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Fibrillogenic propensity of the GroEL apical domain: A Janus-faced minichaperone

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ABSTRACT

The chaperonin GroEL plays an essential role in promoting protein folding and in protecting against misfolding and aggregation in the cellular environment. In this study, we report that both GroEL and its isolated apical domain form amyloid-like fibrils under physiological conditions, and that the fibrillation of the apical domain is accelerated under acidic conditions. We also found, however, that despite its fibrillation propensity, the apical domain exhibits a pronounced inhibitory effect on the fibril growth of β_2-microglobulin. Thus, the analysis of the behaviour of the apical domain reveals how aggregation and chaperone-mediated anti-aggregation processes can be closely related.

Structured summary of protein interactions:
groEL and **groEL** bind by circular dichroism (View interaction)
β2m and **β2m** bind by transmission electron microscopy (View interaction)
β2m and **β2m** bind by fluorescence technology (View interaction)
groEL and **groEL** bind by transmission electron microscopy (View interaction)
groEL and **groEL** bind by nuclear magnetic resonance (View interaction)
groEL and **groEL** bind by fluorescence technology (View interaction)

1. Introduction

Misfolded proteins that escape the surveillance of molecular chaperones can aggregate and form stable highly ordered assemblies known as amyloid fibrils [1]. This process was initially studied because of its links with disease, but it was subsequently recognised as very general, so that even proteins not associated with any known disease may form amyloid-like fibrils depending on the experimental conditions [2,3]. According to the "life on the edge" hypothesis, proteins are expressed in living organisms at levels at which they are only just soluble, and therefore they are readily susceptible to aggregation [4]. It has also been suggested that that almost all proteins have at least one structural segment with a high propensity to aggregate [5].

The generic nature of the phenomenon of protein aggregation raises the question of how molecular chaperones, which are key player in the cellular defenses against aggregation, can control their own propensity to aggregate. This question is especially relevant as many molecular chaperones have been reported to undergo aggregation, such as in the case of the small heat shock proteins (sHsps) [6–8], the chaperone-like casein proteins [9], or the proteins in the crystallin family from human eye lenses [6,10,11].

In this study, we identified the aggregation-prone regions of the chaperonin GroEL, which together with GroES, plays a central role in the cellular quality-control system [12]. It is now well established, both from *in vivo* and *in vitro* studies, that the GroEL/GroES system is capable of assisting the folding process of a variety of different proteins [12–14]. Thus, the GroEL/GroES system has been the subject of a large number of structural and kinetic studies aimed at characterising the molecular mechanism by which it performs its function [15,16]. The oligomeric GroEL consists of 14 identical subunits arranged into two heptameric rings in a back-to-back manner. Each GroEL subunit has three functional domains termed apical, intermediate and equatorial. The equatorial domain

Abbreviations: β_2m, β_2-microglobulin; αBα, α-crystallin domain of αB-crystallin; ThT, thioflavin T; SDS, sodium dodecyl sulphate; CD, circular dichroism

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accounts for most of the contacts between the heptameric rings, the apical domain is involved in the binding of GroES and substrate proteins, and the intermediate domain acts as a hinge region to flank equatorial and intermediate domains [15,17].

The GroEL co-chaperonin, GroES was observed to form amyloid-like fibrils after long incubation in the presence of guanidine hydrochloride [18,19], and thus was correlated with some intrinsic disordered properties [18,19]. As the intrinsic disordered region in many cases is involved in the amyloid-formation of a protein [1], we therefore investigated whether GroEL, as the structural and functional partner of GroES, may also contain aggregation-prone regions so as to fibrillate. We found that the GroEL apical domain, previously defined as minichaperone, has a high propensity to form amyloid-like fibrils when incubated with agitation under both physiological and acidic conditions. Interestingly, despite its amyloidogenic tendency, the apical domain is still capable of inhibiting the seed-dependent fibril growth of β_2m. Therefore, the GroEL apical domain, owing to its dual properties, represents a minichaperone system that contributes to our understanding of the relationship between the generic phenomenon of amyloid formation and chaperone functions.

2. Materials and methods

2.1. Proteins and fibrils preparation

Recombinant human β_2m was produced as described [20]. The isolated GroEL apical domain (GroEL residues 191–376, for simplicity, we use "apical domain" to denote hereafter) was cloned into a PET24a vector (Invitrogen, Carlsbad, CA), yielding an expression plasmid with an N-terminal histidine-tag. Both apical domain and GroEL were produced as described [21,22]. Prior to use, the proteins were dialysed overnight against 20 mM Tris–HCl at pH 7.0. The isolated human αB-crystallin fragment (residues 68–162) was prepared as reported [23].

The β_2m amyloid fibrils were formed at 37 °C by repeated seed-dependent extension reactions, starting with 0.3 mg/ml recombinant β_2m. To prepare the seeds, the β_2m fibrils were ultrasonicated by using a Microson sonicator (Misonix, Farmingdale, NY) with ten 1-s pulses on ice. The frequency and power of output were set to 17–20 kHz and 700 W, respectively.

