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Statistical allosteric coupling to the active site indole ring flip equilibria in the FK506-binding domain

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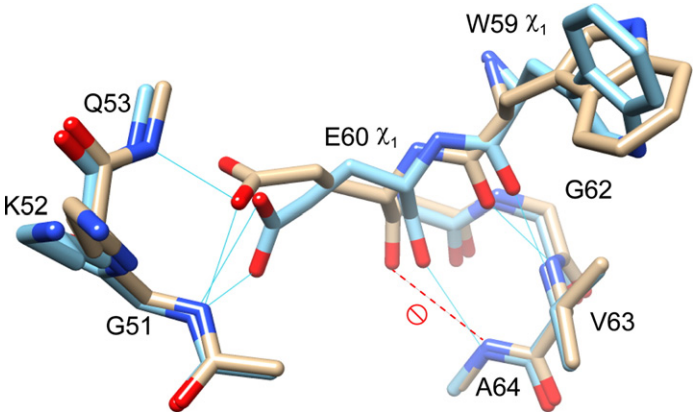
HIGHLIGHTS

- Unlike FKBP12, the active site Trp 90 ring of FKBP51 and FKBP52 does not flip.
- The V101I substitution decreases the indole ring flipping in FKBP12 by 10-fold.
- Transitions of the Trp 59 and Glu 60 sidechains are strongly correlated.
- 1.14 μs of CHARMM27 simulation indicates no concerted transitions for these residues.
- Allosteric analysis of statistically coupled but dynamically uncoupled transitions

GRAPHICAL ABSTRACT



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ABSTRACT

In solution, the Trp 59 indole ring at the base of the active site cleft in the FKBP domain protein FKBP12 is rotated by ~90° at a population level of 20%, relative to its canonical crystallographic orientation. NMR measurements on the homologous FK1 domains of human FKBP51 and FKBP52 indicate no observable indole ring flip conformation, while the V101I variant of FKBP12 decreases the population having a perpendicular indole orientation by 10-fold. A set of three parallel 400 ns CHARMM27 molecular simulations for both wild type FKBP12 and the V101I variant examined how this ring flip might be energetically coupled to a transition of the Glu 60 sidechain which interacts with the backbone of the 50's loop located ~12 Å from the indole nitrogen. Analysis of the transition matrix for the local dynamics of the Glu 60 sidechain, the Trp 59 sidechain, and of the structurally interposed α-helix hydrogen bonding pattern yielded a statistical allosteric coupling of 10 kJ/mol with negligible concerted dynamical coupling for the transitions of the two sidechains.

1. Introduction

The FK506-binding domain protein FKBP12 is best known for its role in mediating the immunosuppressive effects of FK506 and rapamycin. Among the fourteen FKBP domain proteins in the human genome [1], FKBP12.6 and the first FKBP domain of FKBP51 and FKBP52 exhibit the closest sequence homology to FKBP12 and they are believed to provide

Abbreviations: PDB, protein data bank; FKBP, FK506 binding protein; NOESY, nuclear Overhauser enhancement spectroscopy.

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the largest functional overlap [2–5], although the significance of that overlap remains open to debate [6]. While each of these FKBP domains retains the cis–trans isomerization activity on model prolyl peptides found in their eubacterial homologues, genetic and cell biology studies indicate that the primary physiological roles for FKBP12 and FKBP12.6 as well as for FKBP51 and FKBP52 involve protein recognition interactions that contribute to the regulation of various signaling pathways, most notably the FKBP12/FKBP12.6 regulation of ryanodine receptor calcium channels [7–10] and the FKBP51/FKBP52 regulation of steroid receptor complexes [11–14]. Given the current challenges of structurally characterizing the regulatory conformational transitions within these large complexes, useful insights may be derived from a more detailed understanding of the energetically accessible conformational transitions of the isolated FKBP domains which may facilitate coupling to the larger scale transitions of the complex.

The long β_4–β_5 loop (or 80's loop) is known to provide critical protein recognition interactions for various signaling functions of FKBP12 [15–18] in addition to its role in mediating the regulatory interactions of FKBP51 and FKBP52 with the steroid receptor [19]. In characterizing the structural basis for two distinct conformational transitions of FKBP12 centered in this loop which give rise to NMR resonance doubling (τ of 3.0 s at 43 °C [20]) and conformational exchange line broadening (t ~ 120 μs at 20 °C [21–23]), respectively, we reported the crystal structure of the G89P variant [24].

Surprisingly, although the C^{α} atoms of Trp 59 and Gly 89 are separated by 21 Å, the indole ring in the G89P structure is rotated ~90° with respect to its canonical position in the crystal structure of the wild type FKBP12 (Graphical abstract), resulting in the occlusion of most of the active site cleft. This sidechain transition, involving a χ_1 torsional rotation from the gauche$^-$ to trans rotamer and a smaller shift in the χ_2 torsion angle, had previously been reported in the 0.94 Å resolution X-ray structure for the E60Q variant of FKBP12 by Saven and colleagues [25]. Both the G89P and E60Q crystal structures also exhibit an ~1 Å shift in the backbone of the central turn of the α-helix which enables the amide of Ala 64 to form a canonical hydrogen bond to the carbonyl oxygen of residue 60. In contrast to wild type FKBP12, the crystal structures of the highly homologous FKBP12.6 [26,27] exhibit an undistorted α-helix with canonical hydrogen bonding geometry between Glu 60 and Ala 64. This shift of the α-helix in FKBP12.6 has been proposed to be sterically accommodated due to its smaller Phe 59 sidechain at the base of the active site [28].

