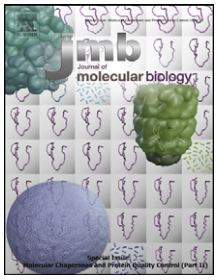
Review



Reaction Cycle of Chaperonin GroEL via Symmetric "Football" Intermediate

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Abstract

Chaperonin GroEL is an essential chaperone that assists in protein folding in the cell. Since one GroEL ring binds one GroES heptamer, the GroEL double ring permits the formation of two types of GroEL:GroES complexes: asymmetric 1:1 "bullet"-shaped and symmetric 1:2 "football"-shaped GroEL:GroES$_2$ complexes. There have been continuing debates about the mechanism and which complex is critical to the chaperonin-assisted folding. In this review, I summarize the recent progress on the football complex.

Protein folding often competes with a side reaction, intermolecular aggregate formation, which is prevented by a variety of chaperones [1–3]. A global aggregation analysis of thousands of *Escherichia coli* proteins, using a reconstituted cell-free translation system, revealed that ~30% of proteins are aggregation prone in the absence of chaperones [4], and most are rescued by one or more conserved chaperones, such as GroEL/GroES (GroE) or DnaK /DnaJ/GrpE (DnaK system) [5].

Chaperonins are a subclass of chaperones that promote protein folding in the cell [6–8]. The best-characterized chaperonin is *E. coli* GroEL and its cochaperonin GroES [3,6–8]. The GroEL subunit consists of three domains: an equatorial ATP-binding domain, an apical domain involved in substrate protein binding, and an intermediate domain with a hinge region connecting the equatorial and apical domains [9,10]. The GroEL subunit forms a heptameric ring, which stacks back to back to form a double-ring structure, and requires the dome-shaped heptameric GroES. GroEL assists in the folding of a wide variety of substrate proteins in the cell in an ATP-dependent manner, with the aid of GroES. Kinetic analyses of ATP hydrolysis by GroEL revealed the nested allosteric behavior of GroEL: intra-ring positive cooperativity and inter-ring negative cooperativity [11]. ATP binding to the GroEL rings induces the positive cooperative upward movement of the intermediate and apical domains, leading to the formation of a GroEL ring that binds GroES (the *cis*-GroEL ring), which has a central cavity for the encapsulation of the substrate protein [10,12,13]. Substrate encapsulation within the chaperonin cavity is critical to the growth of *E. coli* [14]. An *in vitro* experiment revealed that proteins with sizes up to ~60 kDa can be accommodated in the GroE cavity [15].

Elucidating the mechanism by which GroEL assists in protein folding in combination with GroES and ATP is one of the greatest challenges in the chaperone field. Although extensive efforts have been dedicated toward clarifying the mechanism of GroEL, including single-molecule experiments [16,17], how GroES coordinates the reaction cycle of GroEL is still under debate and is discussed here. In this mini-review, I summarize the recent progress on the quaternary structures of the GroEL and GroES complexes, with particular focus on a symmetric intermediate, the so-called "football"-shaped complex. Other details about GroEL have been summarized in previous reviews [6–8,18–20].

Asymmetric "Bullet" and Symmetric "Football" Complexes

An electron microscopy (EM) analysis revealed that the GroEL tetradecamer is a seven-membered ring as viewed from the top and is a rectangular

(square) shape from the side [21,22], which were confirmed by the crystal structure of GroEL [9]. An EM analysis of the chaperonin from a thermophilic eubacterium, *Thermus thermophilus*, which was isolated as a holo-chaperonin complex of GroEL and GroES, showed that the side views of the GroEL:GroES complex resemble bullet and football (American football) shapes [23,24].

Since one GroEL ring binds one GroES heptamer in the presence of ATP or ADP, the GroEL double ring permits the formation of two types of GroEL:GroES complexes (Fig. 1): the asymmetric 1:1 bullet-shaped and the symmetric 1:2 football-shaped GroEL:GroES$_2$ complexes [25]. EM analyses revealed that the bullet-shaped particles are formed in the presence of ADP [26–28], whereas both the bullet-shaped and the football-shaped particles were detected in the presence of either ATP or nonhydrolyzable ATP analogues, such as AMPPNP [27–32]. An analytical ultracentrifugation analysis showed the formation of the football complex in the presence of ATP under equilibrium conditions [33]. In addition, biochemical quantification including chemical crosslinking also revealed the football complex [34–36]. Although the football complex has been detected under a variety of conditions, the percentage of football particles is well correlated to the [ATP]/[ADP] ratio and to the K^+ concentration [37], consistent with the accumulation of the football complex in the presence of nonhydrolyzable ATP analogues. Each of the two folding cavities in the football complex can accommodate the substrate proteins at the same time, as revealed by a stable football complex in the presence of fluoroberyllate (BeFx), which mimics the phosphate moiety of the enzyme-bound nucleotides [32].

