Template-based docking of a prolactin receptor proline-rich motif octapeptide to FKBP12: Implications for cytokine receptor signaling

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Abstract

A conserved proline-rich motif (PRM) in the cytoplasmic domain of cytokine receptors has been suggested to be a signaling switch regulated by the action of the FK506 binding protein (FKBP) family of peptidylprolyl isomerases (O'Neal KD, Yu-Lee LY, Shearer WT, 1995, *Ann NY Acad Sci* 766:282–284). We have docked the prolactin receptor PRM (Ile¹-Phe²-Pro³-Pro⁴-Val⁵-Pro⁶-Gly⁷-Pro⁸) to the ligand binding site of FKBP12. The procedure involved conformational search restricted by NMR restraints (O'Neal KD et al., 1996, *Biochem J* 315:833–844), energy minimization of the octapeptide conformers so obtained, template-based docking of a selected conformer to FKBP12, and energy refinement of the resulting complex. The template used was the crystal structure of a cyclic FK506-peptide hybrid bound to FKBP12. Val⁵-Pro⁶ of the PRM was taken to be the biologically relevant Xaa-Pro bond. The docked conformer is stabilized by two intramolecular hydrogen bonds, N⁷H⁷ → O⁴ and N²H² → O⁸, and two intermolecular ones, Ile⁵⁶:N-H → O=C:Pro⁶ and Tyr₈₂:O-H → O=C:Gly⁷. This conformer features a Type I β-turn and has extensive hydrophobic contacts with the FKBP12 binding surface. The observed interactions support the hypothesis that FKBP12 catalyzes *cis-trans* isomerization in the PRM when it is part of the longer cytoplasmic domain of a cytokine receptor, and suggest a significant role for the PRM in signal transduction.

Keywords: FK506; FK506 binding protein; immunosuppressant; molecular modeling; prolactin receptor; proline-rich motif; rapamycin; signaling

The prolactin receptor, a one-pass transmembrane protein, is a member of the cytokine/hematopoietin superfamily of receptors regulating the growth and differentiation of hematopoietic cells (Bazan, 1989). The earliest events in PRL-R activation are ligand binding to the extracellular domain, receptor dimerization by an unknown mechanism, and subsequent activation of the JAK-STAT signaling cascade. Although the detailed conformation of the ligand-bound extracellular domain of the growth hormone receptor is known from its X-ray crystal structure (De Vos et al., 1992; Somers et al., 1994), little is known about the structure of the intracellular domain and how it functions in signal transduction. The focus of the present work is an eight-residue segment of the cytoplasmic domain designated Box 1 for some cytokine receptors (Murakami et al., 1991) or generalized to the proline-rich motif that defines a

consensus sequence for all members of the cytokine-hematopoietin superfamily (O'Neal & Yu-Lee, 1993). Although a molecular mechanism for the functionally required PRM has not yet been elucidated, it may serve as a binding site for JAK2 (LebRun et al., 1994) or interact with an immunophilin, such as FKBP (O'Neal et al., 1994, 1996). The immunosuppressant drugs FK506 and rapamycin interrupt signaling by the T-cell receptor and cytokine receptor, respectively. The first step in this inhibition is the formation of a tight binary complex of the drug with the cytosolic peptidyl prolyl isomerase, FKBP12,¹ a member of one of three classes of enzymes that isomerize Xaa-Pro peptide bonds (where Xaa = any amino acid) (for a recent review, see Kay, 1996). Although it is thought that these drugs act by mimicking the nat-

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Abbreviations: PRM, proline-rich motif; PRL-R, prolactin receptor; FKBP12, protein of 12 kDa that binds FK506; Xaaⁿ, denotes amino acid in PRM with sequence position *n*; Xaa_n, denotes amino acid in FKBP12 with sequence position *n*.

