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NMR assignments of the FK506-binding domain of FK506-binding protein 35 from *Plasmodium vivax*

Reema Alag · Joon Shin · Ho Sup Yoon*

*School of Biological Science, Nanyang Technological University, 60 Nanyang Drive, Singapore 637665, Singapore
e-mail: hsyoon@ntu.edu.sg

Abstract

PvFKBP35 is a member of the FK506 binding protein family (FKBP) from *Plasmodium vivax*. The FK506-binding domain of PvFKBP35 shows a canonical peptidylprolyl *cis*–*trans* isomerase (PPIase) activity. To understand the role of PvFKBP35 in the parasite, we have performed NMR studies. Here, we report the assignment of the FK506-binding domain of PvFKBP35.

Keywords

Plasmodium vivax • FK506 FKBP • Peptidylprolyl *cis*–*trans* isomerase • Heteronuclear NMR

Biological context

Malaria is a severe problem in many developing countries, responsible for almost 2 million deaths per year (World Health Organization 2005). Human malaria is caused by infection with intracellular parasite *Plasmodium* that is transmitted by Anopheles mosquitoes. After *P. falciparum*, *P. vivax* is the second major malaria causing species which is getting resistance to currently available antimalarial drugs. Identification of the new parasitic proteins that could serve as novel targets to design a novel antimalarial drug has increased in last few years. Since FK506 binding proteins (FKBPs) are involved in many important cellular pathways (Gothel and Marahiel 1999; Ratajczak et al. 2003; Riggs et al. 2004), it could be a good target for anti-malarial therapeutics. Previously FK506 binding protein with molecular weight of 35 kDa [PfFKBP35] has been identified and characterized (Kumar et al. 2005; Yoon et al. 2006; Monaghan and Bell 2005) from *P. falciparum*. We have identified ortholog of PfFKBP35 in *P. vivax* and named it PvFKBP35. Like PfFKBP35, PvFKBP35 contains N-terminal FK506 binding domain (hereafter referred to as PvFKBD), C-terminal tetratricopeptide repeats (TPR) domain and calcium/calmodulin binding motif. FK506 binding domain is also called peptidylprolyl *cis*–*trans* isomerase (PPIase) domain (Kissinger et al. 1995) because it catalyzes *cis*–*trans* isomerization of peptidylprolyl bond and help in correct folding of proteins (Galat 2003). However binding of FK506 inhibits the PPIase activity of the protein. The immunosuppressive activity of the drug involves a different mechanism in which FK506 and FKBP complex binds to calcineurin (CN), a Ca²⁺ calmodulin (CaM)-dependent protein phosphatase, and inhibits its activity. This further increases the phosphorylation of the transcription factor (NF-AT) that fails to translocate from the cytoplasm to the nucleus and ultimately leads to temporary suppression of T-cell activation (Feske et al. 2003). Here we report the ¹H, ¹³C and ¹⁵N backbone and side chain

assignments of 126 amino acid long PvFKBD, which will provide further insight into the structural basis of PvFKBD and could help in designing novel anti-malarial drugs.

Methods and experiments

Expression and purification of FKBD domain of *P. vivax*

The DNA fragment encoding the FK506 binding domain of PvFKBP35 was amplified by Polymerase chain reaction from the genomic DNA of *P. vivax* (a gift from Dr. Zbynek Bozdech). The forward primer contained *Bsa*I restriction enzyme site (5'-CGC CGC GGT CTC GAGGT ATG GAG CAG GAG ACC CTC GAG CAA GTG CAC-3') and reverse primer contained *Bam*HI restriction enzyme site (5'-CGC CGC GGA TCC TTA TTC TCT AAA GCT GAT TAG CTC TAT TTC-3'). The amplified DNA fragment was cloned into the *Bsa*I/*Bam*HI cleaved pSUMO vector which results in pSUMO-FKBD with a hexahistidine-tag and SUMO fusion protein at the N-terminus. The recombinant pSUMO-FKBD plasmid was transformed into *E. coli* BL21 (DE3) cells, grown in M9 medium containing 1 g/l of $^{15}\text{NH}_4\text{Cl}$ or 1 g/l of $^{15}\text{NH}_4\text{Cl}$ and 2 g/l of ^{13}C -glucose at 37°C to prepare uniformly ^{15}N and $^{15}\text{N}/^{13}\text{C}$ -labeled proteins respectively. When absorbance of cell culture reached $\text{Abs}_{600} = 0.6\text{--}0.8$, IPTG was added at final concentration of 1 mM for induction of protein for 3 h. The cells were harvested by centrifugation at 18,600g for 10 min. Cell pellet was resuspended in a resuspension buffer (20 mM NaPO_4 , 500 mM NaCl, 10 mM imidazole) and cells were broken by sonication for 30 min on ice. The cell lysate was cleared by centrifugation at 48,400 $\times$ g for 20 min and purified on a Ni^{2+} -NTA resin as described previously (Kang et al. 2005). For a further purification the eluted fractions from Ni^{2+} -NTA column were loaded onto superdex-200 filtration column. After gel filtration chromatography, the N-terminal hexahistidine-tag and SUMO was cleaved by SUMO protease. NMR samples (0.5 mM) were prepared in a buffer containing 20 mM NaPO_4 (pH 6.8), 50 mM NaCl, and 1 mM DTT, and 0.01% NaN_3 .

