

COMMUNICATIONS

Two-dimensional Crystals of the Molecular Chaperone GroEL Reveal Structural Plasticity

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For two-dimensional (2-D) crystallization we have purified the molecular chaperone GroEL from *Escherichia coli* to homogeneity. The final and important step for crystallization in the purification procedure was an ATP-agarose column, on which the spacer between ATP and agarose was attached to C8 of adenine. Using the mica spreading “negative staining-carbon film” procedure and polyethylene glycol as a precipitant, we obtained four different 2-D periodic arrays. Two of them turned out to be true crystals. One crystal has *P*2 symmetry and lattice constants of $a = 24.3$ nm and $b = 16.9$ nm, the other has essentially *P*4 symmetry and shows smoothly varying local changes in the lattice parameters ($a = b = 23(\pm 1.3)$ nm). Very striking in the *P*4 crystal is the departure within each individual GroEL particle from the GroEL-typical seven-fold symmetry, which seems to be required for GroEL to accommodate to a crystal symmetry.

Keywords: molecular chaperone; GroEL; 2-D crystal; protein structure; protein folding

The *Escherichia coli* protein GroEL belongs to a family of proteins called molecular chaperones (Ellis, 1987) which are considered to be involved in the folding, assembly and transport of proteins (Goloubinoff *et al.*, 1989a,b; Laminet *et al.*, 1990; Buchner *et al.*, 1991; Martin *et al.*, 1991; Mendoza *et al.*, 1991; Viitanen *et al.*, 1991; Zahn & Plückthun, 1992). Homologs of GroEL, called chaperonin 60, have been found in mitochondria and in chloroplasts (Ellis & van der Vies, 1991). Although GroEL is a heat-shock protein, it is also essential for growth at normal temperatures (Fayet *et al.*, 1989). GroEL has a weak Mg^{2+} and K^{+} -dependent ATPase activity (Viitanen *et al.*, 1990), which is partially inhibited by a second chaperonin, GroES (Chandrasekhar *et al.*, 1986), and interacts *in vitro* with non-native states of a multitude of *E. coli* proteins (Viitanen *et al.*, 1992).

Native GroEL protein is a tetradecameric protein, consisting of two stacked rings with seven identical 60 kDa subunits in each ring; each subunit contains two domains of approximately equal size which appear as a double-ring in side view. The seven-fold symmetry is readily apparent in the end-

on views of the native molecule in the electron microscope (Hendrix, 1979; Hohn *et al.*, 1979; Zwickl *et al.*, 1990). In the side-on view, the tetradecameric chaperonin GroEL has a rectangular shape with a characteristic pattern of striations, representing the two double rings. For obtaining more detailed insight into the structure of GroEL by means of electron crystallography, the availability of well-ordered two-dimensional (2-D†) crystals is a prerequisite. Several attempts have been made previously to produce 2-D crystals of GroEL or homologs of it. However, at best, very tiny and rather poorly ordered arrays have been obtained (e.g. Ishii *et al.*, 1991) and their crystalline nature is not beyond doubt. In this communication we describe the methods used to produce such 2-D crystalline arrays and we characterize the major crystal forms we obtained.

For 2-D crystallization we purified the GroEL protein from lysates of cells harboring the multi-copy plasmid pOF39 (Fayet *et al.*, 1989) in two

† Abbreviations used: 2-D, two-dimensional; NS-CF, negative staining-carbon film; PEG, polyethylene glycol.

steps. The first purification step was an ion-exchange chromatography step on a DEAE-Sephacel column (Pharmacia). The proteins were eluted with a 0 to 500 mM-NaCl gradient in a buffer containing 50 mM-Tris·HCl (pH 7.2), 1 mM-DTT, 0.1 mM-EDTA. The second step was gel filtration on a Sephacryl S-300 column (Pharmacia). After this procedure, the protein was assessed to be pure by SDS-PAGE, but under the electron microscope some amorphous material could still be seen. Because this "amorphous" material interferes with crystallization, we have developed a further purification step with an ATP-affinity column.

We tested four ATP-agarose columns (Sigma), which differ in the attachment of the spacer between ATP and agarose, and in spacer length. GroEL bound only to one of these agarose columns, namely the one with the spacer attached to C8 of adenine, but did not bind to ones with the spacer attached to the adenine NH_2 nor to the ribose OH. In the presence of 20 mM- MgCl_2 in a buffer containing 50 mM-Tris·HCl (pH 7.2), 1 mM-DTT, 0.1 mM-EDTA there was quantitative binding of native GroEL. The GroEL was eluted as a sharp peak with the same buffer by leaving out MgCl_2 . We conclude that GroEL binds ATP only in the presence of Mg^{2+} and when ribose-triphosphate and the amino group of the purine ring are accessible.