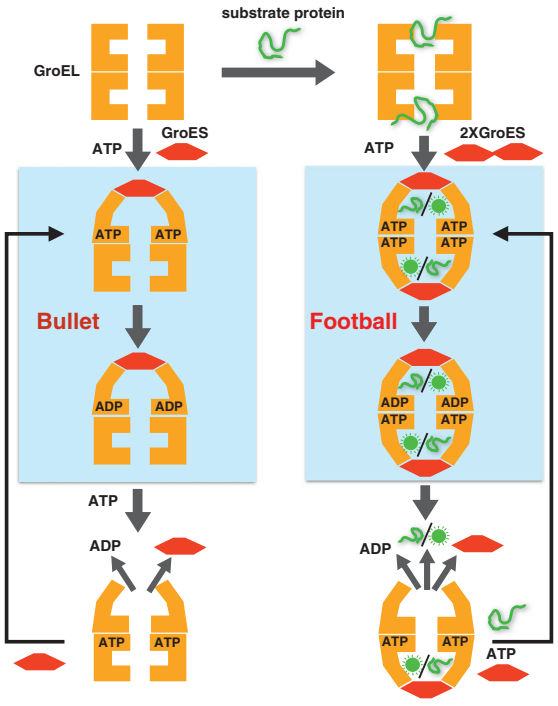

Fig. 1. A GroEL:GroES cycling model including the symmetric football intermediate. The GroEL double ring forms two types of GroEL:GroES complexes: asymmetric 1:1 bullet-shaped and symmetric 1:2 football-shaped GroEL:GroES$_2$ complexes. In the absence of substrate proteins, GroEL:GroES cycling proceeds via the asymmetric bullet intermediate [40]. In the presence of substrate proteins, the symmetric football complex accumulates as the productive intermediate during the GroEL:GroES cycling. Protein folding occurs in both rings of the football complex [32,53].

Revisiting the Chaperonin Reaction Cycle via the Football Complex

During the process to elucidate the GroEL mechanism, the formation of the bullet and football complexes raised the question of which is the critical intermediate complex during the functional GroEL:GroES cycle. In 1994, several reports proposed that the football complex is a catalytic intermediate during the cycle [28,29,38]. However, subsequent arguments about the role of the intermediate complex in the GroEL:GroES reaction cycle have generated wide acceptance of the bullet complex as the functional intermediate [36,39,40]. In the model, GroEL alternates the folding-active rings, in which one GroES ring engages one GroEL ring at a time, resulting in the accumulation of the asymmetric bullet complex as the predominant species (Fig. 1) [40]. The origin of the asymmetric complex-based cycling has been explained by the negative cooperativity between the two GroEL rings [11].

The key experiments to establish the alternation model were a series of studies using an ATPase-deficient GroEL mutant, in which Asp398 is replaced with Ala (D398A) [39,40]. D398A is deficient in ATP hydrolysis (<2% of wild type), but not in ATP binding, and thus forms a long-lived ATP-bound GroEL:GroES complex (asymmetric GroEL/ES complex, called the "ATP bullet"). Rye *et al.* reported that the *trans*-ring of the ATP-bound bullet complex cannot bind either GroES or the substrate protein [40]. ATP hydrolysis in the *cis*-ring of the ATP-bound bullet permits the binding of both ATP and the substrate to the *trans*-ring [39,40].

However, more recent detailed mechanistic analyses of GroEL have changed the situation and prompted reconsideration of the symmetric football complex [41,42]. Koike-Takeshita *et al.* demonstrated that a slow ATP-hydrolyzing GroEL mutant (D398A), which was the key mutant to construct the reaction cycle via the asymmetric complex [39,40], forms a symmetric GroEL:GroES$_2$ complex in the presence of ATP, spurring a revisitation of the