Structural Basis for Peptidomimicry by a Natural Product

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When an exogenous and nonpeptidal natural product is found to influence a biological system, it is logical to ask if the molecule is mimicking an endogenous, possibly even peptidal substance. For example, morphine has long been suspected of mimicking the enkephalin peptides.[1] However, no structural evidence has yet been gathered to support this view. We now provide evidence that the natural product FK506 uses nonpeptidic structural elements to bind to its intracellular receptor FKBP12[2] in a way that closely resembles the binding of a peptide–FK506 hybrid to the same receptor. The possibility that FK506 (Figure 1A) and rapamycin mimic peptide or protein ligands to, or substrates of, FKBP12 was suggested earlier on the basis of several observations.[4] The pyranose rings,[5] α-keto amide functions,[6] and homoprolyl moieties of the natural products show structural similarities to the transition-state structures for *cis–trans* isomerization of leucyl–prolyl and valyl–prolyl substrates, which are optimal rotamase substrates for FKBP12.[5,7] Even more striking is the pair of hydrogen bonds between FKBP12 and FK506 that has been proposed to mimic the antiparallel strand interactions commonly found between peptides and peptide-binding proteins (Figure 1B).[8] A more subtle variation of this mimicry has been noted in the structure of the FKBP12–rapamycin complex.[4,9] However, this early view created a stereochemical puzzle—the "side chain" substituent of FK506 (R in Figure 1B) has nonnatural stereochemistry in comparison to a natural peptide (R′ in Figure 1C).

In order to gain new insights into the binding of natural products and peptides to immunophilin receptors, we have analyzed the interactions of a cyclic peptide–FK506 hybrid with FKBP12 by X-ray crystallography. The current studies focused on three newFKBP12 ligands, compounds **1–3** (Figure 2). We developed these ligands around the α-keto homoprolyl amide moiety found in FK506 and rapamycin, as this element appears to be a critical binding determinant.[10] As a result, we anticipated that this group would serve as an anchor point for an attached peptide. A variety of amino acids were first fused to the dicarbonyl moiety and then homologated sequentially to the carboxyl end of the homoprolyl moiety, optimizing for binding to FKBP12 at each stage. Finally, tethers of variable lengths were introduced via a macrocyclization protocol.[11] The affinities of **1–3** for FKBP12, which were determined by the compounds' abilities to inhibit the rotamase

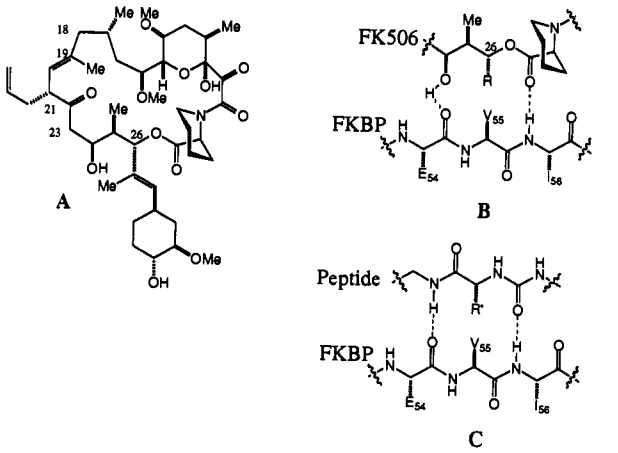

Figure 1. (A) Structure of FK506. (B) FKBP12–FK506 substructure. (C) FKBP12–peptide substructure (model).