2-D crystals of GroEL were prepared by the mica-spreading "negative staining-carbon film" (NS-CF) procedure. This technique was originally described by Horne & Pasqualli-Ronchetti (1974) and has been applied to macromolecules (Harris, 1982). More recently, the technique has been modified by the inclusion of polyethylene glycol (PEG; Wells *et al.*, 1981) and successfully applied to a range of different macromolecules (Harris, 1991; Harris & Horne, 1991). Small (10 μl) volumes of GroEL (0.5 to 1.0 mg/ml in 5 mM-Tris·HCl buffer (pH 8.0)) were mixed with an equal volume of 1% (w/v) ammonium molybdate containing 0.2% (v/v) PEG (M_r 1500) adjusted to pH 8.0 with 1.0 M-NaOH, and spread evenly over the surface of a small piece of freshly cleaved mica. Excess fluid was removed by holding the mica pieces vertically onto a filter paper for five seconds. The remaining layer of fluid was allowed to dry at room temperature. 2-D crystals form at the fluid-mica surface and are evenly spread under appropriate conditions, but considerable aggregation of GroEL was encountered, which often interfered with 2-D crystal formation. The dried layer of protein plus ammonium molybdate-PEG was then coated *in vacuo* with approximately 5 nm of carbon, and the layer of carbon with adsorbed protein was floated onto the surface of a 2% (w/v) solution of aqueous uranyl

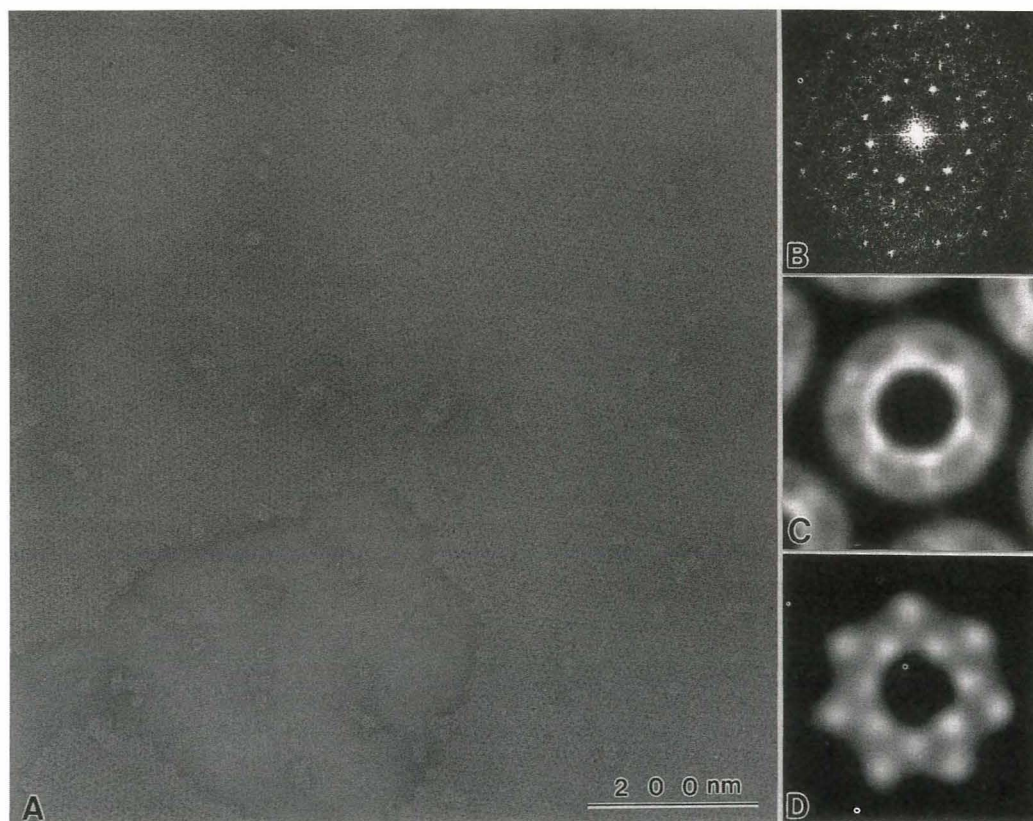


Figure 1. A, Close-packed arrays of GroEL showing individual molecules in the end-on orientation. B, The corresponding power spectrum. C, Correlation averaging (351 motifs) without rotational alignment of the individual molecules produces indistinct and featureless averages. D, Single particle averaging including rotational alignment yields clear averages with 7-fold symmetry. The average is based on 850 individual motifs. The diameter of the GroEL molecule is 13.3 nm.

acetate. Bare 400 mesh grids were then brought from beneath the floating carbon film to pick up the specimen (Harris & Horne, 1991).

