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Crystallization and preliminary X-ray investigation of the *Escherichia coli* molecular chaperone cpn60 (GroEL)

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The *Escherichia coli* molecular chaperone, cpn60 (GroEL), has been purified from an overproducing *E. coli* strain and crystallized. Of the two crystal forms that were obtained, one was found to be suitable for crystallographic and structural studies at low resolution. Preliminary X-ray investigation of the crystals show unit cell dimensions: a = 143.3, b = 154.6 and c = 265 Å, with α = 82°, β = 95° and γ = 107°. The space group is P1 and the crystals diffract to a maximum of 7 Å when using CuK_α X-rays from a rotating anode. Both electron microscopy and non-denaturing electrophoretic analysis of redissolved cpn60 crystals show that cpn60 crystallizes in the native oligomeric form. Comparison between the dimensions of oligomeric cpn60 and the crystallographic unit cell volume suggests that the unit cell contains two oligomeric cpn60 molecules. The V_M value for two cpn60 molecules per unit cell is 3.5 Å^3/Da, corresponding to a water content of 65%. Electrophoretic analysis under denaturing conditions shows that the cpn60 in crystals is heterogeneous, and this probably explains the limited resolution of the diffraction data.

Molecular chaperone, GroEL; *Escherichia coli*; Crystallization; X-ray analysis

1. INTRODUCTION

The *Escherichia coli* cpn60 (GroEL) and its homologues in mitochondria (Hsp60) and chloroplasts (rubisco subunit-binding protein) belong to a class of cellular proteins called molecular chaperones which facilitate the correct folding and assembly of other proteins [1,2]. Cpn60 is constitutively expressed in *E. coli* and is vital for normal growth, but its synthesis is also induced in cells subjected to heat shock [3]. Cpn60 can bind to fully or partly unfolded proteins in vitro [4–6], and if supplemented with MgATP, K^+ and the molecular chaperone, cpn10 (GroES), it can govern the correct re-folding and restore the activity of the bound substrate concomitant with its release from the cpn60/cpn10 holo-chaperonin complex [7,8]. The molecular mass of the monomeric *E. coli* cpn60 is 57.2 kDa, as calculated from the amino acid sequence [2], whereas the functional cpn60 complex has a molecular mass of 700–850 kDa, as determined by biophysical methods [9,10]. Electron microscopic images of cpn60 viewed from above show a seven-membered ring, with a rectangular profile with four stripes in side-view, and it has been proposed that the complex consists of two cpn60 heptamers stacked on top of each other [9–11]. The binding of cpn10 is believed to take place on top of one of the cpn60 heptamers, resulting in a holo-chaperonin complex consisting of one oligomeric cpn60 and one oligomeric cpn10 molecule [11]. Such holo-chaperonin complexes can be isolated directly from *Thermus thermophilus* and have recently been crystallized [12,13]. Upon heat shock treatment in vivo, cpn60 becomes reversibly phosphorylated, and this alters its binding to its substrate [14]. Neither the identity of the phosphorylated amino acid(s) nor the stoichiometry of phosphate incorporation has been determined.

Since the molecular mechanism for function of cpn60 is largely unknown, it is important to elucidate its three-dimensional structure. As an initial step in this direction we here report on the crystallization and preliminary X-ray investigation of three-dimensional crystals of *E. coli* cpn60 (GroEL). We also suggest that the limited resolution of the crystals is due to the presence of two forms of cpn60, as revealed by SDS-polyacrylamide gel electrophoresis.

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Abbreviations: cpn60, chaperonin 60 or GroEL; cpn10, chaperonin 10 or GroES; PEG, polyethylene glycol; OGP, *n*-octyl-β-D-glucopyranoside; MES, 4-morpholinoethanesulphonic acid; Tris, tris[hydroxymethyl]aminomethane; SDS, sodium dodecyl sulphate; LB medium; Luria-Bertani medium.

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2. MATERIALS AND METHODS

2.1. *Purification of* E. coli *cpn60*

Cpn60 was purified from a recombinant *E. coli* strain containing a plasmid, pBS559, with the *groE* operon downstream of tandem bacteriophage λ promotors. It directs increased overproduction of cpn60 and cpn10 following a shift of the temperature of cultures to 42°C [15]. For purification of cpn60, cells were grown in LB broth at 30°C to an optical density of 0.5 at 595 nm. This was followed by treatment at 42°C for 4 h. Cells were lysed by the action of lysozyme and Brij-58 according to [16]. After precipitation of cpn60 from the soluble cell extract with polyethyleneimine and fractionation with ammonium sulphate, it was further purified by chromatography on columns of DEAE-Sephacel and Bio-Gel A-1.5m (B.P.S. and N.E.D., unpublished). The purity of cpn60 was estimated to be better than 95% as judged by SDS-PAGE, according to [17]. A detailed biochemical characterization of the isolated protein is presented elsewhere [15].

