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Effective ATPase activity and moderate chaperonin–cochaperonin interaction are important for the functional single-ring chaperonin system

Melissa Illingworth [a], Jared Salisbury [a], Wenqian Li [b], Donghai Lin [b], Lingling Chen [a, b, *]

[a] Department of Molecular and Cellular Biochemistry, 212 S. Hawthorne Dr., Simon Hall 305B, Indiana University, Bloomington, IN 47405, USA
[b] Department of Chemistry, College of Chemistry and Chemical Engineering, Xiamen University, Xiamen, Fujian, China

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ABSTRACT

Escherichia coli chaperonin GroEL and its cochaperonin GroES are essential for cell growth as they assist folding of cellular proteins. The double-ring assembly of GroEL is required for the chaperone function, and a single-ring variant GroEL^{SR} is inactive with GroES. Mutations in GroEL^{SR} (A92T, D115N, E191G, and A399T) have been shown to render GroEL^{SR}–GroES functional, but the molecular mechanism of activation is unclear. Here we examined various biochemical properties of these functional GroEL^{SR}–GroES variants, including ATP hydrolysis rate, chaperonin–cochaperonin interaction, and in vitro protein folding activity. We found that, unlike the diminished ATPase activity of the inactive GroEL^{SR}–GroES, all four single-ring variants hydrolyzed ATP at a level comparable to that of the double-ring GroEL–GroES. The chaperonin–cochaperonin interaction in these single-ring systems was weaker, by at least a 50-fold reduction, than the highly stable inactive GroEL^{SR}–GroES. Strikingly, only GroEL^{SR}D115N–GroES and GroEL^{SR}A399T–GroES assisted folding of malate dehydrogenase (MDH), a commonly used folding substrate. These in vitro results are interesting considering that all four of the single-ring systems were able to substitute GroEL–GroES to support cell growth, suggesting that the precise action of chaperonin on MDH folding may not represent that on the intrinsic cellular substrates. Our findings that both effective ATP hydrolysis rate and moderate chaperonin–cochaperonin interaction are important factors for functional single-ring GroEL^{SR}–GroES are reminiscent of the naturally occurring single-ring human mitochondrial chaperonin mtHsp60–mtHsp10. Differences in biochemical properties between the single- and double-ring chaperonin systems may be exploited in designing molecules for selective targeting.

1. Introduction

GroEL, the paradigm chaperonin Hsp60 in *Escherichia coli*, is essential for cellular viability [1], as it along with the cochaperonin GroES mediates folding of a range of important cellular proteins [2]. The tetradecameric GroEL is organized into two heptameric rings [3], stacked back-to-back via the equatorial domain (below), to form two functionally correlated folding cavities. Each GroEL monomer is folded into three domains. The apical domains, located on the opening of the folding cavity, form the main binding site for the mis-folded protein substrate, and also contain the binding site for GroES. The equatorial domains form the base of the folding cavity, and they interact extensively across the ring forming the ring–ring interface. The equatorial domains also contain the nucleotide-binding sites. The intermediate domains connect the apical and equatorial domains, transmitting signals between equatorial and apical domains. The double-ring assembly is required for the GroE-mediated protein folding via a two-stroke mechanism [4–8]. In this mechanism, mis-folded proteins characterized by exposed hydrophobic patches are captured into one GroEL cavity via hydrophobic interaction [9]. ATP binding to this substrate-loaded GroEL ring and its subsequent hydrolysis trigger a large conformational change in the ring, leading to capping of the heptameric GroES to this folding-active (cis) ring to sequester the substrate in an enclosed chamber [10]. The folding chamber allows the substrate to fold in a favorable environment for ~15 s [11,12].

* Corresponding author. Department of Molecular and Cellular Biochemistry, 212 S. Hawthorne Dr., Simon Hall 305B, Indiana University, Bloomington, IN 47405, USA.
E-mail address: linchen@indiana.edu (L. Chen).

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The chamber dissociates as a result of ATP binding to the second (trans) GroEL ring via a multi-step allosteric mechanism, releasing the folding substrate outside the GroE system. The second ring then becomes folding active, and the GroE system functions as a two-stroke folding machine. Thus, the allosteric role of a second ring is necessary to complete folding of the substrate protein by ejecting it to the environment for function, and to propel a continuous GroE-reaction cycle.

