Atomic Structure of the Immunophiln FKBP13–FK506 Complex: Insights into the Composite Binding Surface for Calcineurin

L. Wayne Schultz,† Patrick K. Martin,‡ Jun Liang,† Stuart L. Schreiber,*,‡ and Jon Clardy*,†

Department of Chemistry, Baker Laboratory Cornell University, Ithaca, New York 14853-1301
Department of Chemistry, Harvard University Cambridge, Massachusetts 02138

Received November 19, 1993

Understanding the interactions of natural products with their macromolecular targets is an important new area for chemistry, and the immunosuppressant–immunophilin system epitomizes both the vigor and complexity of the venture.[1] The potent immunosuppressant FK506 binds tightly (K_d = 0.4 nM) to the cytosolic 12-kDa protein FKBP12.[2,3] The FKBP12–FK506 complex—but neither molecule alone—blocks the signal from the T cell receptor by inhibiting the phosphatase calcineurin (K_i = 7.9 nM).[4] Defining the molecular features of FKBP12–FK506 that bind to calcineurin, the composite binding surface, is an important but unsolved problem. Analysis of a homologous protein complex, FKBP13–FK506, has provided significant insights into the nature of the composite binding surface. FKBP13 also forms a tight complex with FK506 (K_d = 55 nM).[5] The two proteins are remarkably similar (43% amino acid identity), and the 92-amino acid C-terminal sequence of FKBP13 has 46 identical and 20 related residues when compared with FKBP12.[5] The two proteins show exact identity for all amino acids lining the FK506 binding pocket.[5] However, embedded in this overall similarity are differences that result in a composite binding surface for FKBP13–FK506 that interacts only weakly with calcineurin (K_i = 1500 ± 400 nM), and loop regions near the binding site are primarily responsible for these differences.[6,7] We have determined the three-dimensional structure of FKBP13–FK506 by high-resolution (2.0 Å) X-ray diffraction techniques to define the architecture of FKBP13 and to identify, through a comparison of FKBP13–FK506 with FKBP12–FK506, prominent features of the composite binding surface.

The overall structure of FKBP13–FK506 is very similar to FKBP12–FK506 with a four-stranded β sheet wrapped around a short seven-residue α helix (Figures 1 and 2).[8–12] The 20 N-terminal amino acids are disordered and do not appear in electron density maps. It is possible that these residues form an additional β strand, although none of these residues contribute

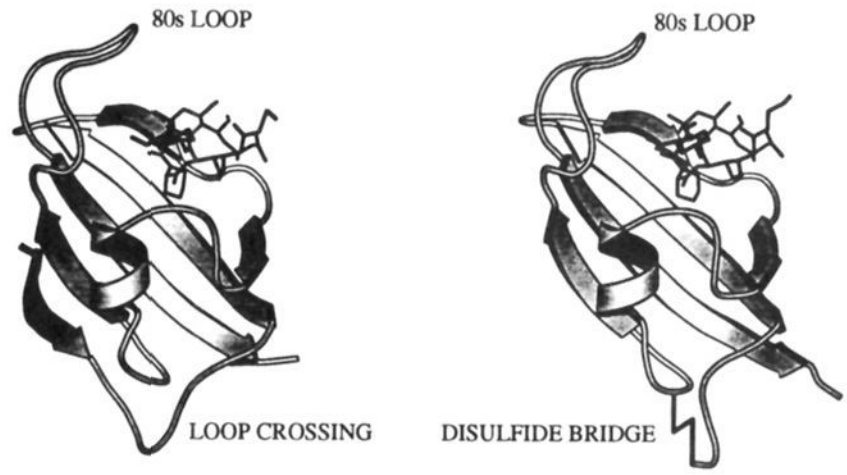

Figure 1. Ribbon diagrams of FKBP12 (left) and FKBP13 (right) with stick models of FK506. Both drawings were prepared from the coordinates of high-resolution structures defined by X-ray diffraction.

to the hydrophobic core of FKBP12 and they probably contribute little to the overall conformation of the complex.[10] The most striking difference between the two proteins is a loop-crossing region. In FKBP12, residues Ser67 to Gln70, from the α helix to the second β strand, form the inner loop, and residues Asp11 to Thr14, following the first β strand, form the outer loop (Figure 1).[10] Such loop crossings between antiparallel β strands are scarce, presumably due to the difficulty of satisfying hydrogen bonds when burying a nonregular main chain protein segment in a hydrophobic protein interior.[13] FKBP12 and FKBP13 solve this dilemma in entirely different ways. FKBP12 uses a series of inter- and intrastrand hydrogen bonds.[10] FKBP13 uses a disulfide link from Cys20 to Cys75 (Figure 1).[5] The strong electron density of the disulfide linkage suggests that it is a tether point for the disordered N-terminal sequence. The disulfide bond in FKBP13 is consistent with its localization in the oxidizing milieu of the lumen of the endoplasmic reticulum (ER).[5] Cytosolic proteins do not contain disulfide bonds due to the reducing nature of their medium.

