Structure of the Human 25 kDa FK506 Binding Protein Complexed with Rapamycin

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The immunosuppressive drugs FK506[1] and rapamycin[2,3] bind to a family of proteins called the FK506 binding proteins or FKBPs.[4] The prototype of the family, the cytosolic human FKBP12,[5,6] has been intensively investigated both structurally[7−11] and biologically.[12−14] FKBP13, which is localized in the endoplasmic reticulum and does not play a role in immunosuppression, has also been characterized.[15,16] FKBP25, a third member of the family, has two domains: a C-terminal domain with 43% sequence identity to FKBP12 and an N-terminal domain with no recognizable homology to any known protein.[17,18] The C-terminal domain of FKBP25 contains a possible nuclear localization sequence, and FKBP25 has been suggested to be a nuclear protein.[19] The C-terminal domain, like the other FKBPs, is a peptidyl-prolyl isomerase (PPIase or rotamase), and this activity is inhibited by FK506 and rapamycin. Unlike the other FKBPs, FKBP25 shows a strong preference for binding rapamycin (K_i = 0.9 nM) over FK506 (K_i = 200 nM). The N-terminal domain does not influence the binding specificity since the C-terminal domain shows the same binding preferences as the full-length protein.[20] In order to understand the structural bases for FKBP25's selectivity, we used X-ray diffraction to characterize the three-dimensional structure of the C-terminal domain of FKBP25 (FKBP25C, residues P109 to D224, see Figure 1) complexed with rapamycin (**1**).

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                1                                                31
hFKBP12   .........G   VQVETISPGD   GRTFPKRGQT   CVVHYTGMLE
hFKBP25   EETLDEGPPK   YTKSVLKKGD   KTNFPKKGDV   VHCWYTGTLQ
          101                                                   140

          32    |----- 40s loop ----|   |-50s loop-|      64
hFKBP12   DGKKFDSS..   .....RDRNK   PFKFMLGKQE   VIRGWEEGVA
hFKBP25   DGTVFDTNIQ   TSAKKKKNAK   PLSFKVGVGK   VIRGWDEALL
          141                                               180

          65              |-------  80s loop -------|     103
hFKBP12   QMSVGQRAKL   TISPDYAYGA   TGHPGIIPP   HATLVFDVEL
hFKBP25   TMSKGEKARL   EIEPEWAYGK   KGQPDAKIPP   NAKLTFEVEL
          181                                               220

hFKBP12   LKLE*
hFKBP25   VDID*
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Figure 1. Alignment of amino acid sequences for C-domain of human FKBP25 and human FKBP12. The number above each row is the position of the nearest amino acid relative to the position in human FKBP12 while the number below each row is for human FKBP25. Critical loop regions are noted, and residues contacting rapamycin in the two different complexes are in bold.

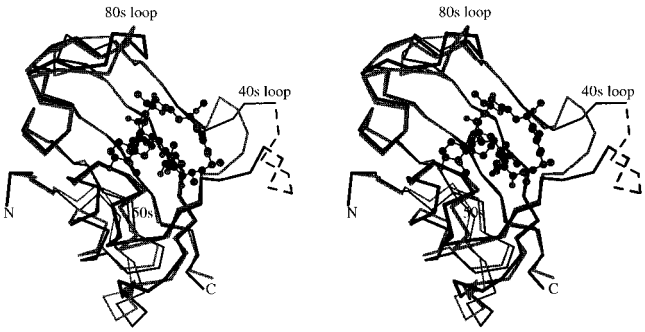
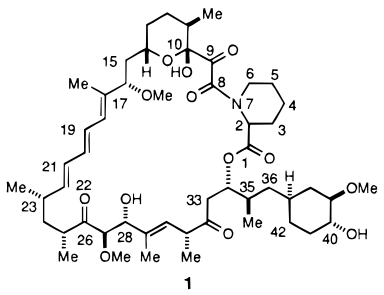


Figure 2. Superposition of the FKBP25 C domain−rapamycin complex and FKBP12 from the structure of the FKBP12−rapamycin complex. Critical loop regions are designated. The disordered region of FKBP25 is drawn as a dashed line.