2.2. *Crystallization of cpn60*

Cpn60 was crystallized by means of vapour diffusion [18] with PEG8000 as the precipitating agent. Two different crystalforms with apparently different morphology were obtained. Crystalform I was grown at 20°C in hanging drops initially containing 50 mM Tris, pH 6.5. Crystalform II was grown in sitting drops after the addition of 0.25% OGP.

2.3. *Electron microscopy*

For electron microscopy, crystals were washed and redissolved by addition of 20 mM Tris, pH 8.0. Samples were negatively stained with 1% uranyl acetate and studied with a Jeol 100 CX microscope equipped with a 60 μm objective aperture and working at 60 kV.

2.4. *X-ray crystallography*

For X-ray analysis, crystals were mounted in glass capillaries sealed at both ends after removal of excess liquid. A Rigaku RU 200BEH rotating anode, with a 0.3×3 mm focal spot, running at 45 kV and 100 mA was used for CuK_α X-ray generation, and a Siemens X-1000 area detector system was used for X-ray measurements. The crystal-to-detector distance was 30 cm and a helium path was used to minimize X-ray absorption by the air. Experiments were performed at room temperature. Crystals were stable in the X-ray beam for 0.5–1 day. The 2θ angle was set to 0°; the frame width and data collection time per frame were 0.15–0.25° and 110–200 s. Frames were transferred to a Vaxstation 3100 m38 where the program XDS [19,20] was used for data reduction. The option in XDS for automatic determination of cell parameters and Bravias lattice (Kabsch, W., personal communication) was used.

2.5. *Electrophoresis*

For electrophoretic analysis, crystals were thoroughly washed and redissolved by addition of the appropriate buffer. SDS-gel electrophoresis was performed at 4°C according to Laemmli [17] using 12–22.5% polyacrylamide gradient gels. Native, non-denaturing electrophoresis was carried out using a Pharmacia Phastsystem and 4–15% polyacrylamide gradient Phastgels. Gels were stained with Coomassie brilliant blue.

3. RESULTS AND DISCUSSION

When analysed by SDS-polyacrylamide gradient gel electrophoresis, purified *E. coli* cpn60 gave two closely spaced stained bands with apparent molecular masses near 65 kDa, see Fig. 1. Since the cpn60 was isolated after heat treatment, the two bands most likely represent the modified and unmodified forms described in [14]. Such heterogeneity was not observed when the protein was analysed using SDS-polyacrylamide gels of uniform acrylamide composition (13%), nor after electrophoresis under non-denaturing conditions where cpn60 migrated as a single band with an apparent molecular mass >600 kDa (not shown). In spite of the heterogeneity revealed by the SDS-polyacrylamide gradient-gel analyses, the protein crystallized and two different crystal forms were obtained.

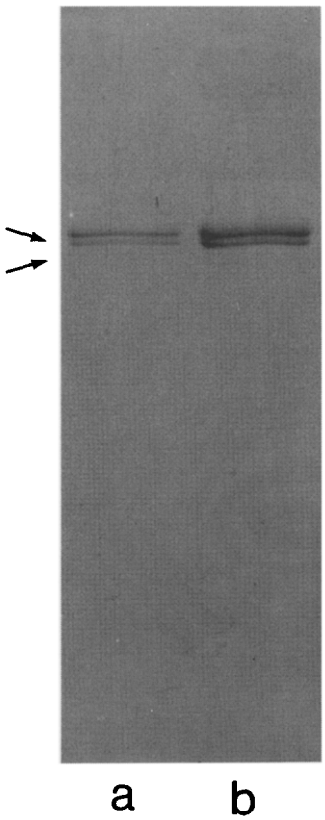

Fig. 1. SDS-polyacrylamide gradient-gel analysis of purified cpn60 (lane a) and redissolved cpn60 crystals (lane b).

3.1. *Crystalform I*

These crystals started to grow in vapour diffusion experiments after about 1 month and appeared as hexagonal discs which in some cases measured up to 0.5 mm in diameter and 0.2 mm in thickness. However, when tested for X-ray diffraction, no or very little diffraction was observed. Since reproducibility of crystallization was poor, no further analysis of this crystalform was carried out.