¹Binding of FKBP12 to the cytoplasmic region of TGF-β type 1 receptor (TβR-1) has been described recently by Wang et al. (1994) and Okadame et al. (1996). This interaction apparently does not involve a proline-rich motif, but rather a membrane-proximal, glycine/serine-rich domain, because a deletion mutant of this membrane does not bind FKBP12. However, there is a conserved proline three residues downstream of the GS domain (Wrana et al., 1994), which FKBP12 may isomerize to facilitate phosphorylation.

ural peptidyl ligands of FKBP (Albers et al., 1990), no such endogenous proteins that could act as ligands have been identified. Results of mutational studies and sequence comparisons have suggested that, in the case of cytokine receptors, the cellular target of FKBP is the PRM, and that rotamase-catalyzed *cis-trans* isomerization of an Xaa-Pro bond in it could act as a switch for receptor activation-inactivation (O'Neal et al., 1995). In PRL-R, the PRM has the sequence Ile¹-Phe²-Pro³-Pro⁴-Val⁵-Pro⁶-Gly⁷-Pro⁸. The abundance of proline residues, with their restricted conformational space and unique ability to exist in *cis* or *trans* configuration, makes the PRM a likely target for rotamases such as FKBP12.

Proline is a unique amino acid in that parts of its backbone and side chain constitute a pyrrolidine ring system. This makes the *cis* and *trans* forms of Xaa-Pro peptide bonds nearly equal in energy, unlike dipeptides without Pro, where the *trans* form is clearly favored over the *cis* (Drakenberg & Forsen, 1971). Another important property of proline is that the ring system restricts the allowed backbone torsion ϕ to a narrow range around -70° (Ramachandran & Sasisekharan, 1968). A consequence of this restriction and the absence of an imide proton is that Pro occurs much more frequently in β turns (Lewis et al., 1973) than in helices or sheets. These conformational properties of prolyl residues are important considerations for the work presented here.

We have recently described the conformational dynamics of the synthetic PRM peptide as determined by NMR and CD spectroscopy and HPLC (O'Neal et al., 1996). A fundamental assumption of that study was that the behavior of the simple octapeptide in water can give clues to its behavior in the much more complex PRL-R in vivo. The PRM peptide contains four prolines in its sequence, and hence 2^4 *cis-trans* isomers about Xaa-Pro bonds are possible. All 16 forms were found to exist in aqueous solution, and their relative concentrations could be estimated. The all-*trans* isomer was suggested to be one of the two isomers functionally present in a biologically relevant form of the receptor. Further evidence suggested that Val⁵-Pro⁶ is the peptide bond most likely to undergo isomerization, and hence to function as the on/off switch of the receptor.

In the present study, we describe the results of a conformational search of the PRM octapeptide based on NMR data (O'Neal et al., 1996) and the docking of a low-energy conformer so obtained to FKBP12. Our objectives were: (1) to arrive at energetically plausible conformations for the PRM in its all-*trans* configuration, and classify them into groups; (2) to select those conformations of greatest structural similarity to that of a known peptide-like ligand of FKBP12 so as to identify potential PRM conformations that might fit snugly in the protein's ligand binding site; (3) to dock the best PRM conformers so identified to FKBP12; and (4) to analyze the intermolecular interactions in the docked "complex" from the point of view of receptor activation and molecular recognition. A probable structure would be useful for understanding rotamase action on the isolated PRM and on the PRL-R. The template used in the present study is a cyclic peptide-FK506 hybrid complexed to FKBP12 (Ikeda et al., 1994). The hybrid was designed by converting the FKBP-binding half of FK506 into a peptide backbone that closely resembles the parent molecule (Fig. 1). The goal was to test the hypothesis that immunosuppressants act by mimicking a natural peptide ligand of FKBP12. Indeed, the hybrid was found to bind FKBP12, although with a low affinity (Ikeda et al., 1994). We consider the X-ray structure of the hybrid bound to FKBP12 to be the best available system for the simulation of peptide binding to FKBP12. Our results described below show that the PRM pep-

Fig. 1. Covalent structures and atom matches of the PRM octapeptide and the FK506-peptide hybrid used as template. **A:** The PRM Ile-Phe-Pro-Pro-Val-Pro-Gly-Pro with the end groups used in the calculations. **B:** The hybrid showing atom naming as in crystal structure (Ikeda et al., 1994). Fragments of the PRM peptide and hybrid are redrawn below each to show atom to atom correspondence between them used in docking.

tide can indeed assume conformations that will fit into the FKBP12 active site in an energetically favorable fashion. We also compare our results with the stabilizing intermolecular FKBP12-peptide

interactions and the intrapeptide interactions found by the Karplus group in a simulation study of the rotamase action of FKBP12 on a peptide ligand (Fischer et al., 1993) and those found in the FKBP12-hybrid complex (Ikeda et al., 1994).