To investigate the fibrillation behaviour of apical domain, we adopted optimised incubation conditions for forming β_2m amyloid fibrils previously established in this laboratory (Table 1). As it was previously reported that agitation of stirring or shaking may shorten the lag phase and promote the fibrillation process of a protein such as insulin or β_2m [24,25], we also examined the effect of the agitation (stirring) in this study. Apical domain was incubated in a physiological buffer at pH 7.0 in the presence of sodium dodecyl sulphate (SDS) and under two acidic conditions at pH 2.5 as shown in Table 1. The sample solutions were incubated in a thermostatic cabinet at 37 °C. As the yield of fibril formation at neutral pH is relatively lower than under acidic conditions, apical domain and GroEL fibrils formed under the neutral condition were further purified in order to be best characterised. Briefly, 900 μl apical domain or GroEL sample solution incubated with agitation at pH 7.0 was spinned down at 17 000*g* for 1 h at 20 °C. The supernatant was carefully removed and the pellet was resuspended with a volume of incubation buffer to optimise a protein concentration for the spectroscopic measurement.

2.2. Fibril assay

The formation of amyloid fibrils was quantitatively characterised using a fluorescent dye, Thioflavin T (ThT) [26]. Five microliters of incubated sample or resuspended fibril solution of apical domain was mixed with 1.0 ml of 5 μM ThT in 50 mM glycine–NaOH buffer (pH 8.5). To monitor the fibrillation kinetics, 5% (v/v) 100 μM ThT was added to the protein solution (a final dye concentration of 5 μM), and the signal change of ThT fluorescence was recorded. Fluorescence was measured with a Jasco FP 6500 spectrofluorometer (Jasco, Tokyo, Japan) at the indicated temperatures. The excitation wavelength was set to 445 nm (slit: 20 nm), and the emission spectra (slit: 10 nm) was scanned three times in the range of 455–550 nm and averaged afterwards. An increase in the fluorescence emission intensity at 485 nm was considered to be indicative of amyloid formation.

2.3. Far-UV circular dichroism (CD) spectroscopy

Far-UV CD spectra were measured in a 1-mm quartz cuvette unless otherwise specified on a Jasco J-720 spectropolarimeter equipped with a constant-temperature water bath. To probe structural changes of apical domain with different SDS concentrations, 167 μM apical domain was aged overnight in the presence of 1 mM SDS without stirring prior to a dilution to a residual SDS concentration of 0.06 mM. The sample was subsequently incubated at room temperature for 12 h followed by the recording of CD spectra. To measure the CD spectra of apical domain fibrils, sample solution was homogenised by sonication with five 1-s pulses on ice before the measurement using a 0.2-mm quartz cuvette.

2.4. ^{1}H NMR spectroscopy

NMR experiments were performed on a Bruker Avance 500 MHz spectrometer. At the indicated temperatures, 270 μl apical domain in 95% H_2O/5% D_2O with a protein concentration of 1 mM was placed in a Shigemi NMR microtube to record the NMR spectra at pH 2.5 and 7.0, respectively. The experimental temperature was maintained with a variable temperature unit. In general, protein samples are invisible to NMR once fibrils formed inside [6]. In order to allow for a structural comparison under different experimental conditions, NMR spectra of apical domain were recorded immediately after the sample solution was prepared without stirring.

2.5. Electron microscopy

The microscopic structures of apical domain fibrils formed at specified conditions were observed using a HITACHI H-7650 transmission microscope (Hitachi, Tokyo, Japan) operated at 80 kV. Five

Table 1
Tested conditions for the fibrillation of apical domain and GroEL.

Solution/buffer	pH	Composition	Static incubation	Agitated incubation (stirring)
HCl (acidic)	2.5	3.2 mM HCl, 0.1 M NaCl	Yes	Yes
Citrate (acidic)	2.5	50 mM citrate buffer, 0.1 M NaCl	Yes	Yes
Sodium phosphate (physiological)	7.0	50 mM sodium phosphate buffer, 0.1 M NaCl, 0.5 mM SDS	Yes	Yes

microliter samples were placed on a 400-mesh copper grid covered by a carbon-coated colloidal film for 60 s. Grids were negatively stained with 5 μl of 2% (w/v) uranyl acetate solution for 60 s. Excess sample solutions were removed with filter paper. The magnification was set to 10 000–30 000.

3. Results

3.1. Theoretical predictions

To study the amyloidogenicity of GroEL, several algorithms including PASTA, TANGO and Zyggregator were employed to generate the aggregation profile of GroEL [27–30]. Fig. 1A shows the prediction result by PASTA. Intriguingly, the aggregation-prone segment generated by PASTA is mainly located within the GroEL apical domain region (residues 260–280) with a score of about 0.05, which is close to the value of highly amylodogenic peptides, such as $A\beta_{1-40}$ [27]. The high aggregation propensity of the apical domain was also found using TANGO and Zyggregator (Fig. S1). Moreover, as shown in Fig. 1B, these aggregation-prone residues mainly compose helix I, the region identified previously to bind substrate proteins [31,32]. Despite having aggregation-prone segments, most proteins with known structures generally do not form fibrils under physiological conditions [4,5]. Therefore, our theoretical aggregation analysis led us to ask whether GroEL or isolated apical domain fragment, shown as a stable, functional form [33,34], may have a propensity to fibrillate. We therefore prepared the isolated apical domain and GroEL and studied their amyloidogenicity.