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The overall structure of the C-terminal domain is very similar to that reported for FKBP12[7−9] and FKBP13[16] (Figure 2). Its predominant secondary structure is a curved β-sheet made from five antiparallel β-strands with β4 and β5 contributing to the binding pocket. A short turn of α-helix, which contributes a tryptophan to the binding pocket, is pressed against this β-sheet. Three loops also contribute to the binding pocket: the 40s loop, which is a bulge in β5, the 50s loop connecting β5 to the α-helix, and the 80s loop, which folds over the bound ligand. FKBP25C has changes in all of the crucial areas relative to FKBP12 and FKBP13.

The 40s loop, a seven-residue insertion in β5 in FKBP12, expands to 15 residues in FKBP25C (Figures 1 and 2). In FKBP25C, the β5 residues before and after the loop (V144 to T147 and L162 to K165) are well ordered and make eight antiparallel β-strand hydrogen bonds to β4. Residue N148 organizes the beginning and end of the loop through side chain to main chain hydrogen bonds (N148 Nϵ2 to K160 O and N148 Oϵ1 to L162 N). Rapamycin helps organize the same region

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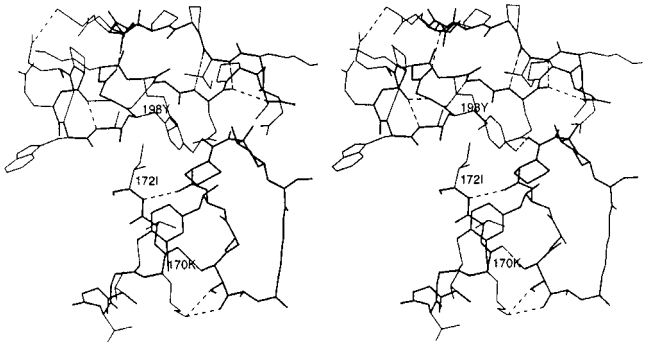

Figure 3. Stereoview of FKBP25C domain−rapamycin interactions involving the 50s loop and the 80s loop.

by contacting residues F145, D146, and L162. In FKBP25C, the 40s loop contains the KKKK motif of a possible nuclear localization signal.[21] The insertion at the tip of the loop in FKBP25C falls in a disordered section (T151 to N158) with no visible electron density (Figure 2). In FKBP12, the 40s loop is flexible in the absence of a ligand and orders upon ligand binding.[7,22] The results for FKBP25C−rapamycin suggest that the end of this longer loop is disordered even when ligand is bound. The pyranose ring and the C10-hemiketal represent an important region of rapamycin and FK506 that interacts with FKBPs. In both FKBP12−FK506 and FKBP12−rapamycin structures, D37 makes a hydrogen bond with the C10-hemiketal group, and the O−H···O^{δ} distance is close to 1.7 Å.[7,8] A triad salt bridge among the side chains of Y26, D37, and R42 observed in crystal structures of FKBP12 complexes could be important in organizing the 40s loop. Although Y26 and D37 have been conserved (Y135 and D146, respectively), N158 (comparing to R42 of FKBP12) has been completely displaced and is localized in the disordered region of FKBP25. The high mobility of the 40s loop affects the hydrogen bond between D146 and the C10-hemiketal group of rapamycin, which becomes less optimal than the corresponding orientations in the FKBP12 complex (the O−H···O^{δ} distance increases to 2.2 Å, and the O−H···O angle contracts from 177° to 105°). Therefore, this hydrogen bond appears to be weakened in the FKBP25C−rapamycin complex and presumably in the FKBP25−FK506 complex as well.

