Crystal Structure of a Symmetric Football-Shaped GroEL:GroES$_2$-ATP$_{14}$ Complex Determined at 3.8 Å Reveals Rearrangement between Two GroEL Rings

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http://dx.doi.org/10.1016/j.jmb.2014.08.017
Edited by J. Buchner

Abstract

The chaperonin GroEL is an essential chaperone that assists in protein folding with the aid of GroES and ATP. GroEL forms a double-ring structure, and both rings can bind GroES in the presence of ATP. Recent progress on the GroEL mechanism has revealed the importance of a symmetric 1:2 GroEL:GroES$_2$ complex (the "football"-shaped complex) as a critical intermediate during the functional GroEL cycle. We determined the crystal structure of the football GroEL:GroES$_2$-ATP$_{14}$ complex from *Escherichia coli* at 3.8 Å, using a GroEL mutant that is extremely defective in ATP hydrolysis. The overall structure of the football complex resembled the GroES-bound GroEL ring of the asymmetric 1:1 GroEL:GroES complex (the "bullet" complex). However, the two GroES-bound GroEL rings form a modified interface by an ~7° rotation about the 7-fold axis. As a result, the inter-ring contacts between the two GroEL rings in the football complex differed from those in the bullet complex. The differences provide a structural basis for the apparently impaired inter-ring negative cooperativity observed in several biochemical analyses.

Introduction

Chaperonins are a subclass of molecular chaperones capable of assisting protein folding in an ATP-dependent manner [1–3]. They have been divided into two distinct classes: group I chaperonins, such as bacterial GroEL, and group II chaperonins, such as archaeal thermosome [3]. Both chaperonin groups share a similar basic architecture that comprises two cylindrical rings, in which the subunits have three domains: an equatorial ATP-binding domain, an apical domain involved in substrate binding, and an intermediate domain with a hinge region connecting the equatorial and the apical domains [4,5].

The best-characterized chaperonin is *Escherichia coli* GroEL and its partner GroES. GroEL and GroES are the only indispensable chaperones for the viability of *E. coli* and assist a wide spectrum of substrates in the cell [6,7]. GroEL consists of two heptameric rings of identical 57-kDa subunits. GroES is a dome-shaped single heptameric ring of identical 10-kDa subunits [4,8,9]. 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*-ring), which has a cavity for the encapsulation of the substrate protein [4]. Since GroEL is a double-ring structure stacked back to

back, two types of GroEL:GroES complexes are possible: asymmetric 1:1 and symmetric 1:2 GroEL:GroES complexes [10–14]. These are referred to as the "bullet" and "football" for the asymmetric and symmetric forms, respectively, based on their appearances in electron microscopic images [10].

Extensive efforts have been dedicated toward clarifying the roles of the asymmetric and symmetric complexes in the productive GroEL cycle. For years, it has been widely accepted that the asymmetric complex is the functional intermediate during the productive GroEL cycle [15,16]. 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 [16]. The origin for the asymmetric complex-based cycling has been explained by the nested allosteric behavior of GroEL: intra-ring positive cooperativity and inter-ring negative cooperativity [17].

However, recent detailed mechanistic analyses have changed the situation and prompted reconsideration of the GroEL cycle, including the symmetric complex [18–24]. Although both the asymmetric and symmetric cycles are possible, the presence of substrate proteins facilitates the formation of the symmetric football complex [20,23], suggesting the critical role of the symmetric cycle in the productive protein-folding cycle of GroEL. It would be of interest to know that the inter-ring negative cooperativity is compromised in the symmetric complex. Therefore, a structural characterization of the symmetric complex is necessary to understand the GroEL mechanism.

We now report the crystal structure of the football GroEL:GroES$_2$ complex using a new GroEL mutant that is deficient in ATP hydrolysis. The football structure revealed unique features of the inter-ring contacts of GroEL and thus provides insights into the differences between the symmetric and asymmetric GroEL cycles.

Results

Crystallization using a long-lived symmetric GroEL:GroES$_2$ complex

The symmetric GroEL:GroES$_2$ complex transiently exists in the GroEL ATPase cycle and decays after ATP hydrolysis [18,19,23,24]. For the crystallization, it was necessary to prepare a long-lived symmetric GroEL:GroES$_2$ complex that maintains the ATP-bound state for at least more than a few days. We previously demonstrated that an ATPase-defective GroEL mutant in which Asp398 was replaced with Ala (GroEL^{398}) [15] forms the symmetric complex in the presence of ATP and GroES [18], but the half-life of the symmetric complex is around 30 min [15,16,18], which is not sufficient for crystallization.

We found that Asp52, which is strictly conserved in chaperonin family including group II chaperonins, of the equatorial domain was also critical for the ATP hydrolysis of GroEL[‡]. Briefly, although ATPase activity of single GroEL mutant, GroEL^{52}, is ~20% of wild-type GroEL, that of a double mutant, in which both Asp52 and Asp398 are replaced with Ala (GroEL$^{52/398}$), is lower than that of GroEL^{398}, less than 0.1% of that of wild-type GroEL. As expected, in the presence of GroES and ATP, GroEL$^{52/398}$ forms a very stable symmetric GroEL:GroES$_2$ complex, in which denatured rhodanese can fold to the native state as wild-type GroEL or GroEL^{398}, indicating that the function of GroEL$^{52/398}$ to encapsulate the substrate proteins is normal. Importantly, GroEL$^{52/398}$ retains the football structure with a half-life of ~150 h (~6 days). The long-lived symmetric complex allowed us to crystallize the symmetric football complex.

Overall structure

The overall structure of the symmetric GroEL$^{52/398}$:GroES$_2$ complex is presented in Fig. 1. The complex shows the football-like shape as observed in the electron microscopy images (e.g., Ref. [25]). The football structure is formed by the back-to-back arrangement of two GroES-bound *cis*-rings, with the longest dimensions of 240 Å (along the 7-fold axis) by 140 Å (normal to the 7-fold axis). The football structure binds 14 ATP molecules at the nucleotide binding sites, where the γ-phosphate binds at the N-terminal end of helix D (residues 89–109) (Supplementary Fig. 1) (see Materials and Methods). The conformations of the two GroES-bound *cis*-rings are similar with root-mean-square deviations (rmsd values) of the C$^{\alpha}$ atoms of 0.6 Å (Supplementary Fig. 2). The structures of the GroES subunit and the three domains in the GroEL subunit are similar, with small rmsd values of 0.2–0.8 Å (Supplementary Fig. 3a and b). The rmsd value for the GroEL subunit is slightly larger (rmsd, 2.0 Å) because there are several variations in the twist angles between the apical domain and the intermediate domain in the GroEL subunit (Supplementary Fig. 3c). As compared with the GroES-bound *cis*-ring in the bullet complex structure (PDB codes: 1PCQ, 1PF9, and 1AON) [4,9], no significant structural differences were observed in the GroEL and GroES subunits (Supplementary Fig. 4). It should be noted the equatorial regions of the *cis*-rings in both the football and bullet structures also are similar with an rmsd of 1.1 Å.

Inter-ring contacts

In the bullet structure, the *en bloc* inward tilt of the *cis*-ring and the complementary outward tilt of the *trans*-ring were observed, as compared with unliganded