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GroEL: A Proteinaceous "Surfactant" ?

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GroEL, a chaperonin from *E. coli*, is responsible for folding and refolding globular proteins *in vitro* [1]. It has also been reported that GroEL improves the ability of a membrane protein synthesized *in vitro* to insert post-translationally into liposomes [2]. Here we investigate the behavior of GroEL towards membrane proteins. One of the membrane proteins studied in this respect is the 105aa S protein, a prototype holin from bacteriophage λ. S accumulates in the cytoplasmic membrane during late gene expression until, at a time programmed into its primary structure, it disrupts the membrane and allows the λ lysozyme, R, to attack the cell wall [3].

In order to characterize how S may be affected by GroEL, solubilized S protein was subjected to detergent removal by dialysis. Interestingly, in the presence of GroEL, S remained in solution after the detergent had been removed while in the control sample (without GroEL) S precipitated. Concerning the question how GroEL interacts with membrane proteins, GroEL was examined in the electron microscope in the absence and presence of S. Briefly, GroEL and GroEL plus S at 0.1 mg protein/ml were negatively stained using a 2% (w/v) aqueous solution of uranyl acetate and omitting any fixation steps [4]. Specimens were observed in a Zeiss 10C operated at 80 kV and images recorded at calibrated magnifications (35,500x and 27,000x). Selected micrographs were digitized using a Leafscan 45 at 20 μm increments corresponding to 0.56 and 0.74 nm/pixel, respectively, and processed using the IMAGIC 5 [5] and EMAN [6] software packages. Fig.1 depicts the results obtained after single particle analysis demonstrating central as well as peripheral protein densities in the samples that contained GroEL+ S. This finding indicates that GroEL may be providing a hydrophobic surface that can bind and prevent the membrane protein from precipitating. It is furthermore conceivable that other S molecules may be recruited to the core of GroEL-bound S giving rise to the peripheral densities. As the potential for high-resolution data retrieval is considerably enhanced using an electron crystallographic approach, the growth of 2-D crystals was attempted. Using the lipid-monolayer technique in the presence of charged nickel-chelating lipids, His-tagged GroEL assembled into 2-D crystals. Crystals were harvested onto holy Formvar/carbon grids and stained using 1% (w/v) uranyl acetate [4]. Micrographs were recorded and digitized as described above. Crystallographic processing was carried out using the software package CRISP [7]. Two different crystals forms were found, one consisting of GroEL in a side-on projection (Fig. 2a), and the other comprising molecules viewed end-on (Fig. 2b). The corresponding projection maps (Fig. 2c and d, respectively) echo the structural features found by single particle averaging. However, in the end-on view (Fig. 2b and d) the central protein deficit is more pronounced compared to Fig. 2a. This difference seems to be due to a more effective stain retention in the densely packed protein crystals compared to single molecules protruding from the support film (compare e.g. single end-on projections in Fig. 2a with b). Co-crystallization trials of GroEL + S are currently under way.

The ability of GroEL to solubilize membrane proteins allows the use of assays that are sensitive to detergent and may make it feasible to attempt 2-D and 3-D crystallography studies. Moreover, this approach may be applicable to other systems requiring detergent-free preparation of membrane

proteins. Finally, these results suggest that GroEL may be involved in the insertion of integral membrane proteins into the lipid bilayer, a role heretofore unsuspected.

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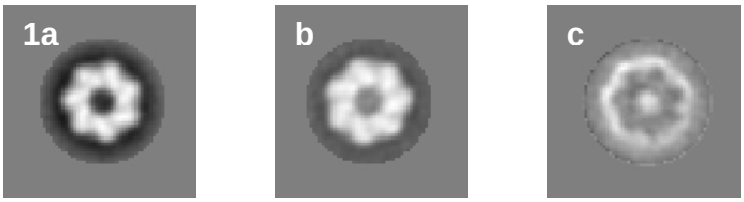

FIG. 1. Single particle averages (end-on views) of (a) GroEL, (b) GroEL+S and (c) difference map (b) minus (a). Box sizes are 36 x 36 nm.

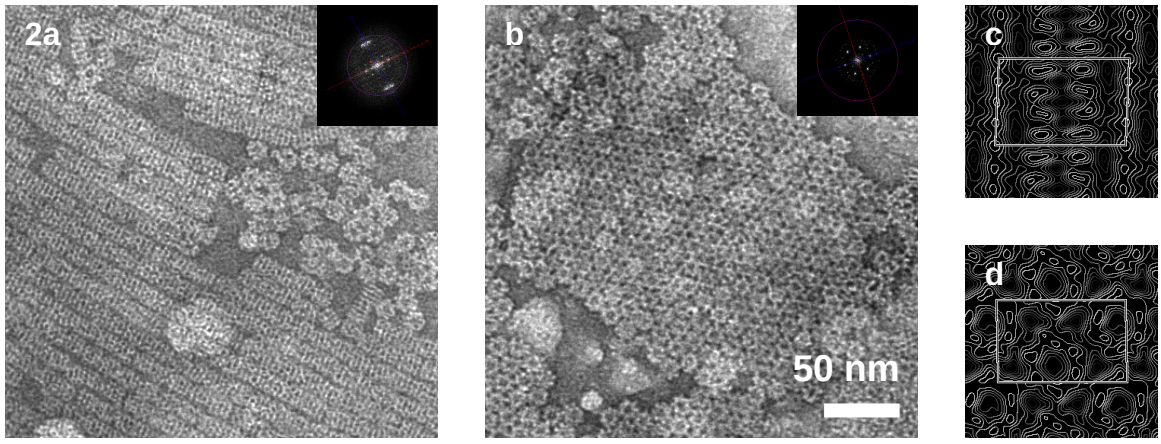

FIG. 2. Electron micrographs of negatively stained 2-D crystals of GroEL (a,b) and corresponding projection maps (c,d). The inserts in (a) and (b) show typical calculated diffraction patterns. Unit cell dimensions are a x b = 21.7 × 14.6 nm ($\gamma = 87.7°$) for side-on view crystals (a,c) and 24.4 × 13.9 nm ($\gamma = 88.3°$) for the end-on view crystals (b,d). Note the presence of end-on projections in (a) that are not part of the lattice. The map in (c) was calculated in p2 two enhance the twofold symmetry. The map in (d) was calculated in p1.

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