The 50s loop of FKBP12 (M49 to I56), which connects β5 to the α-helix, contains several important residues for ligand binding. V55 and I56 contribute to the hydrophobic binding pocket, and Q53, E54, and I56 form three hydrogen bonds with the bound rapamycin molecule through their main chain atoms. All these critical interactions are maintained in the structure of the FKBP25C−rapamycin complex (Figure 3). Interestingly, the substitution of E54 in FKBP12 to K170 in FKBP25 creates two more hydrogen bonds between FKBP25C and rapamycin. In FKBP12−rapamycin, the E54 side chain forms hydrogen bonds with main chain atoms.[9] However, the K170 side chain in FKBP25C takes a different direction and forms hydrogen bonds with the C26 carbonyl and C27 methoxy of rapamycin (distances between N−H^{ζ}···C26−O and N−H^{ζ}···C27−O are 2.08 and 2.09 Å, respectively) while its extended methylene chain provides a hydrophobic surface for C26 to C29 of rapamycin (Figure 3). In this way, K170/E54 substitution could increase the binding of rapamycin to FKBP25C. In contrast, there is no interaction between the residue K170 and FK506 when FK506 is docked into the binding pocket of FKBP25C by superimposing the common ligand binding domains of FK506 and rapamycin.

Changes in the 80s loop (E193 to K213) mainly involve residues near the one-residue, K207, insertion (Figure 1). The Q203 to K207 region adopts a 3_{10} helical conformation rather than a type II turn to accommodate K207 without disturbing the conformations of the flanking sequences (Figure 2). The pyranose ring of rapamycin binds in a hydrophobic cavity which is formed by residues I90, I91, H87, F36, Y82, and D37 (C^{β} only) in FKBP12. In FKBP25C, the cavity is formed by residues A206, I208, F145, Y198, and D146 (C^{β} only). The conformational change of the 80s loop in FKBP25 introduces A206 in place of I90, and the carbonyl oxygen of A206 makes a van der Waals contact (3.8 Å) with the C11 methyl of rapamycin, a residue known to contribute to ligand binding.[23] At the same time, the Q203/H87 change in FKBP25C abolishes all the interactions between the histidine residue and the pyranose ring.

The pipecolinyl ring of rapamycin and FK506 most deeply penetrates into FKBP12, fitting snugly inside a hydrophobic pocket formed by residues Y26, F46, V55, I56, I76, W59, and F99. The indole ring of W59 forms the bottom of the pocket while other residues provide a continuous hydrophobic wall. The ligand-binding pocket of FKBP25C is almost identical to that of FKBP12. However, the hydrophobic packing and interactions in the region could be adversely affected by a single-site substitution of F46 to L162 in FKBP25C. Leucine itself provides a smaller van der Waals surface than phenylalanine. In addition, differences in the 50s loop move V171 away by 0.4 Å from L162 compared to that of V55 relative to F46 in FKBP12. A pronounced gap appears between L162 and V171, but F164 moves up 1.5 Å, compared to the corresponding F48 in FKBP12, to partially close the gap. The packing between the pipecolinyl ring and the hydrophobic pocket is less good in FKBP25 than FKBP12.

In summary, this preliminary analysis suggests that changes in the 50s loop, the flexibility of the 40s loop insertion and its surrounding residues, and the substitution of one residue in the hydrophobic ligand-binding pocket could all be involved in the rapamycin selectivity of FKBP25C. For example, the L162/F46 substitution and the change between D146 of FKBP25 and D37 of FKBP12 may decrease the binding affinity of FKBP25C for both rapamycin and FK506, but these adverse effects could possibly be compensated in the FKBP25C−rapamycin interactions but not the FKBP25C−FK506 interactions by the beneficial effect of K170/E54 substitution. Both site-directed mutagenesis and ligand modifications will be needed to more fully understand this subtle molecular interaction. Such studies are underway and will be reported in due course.

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Supporting Information Available: Text giving experimental procedures for the crystallization, data collection, analysis, and refinement of FKBP25C−rapamycin complex (8 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, can be ordered from the ACS, and can be downloaded from the Internet; see any current masthead page for ordering information and Internet access instructions. The coordinates have been deposited with the Brookhaven Protein Data Bank with identification number 1PBK.

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