ATP Binding Is Critical for the Conformational Change from an Open to Closed State in Archaeal Group II Chaperonin*

Received for publication, May 26, 2003, and in revised form, August 12, 2003

Published, JBC Papers in Press, August 14, 2003, DOI 10.1074/jbc.M305484200

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Group II chaperonins, found in archaea and in eukaryotic cytosol, do not have a co-chaperonin corresponding to GroES. Instead, it is suggested that the helical protrusion extending from the apical domain acts as a built-in lid for the central cavity and that the opening and closing of the lid is regulated by ATP binding and hydrolysis. However, details of this conformational change remain unclear. To investigate the conformational change associated with the ATP-driven cycle, we conducted protease sensitivity analyses and tryptophan fluorescence spectroscopy of a-chaperonin from a hyperthermophilic archaea, Thermococcus strain KS-1. In the nucleotide-free or ADP-bound state, the chaperonin, especially in the helical protrusion region, was highly sensitive to proteases. Addition of ATP and ammonium sulfate induced the transition to the protease-resistant form. The fluorescence intensity of the tryptophan residue introduced at the tip of the helical protrusion was enhanced by the presence of ATP or ammonium sulfate. We conclude that ATP binding induces the conformational change from the lid-open to lid-closed form in archaeal group II chaperonin.

Chaperonins, one of the principal molecular chaperones, capture non-native proteins and promote folding in vivo and in vitro in an ATP-dependent manner. They form large cylindrical complexes composed of two stacked rings of 7–9 subunits, each about 60 kDa in size. There is a large cavity in the center of the complex, where unfolded proteins may be encapsulated and undergo productive folding (1, 2). Based on protein sequence similarity and structural features, chaperonins are grouped into two subfamilies (3, 4). Group I chaperonins are found in bacteria and eukaryotic organelles, mitochondria and chloroplasts. The Escherichia coli chaperonin, GroEL, is the most extensively characterized member of the chaperonins. GroEL facilitates protein folding with a cofactor termed GroES in an ATP-dependent manner. GroES has two functions; it acts as a lid to cap the cavity of GroEL, and then induces the release of bound substrate protein into the GroEL-GroES cavity where it can undergo folding (5).

The group II chaperonins are found in archaea (as thermosome) and in the cytosol of eukaryotic cells (as CCT or TRiC). Group II chaperonins do not have a co-chaperonin corresponding to GroES. The crystal structure of the group II chaperonin from an acidothermophilic archaea, Thermoplasma acidophilum, suggested that the function of GroES is replaced by long helical protrusions from the apical domain (6, 7). These protrusions are thought to function as a “built-in lid” for the central cavity. Presumably, ATP binding drives group II chaperonins from the lid-open, substrate binding conformation, into the lid-closed conformation, where substrate folds within the central cavity (6–9). However, the exact relationship between the nucleotide-bound state and the conformation is still controversial in group II chaperonins. Szpikowska et al. (10) reported that CCT is more resistant to trypsin digestion at the apical domain in the presence of ATP than in the absence of nucleotide and the presence of AMP-PNP, and the AMP-PNP-bound form of CCT is trypsin-sensitive as is the nucleotide-free form. On the other hand, Llorca et al. (11) showed that the binding of AMP-PNP to CCT results in the cavity being closed off by the helical protrusions of the apical domains. Also, they showed that ATP induces an asymmetric structure; one of the rings in the conformation is similar to that of the nucleotide-free CCT, whereas the other presents a more open conformation (12, 13). Gutsche and colleagues (14) pointed out that the binding of ATP or AMP-PNP to the thermosome induces the closed conformation. Using cryo-electron microscopy and three-dimensional reconstruction, three conformational states were found in archaeal chaperonins, regardless of the presence of nucleotides; an open form, a closed form, and a bullet-shaped form with one open and one closed ring (15, 16).

