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Crystallization and preliminary crystallographic studies of the C-terminal domain of human FKBP52

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FKBP52 is a high-molecular-weight immunophilin belonging to the FKBP (FK506-binding protein) family. FKBP52 is one of several chaperone proteins associated with untransformed steroid receptors in steroid receptor–hsp90 heterocomplexes. Here, the C-terminal domain (amino acids 145–459) has been cloned, overexpressed and purified. Crystals were obtained using the hanging-drop vapour-diffusion technique with ammonium sulfate as precipitant in 0.1 *M* Tris pH 8.0 solution. Diffraction data to 2.7 Å were collected from a selenomethionine-containing crystal belonging to space group $C222_1$, with unit-cell parameters $a = 114.4, b = 143.1, c = 171.2$ Å, $\alpha = \beta = \gamma = 90^\circ$. There are three molecules per asymmetric unit.

1. Introduction

FKBP52 is a high-molecular-weight immunophilin possessing peptidylprolyl isomerase (PPIase) activity and is able to bind FK506, a macrolide immunosuppressant. Sequence and hydrophobic cluster analysis suggest that FKBP52 is composed of four different domains (Callebaut *et al.*, 1992). The first domain (amino acids 1–148), which is mainly responsible for the PPIase activity, exhibits 55% amino-acid homology and 49% sequence identity with FKBP12 (Callebaut *et al.*, 1992; Lebeau *et al.*, 1999). The second domain (amino acids 149–263) exhibits 28% sequence identity with FKBP12, which has marginal PPIase activity (Chambraud *et al.*, 1993). The third domain (amino acids 264–400) consists of three tetratricopeptide repeats (TPRs), which bind to hsp90 (Barent *et al.*, 1998; Radanyi *et al.*, 1994). The fourth domain (amino acids 400–458) seems to contain a calmodulin-binding site (Massol *et al.*, 1992).

The molecular chaperone hsp90 in the eukaryotic cytosol interacts with a variety of protein cofactors. Several hsp90-associated cofactors contain multiple copies of the TPR motif (Nair *et al.*, 1996; Pratt & Toft, 1997), a degenerate 34-amino-acid consensus sequence that mediates protein–protein interactions (Fig. 1). The structures of some TPR-containing proteins have been reported, such as Hsp70/90 organizing protein (Hop), cyclophilin 40 (Cyp40), protein phosphatase 5 (PP5) and FKBP51 (Das *et al.*, 1998; Lamb *et al.*, 1995; Scheufler *et al.*, 2000; Sikorski *et al.*, 1990; Sinars *et al.*, 2003; Taylor *et al.*, 2001). Using a yeast two-hybrid screen, the 12 kDa C-terminal domain of human hsp90α has been found to mediate the interaction of hsp90 with TPR-containing sequences from the hsp90 cofactors (Young *et al.*, 1998). It has been supposed that signalling proteins move through the cytoplasm by diffusion and then become trapped at their sites of action by protein–protein interactions. Steroid receptors, such as glucocorticoid receptor (GR), can translocate to the nucleus along microtubular tracts as a GR–hsp90–immunophilin heterocomplex. This signalling protein–hsp90 heterocomplex is assembled by a multiprotein chaperone comprising hsp90, hsp70, Hop, hsp40 and p23 (Pratt *et al.*, 1999). FKBP52 binds to hsp90 *via* the TPRs. It has been demonstrated that there is one binding site for the FKBP52/dimer of hsp90 (Silverstein *et al.*, 1999). In the heterocomplex, FKBP52 has been shown to be the connector protein that links the signalling protein to the movement machinery by interacting with molecular motors, such as dynein (Pratt *et al.*, 1999). FKBP51, FKBP52 and Cyp40 bind competitively to hsp90 through their TPR domains and any one of the three immunophilins can be isolated in mature steroid receptor complexes.

Despite the overall similarity of FKBP51 and FKBP52 (Fig. 2), these two immunophilins associate differentially with steroid receptors. FKBP51 associates preferentially with progesterone receptor (PR) and glucocorticoid receptor. Estrogen receptor (ER) has a relatively higher preference for FKBP52 than PR and GR (Barent *et al.*, 1998). The difference has been found to relate to both the hsp90-binding TPR domain and to the poorly conserved C-terminal sequences by generating a series of chimeras and mutants. Studies of FKBP chimeric constructs and truncation

mutants have shown that the TPR domain of FKBP51 requires an appropriate downstream sequence for hsp90 binding, but that the TPR domain of FKBP52 does not. The mutants in the C-terminal half of Hsp90 that have been proved to interact with immunophilins have different effects on the binding of hsp90 by FKBP52 compared with FKBP51 (Barent *et al.*, 1998; Cheung-Flynn *et al.*, 2003). The crystal structure of human FKBP51 has been solved recently (Sinars *et al.*, 2003), but there is no corresponding complete structure of FKBP52. The structural alterations between these two proteins may provide clues to an explanation of the differential effects on binding steroid receptors and Hsp90.

