crystallization papers

Crystallization and preliminary X-ray diffraction analysis of FKBP52 N-terminal domain

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FKBP52 is a FK506-binding protein which was first discovered in the heterocomplex composed of HSP90 and inactive steroid receptor. Here, the N-terminal domain (residues 1–140) of FKBP52 has been overexpressed, purified and crystallized using the hanging-drop vapour-diffusion technique. Crystals with a 2.4 Å resolution limit were obtained using ammonium sulfate as precipitant at pH 8.5. The crystals belong to space group $P2_1$, with unit-cell parameters $a = 27.8$, $b = 58.4$, $c = 70.9$ Å, $\beta = 98.3°$. Assuming two molecules per asymmetric unit, the solvent content is calculated to be 40%.

1. Introduction

FKBP52 is an immunophilin belonging to the FK506-binding protein (FKBP) family, which was first discovered as a component of the untransformed steroid receptor and HSP90 heterocomplex (Sanchez, 1990). Sequence and hydrophobic cluster analysis suggested that FKBP52 is composed of four different domains, each separated by short hydrophilic linker sequences (Callebaut et al., 1992). It was suggested that two of these domains are structurally related to FKBP12. The first domain (amino acids 1–148, defined as FKBP52-I) exhibits 55% homology and 49% sequence identity with FKBP12, is responsible for the peptidyl–prolyl isomerase (PPIase) activity of FKBP52 and is able to bind FK506 (Chambraud et al., 1993; Pirkl et al., 2001; Tai et al., 1993; Yem et al., 1992).

FKBP12 complexed with FK506 is able to inhibit the phosphatase activity of calcineurin, but both FKBP52 and FKBP52-I are unable to inhibit calcineurin when complexed with FK506 (Lebeau et al., 1994; Wiederrecht et al., 1992). In addition to binding to FAP48, FKBP52 also interacts with cytoplasmic dynein and phytanoyl-CoA α-hydroxylase (PAHX) via the FKBP52-I domain. FK506 competes with the binding of FAP48, but does not affect the binding of dynein and PAHX. It has been shown that FKBP12 does not interact with dynein and PAHX (Chambraud et al., 1996, 1999; Galigniana et al., 2001; Silverstein et al., 1999).

In spite of its high homology with FKBP12, FKBP52-I is functionally distinct from FKBP12. The NMR structure of FKBP52-I has been determined (Craescu et al., 1996). Here, we report the crystallization and preliminary X-ray analysis of the N-terminal domain of FKBP52 (FKBP52-N, amino acids 1–140, in which the hydrophilic hinge sequence of FKBP52-I is truncated). The determination of the crystal structure of FKBP52-N is expected to provide insight into the function of the FKBP52-I domain.

2. Materials and methods

2.1. Protein expression and purification

The coding sequence for FKBP52-N (residues 1–140) was amplified using PCR and cloned into the pET28a(+) plasmid (Novagen Inc.). The plasmid was then transformed into Escherichia coli strain BL21(DE3) plysE and transformants were selected on LB agar plates containing 25 µg ml^{-1} kanamycin. The cells were cultured at 310 K in LB medium containing 50 µg ml^{-1} kanamycin. When the culture density (A_{600}) reached 0.6–0.7, the culture was induced with 0.5 mM IPTG and grown for an additional 5 h before the cells were harvested.

The bacterial cell pellet was resuspended in lysis buffer (25 mM Tris–HCl pH 8.0, 500 mM NaCl, 10 mM imidazole, 1 mM PMSF) and homogenized by sonication. The lysate was centrifuged at 20 000g for 25 min to remove the cell debris. The supernatant was applied to an Ni^{2+} column (1 ml Ni^{2+}–NTA agarose). The contaminating protein was washed with the same lysis buffer. The target protein was eluted with 25 mM Tris–HCl pH 8.0, 500 mM NaCl, 250 mM imidazole, 1 mM PMSF and the purified protein was analyzed on SDS–PAGE.

2.2. Crystallization

The purified protein was exchanged into 10 mM sodium cacodylate pH 6.5 buffer and concentrated to 20–30 mg ml^{-1}. Initial crystallization conditions were screened using an ammonium sulfate kit (which we had prepared by varying the buffer pH over the range 4.6–8.5

and the ammonium sulfate concentration over the range 0–3 M) at 291 K in hanging drops. The conditions yielding small crystals were further optimized by variation of the precipitant and protein concentration and the buffer pH. The best crystals were obtained using vapour diffusion against 1.5–2.5 M ammonium sulfate in 0.1 M Tris–HCl buffer pH 8.5. 1 µl protein solution was mixed with 1 µl reservoir solution and the mixture was equilibrated against 500 µl reservoir solution at 291 K. Rod-like crystals appeared after 10 d.

2.3. Data collection and processing

Data were collected to 2.4 Å using a 345 mm MAR Research image-plate system mounted on a Rigaku RU-2000 rotating-anode generator operated at 48 kV and 98 mA (Cu $K\alpha$; λ = 1.5418 Å). During data collection, the crystal was maintained at 100 K using an Oxford Cryosystems Cryostream and with 20% glycerol added to the mother liquor as a cryoprotectant. Data processing was performed with *DENZO* and *SCALEPACK* (Otwinowski & Minor, 1997) with no σ-cutoff.

3. Results and discussion

We obtained the crystals from a solution containing 1.5–2.5 M ammonium sulfate and 0.1 M Tris–HCl pH 8.5, but the crystals were twinned and were not suitable for X-ray diffraction. A single crystal was sliced from a cluster of crystals and diffracted to 2.4 Å. A data set was collected from this crystal. The

Table 1
Data collection and processing statistics.

Values in parentheses correspond to the highest resolution shell (2.49–2.4 Å)

Space group	$P2_1$
Unit-cell parameters	
a (Å)	27.8
b (Å)	58.4
c (Å)	70.9
β (°)	98.3
Resolution limits (Å)	940–2.4 (2.49–2.4)
Total observations	96262
Unique reflections	8929 (889)
Average $I/\sigma(I)$	17.3 (9.3)
Redundancy	10.8 (8.4)
Completeness (%)	99.9 (99.9)
χ^2 value	0.955
R_{merge}† (%)	9.5 (29.4)

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

crystals belong to space group $P2_1$, with unit-cell parameters a = 27.8, b = 58.4, c = 70.9 Å, β = 98.3°. Assuming two molecules in the asymmetric unit, the solvent content is calculated to be about 40%. Selected data statistics are reported in Table 1.

The molecular-replacement method is being used to solve the structure of the FKBP52 N-terminal domain, with the structure of FKBP12 as a search model; crystallization of the FKBP52 N-terminal domain complexed with small ligands is under way.

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