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Structural and functional aspects of allostery in chaperonins

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Protein folding in vivo and in vitro is facilitated by a family of proteins named 'molecular chaperones'. Our research focuses on a subfamily of molecular chaperones named chaperonins which are divided into type I found in eubacteria, mitochondria and chloroplasts and type II found in archaea and the eukaryotic cytosol. Type I chaperonins, such as GroEL from E. coli, consist of 14 identical subunits that form two heptameric rings. They have helper-proteins such as GroES from E. coli. Type II chaperonins consist of two eight- or nine-membered rings that are made up of two types of subunits in the case of the archaeal thermosome or eight different subunits in the case of the cytoplasmic eukaryotic chaperonin containing TCP-1 (CCT). Both type I and type II chaperonins assist protein folding in an ATP-regulated manner. The ATPase activity of chaperonins involves complex allosteric regulation. The focus of our research is to understand the molecular basis of allosteric transitions in chaperonins and how they relate to their function. Steady-state and transient kinetic data led us to propose a nested allosteric model for GroEL. According to this model, each ring of GroEL is in equilibrium between a T state (with low affinity for ATP and high affinity for protein substrates) and an R state (with high affinity for ATP and low affinity for protein substrates). A second level of allostery is reflected in inter-ring negative cooperativity. These allosteric states of GroEL in the presence of ATP have been visualized using cryo-EM. Kinetic data for CCT shows that it also undergoes two ATP-induced allosteric transitions which, in contrast to GroEL, may not be concerted. Specific questions we are addressing are:

What is the relationship between allostery in the GroE system and its folding function?

Recently, we reported the first data on the relationship between GroEL-assisted protein folding rates and allostery in GroEL. Using different cooperativity mutants of GroEL, a linear relationship was found between the folding rate of mouse dihydrofolate reductase (mDHFR) and the rate of the T to R transition. We also found linear relationships between the folding rate of mDHFR and the extent of inter-ring negative cooperativity. These experiments demonstrated, for the first time, that protein folding by GroEL is coupled to cooperative ATP binding. In addition, we have been analysing the importance of allostery in GroEL in vivo by generating E. coli strains which express only plasmid-derived GroEL (wild-type or different mutants which are defective in their allosteric properties) and characterizing their phenotypes.

What is the pathway(s) of ATP-induced allosteric transitions of GroEL?

The atomic-resolution structures of the relatively stable end states of several allosteric proteins are known but the pathways by which they interconvert are generally not known. We are trying to address this issue using GroEL as a model system. One approach is to employ linear free energy relationships of physical organic chemistry such as the Brönsted plot. Our data so far suggests that in the transition state of the T to R reaction of GroEL the inter-subunit R197-E386 salt-link is broken thus enabling rotation of subunits in the plane of the ring but that the upward shift of the apical domains has not yet taken place. Our data also led to deriving a kinetic criterion for concerted allosteric transitions. We are also interested in theoretical methods that may shed light on pathways of information transfer in this molecule.

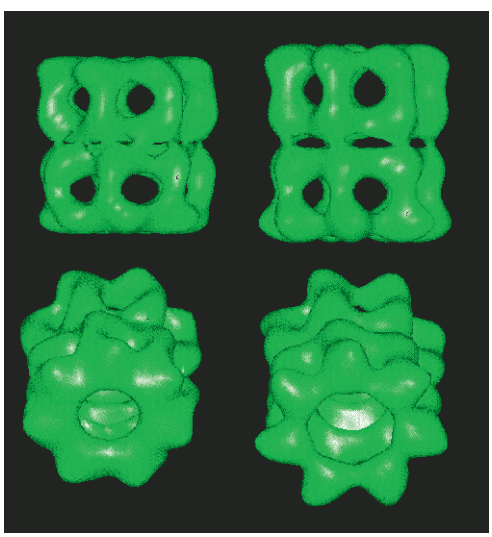

Fig. 1 *Cryo-EM images of the TT (left) and RR (right) allosteric states of GroEL at 30Å resolution (Prof. H. Saibil, Birkbeck College).*

What is the mechanism and function of allostery in CCT?

Recently, we showed that CCT displays positive intra-ring cooperativity and negative inter-ring cooperativity with respect to ATP. Virtually nothing is known about the mechanism of allosteric transitions in CCT and the role of allostery in its function. One attractive hypothesis is that the subunit heterogeneity of CCT facilitates sequential progression of conformational changes around the ring which, in turn, facilitates sequential folding of protein domains. We are carrying out kinetic experiments on ATP binding to CCT to examine such a model. We are also carrying out genetic and biochemical experiments on CCT from yeast in collaboration with Prof. K. Willison from Chester Beatty Laboratories, London.

Selected Publications

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Acknowledgements

Our work is supported by The Israel Science Foundation and the Minerva Foundation. A. H. holds the Carl and Dorothy Bennett Professorial Chair in Biochemistry.