3.2. Fibrillation of apical domain in a physiological buffer

Fibrillation of apical domain was firstly monitored by ThT assay [26] in a physiological buffer (Table 1) in the presence of SDS. As shown in Fig. 2A, when incubated at pH 7.0 without stirring, apical domain showed a lack of fluorescence signal increase by ThT assay (dash black *vs* solid red). However, purified fibrils from agitated sample showed a remarkable ThT fluorescence signal increase compared with statically incubated sample (solid black vs red). Furthermore, transmission electron microscopy (TEM) observations revealed an apparent fibrillar structure formed under the agitated condition (Fig. 2B), suggesting that apical domain forms an amyloid-like structure. It should be noted that compared with the purified fibrils, ThT assay of bulk incubation solution of apical domain (without spinning down the fibrils) showed a less pronounced fluorescence signal increase (dash red vs solid black), indicating a relatively low population of fibrils in physiological buffer. Fibrillation behaviour of GroEL was also investigated. In contrast to apical domain, purified GroEL fibrils exhibited a marginal increase of fluorescence signal (Fig. S2A). Interestingly, TEM measurement of GroEL sample also showed distinguishable amyloid-like formation in the solution (Fig. S2B).

To investigate the secondary structure of the protein samples, we measured the far-UV CD spectra of apical domain under different conditions. As shown in Fig. 2C, the overall CD spectra of the native apical domain displayed a helix-abundant structure with two minima at around 208 and 220 nm. In the presence of 0.5 mM SDS, the CD spectra showed a more negative spectral minimum at around 207 nm (open circles), suggesting that the domain core was retained with C-terminal helices partially unfolded [33]. After overnight incubation with agitation, the overall CD spectrum of apical domain fibrils showed a high content of β-sheet composition similar to that observed in GroES [18] with a negative spectral minimum around 225 nm (open triangles). The key role of agitation in the fibrillation of apical domain was further corroborated by recording far-UV CD spectra of SDS-aged apical domain without agitation (Fig. 2D). In contrast to that obtained from the sample incubated with agitation (open triangles in Fig. 2C), CD spectra of apical domain incubated overnight without agitation at 1 mM SDS (open triangles in Fig. 2D) showed a partially disordered structure. When stock solution at 1 mM SDS was diluted to a residual SDS concentration of 0.06 mM, we observed a CD spectrum (open circles in Fig. 2D) of apical domain superimposable to that of 0.06 mM SDS-aged sample (filled circles in Fig. 2D) indicating that submicellar concentration of SDS (1 mM) causes a partial albeit reversible structural change of apical domain ready for its later fibrillation if the agitation is introduced.

3.3. Fibrillation of apical domain under acidic conditions

We also investigated acidic conditions, since these may accelerate the fibrillation of β_2m in our protocol. As shown in Fig. 3A, when incubated in a dilute HCl solution (pH 2.5) for 12 h at 37 °C, the ThT fluorescence signal of apical domain was increased

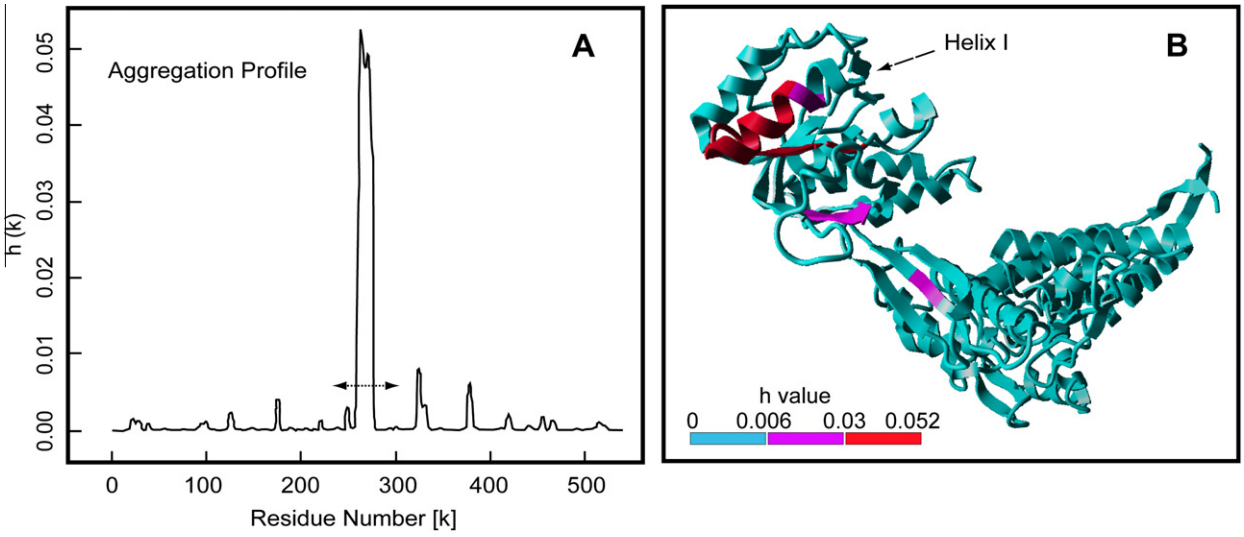

Fig. 1. Fibrillogenic propensity of the GroEL apical domain. (A) Aggregation propensity profile of GroEL by PASTA with aggregation-prone regions indicated by an arrow. (B) A coloured GroEL subunit based on the h value predicted by PASTA.

