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Crystallization of the chaperonin GroEL–GroES complex from *Thermus thermophilus* HB8

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The chaperonin GroEL–GroES (GroEL/ES) complex from a thermophilic eubacteria, *Thermus thermophilus* HB8, has been purified and crystallized. The GroEL/ES complex is known to be composed of 14 identical GroEL subunits (58 kDa) and seven identical GroES subunits (11 kDa). The GroEL/ES complex crystals belong to the triclinic space group $P1$, with unit-cell parameters $a = 140.4$, $b = 156.4$, $c = 273.1$ Å, $\alpha = 82.9$, $\beta = 85.4$, $\gamma = 68.5^\circ$. The crystal asymmetric unit contains two molecules (MW = 885 kDa). One data set to 3.0 Å resolution, with 383 652 independent observations (89.3% complete) and an R_{merge} of 0.08, has been collected from a single crystal. A molecular-replacement solution was obtained using the structure of the GroEL/ES complex from *Escherichia coli* as a search model.

1. Introduction

Molecular chaperones assist in the folding of many cellular proteins. Chaperonins, a family of molecular chaperones, mediate the folding of ~10% of newly translated polypeptides in an ATP-dependent manner (Hartl, 1996). Chaperonins are essential and abundant proteins and have been isolated from many organisms. The mechanisms of chaperonin action have been studied mainly using the chaperonin GroEL and its co-chaperonin GroES from *Escherichia coli*. GroEL comprises two heptameric rings (*cis*-ring and *trans*-ring) of identical 57 kDa subunits stacking back to back. GroES, a dome-shaped heptameric ring composed of seven identical 10 kDa subunits, binds to the GroEL *cis*-ring and forms the *cis*-cavity. The chaperonin reaction cycle begins by the binding of the unfolded proteins to the free end (the *trans*-ring) of the GroEL/ES complex. The subsequent binding of seven ATP molecules and GroES to the ring encapsulates the substrate proteins into the *cis*-cavity. The *cis*-cavity affords the space for the substrate proteins to fold during the hydrolysis of the seven ATP molecules bound in the ring. On completion of the hydrolysis, binding of a further seven ATP molecules and a unfolded protein molecule to the opposite ring (*trans*-ring) triggers the release of GroES, seven ADP molecules and the folded or partially folded substrate protein from the cage. Among the intermediates of this cycle, only the structure of the $GroEL_{14}$–$GroES_7$–ADP_7 complex from *E. coli* has been solved at high resolution (3.0 Å; Xu *et al.*, 1997), although the structure of the $GroEL_{14}$–ADP_7–$GroES_7$–ATP_7 complex from *E. coli* has been solved by cryo-electron microscopy (12.5 Å; Ranson *et al.*, 2001). The crystallized GroEL/ES complex was reconstituted from independently purified GroEL and GroES and ADP molecules without the substrate proteins, because GroEL and GroES from *E. coli* are easily separated during purification. In order to fully elucidate and generalize the chaperonin mechanisms, it is necessary to solve the structures of the GroEL/ES complexes from different organisms, as well as solving different intermediate states and the complex containing substrate protein.

Here, we report the crystallization of the native intact GroEL/ES complex from *Thermus thermophilus* HB8 purified from intact cells (Taguchi *et al.*, 1991). The crystals of the complex contained substrate proteins and diffracted anisotropically to 3.0 Å. The comparison of the GroEL/ES complex structure from *T. thermophilus* with that from *E. coli* should help in the elucidation of the universal chaperonin mechanisms common to all organisms.

2. Material and methods

2.1. Expression and purification

The GroEL/ES complex was purified from intact *T. thermophilus* HB8 cells cultured at 348 K under strong aeration in a medium containing 10 g yeast extract, 10 g polypeptone and 2 g NaCl per litre (Taguchi *et al.*, 1991). Cells were harvested at the late log phase by centrifugation (277 K, 30 min). Cells were lysed by sonication in 50 m*M* Tris–HCl pH 8.0,

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5 mM $MgCl_2$ (buffer A). All subsequent steps were performed at 277 K. After centrifugation and addition of Triton X-100 (to a final concentration of 3.0%), the supernatant was applied to a DEAE-Sephacel column (Amersham Biosciences, Sweden) equilibrated with buffer B (buffer A plus 0.1% Triton X-100). The column was subsequently washed with four column volumes of buffer B before protein was eluted with a linear gradient of 0–100% buffer B containing 0.5 M NaCl. The chaperonin fractions were pooled and concentrated to minimal volume using an Amicon cell (Millipore, CA, USA) with a 100 kDa cutoff filter. The concentrated protein was applied to a Superdex S-200 gel-filtration column (Amersham Biosciences, Sweden) equilibrated with two column volumes of buffer B containing 50 mM NaCl and subsequently eluted using the same buffer. Fractions containing the chaperonin were pooled and concentrated using a Vivaspin (Vivascience, Germany) 100 kDa molecular-weight cutoff filter. The protein was finally concentrated to 10 mg ml^{-1} and then used for crystallization trials.

2.2. Initial crystallization

Crystals were grown by the hanging-drop vapor-diffusion method using Linbro plates (Hampton Research, USA). 1 µl of protein sample was mixed with 1 µl of reservoir solution and left to equilibrate at 293 K. Initial crystals were obtained from a reservoir solution containing 0.1 M MES pH 6.0, 0.1 M NaCl, 0.1 M $MgCl_2$, 10% PEG 6000. Crystals appeared after 1 d and stopped growing within a week.