* This work was supported by grant-in-aid for scientific research on priority areas (15035008, 14037216, and 15032212) and a grant of the National Project on Protein Structural and Functional Analyses from the Ministry of Education, Science, Sports and Culture of Japan. The work reported here is a part of the 21st Century COE (Center of Excellence) program of “Future Nano-Materials” research and education project, which is financially supported by the Ministry of Education, Science, Sports, Culture, and Technology through Tokyo University of Agriculture & Technology. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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††† The abbreviations used are: CCT, chaperonin-containing t-complex polypeptide-1; TRIC, TCP1-ring complex; T. KS-1, hyperthermophilic archaea Thermococcus sp. strain KS-1; aWT, wild type a T. KS-1 chaperonin; aG65S, G65S mutant a T. KS-1 chaperonin; aG65C, G65C mutant a T. KS-1 chaperonin; aL265W, L265W mutant a T. KS-1 chaperonin; aG65S/L265W, double mutant a T. KS-1 chaperonin; aG65C/I125T, G65C/I125T double mutant a T. KS-1 chaperonin; aG65S/I125T, G65S/I125T double mutant a T. KS-1 chaperonin; GFP, green fluorescent protein; DTT, dithiothreitol; native PAGE, PAGE without SDS; PK, proteinase K; AMP-PNP, adenosine 5′-(6-aminohexyl)-triphosphate.
The chaperonin from a hyperthermophilic archaea, Thermococcus sp. strain KS-1 (T. KS-1), is one of the most studied group II chaperonins (17–23). T. KS-1 chaperonin is composed of two highly homologous subunits, α and β. Although the natural chaperonin isolated from T. KS-1 is a hetero-oligomer, each of the recombinant α and β subunits forms a double-ring homo-oligomer and functions as a chaperonin in vitro (17–19). Recent experimental results using recombinant and natural chaperonins suggest that the protein folding occurs within the cis-cavity (21). However, details of conformational changes in the protein folding cycle of group II chaperonins remain unclear.

In this report, we analyzed the protease sensitivity and tryptophan fluorescence spectroscopy of T. KS-1 α-chaperonins to study the conformational changes associated with the functional ATPase cycle. In the nucleotide-free or ADP-bound state, the chaperonin was highly sensitive to proteases. The amino acid sequences of the digested fragments suggested that the proteases firstly attacked the helical protrusions, which composed the built-in lid. Addition of ATP induced the transition to the relatively protease-resistant form. The fluorescence intensity of the introduced tryptophan residue was enhanced by the addition of ATP and AMP-PNP. The findings support the notion that ATP binding induces conformational change from an open to closed state in archaeal group II chaperonin.

MATERIALS AND METHODS

Bacterial Strains, Plasmids, Reagents, and Buffers—The E. coli strains used in this study were DH5α for plasmid preparation and BL21(DE3) for protein expression. The plasmids pKE1e2 and pKE1e1–4 were used as a template for mutagenesis and for expression of αWT and α6G6S, respectively (18, 20). The restriction endonucleases were products of Takara (Kyoto, Japan). Nucleotides and thermolysin were purchased from Wako Chemicals (Tokyo, Japan). Other reagents or enzymes were used as the biochemical research grade. The assay buffer used in this work is TMN buffer (50 mM Tris-HCl, pH 7.5, 100 mM NaCl, and 25 mM MgCl2) or TKM buffer (50 mM Tris-HCl, pH 7.5, 100 mM KCl, and 25 mM MgCl2), with exceptions specially described.

Site-directed Mutagenesis—The tryptophan mutants were constructed using a QuikChange site-directed mutagenesis kit (Stratagene, CA). The 30-nucleotide primers 5′-CCAGCGGGCAGACTGGAGAGGTTGCCACTGTGCTGGGCTTG-3′ and 5′-CAAGGAGGCTCTCCTGTTCTTGGCGGCGGCTTG-3′ (mutated nucleotides are underlined), containing a cleavage site of FokI, were used for the codon substitution, CTC to TGG (Leu to Trp) on pKE1e2 or pKE1e1–4. The mutants were selected by FokI digestion and were confirmed by DNA sequencing. DNA manipulation was carried out as described by Sambrook et al. (24).

Purification of Chaperonins—The α-chaperonins of wild and mutants were expressed in E. coli BL21(DE3) cells transformed with expression plasmids. They were cultured aerobically in 2xYT medium (18 g of tryptone, 10 g of yeast extract, and 5 g of NaCl per liter) containing 75 μg/ml kanamycin. The harvested cells were suspended in 50 mM Tris-HCl, pH 7.5, and disrupted by sonication. The suspension of disrupted cells was centrifuged at 25,000 × g for 30 min at 4 °C, and MgCl2, glycerol, and dithiothreitol (DTT) were added to the supernatant to 25 mM, 5% (v/v), and 1 mM, respectively. The cell extract was then subjected to heat treatment at 70 °C for 30 min, and denatured proteins were removed by centrifugation (25,000 × g, 30 min, 4 °C). The supernatant was applied to a DEAE-Tocracolumn (Tosoh, Tokyo, Japan) equilibrated with buffer A (50 mM Tris-HCl, pH 7.5, and 25 mM MgCl2) containing 5% (v/v) glycerol and 1 mM DTT. Proteins were eluted with a linear gradient of 0–500 mM NaCl in the same buffer. Fractions containing the chaperonins were collected and dialyzed and concentrated by ultracentrifugation. The fractions containing the chaperonins were collected and dialyzed and concentrated by ultrafiltration (Centriprep YM-10, Millipore, MA). The protein was applied to an UNO Q8 column (Bio-Rad, CA) equilibrated with buffer A and eluted with a linear gradient of 0–500 mM NaCl. The concentrated fractions containing α-chaperonins were loaded onto a gel filtration column (HiLoad 26/60 Superdex 200 prep grade, Amersham Biosciences) equilibrated with buffer A containing 150 mM NaCl. Puriﬁed preparations were concentrated by ultrafiltration, supplemented with glycerol to 20% (v/v), and stored at −80 °C.