Grids were examined in a Philips EM420 transmission electron microscope at an accelerating voltage of 100 kV and micrographs were recorded at nominal magnifications of $\times 36,000$ and $\times 49,000$. For image processing, micrographs containing 2-D arrays were preselected by laser diffractometry, judging defocus, astigmatism and crystalline order. Selected images were digitized using an EIKONIX camera Model 1412 at a step size of $15\ \mu\text{m}$, corresponding to $0.42\ \text{nm}$ and $0.31\ \text{nm}$ at the specimen level; $2048\ \text{pixel} \times 2048\ \text{pixel}$ arrays were taken

and processed using standard correlation averaging methods (Saxton & Baumeister, 1982) as implemented in the SEMPER image processing system (Saxton *et al.*, 1979). Some of the images were also subjected to distortion analysis (Dürr, 1991) and to unbending procedures (Dürr *et al.*, 1991).

When examining the grids, an unusual variety of different 2-D periodic arrays of GroEL was observed. Very abundant is an undulatory type of array with the GroEL complexes in the side-on orientation (data not shown). Since it turned out difficult to define a unit cell, (there are apparently slips between rows and there is also disorder within rows) we have not analyzed further this para-

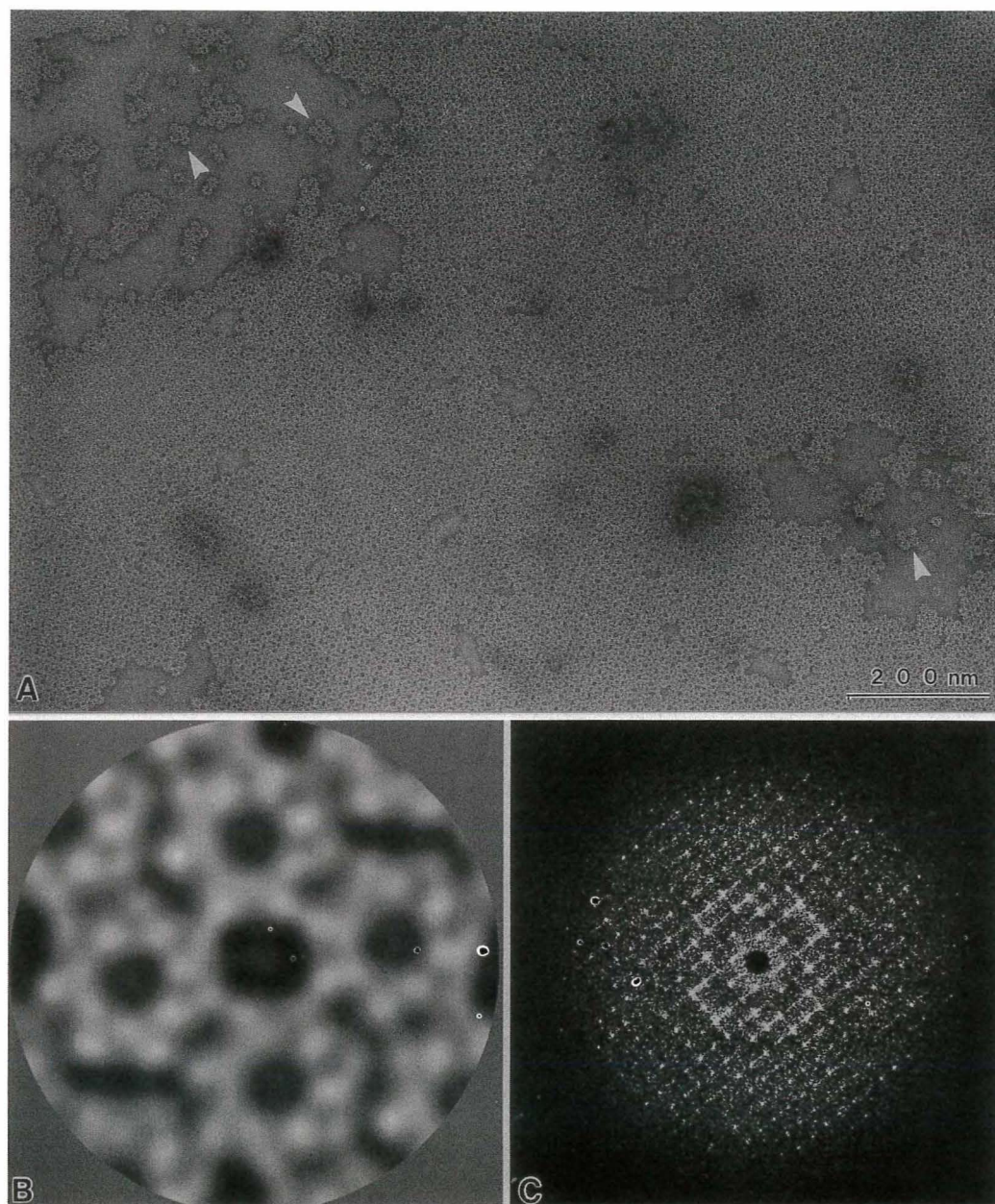


Figure 2. A, 2-D crystalline patches of GroEL. The lattice has $P4$ symmetry, although the lattice constants are subject to significant local variations. Possibly groups of 4 molecules (white arrows) act as disperse nucleation structures. B, Correlation average derived from 350 individual motifs; no symmetry has been imposed. C, The corresponding power spectrum shows somewhat blurred spots reflecting substantial disorder.

crystalline array. Also very abundant were extensive pseudo-hexagonal arrays of GroEL in the end-on orientation (Fig. 1). These, however, turned out to be merely close-packed arrays: when treated as crystalline arrays correlation averages yielded more or less featureless rings (Fig. 1C); however, when single-particle averaging techniques were applied to the same data, i.e. when translational and rotational alignment of each individual molecular image was performed, the sevenfold symmetry was clearly revealed (Fig. 1D).