Results and discussion

PRM minima

The ranges and intervals of ϕ and ψ used in the conformational search were determined in part by NMR coupling constants; they define a total of >11 million conformations. The entire search was performed in two separate streams, each containing one-half of the total number of starting conformations. Group 1 was characterized by negative ϕ values at Gly⁷, and Group 2 by positive values (Table 1). The end-to-end distance restriction of ≤ 6.0 Å suggested by previous NMR data reduced this vast number to 3,951 conformers in Group 1 and 6,885 in Group 2. The results are summarized in Table 2. These 10,836 conformations were treated as starting points for energy minimization. Prior to minimization, the correct side chains of the PRM peptide (IFPPVPGP) were introduced in extended conformations at all α carbons of the (Gly)₈ octapeptide used in the conformational search, without affecting the backbone. Many of the resulting conformers did not minimize because of unresolvable steric clashes. Group 1 yielded 987 minima and Group 2 gave 1,000. Both sets were sorted based on energy and saved for further analysis. Interestingly, the internal energies of these two groups of conformations were very similar: the lowest and highest were 289.6 and 309.6 kcal/mol for Group 1, and 290.3 and 309.0 kcal/mol for Group 2.

In order to gain insight into the conformational variation represented by this large number of minima, we examined their backbone torsion angles. Because of the obvious conformational difference between the two groups, they are analyzed separately (Fig. 2). The distribution of ϕ for Phe² shows two bands around the starting ϕ values of -153° and -87° (Fig. 2A,C, panels F), and two bands around starting values of -152° and -88° (Fig. 2A,C, panels V), as expected. The ϕ of Gly⁷, Group 1 (Fig. 2A, panel G) shows similar distribution around -167° and -73° . However, in Group 2, ϕ Gly⁷ (Fig. 2C, panel G) shows distribution only around the higher of the original two values of 34° and 86° . Apparently, the conformations starting with $\phi = 34^\circ$ did not lead to any acceptable minimum. The prolyl ϕ s in both Figure 2A and C display a tight distribution around -70° , as expected (Ramachandran & Sasisekharan, 1968).

Table 1. Ranges of backbone torsion angles ($^\circ$) used in the conformational search of PRM peptide derived from NMR data

Residue	ϕ	ψ Range	ψ Increment
Phe 2	$-153, -87$	-90 to 180	30
Pro 3	-75	-90 to 180	30
Pro 4	-75	-90 to 180	30
Val 5	$-152, -88$	-90 to 180	30
Pro 6	-75	-90 to 180	30
Gly 7	$-167, -73,^a 34, 86^b$	-150 to 180	30

^aGroup 1.

^bGroup 2.

Table 2. Summary of results from conformational search and molecular mechanics of the octapeptide

Property	Group 1	Group 2
Conformational search		
Starting ϕ for Gly ⁷ ($^\circ$)	-167.0 and -73.0	$+34.0$ and $+86.0$
Total possible conformers	5,668,704	5,668,704
C $\alpha_1 \cdots C\alpha_8$ limit (Å)	≤ 6.0	≤ 6.0
Number starting minimization	3,951	6,885
Energy minimization		
Number of minima saved	987	1,000
Lowest energy (kcal/mol)	289.6	290.3
Highest energy (kcal/mol)	309.6	308.0
ϕ_{Gly^7} of lowest ($^\circ$)	-180	118
Superimposing and docking		
Best RMS with hybrid (Å)	0.48	0.99
Energy rank order within group	826	838
Energy of best RMS (kcal/mol)	308.28	307.03
Highest RMS with hybrid (Å)	2.05	2.01

The distribution of ψ values shows much greater variation (Fig. 2B,D), in accordance with the greater freedom this torsion angle was permitted during the conformational search (Table 1). Although ψ was varied uniformly at 30° intervals from -90° to $+180^\circ$ (from -150° for Gly⁷), clustering is observed around some but not all of these values. This reflects the intrinsic dependence of ψ on ϕ for sterically allowed conformations, and the effect of the end-to-end distance restriction in our conformational search. The peptide torsion angle ω in these 1,987 conformations is distributed tightly around *trans* = 180° (not shown), attesting to good planarity for all of the peptide bonds.

