Communication

ATP Binding Induces Large Conformational Changes in the Apical and Equatorial Domains of the Eukaryotic Chaperonin Containing TCP-1 Complex*

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The chaperonin-containing TCP-1 complex (CCT) is a heteromeric particle composed of eight different subunits arranged in two back-to-back 8-fold pseudo-symmetrical rings. The structural and functional implications of nucleotide binding to the CCT complex was addressed by electron microscopy and image processing. Whereas ADP binding to CCT does not reveal major conformational differences when compared with nucleotide-free CCT, ATP binding induces large conformational changes in the apical and equatorial domains, shifting the latter domains up to 40° (with respect to the inter-ring plane) compared with 10° for nucleotide-free CCT or ADP-CCT. This equatorial ATP-induced shift has no counterpart in GroEL, its prokaryotic homologue, which suggests differences in the folding mechanism for CCT.

Chaperonins are ubiquitous oligomeric double-ring assemblies that mediate ATP-dependent protein folding, both in vitro and in vivo, through the binding of unfolded or misfolded polypeptides followed by their subsequent release into a folded or “committed to fold” conformation (1, 2). Chaperonins are classified into two distinct groups (3): group I (GroEL-like), present in eubacteria and endosymbiotic organelles, and group II (CCT/TF55), present in archaebacteria and the eukaryotic cytosol (4, 5). Both groups share limited but significant sequence homology (4–6).

Bacterial GroEL is the most studied chaperonin from the biochemical and structural viewpoint. The three-dimensional structure of GroEL at atomic resolution (7) and the changes it undergoes upon binding of ADP and its co-chaperonin, GroES (8) are known. Compared with GroEL, there is a relative paucity of structural information relating to CCT (chaperonin-containing TCP-1).1 Unlike GroEL, a homo-oligomeric complex composed of two stacked 7-mer rings, CCT is a hetero-oligomer assembled into two rings each composed of eight different subunits (9–11). Substrate-wise, GroEL is relatively nonspecific and mediates the folding of a wide variety of proteins, whereas CCT is more specific, specializing in the folding of the cytoskeletal proteins, actin and tubulin (5). Similar to GroEL, CCT binds non-native forms of these substrates, which in the presence of ATP are discharged in a state committed toward the native fold (12).

Despite these similarities, there is growing evidence that CCT mediates the folding of substrates through a mechanism different from that of GroEL. For example, CCT folds substrates without the assistance of a co-chaperonin (which is essential in GroEL-mediated folding). It has also been shown that the group II archaeosome undergoes an ATP-dependent disassembly into free subunits during its functional cycle (13) and that CCT displays a nucleotide-dependent single ring disassembly and reassembly activity (14). Recently, the discovery of a novel helix-turn-helix motif in the crystal structure of the apical domain of the thermosome (15), which seems to be characteristic of all group II chaperonins, has been suggested to have a role in substrate binding and to act as a co-chaperonin in controlling the access of the substrate to the cavity. Hence, many questions remain to be answered with respect to the CCT reaction cycle.

The conformational analysis of nucleotide-bound GroEL by electron microscopy has provided valuable structural information on the molecular mechanism of the GroEL reaction cycle (16, 17). Electron microscopy (EM) of negatively stained specimens is a powerful technique for studying both the structural and conformational changes at medium to low resolution (20–30 Å) of large oligomeric complexes. One of its advantages is that it provides a high contrast background that allows images to be observed at very low underfocus values without significant loss of contrast, thereby extending the resolution of the structural details to the limits set by the contrasting agent, normally 10–15 Å (18). The use of negative staining also has disadvantages; the most important being the non-imaging of internal protein structures that are inaccessible to heavy metal salts (i.e. virus). Fortunately, the structures of many of the chaperonins are quite open and porous, with large openings (entrance of the cavity and portals; see Ref. 7) where the heavy metal salts can penetrate and contrast the internal regions. In the case of GroEL, this technique has revealed the structures of GroEL, GroEL-GroES asymmetric (19), and symmetric complexes (20), and the conformational changes generated upon nucleotide and GroES binding (17, 19, 21). The data were later confirmed by cryo-EM (16, 22) and x-ray crystallography (7, 8).