Less frequently than these two types of arrays, which are not useful for electron crystallographic purposes, we found two types of arrays which turned out to be truly crystalline. One of them has *P2* symmetry with lattice constants of $a = 24.3$ nm and $b = 16.9$ nm (data not shown). Unfortunately this crystal modification is often very weakly stained and the images thus generated are probably dominated by positive staining and therefore difficult to interpret. More useful for electron crystallographic purposes is a lattice, which has basically *P4* symmetry (Fig. 2) although it shows smoothly varying local changes in the lattice parameters ($a = b = 23(\pm 1.3)$ nm). These changes are not due to an unintentional tilting of the specimen; it rather appears from distortion analysis that the unit cell area, and thus the packing density, is subject to a significant local variation (Fig. 3). Very striking is

the departure within each individual complex from the sevenfold symmetry. This sevenfold symmetry, however, is clearly visible in the individual molecules found in the immediate vicinity of these arrays (see also Fig. 1), indicating that the deviations from rotational symmetry are related to the crystallization.

Figure 4 shows a single particle average, side-by-side with a single GroEL complex extracted from the lattice average. It appears that the departure from the sevenfold symmetry is required for the particle to accommodate to the crystal symmetry. The changes within the GroEL complex, but perhaps also the local changes in the crystal packing, reflect an unusual plasticity of the GroEL molecule, allowing for large domain or subunit movements which may be of importance for the biological function of the molecule. Presumably the periodic binding and discharging of partially unfolded substrate requires such major conformational changes. Furthermore, structural changes and changes of dissociation constant of GroEL and substrate with temperature were also recently observed by spectroscopic techniques (R. Zahn & A. Plückthun, unpublished results).

For obtaining large and well-ordered 2-D crystals for high-resolution electron crystallography and probably also for three-dimensional crystallization for X-ray crystallography, it will be mandatory to

