Structural Insights into Substrate Binding by \(Pv\)FKBP35, a Peptidylprolyl cis-trans Isomerase from the Human Malarial Parasite \textit{Plasmodium vivax} \\

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The immunosuppressive drug FK506 binding proteins (FKBPs), an immunophilin family with the immunosuppressive drug FK506 binding property, exhibit peptidylprolyl cis-trans isomerase (PPIase) activity. While the cyclophilin-catalyzed peptidylprolyl isomerization of X-Pro peptide bonds has been extensively studied, the mechanism of the FKBPs-mediated peptidylprolyl isomerization remains uncharacterized. Thus, to investigate the binding of FKBPs with its substrate and the underlying catalytic mechanism of the FKBPs-mediated proline isomerization, here we employed the FK506 binding domain (FKBD) of the human malarial parasite \textit{Plasmodium vivax} FK506 binding protein 35 (FKBP35) and examined the details of the molecular interaction between the isomerase and a peptide substrate. The crystallographic structures of apo \(Pv\)FKBD35 and its complex with the tetrapeptide substrate succinyl-Ala-Leu-Pro-Phe-p-nitroanilide (sALPFp) determined at 1.4 Å and 1.65 Å resolutions, respectively, showed that the substrate binds to \(Pv\)FKBD35 in a cis conformation. Nuclear magnetic resonance (NMR) studies demonstrated the chemical shift perturbations of D55, H67, V73, and I74 residues upon the substrate binding. In addition, the X-ray crystal structure, along with the mutational studies, shows that Y100 is a key residue for the catalytic activity. Taken together, our results provide insights into the catalytic mechanism of \(Pv\)FKBP35-mediated cis-trans isomerization of substrate and ultimately might aid designing substrate mimetic inhibitors targeting the malarial parasite FKBPs.

A member of the immunophilin family, the FK506 binding proteins (FKBPs) that bind to the immunosuppressive drugs FK506 and rapamycin with high affinity belongs to the peptidylprolyl cis-trans isomerase (PPIase) family (1), which also includes cyclophilins and parvulins (2, 3). PPIase proteins catalyze the cis-trans isomerization of the peptidylprolyl bond. The cis-trans isomerization of the peptidylprolyl bond is one of the rate-limiting steps in protein folding due to a large energy barrier of 14 to 24 kcal/mol (4). Conformation of the protein backbone is usually defined by three torsion angles, \(\phi\), \(\psi\), and \(\omega\). Values of \(\omega\) are 0° in cis conformation of the peptide bond and 180° in trans conformation. These cis (0°) and trans (180°) forms are separated by a rotational barrier of the perpendicular high-energy state where \(\omega\) is 90°. Therefore, the transition state of cis-trans isomerization is approximately by a high energy, “twisted” syn-90 state (5, 6). FKBPs act as a catalyst and accelerate the cis-trans isomerization by reducing the energy barrier of the reaction (1) and help in correct folding of proteins (5). FK506 binding to FKBP inhibits its PPIase activity (7), while its immunosuppressive function is a result of the inhibition of calcineurin activity after the FKBP-FK506 binary complex binds to calcineurin (8, 9). Inhibition of PPIase activity by FK506 suggests that both the substrate and FK506 bind to the same binding pocket of the protein.

The physiological role of the PPIase activity was first established by showing that cyclosporine (CsA) slows down the folding of procollagen I into triple-helix collagen (10). Later it was found that Cyp20, a component of the mitochondrial folding machinery, cooperates with Hsp70 and Hsp60 in protein folding (11). However, a major physiological significance of the PPIase-catalyzed cis-trans isomerization was recognized after the discovery of the catalytic properties of Pin1 (6, 12). Unlike other PPIases, Pin1 isomerizes only phosphorylated Ser/Thr-Pro motifs, indicating its important role in cellular signaling pathways, where it can regulate the conformation of its substrates after phosphorylation to control protein function (6, 13). Recent studies showed that the peptidylprolyl cis-trans isomerization may function as an effective and reversible molecular switch that controls the kinetics of auto-inhibition of a signaling molecule, Crk adaptor protein (14). It is also responsible for the opening of the pores of the neurotransmitter-gated ion channel (15). PPIases act as a catalyst for cis-trans isomerization and increase the reaction rate by several orders of magnitude.