When the two sets of minima were superimposed on the bound conformation of the hybrid, the RMS deviations varied from 0.48 Å to 2.05 Å within Group 1, and from 0.99 Å to 2.01 Å within Group 2. The lowest value of 0.48 Å was for conformation #826 from Group 1. Indeed, as the rank order indicates, it has a high internal energy of 308.28 kcal/mol. These two lowest RMS conformers are shown in stereo in Figure 3. Interestingly, the peptide conformer with the lowest RMS deviation from the X-ray structure of the related FKBP-peptide complex is one of the least preferred solution conformations, suggesting that FKBP induces a conformation of PRM competent for binding. The Group 1 #826 conformer is characterized by two internal hydrogen bonds (see Table 4 for details). Of the two, the N⁷H⁷-O⁴ hydrogen bond and the backbone torsion angles at residues Val⁵ and Pro⁶ (Table 3) are characteristic of a Type I β -turn. The second hydrogen bond from Phe²-NH to Pro⁸-CO seems to hold the ends of the conformer together in a quasi-cyclic shape. This feature may have functional implications, as described later. The other low RMS conformer shown (Group 2, #838) is also characterized by an internal hydrogen bond: Val⁵-NH to Phe²-CO. This hydrogen bond and the values of ϕ and ψ at residues Pro³ and Pro⁴ (Table 3) are characteristic of a Type I β turn (Lewis et al., 1973).

The docked complex

The conformer #826 from Group 1 was chosen for further analysis. This conformer was used to replace the peptide-FK506 hybrid in

Fig. 2. Backbone torsion angles ϕ and ψ for the PRM minima. Along the abscissa is the conformer number (ranked in increasing order of energy) and along the ordinate is the value of the torsion angle. **A,B:** ϕ , ψ for Group 1. **C,D:** ϕ , ψ for Group 2. Single-letter amino acid code is used in each panel to indicate the residue that corresponds to it.

Fig. 3. Stereo view of the lowest-energy conformation from each of the two groups of PRM minima. **A:** Group 1, #826. **B:** Group 2, #838. Interatomic distances for Pro 4-C=O...H-N-Gly 7 and Pro 8-C=O...H-N-Phe 2 of conformer #828, and for Phe 2-C=O...H-N-Val 5 of conformer #838 are somewhat greater than for corresponding interactions in the docked conformer.

the active site of FKBP12, and the resulting complex was energy minimized as described in Materials and methods. At the end of the minimization, the average absolute derivative was 0.0664 and the RMS derivative was 0.1, demonstrating a well-minimized structure. The docked complex is shown in Figure 4. The protein is shown as a green ribbon, with the catalytic site cavity at top left. The PRM

octapeptide is shown as a CPK model. At the top left corner of the PRM is the Ile¹ side chain, and its oxygen atom is clearly visible as a red sphere. At the bottom part of the PRM, the Phe² ring is seen. At the right hand edge of PRM are the Val⁵ (top) and Pro⁶ (bottom) residues. In this view, residues 1–5 form the front of the PRM, behind which residues 6–8 are partially hidden.

Table 3. Backbone torsion angles (°) for the Group 1 and Group 2 minima best matching the hybrid, and for the Group 1 minimum after docking to FKBP12

Residue	Best RMS of Group 1				Best RMS of Group 2		Bound hybrid (X ray)	
	(#826, RMS = 0.48 Å)		After docking		(#838, RMS = 0.99 Å)			
	ϕ	ψ	ϕ	ψ	ϕ	ψ	ϕ	ψ
Ile 1	...	161	...	135	...	158		
Phe 2	-82	140	-79	124	-88	137		
Pro 3	-67	-26	-53	-56	-64	-46		
Pro 4	-88	160	-68	152	-82	-1		
Val 5	-51	-41	-60	-36	-146	145	26	-107
Pro 6	-70	-10	-72	-9	-78	165	-127	10
Gly 7	-80	145	-69	155	108	148	-74	148
Pro 8	-71	...	-68	...	-71	...	-70	130

^aNumbers shown in bold are characteristic of torsion angles of residues in type I β -turns.

Fig. 4. Best fit for the PRM peptide (conformer #826, Group 1) in the active site of FKBP12 obtained by docking. A backbone ribbon is shown for the protein, whereas for PRM, all atoms are shown (CPK). C, green; O, red; N, blue; H, white.

