FOR THE RECORD

Modeling of possible subunit arrangements in the eukaryotic chaperonin TRiC

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Abstract

The eukaryotic cytosolic chaperonin TRiC (TCP-1 Ring Complex), also known as CCT (Cytosolic Chaperonin containing TCP-1), is a hetero-oligomeric complex consisting of two back-to-back rings of eight different subunits each. The general architecture of the complex has been determined, but the arrangement of the subunits within the complex remains an open question. By assuming that the subunits have a defined arrangement within each ring, we constructed a simple model of TRiC that analyzes the possible arrangements of individual subunits in the complex. By applying the model to existing data, we find that there are only four subunit arrangements consistent with previous observations. Our analysis provides a framework for the interpretation and design of experiments to elucidate the quaternary structure of TRiC/CCT. This in turn will aid in the understanding of substrate binding and allosteric properties of this chaperonin.

Keywords: chaperonin; chaperone; TRiC; CCT; GroEL; protein folding; ring complex

Ring-shaped protein complexes play important roles in cellular function. In the mitochondria, membrane-associated protein rings drive the synthesis of ATP (Boyer 1997). The AAA+ (ATPases Associated with various cellular Activities) proteins form oligomeric rings that play a variety of roles in protein disaggregation, protein degradation, protein complex disassembly, and DNA replication (Davey et al. 2002; Hanson and Whiteheart 2005). Regulated proteases such as HsV, ClpP (Wang et al. 1997), and the proteasome (Groll et al. 1997) form back-to-back rings of proteolytic subunits. Chaperonin complexes are stacked-ring structures that play a critical role in cellular protein folding (Frydman 2001; Spiess et al. 2004). All these ring complexes contain

varying numbers of subunits and can be either homo-oligomeric or hetero-oligomeric in nature. While crystal structures have revealed the subunit arrangement of many of these ring complexes, the arrangement of subunits in the eukaryotic chaperonin TRiC remains an open question.

Chaperonins are essential cytosolic chaperones that assist in the folding of newly translated proteins (Hartl and Hayer-Hartl 2002; Spiess et al. 2004). These enzymes can be divided into two classes: type I chaperonins require a lid-like cofactor and are found in bacteria and organelles of bacterial origin, while type II chaperonins have a built-in lid and are found in the eukaryotic cytosol and archaea. The general architecture of chaperonins has been determined by biochemical and structural approaches to consist of two rings stacked back-to-back, each enclosing a large central cavity (Braig et al. 1994; Ditzel et al. 1998; Saibil 2000).

The eukaryotic chaperonin TRiC, also called CCT, folds a variety of cellular substrates, including actin and tubulin (Dunn et al. 2001), which cannot fold spontaneously or be folded by other chaperone systems (Tian et al. 1995). Encapsulation of folding substrates within the

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TRiC cavity is required for productive folding, suggesting that substrates may fold within the cavity (Meyer et al. 2003). Whereas type I chaperonins are primarily homooligomeric, type II chaperonins from archaea and eukaryotes are mostly hetero-oligomeric. Archaeal complexes range from one to four different subunits, while TRiC contains eight paralogous subunits that are conserved throughout eukaryotic organisms (Leroux and Hartl 2000; Archibald et al. 2001; Valpuesta et al. 2002).

Previous experiments suggest that each of the eight subunits occurs once in each ring. All eight subunits copurify in one complex, and immunoprecipitation experiments with subunit-specific antibodies recover all eight subunits (Frydman et al. 1992; Lewis et al. 1992). Additionally, a study that analyzed free dimeric and trimeric subcomplexes of TRiC subunits demonstrated that each free subunit associates with only one or two other types of subunits, suggesting a defined order of subunits within a ring (Liou and Willison 1997). Together, these results suggest a fixed arrangement of subunits within each ring of TRiC. However, the arrangement of the eight subunits within each ring and the alignment of the two rings in the complex have not been clearly defined.