An overlay of the binding domains of FKBP12–FK506 and FKBP13–FK506 is presented in Figure 3. The conserved aromatic residues lining the binding pocket (Tyr26, Phe36, Trp59, and Phe99) and FK506 are virtually superimposable. The overall root mean square deviation between FKBP12– and FKBP13–FK506 for all FK506 atoms and all FKBP atoms within 3.5 Å of FK506 is 0.55 Å. The somewhat poorer binding of FK506 with FKBP13 does not find a simple explanation in the structure and is presumably due to the cumulative effect of many small distortions.

The poorer calcineurin inhibition is due to changes in the protein structure. A series of mutagenesis experiments have identified two residues in FKBP12 at the end of the 80s loop, Gly89 and Ile90 (corresponding to Pro97 and Lys98 in FKBP13), as important for calcineurin binding.[6,7] Changing these two residues, the P89G/K90I mutant of FKBP13, resulted in a chimeric FKBP–

† Cornell University.
‡ Harvard University.

(1) Rosen, M. K.; Schreiber, S. L. *Angew. Chem., Int. Ed. Engl.* **1992**, *31*, 384–400.
(2) Siekierka, J. J.; Hung, S. H. Y.; Poe, M.; Lin, C. S.; Sigal, N. H. *Nature* **1989**, *341*, 755–757.
(3) Harding, M. W.; Galat, A.; Uehling, D. E.; Schreiber, S. L. *Nature* **1989**, *341*, 758–760.
(4) Liu, J.; Farmer, J. D.; Lane, W. S.; Friedman, J.; Weissman, I.; Schreiber, S. L. *Cell* **1991**, *66*, 807–815.
(5) Jin, Y.-J.; Albers, M. W.; Lane, W. S.; Bierer, B. E.; Schreiber, S. L.; Burakoff, S. J. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 6677–6681.
(6) Yang, D.; Rosen, M. K.; Schreiber, S. L. *J. Am. Chem. Soc.* **1993**, *115*, 819–820.
(7) Rosen, M. K.; Yang, D.; Martin, P. K.; Schreiber, S. L. *J. Am. Chem. Soc.* **1993**, *115*, 821–822.
(8) VanDuyne, G. D.; Standaert, R. F.; Karplus, P. A.; Schreiber, S. L.; Clardy, J. *Science* **1991**, *251*, 839–842.
(9) VanDuyne, G. D.; Standaert, R. F.; Schreiber, S. L.; Clardy, J. *J. Am. Chem. Soc.* **1991**, *113*, 7433–7434.
(10) VanDuyne, G. D.; Standaert R. F.; Karplus, P. A.; Schreiber, S. L.; Clardy, J. *J. Mol. Biol.* **1993**, *229*, 105–124.
(11) Michnick, S. W.; Rosen, M. K.; Wandless, T. J.; Karplus, M.; Schreiber, S. L. *Science* **1991**, *251*, 836–839.
(12) Moore, J. A.; Peattie, D. A.; Fitzgibbon, M. J.; Thomson, J. A. *Nature* **1991**, *351*, 248–250.
(13) Ptitsyn, D. B.; Finkelstein, A. V. *Q. Rev. Biophys.* **1980**, *13*, 339–368.
(14) Single crystals of the complex were obtained after three generations of macroseeding using the hanging-drop method and belong to space group $P2_12_12$ with cell constants a = 74.26(1) Å, b = 74.06(1) Å, and c = 39.24(3) Å with two FKBP13–FK506 complexes in the asymmetric unit. Data were collected from a crystal with dimensions 0.2 × 0.08 × 1.1 mm³ with a SDMS Mark II detector and graphite-monochromated Cu Kα radiation. The structure was solved by molecular replacement using X-PLOR 3.1.[15] The coordinates of FKBP12 from the FKBP12–FK506 complex were used as the model.[8] Alternating cycles of simulated annealing and conventional least-squares refinement in X-PLOR were followed by manual adjustments in CHAIN[16] to refine the model. The current model includes the two FKBP13–FK506 complexes (residues 20–120) and 112 water molecules and has an R factor of 0.176 for the data from 8.0 to 2.0 Å ($I/\sigma > 2.0$). The R_{free} for a 10% test set is 0.289.[15] The root mean square deviations of bond lengths, bond angles, and dihedral angles (planarity and chirality) from their ideal values are 0.016 Å, 3.4°, and 1.5°, respectively. The root mean square deviations between the two complexes are 1.04 Å for all atoms and 0.30 Å for Cα atoms (Figure 2).
(15) Brünger, A. T.; Kuriyan, J.; Karplus, M. *Science* **1987**, *235*, 458–460.
(16) Sack, J. S. J. *Mol. Graphics* **1988**, *6*, 244.

