The Stereochemistry of Chaperonin Assisted Protein Folding

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Chaperonins are large double toroidal molecular assemblies that proofread and edit the proper folding of proteins in an ATP dependent manner. GroEL, the archtype and best studied of the class I chaperononins, is an essential protein in *E. coli*. It is composed of two rings, each composed of seven 60-kD subunits, stacked back-to-back. Non-native polypeptides, but not most native proteins, are bound to the opening of the central cavity. ATP and GroES, a ring-like co-chaperonin of seven 10-kD subunits, bind asymmetrically to one ring (the *cis* ring) creating a large folding chamber into which the non-native polypeptide is released to fold in isolation. During a 15-second folding half-cycle, the seven bound ATP molecules are hydrolyzed, weakening the *cis* assembly. When ATP and GroES bind to the opposite ring, the weakened ADP *cis* complex collapses, releasing ADP, GroES, and a folded polypeptide. Thus, the products formed in one ring are expelled upon loading the reactants in the second ring as in a two-stroke engine. Three crystal structures, unliganded GroEL (2.8 Å), GroEL/ATP-γS_{14} (2.4 Å), and GroEL/GroES/ADP_7 (3.0 Å) reveal the mechanism for (1) the highly cooperative formation of the *cis* ring, (2) unfolding of a misfolded polypeptide, (3) the release of the polypeptide into the *cis* folding chamber, (4) the disassembly of the *cis* complex and release of folded protein into the environment, and (5) the nearly absolute negative cooperatively that underlies the "two stroke engine" behavior of the double toroid. The size of the unit cell (1.24×10^7 $Å^3$) and mass of asymmetric unit (nearly 10^6 Da) require the use of very bright, well conditioned synchrotron beams such as those provided by ID19 at the Advanced Photon Source (APS) and X25 at the National Synchrotron Light Source (NSLS).