The importance of the double-ring assembly is further supported by the observations that a single-ring GroEL variant [13], termed GroEL^{SR} here, which has four mutations (R451A/E461A/S463A/V464A) to disrupt the inter-ring contact, cannot complement GroEL to support cell growth [14]. Although GroEL^{SR} binds ATP and GroES like the double-ring GroEL and the substrate folds within the GroEL^{SR}–GroES chamber, the folding substrate is not released from the GroE system [15–18], due to the lack of a second ring to allosterically dissociate the stable GroEL^{SR}–GroES complex ($t_{1/2} = 300\ min^{-1}$) [13].

Several approaches have been taken to activate the single-ring GroEL^{SR}–GroES. In our earlier studies [19], we took a biochemical/structural approach to directly weaken the GroEL^{SR}–GroES interaction, in order to render the stalled GroEL^{SR}–GroES recyclable. A straightforward point mutation on the GroEL-interacting residues (I25 or L27) in *groES* abruptly disrupts the GroEL–GroES interaction, most likely due to the seven-fold amplified mutational effect in heptameric GroES. To weaken GroEL–GroES interaction in a controlled manner, we concatenated seven copies of *groES* to generate a gene *groES*^{7}, with specific cloning sites so that the number of mutations can be varied at the protein level. We identified GroES^{7} variants that collaborate with GroEL^{SR} to undergo a continuous folding reaction, and to complete mediating folding of malate dehydrogenase (MDH), whose folding requires chaperone function of GroEL–GroES. We found that the ATP hydrolysis rate of these active single-ring GroEL^{SR}–GroES^{7} systems is comparable to the double ring GroEL–GroES. In these studies, no additional mutations were incorporated into GroEL^{SR}, so the intrinsic properties of GroEL, including ATP binding/hydrolysis, bindings of mis-folded proteins and GroES, and allosteric mechanisms within the GroEL ring, were presumably maintained.

Mutations in GroEL^{SR}, obtained via genetic screen, have been shown to activate the single-ring GroEL^{SR}–GroES [20]. The mutated residues, A92T, D115N, E191G, and A399T, are not located in the region that directly interacts with GroES, therefore, their mutations may not affect the highly stable GroEL^{SR}–GroES interaction. How these mutations lead to dissociable GroEL^{SR}–GroES systems to release folding substrate is not known. To investigate the activation mechanism, in this study we characterized various biochemical properties of these functional, single-ring GroEL^{SR}–GroES variants.

2. Materials and methods

2.1. Protein expression and purification

E. coli BL21(DE3) cells were used to express GroEL or GroES via pTrc-*groEL* or pET3a-*groES*, respectively. *E. coli* MGM100 cells [21], whose chromosomal *groELES* operon is under the arabinose-dependent P_{BAD} control, were used to express GroEL^{SR} or GroEL^{SR} (via pTrc-*groEL*^{SR}) variants. Point mutation was incorporated into *groEL*^{SR} using the QuikChange kit (Stratagene) to generate GroEL^{SR} variants. BL21DE3 cells were grown in LB with ampicillin (100 μg/ml) while MGM100 cells were grown in LB with ampicillin (100 μg/ml), kanamycin (50 μg/ml) and 0.2% glucose. Conditions for cell growth, induction of protein expression, and protein purification are described in Ref. [19].

2.2. ATPase activity assays via malachite green

GroEL, GroEL^{SR}, and GroES proteins were dialyzed into TEA reaction buffer (50 mM triethanolamine pH 7.5, 50 mM KCl, and 20 mM $MgCl_2$), to 0.125 μM tetradecameric GroEL or 0.25 μM heptameric GroEL^{SR}, and 0.3 μM heptameric GroES. ATPase activity was measured via malachite green as described in Ref. [19]. Absorption at 660 nm (A_{660}) was measured, and the final A_{660} values were averaged over three readings. The amount of hydrolyzed free phosphate was derived from a standard curve, and the hydrolysis rate was normalized to GroEL or GroEL^{SR} monomer and expressed in PO_4 per minute per GroEL monomer or min^{-1}. At least three independent experiments were performed.

