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Molecular & Biochemical Parasitology 141 (2005) 163–173
MOLECULAR & BIOCHEMICAL PARASITOLOGY

The FK506-binding protein of the malaria parasite, *Plasmodium falciparum*, is a FK506-sensitive chaperone with FK506-independent calcineurin-inhibitory activity☆

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Received 21 October 2004; received in revised form 17 December 2004; accepted 17 February 2005
Available online 19 March 2005

Abstract

We have identified an immunophilin of the FKBP family in *Plasmodium falciparum* that contains a conserved peptidyl prolyl isomerase (PPIase) and tetratricopeptide repeat (TPR) domains. The 35 kDa protein was named FKBP35 and expressed in bacteria. Recombinant FKBP35 exhibited potent PPIase and protein folding activities against defined substrates in vitro, suggesting that it is a parasitic chaperone. Both activities were inhibited by macrolide immunosuppressant drugs, ascomycin (a FK506 derivative) and rapamycin, but not by cyclosporin A, providing biochemical evidence of its inclusion in the FKBP family. Interestingly, FKBP35 inhibited purified plasmodial calcineurin (protein phosphatase 2B) in the absence of any drug. In the parasite's cell, FKBP35 exhibited a stage-specific nucleocytoplasmic shuttling and did not co-localize with calcineurin. FKBP35 associated with plasmodial heat shock protein 90 (Hsp90), another member of the chaperone superfamily, via the TPR domain. Geldanamycin, a Hsp90 inhibitor, and ascomycin inhibited *P. falciparum* growth in a synergistic fashion. Extensive search of the *P. falciparum* genome revealed no other FKBP sequence, implicating PfFKBP35 as a highly significant antimalarial drug target. Thus, the single FKBP of *Plasmodium* is an essential parasitic chaperone with a novel drug-independent calcineurin-inhibitory activity.

Keywords: FK506; FKBP; Ascomycin; Rapamycin; PPIase; Chaperone; Malaria; Calcineurin

1. Introduction

The immunophilin superfamily consists of highly conserved, ubiquitously expressed proteins that possess rotamase or peptidyl prolyl *cis–trans* isomerase (PPIase) activity and are considered to play an essential role in the folding of client proteins by accelerating the isomerization of X-Pro peptide bonds, a rate-limiting step in protein folding pathway [1–3]. The majority of immunophilins can be classified into two large families, viz. cyclophilins (CyPs) and FK506-binding proteins (FKBPs), and each family binds to specific immunosuppressant molecules of fungal origin [4]. The CyPs bind cyclosporin A (CsA), a cyclic undecapeptide [5,6]; in contrast, the FKBPs specifically bind macrolides such as FK506 (tarcolimus) and rapamycin (sirolimus) that are structurally unrelated to CsA [7]. In both families, the drugs bind to the PPIase domain that is roughly 100 amino acids long, leading to inhibition of the PPIase activity. Thus, the immunophilin PPIase domain is synonymous with drug-binding domain; the FKBP PPIase domain, for example, is also referred to as FK506-binding domain or FKBD (Figs. 1 and 2).

The immunosuppression activity of the drugs, however, involves a mechanism different from PPIase inhibition, in which the cytosolic CsA–CyP or FK506–FKBP com-

☆ While our paper was in the final stages of acceptance, it came to our attention that Monaghan and Bell have recently published their results on PfFKBP35 that are very similar to ours: Monaghan P, Bell A. A Plasmodium falciparum FK506-binding protein (FKBP) with peptidyl prolyl *cis–trans* isomerase and chaperone activities. Mol Biochem Parasitol 2005;139:185–95.

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doi:10.1016/j.molbiopara.2005.02.007

Fig. 1. The PPIase catalytic domain (PPIase/FKBD), the three TPR motifs (-1, -2, -3), and prospective CaM-binding domain (CaM-BD) of relevant FKBPs are shown. The extra C-terminal sequence in human FKBP38 (accession number: AAO39020) contain the underlined hydrophobic mitochondrial targeting sequence [54], absent in the others. Within the PPIase domain, the asterisked residues are conserved in all four FKBPs; the shaded ones are important for FK506-binding and thus, for inhibition of PPIase activity and calcineurin (see text for details). Within the TPR domain, residues important for Hsp90-binding are shaded; those that are absolutely essential for binding are additionally marked by overhead dots [50]. For CyP40, only the TPR domain is shown, because its PPIase domain has no homology with the FKBP PPIase domains. Due to the degeneracy of TPR motifs, their amino acid identities are poor, and are not marked. The plasmodial sequences are described in detail under Section 3; accession numbers of FKBP 12 and CyP40 are NP_463460 and Q08752, respectively. The deletions of PfFKBP35 are demarcated with triangles (also see Fig. 2). All recombinants, including the deletions contained N-terminal His-tag for purification.

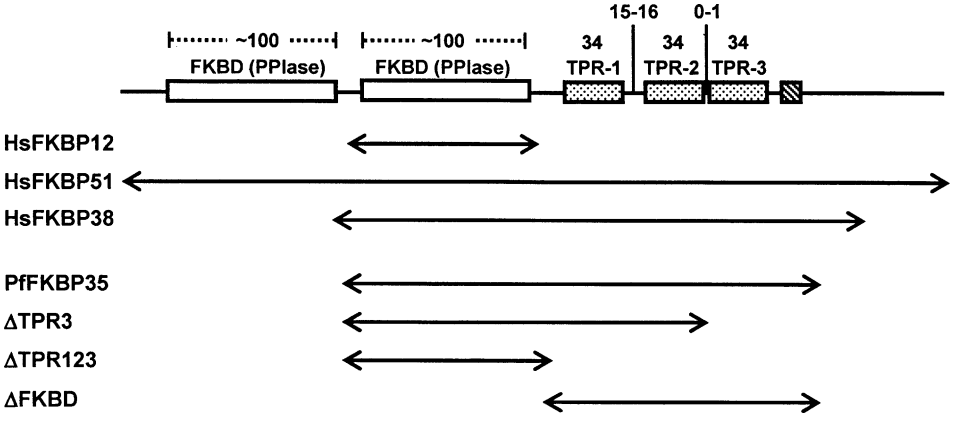

Fig. 2. Domain arrangement in FKBPs and the PfFKBP35 deletion mutants. The modular PPIase/FK506-binding domain, TPRs (box) and CaM-BD are indicated, respectively, by white, speckled and striped boxes. The approximate lengths and domain content of a few representative FKBPs are indicated graphically; overhead numbers are average lengths of the domains in number of amino acids. FKBPs may contain additional domains that are not shown. Recombinant mutants of PfFKBP35 were constructed in which the deletions extended from the C-terminus and included the third TPR (ΔTPR3) or all three TPRs (ΔTPR123). The N-terminal deletion (ΔFKBD) removed the PPIase/FKBD domain only, and therefore, contained the rest of the protein including the TPRs and CaM-BD. The exact deletion points are shown in Fig. 1.