Fig. 5. FKBP12-PRM putative complex in the same orientation as in Figure 4, showing hydrophobic interactions. The α -carbon trace of FKBP12 and the aromatic side chains Tyr₂₆, Phe₃₆, Phe₄₆, Trp₅₉, Tyr₈₂, Phe₉₉ lining the binding pocket are shown.

Fig. 6. Intra- and intermolecular hydrogen bonding networks in the FKBP12-PRM complex. The PRM (thick stick representation) has the same coloring scheme as in Figure 4; interacting FKBP12 residues are shown with thin sticks. See Figure 4 legend and Table 4 for further details.

The hydrophobic component

The α -carbon trace of FKBP12 is shown in Figure 5, with the side chains of aromatic residues lining the binding site in green and the PRM (heavy atoms only) shown in white. Stick representation is used for the aromatics and the PRM. The orientation in this figure is the same as in Figure 4. FKBP12 has a very hydrophobic ligand binding site and its interactions with hydrophobic parts of ligands such as FK506 and rapamycin stabilize those complexes (Van Duyn et al., 1993). This property is also true for the putative FKBP12-PRM complex (Fig. 5). When folded in a β -turn, PRM fits snugly into the binding site of the enzyme, and the side chains of Pro⁴, Val⁵, and Pro⁶ are located well inside the FKBP12 hydrophobic pocket, very near several aromatic rings, especially those of Tyr₂₆, Tyr₈₂, Phe₃₆, Phe₄₆, Phe₉₉, and Trp₅₉. This mutual burial of hydrophobic groups would make a significant favorable contribution to the $\Delta G_{\text{binding}}$. If the Trp₅₉ ring system is viewed as forming a flat bottom of the FKBP12 binding pocket, PRM Pro⁶ sits directly over it. Even the PRM Phe² ring, which is just outside the pocket, is within <4 Å of Phe₄₆ (ring C–ring C distance). These results indicate that PRM is capable of binding to FKBP12 in an orientation similar to that suggested by our previous studies (O'Neal et al., 1996).

β -Turns and hydrogen bonding

In addition to these hydrophobic contacts, the PRM forms a number of intramolecular hydrogen bonds within itself and intermolecular bonds with FKBP12. These interactions, which would contribute to the stability of the conformation of PRM and of the complex, are illustrated in Figure 6. The intra- and intermolecular hydrogen bonds are indicated by green dashed lines. The overall shape of the PRM in this docked conformation can be described as a hairpin. The segment Pro⁴–Val⁵–Pro⁶–Gly⁷ forms a classical Type I β turn with ϕ , ψ of -60° , -36° for Val and -72° , -9° for Pro, the middle two residues (Table 3), very close to the ideal values of -60° , -30° and -90° , 0° for that type of turn (Venkatachalam, 1968). The 4–7 turn also contains its characteristic hydrogen bond Gly⁷–NH $\rightarrow$ O=C–Pro⁴. The hydrogen bond distances (H $\cdots$ O) and other details related to the PRM conformation and the FKBP12–PRM complex are given in Table 4 and described below.

Intramolecular hydrogen bonds

A conventional description for backbone hydrogen bonds is given in terms of the number of atoms in the "ring" closed by the NH $\cdots$ O bond. In that notation, the β -turn hydrogen bond of the type Gly⁷–NH $\cdots$ O=C–Pro⁴ closes a 10-atom ring. An equivalent of this hydrogen bond is present in the hybrid (Ikeda et al., 1994): N¹H¹ $\rightarrow$ O⁴ (Fig. 1B). Because of the different covalent structure of the hybrid, the hydrogen bond closes an 11-membered ring, rather than the 10-membered ring characteristic of β -turns. In the docked conformation, the PRM also displays a second internal hydrogen bond: Phe²–NH $\rightarrow$ O=C–Pro⁸, which has no reported counterpart in the simulation by Fischer et al. (1993), or in the hybrid crystal structure by Ikeda et al. (1994). This interaction presumably helps to stabilize the PRM in its docked, quasi-cyclic conformation. Thus, the two internal hydrogen bonds observed in the PRM conformer before docking are retained in the docked conformation.

Table 4. Interatomic interactions found in the docked FKBP12–PRM complex (Group 1, #826, RMS = 0.48 Å)

Donor	Acceptor	Hydrogen bond distance (Å)	Atoms in ring	Remark
A. Intramolecular interactions				
Gly 7–NH	Pro 4=O	2.00	10	a,b
Phe 2–NH	Pro 8=O	1.99	23	c
B. Intermolecular interactions				
Ile 56–NH	Pro 6=O	2.01		a,b
Tyr 82–OH	Gly 7=O	1.92		a,b
Pro 8–N ^d	Glu 54=O	No hydrogen bond		a,b

^aPresent in hybrid–FKBP12 complex (Ikeda et al., 1994).