Because of the instability of FKBP52, full-length FKBP52 was divided into two parts, the N-terminal domain (FKBP52-N, amino acids 1–140, containing the first domain) and the C-terminal domain (FKBP52-C, amino acids 145–459, containing three C-terminal domains). We have already obtained the 2.4 Å X-ray crystal structure of FKBP52-N (Li *et al.*, 2003). Here, we report the crystallization and preliminary crystallographic studies of FKBP52-C.

2. Materials and methods

2.1. Selenomethione-protein expression and purification

The FKBP52-C gene was amplified by PCR, cloned into the pET28a(+) plasmid (Novagen Inc.) and transformed into the methionine-deficient *Escherichia coli* strain B834(DE3). Transformed cells were cultured in 5 ml LB medium containing 50 µg ml^{-1} kanamycin overnight. 65 ml adaptive medium [15%(*v*/*v*) 5×M9, 15% LB medium, 5% glucose, 50 µg ml^{-1} kanamycin] was inoculated using the overnight culture. When the culture density reached A_{600} = 0.6−0.7, pelleted cells from 30 ml of adaptive culture were resuspended in 500 ml of expression medium (20% 5×M9, 3% glucose, 6.5% YNB, 50 µg ml^{-1} kanamycin). Cells were grown for 5 h at 310 K before addition of L-selenomethionine (SeMet) at 15 mg l^{-1}, lysine, threonine and phenylalanine at 25 mg l^{-1} and leucine, isoleucine and valine at 12.5 mg l^{-1}. Induction with 1 mmol l^{-1} isopropyl-β-D-thiogalactopyranoside (IPTG) was performed 15 min later and cell growth continued for 12–14 h at 289 K. Cells were harvested by centrifugation and stored at 253 K.

The bacterial cell pellet was resuspended in lysis buffer [25 m*M* Tris–HCl pH 8.0, 500 m*M* NaCl, 10%(*v*/*v*) glycerol, 10 m*M* imidazole] and sonicated. The supernatant was applied to an Ni^{2+} column (1 ml Ni^{2+}–NTA agarose). The column was thoroughly washed with the same lysis buffer. The target protein was then eluted with elution buffer (25 m*M* Tris–HCl pH 8.0, 500 m*M* NaCl, 10% glycerol, 200 m*M* imidazole). The protein was further purified by gel filtration on a Superdex200 HR 10/30 (Amersham Pharmacia, USA) column run in 20 m*M* Tris pH 8.0, 0.1 *M* NaCl. The pooled fractions were loaded onto a Resource Q (Amersham Pharmacia, USA) ion-exchange chromatography column run in 20 m*M* Tris pH 8.0 and developed with a 0–250 m*M* NaCl gradient. The purity of FKBP52-C was estimated by SDS–PAGE to be greater than 95%.

2.2. Crystallization

The purified SeMet-containing protein was concentrated to ∼10 mg ml^{-1} in 100 m*M* NaCl, 20 m*M* Tris–HCl pH 8.0, 5 m*M* DTT. Crystallization was performed at 291 K using the hanging-drop vapour-diffusion method. Each drop contained 1.5 µl protein solution and 1.5 µl reservoir solution. Initial screening used the sparse-

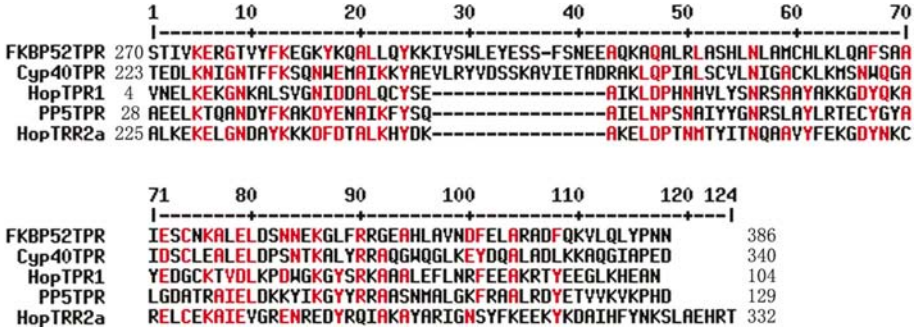

Figure 1
Sequence alignment of the TPR domains of FKBP52, Cyp40, Hop and PP5. Amino acids with high consensus are shown in red.