2.3. Optimization of crystallization conditions

Crystals from the initial conditions were small and diffracted very poorly. Screening for an alternative pH, PEG type and PEG concentration improved the size and the diffraction quality of the crystals. Additive Screens 1, 2 and 3 from Hampton Research were used for further optimization: 1 µl of each additive was mixed with 9 µl of reservoir solution and used to prepare drops as described above. The ratio of the sample volume to the reservoir volume was then optimized to 2:1. The final optimized crystallization conditions after iterative optimization cycles were 0.1 M MES pH 6.5, 0.1 M NaCl, 0.1 M $MgCl_2$, 3–5% PEG 4000, 10% dimethyl sulfoxide. Crystals appeared after 1 d and reached maximum dimensions of 0.05 × 0.3 × 0.7 mm within a week.

2.4. X-ray data collection

Prior to data collection, crystals were soaked in cryosolutions with increasing concentrations of PEG 4000 (3.6–6.4%) and MPD (25%) and frozen in liquid nitrogen. Data collections from frozen crystals were performed at 100 K. Diffraction data to 3.0 Å were collected at beamline ID14/EH2 of the European Synchrotron Radiation Facility (ESRF) using an ADSC Quantum 4 CCD detector. The beam size, oscillation range, exposure time and crystal-to-film distance were 50 × 50 µm, 0.25°, 10 s per frame and 270 mm, respectively. Image data were processed using the program packages *DENZO* and *SCALEPACK* (Otwinowski & Minor, 1997). All data better than $-3.0\sigma(I)$ were used for scaling.

3. Results and discussion

Initial crystals diffracted weakly (to 5 Å) and were too small to collect a complete data set from a crystal with space group $P1$ owing to radiation damage caused by the long exposure time. Both the size and the diffraction resolution of the crystals were then improved by optimization of the crystallization conditions. Additive screening identified that dimethyl sulfoxide was very effective at improving both crystal quality and size. Moreover, the selection of the cryoprotectant proved to be critical. MPD instead of glycerol or sugars improved the diffraction resolution from 3.6 to 3.0 Å.

The optimized crystals of the GroEL/ES complex from *T. thermophilus* belonged to space group $P1$, with unit-cell parameters a = 140.4, b = 156.4, c = 273.1 Å, α = 82.9, β = 85.4, γ = 68.5°. We have collected one data set from one of the largest crystals obtained (Fig. 1) by changing the orientation of the crystal in the X-ray beam every 30°. The initial images clearly show anisotropic diffraction to 3.0 Å, although later images showed some radiation damage. The data were integrated and scaled to 3.0 Å resolution (Table 1). We have obtained a data set with 614 927 total observations and 383 652 independent observations in the resolution range 40–3.0 Å with an overall completeness of 89.3% (82.9% for the last shell) and an R_{merge} of 8%. The overall $I/\sigma(I)$ is 9.6. The lower redundancy of the data can be overcome by the use of the 14-fold non-crystallographic symmetry. With two molecules in the asymmetric unit, the V_M (Matthews, 1968) of the crystals is 3.1 Å^3 Da^{-1} and the solvent content is 61%. The relatively high V_M and solvent-content values may arise because of the inherently large *cis*-cavity inside the molecule.

Molecular-replacement trials were performed with the program *AMoRe* (Navaza, 1994) using the structure of the *E. coli* GroEL/ES complex (PDB code 1aon; Xu *et al.*, 1997) as a search model. A clear peak was found with a correlation coefficient of 66.2 and an R factor of 41.7% (15–5 Å resolution). There were no unfavourable molecular contacts observed in the crystal packing (Fig. 2). Model building by manual fitting to the electron-density map using the program O (Jones *et al.*, 1991) is now under way. The application of the 14-fold non-

Table 1
Data statistics.

Statistics for the last shell (3.11–3.00 Å) are given in parentheses.

Space group	$P1$
Resolution (Å)	3.0
Unit-cell parameters (Å, °)	a = 140.4, b = 156.4, c = 273.1, α = 82.9, β = 85.4, γ = 68.5
Total observations	614927
Unique observations	383652
Completeness (%)	89.3 (82.9)
R_{merge} (%)	8.2 (64.2)
Average $I/\sigma(I)$	9.6 (1.2)

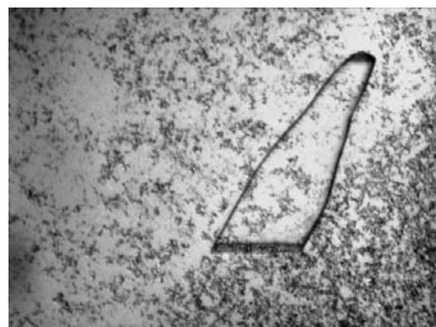

Figure 1
Crystal of the GroEL/ES complex from *T. thermophilus*. The crystal has approximate dimensions of 0.05 × 0.3 × 0.7 mm.

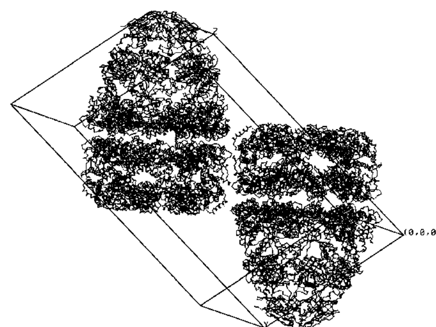

Figure 2
Crystal packing of the molecular-replacement solution.

crystallographic symmetry will help in the refinement stage.

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