^bFound by Fischer et al. (1993) in the peptide–FKBP12 system.

^cNot reported by others.

^dNo donor proton (Pro).

Intermolecular hydrogen bonds

The PRM peptide is capable of forming two hydrogen bonds with FKBP12: Pro⁶–C=O to Ile₅₆–NH and Gly⁷–C=O to Tyr₈₂ side-chain OH (Table 4; Fig. 6). Both of these are anticipated, based on their presence in the structure of the FKBP12–hybrid complex (Ikeda et al., 1994) and in the simulation (Fischer et al., 1993). A third intermolecular hydrogen bond (acceptor: Glu⁵⁴–C=O) has been reported in the simulation study (Fischer et al., 1993) (donor: NH of the C-terminal amide group) and in the crystal structure analysis of the hybrid complex (Ikeda et al., 1994) (donor: NH of the modified lysine residue). The equivalent of these donor residues in the PRM would be Pro⁸, which lacks an amide hydrogen. It must be noted, however, that the relevant short segments of the protein and the ligand backbones run nearly perpendicular to each other in both the FKBP12–hybrid and the FKBP12–PRM complexes. If the position were occupied by a non-proline amino acid, this residue might be involved in the expected hydrogen bond interaction with FKBP12, possibly leading to an even tighter complex. Although the docked PRM does not show a hydrogen bond to Pro⁸, it is significant that this residue is highly conserved in cytokine receptors.

Other docking modes

The results described so far have been interpreted within the context of the possibility that *cis*–*trans* isomerization of the Val⁵–Pro⁶ peptide bond acts as a conformational switch for the cytokine receptors. However, we also consider two other possibilities. For *cis*–*trans* isomerization of short proline-containing peptides by FKBP12, it has been reported that the Xaa–Pro sequence (where Xaa = Phe) is isomerized efficiently by the rotamase (Harrison & Stein, 1990b). Thus, there is the possibility that Phe²–Pro³ of the PRM might be the peptide unit that functions as the proposed molecular switch.

The same procedure as described in Materials and methods for superimposing the PRM minima on the hybrid was followed, but using an alignment that places the ring N of Pro³ on the homopropyl N of the FK506–peptide hybrid (a frame shift of three residues compared to the previous overlaying scheme in Fig. 1B). As in the

previous case, the lowest RMS conformer was docked to FKBP12 following an identical procedure. Docking this conformer of PRM in the active site generated a less favorable fit. This was evidenced by the calculated protein PRM interaction energy of 15.7 kcal/mol, compared with -15.2 kcal/mol for the docking experiment using Pro⁶. A significant fraction of this energy came from the repulsive van der Waals interaction of $+17.9$ kcal/mol, compared with the stabilizing energy of -12.0 kcal/mol in the previous case. Thus, the Phe²-Pro³ binding mode appears to be much less favorable than the Val⁵-Pro⁶ mode. Furthermore, Pro³ is not absolutely conserved in the PRM consensus sequence (O'Neal & Yu-Lee, 1993).

Similarly, we also considered the possibility that rapamycin bound to FKBP12 might be a good template for modeling peptide binding, especially because the X-ray structure of the complex is known (Van Duyne et al., 1993) and because rapamycin inhibits the signal transduction via cytokine receptors. The best overlays of the PRM minima on bound rapamycin were not as good as with the hybrid template, judging from the RMS values. Moreover, when PRM was docked with FKBP12 using rapamycin as the template, the FKBP12-PRM interaction energies were less stabilizing than those reported above with the FKBP12-hybrid template.

It is clear from the results presented that the PRM octapeptide has one or more low-energy conformations, consistent with NMR data, that can fit snugly into the active site of FKBP12. This supports our hypothesis that the PRM, when it is part of the intracellular domain of the PRL-R or other members of the cytokine receptor superfamily, is a feasible target for FKBP12. But, in order for this binding to act as a conformational switch, turning signaling on/off, it is necessary for the peptide to undergo catalyzed isomerization from *trans* to *cis* configuration. This possibility is now considered in light of our results.

