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Letter to the Editor: Backbone H^N, N, C^α, C' and C^β chemical shift assignments and secondary structure of FkpA, a 245-residue peptidyl-prolyl *cis/trans* isomerase with chaperone activity

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Biological context

FkpA is a 245-residue periplasmic peptidyl-prolyl *cis/trans* isomerase (PPIase) from *E. coli* with chaperone activity, which is induced by heat shock (Horne and Young, 1995; Missiakas et al., 1996). Previous studies suggested that the chaperone activity of FkpA is independent of its PPIase activity (Ramm and Pluckthun, 2000, 2001) as it is also observed for proteins devoid of *cis*-prolines. Overexpression of FkpA suppresses the formation of inclusion bodies from a defective folding variant of the maltose-binding protein and promotes the reactivation of denaturated citrate synthase (Arie et al., 2001). Coexpression of FkpA can dramatically improve functional periplasmic production of single-chain fragments of antibodies, even those not containing *cis*-prolines (Bothmann and Pluckthun, 2000). The folding-assisting function of FkpA is hypothesized to be due to its interaction with early folding intermediates preventing their aggregation, and its ability to reactivate inactive proteins, possibly by binding to partially unfolded species (Ramm and Pluckthun, 2000).

Amino acid sequence alignment and homology modelling indicated that the FkpA dimer is comprised of two domains, the N-terminal dimerization domain and the C-terminal FK506-binding domain (FKBP). The high PPIase enzymatic activity towards proteins (among the highest in bacteria) and the chaperone function of the dimeric FkpA are suggested to be mechanistically related and confined to the FKBP domain (Ramm and Pluckthun, 2001). Nevertheless, protein binding and the occupation of the active site can be independent, and thus these two functions are not identical. The additional N-terminal domain appears to be utilized to mediate the dimerization, which places the two active sites of the FKBP domains in juxtaposition, such that they can simultaneously interact with a protein substrate (Ramm and Pluckthun, 2001; Riboldi-Tunnicliffe et al., 2001). The amino acid sequence of FkpA shows similarity to MIP (macrophage infectivity potentiator) proteins produced by a number of pathogenic bacteria (Rahfeld et al., 1996). Due to the availability of the high resolution X-ray structure of the MIP, the side chain assignment of FkpA is not essential for characterization of interactions of FkpA with polypeptide substrates in order to reveal mechanistical details of its combined chaperone and PPIase activity.

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Experiments and methods

The chemical shift assignments were obtained in turn from triple-resonance spectra of the mature full length FkpA protein (245 amino acid residues) and an engineered variant, the so-called 'shortened FkpA' (sFkpA) lacking the first presumably disordered 9 N-terminal and 18 C-terminal amino acid residues of the mature FkpA, which are dispensable for its functionality (Ramm and Pluckthun, 2001).

The mature full length FkpA protein was prepared as described in (Ramm and Pluckthun, 2000) using M9 D_2O minimal media supplemented with $^{15}NH_4Cl$ (99%, 1 g/L) and $^{13}C_6$-glucose (99%, 4 g/L). Overproduction of the 'shortened' sFkpA (residues 10-227), also containing the C-terminal His_6-tag, was carried out in the *E. coli* strain BL21 at 25 °C. The final NMR sample conditions for both constructs were 0.6 mM of protein per monomer in 20 mM Mes buffer at pH 6.0 with 50 mM NaCl.

All NMR spectra of both constructs, FkpA (residues 1-245) and sFkpA (residues 10-227), were acquired using TROSY (Pervushin et al., 1997) at

