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Expression, purification, and molecular characterization of *Plasmodium falciparum* FK506-binding protein 35 (PfFKBP35)

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Abstract

The immunosuppressive drug FK506 binds its targets FK506-binding protein (FKBP) family and modulates cellular processes. Recent studies demonstrated that FK506 shows anti-malaria effects. Newly identified FK506-binding protein 35 from *Plasmodium falciparum* (PfFKBP35) is assumed to be the molecular target of FK506 in the parasite. Currently, molecular and structural basis of growth inhibition of the parasite by FK506 remains unclear. In this study, to examine characteristics of PfFKBP35 and also understand its molecular mechanism of the inhibition by FK506, we have cloned, expressed, and purified the full-length PfFKBP35 and its FK506-binding domain (FKBD). We demonstrate that the full-length PfFKBP35 and the FKBD were properly folded, and suitable for biochemical and biophysical studies. PfFKBP35 showed a basal activity in inhibiting the phosphatase activity of calcineurin in the absence of FK506, but the presence of FK506 greatly enhanced its calcineurin-inhibitory activity. Our NMR data indicate that the FKBD binds FK506 with a high affinity.

Keywords: FK506; FKBP; Calcineurin; Malaria; *Plasmodium falciparum*; NMR

Human malaria still remains a major threat to the public health of countries in the tropical and subtropical regions of the world [1]. About 40% of the world's population lives in areas where malaria is transmitted [1–3]. 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. In recent years, extensive efforts have been made for the development of various tools and drugs to prevent the infection by *P. falciparum* [4]. The variation of parasite antigens hinders the development of vaccine against the parasite. In the midst of efforts to develop potential vaccines against the parasite, the identification of molecular targets, and attempts to develop inhibitors against authentic targets may serve as an alternative option in combating malaria [3].

Previous studies demonstrated that the immunosuppressive drug FK506 shows an anti-malarial effect [5], suggesting that the parasite may contain a potential FK506-binding protein as the molecular target of the drug. Recent efforts, mainly through a genomic analysis, resulted in the identification of a FKBP family protein (PfFKBP35) in *P. falciparum* [3,6]. PfFKBP35 shows a high similarity to FKBP12 in the catalytic core domain, whereas the overall structural architecture resembles the multiple tetratricopeptide repeat (TPR)[1]-containing FKBP family including FKBP38, FKBP51, and FKBP52 [7]. The canonical FKBP family proteins possess peptidylprolyl *cis–trans* isomerase activity, FK506-binding

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[1] *Abbreviations used:* TPR, tetratricopeptide repeat; FKBD, FK506-binding domain CBD, calmodulin-binding domain; NTA, nitriloacetic acid; IPTG, isopropyl-thio-β-D-galactopyranoside; MALDI-TOF/TOF, matrix-assisted laser desorption/ionisation-time of flight; MS, mass spectrometry; α-CHCA, α-cyano-4-hydroxycinnamic acid; HSQC, ^{1}H-^{15}N heteronuclear single quantum correlation spectroscopy.

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Fig. 1. Comparison of PfFKBP35 with other FKBP family proteins. (a) The diagram of PfFKBP35, its FKBD, and human FKBP38. The diagram shows domain layout of PfFKBP35. Compared to human FKBP38 (AAB00102), PfFKBP35 contains no transmembrane domain: FKBD, FK506-binding domain; TPR, tetratricopeptide repeat; CBD, calmodulin-binding domain; TM, transmembrane domain. (b) The amino acid alignment of human FKBP12 and the FKBD of PfFKBD35, human FKBP38, and human FKBP52 (A46437) is shown. ∇, represent aromatic residues in the FK506-binding pocket. ●, represent residues involved in interaction with FK506 through hydrogen bonds. The protein sequences were aligned by Vector NTI (InforMax).

activity, and chaperon activity [6]. PfFKBP35 as one of the multiple TPR-containing FKBP family members contains a FKBD (FK506-binding domain), a tripartite TPR domain, and one putative calmodulin-binding domain (CBD) (Fig. 1). It was demonstrated that FKBP38 and FKBP52, which show similar structural characteristics to that of PfFKBP35, interact with proteins in the cell cycle or apoptosis, and regulate their activities [8–10]. This suggests that PfFKBP35 may play an important role in the pathogenesis of *P. falciparum* in humans. Currently, molecular basis of the growth inhibition of the parasite by FK506 remains unclear. Towards a better understanding on the biological function of PfFKBP35, in this study, we have cloned, expressed, purified PfFKBP35, and subsequently performed biochemical and biophysical characterizations. Our results demonstrate that the purified proteins were properly folded and showed a basal level of inhibitory activity on calcineurin. NMR analysis showed that the FKBD of PfFKBP35 binds FK506 with a high affinity.

Materials and methods

Materials

Antibody against His-tag was purchased from Santa Cruz Biotech (Santa Cruz, CA, USA). Ni^{2+}–nitriloacetic acid (NTA) resin was purchased from Qiagen (Hilden, Germany). Protein molecular weight marker was from Bio-Rad Laboratories (Hercules, CA, USA). HiPrep 26/60 Sephacryl S-200 column was from Amersham Biosciences (Buckinghamshire, UK). Chemicals were purchased from Sigma–Aldrich (St. Louis, MO, USA). *Escherichia coli* BL21 (DE3) cells and kanamycin were from Invitrogen (Carlsbad, CA, USA). The vector pET29b was from Novagen (Madison, WI, USA). Isopropyl-thio-β-D-galactopyranoside (IPTG) was from Promega (Madison, WI, USA). C_4 and C_{18} ZipTips were from Millipore (Billerica, MA, USA).

Construction of bacterial expression vectors for PfFKBP35 and its FKBD

The coding sequence for PfFKBP35 was amplified using the genomic DNA of *P. falciparum* 3D7 (A kind gift from Dr. Peter Preiser) as a template. The following primers were used for the cDNA amplification: forward primer contains *Nde*I site (5′-gctatctcatatgactaccgaacaagaattt-3′); reverse primer contains *Xho*I site (5′- agctagactcgagatttgcactatt tttttttc-3′). The amplified DNA fragment was digested with *Nde*I and *Xho*I and the resulting product was inserted into pET29b to generate pET29-FKBP35 with a hexahistidine tag at the C-terminus. The FKBD (M1-E127) of PfFKBP35 was also sub-cloned into pET29b using the same restriction enzymes and using pET29-FKBP35 as a template. The primers used were as follows: forward primer (5′-gctatctcatatgactaccgaacaagaattt-3′); reverse primer (5′-agctaga*ctcgag* ttctctaaagcttaataattc-3′) [11].