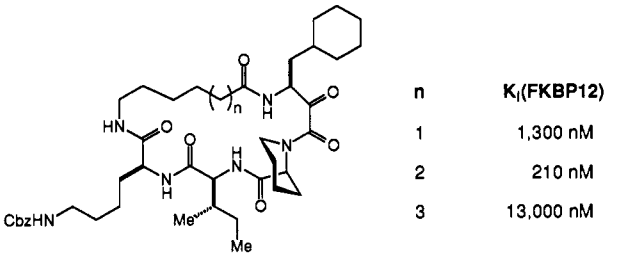

Figure 2. Structure of cyclic peptide–FK506 hybrids **1–3**, and their inhibitory constants (K_i) for FKBP12 rotamase inhibition.

activity of FKBP12,[12] are seen to be dependent upon the length of the tether (Figure 2). Not surprisingly, they are also considerably lower than the affinity of FK506, which makes many additional contacts to the protein. We chose the highest affinity ligand, **2**, for further structural analysis.

The X-ray crystallographic studies are described in Figure 3. The overall protein topology of the FKBP12–**2** complex is essentially identical to that observed in the FKBP12–FK506 complex: the protein folds as a five-stranded antiparallel β-sheet with a right-handed twist wrapping around a short α-helix (Figure 3A).[8] Two complexes exist in the asymmetric unit. The positions of the main-chain atoms in the two complexes do not differ significantly (root mean square deviation of 0.67 Å), nor do they differ significantly from those of the FKBP12–FK506 complex(0.54 and 0.74 Å).[8] The ligand binds in the hydrophobic pocket between the β-sheet and the α-helix. The conformations of the two ligands in the asymmetric unit are similar and differ only in the orientation of the Cbz fragment.

The structure reveals the anticipated pair of hydrogen bonds between the main-chain NH of Ile56 and the homoprolyl C=O of the ligand (**2**) and between the main-chain C=O of Glu54 and the lysine NH of **2** (Figure 3B). Thus, a dipeptide fragment of **2** binds to FKBP12 by forming a pair of hydrogen bonds characteristic of antiparallel β-sheets. However, an unanticipated feature of the binding of FK506 to FKBP12 is revealed by examining the superposition of a portion of the two ligands in this region (cf. Figure 3B; see also Figure 4A,B). The trisubstituted olefin at C26 of FK506, which was earlier considered to be the "side chain" substituent (cf. R in Figure 1B), adopts a nearly identical orientation and occupies a nearly identical region of space with the isoleucine amide of **2**. In addition, the C24 and C25 ring atoms and the C25 methyl of FK506, which were earlier considered to be part of the FK506 "main chain", adopt a nearly

