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Minireview

The GroEL/GroES *cis* cavity as a passive anti-aggregation device

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ABSTRACT

The GroEL/GroES chaperonin folding chamber is an encapsulated space of ~65 Å diameter with a hydrophilic wall, inside of which many cellular proteins reach the native state. The question of whether the cavity wall actively directs folding reactions or is playing a passive role has been open. We review past and recent observations and conclude that the chamber functions as a passive "Anfinsen cage" that prevents folding monomers from multimolecular aggregation.

1. Introduction

Chaperonins are large oligomeric double ring assemblies that carry out an essential function in the cell assisting many newly-translated proteins to fold to their native forms [1–3]. The bacterial chaperonin, GroEL, the most studied of this family, is a tetradecamer of 57 kDa subunits, assembled as two back-to-back seven-membered rings, each with a central cavity containing a hydrophobic lining to which a non-native polypeptide substrate can bind [4]. The co-chaperonin GroES, a seven-membered ring of 10 kDa subunits, associates as a "lid" structure with either end of GroEL in an ATP-dependent manner to form an enclosed cavity with a now hydrophilic wall character where folding of non-native substrate proteins takes place [5–10] (see Fig. 1).

While the steps of the ATP-driven GroEL/GroES reaction cycle have been generally understood for nearly 10 years, how this system acts on substrate polypeptides to assist their proper folding has remained unclear. It has been established, for example, that non-native proteins are bound by an open ring, typically of an asymmetric GroEL/GroES/ADP "bullet" complex ([11]; see Fig. 1, panels a and b], via hydrophobic contacts. Yet whether such binding mediates polypeptide unfolding, effectively taking a misfolded protein back to the top of its energy landscape, has been unclear. In the subsequent step of the reaction, GroES binding to the same ring as polypeptide and ATP releases substrate from the cavity wall into a now encapsulated hydrophilic chamber ([12–14]; Fig. 1c). The fate of substrate during this sequence of ATP-mediated freeing of the apical domains, GroES collision, and large forceful rigid body movements to produce the domed end-state, has also been under study. Finally, protein folding proceeds within the GroEL/GroES/ATP *cis* folding chamber, the longest-lived state in the reaction cycle (Fig. 1c). Does the GroEL cavity wall actively direct or modify this reaction, or does it simply passively contain the folding polypeptide? The first questions, concerning GroEL actions on polypeptide during the steps of polypeptide binding and *cis* complex formation, are beginning to be resolved, and we review current understanding of them at length elsewhere. The present discussion focuses on the last question concerning the mechanism by which the *cis* GroEL/GroES folding chamber, a unique encapsulated hydrophilic cavity, supports productive folding.

Our thesis, derived from recent experiments coupled with consideration of past observations, is that the *cis* chamber is, as John Ellis termed it in 1993, a passive "Anfinsen folding cage", where a non-native polypeptide chain is isolated as a monomer and employs the information intrinsic to its primary structure, in the absence of external information, to fold to its energetic minimum, the native state [15]. The polypeptide may be subject to kinetic er-

Abbreviations: DM-MBP, double mutant of maltose-binding protein; GSH/GSSG, reduced and oxidized glutathione, respectively; TG, trypsinogen; BSA, bovine serum albumin; NMR, nuclear magnetic resonance; DHFR, dihydrofolate reductase; GFP, green fluorescent protein; *R. rubrum*, *Rhodospirillum rubrum*

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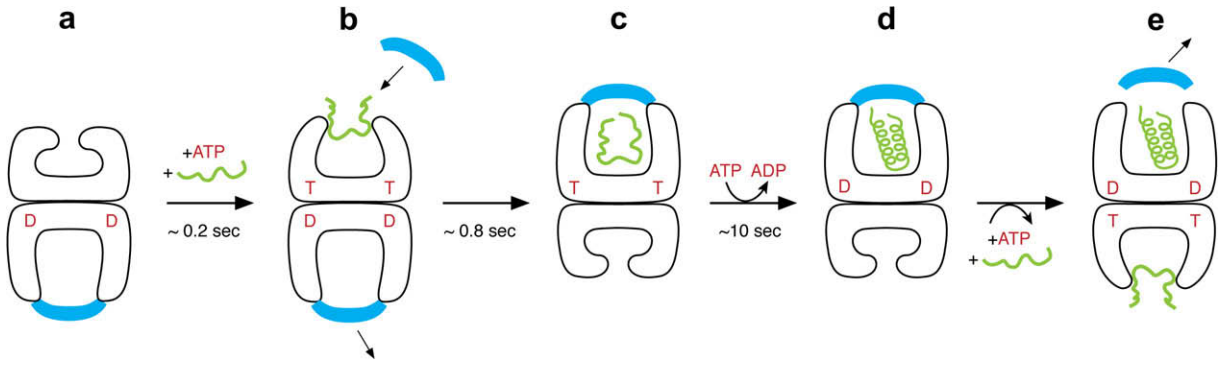

