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^{1}H, ^{13}C, and ^{15}N resonance assignments of FK506-binding domain of *Plasmodium falciparum* FKBP35

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Abstract The immunosuppressant FK506 binds *Plasmodium falciparum* FK-506 binding protein 35 (PfFKBP35) and shows anti-malarial activity. To understand molecular mechanism of the drug on the parasite, we have done NMR studies. Here, we report the assignment of FK506-binding domain of PfFKBP35.

Keywords *Plasmodium falciparum* • FK506 • FKBP • Heteronuclear NMR

Biological context

Human malaria still remains a major threat to the public health of countries in the tropical and subtropical regions of the world. Human malaria is caused by infection with intracellular parasites *Plasmodium* that are transmitted by Anopheles mosquitoes. *Plasmodium falciparum* is the most lethal pathogen among the four species of *Plasmodium* that infect human beings. Previous studies demonstrated that the immunosuppressive drug FK506 shows an anti-malarial effect, suggesting that the parasite may contain a potential FK506 binding protein as the

molecular target of the drug. Recent efforts, mainly through genomic analysis, resulted in the identification of a FKBP family protein (PfFKBP35) in *Plasmodium falciparum* (Kumar et al. 2005; Monaghan and Bell 2005). PfFKBP35 shows a high sequence similarity to FKBP12 in the catalytic core domain, whereas the overall structural architecture resembles the multiple tetratricopeptide repeat (TPR)-containing FKBP family including FKBP38, FKBP51, and FKBP52. PfFKBP35 contains a FKBD (FK506 binding domain), a tripartite TPR domain, and one putative calmodulin binding domain (CBD) (Kumar et al. 2005; Monaghan and Bell 2005). The unique structural feature leads us to speculate that PfFKBP35 may play an important role for the pathogenesis of *Plasmodium faciparum* in humans, because FKBP38 and FKBP52, which show similar structural characteristics, interact with proteins in the cell cycle or apoptosis and regulate their activities (Gkika et al. 2006; Kang et al. 2005). Currently, molecular basis of the growth inhibition of the parasite by FK506 remains unclear. Towards a better understanding on the biological function of PfFKBP35, we performed the NMR study on the FKBD of PfFKBP35. Here we report the ^{1}H, ^{13}C, and ^{15}N resonance of FKBD of PfFKBP35.

Methods and experiments

Protein preparation

The DNA fragment encoding PfFKBP35 was amplified from the genomic DNA of *Plasmodium falciparum* library (A kind gift from Dr. Peter Preiser). The cDNA was digested with *Nde I* and *Xho I* and the resulting product was inserted into pET29b to generate pET29-FKBP35, encoding a C-terminal hexahistidine-containing fusion protein. The FKBD (M1-

R127) of PfFKBP35 was also sub-cloned into pET29b using the same restriction enzymes and using pET29-FKBP35 as a template (Yoon et al. 2007). The $^{13}C/^{15}N$ and ^{15}N uniformly labeled FKBD were purified by Ni^{2+}-NTA and gel filtration, The protein samples were in the buffer containing 20 mM Na–PO_4, pH 6.8, 50 mM NaCl, 1 mM DTT, 0.1 mM EDTA with concentration ranging from 0.5 mM to 1 mM for NMR study.

NMR spectroscopy

All NMR spectra were recorded at 298 K on Bruker AV600 spectrometer equipped with a cryoprobe accessory. Backbone ^{1}H, ^{15}N and ^{13}C resonance were assigned using data from 2D $^{1}H–^{15}N$ HSQC, 3D HNCA, HNCACB, CBCACONH, HNCOCA spectra. The side chain ^{1}H and ^{13}C were obtained from 3D HCC(CO)NH-TOCSY, 3D (H)CC(CO)NH-TOCSY, HCCH-TOCSY, (H)CCH-TOCSY, 3D ^{15}N-^{1}H-NOESY-HSQC (Sattler et al. 1999, Simon and Sattler 2004). All spectra were processed with NMRPipe (Delaglio et al. 1995) and NMRView (Johnson 2004).