2.3. MDH refolding assay

GroEL, GroEL^{SR}, and GroES proteins were dialyzed into TEA reaction buffer. Malate dehydrogenase (Roche) was unfolded in TEA buffer including 3 M GdmHCl to a final concentration of 36.7 μM (monomeric MDH) for 60 min prior to the experiments. MDH refolding assay via monitoring the enzymatic activity of the refolded MDH at A_{340}, was described in Ref. [19]. The final protein concentrations were 1 μM of GroEL or 2 μM of GroEL^{SR}, 4 μM of GroES, and 0.7 μM of monomeric MDH. The enzymatic activity of native MDH was set to 100%, and at least three independent experiments were performed.

2.4. Chaperonin–cochaperonin binding via microscale thermophoresis (MST) assay

GroES was fluorescently labeled with DyLight™ 650 NHS Ester Amine Reactive Dye (ThermoScientific) according to manufacturer's protocol. The labeled GroES was separated from the free dye using MidiTrap (GE Healthcare) followed by dialysis (to 50 mM TrisCl pH 7.5, 100 mM KCl, 10 mM $MgCl_2$, and 1 mM EDTA), and its concentration was measured using the Bradford assay. For each unlabeled proteins (GroEL, GroEL^{SR}, and GroEL^{SR} mutants) a serial dilution of 15 samples were prepared in the binding buffer (50 mM TrisCl pH 7.5, 100 mM KCl, 10 mM $MgCl_2$, 1 mM EDTA, 2 mM ADP, and 0.5 mg/mL BSA). 10 μl of the unlabeled protein was incubated with 10 μl of labeled GroES for 30 min, and the solution was loaded into a glass capillary (NanoTemper Technologies) for MST measurements. Three identical series of dilution were prepared, and their data were averaged. The thermophoresis measurements were carried out using NanoTemper Monolith NT115 (NanoTemper Technologies) with 100% LED power and 40% IR-Laser power. Initial MST data were processed using Monolith NT115, and dissociation constant (K_d) was determined using KalidaGraph by fitting the following equation:

$$y = \frac{m1 + (m2 - m1)}{\left(1 + \frac{m3}{x}\right)} \quad (1)$$

where m1 is the thermophoresis reading of the labeled GroES in the absence of the unlabeled titrating protein, m2 is the thermophoresis reading when all the labeled GroES was bound with the unlabeled titrating protein, and m3 is the K_d.

2.5. E. coli MGM100 in vivo complementation assay

The MGM100 *E. coli* cell strain was obtained from the *E. coli* Genetic Stock Center at Yale University. MGM100 cells (kanamycin resistant, Kan^{R}) were propagated in LB media containing 50 μg/mL kanamycin and 0.2% arabinose. Plasmids with *lac* promoter were

used to express chaperonins and cochaperonins in MGM100 cells. GroEL and GroEL^{SR} were expressed using pTrc plasmid (ampicillin resistant, Amp^{R}), and GroES was expressed using pBbE5c plasmid (chloramphenicol resistant, Cam^{R}), which belongs to the BglBrick series [22]. $CaCl_2$ competent MGM100 cells were co-transformed with both plasmids and plated onto LB agar containing 50 μg/mL kanamycin, 100 μg/mL ampicillin, 50 μg/mL chloramphenicol, and 0.2% w/v arabinose. Cultures (5 mL of LB liquid with antibiotics and arabinose as above) from single colonies were grown at 37 °C with shaking. After an 18-h growth, absorbance at 600 nm was measured on a 1:10 dilution of culture. All cultures were normalized to the absorbance reading of 0.6, and serial dilutions (10^{-1}–10^{-7}) were prepared with LB without antibiotics or sugar. 5 μl of each dilution were pipetted onto LB agar containing three antibiotics as above, 0.2% w/v glucose and 0.1 μM IPTG. Plates were incubated at 37 °C or 42 °C for 18 h.