3.2. *Crystalform II*

The addition of 0.25% OGP seemed to be crucial for appearance of this second crystalform. Crystals began to grow after 3–4 days, reaching maximum size after about 1 month. Normally, they had the dimensions of $1.0 \times 0.5 \times 0.5$ mm, but occasionally growth in one direction extended to 3 mm. When analysed using a rotat-

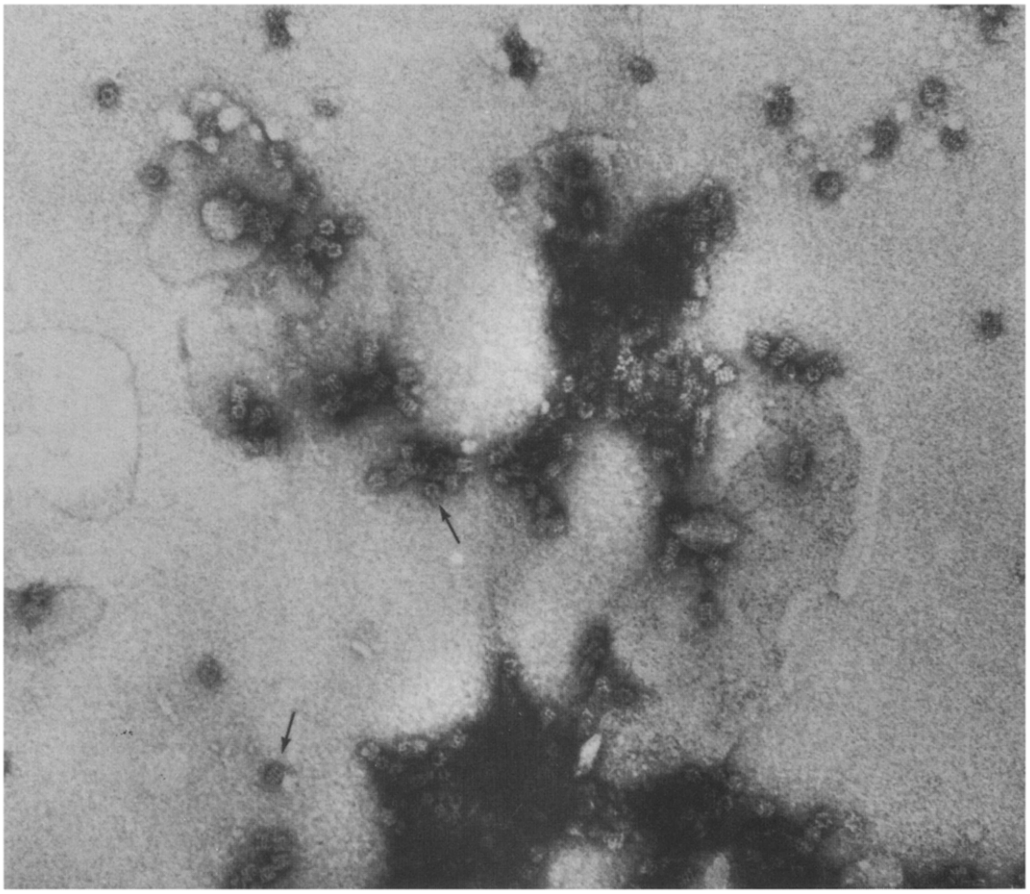

Fig. 2. Electron micrograph showing negatively stained cpn60 protein particles obtained from redissolved crystals. Magnification ×120,000.

ing anode, some crystals showed reflections out to 7 Å, however, initial crystallographic characterization was made with data collected to 8.9 Å. Cell parameters and Bravias lattice were determined in XDS by using the subroutines COLSPOT and IDXREF. Of 2,500 collected spot positions from 160 frames, 2,292 were successfully indexed. Calculated standard deviations of spot positions from calculated positions in pixels and degrees were 1.22 and 0.05, respectively. A post-refinement of cell and camera parameters using 7,028 reflections from 475 frames gave standard deviations of 0.96 pixels and 0.04°. The unit cell size was large with dimensions: $a = 143.3$ Å, $b = 154.6$ Å and $c = 265$ Å with $\alpha = 82°$, $\beta = 95°$ and $\gamma = 107°$. The space group was determined as P1, since the next best Bravias lattice solution was a monoclinic cell with a poor quality of fit. Since the stability of the crystals in the X-ray beam is limited, several will need to be analysed to collect a complete data set. Even at resolution of 8.9 Å, analysis of a complete data set (in progress), should provide valuable information about the internal structural symmetry of the cpn60 oligomeric complex and the symmetry between the complexes in the crystallographic asymmetric unit.

The protein composition of the cpn60 crystals is shown in Fig. 1, where washed, redissolved crystals were analysed by SDS-polyacrylamide gradient-gel electrophoresis. The crystals gave rise to 65 kDa protein bands which co-migrated with those from purified protein and represent different monomeric forms of cpn60. Although this shows that the crystals are indeed composed of cpn60, it does not give any information about the oligomeric state of the protein in the crystals. That cpn60 most likely crystallises in the native oligomeric form is demonstrated in Fig. 2, which shows an electron micrograph derived from crystals that had been washed, redissolved and their content negatively stained. Two different aspects of the protein were observed; both clearly show the characteristic symmetrical and morphological features of native oligomeric cpn60 [9,10]. One view shows a 7-fold ring structure for the cpn60 oligomer viewed from the top while the second represents a side-view with its rectangular shape and four stripes [11]. Furthermore, when the protein content of washed, redissolved crystals was analysed by electrophoresis under non-denaturing conditions, only a single protein band was detected, which migrated with an apparent molecular mass >600 kDa (not shown). These analyses strongly suggest that the cpn60 protein crystallized in its native oligomeric form.