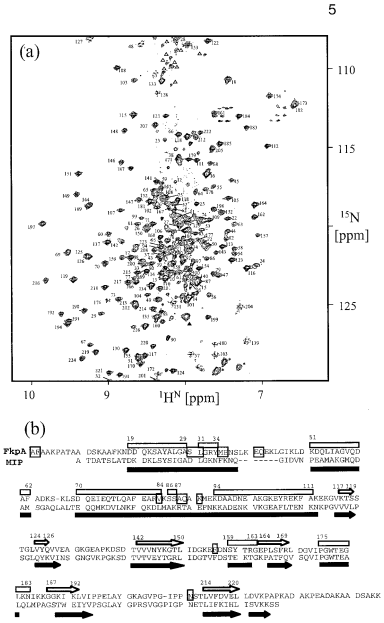

Figure 1. (a) 2D [^{15}N,^{1}H^{N}]-TROSY spectrum of sFkpA (residues 10-227) measured using the Avance 900 spectrometer at 37 °C. The cross-peaks are annotated according to residue numbering of the full length FkpA. The cross-peaks stemming from the guanidinum group of Arg side-chains are labeled with asterisks. The cross-peaks marked with open triangles are degradation products which appear with the aging of the protein samples (see Supplementary Material). (b) The polypeptide sequence and secondary structure of FkpA (open bars and arrows for α-helices and β-sheets) identified using the chemical shift index (CSI) based on the experimental $^{13}C^{\alpha}$ chemical shifts. The sequence of FkpA is aligned with the MIP (Macrophage Infectivity Potentiator, PDB code 1FD9), for which the secondary structure elements derived from the crystal structure are indicated with solid arrows and bars. Residues of FkpA with ambiguously assigned backbone resonances are enclosed in boxes.

25 °C and 37 °C on Bruker Avance 600 and 900 MHz spectrometers. The NMR experiments include 2D [^{1}H,^{15}N] TROSY, 3D TROSY-versions of HNCA, HNCACB, HNCO, HN(CA)CO and 3D ^{15}N resolved TROSY-NOESY (τ_{mix} = 100 ms).

Extent of assignment

The $^{1}H^{N}$, ^{15}N, $^{13}C'$, $^{13}C^{\alpha}$ and $^{13}C^{\beta}$ resonances were sequence-specifically assigned by identification of sequentially connected [$^{1}H^{N}$, ^{15}N] fragments of the TROSY-HNCA, TROSY-HNCACB, TROSY-HNCO and TROSY-HN(CA)CO spectra supported by TROSY-NOESY connectivities followed by global fragment mapping with the program MAPPER (Guntert et al., 2000). With this approach ca. 60% of all resonances of the full length FkpA were sequence-specifically assigned at 25 °C. Genetical removal of the first 9 N-terminal and 18 C-terminal amino acid residues in the so-called 'shortened FkpA' (sFkpA), together with the increase of temperature to 37 °C and the use of the ultra high field NMR spectrometer operating at 900 MHz permitted a largely complete backbone resonance assignement of sFkpA. The spectra of sFkpA were then aligned and compared to the corresponding spectra of FkpA showing in most cases no significant perturbations of chemical shifts. This promoted the assignment of backbone resonances of FkpA to 94%. Figure 1a shows the 2D [$^{1}H^{N}$, ^{15}N] TROSY spectrum of sFkpA.

In Figure 1b the polypeptide sequence of FkpA from *E. coli* is aligned with the sequence of the MIP from *Legionella pneumophila*, a PPIase for which the crystal structure was recently determined (PDB code 1FD9) (Riboldi-Tunnicliffe et al., 2001). Despite of the modest sequence identity of 34% the secondary structure elements closely correspond to each other in the aligned sequences of both proteins, indicating possible 3D structural similarity. The presented backbone assignment should serve as a platform to investigate atomic resolution details of chaperone/protein substrate interactions.

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Data deposition. The chemical shifts are available at BMRB database, accession code 5863.

References

Arie, J.P., Sassoon, N. and Betton, J.M. (2001) *Mol. Microbiol.*, **39**, 199–210.

Bothmann, H. and Pluckthun, A. (2000) *J. Biol. Chem.*, **275**, 17100–17105.

Güntert, P., Salzmann, M., Braun, D. and Wüthrich, K. (2000) *J. Biomol. NMR*, **18**, 129–137.

Horne, S.M. and Young, K.D. (1995) *Arch. Microbiol.*, **163**, 357–365.

Missiakas, D., Betton, J.M. and Raina, S. (1996) *Mol. Microbiol.*, **21**, 871–884.

Pervushin, K., Riek, R., Wider, G. and Wüthrich, K. (1997) *Proc. Natl. Acad. Sci. USA*, **94**, 12366–12371.

Rahfeld, J.U., Rucknagel, K.P., Stoller, G., Horne, S.M., Schierhorn, A., Young, K.D. and Fischer, G. (1996) *J. Biol. Chem.*, **271**, 22130–22138.

Ramm, K. and Pluckthun, A. (2000) *J. Biol. Chem.*, **275**, 17106–17113.

Ramm, K. and Pluckthun, A. (2001) *J. Mol. Biol.*, **310**, 485–498.

Riboldi-Tunnicliffe, A., Konig, B., Jessen, S., Weiss, M.S., Rahfeld, J., Hacker, J., Fischer, G. and Hilgenfeld, R. (2001) *Nat. Struct. Biol.*, **8**, 779–783.