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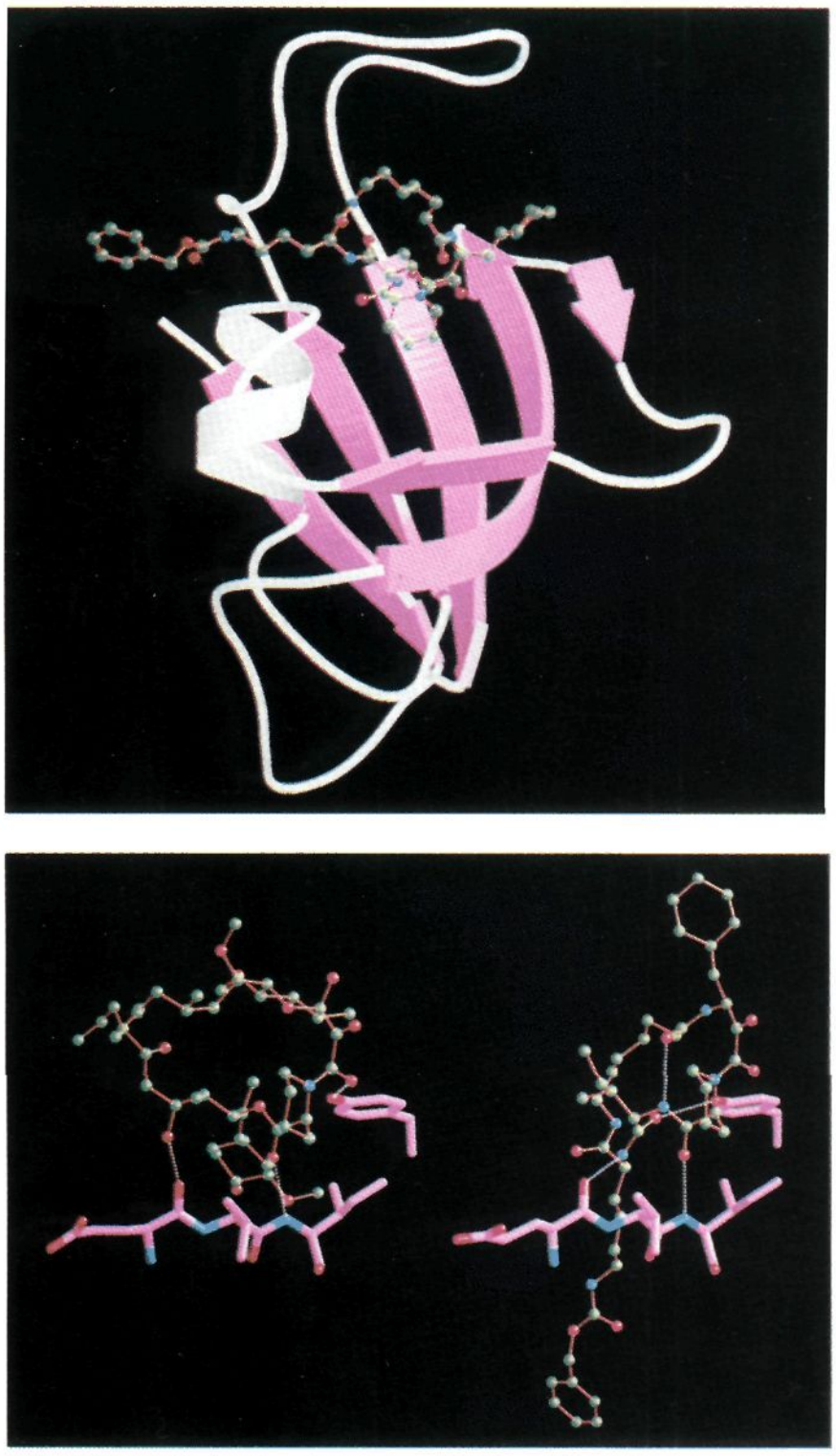

Figure 3. (A, top) Richardson ribbon diagramm of the FKBP12–**2** complex. (B, bottom) Comparison of the contact surfaces of FKBP12–FK506 (left) and FKBP12–**2** (right). Dashed lines indicate hydrogen bonds between the receptor and its ligands. (Experimental procedures and summary of statistics are provided in the supplementary material; the coordinates will be deposited in the Brookhaven Protein Data Bank.)

identical orientation and occupy a nearly identical region of space with the isoleucine side chain of **2** (Figure 3B). The structure therefore suggests that the trisubstituted olefin of FK506 is an isosteric amide replacement and its ring atoms are a mimic of an amino acid (e.g., isoleucine) side chain. Reversing the roles of these two groups in FK506 resolves the stereochemical puzzle referred to earlier, since the stereochemistry at C26 now corresponds to the natural *S*-stereochemistry of an amino acid (Figure 4A,B).

The phenolic hydrogen of Tyr82 forms a hydrogen bond to the isoleucine C=O of **2** (cf. Figures 3B and 4B). In the FK506 complex, this same phenolic hydrogen forms a hydrogen bond to the amide carbonyl of the dicarbonyl unit (cf. Figures 3B and 4B). Of course, the methyl group of FK506's C26 trisubstituted olefin cannot accept a hydrogen bond. An intramolecular hydrogen bond between the linker carbonyl and the isoleucine NH of **2** suggests that peptides might bind to FKBP12 with a β-turn, as shown in Figure 4B. This structure is remarkably