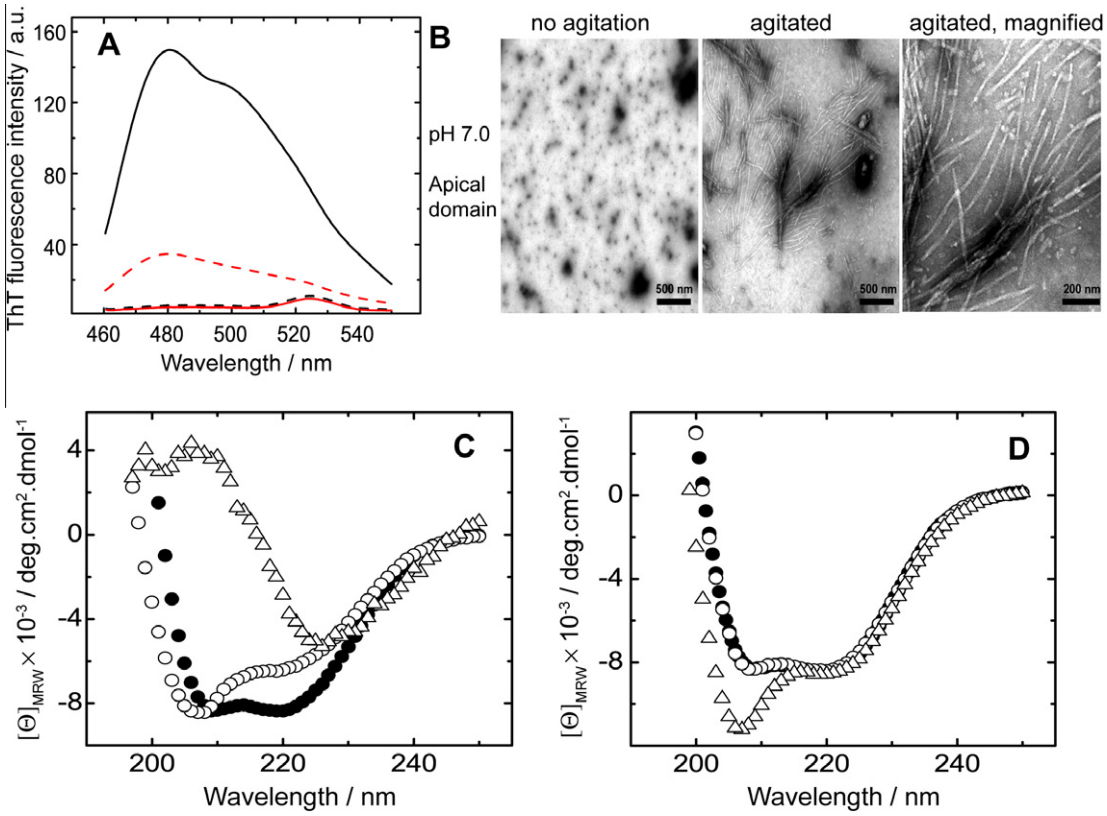

Fig. 2. Fibrillation of apical domain under a physiological condition. (A) ThT fluorescence spectra of 0.3 mg/ml apical domain at time 0 (dash black line) and incubated without agitation (solid red line) or incubated with agitation (bulk solution: dash red line; purified fibrils resuspended in 100 μl buffer: solid black line) after 30 h at 37 °C. (B) TEM observation of apical domain. Representative microscopic pictures are shown. (C) Far-UV CD spectra of 0.3 mg/ml apical domain solution: native state (●), incubated after 1 h (○) and purified fibrils from 24 h (△) incubation with agitation at 37 °C. (D) Far-UV CD spectra of 10 μM apical domain in neutral incubation buffer with 0.06 mM SDS (●), 1 mM SDS (△) and 0.06 mM SDS by dilution from the stock sample of apical domain in 1 mM SDS (○).

more than four-fold, suggesting the formation of amyloid-like structures. Moreover, sample incubated without agitation showed marginal differences of fluorescence signal increase with that incubated with agitation (solid black vs red), implying that a similar fibrillation process was involved for both conditions. A substantial increase of fluorescence signal was also observed for sample incubated in citrate buffer (Fig. S3), suggesting that the acidic conditions may promote fibril formation of apical domain. Interestingly, we also observed a ThT fluorescence increase for full-length GroEL (Fig. 3F), but unlike apical domain, the agitation greatly contributes to its signal increase. The amyloid-like fibrils of apical domain were further visualised by TEM. As shown in Fig. 3B, although GroEL also showed a remarkable ThT signal increase at acidic pH, large aggregate-like structures instead of amyloid-like fibrils were observed. In contrary, apical domain can still form clear amyloid-like fibrils when incubated with agitation, but not without, for both acidic conditions, suggesting a crucial role of agitation in terms of apical domain fibrils-formation. The amyloid-like fibrils of apical domain are relatively short compared to those of β_2m (Figs. 3B and 5B).

