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Letter to the Editor: Assignment of the ^{1}H, ^{13}C and ^{15}N resonances of the PPIase domain of the trigger factor from *Mycoplasma genitalium*

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Biological context

Every cell contains a diverse set of cellular factors which aid protein folding. These proteins can be divided into two main classes: chaperones and isomerases. Chaperones have been shown to bind to unfolded proteins and to nascent protein chains emerging from the ribosome. Their key role is to reduce the rate of protein aggregation and to release a properly folded protein. Two classes of isomerases are important in the folding process, disulfide isomerases and peptidyl-prolyl cis/trans-isomerases (PPIases). PPIases catalyze the slow isomerization of Xaa-Pro peptide bonds, thus accelerating the folding of proline-containing polypeptides. PPIases are divided into three families: cyclophilins, FK506-binding proteins (FKBPs), and parvulins. Cyclophilins and FKBPs are called immunophilins since they are receptors for the immunosuppressant drugs cyclosporin A (CsA) and FK-506, respectively. Both immunosuppressants also effectively inhibit PPIase activity in vitro, however, peptidyl-prolyl isomerisation and immunosuppression are unrelated processes.

The trigger factor was first discovered in 1987 as a protein involved in *E. coli* secretatory protein export (Crooke and Wickner, 1987). It was later rediscovered in the context of its association with chaperones (Stoller et al., 1995; Valent et al., 1995; Hesterkamp et al., 1996) and was also found to possess PPIase activity (Hesterkamp et al., 1996). This activity is confined to a domain with weak homology to FKBPs (Stoller et al., 1995), which binds neither FK-506 nor CsA (Stoller et al., 1996). Next to this FKBP-like domain, intact trigger factor features an N-terminal ribosome binding domain and a C-terminal domain of currently unknown function (Stoller et al., 1995). PPIase activity of the FKBP-like domain is quite low, however, the full length trigger factor is able to catalyze protein folding as judged by the RNAse T1 refolding assay (Stoller et al., 1995) at significantly increased rate when compared to other PPIases (Scholz et al., 1997). This is due to the high affinity of the intact protein towards unfolded peptide chains (Scholz et al., 1998), which can thus be regarded as a ribosome-associated multifunctional folding enzyme that interacts with the nascent peptide chain.

To date trigger factor homologues have been found in all sequenced procaryotic genomes. In *M. genitalium*, it is the only gene that can be associated with PPIase activity and is therefore presumed essential for cell survival (Bang et al., 2000).

Methods and experiments

The *Mycoplasma genitalium* trigger factor PPIase domain was overexpressed in *E. coli* and purified as described by Bang et al. (2000).

All NMR measurements were conducted on samples containing ca. 2 mM protein and 50 mM sodium

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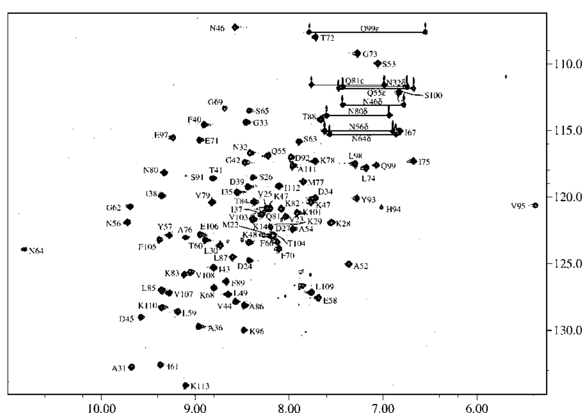

Figure 1. Annotated $^{1}H,^{15}N$-HSQC spectrum of the trigger factor recorded at 298 K and a ^{1}H resonance frequency of 800 MHz. The assignment of the peaks is indicated with their one-letter amino acid code and number.

phosphate buffer, pH 6.5, at 298 K. The following experiments were carried out: (a) ^{15}N-labelled sample in H_2O/D_2O 90/10: 3D ^{15}N-NOESY-HSQC (80 ms mixing time), 3D ^{15}N-TOCSY-HSQC (60 ms mixing time), 3D HNHA, 3D HSQC-NOESY-HSQC (100 ms mixing time). (b) ^{13}C-^{15}N-labelled sample in H_2O/D_2O 90/10: 3D HNCO, 3D HNCA, 3D CBCA(CO)NH, 3D HNCACB, 3D CC(CO)NH-TOCSY, 3D H(CC)(CO)NH-TOCSY, 3D HBHA-CONH. (c) ^{13}C-^{15}N-labelled sample in D_2O: 3D HCCH-TOCSY, 3D ^{13}C-NOESY-HMQC (80 ms mixing time) (aliphatic region), 3D ^{13}C-NOESY-HMQC (80 ms mixing time) (aromatic region). (d) unlabelled sample in D_2O: 2D NOESY (80 ms mixing time), TOCSY. All heteronuclear experiments were acquired as gradient versions with sensitivity enhancement (Sattler et al., 1999). Spectra were recorded on Bruker DRX 800 or DRX600 instruments (Bruker Analytik GmbH, Rheinstetten, Germany). Time-domain data was Fourier transformed after zero-filling, apodization and linear prediction using XWINNMR 2.6 (Bruker). All assignments were accomplished using XEASY (Bartels et al., 1995). Resonance positions were referenced to internal DSS.

Extent of assignments and data deposition

^{1}H, ^{13}C and ^{15}N backbone resonances of most non-proline residues were assigned, excluding residues 1–11 (including the His_6-tag added for purification), 15 and 20. For all other residues, the H_α, H_β, C_α and C_β assignment is complete with the exception of residue 51. Other backbone assignments are not complete for residues 14, 50, 51 and 67. All ^{1}H and ^{13}C signals for the two prolines could be assigned. Most side-chain proton and carbon shifts and all asparagine and glutamine amides were completely assigned. The chemical shift values of proton, nitrogen and carbon resonances were deposited in the BioMagResBank in Madison, WI, U.S.A. (accession number BMRB-4953).

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References

Bang, H., Pecht, A., Raddatz, G., Scio, T., Solbach, W., Brune, K. and Pahl, A. (2000) *Eur. J. Biochem.*, **267**, 3270–3280.

Bartels, C., Xia, T.-H., Billeter, M., Güntert, P. and Wüthrich, K. (1995) *J. Biomol. NMR*, **6**, 1–10.

Crooke, E. and Wickner, W. (1987) *Proc. Natl. Acad. Sci. USA*, **84**, 5216–5220.

Hesterkamp, T., Hauser, S., Lutcke, H. and Bukau, B. (1996) *Proc. Natl. Acad. Sci. USA*, **93**, 4437–4441.

Sattler, M., Schleucher, J. and Griesinger, C. (1999) *Prog. NMR Spectrosc.*, **34**, 93–158.

Scholz, C., Stoller, G., Zarndt, T., Fischer, G. and Schmid, F.X. (1997) *EMBO J.*, **16**, 54–58.

Scholz, C., Mücke, M., Rape, M., Pecht, A., Pahl, A., Bang, H. and Schmid, F.X. (1998) *J. Mol. Biol.*, **277**, 723–732.

Stoller, G., Rücknagel, K.P., Nierhaus, K.H., Schmid, F.X. and Rahfeld, J.U. (1995) *EMBO J.*, **14**, 4939–4948.

Stoller, G., Tradler, T., Rücknagel, K.P., Rahfeld, J.U. and Fischer, G. (1996) *FEBS Lett.*, **384**, 117–122.

Valent, Q.A., Kendall, D.A., High, S., Kusters, R., Oudega, B. and Luirink, J. (1995) *EMBO J.*, **15**, 5494–5505.