NMR spectroscopy

All 2D and 3D heteronuclear NMR experiments were performed at 298 K on a Bruker Avance 700 MHz equipped with a cryoprobe. Backbone assignments were obtained from the spectra of 2D [$^{15}\text{N}/^1\text{H}$]-HSQC, HNCA CB, CBCA (CO) NH, HNCO, HN (CO) CA (Satller et al. 1999). HCCH-TOCSY, ^{13}C -HSQC-NOESY, ^{15}N -HSQCNOESY were used for side chain assignments. Aromatic ring resonances were assigned from ^{13}C -HSQC-NOESY, ^{15}N -HSQC-NOESY (Satller et al. 1999). All Spectra were processed with NMRPipe (Delaglio et al. 1995) and analyzed using SPARKY (T.D. Goddard and D.G. Kneller, UCSF, San Francisco, CA, USA).

Extent of assignment and data deposition

2D ^{15}N - ^1H -HSQC assigned spectrum of PvFKBD (M1- E126) is shown in Fig. 1. Except two nonproline residues (M1 and E2), ^1H and ^{15}N backbone chemical shifts were assigned for all residues (98.4%). Chemical shifts for $^{13}\text{C}^\alpha$, $^{13}\text{C}^\beta$ were assigned for all residues (100%) and ^{13}C chemical shifts were assigned for 121 residues (96%) out of 126 residues. The assignments have been deposited in the BioMagResBank (<http://www.bmrb.wisc.edu>) under BMRB accession number 16260. Sequence alignment between PvFKBD, *P. falciparum* FKBD (PfFKBD) and

human FKBP12 (HsFKBP) reveals that PvFKBD shows 81% sequence identity to PfFKBD and 44% identity to HsFKBP (Fig. 2). Key aromatic residues in the active site involved in FK506 binding are well conserved in *P. vivax*. The secondary structure elements (Fig. 3) of the PvFKBD protein were estimated from the analysis of H^α , C^α , C^β and CO chemical shifts using TALOS (Delaglio et al. 1995). Chemical shift index (CSI) analysis predicts that the structure of PvFKBD mainly composed of β -sheets with short stretches of α helices, which is similar to the other canonical FKBP proteins like *Plasmodium falciparum* FKBP35 (Kang et al. 2008) and human FKBP12 (Michnick et al. 1991). Solution structure determination of PvFKBD is underway and will provide more information and structural insights after completion.

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List of Figures

- Figure 1 2D $^1\text{H} - ^{15}\text{N}$ – HSQC spectrum of PvFKBD. The spectrum was recorded at 298 K on a Bruker Avance 700 MHz spectrometer. The assignments for resolved backbone residues are labeled with one letter amino acid code and residue number
- Figure 2 Sequence alignment of PvFKBD, FK506 binding domain of *P. vivax* (EDL44272), PffFKBD, FK506 binding domain of *P. falciparum* (AAN36539) and human FKBP12 (AAA35844), protein sequence alignment was generated by Vector NTI Align X (Informax)
- Figure 3 Chemical shift index (H^α , C^α , C^β and CO) for PvFKBD, β -strands are indicated by +1, α -helices are indicated by -1 and random coil by 0. The inferred β -strands are represented by *arrows* and helical regions by *cylinder*

Figure 1

Figure 2

Figure 3