1. Introduction

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 C2221, with unit-cell parameters a = 114.4, b = 143.1, c = 171.2 Å, α = β = γ = 90°. There are three molecules per asymmetric unit.
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) M9, 15% LB medium, 5% glucose, 50 μg ml⁻¹ kanamycin] was inoculated using the overnight culture. When the culture density reached A₆₀₀ = 0.6–0.7, pelleted cells from 30 ml of adaptive culture were resuspended in 500 ml of expression medium (20% 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 mM Tris–HCl pH 8.0, 500 mM NaCl, 10% (v/v) glycerol, 10 mM imidazole] and sonicated. The supernatant was applied to an Ni²⁺ column (1 ml Ni²⁺–NTA agarose). The column was thoroughly washed with the same lysis buffer. The target protein was then eluted with elution buffer (25 mM Tris–HCl pH 8.0, 500 mM NaCl, 10% glycerol, 200 mM imidazole). The protein was further purified by gel filtration on a Superdex200 HR 10/30 (Amersham Pharmacia, USA) column run in 20 mM 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 mM Tris pH 8.0 and developed with a 0–250 mM 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 mM NaCl, 20 mM Tris–HCl pH 8.0, 5 mM 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-
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, \( \lambda_1 \) = 0.9798, \( \lambda_2 \) = 0.9800 and \( \lambda_3 \) = 0.9000 Å). 25% glycerol added to the mother liquor was used as a cryoprotectant. The crystal diffracted to 2.7 Å. Data processing and scaling were performed using HKL2000 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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### References


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**Table 1**

Data-collection and processing statistics.

Values in parentheses are for the highest resolution shell.

<table>
<thead>
<tr>
<th></th>
<th>SeMet ( \lambda_1 )</th>
<th>SeMet ( \lambda_2 )</th>
<th>SeMet ( \lambda_3 )</th>
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<tr>
<td>Wavelength (Å)</td>
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<td>0.9800</td>
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<td>Resolution range (Å)</td>
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<td>50–2.7</td>
<td>50–2.8</td>
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<td>100.0 (99.9)</td>
<td>100.0 (100.0)</td>
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<td>291742</td>
<td>258271</td>
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<td>38872</td>
<td>34752</td>
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<td>7.5</td>
<td>7.4</td>
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<td>( R_{exp} ) (%)</td>
<td>6.4 (32.9)</td>
<td>5.0 (34.4)</td>
<td>6.2 (42.2)</td>
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<td>Unit-cell parameters (Å, °)</td>
<td>( a = 114.4, b = 143.1, c = 171.2, \alpha = \beta = \gamma = 90° )</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\( R_{merge} = \sum |I_i - \langle I_i \rangle|/I_i \).