STRUCTURAL ANALYSIS OF RAPAMYCIN'S ROLE IN BINDING FKBP12 AND FRAP

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The immunosuppressive and cell cycle arrest agent rapamycin works by binding together two proteins: the FK506 binding protein (FKBP12) and the FKBP-rapamycin associated protein (FRAP). A 2.7Å resolution crystal structure of the triple complex of human FK506 binding protein (FKBP12), rapamycin, and FKBP12-rapamycin binding domain (FRB) of FRAP, reveals two proteins bound together through rapamycin's ability to simultaneously occupy two different hydrophobic binding pockets. FKBP12 has a hydrophobic binding pocket between a large β-sheet and a short amphipathic α-helix. FRB is a four-helix bundle, and the crossing region between two antiparallel helices, α1 and α4, forms a hydrophobic binding pocket. The structure shows extensive interactions between rapamycin and both proteins but only modest interactions between proteins. The structure also provides, among other things, an understanding of the critical nature of the Ser^{2035} residues, and the first structural information about the growing family of proteins related to the ataxia telangiectasia mutant (ATM) gene product.

Crystals of the triple complex form in space group $P2_12_12_1$ with a=44.63, b=52.14, and c=102.53 Å and one FKBP12-rapamycin-FRB complex in the asymmetric unit. Data were collected to 2.7 Å with a rotating anode source. A phasing model was found using MR (FKBP12) and SIRAS ($HgCl_2$ derivative). The current model has R=19.3% (R_{free}=29.9%) for the 6206 reflections between 8 and 2.7 Å and good geometry (r.m.s. deviations of 0.008 Å for bond lengths and 1.48° for bond angles) for a model with 1639 protein atoms, 65 rapamycin atoms, and 23 solvent atoms--not counting hydrogens.