Far-UV CD spectra of apical domain at acidic pH were also recorded. As already shown in Fig. 2C, the native protein showed CD spectra minima at around 208 and 220 nm (Fig. 3C). After incubated for 1 h, we observed a disappearance of the double-well spectra minima (208 and 220 nm) suggesting the disruption of the helical structure. After we incubated the apical domain at pH 2.5 with agitation for 12 h, the overall CD spectra resembled the formation of a β-sheet-rich structure with an observable negative peak at 216 nm. The overall secondary structural change was further reflected in the 1D-^{1}H NMR spectra. At both 25 °C (experimental temperature) and 37 °C (physiological temperature), apical domain at pH 7.0 showed widely distributed resonance signals (Fig. 3D and Fig. S4) including backbone amide ^{1}Hs (7–10 ppm) and up-field aliphatic ^{1}Hs (0–2 ppm) (red curve), indicating the presence of the natively folded form of the protein [35]. However, at pH 2.5, apical domain exhibited an NMR spectrum typical for an unfolded protein, and the up-field shifted resonances observed in the native state at pH 7.0 (red curve) were vanished (black curve), indicating a disruption of the tertiary structure under acidic conditions.

The fibrillation kinetics of apical domain was monitored by adding dye directly in the solution. During the first 4–5 h of incubation, we observed a sharp fluorescence increase without any observable lag phase (Fig. 3E), suggesting a presence of fast or heterogeneous nucleation which may be induced by bubbles from stirring during the apical domain fibrillation. The ThT fluorescence signal increase was slowed down dramatically after 5 h of incubation, and finally reached a plateau after overnight incubation. The fast nucleation process involved in the apical domain fibrillation was further corroborated by TEM observations. As shown in Fig. S5, during the early stage of incubation with agitation (<4 h), we did not observe any apical domain fibrils in comparison with the overnight incubated sample (17 h).

3.4. Janus-faced properties of the apical domain minichaperone

As the GroEL apical domain harbours the substrate protein binding sites [31,32], it is not surprising that the isolated apical domain still possesses the chaperone activity [36,37]. We hypothesise that apical domain may retain some structural integrity even in the