Using the same methodology for GroEL (electron microscopy of negatively stained specimens followed by image processing), we have studied the conformational changes induced in CCT upon

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1 The abbreviations used are: AMP-PNP, adenylyl-imidodiphosphate; CCT, chaperonin-containing TCP-1 complex; EM, electron microscopy.
the equatorial domain, which is involved in the inter- and intra-ring interactions and also contains the ATP binding site; the apical domain, which interacts with incoming substrate and with the co-chaperonin GroES; and the intermediate domain, which acts as a hinge region between the equatorial and intermediate domains (7). These three domains can be unambiguously located in the side views of negatively stained GroEL (see Fig. 3D). The projection shows four striations, two per GroEL ring, with the outer striations representing the mass projected by the apical domains and the inner striations representing those of the equatorial domains. The intermediate domain is located in the small mass connecting the outer part of the inner and outer striations. The CCT side view also shows two outer and two inner striations bound in the outside by a small mass that can be assigned to the apical, intermediate, and equatorial domains, respectively.

Besides these similarities there are also differences between GroEL and CCT especially in the general shape of CCT, which has the topology of a slightly elongated barrel (160 × 150 Å), compared with the rectangular shape of GroEL. The projection structure of the intermediate domain of CCT is bigger than that of GroEL, whereas the apical domains of CCT are smaller than their GroEL counterparts. However, the most significant differences between the structures lie in their equatorial domains. The equatorial mass in GroEL is evenly distributed throughout the inner striations (see Fig. 3D), whereas the mass in CCT is concentrated mainly in the outer parts of the inner striations, with a protrusion pointing to the interior of the oligomer (Figs. 1A and 3A).

The average image of the front view reveals, as described previously, eight well separated subunits comprising a ring of 150-Å diameter surrounding a cavity of approximately 50-Å diameter (Fig. 1B). Each of the subunits displays a bilobal structure, a feature that has not been described for any other chaperonins studied to date. When CCT is stained with uranyl acetate (which results in less height contrast than the particles stained with a mixture of uranyl acetate and glucose), the average front image obtained reveals a particle without visible bilobes and with a larger cavity (result not shown). This indicates that the bilobes are located in the equatorial domain, which is the widest part of each ring. Identical results were obtained with human CCT (results not shown), suggesting that the bilobal feature is a characteristic specific to the CCT subunits. The front view obtained in the absence of glucose also shows a larger cavity (result not shown), suggesting that the inner part of each subunit (stained in the presence of glucose) forms part of the equatorial domain, which correlates well with the barrel-shaped side views of CCT (Fig. 1A).

Apo-CCT was incubated with 5 mM of ADP and the side and front views independently processed (Fig. 1, C and D, respectively) and compared with nucleotide-free CCT. The average side view of ADP-CCT (Fig. 1C) reveals that ADP binding does not induce any substantial change in the CCT structure at the resolution of this work (20 Å). The average front view of ADP-CCT (Fig. 1D) shows similar features to its nucleotide-free counterpart, which confirms that no conformational changes are seen in CCT upon ADP binding.

Nucleotide-free CCT was incubated with 5 mM ATP or 10 mM AMP-PNP to fill all the available nucleotide binding sites. Both front and side views of CCT were independently processed and classified (Fig. 2). As in the case of nucleotide-free CCT or ADP-CCT, side views were less frequent, but the presence of both types of nucleotides increased the frequency of their occurrence. The side view images in the presence of ATP (Fig. 2A) or AMP-PNP (Fig. 2B) are almost identical and show clear conformational changes when compared with nucleotide-free...
CCT (Fig. 1A) or CCT-ADP (Fig. 1C), in which no changes were detected. The side views are clearly asymmetric, with the lower ring differing slightly from those of the nucleotide-free sample (Fig. 1A). The top ring reveals a large conformational change affecting both the apical and equatorial domains (Fig. 2, A and B). Therefore, under physiological concentrations of ATP or high levels of AMP-PNP CCT displays a large rearrangement in one of its rings (with some changes in the other).

In its folding cycle, GroEL displays a positive and concerted intra- and negative inter-ring co-operativity with respect to nucleotide binding, so that the double ring is in equilibrium between three different allosteric states: TT, TR, and RR (34). In the case of CCT, Lin and Sherman (35) have examined genetic interactions between mutations in the ATP binding sites of four CCT subunits in *Saccharomyces cerevisiae* and have proposed a simple sequential mechanism of co-operative ATP binding and hydrolysis progressing in one direction around the CCT ring. Perhaps a consequence of such a mechanism is a difference in the inter-ring signaling behavior between GroEL and CCT. The fact that an asymmetric CCT particle is obtained, even at high AMP-PNP concentrations, can be explained in either of two ways: (i) only one ring binds nucleotide (equivalent to the TR state in GroEL) due to a highly negative co-operative activity between the two rings or (ii) both rings bind nucleotide (equivalent to the RR state in GroEL), and the particle remains asymmetric due to inter-ring signaling.