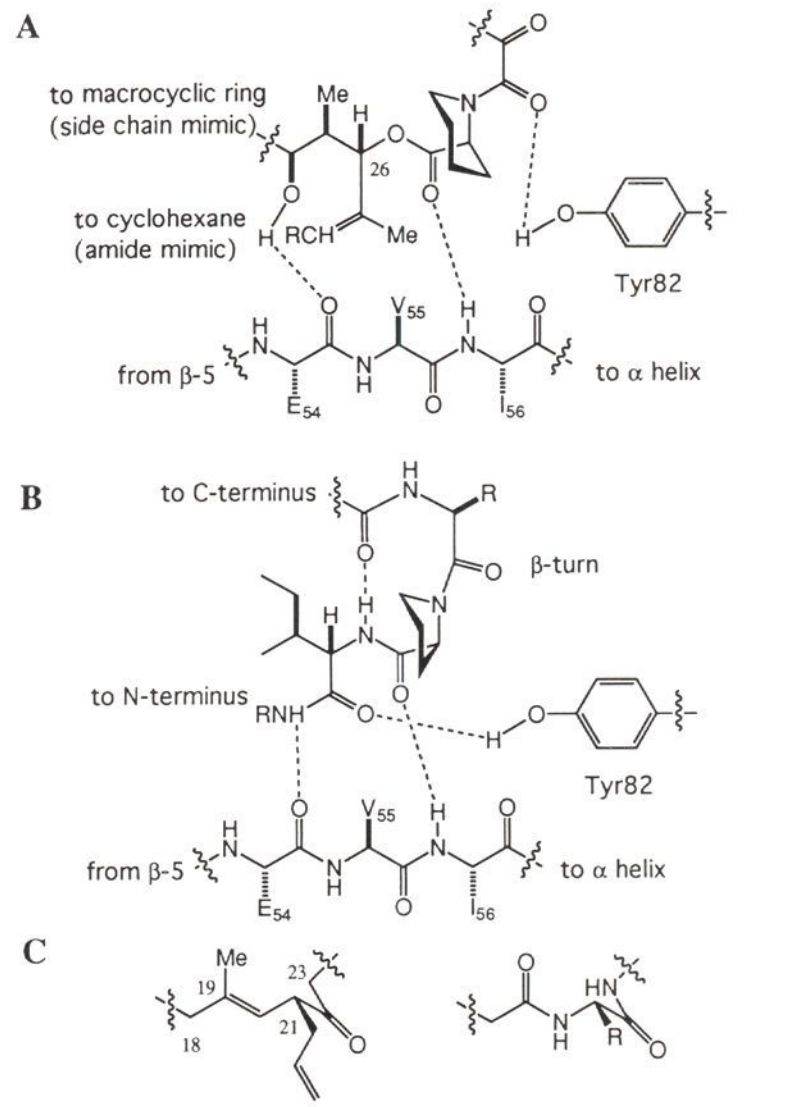

Figure 4. (A) Schematic of peptidomimicry exhibited by FK506. (B) Model of a peptide bound to FKBP12. (C) Hypothetical peptidomimicry of the calcineurin-binding region of FK506.

similar to that predicted earlier by Karplus and co-workers in the course of their modeling studies.[13]

FK506 makes extensive contact with FKBP12. This study provides evidence that much of this contact mimics that of the peptide moiety of a peptide hybrid. But FK506, like CsA and rapamycin, is unusual in that it contains a second protein-binding surface, which includes the C18–C23 fragment shown in Figure 4C (that protein is calcineurin in the cases of FKBP12–FK506 and cyclophilin–CsA[14] and FRAP in the case of FKBP12–rapamycin[15]). We speculate, on the basis of the finding that FK506 uses a trisubstituted double bond to mimic a trisubstituted amide unit of a peptide, that this region of the FK506 "effector element" may also function as a peptide mimic when bound to calcineurin (note resemblance to the dipeptide fragment in Figure 4C).

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Supplementary Material Available: Addendum to the caption for Figure 3, describing experimental procedures and crystal data (2 pages). This material is contained in many libraries on microfiche, immediately follows the article in the microfilm version of the journals, and can be ordered from the ACS; see any current masthead page for ordering information.

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