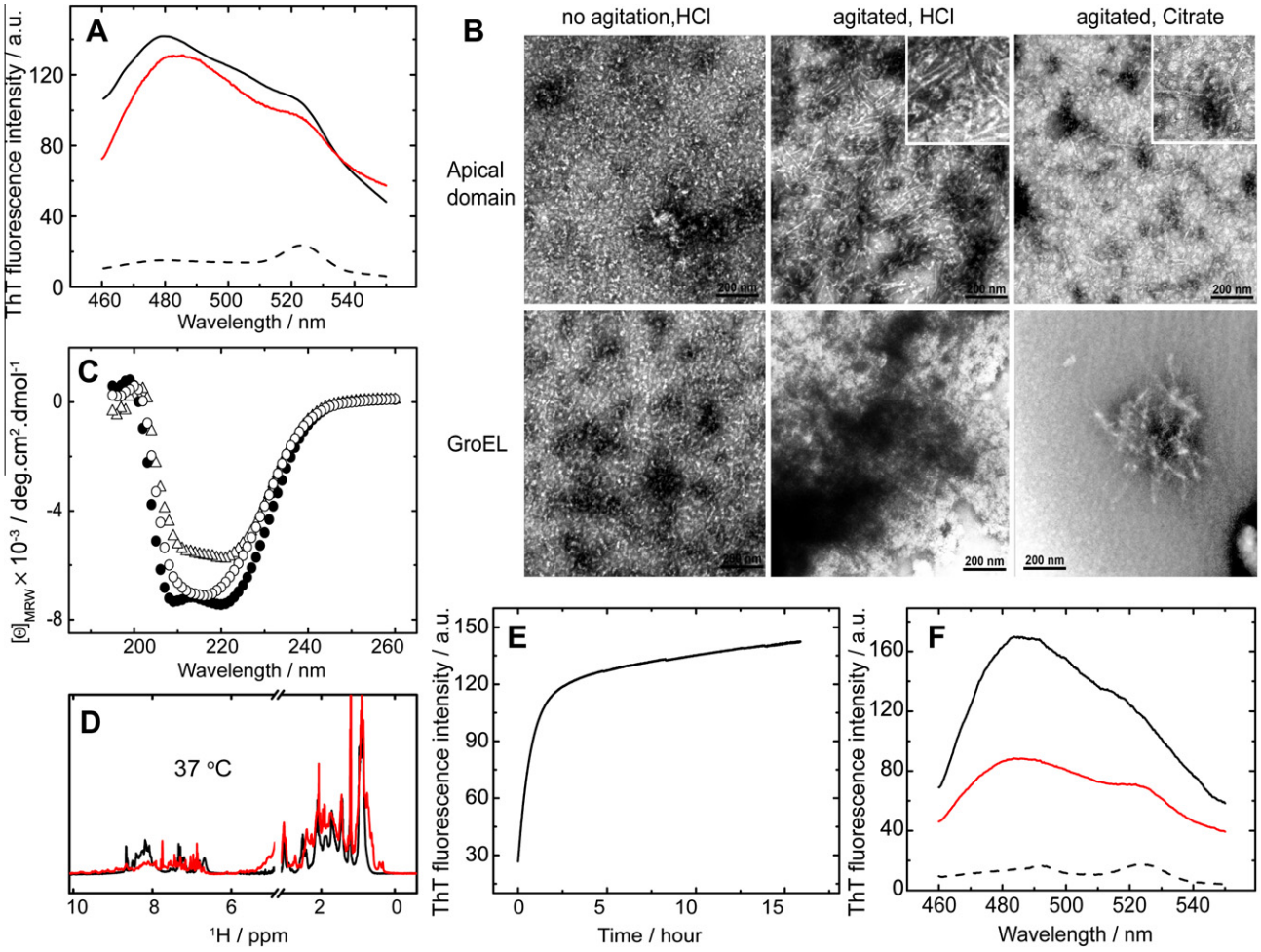

Fig. 3. Fibrillation of apical domain under acidic conditions. ThT fluorescence spectra of 0.3 mg/ml apical domain (A) and GroEL (F) at time 0 (dotted line) and incubated without agitation (red line) or incubated with agitation after 12 h (solid black line) at 37 °C. Incubation buffer: HCl, pH 2.5. (B) TEM observation of apical domain and GroEL. Representative microscopic pictures are shown (inset: magnified portion of images). (C) Far-UV CD spectra of 0.3 mg/ml apical domain: native state (●), incubated (HCl, pH 2.5) after 1 h (△) and 12 h (○) under an agitated condition at 37 °C. (D) Magnified 500-MHz 1D-^{1}H NMR spectra of apical domain at 37 °C. Red: pH 7.0; Black: pH 2.5. (E) The ThT fluorescence signal of 0.3 mg/ml apical domain under an agitated condition in HCl buffer changes with time at 37 °C.

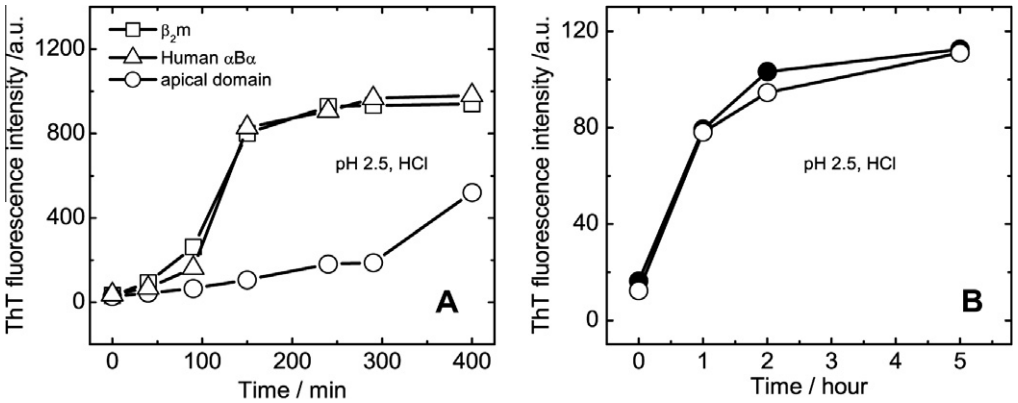

Fig. 4. Inhibitory effects of apical domain under acidic conditions. (A) Seed-dependent fibril growth of β_2m by incubating 0.3 mg/ml apical domain (○) or human αBα (△) with 0.3 mg/ml β_2m at pH 2.5 without stirring. 0.3 mg/ml β_2m (□) was used as a control. Samples were seeded with 5 μl sonicated β_2m fibrils and subsequently monitored by ThT assay. (B) Examination of cross-seeding of 0.3 mg/ml apical domain in the same buffer as A with (○) or without (●) β_2m fibrils.

fibrillation buffer, and hence exhibit some molecular chaperone activity. To address this question in the context of the present amyloidogenic study, we used an established β_2m seed-dependent fibril growth experiment to explore the chaperoning capability of apical domain.

As shown in 4A, we initiated the fibril growth of β_2m by mixing β_2m and preformed β_2m seeds with or without chaperone fragments, apical domain and α-crystallin domain from human αB-crystallin (human αBα), in HCl at pH 2.5, and monitored the β_2m fibril growth by ThT assay. When chaperone fragments were ab-

sent, we observed a typical fibril growth process of β_2m (Fig. 4A). Interestingly, in the solution of the equi-stoichoimetric apical domain and β_2m, we did not observe any significant ThT signal increase during the early 4-h incubation compared with the β_2m control (open circles *vs* squares) demonstrating the inhibitory effect of apical domain on the β_2m fibril growth. In contrast to apical domain, human αBα, the highly conserved domain of sHsps [38] which was identified to retain some chaperone activity under physiological conditions [23], did not show any pronounced inhibitory effect on the fibril growth of β_2m (open triangles *vs* squares). The inhibitory effect of apical domain on the β_2m fibril growth continued to some extent at 5-h incubation in HCl (with apical domain included, fluorescence intensity at 485 nm was 20% compared with the control). After 5-h incubation, we again observed a pronounced increase in ThT fluorescence, indicative of the fibril growth in HCl. Since apical domain readily fibrillates by itself at acidic pH, this may ultimately lead to its loss of the inhibitory activity.

