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Review

Dynamics, flexibility, and allostery in molecular chaperonins

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ABSTRACT

The chaperonins are a family of molecular chaperones present in all three kingdoms of life. They are classified into Group I and Group II. Group I consists of the bacterial variants (GroEL) and the eukaryotic ones from mitochondria and chloroplasts (Hsp60), while Group II consists of the archaeal (thermosomes) and eukaryotic cytosolic variants (CCT or TRiC). Both groups assemble into a dual ring structure, with each ring providing a protective folding chamber for nascent and denatured proteins. Their functional cycle is powered by ATP binding and hydrolysis, which drives a series of structural rearrangements that enable encapsulation and subsequent release of the substrate protein. Chaperonins have elaborate allosteric mechanisms to regulate their functional cycle. Long-range negative cooperativity between the two rings ensures alternation of the folding chambers. Positive intra-ring cooperativity, which facilitates concerted conformational transitions within the protein subunits of one ring, has only been demonstrated for Group I chaperonins. In this review, we describe our present understanding of the underlying mechanisms and the structure–function relationships in these complex protein systems with a particular focus on the structural dynamics, allostery, and associated conformational rearrangements.

1. Introduction

Most proteins require assistance to fold into their three-dimensional native state and achieve their functional activity [1]. This is due to the extremely crowded environment within the cell, which renders newly synthesized proteins prone to form toxic aggregate species. Given the need to minimize aggregation, nature has developed quality control mechanisms, including a complex system of chaperone surveillance that ensures protein homeostasis, or proteostasis [2,3]. The chaperonins, a critical group of molecular chaperones, are large double-ring complexes of 800–1000 kDa built of 7–9 subunits per ring (Table 1). The chaperones in this family facilitate protein folding by providing a protective chamber where non-native substrate proteins can enter and (re)-fold, in isolation from the cell environment to avoid destructive molecular interactions. To enable encapsulation and subsequent release of the substrate protein, chaperonins undergo a series of ATP-dependent conformational transitions [4,5].

Chaperonins are classified in two distantly related structural groups (Table 1); Group I is found in bacteria (GroEL; from growth essential large) and eukaryotic organelles (Hsp60; heat-shock protein 60), while Group II is expressed in Archaea (thermosome) and in the eukaryotic cytosol (chaperonin containing TCP1 (CCT), or TCP1 ring complex (TRiC)). Their gene family is extensive and complex [6–8], but the overall architecture is largely conserved. The main structural difference between Groups I and II is the lid arrangement that seals off the central chamber (Fig. 1). Group I chaperonins cooperate with Hsp10 (GroES in *Escherichia coli*), which provides a lid that covers the folding chamber to create the closed conformation, whereas in the Group II variants, the lid that seals the central chamber is formed by a built-in unit made of a long α-helix attached to the apical domain in each subunit (Fig. 1). Despite the differences in lid arrangement and the divergent amino acid sequences, which show pairwise identity of only ~20% (Fig. S1), their structural similarity at the subunit and oligomeric levels is striking (Fig. 1).

The main steps in the reaction cycle have been well established over the last two decades by extensive functional and structural studies including X-ray crystallography [9,10], electron microscopy (EM) [11,12], and to a lesser degree, NMR [13] and SAXS [14]. These tools have been decisive in determining the structure of the chaperonins in several states along their functional cycle. The exponential increase in computational power during the last decade has opened for extensive simulation studies providing a more

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Table 1
Chaperonin classification (adapted from [8]).

Occurrence	Group I		Group II	
	Bacteria	Eukaryotic organelles	Archaea	Eukaryotic cytosol
Name	GroEL	Hsp60	Thermosome	CCT (TRiC)
Co-chaperone	GroES	Hsp10	–	–
# Subunit types	1	1	1–3	8
Oligomerization	2 × 7	2 × 7	2 × 8/2 × 9	2 × 8

complete view of possible trajectories between the stable end-states, transient conformations, and detailed mechanisms underlying ligand-induced conformational transitions [15]. In this review, we present an overview of the structure–dynamics–function relationships in this class of molecular chaperones. We start by surveying current knowledge of the GroEL functional cycle, with associated conformational transitions and models of allostery. We then present the less-characterized Group II chaperonins, the thermosome and CCT.

2. Group I chaperonins: GroEL–GroES

2.1. Overall architecture

GroEL is an oligomer composed of two chemically identical homoheptameric rings stacked back-to-back [9,16,17]. In its open, substrate-receptive state, the two rings form a ~150 Å-long cylindrical structure with a diameter of ~145 Å (Fig. 1A) [9,18]. At each end of the cylinder, the structure forms a ~45 Å deep and wide cavity that constitutes the folding chamber [9]. In the *folding active* state, the chamber is extended to a ~55 Å wide and ~80 Å deep cavity capped by the co-chaperone GroES (which is also a ring-shaped heptameric structure; Fig. 1A). This provides a confined, protective hydrophilic environment in which non-native substrate proteins can refold without inappropriate interactions in the crowded cell environment [16,19,20].