3. Results

3.1. ATP hydrolysis activity of GroEL^{SR} mutants

GroES decreases the ATP hydrolysis rate of GroEL by ~50% [11,23–25] (also Fig. 1), and exerts a greater inhibitory effect on GroEL^{SR} than GroEL, as it decreases ATP hydrolysis activity of GroEL^{SR} to 5–10% of the intrinsic rate of GroEL^{SR} [13] (also Fig. 1). The diminished ATPase activity arrests the chaperone reaction cycle, trapping the folding substrate within the chaperonin system, resulting in nonfunctional GroEL^{SR}–GroES. Presumably, the functional GroEL^{SR} variants should maintain some levels of ATPase activity in the presence of cochaperonin GroES, for a continuous reaction cycle.

In the functional GroEL^{SR}–GroES variants, the intrinsic ATP hydrolysis rate of GroEL^{SR} was affected by mutations to various degrees (Fig. 1), although none of the mutated residues are directly within the ATP binding site. Mutations of D115N and E191G have small yet noticeable effects (within 10%) on decreasing and increasing, respectively, the ATPase rate, while A399T mutation reduced the GroEL^{SR} ATPase rate by 20%. Notably, A92T mutation almost abolished ATPase activity of GroEL^{SR} as reported [26]. These GroEL^{SR} mutants also responded differently to the presence of cochaperonin GroES. GroES reduced ATPase activities of GroEL^{SR}D115N and GroEL^{SR}A399T by ~10–15% and GroEL^{SR}E191G by ~50%, when compared to their respective rates in the absence of the cochaperonin. In contrast to the inhibitory effect, GroES stimulated and re-activated the diminished ATP hydrolysis rate of GroEL^{SR}A92T (Fig. 1, also [26]). Despite different GroES effects, all four single-ring GroEL^{SR}–GroES systems revitalized the stalled ATP activity of GroEL^{SR}–GroES. Intriguingly, the four single-ring systems hydrolyzed ATP at rates (0.32–0.62 min^{-1}) comparable to that of the conventional double-ring GroEL–GroES system (0.41 min^{-1}).

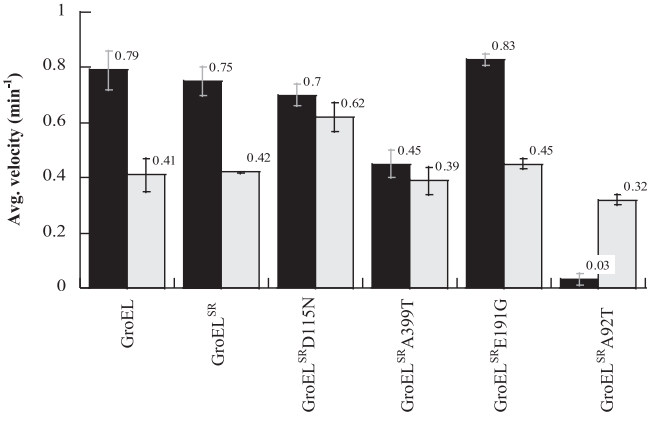

Fig. 1. ATP hydrolysis activities of the GroEL^{SR} mutants without (black bars) and with GroES (gray bars) via malachite green. GroEL^{SR} hydrolyzes ATP at a similar rate as GroEL. When compared to GroEL^{SR}, D115N and E191G mutations decreased and elevated, respectively, the ATPase activity by ~10%, and A399T mutation reduced by ~45%. The A92T mutation almost abolished the ATPase activity. GroES inhibited the ATP hydrolysis rate of both GroEL^{SR}D115N and GroEL^{SR}A399T by ~20%, and of GroEL^{SR}E191G by ~45%, while GroES stimulated GroEL^{SR}A92T by ~10 fold.

3.2. Interaction between the single-ring GroEL^{SR} variants and GroES

The diminished ATPase activity of the GroEL^{SR}–GroES system is due to formation of a highly stable GroEL^{SR}–GroES complex with a half life $t_{1/2}$ of ~300 min [13], whose dissociation is mediated via the allosteric effect from the absent second ring as stated above. Consistently, we found that in the presence of ADP GroEL^{SR} bound tightly with GroES via microscale thermophoresis (MST), with a dissociation constant (K_d) of 6.69 (±2.11) nM in agreement with the reported values of K_d~10 nM [27]. Note that our MST derived K_d of 3.84 (±0.77) nM for binding of GroES to the double ring GroEL in the presence of ADP compares well with the 0.3–3 nM range derived from various techniques [24,28,29].