While extensive studies have been done on cyclophilins (16, 17) and Pin1-mediated (18) cis-trans isomerization, to our knowledge, no structural study has been performed on FKBPs with their in vitro peptide substrate succinyl-Ala-Leu-Pro-Phe-p-nitroanilide (sALFPp). We previously showed that \(Pv\)FKBD35 (the FK506 binding domain of \textit{Plasmodium vivax} FKBP35) possesses a canonical PPIase activity like HsFKBP12 (human FKBP12) (19). A preliminary search in plasmoDB (20) using “Ala-X-Pro-Phe,” where X represents any hydrophobic amino acid, resulted in 629 pro-
teinss across seven different *Plasmodium* species (see Table S1 in the supplemental material), and the probable recognition of FKBP's by these proteins could be taken up for future interest. Further, a study on the identification of unique proline-containing motifs across proteins in the *Plasmodium falciparum* proteomes (21) has proposed that these motifs could serve as possible leads toward developing peptidomimetic antimalarial drugs. In this direction, we have performed X-ray crystallographic studies of both apo *PvFKBD35* and its complex with the peptide sALPP. as well as nuclear magnetic resonance (NMR) titration and site-specific mutational studies. Our data presented here provide molecular insights into the FKB-mediated peptidylprolyl *cis-trans* isomerization, the first of its kind.

**MATERIALS AND METHODS**

**Sample preparation.** The coding sequence for *PvFKBD35* (M1-E126) was amplified and cloned into the pSUMO-*PvFKBD35* plasmid with N-terminal hexahistidine and SUMO protein. The resulting pSUMO-*PvFKBD35* plasmid was used as the template to generate single-amino-acid-substitution mutants (Y100F, Y100A, Y100W, Y100R, Y100P, Y100E, Y100L) using the Quick Change site-directed mutagenesis kit (Stratagene, La Jolla, CA). The pSUMO-*PvFKBD35* construct was transformed in *Escherichia coli* BL21(DE3) cells, cells were grown into LB medium and M9 medium containing 1 g/liter of 15NH4Cl for X-ray crystallography and NMR studies, respectively, and protein was purified as described before (19). In short, protein expression was induced by adding 1 mM isopropyl-β-D-thiogalactoside (IPTG) when the cell density reached 0.6 to 0.8. The cells were harvested by centrifugation at 18,600 × g for 10 min, resuspended in the lysis buffer (20 mM NaPO4, 500 mM NaCl, pH 7.6), and broken by sonication for 20 to 30 min on ice. The cell lysate was cleared by centrifugation at 48,400 × g for 20 min and purified by Ni2+-nitrilotriacetic acid (NTA) resin. For a further purification, Ni2+-NTA column elution fractions were loaded onto a Superdex-200 filtration column (GE Healthcare, Singapore). The N-terminal SUMO tag was removed by sumo protease and passed through an Ni2+-NTA column to obtain the pure protein near homogeneity. The NMR samples were prepared in a buffer containing 20 mM NaPO4 (pH 6.8), 20 mM NaCl, 1 mM dithiothreitol (DTT), and 0.01% NaN3. For X-ray crystallography, the protein was concentrated to 10 mg/ml in 10 mM phosphate buffer, 137 mM NaCl, and 2.7 mM KCl (pH 6.8), as described before (19).

**Peptide synthesis.** Solid-phase peptide synthesis method with 9-fluorenylemethoxycarbonyl (Fmoc) chemistry and Wang resin was used on a tetrapeptide Ala-Leu-Pro-Phe (ALPF) as described elsewhere (22, 23). In short, protein expression was induced by adding 1 mM isopropyl-β-D-thiogalactoside (IPTG) when the cell density reached 0.6 to 0.8. The cells were harvested by centrifugation at 18,600 × g for 10 min, resuspended in the lysis buffer (20 mM NaPO4, 500 mM NaCl, pH 7.6), and broken by sonication for 20 to 30 min on ice. The cell lysate was cleared by centrifugation at 48,400 × g for 20 min and purified by Ni2+-nitrilotriacetic acid (NTA) resin. For a further purification, Ni2+-NTA column elution fractions were loaded onto a Superdex-200 filtration column (GE Healthcare, Singapore). The N-terminal SUMO tag was removed by sumo protease and passed through an Ni2+-NTA column to obtain the pure protein near homogeneity. The NMR samples were prepared in a buffer containing 20 mM NaPO4 (pH 6.8), 20 mM NaCl, 1 mM dithiothreitol (DTT), and 0.01% NaN3. For X-ray crystallography, the protein was concentrated to 10 mg/ml in 10 mM phosphate buffer, 137 mM NaCl, and 2.7 mM KCl (pH 6.8), as described before (19).