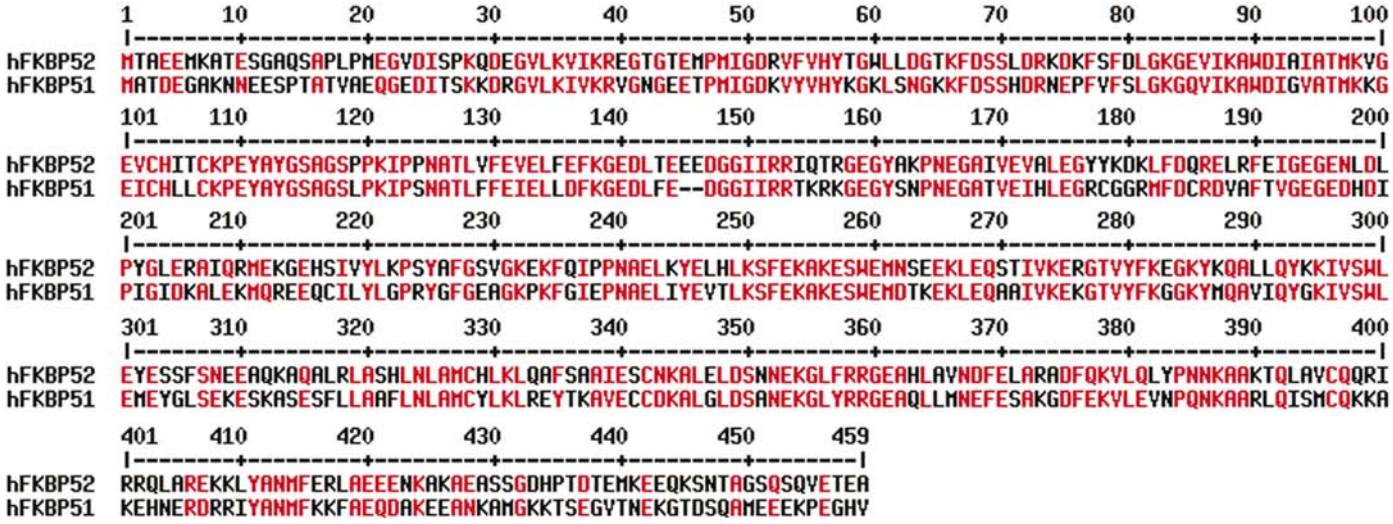

Figure 2
Sequence alignment of human FKBP52 (hFKBP52) and human FKBP51 (hFKBP51). Amino acids with high consensus are shown in red. Human FKBP52 shares 60% amino-acid sequence identity and 75% similarity with human FKBP51. FKBP52-C contains residues 145–459.

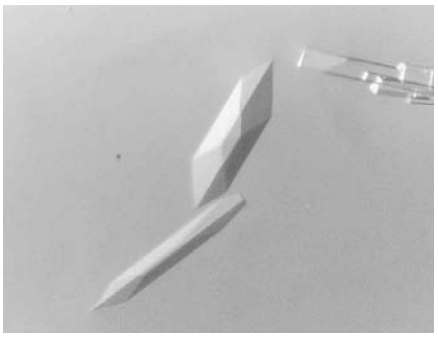

Figure 3
Crystals of FKBP52-C. A single crystal had dimensions 0.4 × 0.1 × 0.1 mm.

Table 1
Data-collection and processing statistics.

Values in parentheses are for the highest resolution shell.

	SeMet λ_1	SeMet λ_2	SeMet λ_3
Wavelength (Å)	0.9798	0.9800	0.9000
Resolution range (Å)	50–2.7	50–2.7	50–2.8
Completeness (%)	100.0 (99.8)	100.0 (99.9)	100.0 (100.0)
Total reflections	291352	291742	258271
Unique reflections	38801	38872	34752
Redundancy	7.5	7.5	7.4
$R_{\rm merge}$† (%)	6.4 (32.9)	5.0 (34.4)	6.2 (42.2)
$\langle I/\sigma(I)\rangle$	16.5 (5.9)	17.0 (6.5)	16.3 (5.7)
Space group	$C222_1$		
Unit-cell parameters (Å, °)	a = 114.4, b = 143.1, c = 171.2, $\alpha = \beta = \gamma = 90°$		

† $R_{\rm merge} = \sum |I - \langle I\rangle|/I$.

matrix (Jancarik & Kim, 1991) screening kits from Hampton Research Crystal Screen Kits I and II (Riverside, CA, USA). The optimized reservoir solution consisted of 2.2–2.4 *M* ammonium sulfate and 2–4%(*v*/*v*) ethanol in 0.1 *M* Tris–HCl pH 8.0. Crystals were obtained within a week (Fig. 3).

2.3. Data collection and processing

A data set was collected from a single SeMet FKBP52-C derivative crystal at 90 K using a MAR Research CCD detector and synchrotron radiation (SPring-8, beamline BL41XU, λ_1 = 0.9798, λ_2 = 0.9800 and λ_3 = 0.9000 Å). 25% glycerol added to the mother liquor was used as a cryprotectant. The crystal diffracted to 2.7 Å. Data processing and scaling were performed using *HKL*2000 and *SCALEPACK* (Otwinowski & Minor, 1997).

3. Results and discussion

We initially obtained crystals using a solution containing 2.3–2.4 *M* ammonium sulfate and 0.1 *M* Tris–HCl pH 8.0, but these crystals were not suitable for X-ray diffraction. We then tried to add organic reagents to the reservoir solution, such as glycerol, ethanol, ethylene glycol and isopropanol. Crystals grown from the optimized reservoir solution [0.1 *M* Tris–HCl pH 8.0, 2.2–2.4 *M* ammonium sulfate, 2–4%(*v*/*v*) ethanol] were more suitable for X-ray diffraction and diffracted to 2.7 Å. Assuming the presence of three molecules in the asymmetric unit, the calculated solvent content is about 55%. Data statistics are given in Table 1. The structure of FKBP52-C has been determined (PDB code 1p5q) and will be published elsewhere.

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