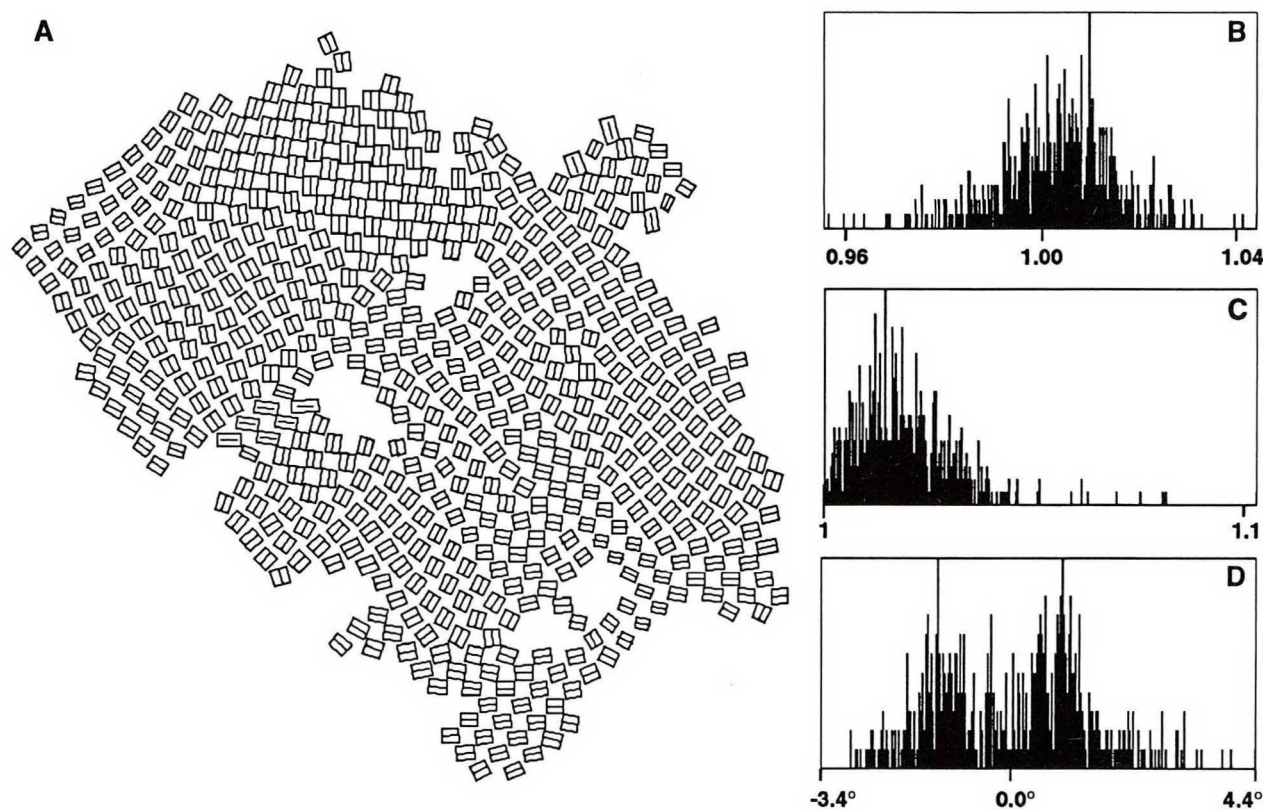


Figure 3. Distortion analysis of a crystal fragment from Fig. 2. A, Distortion map. The rectangular boxes indicate local changes in unit cell size, and maximum elongation direction (magnified 6 times). The histograms give a quantitative measure of the distortion parameters: B, the magnification of the unit cells; C, unit cell elongation factors; D, unit cell rotation.

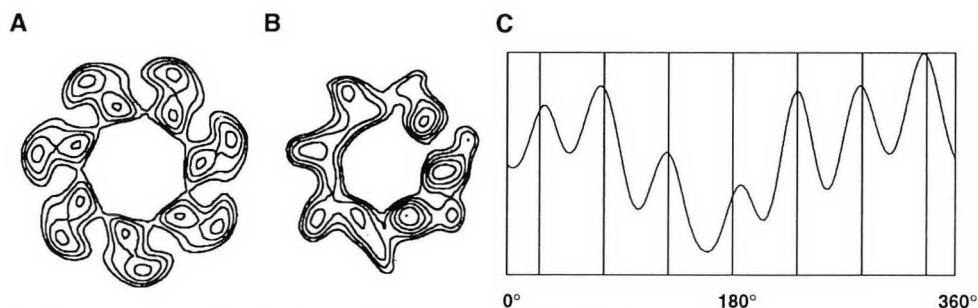


Figure 4. A, Contour plots of a single particle average of GroEL (340 motifs). The 7-fold symmetry is obvious. B, A single GroEL complex extracted from the lattice average (see Fig. 2). C, The rotational correlation function of (B), which was generated by extracting a single "subunit" as a reference motif, emphasizes the deviation from the 7-fold symmetry.

identify the parameters which play a role in GroEL conformation and interaction. It will be of utmost importance on the one hand to minimize self-association of GroEL and on the other hand to "condition" the molecule such that those interactions are promoted which enable the molecule to accommodate in a lattice rather than forming close-packing or paracrystalline arrays.

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