A detailed understanding of the subunit arrangement within the chaperonin is essential for understanding the mechanism of TRiC, since different subunits within the complex appear to play different roles in both substrate binding and the ATPase cycle (Lin et al. 1997; Llorca et al. 1999, 2000; McCallum et al. 2000; Feldman et al. 2003; Etchells et al. 2005; Rivenzon-Segal et al. 2005). For other ring complexes such as GroEL (Braig et al. 1994), the archaeal thermosome (Ditzel et al. 1998), and the proteasome (Groll et al. 1997), the precise arrangement of subunits was revealed by high-resolution structures obtained by X-ray crystallography. Thus far, TRiC has proven refractory to attempts at high-resolution structural analysis. To provide a theoretical framework both to analyze existing data and to guide future experiments, we have constructed a simple model for TRiC and enumerated the possible arrangements of its stacked chaperonin rings. By combining this analysis with existing data, we find that only four structures are consistent with previous literature. Our results demonstrate several important properties of the chaperonin architecture that may aid future experiments to resolve this problem.

Results

Model for subunit arrangement in TRiC/CCT

To examine the possible inter-ring interactions in TRiC, we constructed a simple reference model consisting of a double-ring complex containing eight different subunits per ring (Fig. 1A). Individual subunits placed within the

Figure 1. (A) Model of subunit interactions. We arbitrarily labeled the eight subunits of the top ring of the model 1 through 8 in a clockwise orientation. In the bottom ring, the subunits are labeled 1' through 8' in a clockwise fashion. The possible alignments of the two rings are generated by stepwise rotation. (B) Possible arrangements of the inter-ring interactions. Using the top ring as a reference, we have listed all of the possible interfaces between the two rings. Each potential alignment (I–VIII) appears as a row listing the subunits in the bottom ring, and their binding partners in the top ring are listed in the column heading.

double-ring structure enumerate possible alignments of the two rings. For simplicity in our model, we arbitrarily numbered the subunits in the top ring from 1 through 8 in a clockwise orientation; then we numbered the bottom ring in a similar fashion. Based on previous data (Frydman et al. 1992; Lewis et al. 1992; Liou and Willison 1997; Ditzel et al. 1998), we make three assumptions. First, each ring consists of one copy of each of the eight individual subunits. This is supported by the observation that all subunits copurify in the intact TRiC/CCT complex and that immunoprecipitation with antibodies directed against one subunit will copurify the other subunits in the complex (Frydman et al. 1992; Lewis et al. 1992). Second, the arrangement of the subunits within each ring is fixed. In support of this assumption, analysis of “microcomplexes” formed by pairs of TRiC subunits (Liou and Willison 1997) indicated that each subunit associates with only one or two partners; further, the observed partners were consistent with a fixed ring structure. Finally, we assume that the rings, with identical subunit arrangements, are stacked back-to-back and in register in the complex, as seen in the crystal structure of the α – β thermosome from *Thermophilus acidophilus* (Ditzel et al. 1998). This assumption is based on the high degree of homology between thermosome and TRiC subunits, which suggests similarities between the overall structure of these complexes. The eight TRiC/CCT proteins from the yeast *Saccharomyces cerevisiae* have pairwise amino acid identities to the thermosome subunits ranging from 30% to 38%, while the eight CCT proteins themselves range from 25% to 35% pairwise amino acid identity. Indeed, electron microscopy and X-ray diffraction

studies on TRiC also yield architectures similar to that of the thermosome (Llorca et al. 1998; Meyer et al. 2003), though they lack the resolution to identify individual subunits.

Applying these assumptions to our model yields eight possible registers for the rings, as shown in the matrix of Figure 1B. Each orientation is generated by stepwise rotation of the bottom ring with respect to the top and is listed as a row in the matrix (Fig. 1B, I–VIII). Each subunit in the bottom ring interacts with the top ring subunit listed in the column headings. Enumerating all possible arrangements indicates that the presence of a fixed order within one ring produces symmetry in the binding partners across the inter-ring interface. Any subunit type in the top ring binds the same partner in the bottom ring as its cognate in the bottom ring binds in the top ring. Therefore, with a fixed-ring arrangement, each type of TRiC subunit has the same three binding partners in both rings: left and right within a ring, and one across the inter-ring interface. In four alignments (alignments I, III, V, and VII; Fig. 1B), two subunits form homo-oligomeric inter-ring interactions that stack cognate subunits from the two rings. In these alignments, the homo-oligomeric pairs are diametrically opposed in a 1,5 orientation. The other four alignments (alignments II, IV, VI, and VIII; Fig. 1B) consist entirely of hetero-oligomeric interfaces between the two rings.

