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Crystallization of the cpn60/cpn10 complex ('holo-chaperonin') from *Thermus thermophilus*

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A stable complex of the chaperonins, cpn60 and cpn10 (*Escherichia coli* GroEL and GroES homologues), from the extremely thermophilic bacterium *Thermus thermophilus* has been isolated and crystallized. The crystals have dimensions up to 30 × 200 × 200 μm. Ultra-thin sections of the crystals estimated by electron microscopy showed a rectangular lattice with unit cell parameters of a=17 nm, b=27 nm, γ=90°.

Molecular chaperone; Chaperonin; GroE; *Thermus thermophilus*; Crystallization; Electron microscopy

1. INTRODUCTION

Chaperonins [1] are the ubiquitous heat-shock proteins of bacteria, mitochondria and chloroplasts possessing molecular chaperone activities [2,3] thus participating in the folding, assembly or membrane translocation of some proteins (see [4–6] for recent reviews). Two chaperonin species isolated from many sources, cpn60 and cpn10, correspond to *Escherichia coli* GroEL and GroES, respectively. GroEL-like proteins exist as large particles composed of 14 subunits of about 60 kDa arranged into two stacked rings with the seven-fold rotational symmetry [7–10]. *E. coli* GroES [11] and, perhaps, homologous proteins from other sources are ring-like structures which are most likely composed of 7 monomers of about 10 kDa [12–15].

It has been shown previously that *E. coli* GroEL and GroES bind to each other in the presence of Mg^{2+} and adenine nucleotides [11,16,17], and that the complex formation is vital for their functioning as a molecular chaperone [16–20]. The knowledge of the detailed structure of chaperonin complexes is thus of great importance for understanding the mechanisms of their action. Recent electron microscopic studies have revealed a similar oligomeric structure of the reconstituted GroEL/GroES complexes from *E. coli* and stable (non-dissociating in the absence of nucleotide) cpn60/cpn10 complexes isolated from an extremely thermophilic bacterium *Thermus thermophilus* [15,21] and our unpublished data). Crystallization of the complex from *Th. thermophilus* allows us to begin the study of the three- dimensional structure of chaperonins. This paper describes crystallization conditions and electron microscopy-estimated properties of the crystals obtained.

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Abbreviations: cpn60 and cpn10, chaperonin 60 and chaperonin 10, respectively; TEA, triethanolamine; EDTA, ethylene diamine tetraacetic acid; DTT, dithiothreitol.

2. MATERIALS AND METHODS

2.1. *Purification*

The chaperonin complex was isolated from a crude ribosome wash fraction of *Th. thermophilus* by a series of successive chromatographic procedures performed on a DEAE-Toyopearl 650 M column, pH 8.0 and a Butyl-Toyopearl 650 S column (Toso, Japan), on Mono Q HR, pH 5.3 and Superose 6 HR columns (Pharmacia, Sweden). Buffer A containing 100 mM KOAc, 20 mM TEA pH 7.5, 0.1 mM EDTA, 1 mM DTT, 3 mM $Mg(OAc)_2$ was used for the final chromatography. Fractions were analyzed by SDS-PAGE and immunoblotting using highly specific antibodies to GroEL.

2.2. *Crystallization*

Crystals have been obtained using the hanging drop/vapor diffusion technique in the presence of 10% polyethyleneglycol (PEG-6000) in buffer A. For determination of their composition, the crystals were washed thoroughly by the precipitant solution, dissolved and analyzed by SDS-PAGE.

2.3. *Electron microscopy*

Crystals were fixed for 3–4 days at 4°C by vapor diffusion of 0.025% glutaraldehyde from the precipitant solution and postfixed at 4°C for 1–2 h by 0.25% glutaraldehyde in precipitant solution, dehydrated in graded ethyl alcohols and embedded in Epon-812. 50 nm sections obtained by a SuperNova ultratome (Reichert-Jung, Austria) using a diamond knife were stained by aqueous solutions of uranyl acetate and lead citrate. Specimens were examined with a JEM-100 C electron microscope (JEOL Japan) at 80 kV, 25 or 50 μm objective aperture and magnification of × 10,000–40,000. Micrographs were tested for optical diffraction.