When CCT-ATP and CCT-AMP-PNP side views are compared with GroEL-ATP (Fig. 3), it is clear that the major conformational changes are occurring in the top rings of CCT, with changes in the equatorial domains and in the apical domains (Figs. 2, A and B, and 3B). Previous work with ATP-bound GroEL, using the same methodology, showed major conformational changes in the apical domains of the projected images, which was later confirmed by x-ray crystallography. In the same way, and due to the similar topologies of GroEL and CCT, the enlargement of the cavity of the top ring upon ATP binding (Fig. 2A and 3B) can be related to an upward and outward movement of the apical domains (Fig. 3E). However, the most striking conformational change induced by ATP is localized in the equatorial domains of the top ring of CCT, which undergo a large movement toward the intermediate domain, striking an angle of approximately 40° with the inter-ring plane (Fig. 3, B and C), a major shift compared with the angle of 10° found in nucleotide-free CCT (Fig. 3A). This is a feature that has not been observed for GroEL upon ADP and GroES binding (8).

With GroEL, the apical domains experience dramatic outward and upward movements using the intermediate domain as a hinge and the equatorial domain as a platform (Fig. 3, E and F). The result of these changes in the apical domains of GroEL is to decrease the binding affinity toward substrate and to allow GroES binding so that the substrate is liberated into the cavity. The small conformational movements detected in the equatorial domains of GroEL, upon binding of ADP and GroES, are related to the allosteric signaling between the two rings of the oligomer, a mechanism that forms an integral part of the GroEL folding cycle. This suggests that the behavior of the apical and equatorial domains of CCT upon ATP or AMP-PNP binding may represent some differences in mechanism compared with the functional cycle of GroEL.

The fact that ATP binding induces large conformational changes in both the apical and equatorial domains, suggests that the CCT protein folding cycle requires not only changes in the apical domains but also major inter-ring movements, which may lead to subunit disassembly. This phenomenon has been observed as part of the folding cycle of the archaeosome from *Sulfolobus shibatae* (13). When the structure of the apical domains of GroEL (7) and the thermosome from *T. acidophilum* (15) are compared, it is observed that the thermosome lacks two helices (helix 11 and 12) present in GroEL. This region of GroEL contains six important contacts between the apical domain and the equatorial domain of the adjacent subunit, suggesting that these interactions are weaker in group II chaperonins.

Front views of CCT-ATP or CCT-AMP-PNP were also aligned, processed, and classified. In both cases, the image processing generated two different homogeneous populations for CCT-ATP (Fig. 2, C and D) and CCT-AMP-PNP (results not shown; similar to CCT-ATP). One of the average images (Fig. 2C) is very similar to that of nucleotide-free CCT (Fig. 1B); however, the second average image (Fig. 2D) appears different from the others (Figs. 1B and 2C). The outer parts of the bilobal subunits are well contrasted, whereas the inner parts are not. This could be the result of the asymmetric CCT-ATP particle contacting the grid with either the slightly modified (Fig. 2C) or with the highly modified ring (Fig. 2D). The mix of uranyl acetate and glucose contrasts only one of the rings and is therefore capable of detecting the asymmetry of the particle (unlike the projections obtained from frozen-hydrated specimens). Hence, in the first image the mass observed in the bottom ring is the projection of the apical, intermediate, and equatorial domains of the slightly modified ring (Fig. 2, A and B). In the second image, the movement of the apical and equatorial domains toward the outer part of the ring (top rings, Fig. 2, A and B) concentrates the contrast in this region, resulting in a less contrasted inner area that corresponds to the central part of the equatorial domains. The asymmetry of the CCT-ATP (or CCT-AMP-PNP) side views correlates with the appearance of two distinct front views and explains why only one population is seen with nucleotide-free CCT (Fig. 1B) or with CCT-ADP particles (Fig. 1D) when subjected to the same classification procedures.

Previous experiments have suggested that ATP binding to CCT may cause conformational changes due to the increase of side views detected by EM (9, 11). Also, non-denaturing isoelectric focussing analysis of CCT suggests that there are two distinct CCT populations (36). More recently, biochemical analyses have also pointed to major conformational changes in the
presence of ATP (37). The present EM characterization of the CCT complex is the first report of the asymmetry induced in CCT upon ATP binding and gives the first glimpse of the nature of the ATP-induced conformational changes and their functional implications in the mechanism of action of this complex folding machine.

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