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Allostery in chaperonin function

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In recent years it has become apparent that both in vivo and in vitro folding of many proteins is facilitated by one or more members of a class of proteins collectively named 'molecular chaperones'. Under certain circumstances, proteins may unfold and form insoluble aggregates (as in a hard-boiled egg or in β -amyloid plaques in Alzheimer's disease). Protein aggregation can also take place under normal physiological conditions. By analogy to human chaperones, molecular chaperones prevent 'improper interactions' between potentially complementary surfaces that may lead to formation of protein aggregates. Our research focuses on the GroE molecular chaperone system from *Escherichia coli* which comprises GroEL and its helper-protein GroES (Horovitz, 1998). GroEL consists of two heptameric rings of identical subunits stacked back-to-back. It assists protein folding in an ATP-regulated manner. Each subunit of GroEL contains an ATP binding site (the entire GroEL molecule therefore has 14 ATP binding sites). Binding of ATP to GroEL triggers large conformational changes and involves both positive cooperativity within rings and negative cooperativity between rings (Yifrach & Horovitz, 1996). We are trying to understand the molecular basis of the ATP-induced conformational changes in GroEL (and also other chaperonins) and their role in GroEL-assisted protein folding both in vitro and in vivo.

Steady-state kinetic studies carried out by us during the past several years suggested a nested model for cooperativity in GroEL function. According to this model, each ring of GroEL is in equilibrium between a tense (T) state, with low affinity for ATP and high affinity for non-folded proteins, and a relaxed (R) state with high affinity for ATP and low affinity for non-folded proteins (Yifrach & Horovitz, 1996). The GroEL double-ring structure is thus in equilibrium between the TT, TR and RR states. The model was extended to include the effects of GroES. It was shown that binding of GroES to one ring of GroEL facilitates the T to R transition of the other ring, thereby promoting protein release from that ring (Inbar & Horovitz, 1997). Cooperativity in GroEL with respect to ATP was also analysed using transient kinetics (Yifrach & Horovitz, 1998a). The steady-state and transient kinetic data were combined in order to obtain information

regarding the pathway and transition state of the allosteric transition (Yifrach & Horovitz, 1998b).

The relationship between cooperative ATP binding by GroEL and the kinetics of GroE-assisted folding of two substrates with different GroES dependence, mouse dihydrofolate reductase (mDHFR) and mitochondrial malate dehydrogenase (MDH), was recently examined using cooperativity mutants of GroEL (Yifrach & Horovitz, 1999). Strong intra-ring positive cooperativity in ATP binding by GroEL was found to decrease the rate of GroEL-assisted mDHFR folding owing to a slow rate of the ATP-induced transition from the protein acceptor (T) state to the protein release (R) state. Inter-ring negative cooperativity in ATP binding by GroEL was found to affect the kinetic partitioning of mDHFR, but not of mitochondrial MDH, between folding in solution and folding in the cavity underneath GroES. These experiments demonstrate, for the first time, that protein folding by GroEL is coupled to cooperative ATP binding.

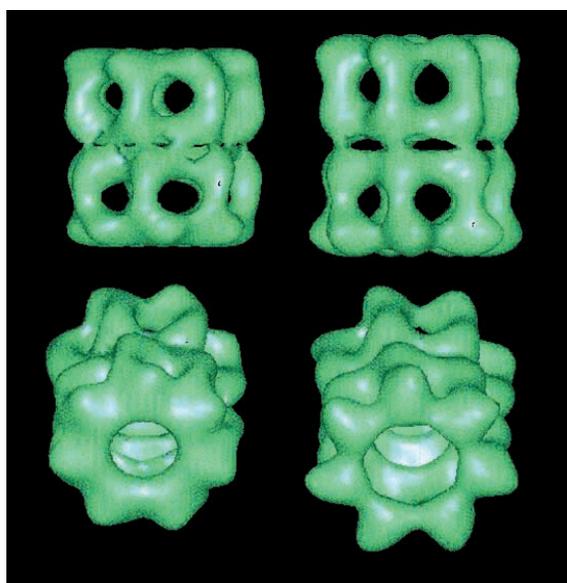


Fig. 1. Side and bottom views of the TT (left) and RR (right) states of GroEL at 30 Å resolution obtained by Helen Saibil and co-workers (Birkbeck College, London) using electron cryo-microscopy and single particle reconstruction.

The above-described in vitro work is complemented by recent in vivo studies using *E. coli* strains in which the *groEL* gene is deleted and the only GroEL protein present in the cells is plasmid-derived. The phenotypes of cells containing GroEL mutants with altered allosteric properties are being characterized. Other work in progress concerns analysis of the allosteric mechanism of the eukaryotic homologue of GroEL, CCT, which also has a double-ring structure but contains eight different subunits in each ring. It is likely that the mechanism of this chaperonin will be quite different from that of GroEL.

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