There are three factors that help lower the free energy barrier of FKBP12's rotamase action by preferential transition-state binding through nonbonded interactions (Harrison & Stein, 1990a; Fischer et al., 1993; Orozco et al., 1993). The bound conformation of the PRM obtained above provides evidence that the process of autocatalysis is possible when Pro⁶ is the target residue. Substrate autocatalysis takes place when the transition state of the peptide is stabilized by an intramolecular hydrogen bond between the amide NH of the residue following the target Pro and the lone electron pair of the imide N of the target Pro. This interaction occurs in our final docked model (Gly⁷ N-H...N-Pro⁶ = 2.29 Å). Because substrate autocatalysis contributes significantly to the reduction of transition-state energy barrier, and the residue following Pro³ in the PRM happens to be another proline that lacks the imide hydrogen, this structural evidence implies that the Pro³ is a less attractive substrate than Pro⁶ for *cis-trans* isomerization. Second, the PRM conformation, in relation to the structure of the binding pocket of FKBP12, also indicates that imide desolvation is feasible. By burying the imide carbonyl of Pro⁶ within the hydrophobic residues of FKBP12, hydrogen bonding to this oxygen atom cannot occur. Hydration of this C=O group raises the C-N π bond overlap, resulting in an elevated *trans-cis* energy barrier, and a significant lowering of the isomerization rate. Because the docking was done with this peptide in the *trans* configuration (which represents the product of the reaction), certain interactions are expected to be less favorable than if the peptide were *cis*. Thus, the structural and energetic data presented here strongly favor the possibility that the PRM peptide is capable of binding at the active site of FKBP12 and undergoing *cis-trans* isomerization.

Materials and methods

PRM peptide

The covalent structure of the PRM octapeptide used in the calculations is shown in Figure 1. Its sequence, Ile¹-Phe²-Pro³-Pro⁴-Val⁵-Pro⁶-Gly⁷-Pro⁸, corresponding to residues 267-274 of human PRL-R (O'Neal et al., 1995), consists entirely of nonpolar residues, but is remarkably water soluble (>23 mg/mL). The N-terminal amino group is protonated and the C-terminus is capped as a neutral amide. The molecule has a charge of $+1.0$. The choice of end groups makes the peptide correspond exactly to the one used in the NMR study (O'Neal et al., 1996).

FKBP12

The structure of the rotamase/immunophilin FKBP12 has been determined by X-ray crystallography and by NMR in the free form and complexed with various ligands (Michnick et al., 1991; Van Duyne et al., 1991, 1993). The atomic coordinates of the FKBP12 structure we have used are those of its complex with a cyclic FK506-peptide hybrid determined at 2.0 Å resolution by Ikeda et al. (1994), and were generously supplied by these authors. In our calculations, Asp and Glu side chains were assigned a net charge of -1.0 each, and the side chains of Arg and Lys, $+1.0$ each; partial charges following the AMBER scheme were assigned (Weiner et al., 1984, 1986). The net charge on FKBP12 was $+1.0$.

Conformational search and energy minimization

The allowed backbone conformational space of an octapeptide (described by the ranges of the torsion angles ϕ , ψ , and ω) is enormous. Sampling these angles for eight residues at 30° intervals would involve generating and analyzing on the order of 10^{26} mathematically possible conformations. The goal of the conformational search was to eliminate most of these possibilities, and to identify a much smaller number of conformations for further analysis. Our conformational search involved the use of NMR data obtained in earlier studies (O'Neal et al., 1996) and a hard sphere approach (Ramachandran & Sasisekharan, 1968). In order to obtain an inclusive set of starting PRM conformers and to reduce the possibility of ruling out important conformations due to steric clashes involving side-chain atoms, the conformational search was conducted on (Gly)₈. The all-*trans* configuration was used, because it has been suggested to be a biologically relevant form of the PRM peptide (O'Neal et al., 1996). Backbone conformational space was restricted as follows. The available $^3J_{\text{NH-C}^\alpha\text{H}^\beta}$ coupling constants measured in water (O'Neal et al., 1996) for residues Phe², Val⁵, and Gly⁷ were converted to ϕ backbone angles and used to restrict the conformations of the corresponding residues (Table 1). The ϕ of Pro (residues 3, 4, 6, and 8) was fixed at -75° . The ψ angle, for which no NMR constraints were available, were varied over the entire allowed range at 30° increments for all the residues (Ramachandran & Sasisekharan, 1968). Gly⁷ has four different allowed values for ϕ , whereas the other residues have only two each; also, its allowed range for ψ is larger than those for the other residues (Table 1). Thus, these values are a direct consequence of the higher conformational flexibility allowed for Gly due to its lack of a side chain. NMR data from a ROESY experiment indicated an Ile¹...Pro⁸ through space interaction in the PRM peptide (M.V. Chari & H. Williams, unpubl. results). Thus, in the conformational search, the C $^\alpha$ ₁...C $^\alpha$ ₈ distance was restricted to ≤ 6.0 Å.