Since sometimes cross-seeding occurs between two amyloidogenic proteins [39], which may complicate the interpretation of the inhibitory effect of apical domain, we also monitored and compared the fibrillation process of apical domain with and without β_2m fibrils. The fibrillation processes that we observed were virtually identical (Fig. 4B) indicating the absence of crossing-seeding in apical domain fibrillation. Moreover, the apical domain fibrils give rise to much lower ThT fluorescence signal intensities than β_2m (Fig. 4A control *vs* Fig. 4B), and hence we conclude that the inhibition of apical domain on the β_2m fibrillation contributes to the low ThT fluorescence signal intensity during the early stage of incubation.

The chaperoning capability of apical domain is getting more evident under the milder physiological conditions (Fig. 5A). When incubated without agitation, there are no observable amyloid properties of apical domain at neutral pH by ThT assay (Fig. 2A) and electron microscopy (Fig. 2B). Therefore, a concentration-dependent inhibitory effect of apical domain on the fibril growth of β_2m was observed (Fig. 5B–D). Particularly, in the presence of equal amount of apical domain, β_2m seed-dependent fibril growth could be fully inhibited (Fig. 5D). Intriguingly, in the presence of GroEL, we also did not find any fibril formation of β_2m, but well-distributed oligomers likely from GroEL (Fig. S2B) were observed (Fig. 5E).

4. Discussion

In this study, we found that the isolated GroEL apical domain, which harbours the substrate protein binding sites, possesses dual properties. In addition to its propensity to form amyloid-like fibrils, the apical domain still retains a partial chaperone activity capable of inhibiting the fibril growth of β_2m. Interestingly, GroEL was also observed to form fibrils in the physiological buffer (Fig. S2). These seemingly paradoxical properties of such an essential chaperone system could be considered from several different points of view.

Firstly, by the combination of theoretical predictions and biochemical assays, we have identified that the amyloid-prone segment of GroEL lies in its apical domain region representing the general feature of amylome theory [1,5]. In the cell, GroEL is constitutively expressed in a complex protein network. Therefore the amyloid propensity of apical domain for the intact GroEL may be suppressed to a great extent due to the structural constraints imposed on the apical domain region by intra- or inter-molecular interactions [40]. However, as we have shown here, when apical domain is present as an isolated form, its fibrillation tendency becomes detectable under the physiological conditions (Fig. 2), and even more under acidic conditions (Fig. 3).

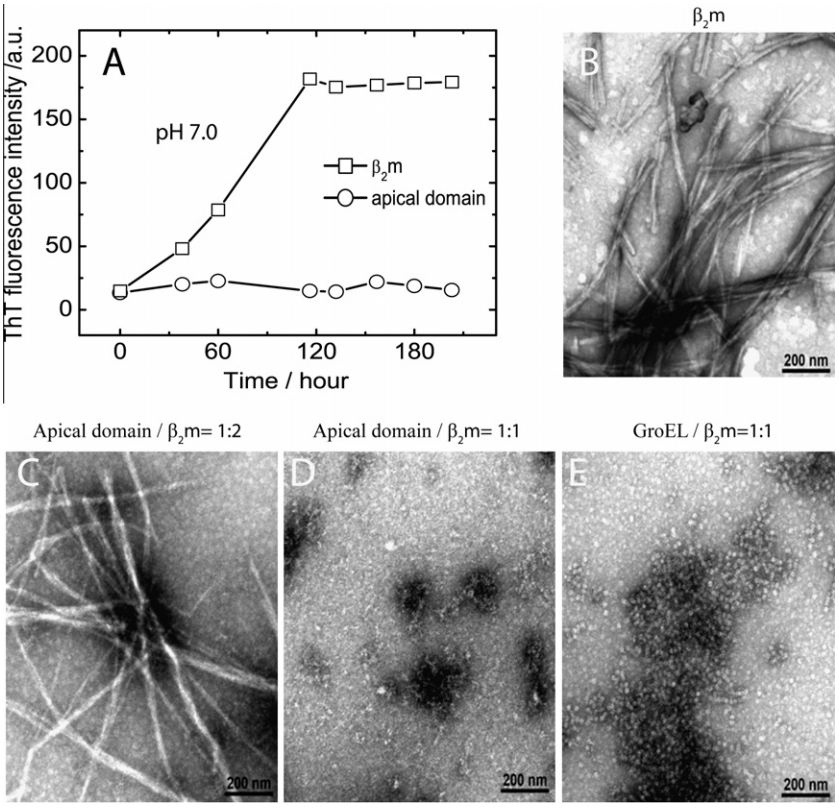

Fig. 5. Inhibitory effects of apical domain in the physiological buffer. (A) Seed-dependent fibrils growth of β_2m by incubating 0.3 mg/ml apical domain (○) with 0.3 mg/ml β_2m at pH 7.0. 0.3 mg/ml β_2m (□) was used as a control. (B–D) Amyloidogenic properties of 0.3 mg/ml β_2m or mixed with apical domain at different weight ratio or (E) GroEL under the same incubation conditions in (A).

