Structural Basis for Peptidomimicry by the Effector Element of Rapamycin

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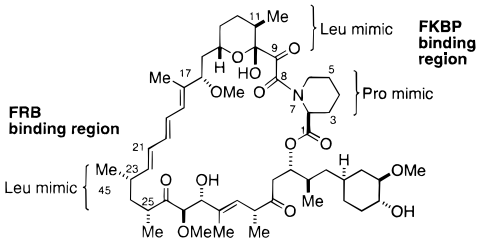
The remarkable natural product rapamycin (**1**) was first identified as an antifungal compound[1] and later shown to be a potent G1/S cell cycle arrest agent with potential therapeutic applications.[2] Rapamycin's unique mechanism of action is now understood in considerable detail. It forms a tight complex (Kd = 0.2 nM) with the cytoplasmic FK506 binding protein (FKBP12), and the rapamycin-FKBP12 complex in turn binds (Kd = 2 nM) to the FKBP-rapamcyin associated protein (FRAP).[2,3] The inhibition of FRAP by FKBP12-rapamycin blocks multiple downstream signaling pathways and leads to cell cycle arrest.[2] FRAP, a member of a rapidly growing class of kinases involved in cell cycle progression and checkpoints,[4]



has 2549 residues, and only a small 100-residue domain, the FKBP-rapamcyin binding (FRB) domain, interacts with rapamycin in the ternary FKBP12-rapamycin-FRAP complex.[5] Atomic resolution three-dimensional structures of FKBP12-rapamycin[6] and FKBP12-rapamycin-FRB[7] have revealed the structural basis for rapamycin's ability to bind both proteins.

An intriguing question for any natural product is the extent to which it mimics a peptidic ligand or substrate, and earlier work addressed the peptidomimicry of rapamycin and its closest relative FK506.[8,9] Rapamycin (**1**) has two distinct regions: one binds FKBP12, and the other, called the effector region, binds FRB. FKBP12 is a peptidyl-prolyl isomerase, and its kinetic preference for leu-pro suggests that the FKBP-binding portion of rapamcyin (**1**) is a leu-pro mimic.[8] More recently FKBP12 has been found to bind to a cytoplasmic domain of TGFβ receptors,[10,11] and binding requires a leu-pro sequence.[12] The effector region of rapamycin (**1**) binds FRB in a shallow hydrophobic pocket, and the degree of peptidomimicry of this region is unknown. FRB appears to bind an as yet unidentified ligand that regulates FRAP's kinase activity,[13] and rapamycin's effector region may resemble this ligand. An atomic resolution structural analysis of FRB has provided an interesting peptidomimetic analysis of the most deeply buried atoms of rapamycin.

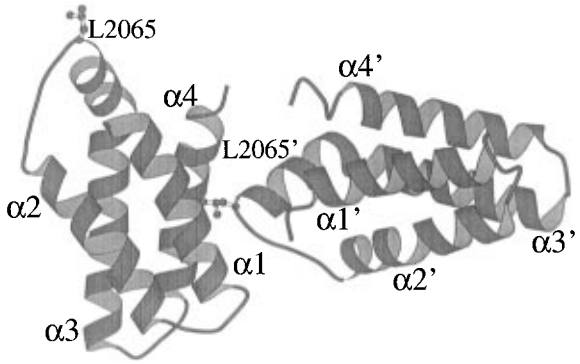


Figure 1. A ribbon diagram of the asymmetric unit of FRB. Note the four helix bundle motif of FRB, the side chain of Leu2065′, and the binding pocket formed by the crossing of helices α1 and α4.[19]

Single crystals of FRB were grown by vapor diffusion in space group $P2_12_12_1$ with $a = 29.94$, $b = 59.21$, $c = 123.54$ Å and two molecules ($Z = 8$) in the asymmetric unit. A set of 2.33 Å resolution intensities was collected at the F2 station of CHESS using 1.01 Å X-rays.[14] The structure was solved using molecular replacement techniques and refined using a simulated annealing procedure to a final *R*-factor of 22.4% with a free-*R*-factor of 34%.[14] A ribbon diagram of the final model is given in Figure 1.