**Crystallization of apo *PvFKBD35* and cocrystallization of *PvFKBD35* with peptide substrate sALPP.** For apo *PvFKBD35*, an automated initial crystallization screen of 672 conditions was performed using the CyBio Crystal Creator (Jena Biosciences). Crystal drops were prepared by mixing 0.2 µl protein solution (10 mg/ml in 10 mM phosphate buffer, 137 mM NaCl, 2.7 mM KCl, pH 6.8) with an equal volume of precipitant solution and equilibrated by sitting-drop vapor diffusion against 0.1 ml precipitant solution at 291 K. Crystals in Crystal Screen condition 6 (0.1 M Tris-HCl [pH 8.5], 30% [wt/vol] polyethylene glycol 4000, 0.2 M magnesium chloride hexahydrate; Hampton Research) showed suitable ordered diffraction for structural studies. *PvFKBD35* and sALPP complex initial crystals were produced using the ammonium sulfate buffer grid screen from Hampton Research followed by optimization in 2.85 M ammonium sulfate and 0.1 M MES buffer, pH 5.5. The concentrated protein sample was mixed with the peptide in a molar ratio of 1:5 and incubated overnight at 4°C prior to crystallization. Crystals were set up by the hanging-drop vapor diffusion method using 2 µl of complex protein solution mixed with an equal volume of reservoir solution equilibrated against 1 ml reservoir solution. Crystals 0.4- by 0.4- by 0.2-mm3 in size appeared after 10 days at 18°C.

**Data collection and structure determination.** A single crystal of *PvFKBD35* was transferred into a cryoprotectant composed of 25% (vol/vol) glycerol in addition to the components of the reservoir solution. Diffraction intensities were collected at the National Synchrotron Radiation Research Center (NSRRC; Hsinchu, Taiwan) to a resolution of 1.4 Å. Similarly, a crystal of the sALPP-bound form of *PvFKBD35* was transferred into a cryo-protectant composed of 50% mineral oil and paratone mixture and flash cooled in liquid nitrogen. Single-wavelength data sets of reservoir solution equilibrated against 1 ml reservoir solution. Crystals 0.4- by 0.4- by 0.2-mm3 in size appeared after 10 days at 18°C.

**TABLE 1 X-ray crystallographic data collection and refinement statistics for the apo- and sALPP-bound forms of *PvFKBD35***

| Statistic | Apo *PvFKBD35* | *PvFKBD35*-sALPP complex |
|-----------|----------------|--------------------------|
| Space group | P 1 2 1 | P 1 2 1 |
| Unit cell a, b, c (Å) | 54.9, 41.8, 55.3 | 53.12, 45.79, 54.78 |
| α, β, γ (°) | 90.0, 106.4, 90.0 | 90.0, 112.47, 90.0 |
| Resolution (Å) | 22.66–1.42 (1.47–1.42) | 33.5–1.65 (1.68–1.65) |
| Completeness (%) | 97.9 (88.3) | 92.7 (88.8) |
| Redundancy | 5.8 (4.4) | 10.7 (10.9) |
| Rmerge (%) | 5.5 (53.2) | 5.2 (34.7) |
| I/σ(I) | 40.3 (2.0) | 25.4 (5.0) |
| No. of reflections (working set/test set) | 42,266/2,249 | 27,158/954 |
| R factor (Rfree) | 0.171/0.219 | 0.174/0.206 |
| No. of atoms of protein/water | 1,947/278 | 1,930/301 |
| Average B factor (Å2 ) | 22.02 | 31.31 |
| RMS deviation | Bond length (Å) | 0.014 | 0.013 |
| Bond angle (°) | 1.302 | 1.458 |
| Ramachandran (%) | Most favored | 94.6 | 99.59 |
| Additionally allowed | 5.4 | 0.41 |
| Generously allowed | 0.0 | 0.0 |
| Disallowed | 0.0 | 0.0 |