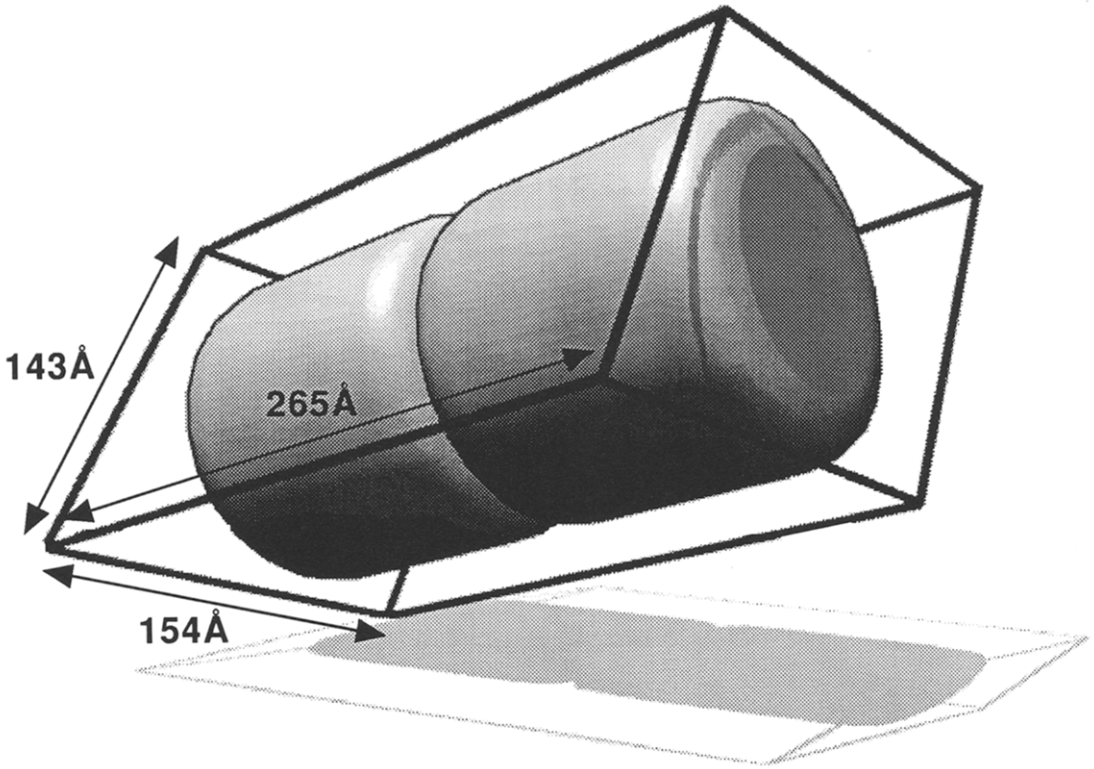

Fig. 3. Possible arrangement of two oligomeric cpn60 molecules in the crystallographic unit cell. Graphic illustration was done using the program Swivel 3D Professional for Macintosh.

The information about the oligomeric state of cpn60 in the crystals can be used to predict how many cpn60 molecules are present in the crystallographic unit cell. Based on electron microscopic analysis [9–11], oligomeric cpn60 has a cylindrical overall shape with a diameter of 13 nm and a height of 13 nm. As seen in Fig. 3, the unit cell cannot contain more than two oligomeric cpn60 molecules. Given the length of the unit cell *c*-axis (265 Å), it is possible that two cpn60 molecules are packed after each other along this axis. The V_M value [21], calculated for two cpn60 oligomers, with molecular masses of 800 kDa (14 × 57.2 kDa), is 3.5 Å^3/Da. This value is within the range normally found for protein crystals [21] and corresponds to a water content in the crystals of 65%.

Although the crystallization of cpn60 in Crystalform II is very reproducible and results in the formation of large crystals, they show diffraction only to a maximum of 7 Å. A likely explanation for this is the presence of two forms of cpn60 monomers, as revealed by high-resolution SDS-polyacrylamide gradient-gel analysis. Work is currently in progress to obtain crystals from electrophoretically pure cpn60 preparations, with the expectation that they will diffract X-rays to higher resolution. Such crystals might form the basis for future high-resolution structural studies of the native unmodified cpn60 and/or the post-translationally modified complex.

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