FRB is an X-shaped four helical bundle with three underhand loops and approximate dimensions of 30 × 45 × 30 Å (Figure 1). Helices α1 and α2 are parallel as are helices α3 and α4, and the crossing angle between α1-α2 and α3-α4 pairs is 40°. The overall geometry of FRB in this structure is remarkably similar to that in the FRB-rapamycin-FKBP12 structure[7] with a mean deviation of only 0.61 Å for main-chain atoms and 1.11 Å for side-chain atoms. The greatest differences occur in the

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(14) Single crystals of FRB were grown at 4 °C by the hanging-drop vapor diffusion method using 10 mg/mL for FRB, pH 8.0, 50 mM Tris-EDTA buffer containing 20% (w/v) 2-MPD and 10% (w/v) PEG 4000 as precipitant. A crystal of 0.3 × 0.3 × 0.05 mm mounted in a nylon loop was used for data collection at 100 K. Data were collected using a Princeton 1k CCD detector at the F2 station of CHESS and were reduced using DENZO and SCALEPACK,[15] and R-sym was 3.2% for the 10 to 2.33 Å data with 1.70 redundancy and 72% completeness. A phasing model was found using molecular replacement as implemented in AMoRe[16] using the coordinates of FRB from ref 7 as a model. Since one of the unit cell lengths was shorter than the shortest dimension of the model, a 25 Å cutoff was applied to the rotation function search, and the FRB model omitted loops and side chains. After rigid body refinement and several cycles of positional refinement (X-PLOR),[17] loop regions could be traced and side chains were assigned. CHAIN[18] was used for model fitting and building the structure. Some N-terminal residues (6 and 7 for the two molecules) and one C-terminal residue were not fitted. The final model contains 66 waters, and has a final *R*-factor of 22.4% (10−2.33 Å, 7218 reflections with $2\sigma(F_o)$) and an *R*-free of 33.8%. The rms deviations from ideality are 0.016 Å for bond distances and 3.4° for bond angles. Coordinates have been deposited with the PDB with accession code 1aue.
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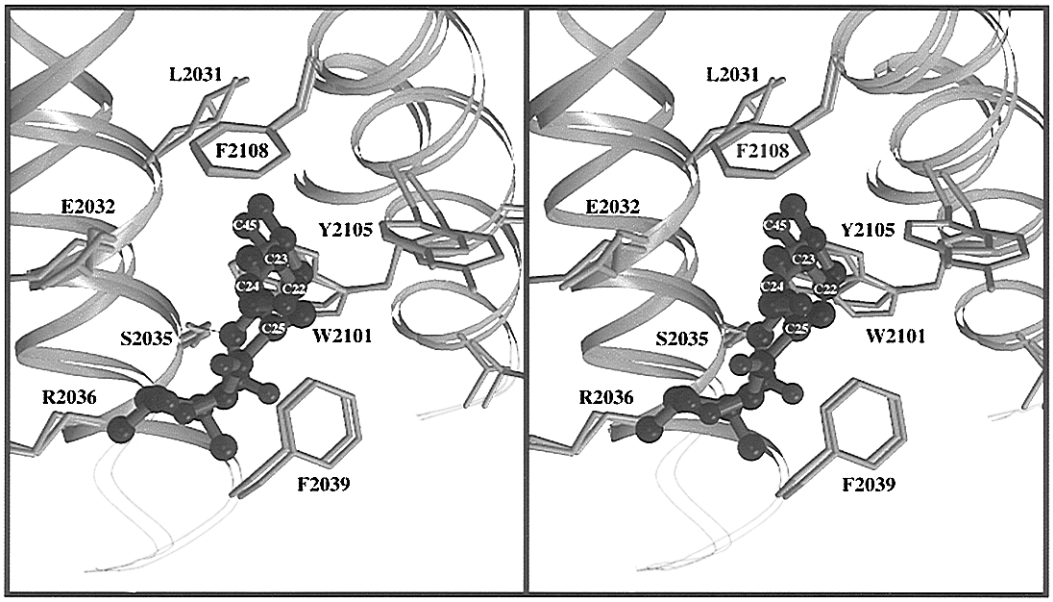