* Values in parentheses refer to the corresponding values of the highest-resolution shell.
beit for few terminal atoms of the succinic acid and p-nitroanilide moieties. Upon closer investigation, it was observed that these two moieties are exposed to the surface (see Fig. S1 in the supplemental material), while the remaining residues of the peptide are sandwiched in the dimer interface. An occupancy refinement using the program PHENIX (27) could not help improve the density, indicating the high mobility of these groups. Iterative cycles of model building were carried out using the program COOT (28) followed by refinement using REFMAC5 (29) of the CCP4 suite (30) and PHENIX (27), for the apo- and peptide-bound structures, respectively. The geometry of the final model was checked with PROCHECK (31), and the figures are drawn using the program PyMOL (32). Atomic coordinates and structure factors for the apo PvFKBD35 and sALPFp-bound forms have been deposited in the RCSB Protein Data Bank with PDB identifiers of 3NI6 and 4ITZ, respectively.

**RESULTS**

**Crystal structure of apo PvFKBD35.** The crystallographic structure of apo PvFKBD35 shows two molecules per asymmetric unit. A total of 245 residues, eight glycerol molecules, and 278 water molecules were included in the final model. The N-terminal residues 1 and 2 of molecule A and 1 to 5 of molecule B are missing from the final model, presumably due to their high mobility in the crystal. Details of data collection and refinement statistics are given in Table 1. The structure of the monomer consists of six antiparallel $\beta$-strands and a short central $\alpha$-helix (Fig. 1A). The two molecules in the asymmetric unit have nearly identical structure (root mean square [RMS] deviation is 0.46 Å for 121 $\alpha$-car-
bon atoms). The two monomers of \( P.\) \( \text{FKBD35} \) assemble to form a dimer through interactions between \( \beta_3-\beta_4 \) loops and \( \beta_5-\beta_6 \) loops (Fig. 1A). There are hydrogen bonds between the main chain N and O atoms and involve residues R60, E103, G104, and G106 of one \( P.\) \( \text{FKBD35} \) molecule and residues Q70, G111, G106, and G104 of the other molecule (Fig. 1B). The tertiary structure of \( P.\) \( \text{FKBD35} \) shows one disulfide bond formed by Cys105-Cys105 of both molecules (Fig. 1A and B). But native polyacrylamide gel electrophoresis (PAGE) and gel filtration chromatography of the protein sample and dissolved crystals revealed only the presence of monomeric species, indicating that the disulfide bond between two molecules could be a crystallization artifact (data not shown).

A superimposition of the crystal structures of the apo form of \( P.\) \( \text{FKBD35} \) and FK506-bound \( P.\) \( \text{FKBD35} \) (PDB identifier 3IHZ) gives an overall RMS deviation of 0.743 Å for 121 equivalent \( \alpha \)-carbon atoms. Most of the secondary structural elements of the apo enzyme are superimposed in FK506-bound \( P.\) \( \text{FKBD35} \) without major differences, except differences observed in the loops that connect strand \( \beta_3 \) to strand \( \beta_4 \) and strand \( \beta_5 \) to strand \( \beta_6 \) (Fig. 1C).

**Crystal structure of \( P.\) \( \text{FKBD35} \) complexed with substrate sALPFp.** \( P.\) \( \text{FKBD35} \) cocystalized with the substrate tetrapeptide, sALPFp, diffracted to a 1.65 Å resolution with two molecules in the asymmetric unit. The initial phases of the peptide-bound structure of \( P.\) \( \text{FKBD35} \) were determined using the molecular replacement technique. The detailed statistics of data collection, phasing, and refinement are given in Table 1.

The final refined crystallographic structure of the sALPFp-bound \( P.\) \( \text{FKBD35} \) complex from \( P.\) \( \text{vivax} \) contains a total of 242 amino acids belonging to two independent molecules present in the asymmetric unit; a tetrapeptide, sALPFp (Fig. 2A); 301 water molecules; and a sulfate ion. The two \( P.\) \( \text{FKBD35} \) monomers present in the asymmetric unit can be superimposed with an average RMS deviation of 0.278 Å for 121 equivalent \( \alpha \)-carbon atoms. Most of the secondary structural elements of the apo enzyme are superimposed in FK506-bound \( P.\) \( \text{FKBD35} \) without major differences, except differences observed in the loops that connect strand \( \beta_3 \) to strand \( \beta_4 \) and strand \( \beta_5 \) to strand \( \beta_6 \) (Fig. 1C).