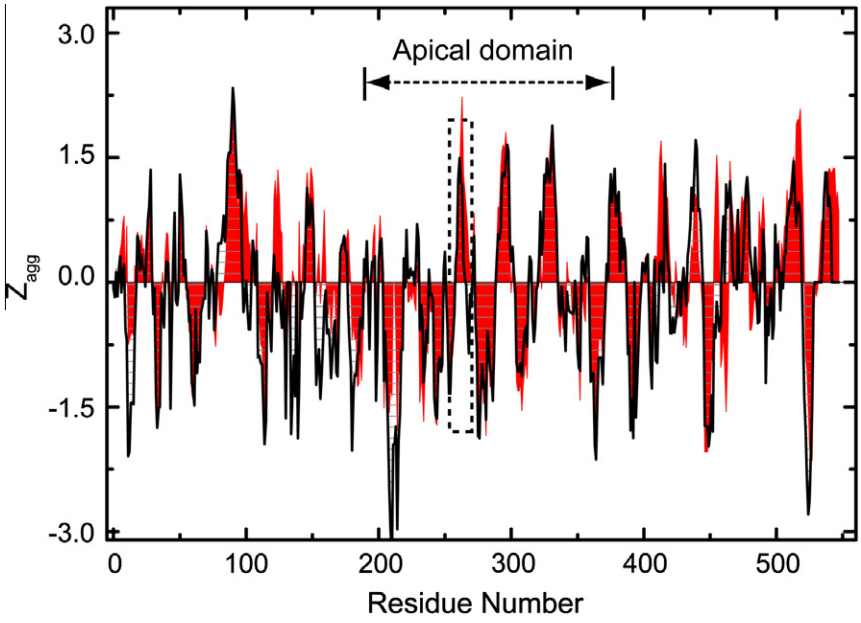

Fig. 6. Comparison of the aggregation propensity profiles obtained with Zyggregator [28] for the *E. coli* GroEL sequence studied in this work (red), and for the consensus sequence (black with grey bar). The consensus sequence is obtained from an analysis of 1003 homologous GroEL sequences. The shaded area corresponds to the helix I region, which plays a key role in the capture of misfolded proteins [31].

Since the sequence conservation of the apical domain is generally low [41], a question arises as to whether the amyloidogenecity of this domain that we described holds for the ubiquitously distributed chaperonin family. Therefore, we further performed aggregation-propensity analyses on 1003 homologous GroEL sequences. Interestingly, from the consensus of the aggregation propensity profiles, the apical domain (Fig. 6), including helix I (Fig. S6), was also identified as one of the most aggregation-prone regions, suggesting a common sequence motif for aggregation within this chaperone family.

Lastly, as SDS is reminiscent of biological membranes in some of their characteristics, the identified amyloidogenicity of the apical domain and GroEL may suggest some physiological implications such as gain-of-function [42] in the cell. This view is supported by a recent report showing that a chaperonin from *Sulfolobus shibatae*, a hyperthermophilic archaeon living in acidic hot springs, could form filaments in physiological buffers and resemble some cytostructures [43]. Experiments to explore the fibrillation or aggregation mechanism of GroEL in a physiological context of cellular environment will further advance our understanding of the functional role endowed by this essential chaperonin system.

Taken together, we hypothesis that the Janus-faced property of apical domain originates from the aggregation propensity of its amino-acid sequence as well as of its three-dimensional structure. The highly aggregation-prone hydrophobic residues of the apical domain, which are structurally buried in the native form [31–33], are exposed in the presence of SDS or under acidic conditions and stirring promotes the inter-molecular hydrophobic interaction so that apical domain starts to fibrillate. Meanwhile, the chaperone activity of apical domain may be not fully diminished at submicellar SDS concentration or during the early stage of acidic conditions. Therefore apical domain still retains the chaperoning capability to inhibit the fibril growth of β_2m to some extent.

The detailed mechanism by which apical domain inhibits β_2m fibrillation has not been resolved in this study. We have found no detectable protein-protein interactions between apical domain and β_2m (data not shown), and hence apical domain may bind to the aggregated or nucleating species of β_2m in solution [44]. Another possibility is that apical domain may bind SDS efficiently in solution so that the effective SDS concentration is reduced insufficiently to induce the fibrillation of β_2m. Furthermore, another chaperone fragment used here, the α-crystallin domain from human αB-crystallin shows a loss of chaperone activity under acidic conditions in contrast to the full-length αB-crystallin, which is still capable to inhibit the insulin fibril growth at an acidic pH 2.5 [45]. These results suggest that the GroEL apical domain may serve as a minichaperone system with some potential application to develop therapeutic agents towards human β_2m-related deposition diseases.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.febslet.2012.03.019.

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