0002-7863/94/1516-3129$04.50/0

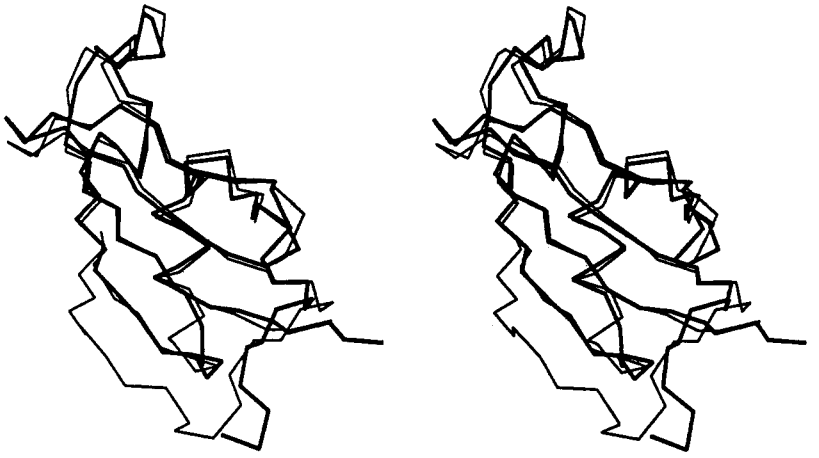

Figure 2. A stereoview of the Cα overlay of the FKBP13 (heavy line) and FKBP12 (light line) complexes. The root mean square deviation for the Cα atoms (12–107 in FKBP12 or 20–120 in FKBP13) of FKBP12–FK506 and FKBP13–FK506 is 1.34 Å.

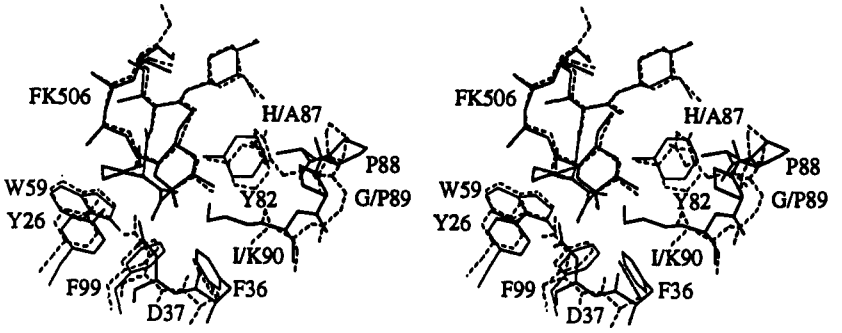

Figure 3. A stereoview of the FKBP–FK506 binding region. The FKBP13–FK506 structure is shown with solid lines, and the FKBP12–FK506 structure, with dashed lines. FKBP12 numbering is used.

FK506 complex that inhibited calcineurin almost as well as FKBP12–FK506 (K_i = 13 ± 1 nM).[7] In FKBP12, Ile90 contributes to a hydrophobic groove created by Phe36 and the hemiketal ring of FK506 (right-hand portion of Figure 3). In the structure of the FKBP13–FK506 complex, a lysine projects out from the 80s loop and covers this groove, thereby completely disrupting the hydrophobic pocket (Figure 3).

Understanding how the small molecule, FK506, and two large molecules, FKBP12 and calcineurin, form a mutually compatible binding surface where each of the three pairwise interactions is satisfied represents a formidable challenge, but continued structural investigations of members of the FKBP family of proteins, both natural and unnatural, will provide valuable insights.

Acknowledgment. Work was supported by NIH Grants CA 59021 (J.C.) and GM38627 (S.L.S.). L.W.S. and P.K.M. were supported by an NIH Biotechnology Training Grant and an NIH Postdoctoral Fellowship, respectively.

Supplementary Material Available: The X-ray crystallographic parameters for FKBP13–FK506 have been deposited with the Protein Data Bank.[14]