Since the single-ring variants were able to continue the ATPase cycle in the presence of GroES, we expected that their interactions with GroES were reduced. The D115N mutation weakened the affinity for GroES by ~50 fold (with K_d = 191 ± 56.4 nM), the A399T mutation by ~90 fold (with K_d = 299 ± 45.4 nM), and the E191G mutation by more than 300 fold (with K_d = 1360 ± 87 nM). Most strikingly, the A92T mutation drastically reduced the GroEL^{SR}–GroES interaction affinity, as indicated by the >1600 fold reduction in K_d (10,843 ± 5005 nM). In sum, all these GroEL^{SR} variants displayed reduced affinities for GroES, ranging from 50 to 1600 folds of reduction (Fig. 2), indicating that GroES cycled between association with and dissociation off the single-ring GroEL^{SR} variants.

3.3. Refolding of MDH by the single-ring systems

The inability of GroEL^{SR}–GroES to assist protein folding is because the highly stable GroEL^{SR}–GroES complex traps and prevents the release of folding substrate protein from the chaperonin system, although the mis-folded substrate protein can complete folding within the enclosed folding cavity [15–18]. Since mutations in GroEL^{SR} reduced the GroEL^{SR}–GroES interaction to various degrees, we expected that when GroEL^{SR}–GroES dissociated the folding substrate protein could be discharged outside the chaperonin system into aqueous environment, resulting in the well-folded, functional substrate protein.

To test whether the above single-ring systems assisted protein folding, we used malate dehydrogenase (MDH), because spontaneous folding of MDH is inefficient and the chaperonin system is required for the productive folding [30]. GroEL^{SR}A399T–GroES exhibited the best activity in folding MDH, with a small delay in initial stage of folding, a slow folding kinetics, and a small decrease in the final folding yield (65.6%) when compared with the double ring GroEL–GroES (75.5%) (Fig. 3). GroEL^{SR}D115N–GroES started folding of MDH with comparable kinetics as GroEL–GroES, and the reaction appeared to slow down slightly until reaching the maximum folding rate at ~10 min. Interestingly, MDH folding mediated via GroEL^{SR}D115N–GroES decreased slightly after reaching the maximum rate, while that via GroEL–GroES and GroEL^{SR}A399T–GroES maintained the maximum rate. Strikingly, both GroEL^{SR}A92T–GroES and GroEL^{SR}E191G–GroES did not productively assist MDH folding, as the MDH folding activities were similar to that of the highly stable, inactive GroEL^{SR}–GroES. The

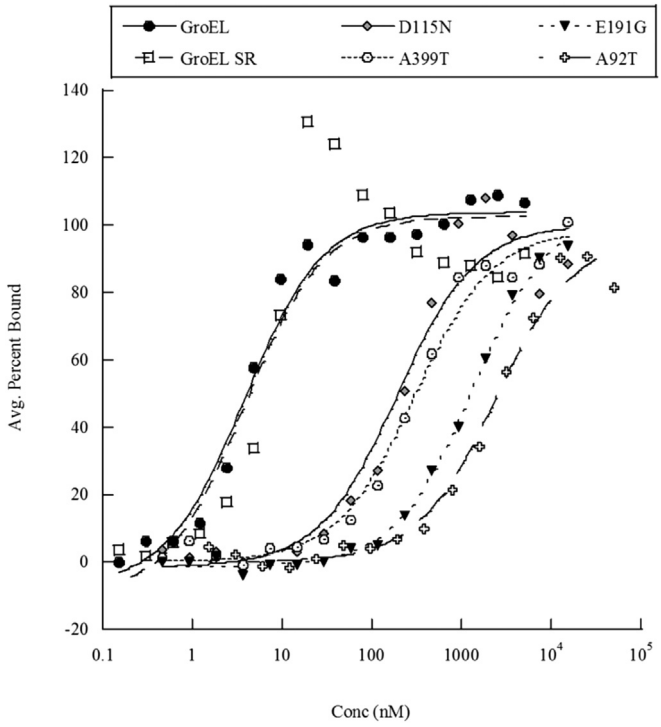

Fig. 2. Binding affinities between GroEL^{SR} variants with GroES by MST. GroES was labeled and kept at a constant concentration of 10 nM, while unlabeled GroEL or GroEL^{SR} was titrated. To obtain the percent bound, the value of the bottom plateau of the curve was subtracted from each raw fluorescence value and then divided by the amplitude. Data of each sample were fit into Equation (1) to generate the titration line and to derive the dissociation constant (K_d). The derived K_d values are summarized in Table 1.