**FIG 2** Crystal structure of sALPFp-bound \( P.\) \( \text{FKBD35} \). (A) The cartoon diagram of the two monomers of \( P.\) \( \text{FKBD35} \)-sALPFp in the asymmetric unit, with color code and orientation similar to those described in Fig. 1A. The sALPFp tetrapeptide is represented as sticks within the pink-colored circle. (B) Cartoon representation of the complex formed between \( P.\) \( \text{FKBD35} \) and sALPFp (in green sticks), with the secondary structures labeled in black. (C) Electrostatic surface potential representation of \( P.\) \( \text{FKBD35} \), with sALPFp shown as green-colored sticks. It could be seen that the proline of sALPFp docks itself deep into the hydrophobic pocket.
the main chain nitrogen of I74, and the nitrogen of p-nitroanilide interacts with the side chain oxygen of D55. The Y100 forms a strong hydrogen bond (36) with a distance of 2.26 Å from the carbonyl oxygen of proline, while the D55 forms a weak one (36) at 3.6 Å from the nitrogen of p-nitroanilide (Table 2). Such short, strong hydrogen bonds observed in protein structures (37) are believed to play an important role in the enzyme catalysis, implying the importance of Y100. In addition, main chain nitrogen of alanine and carbonyl oxygen of phenylalanine is stabilized by three water molecules. Nonpolar contacts (Fig. 4A and Table 2) with other key active-site residues, such as Y43, F54, D55, V73, W77, C105, I109, and F117, also stabilize the substrate interactions. The D55 OD2 of PvFKBD35 is just 3.14 Å away from the beta carbon of the substrate proline and 3.6 Å from the nitrogen atom of p-nitroanilide, which could have probably resulted in their peak shifts during NMR titration (Fig. 4B). The residues of PvFKBD35 involved in sALPFp interactions are mostly coming from the β4-α1 loop (V73 and I74) and β5-β6 loop (C105, I109, Y100) (Fig. 4A). The leucine and proline, the two key residues of the substrate, form strong hydrogen bonding interaction with Y100 and I74 (Fig. 4A) and are buried deep inside the nucleus of the FKBP active-site pocket (Fig. 2C). We further investigated the molecular interaction between the active-site residues and the substrate peptide by NMR spectroscopy. To this end, the 15N-labeled protein was titrated with various concentrations of the peptide, and amide chemical shifts of PvFKBD35 were investigated on 2D 1H-15N HSQC. The chemical shift perturbations were detected mainly in four residues, D55, H67, V73, and I74 (Fig. 4B; see also Fig. S2 in the supplemental material). The chemical shift perturbations of D55, V73, and I74 are due to substrate binding and consistent with the crystallographic structure of the protein-substrate complex. However, in the crystal structure, the residue H67 is ~10 Å away from the substrate located at the C-terminal end of the β4 strand. Thus, one possibility for the chemical shift perturbation of H67 might be an indirect effect of the substrate.

The interaction of sALPFp with PvFKBD35 (Table 2) is shown in Fig. 4A. The hydrogen bonded (blue dashes) and nonbonded (gray dashes) interactions made by sALPFp (green sticks) with PvFKBD35, revealing the binding of the proline (P3) region into the deep active-site pocket. The three α angles corresponding to the peptide bonds are also labeled and shown by pink dots. (B) Chemical shift perturbations of PvFKBD35 (0.35 mM) upon adding the substrate peptide. Residues showing perturbations upon addition of the peptide are labeled.

### Table 2: Interactions between PvFKBD35 and sALPFp

| Type of contact | PvFKBD35 atoms | Donor-acceptor distance (Å) |
|----------------|----------------|-----------------------------|
| Polar          |                |                             |
| A1amino        | H2O            | 2.77                        |
| L2 carbonyl oxygen | I74 N      | 2.92                        |
| P3 carbonyl oxygen | Y100 OH      | 2.26                        |
| P3 amino       | Y100 OH        | 3.43                        |
| F4 carbonyl oxygen | H2O         | 2.65                        |
| F4 carbonyl oxygen | H2O         | 2.51                        |
| p-Nitroanilide N1 | D55 OD2   | 3.60                        |
| Nonpolar       |                |                             |
| L2             | V73, I74, Y100 |                             |
| P3             | Y43, D55, W77, Y100, F117 |                         |
| F4             | C105, I109      |                             |
| p-Nitroanilide | F54            |                             |
binding-induced conformational changes in the β4-α1 loop (Fig. 3B).

