revealed that each monomer consists of an N-terminal dimerization module, a long (6.5 nm) connecting α-helix and the C-terminal peptidyl prolyl *cis*/*trans* isomerase (PPIase) domain (Riboldi-Tunnicliffe et al., 2001). The fold of the C-terminal domain (residues 100–213) resembles the human FK506-binding protein (FKBP12). Moreover, it has been shown that this domain is responsible for the binding of the immunosuppressants FK506 and rapamycin which efficiently inhibit PPIase activity (Fischer et al., 1992).

Infection studies in *Acanthamoeba castellanii*, *Hartmannella vermiformis*, human mononuclear phagocytes, and lung epithelial cells showed that Mip-negative mutants of *Legionella* are 10- to 100-fold less infective than their isogenic Mip-positive parental strains. Moreover, it has been demonstrated that Mip potentiates infection in the guinea pig animal model (Wintermeyer et al., 1995). It could be demonstrated that both, the PPIase activity and the full-length structure of the Mip dimer, are crucial for full virulence of *Legionella* (Köhler et al., 2003), each representing an independent target for new drugs.

In order to examine the effects of amino acid exchanges in the PPIase domain and to study the binding mechanisms of specific inhibitors FK506 and rapamycin, we have assigned the resonances of the monomeric $Mip^{(77–213)}$ containing the PPIase domain.

Experiments and methods

Escherichia coli harbouring a plasmid coding for $Mip^{(77–213)}$, the PPIase domain of Mip, were used to overproduce the PPIase domain in ^{13}C, ^{15}N labeled medium (Martek M9). $Mip^{(77–213)}$ was purified from these bacteria as described previously (Köhler et al., 2003).

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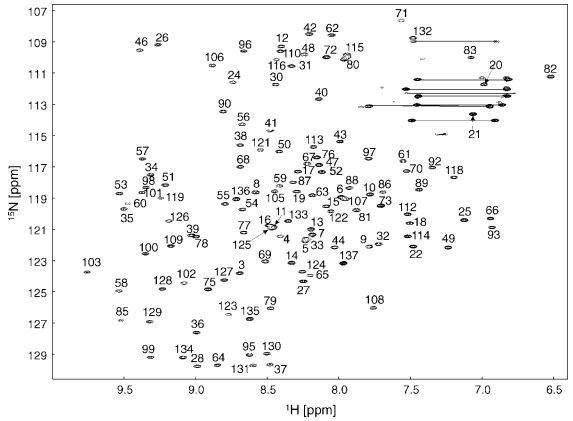

Figure 1. 2D ^{1}H-^{15}N HSQC spectrum of Mip$^{(77–213)}$ recorded at 298 K. The peaks are annotated from 1 to 137 according to the residue numbering of Mip$^{(77–213)}$. The amide side-chain peaks of asparagine and glutamine residues are connected by horizontal lines.

The sample used for NMR experiments contained 2.5 mM ^{13}C/^{15}N-labeled monomeric Mip$^{(77–213)}$ dissolved in 20 mM phosphate buffer in 90% H_2O/10% D_2O (pH 6.5). NMR spectra were recorded with a 700 MHz Bruker Avance spectrometer at 298 K. NMR data were processed using NMRPipe (Delaglio et al., 1995). Peak-picking and visualization of transformed data were carried out using NMRView in conjunction with Smartnotebook (Slupsky et al., 2003).

Sequence-specific backbone assignments were obtained from 3D HNCO, HNCA, HNCACB, and CBCA(CO)NH spectra. For aliphatic side-chain ^{1}H and ^{13}C assignments, HNHA, HBHA (CO)NH, C(CO)NH, and HCCH-TOCSY were used. Amino groups were assigned from 3D CBCA(CO)NH, 2D HSQC, and 3D ^{15}N-NOESY spectra. Aromatic ^{13}C resonances were assigned from ^{15}N-edited NOESY and ^{13}C 2D HSQC and 3D NOESY, centered at the aromatic frequency. The secondary structure was predicted using consensus ($^{1}H^{\alpha}$, $^{13}C^{\alpha}$, ^{13}CO) chemical shift indexing (CSI).

Extent of the assignment and data deposition

All ^{13}CO backbone resonances were assigned except for Phe^{1} and the residues followed by a proline. All other backbone resonances, ^{1}H, ^{15}N, $^{13}C^{\alpha}$ as well as all $^{13}C^{\beta}$ and $^{1}H^{\beta}$ resonances of Mip$^{(77–213)}$, were assigned, except for the resonances of the N-terminal Phe^{1}, ^{1}HN, and ^{15}N of Asn^{2} and $^{1}H^{\beta}$ of Lys^{44}. More than 80% of the aliphatic side-chain protons, 94% of the side-chain carbons, and 84% of the aromatic ring protons and corresponding carbons were assigned. Spectra quality is exemplified by a section from the 2D ^{15}N-HSQC spectrum (Figure 1). The CSI-derived secondary structure elements and the secondary structure from the crystal structure of full-length Mip (PDB code 1FD9) match very well. On the basis of the crystal structure, the strong shift (5.16 ppm) of the indol proton resonance of Trp^{86} could be explained as this proton is located directly above the center of the aromatic ring of Phe^{126}. Trp^{86} forms the base of the binding pocket for FK506 and, presumably, for rapamycin. This indole resonance in particular may serve as a very sensitive marker for structural rearrangements of the binding pocket due to mutations or for binding of inhibitors.

The chemical shifts are available at BMRB database accession code 6334.

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