Assignment and data deposition

The backbone amide assignment is summarized in Fig. 1. Total observable backbone $^{1}H–^{15}N$ correlations spanning residues 3–127 (which includes three prolines) were assigned. For the carbon resonances, 126 C^{α} and 122 C^{β} (14 Glycines without C^{β}) resonances have been assigned except M1. The unassigned residues are M1, T2 and the C-terminal His-tag. Excluding the C-terminal His-tag residues, assignment of the side chains are about 90% complete. The backbone chemical shifts analysis using program CSI (Wishart and Sykes 1994) suggests that FK506-binding domain of PfFKBP35 shares the similar secondary structure with FKBP12, which contains at least six β-strands and one α-helix. NOE data

from the ^{15}N-NOESY-HSQC are consistent with this prediction. We believe that the quality of the NMR data is sufficient for the structure determination of FK506-binding domain of PfFKBP35, which is currently in progress. The assignments have been deposited with BMRB accession number 15038.

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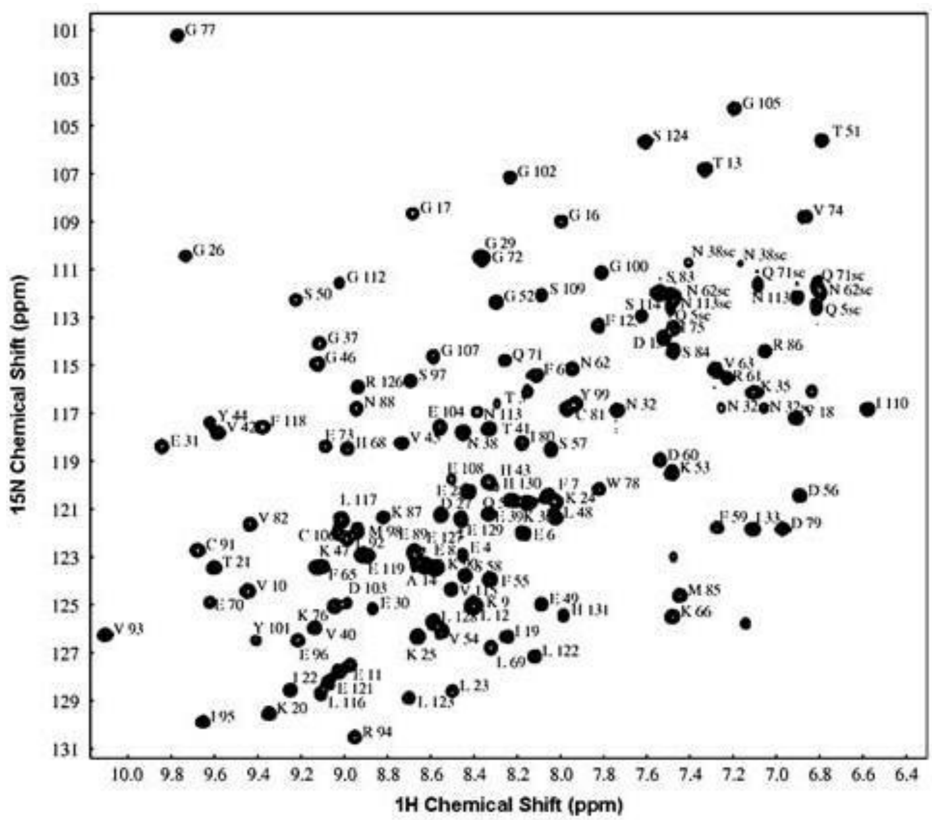
15N Chemical Shift (ppm)
101 103 105 107 109 111 113 115 117 119 121 123 125 127 129 131
10.0 9.8 9.6 9.4 9.2 9.0 8.8 8.6 8.4 8.2 8.0 7.8 7.6 7.4 7.2 7.0 6.8 6.6 6.4
1H Chemical Shift (ppm)

Fig. 1