The GroEL monomer has 547 residues and folds into three distinct structural domains: the *equatorial*, *intermediate*, and *apical* domain (Fig. 1A). The equatorial is a solid α-helical domain that provides most intra-ring subunit contacts and all inter-ring contacts [9,16] (Fig. 1A). It also contains the nucleotide binding site which is located at its top, facing the adjacent subunit [16]. The apical domain is situated at the end of the cylinder and forms the entrance to the folding chamber. It contains exposed hydrophobic residues responsible for interactions with non-native substrate proteins, as well as a number of charged residues that facilitate inter-subunit salt bridges. The intermediate domain acts as a hinge between the apical and equatorial domains, providing flexibility to the GroEL assembly and facilitating large-scale conformational changes [16].

2.2. Allostery

GroEL-assisted protein folding is precisely regulated by ATP binding and hydrolysis, the main facilitators of the large-scale structural changes responsible for the cycling between substrate folding and release states [21]. The high cooperativity of ATP binding and hydrolysis in the GroEL/ES system, revealed by early functional and kinetic studies [22–24], was later associated to

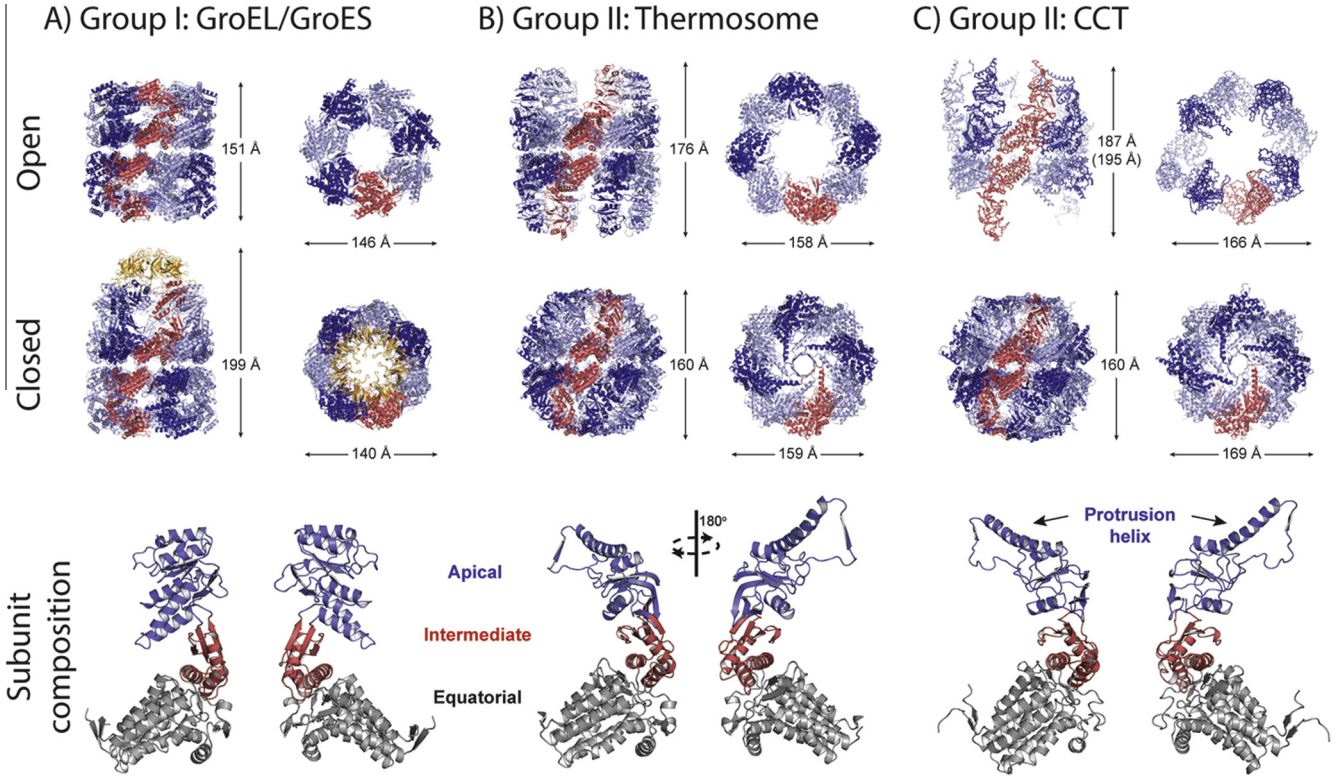

Fig. 1. Overview of chaperonin structures. Major conformational species of Group I and II chaperonins are shown in cartoon representation. (A) GroEL/ES, (B) thermosome, and (C) CCT are shown in columns one to three, respectively. The individual subunits are coloured alternating dark and light blue, and the co-chaperone GroES is shown in orange. Two inter-ring adjacent subunits are highlighted in red colour to illustrate the interaction relationship between the rings (1:2 for GroEL, and 1:1 for thermosomes and CCT). Closed (folding active) and open (folding inactive) structures are shown in rows one and two, respectively. The atomic structures of individual subunit structures (closed form) are shown in the third row from two angles. The subunit is colored according to their domain composition: blue (apical domain), red (intermediate domain), and grey (equatorial domain). The protrusion helix of CCT is labelled to indicate the dominating structural deviation between Group I and II. PDB codes: GroEL/ES: 1XCK (open) and 1SX4 (closed); thermosome: 3KFK (open, Δlid) and 1A6D (closed); CCT: 2XSM (open) and 4V8R (closed). Reported dimensions are calculated from the coordinates provided in the respective PDB structure and might deviate from those reported in their original paper.