Role of Y100 in catalysis. The structural analysis of PrFKBD35 in complex with sALPFp reveals the importance of Y100 in the cis-trans isomerization of the peptidylprolyl bond. It could be observed (Fig. 4A, Table 2) that Y100 is the only residue in the PrFKBD35 active site that plays a key role by anchoring the leucine and proline, the two key residues of the substrate peptide. Interestingly, Y100 did not show as much perturbations as D55, V73, and I74 from NMR titration (Fig. 4B), consistent with a comparably holds on to its position in the active site and need not require much perturbation upon substrate binding. To further validate the role of the Y100 side chain on PPIase activity, mutagenesis studies were performed using Y100F, Y100W, Y100R, Y100P, Y100E, Y100L, and Y100A mutants. The data showed that the Y100F and Y100W mutants maintain activity similar to that of the wild type. The Y100R mutant retains 70% activity, while the Y100P and Y100E mutants have 60% and 50% of the wild-type activity, respectively. The Y100L and Y100A mutants show 20 to 30% activity (Fig. 5), substantiating the fact that the aromatic residue at the Y100 position plays an important role for the PPIase activity, corroborating with the short hydrogen bond (2.26 Å) observed in the crystal structure.

Comparison of the substrate- and inhibitor-bound forms of PrFKBD35. The substrate-bound state of the PrFKBD35-sALPFp complex enables, for the first time, a comparison with the inhibitor (FK506)-bound state of FKBP (19). The three PrFKBD35 structures in apo-, FK506-, and sALPFp-bound forms possess similar resolutions, 1.42 Å, 1.67 Å, and 1.65 Å, respectively, and cell parameters which validate the comparison. The RMS deviation values calculated by superposition of the main chain atoms of the PrFKBD35-sALPFp-bound structure with the apo- and FK506-bound forms are 0.360 Å and 0.645 Å, respectively, for the 121 equivalent α-carbon atoms (Fig. 3B and 6A). Minor differences could be observed in the ligand-flanking loop regions of β3-β4, β4-α1, and β5-β6. The comparison reveals that both the substrate and the inhibitor FK506 are located in the ligand binding pocket, formed within α1 and the β-sheet platform (Fig. 6B and C). The proline of the substrate superimposes over the pipecolic ring of FK506 (Fig. 6B) just above W77, which forms the platform for this hydrophobic active site. A comparison of the active-site residues in the apo-, FK506-, and sALPFp-bound forms (Fig. 6C) indicates subtle changes in W77 and C105, the two residues positioned at the perpendicular ends of the active site apart from other residues, notably V73 and I109. A directional motion, though negligible, can be observed in these four residues, where it closes in toward the pocket in the sALPFp structure and moves away in the FK506 structure (Fig. 6C).

DISCUSSION

In our present study, we examined the binding of PrFKBD35 with its peptide substrate sALPFp and also compared the modes of binding between the substrate and its inhibitor, FK506. Our structural studies together with NMR titration and mutational data highlight the roles of D55, I74, and Y100 in the substrate binding in cis conformation. Previous studies demonstrated that most of the mutations on Y82 in HsFKBP12, which is equivalent to Y100 in PrFKBD35, resulted in a decrease in the PPIase activity of

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**FIG 5** PPIase activity of wild-type PrFKBD35 and the Y100 mutants. PPIase assay was performed at 4°C, as described in Materials and Methods, and change in the absorbance was measured at 390 nm for 5 min.

**FIG 6** Comparison of FK506 and sALPFp binding to PrFKBD35. (A) Shown is a superposition of the Cα traces of FK506-bound PrFKBD35 (colored in white) with the sALPFp complex (colored in brown). The ligand-flanking loops of the FK506- and sALPFp-bound structures are colored in purple and green, respectively. The bound FK506 and sALPFp are represented in yellow and green sticks, respectively. (B) A closer view of the superposition of the FK506 (yellow sticks) over the sALPFp (green sticks) shows that the pipecolic moiety of FK506 overlays itself on the proline residue of substrate (within the blue circle). The cartoon representation of the PrFKBD35-sALPFp (in orange) and W77 (in stick mode), which forms the base of the pocket, is shown for reference. (C) The common residues that are involved in the binding of FK506 (in cyan) and sALPFp (in orange) in the PrFKBD35 structure are shown in stick mode, while those of the apo structure are shown in white. The figure enables us to visualize the subtle changes in the active-site residues due to ligand binding.
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