Fig. 1. Chaperonin reaction cycle. An asymmetric GroEL-GroES-ADP complex (a) is the normal acceptor state for ATP (red; also indicated as T) and non-native polypeptide (green), binding them (b) in the open ring opposite the one bound by GroES (blue) and ADP (red D). ATP binding produces small rigid body apical domain movements in the bound ring (b), enabling GroES binding, attended by large rigid body movements that produce the stable folding-active *cis* complex end-state (c). This folding-active state is the longest-lived state of the reaction cycle, ~10 s, followed by ATP hydrolysis (c → d), which then gates the entry of ATP and polypeptide into the opposite *trans* ring, rapidly discharging the *cis* ligands (e) and initiating a new folding-active cycle on the ATP/polypeptide-bound ring.

rors during this process, particularly at physiologic temperature, taking it off the productive pathway, but its confinement as a monomer protects it from multimolecular aggregation, enabling kinetically unproductive monomeric states to ultimately redirect themselves, through the energetic action of thermal fluctuations, onto a productive pathway to the native state. The major action of the chamber is thus to prevent protein aggregation, which comprises, when reversible, a set of off-pathway diversions that slows productive folding, and when irreversible, an off-pathway end-state that diminishes yield and produces potentially harmful structures.

In support of the foregoing conclusion about the *cis* chamber, we summarize below a number of key observations concerning the GroEL/GroES reaction. We focus first on those derived from studying the machine's action under so-called non-permissive conditions, where polypeptide substrate cannot reach the native state without the presence of the complete GroEL/GroES/ATP system, then on studies under permissive conditions, where the same protein substrates can reach the native state either while inside the GroEL/GroES cavity or while folding free in solution.

2. Non-permissive conditions

2.1. Requirement for the GroES "lid"

The first in vitro reconstitution of the chaperonin reaction showed that both GroES and ATP had to be added to a binary complex of GroEL and *Rhodospirillum rubrum* Rubisco in order to recover native active Rubisco enzyme [16]. Notably that first experiment, and many that have followed, was carried out under so-called non-permissive conditions, in particular involving a concentration of substrate protein (in that case ~100 nM) and temperature (25 °C), where, in the absence of the GroEL/GroES system, the substrate protein quantitatively aggregated, and where, for any recovery of native protein, the complete chaperonin system was required. In this latter regard, although GroEL alone at stoichiometric or greater concentration could forestall aggregation, recovery of the native state required GroES, whose binding to GroEL was known to be ATP-dependent.

The role of GroES as an encapsulating agent was deduced from EM and biochemical studies [5,6], and it was established that substrate proteins as large as the *R. rubrum* Rubisco subunit (51 kDa) could be refolded in the *cis* chamber formed when GroES bound to GroEL [8,12]. One obvious well-commented role of this chamber, which measures ~65 Å in both height and diameter (Fig. 2), was to provide a space where proteins of ~20–60 kDa, the typical size of authentic *cis* substrates, could be confined as single molecules and would be unable to aggregate [10]. In addition, it seemed unlikely that exposed hydrophobic surfaces of these non-native states, surfaces that originally recruited them to the hydrophobic lining of an open ring, would interact with the *cis* cavity wall, because the wall of the *cis* chamber was observed by X-ray studies to have been switched by rigid body movements, which occur during ATP/GroES binding, to a polar character, exposing 315 electrostatic side-chains and only 14 hydrophobic ones [10,17].

3. Single round refolding mediated by SR1-GroES

The importance of the *cis* chamber to productive folding is highlighted by the observation that a single ring version of GroEL, called SR1, which binds GroES in the presence of ATP but then does not release it, is fully productive, with kinetics and extent of native state recovery in this stable folding chamber that are virtually identical to those of the cycling GroEL/GroES reaction, where GroES and substrate polypeptide are discharged from GroEL approximately every 10 s [8,12]. The *cis* chamber of SR1/GroES is stable because the normal allosteric signal for discharge of the *cis* ligands, ATP binding to the opposite (*trans*) GroEL ring, cannot occur – there is no *trans* ring. Importantly, the length of time required for full recovery of the native state of many substrate proteins, both by the cycling reaction and by SR1/GroES, is greater than 15 min. In the case of the SR1/GroES-mediated reaction, this implies that a significant fraction of the non-native states inside the *cis* chamber of SR1/GroES can spend a relatively large amount of time exploring their folding free energy landscapes without becoming irreversibly trapped. They clearly do not have any requirement to be re-bound by an open ring during this time, because they continue to form the native state at the same rate as in a cycling reaction and ultimately, as in the cycling reaction, they yield the native form with high efficiency, approaching 90–100% for many substrates.

Notably, there is just one round of ATP turnover by an SR1/GroES complex, occurring in the first 10 seconds after its formation, after which the complex is stable as an ADP-SR1/GroES complex [6]. Thus there is no ongoing involvement of ATP in the folding process inside this complex, and the polypeptide chain has to rely on the energy of thermal fluctuations to drive conformational changes, essentially as it would if free in solution. The difference is that, in this chamber, it cannot aggregate. It can remain unfolded or misfolded as a monomer and thus is free of the complications of an additional set of kinetic mis-steps involving multimolecular associations.