3. RESULTS AND DISCUSSION

Unlike the *E. coli* GroEL and GroES, the complex of

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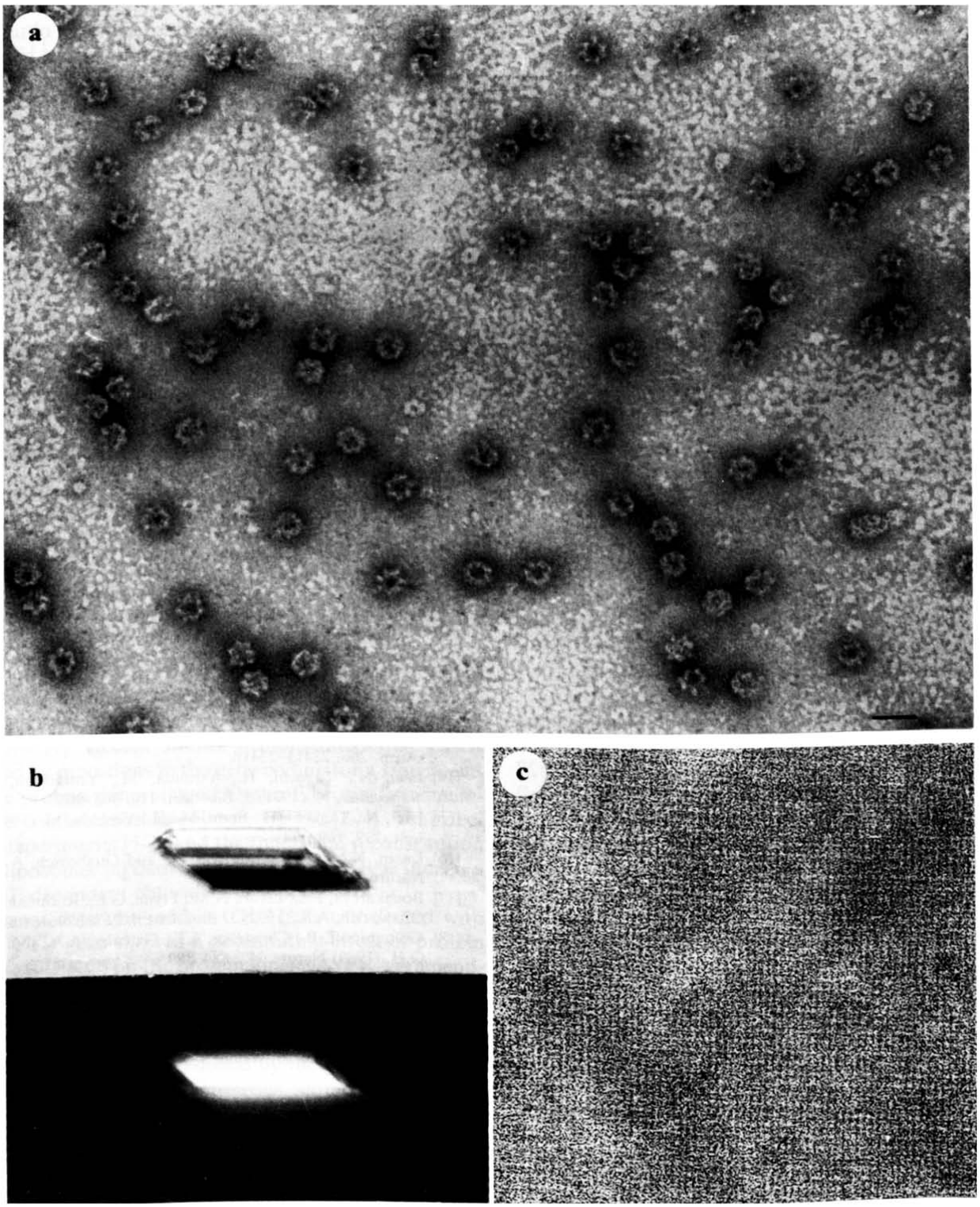

Fig. 1. Characterization of crystals of *Th. thermophilus* chaperonin complexes. (a) Electron micrograph of a cpn60/cpn10 complex preparation negatively stained by uranyl acetate using the single-layer carbon technique. Bar = 200 nm; (b) General view of a 100 μm crystal observed by light microscopy (non-polarized and polarized light). (c) Electron micrograph of a positively stained ultra-thin section of an embedded crystal.

which can be observed only in the presence of ATP or ADP, the *Th. thermophilus* cpn60 and cpn10 are isolated coupled together as a 'holo-chaperonin' [15] in the absence of nucleotides. Thus, in our case, the SDS-PAGE analysis of the final preparation of GroEL-like protein from *Th. thermophilus* demonstrated the presence of

both polypeptides of relative molecular masses slightly less than that of GroEL and GroES, respectively, in a proportion similar to that in the *E. coli* GroEL/GroES complex (data not shown). An affinity-purified antibody to *E. coli* GroEL interacted strongly with the larger polypeptide in the preparation. The native molecular mass of the complex estimated by size-exclusion chromatography on a Superose 6 HR column coincided with that of the *E. coli* GroEL particle (not shown).

Electron microscopy of the specimen negatively stained by uranyl acetate demonstrated both the sevenfold symmetry 'stars' and the 'bullet-like' [15] or 'dome-like' [21] structures (Fig. 1a) corresponding to the top and side views of the complex, respectively. The characteristic shape of the side projection confirms the associated state of two chaperonin species in our preparation. The side views have an average diameter of approximately 15 nm and look similar to those in the preparations from other sources including *E. coli* [7–10,13–15].

Crystals in the form of plates appeared in the drops within 3 days and slowly grew up to the size of about $200 \times 200 \times 30$ μm. Though the crystals were thin, they rotated the plane of polarized light (Fig. 1b). The presence of both the cpn60 and cpn10 in the ratio typical for the complex was detected using SDS-PAGE of the dissolved crystals (not shown). The crystals looked too thin to be suitable for X-ray studies. So, we used ultrathin section techniques for determining their ordering.

Fig. 1c shows an ultrathin section of cpn60/cpn10 crystal. The well ordered lattice is clearly seen. The periodicity parameters of the lattice are: a=17 nm, b=27 nm, γ=90°. These parameters correspond to the overall dimensions of the chaperonin complex (diameter 15 nm, length 18 nm). The resolution limit of 5 nm makes it impossible to precisely determine the orientation of the complexes relative to the plane of the section. However, the lattice parameters do not contradict an antiparallel side orientation of two 'holo-chaperonin' particles on the observed projection of the unit cell. It is clear that the recently reported two-dimensional crystals of the *Th. thermophilus* cpn60/cpn10 complex [14] had different parameters of periodicity and our crystals have a more tight arrangement.

Thus, three-dimensional crystals of the *Th. thermophilus* cpn60 and cpn10 complex have been obtained for the first time. Our data demonstrate a tight packing of the oligomers in such a crystalline form. The crystals we obtained may be promising for future structural studies of the chaperonins by X-ray analysis.

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