The *Search_Compare* program of the Biosym modeling software version 9.50 (Molecular Simulations, Inc., San Diego, California) was employed. It generates conformations restricted by distance and torsion angle ranges and increments, and examines them for steric clashes. The sterically allowed conformations of (Gly)₈ obtained in the above step were then converted to corresponding conformations of the PRM sequence IFPPVPGP by attaching L-residue side chains in extended conformation, without altering the backbone structure. These conformations were then subjected to geometry optimization in the CVFF force field (Dauber-Osguthorpe et al., 1988) using the *Discover* program (Molecular Simulations, Inc.). Cross terms of CVFF were not turned on. No cutoffs were used for nonbonded or electrostatic interactions. For each conformation, 50 steps of steepest descent minimization were run. No distance or torsion constraints were imposed during minimization. Thus, in theory, the minima need not satisfy the original NMR-derived restraints. However, during a total of 50 steps, the starting structure is not likely to drift substantially.

Docking

The conformations that successfully completed minimization were sorted based on energy and used for docking. First, hydrogen atoms were added to the FKBP12 structure, which was then energy minimized (100 steps of steepest descent). The force field employed was the all-atom form of *AMBER*, developed by Kollman and coworkers (Weiner et al., 1984, 1986). The total potential energy was calculated as the sum of (1) penalties for deviations of bond lengths, angles, and dihedral angles from their respective ideal values, (2) nonbonded and electrostatic energies, and (3) the nonelectrostatic contribution to hydrogen bond energy. Solvent was not included explicitly in our calculations; instead, a distance-dependent dielectric ($\epsilon = r_{ij}$, where ϵ is the dielectric constant for the electrostatic interaction of atoms i and j separated by a distance r_{ij}) was employed in the electrostatic term above (Weiner et al., 1984). This term accounts for the presence of polar water indirectly, and prevents the formation of spurious electrostatic contacts. The PRM minima were then superimposed on the FK506-peptide hybrid bound in the catalytic site of FKBP12. The covalent structures of the PRM (upper panel) and the hybrid (lower panel) and their superimposed segments are shown in Figure 1. Atoms in corresponding positions of the two molecules are indicated by the stick diagrams. The 17 hybrid atoms shown in this figure correspond to a peptide backbone, and include atoms important for interaction with FKBP12 in the complex (Ikeda et al., 1994). The backbone nitrogen (N6) of Pro⁶ in PRM corresponds to the ring nitrogen (N7) of the homoproline in the hybrid. RMS deviations between each PRM minimum and the hybrid were calculated. Docking the PRM structure thus obtained with FKBP12 generated an FKBP12-PRM "complex." The total energy of this system (sum of the internal energies of protein and ligand plus their interaction energy) was minimized successively by Steepest Descent and Conjugate Gradient minimizers (*Discover*) until the final maximum derivative of energy was below 1.0. During minimization, no distance restraints were in effect either on FKBP12 or the PRM.

A crucial step in the procedure described above is the selection of the starting point for docking. We used two criteria for selecting the starting PRM conformer: consistency with available NMR data (O'Neal et al., 1996) and relatively low energy. In addition, to increase the likelihood of leading to a good docked fit under our controlled minimization procedure, it was important to start not far

from a stable minimum. This was ensured by selecting starting conformations that were close (i.e., low in RMS) to that of the peptide half of the bound hybrid. This procedure of considering a large number of minima as candidates for docking is an indirect way of exploring the conformational flexibility of the bound PRM. An alternative approach would be to consider a single starting point—an obvious one being the conformation of the bound hybrid—and to explore conformational space by long and CPU-intensive molecular dynamics simulations.

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