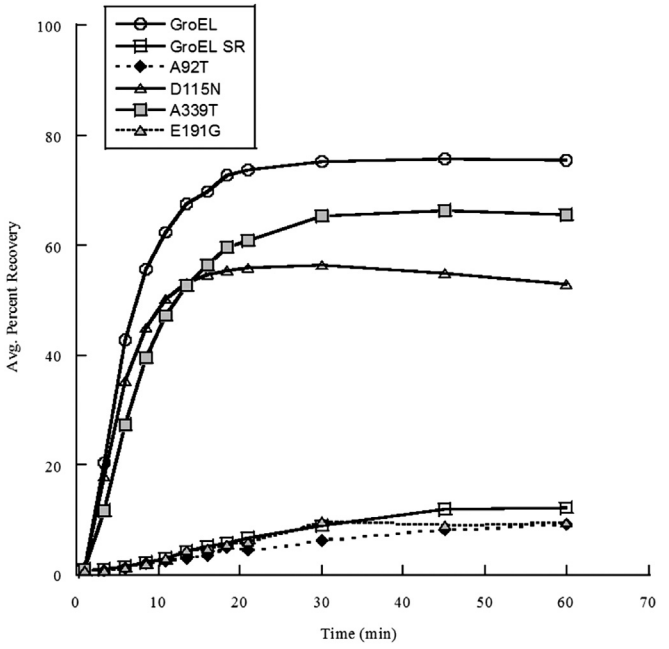

Fig. 3. Folding of MDH by the GroEL^{SR}–GroES variants. Shown are the average MDH activities over 60 min of refolding. MDH activity was evaluated via its enzymatic reduction of mesoxalic acid using electrons from NADH. Conventional double-ring GroEL–GroES had a burst of activity that reached a plateau after 20 min, while GroEL^{SR}–GroES displayed low activity in refolding MDH. GroES paired with GroEL^{SR}D115N and GroEL^{SR}A399T displayed the similar MDH refolding curve as GroEL, however with lower plateau values. GroEL^{SR}A92T and GroEL^{SR}E191G with GroES were similar to GroEL^{SR}–GroES in their low ability to fold MDH. The average folding yields of MDH after 60 min are listed in Table 1.

inability of both systems to mediate MDH folding is surprising, considering that each chaperonin–cochaperonin pair resumed the association–dissociation cycling in both systems (above) thus the folding substrate was presumably allowed to be released from the chaperonin system for its enzymatic function.

3.4. In vivo chaperone function via cell growth by the single-ring systems

GroEL–GroES is required for cellular viability [1] as the chaperonin system assists folding of a range of essential cellular proteins [2]. Since the highly stable GroEL^{SR}–GroES cannot dissociate to release folding substrate proteins [15–18], the system cannot substitute GroEL–GroES to support cell growth [14].

We evaluated whether the single-ring systems could functionally complement GroEL–GroES to sustain cell growth under both the optimal growth temperature (37 °C) and the heat shock condition (42 °C). As expected from their recovered MDH folding activity, both GroEL^{SR}A399T–GroES and GroEL^{SR}D115N–GroES single-ring systems supported cell growth in the same manner as the conventional double-ring GroEL–GroES at 37 °C and 42 °C (Fig. 4). Surprisingly, in contrast to its inability to fold MDH, GroEL^{SR}A92T–GroES complemented GroEL–GroES at both 37 °C and 42 °C, as cells expressing them grew as vigorously as those expressing GroEL–GroES. GroEL^{SR}E191G–GroES could complement GroEL–GroES to a lesser extent at 37 °C, but not at 42 °C, an observation may be correlated with the findings that the double-ring GroELE191G mutant is temperature sensitive and does not support cell growth at 42 °C [31]. In summary, all four single-ring variants collaborate with GroES as functional chaperonin system at 37 °C, and three of them are functional under heat shock (42 °C) condition.

