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Crystallization and preliminary X-ray diffraction analysis of the 10 kDa C-terminal subdomain of 70 kDa heat-shock cognate protein

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The 70 kDa heat-shock cognate protein (Hsc70) is a cytosolic molecular chaperone. It is composed of a 44 kDa N-terminal nucleotide-binding domain, an 18 kDa peptide-binding subdomain and a 10 kDa C-terminal subdomain. Single crystals of recombinant 10 kDa subdomain of rat Hsc70 have been obtained using ammonium sulfate as a precipitant at room temperature. The crystals diffract beyond 3.5 Å using a synchrotron-radiation source at a wavelength of 1.0 Å. The crystals belong to the hexagonal space group $P6_122$ or $P6_522$, with unit-cell parameters $a = b = 119.0$, $c = 166.4$ Å.

1. Introduction

The 70 kDa heat-shock cognate protein (Hsc70), a member of the hsp70 family, is constitutively expressed in the cytosol of vertebrate cells. It is clear that Hsc70 and other members of the hsp70 family share the same domain structures. The 44 kDa N-terminal region is a nucleotide-binding domain and possesses an intrinsic ATPase activity (Chappell *et al.*, 1987). The three-dimensional structure of the N-terminal domain has been determined (Flaherty *et al.*, 1990). The 30 kDa C-terminal domain is responsible for binding unfolded proteins. It can further be divided into an 18 kDa N-terminal peptide-binding subdomain and a 10 kDa C-terminal subdomain (Wang *et al.*, 1993; Tsai & Wang, 1994). The structures of the 30 kDa domain of DnaK (a bacterial Hsc70) and the 18 kDa subdomain were recently determined by X-ray diffraction (Zhu *et al.*, 1996) and NMR spectroscopy (Wang *et al.*, 1998; Morshauser *et al.*, 1999), respectively. The secondary structure of the 10 kDa subdomain of DnaK was also characterized (Bertelsen *et al.*, 1999).

Substantial evidence supports the view that Hsc70 is a molecular chaperone (for reviews, see Hartl, 1996; Bukau & Horwich, 1998) that works in concert with other cellular proteins to exert its functions, which include the dissociation of clathrin from coated vesicles (Schlossman *et al.*, 1984), transporting proteins into organelles and folding of denatured proteins (Hartl, 1996; Bukau & Horwich, 1998). Evidently, the 10 kDa subdomain of Hsc70 is responsible for a number of co-chaperones interacting with the Hsc70. For instance, Demand *et al.* (1998) showed that the 10 kDa subdomain of Hsc70 provides binding sites for both Hsp40 (Hdj-1) and Hop (p60/Sti1). It was also demonstrated that the 10 kDa subdomain was responsible for the interaction of Hsc70 with a group of proteins containing tetratricopeptide repeats (TPR; Liu *et al.*, 1999). Therefore, the 10 kDa subdomain of Hsc70 may participate in interactions with many specific target proteins to facilitate the chaperone activity, even though it has been considered to be a portion of the peptide-binding domain (Zhu *et al.*, 1996).

Despite the availability of the three-dimensional structure of the C-terminal domain of DnaK (Zhu *et al.*, 1996), the structure of the vertebrate 10 kDa C-terminal subdomain remains elusive. This may be because of the lack of homology in this region between Hsc70 and DnaK. Moreover, the crystal structure of the 30 kDa domain of DnaK reported by Zhu *et al.* (1996) did not contain the last C-terminal 30 amino acids, to which specific roles have been attributed (Liu *et al.*, 1999). In order to investigate the structure and the functional significance of the 10 kDa subdomain of vertebrate Hsc70, we have crystallized this subdomain including the the last C-terminal 30 amino acids. We report here the preliminary X-ray analysis.

2. Materials and methods

Cloning, expression and purification of the recombinant 10 kDa C-terminal subdomain (Hsc10) of rat (*Rattus norvegicus*) Hsc70 was carried out as described previously (Hu & Wang, 1996). Briefly, the cDNA fragment of rat Hsc70 corresponding to amino-acid residues 541–646 was inserted into the pET-15b vector (Novagen). The recombinant protein was expressed in *Escherichia coli* BL21(DE3) and purified using Qiagen Ni-NTA Superflow resin.

Figure 1
A crystal of the 10 kDa C-terminal subdomain of rat Hsc70.

The protein, including the His tag, contains 125 amino-acid residues and has a molecular weight of 13 534 Da. In the first trial for crystallization, the His tag was removed by thrombin digestion. However, no protein crystals were obtained from Hsc10 without the His tag. Therefore, the 10 kDa subdomain with the His tag at the N-terminus was used for crystallization.