Figure 2. A stereoview of the binding pocket of FRB. Side chains contributing to the binding pocket are shown in light blue for the FKBP12-rapamycin-FRB complex and in light red for the FRB-FRB complex. Rapamycin from the ternary complex has black atoms and light blue bonds, and the selected atoms from FRB are shown in CPK colors with light red bonds.[19]

loop connecting α2 to α3 and the N-terminal portion of α3 (Figure 1) where mean differences between main-chain atoms for residues 2059 to 2065 are 1.39 Å. In the ternary complex, this region is exposed to solvent, while in the FRB structure, Leu2065 at the end of the loop interacts with a neighboring FRB (Figure 1).

Rapamycin binds to FRB in a shallow hydrophobic pocket at the crossing point of helices α1 and α4. The residues that most strongly interact (Tyr2105, Leu2031, Ser2035, Phe2039, Trp2101, and Phe2108) are hydrophobic, mostly aromatic, and conserved in the close homologs of FRAP such as RAFT, TOR1, and TOR2.[4,7] The atoms from rapamycin involved in bonding are primarily those at the end of the triene arm (C19, C20, C21, C22, C23, and C45, the CH_3 attached to C23, see **1**).[7] The C45 methyl is the most deeply buried and fits into a crease between Phe2108 and Leu2031 (Figure 2).[7]

While the original motivation for the structural analysis of unliganded FRB was assessing whether FRB's hydrophobic binding pocket partially collapses in the absence of ligand and rapamycin's effect on the conformation of FRB, the occupation of the binding pocket by the Leu2065 side chain indicates a strong preference for a filled binding pocket. The two crystallographically independent FRBs have their binding pockets filled by independent Leu2065 side chains in two slightly different manners. If the two FRBs that contribute the binding pocket are overlapped, the two FRBs that present Leu2065 side chains differ by a rotation about the buried side chain of 25°. The two presenting FRBs pivot about the Leu2065 side chain in a manner resembling a tiny ball and socket joint, and the pivoting motion has virtually no effect of the side chain atoms in the pocket [Figure 2]. The two independent binding modes result in slightly more solvent accessible surface area being buried in an FRB−FRB interaction than in a rapamycin-FRB interaction (960 Å^2 vs 805 Å^2). The Leu2065 side chain forms van der Waals contact with Leu2031, Tyr2105, and Phe2108, while the main chain NH forms a hydrogen bond (2.83 Å) with the hydroxyl of Ser2035—all residues that interact with rapamycin. In one of the two binding pockets, the side chain of Glu2032 forms hydrogen bonds with the amide NH of Lys2066 and the side-chain hydroxyl of Thr2064.

The most striking feature of the structure is the congruence between the deeply buried atoms of rapamycin and the side chain of Leu2065 (Figure 2). The atom pairs Cδ1−C45, Cδ2−C22, Cγ−C23, Cβ−C24, and Cα−C25 have a mean deviation of 0.8 Å if the two binding pockets are superimposed (Figure 2). In addition, C26 and C27 of rapamycin correspond to main-chain atoms of Thr2064 and Leu2065, while the carbonyl oxygen attached to C26 corresponds to the carbonyl oxygen of Thr2064 (Figure 2). This correspondence strongly suggests that a portion of rapamycin's effector domain mimics a leucine side chain. On this basis, we predict that the endogenous ligand(s) of FRB will use a leucine residue as an important binding element.

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Supporting Information Available: Tables of supporting X-ray data (4 pages). See any current masthead page for ordering and Internet access instructions.

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