4. Discussion

Most often bacterial chaperonins, represented by the prototypic *E. coli* GroEL, assemble into a double-ring conformation to function as a two-stroke engine to assist protein folding; however, human mitochondrial chaperonin exists as a single-ring assembly and may function as a one-stroke folding machine. The ability of chaperonin to adopt different quaternary conformations is mechanistically important, providing a broad understanding of chaperonin evolution from bacteria to mitochondria, and may be exploited in

Table 1
Biochemical characterizations of the functional single-ring GroEL^{SR}–GroES systems.

	GroEL	GroEL^{SR}	GroEL^{SR} D115N	GroEL^{SR} A399T	GroEL^{SR} E191G	GroEL^{SR} A92T
ATPase activity (min^{-1})	0.79 ± 0.07	0.75 ± 0.05	0.70 ± 0.04	0.45 ± 0.05	0.83 ± 0.02	0.03 ± 0.02
ATPase activity with GroES (min^{-1})	0.41 ± 0.06	0.04 ± 0.004	0.62 ± 0.05	0.40 ± 0.05	0.43 ± 0.02	0.32 ± 0.02
Binding Affinity for GroES K_d (nM)	3.84 ± 0.77	3.69 ± 2.11	191 ± 56.4	299 ± 45.4	1360 ± 87	10,843 ± 5005
Maximal MDH yield	75.5% ± 3.0%	12.3% ± 2.0%	52.9% ± 9.0%	65.5% ± 8.0%	9.4% ± 5.0%	9.2% ± 4.0%