Crystals of the 10 kDa subdomain were grown by the hanging-drop vapour-diffusion method from a 20 mg ml^{-1} protein solution containing 0.1 *M* NaCl in 50 m*M* Tris–HCl pH 7.9. In the initial experiments, a crystallization kit (Hampton Research, Screen I) was used for screening. Only probe No. 47 (2.0 *M* ammonium sulfate, 0.1 *M* sodium acetate pH 4.6) yielded microcrystals at room temperature, but they were unusable. In the second stage, the amount of precipitant and pH value were varied. The best crystals were obtained with 2.5 *M* ammonium sulfate and 1% 2-propanol. Small lemon-shaped crystals (Fig. 1) appeared in 3 d and grew to maximum dimensions of 0.8 × 0.5 × 0.3 mm in two weeks.

For X-ray analysis, oscillation diffraction images were taken at the BL6B synchrotron beamline of the Photon Factory at the High Energy Accelerator Research Organization (KEK), Tsukuba, Japan. A single crystal was frozen at 110 K using 12% sucrose as a cryoprotectant to prevent ice formation during data collection at cryogenic temperature. A screenless Weisenberg camera (Sakabe *et al.*, 1997) with a 400 × 800 mm imaging-plate with a radius of 573 mm was used. The space between the beam collimator and the camera cassette was filled with helium gas to reduce the background of the imaging plate during data collection. The wavelength of the X-ray beam was adjusted to 1.0 Å. The data were collected from 0 to 90° and the oscillation range was 2.5°. The diffraction data were indexed and integrated using the program *DENZO* and *SCALEPACK* (Otwinowski & Minor, 1997). The data were obtained from a total of 51 961 reflections and reduced to 8106 unique structure factors with an $R_{\rm merge}$ of 4.8% and a completeness of 87.2%. The data statistics are given in Table 1.

Table 1
The diffraction data statistics of the 10 kDa subdomain crystal.

Values in parentheses are for the outermost resolution shell.

Synchrotron-radiation source	BL-6B (PF)
Wavelength (Å)	1.0
No. of imaging plates	36
Total No. of reflections	51961
No. of unique reflections	8106
Redundancy	6.4
Space group	$P6_122$ or $P6_522$
a (Å)	119.0
c (Å)	166.4
Resolution (Å)	35–3.5
Mosaicity of crystal (°)	0.56
Completeness	87.2 (82.6)
$I/\sigma(I)$	20.5 (4.2)
$R_{\rm merge}$† (%)	4.8 (13.7)

† $R_{\rm merge}(I) = \sum_h \sum_i |I_i - I|/\sum_h \sum_i I$, where I is the mean intensity of the i observations of reflection h.

3. Results

The crystals of the 10 kDa C-terminal subdomain of Hsc70 have hexagonal unit-cell parameters a = 119.0, c = 166.4 Å and diffract beyond 3.5 Å resolution. The systematic absences (00l except for l = 6n) and Laue symmetry of the diffraction pattern (6/*mmm*) indicate that the space group is either $P6_122$ or $P6_522$. Self-rotation studies (Tong & Rossmann, 1997) using data in the resolution range 10–3.5 Å with a Patterson cutoff radius of 20.5 Å indicate that there is a non-crystallographic pseudo-fivefold symmetry axis approximately along the crystallographic sixfold symmetry axis in the unit cell (Fig. 2). Based on calculation of the Matthews coefficient (Matthews, 1968), it is likely that there are four or five molecules per asymmetric unit.