The E60Q substitution partially disrupts the hydrogen bonding interactions that occur between wild type Glu 60 sidechain and the backbone amides of the 50's loop in the wild type structure. In the E60Q structure, a flip of the (ψ_{52}, ϕ_{53}) torsion angles brings the carbonyl oxygen of Lys 52 into a hydrogen bond with the sidechain amide of Gln 60. In a 12 ns CHARMM27 molecular simulation analysis by Park and Saven [29], they found that the Trp 59 indole ring in wild type FKBP12 flipped to a perpendicular orientation after ~8 ns. Although the absence of a return transition precluded any conclusions about the predicted equilibrium for the indole ring flip, their molecular dynamics study suggested the energetic accessibility of this transition.

In contrast to the crystal structure of the E60Q variant, the backbone geometry in the 50's loop is not disrupted in the G89P structure. The sidechain carboxylate of Glu 60 in the G89P structure maintains hydrogen bonding interactions with the backbone amides of the 50's loop, but in contrast to the gauche$^-$ χ_1 rotamer seen in the crystal structure of wild type FKBP12, Glu 60 adopts a trans χ_1 rotamer conformation in the G89P structure. This extended sidechain conformation enables the backbone of Glu 60 to shift toward the indole ring allowing for the formation of a canonical α-helical hydrogen bonding geometry between its carbonyl oxygen and the amide of Ala 64.

Following up on our initial observation that the indole H^N resonance of Trp 59 in wild type FKBP12 exhibits NOE crosspeaks to methyl resonances that are incompatible with the canonical crystal structure, we carried out quantitative NOE buildup analysis to show that the crosspeak pattern is consistent with a 20% population of the rotated indole conformation [24]. In the present study, we wished to establish whether this indole ring reorientation is suppressed in the FK1 domains of FKBP51 and FKBP52 since the marked alteration of the active site geometry offers an opportunity for the design of selective inhibitors to facilitate discrimination among the FKBP domain proteins. Furthermore, the presence of trans χ_1 rotamers for both Trp 59 and Glu 60 in the G89P structure, in contrast to the gauche$^-$ rotamers at both positions in the wild type crystal structure, is suggestive of an energetic coupling between the interactions of the 50's loop backbone and the Trp 59 indole ring which might enable binding interactions at the 50's loop to allosterically modulate the geometry of the active site.

Our ^{15}N NMR relaxation analysis indicated that the indole ring flip may occur at a rate similar to that of the global molecular tumbling of the protein [24]. Since it appeared likely that the sidechain rotamer transition of Glu 60 and the shifting of the hydrogen bonding geometry in the central α-helix might occur at least as rapidly, we investigated whether molecular simulations on the μs timescale could usefully sample these three distinct local transitions to examine the degree of thermodynamic coupling between them and to analyze the degree to which this allosteric process arises from concerted transitions. The integration of thermodynamic analysis with the various mechanistic structural models of protein allostery continues to present a significant challenge for the field [30,31]. The role played by concerted conformational transitions has long remained a central aspect of the mechanistic analyses. Unfortunately, the kinetics underlying many of the best studied allosteric systems are too slow to allow for unbiased molecular dynamics simulations to provide estimates of the transition rates and equilibria. The comparatively rapid kinetics of the indole ring flip in FKBP12 provides an allosteric system that is amenable to such a molecular simulation analysis.

2. Materials and methods

2.1. Protein preparation

DNA sequences for the genes encoding the wild type [20] and the V101I variant of FKBP12 as well as the FK1 domains of FKBP51 and FKBP52 [32] were chemically synthesized (Genscript), with codon optimization for the expression in *Escherichia coli*. The gene sequences were cloned into the expression vector pET11a and then the plasmids were transformed into the BL21(DE3) strain (Novagen) for expression. The protein expression and purification procedure were carried out as described for FKBP12 [20,33] as well as for the FK1 domains of FKBP51 and FKBP52 [32]. For the selective ^{13}C-methyl labeled samples, 85 mg/L of [3-^{2}H, 4-^{13}C] α-ketoisovalerate and 50 mg/L of [3-$^2H_2$, 4-^{13}C] α-ketobutyrate [34] were supplemented into a medium for U-^{2}H,^{15}N enriched sample growths as previously described [24]. For back exchanging the amide hydrogens, 1 mM tris(2-carboxyethyl)phosphine and solid Tris base were added to a solution of the purified protein to obtain a pH value above 9, and the samples were incubated at 25 °C for 3 h and then neutralized with solid monobasic sodium phosphate. All protein samples were concentrated via centrifugal ultrafiltration and then equilibrated into a pH 6.50 buffer containing 25 mM sodium phosphate, 2 mM dithiothreitol and 2 mM tris(2-carboxyethyl)phosphine by a series of centrifugal concentration steps.

2.2. NMR spectroscopy

NMR data for the wild type and V101I variant of FKBP12 were collected on a Bruker Avance 600 MHz spectrometer and the NMR data for the FK1 domains of FKBP51 and FKBP52 were collected on a Bruker 800 MHz spectrometer at 25 °C. Resonance assignments for the wild type FKBP12 (BMR Data Bank accession numbers 19240 and 19241 [20]) and FK1 domains of FKBP51 and FKBP52 (BMR Data Bank accession numbers 19787 and 19788 [32]) have previously been reported. The indole ring