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Letter to the Editor: Assignment of ^{1}H, ^{13}C and ^{15}N resonances of FKBP from *Methanococcus thermolithotrophicus*

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Biological context

The *cis-trans* isomerization of the peptidyl prolyl bond is a rate-limiting step of protein folding. Peptidyl prolyl *cis-trans* isomerase (PPIase) catalyzes this step and is proposed to accelerate the protein folding in vivo. FK506 binding protein (FKBP) and cyclophilin (CyP) are two major families of PPIases, and also the natural targets of the immunosuppressants cyclosporine and FK506, respectively. In addition to PPIase activity, some FKBPs and CyPs have been reported to exhibit chaperone-like activity. Despite the similarities in function, these two proteins show little sequence homology to each other. FKBP and CyP are ubiquitous proteins in nature and many organisms have both of these proteins. However, the genome projects revealed that some thermophilic and hyperthermophilic archaea lack the genes of CyP homologues and have only FKBP as PPIase (Furutani et al., 2000). These FKBPs lack sequences at the N-terminus, where one β-strand is contained in human FKBP12 (hFKBP12), and have two insertion sequences in the regions corresponding to the bulge and the flap of hFKBP12. The insertion in the bulge (12–13 residues) is unique to archaeal FKBPs while similar insertion in the flap (44–49 residues) is also found in some bacterial FKBPs.

Recently it has been reported that both of the insertion sequences are important for the PPIase activity of FKBP from a thermophilic archaeon, *Methanococcus thermolithotrophicus* (MTFK) (Furutani et al., 2000). MTFK also exhibits chaperone-like activity that abates the aggregation of folding intermediates of rhodanese and elevates the final yield of active protein. This activity is suppressed in mutants without the insertion in the flap.

There have been reported many crystal and NMR structures of FKBPs in the domain *Eucarya*, mostly from human. On the other hand, no structure is solved in the domain *Bacteria* or *Archaea* and the structural features of these insertions remain unknown. We have assigned the chemical shifts of MTFK and predicted its secondary structure. This is the first report of the secondary structural elements of FKBP from *Archaea*.

Methods and results

MTFK was overproduced in *Escherichia coli* strain BL21(DE3)/pLysS transformed with ptFK plasmid by using the T7 promotor/T7 polymerase expression system (Furutani et al., 2000). For the uniform ^{15}N labeling of MTFK, $^{15}NH_4Cl$ and ^{15}N-labeled algal amino acid mixture were used, and for the uniform $^{15}N/^{13}C$ labeling, $^{15}NH_4Cl$, $^{15}N/^{13}C$-labeled algal amino acid mixture and [U-$^{13}C_6$]-glucose were used. The harvested cells were lysed by sonication and incubated at 80 °C for 30 min. The supernatant was fractionated by adding $(NH_4)_2SO_4$. The fractions containing MTFK were loaded onto a Phenyl Sepharose (Pharmacia) column. The column was eluted with a 1.8–0 M reverse gradient of $(NH_4)_2SO_4$ followed by

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a 20–50% gradient of ethylene glycol. Fractions containing MTFK were pooled and concentrated by ultrafiltration (Centriprep 10, Amicon). Ethylene glycol was removed with a desalting column (Ampure-SA, Amersham). Purity was confirmed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and mass spectrometry.

The NMR sample (0.4 ml) contained 4.5 mM ^{15}N- or 8 mM ^{15}N/^{13}C-MTFK in 90% H_2O/10% D_2O with 50 mM potassium phosphate buffer at pH 7.0 (not corrected for isotope effects). All NMR spectra were recorded at 25 °C. ^{15}N-HSQC, ^{15}N-TOCSY-HSQC and ^{15}N-NOESY-HSQC experiments were performed with ^{15}N-MTFK on a Varian UnityINOVA 500 spectrometer equipped with a Narolac z-axis gradient probe. Triple resonance experiments were performed with ^{15}N/^{13}C-MTFK on a Varian UnityINOVA 750 spectrometer equipped with a Varian z-axis gradient probe. All the experiments other than ^{15}N-TOCSY-HSQC (Zhang et al., 1994) were carried out with pulse sequences in the ProteinPack suite of pulse programs (Varian Inc.).

NMR data were processed on Silicon Graphics workstations with NMRPipe software (Delaglio et al., 1995). ^{1}H chemical shifts were referenced to internal sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS). ^{13}C and ^{15}N chemical shifts were referenced indirectly to DSS by multiplying the spectrometer frequency corresponding to 0 ppm in the ^{1}H spectrum by the ^{13}C/^{1}H and ^{15}N/^{1}H frequency ratios (Wishart et al., 1995).

The strip plots of the spectrum were printed using the P-ROI system (Kohda, 1998) written in Tcl/Tk and nmrWish, which is a companion to the NMRPipe system. The analysis on a computer display was achieved using SPARKY 3 (Goddard, T.D. and Kneller, D.G., University of California, San Francisco, CA). The chemical shift indices were calculated with the CSI program (Wishart and Sykes, 1994) and the torsion angles phi and psi were predicted by use of the TALOS program (Cornilescu et al., 1999).

Extent of assignments, secondary structure prediction and data deposition

The backbone assignments ($^{1}H^{N}$, ^{15}N, $^{13}C^{\alpha}$, $^{13}C^{\beta}$, $^{13}C'$ and $^{1}H^{\alpha}$) were made for all residues other than C′ of E154. All non-aromatic side-chain carbons beyond the β-position were assigned except for $^{13}C^{\epsilon}$ of K10, K34, K141 and $^{13}C^{\epsilon}$ of all methionines. $^{15}N^{\delta}$ of asparagines and $^{15}N^{\epsilon}$ of glutamines were also assigned. Non-exchangeable and non-aromatic protons were assigned except for $^{1}H^{\epsilon}$ of K10, K34, K141 and $^{1}H^{\epsilon}$ of all methionines.

The secondary structure of MTFK was predicted from the chemical shifts of backbone atoms by use of the CSI and TALOS programs. The prediction indicates that the secondary structural elements of MTFK are located similarly to those of hFKBP12 except for the β-strand at the N-terminus that is absent in MTFK. Thus, MTFK would have a similar fold to hFKBP12. MTFK has two insertion sequences in the bulge (13 amino acids) and the flap (44 amino acids) regions (Furutani et al., 1998). The insertion in the bulge, which is uniquely found in FKBPs from archaea and is important for the PPIase activity of MTFK, is predicted to contain an α-helix. The insertion in the flap, which is found in FKBPs from archaea and bacteria and is important for both the PPIase and chaperone-like activities of MTFK, is predicted to contain one α-helix and two or three β-strands.

Chemical shift assignments have been deposited in the BioMagResBank database (accession no. 4668).

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