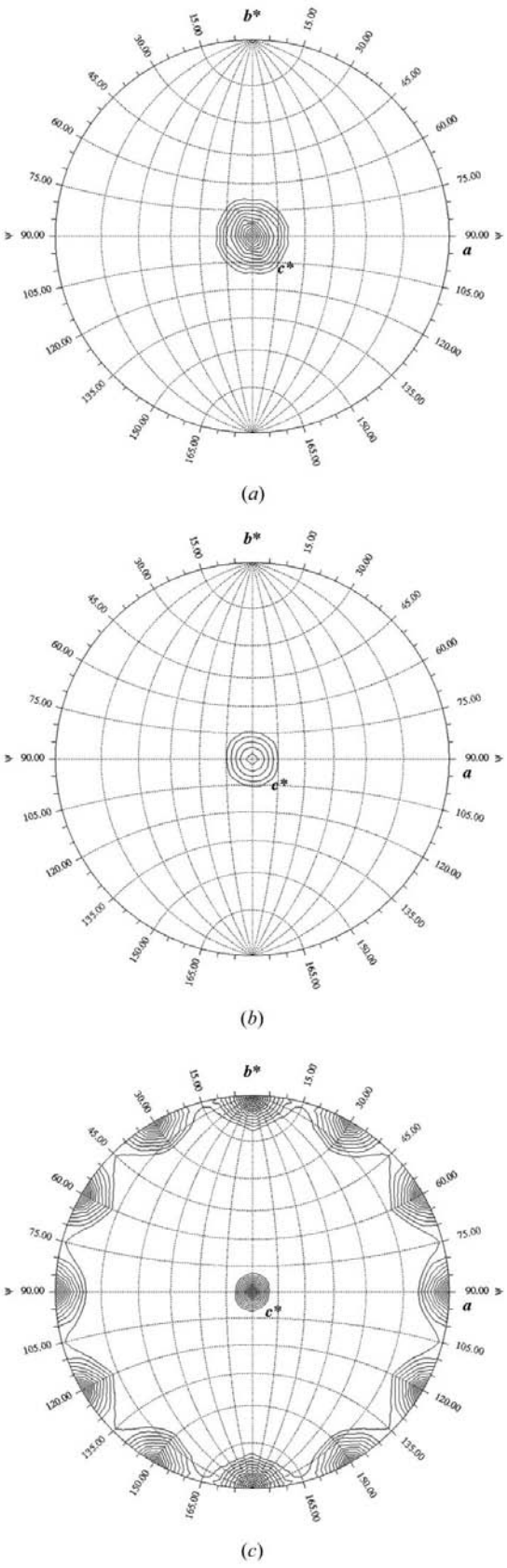

Figure 2
The self-rotation function of the 10 kDa C-terminal subdomain crystal at (*a*) κ = 60, (*b*) κ = 72 and (*c*) κ = 180°. The pseudo-fivefold symmetry axis is parallel to the c^* axis.

Assuming five molecules per asymmetric unit, V_M is 2.51 Å^3 Da^{-1} with an estimated solvent content of 51%. Assuming four molecules per asymmetric unit, V_M is 3.14 Å^3 Da^{-1} with an estimated solvent content of 61%. 2.51 Å^3 Da^{-1} is a reasonable value for V_M. However, 3.14 Å^3 Da^{-1} is also a plausible value for this crystal, as it is a significantly large crystal and diffracts only to 3.5 Å, suggesting the presence of a high percentage of solvent in the crystal.

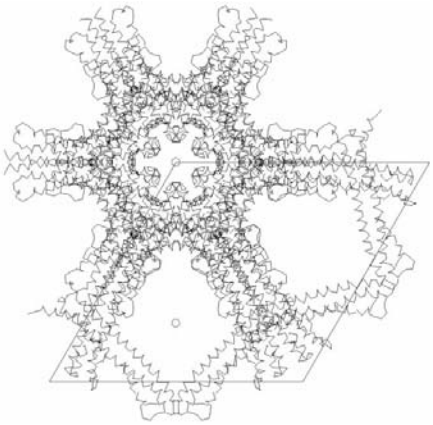

Figure 3
Crystal packing of the 10 kDa C-terminal subdomain of rat Hsc70 viewed down the crystal *c* axis.

During revision of this manuscript, the structure of the 10 kDa C-terminal subdomain of Hsc70 was determined (unpublished results) using multiwavelength anomalous diffraction (MAD) phasing (Hendrickson, 1991) applied to the selenomethionyl analogue (Hendrickson *et al.*, 1990). The asymmetric unit contains four molecules forming two dimers that assemble as a cross-like shape. From the crystal packing (Fig. 3), there is an imperfect five-fold screw axis approximately parallel to the *c* axis. This phenomenon provides an explanation for the significant peak at 72° projection in the self-rotation function study.

The sequences of the 10 kDa subdomains are quite different between rat Hsc70 and DnaK. However, it might still be possible that this subdomain plays a similar functional role in the two proteins. The structures of the C-terminal 10 kDa subdomain of rat Hsc70 and the peptide-binding domain of DnaK (Zhu *et al.*, 1996) have been superimposed, which indicates a large conformation disparity (data not shown). Previously, a preliminary molecular-replacement study had been performed using the information obtained from the crystal structure of DnaK, but failed to yield a solution. The result also suggested that the structure of the 10 kDa C-terminal subdomain of Hsc70 had a conformation that differed significantly from that of DnaK. Structure refinement and analyses are currently in progress.

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