In the archaeal thermosome (Ditzel et al. 1998), all of the α and β subunits form homo-oligomeric interfaces between the two rings. The analysis in Figure 1B shows that this scenario is impossible in TRiC if the arrangement of subunits within one ring is fixed. Theoretically, back-to-back, homo-oligomeric rings of TRiC subunits would be consistent with the subunit order proposed by Liou and Willison (1997) if one ring had a clockwise arrangement of subunits and the opposite ring had a counterclockwise arrangement of subunits. This would require, however, that the same lateral intra-ring interfaces of each subunit have the ability to bind to different partner subunits in the top and bottom rings. For this reason, we suggest that the fixed ring order is more likely.

Application of experimental data to the model

We next combined our model with previous experimental evidence to eliminate incompatible architectures. On the basis of microcomplexes consisting of two or three TRiC subunits found in solution, Liou and Willison (1997) have proposed the order α , ε , ζ , β , γ , θ , δ , η for the individual subunits within one TRiC ring. Because each ring possesses a defined apical to equatorial axis, the clockwise and counterclockwise arrangements of this order define unique structures (Cowan and Lewis 2001). Arbitrarily mapping the α subunit to position 1 in our model (Fig. 1A) using these ring orders narrows the possible arrangements of the complex from 40,320 (eight alignments

of seven factorial, or 5040, possible rings) to 16 (alignments I–VIII with clockwise and counterclockwise arrangements).

One of the few experimental results to address the relative register of the TRiC rings is an electron microscopy (EM) reconstruction of the chaperonin complex bound to an α subunit-specific antibody. When two antibodies were bound to one complex, each ring of TRiC was associated with one antibody, and the two antibodies were located nearly opposite each other across the complex (Grantham et al. 2000). This finding suggests that the α subunits may be aligned $\sim 180^\circ$ out of phase with each other. If we maintain the correspondence between the α subunit and position 1, then alignments IV, V, or VI (Fig. 1B) are the most likely.

A second EM reconstruction of TRiC in complex with prefoldin, a chaperone that cooperates with TRiC, showed that, when two prefoldin molecules were bound to one TRiC complex, the prefoldin-interacting TRiC subunits were stacked on top of each other in a 1,4 orientation within each ring (Martin-Benito et al. 2002). If the binding of prefoldin to TRiC is subunit-specific, then this result suggests that two of the TRiC subunits that are arranged in a 1,4 orientation within one ring may contact the corresponding two subunits in the opposite ring. Examination of the alignments in Figure 1B shows that it is possible for structures to contain back-to-back 1,2 and 1,4 partners (alignments II, IV, VI, and VIII) or back-to-back 1,3 and 1,5 partners (alignments I, III, V, and VII), but not both. If the prefoldin binding sites are directly on top of each other in the two rings, these conditions limit the possible arrangements of the two rings to alignments II, IV, VI, or VIII (Fig. 1B).

Using the three experimental observations described above as constraints limits the possible arrangements of the TRiC complex to either alignment IV or alignment VI. Mapping the clockwise and counterclockwise arrangements of Liou and Willison onto these alignments yields four structures consistent with previous experimental observations (Fig. 2). Among these four possible structures, two pairs are mirror images of each other. Alignment IV with the clockwise subunit arrangement and Alignment VI with the counterclockwise subunit arrangement produce the same binding partners and inter-ring interfaces for each subunit. However, the lateral interfaces between subunits within a ring are reversed in these arrangements and thus consist of different amino acids in the protein structure. The same is true of alignment IV with the counterclockwise arrangement and alignment VI with the clockwise arrangement.

Discussion

We here present a simple model for the possible subunit arrangement in the eukaryotic chaperonin TRiC. We find

Figure 2. Models of compatible TRiC structures. With the use of experimental data from three studies (Liou and Willison 1997; Grantham et al. 2000; Martin-Benito et al. 2002), only four possible structures are consistent with the conclusions drawn therein. We reference the CCT- α subunit to position 1 and use alignments IV and VI with clockwise and counterclockwise subunit arrangements to construct the maps corresponding to these structures.

that if the arrangement of subunits within each ring is fixed, the inter-ring interface for any individual subunit is identical in both rings for all possible arrangements. For example, if subunit 1 in the top ring is rotated to bind across the interface of subunits 2' and 3' in the bottom ring, then subunit 1' in the bottom ring binds across the interface of subunits 2 and 3 in the top ring with an identical conformation (Fig. 1A). A fixed order of subunits within each ring also limits the number of subunits that can interact in a *trans* fashion across the ring interface, as it excludes entirely homo-oligomeric, back-to-back stacking. The fixed ring structure constrains both copies of each type of subunit to have the same three binding partners (or possibly four if the rings are aligned out of phase): one to the left within the ring, one to the right within the ring, and one partner in the opposing ring. Our model demonstrates that a specific intra-ring organization also forces specific inter-ring organization, regardless of the register between the two rings. In such a system, both the intra-ring and inter-ring interfaces could evolve unique contacts to stabilize the complex and transmit allosteric information. Our simple analysis thus provides a framework to design and interpret experiments aimed at elucidating the quaternary subunit arrangement of TRiC.