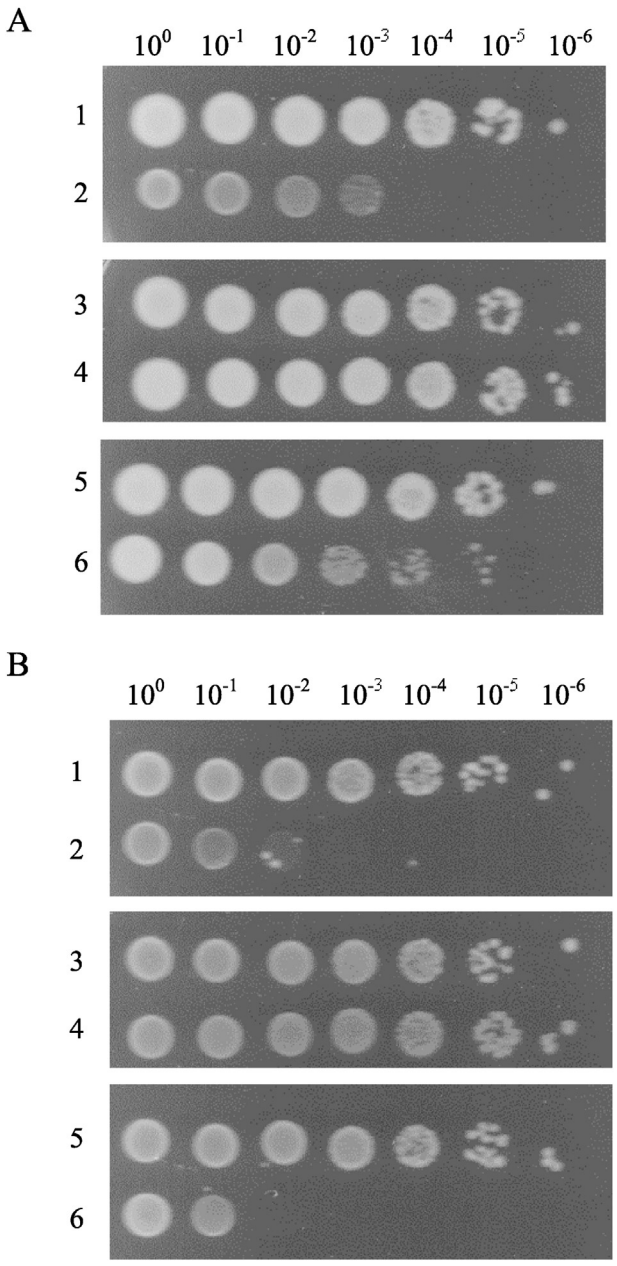

Fig. 4. Complementation of GroEL^{SR}–GroES variants for GroEL–GroES in supporting cell growth using *E. coli* MGM100 at the optimal growth temperature 37 °C (A) and under heat stress condition at 42 °C (B). Cells were serially diluted and plated, with added glucose to repress the chromosomal *groESEL* and added IPTG to express plasmid-encoded genes with *lac* promoters. Cells complemented with separate plasmids for *groEL* and *groES* (Line 1) exhibited growth level to dilution 10^{-6} for normal and heat shock temperatures, while those with *groEL*^{SR} and *groES* exhibited poor growth level to 10^{-3} (Line 2). Lines 3, GroEL^{SR}D115N–GroES; 4, GroEL^{SR}A399T–GroES; 5, GroEL^{SR}A92T–GroES; 6, GroEL^{SR}E191G–GroES.

medical applications via selective differentiation between the bacterial and human mitochondrial systems. However, the principles that allow chaperonin in different conformations, single or double ring, to execute their biological function are still emerging. In this study, we investigated factors important for a functional single-ring system and compare them with the double-ring counterpart. The single-ring GroEL^{SR}–GroES is nonfunctional as the chaperone cycle is stalled and the folding substrate is trapped due to the highly stable GroEL^{SR}–GroES. Mutations leading to active GroEL^{SR}–GroES can be divided into two groups: mutations to directly weaken the GroEL^{SR}–GroES interaction via the GroEL^{SR}–GroES interacting residues [19], and mutations outside the GroEL^{SR}–GroES interface which work presumably via an allosteric effect [20]. The allosteric activation mechanisms are not clear, and here we examined several aspects of the functional single-ring systems.

Several biochemical properties are important for the single-ring chaperonin systems to be functional. We found that the functional GroEL^{SR}–GroES variants revived the stalled ATP reaction of GroEL^{SR}–GroES, and hydrolyzed ATP at a rate comparable to that of the double ring GroEL–GroES. We also found that the GroEL^{SR} variants had much weaker binding affinity than GroEL^{SR} for GroES, with at least 50-fold higher K_d values. Interestingly, not all the functional GroEL^{SR}–GroES systems mediated folding of MDH effectively, suggesting that the ability to assist *in vitro* folding of MDH, despite its being commonly used as the folding substrate in the chapreonin studies, may not be correlated with the *in vivo* chaperone function of chaperonins. Thus, both the revived ATP hydrolysis and the reduced chaperonin–cochaperonin interaction are reliably indicative for the GroE-derived single-ring systems to function as folding chaperones.

The unique biochemical properties of the functional single-ring chaperonin systems may present opportunities to distinguish themselves from the conventional double-ring chaperonin system. In the two-stoke mechanism of the double-ring system, the inter-ring communication is far reaching, and consists of many allosteric conformations. For example, binding of ATP to the trans ring is transmitted across the ring–ring interface to the substrate-loaded and GroES-bound GroEL ring to weaken and dissociate GroES from the other GroEL ring [12,16,32]. This long-range allostery, starting from ATP binding to one ring to GroES dissociation from the other ring, consists of many intermediate conformations that may be stabilized by compounds that result in functional inhibition. Such compounds may not affect the single-ring chaperonin systems such as the human mitochondrial mtHsp60–mtHsp10, because the single-ring systems do not progress through the inter-ring allosteric communication path and the associated intermediate conformations. These compounds may be developed to reagents to inhibit the double-ring chaperonin system within a concentration range that has minimal effect on the single-ring system including mtHsp60–mtHsp10. Such reagents may be of therapeutic significance in anti-bacterial applications, because bacterial chaperonins form the double-ring structure while the human mitochondrial homolog exists as a single-ring assembly.

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Conflict of interest

The authors declare that there are no conflicts of interest.

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2015.08.034.

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