Defining the subunit arrangement of TRiC is key to understanding its function. It has been proposed that the relative arrangement of TRiC subunits around the ring leads to a specific orientation of the bound substrate, as suggested by EM of substrate bound to the complex (Llorca et al. 1999, 2000, 2001), possibly affecting the

substrate's folding pathway. In addition, the arrangement of subunits may be important for the allosteric regulation of the ATPase activity of TRiC. TRiC displays "nested cooperativity" in which subunits within one ring display positive cooperativity in ATP binding and hydrolysis, while the two rings exhibit negative cooperativity with each other (Kafri et al. 2001; Kafri and Horovitz 2003). Furthermore, sequential ATP binding to subunits within a ring has been proposed (Lin et al. 1997; Kafri and Horovitz 2003; Rivenzon-Segal et al. 2005). This model requires structural communication between subunits both within a ring and across the ring interface.

The conclusion that intra-ring subunit order and inter-ring register are constant for all subunits provides a possible mechanism for the production of new TRiC complexes. Any free, single ring could template the formation of a ring with identical subunit arrangement using the specificity of inter-ring interactions to bind free subunits of its inter-ring interaction partner. In rabbit reticulocyte lysate, Liou and coworkers (Liou et al. 1998) suggested that *in vitro* translated TRiC subunits were incorporated through a single-ring intermediate.

Additional experimental data are necessary to define the arrangement of subunits within TRiC. Mutational analysis of the chaperonin is hampered by both the lack of a recombinant expression system and the fact that all eight subunits are essential in every eukaryote studied to date. Given that a model for the arrangement of subunits in one ring exists, identification of one pair of subunits that interact across the ring interface should be sufficient to specify the register of the rings. Nearest neighbor data are insufficient, however, to distinguish between the clockwise and counterclockwise ring arrangements as both produce the same binding partners. Subunit-specific antibodies have been used to align images of TRiC rings from electron microscopy experiments (Llorca et al. 1999; Rivenzon-Segal et al. 2005). While images with one or two subunit-specific antibodies will yield identical results in either arrangement, collecting images of TRiC rings bound to three subunit-specific antibodies with unequal spacing in the ring would be sufficient to discriminate between the clockwise and counterclockwise orientations.

Our analysis can extend to any hetero-oligomeric assembly of back-to-back ring structures with three or more subunits. For assemblies with an even number of subunits, either two subunits will form a homo-oligomeric interface across the rings or no subunits will form such an interface. Assemblies that contain an odd number of subunits will have one subunit that forms a homo-oligomeric interface across the rings in any possible register. For example, the yeast proteasome consists of two stacked, seven-membered, hetero-oligomeric rings with a fixed ring arrangement (Groll et al. 1997). In the

inner β rings, only the $\beta 1$ subunits are juxtaposed back-to-back across the interface. While the α rings are separated by the β rings and form no contacts, only the $\alpha 4$ subunits are directly aligned across the rings.

In bacteria and some archaea, chaperonins are homo-oligomeric complexes. How did distinct subunits and subunit interfaces emerge in the evolution of the hetero-oligomeric, eukaryotic chaperonin? In general, back-to-back assembly of hetero-oligomeric complexes may be easier to achieve than one might assume. By fixing the order of subunits within a single ring of any size, each type of subunit is constrained to have one specific type of binding partner across the inter-ring interface. Such a hetero-oligomeric complex would in turn develop more specialized interactions in inter-ring interfaces. Mutations may occur uniquely on one side of the inter-ring interface in a hetero-oligomeric complex, whereas in a homo-oligomeric interface any mutation will appear on both sides simultaneously. This would provide a mechanism for hetero-oligomeric complexes to evolve a unique, specialized inter-ring interface.

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