Examination for Nucleotide Contamination—The purified chaperonins were shown to be nucleotide-free by the following procedure. The purified proteins were denatured with ice-cold perchloric acid and then centrifuged to remove proteins. Aliquots of the supernatant were applied to a TSK ODS-80Ts column (4.6 mm × 15 cm, Tosoh) equilibrated with 30 mM sodium phosphate buffer, pH 6.8, containing 2% (v/v) acetonitrile. The eluate was monitored with an on-line UV spectrophotometer at 259 nm (Waters Alliance high-performance liquid chromatography system, Tokyo, Japan).

Proteolysis of a Subunit Chaperonins and N-terminal Sequencing—The α-chaperonin (50 or 250 nM) was incubated with or without nucleotide (to a ﬁnal concentration of 1 mM) at 65 °C for 5 min, being continuously stirred. Proteolytic digestion was carried out with thermolysin (1 ng/μl) or protease K (40 ng/μl) at 65 °C for 5 or 10 min. An aliquot of the reaction mixture was precipitated using 30% (w/v) trichloroacetic acid and placed on ice for more than 5 min. The precipitate was centrifuged and subjected to SDS-PAGE after a wash with ice-cold water at 60 °C.

Protease K-digested protein obtained by trichloroacetic acid precipitation was subjected to SDS-PAGE and electroblotted onto a polyvinylidene diﬂuoride membrane. The blotted membrane was stained with Coomassie Brilliant Blue R-250. The bands were excised and analyzed with a peptide sequencer.

Fluorescence Measurements—The ﬂuorescence spectra of tryptophan mutants were measured at 65 °C with a spectrophotometer, RF-5300PC (Shimadzu, Kyoto, Japan). The excitation wavelength was set at 295 nm, and emission was recorded from 300 to 450 nm (bandwidth = 1.5 nm for excitation and 3.0 nm for emission). α-Chaperonin (250 nM) was preincubated with or without nucleotide (1 mM) for 5 min at 65 °C, and the mixture was stirred throughout the measurement. All spectra were obtained from the average of ﬁve scans.

Miscellaneous Methods—Proteins were analyzed by electrophoresis either on 10% (w/v) or 15% (w/v) SDS-PAGE (25) or on 6% (w/v) polyacrylamide gels without SDS (native-PAGE). Gels were stained with Coomassie Brilliant Blue R-250. Protein concentrations were measured using a commercial kit, Bio-Rad Protein Assay (Bio-Rad, CA) with bovine serum albumin as the standard (26).

RESULTS AND DISCUSSION

The Absence of Potassium Ion Inhibits ATP Hydrolysis and Protein Folding Activity by αWT—The structural changes of chaperonin in the protein folding cycle are controlled by ATP binding and subsequent hydrolysis. Generally, potassium ion is required for efficient chaperonin-catalyzed ATP hydrolysis (27, 28). The hydrolysis of ATP by αWT was also highly dependent on the presence of potassium ion. When potassium ion is replaced by sodium ion, the ATPase activity of αWT decreases by a factor of about 6 (data not shown). Consistently, the protein folding activity of αWT also dropped significantly in the absence of potassium ion as observed in the GFP refolding experiment (data not shown). Multiple rounds of binding, hydrolysis, and release of nucleotides, which are required for efficient refolding of GFP, are hindered in the absence of potassium ion. Thus, we expected the chaperonin conformation to be fixed in the ATP-bound state using potassium free buffer.

At Least Two Conformational States Exist in αWT—Figs. 1 and 2 show the SDS-PAGE analyses of protease-treated αWT and α6G6S. αWT was subjected to thermolysis digestion with or without nucleotides in the TMN and TKM buffer (Fig. 1A). In the presence of ATP or AMP-PNP, a non-hydrolyzable analogue of ATP, αWT was relatively thermolysin-resistant, and polypeptides of about 60 kDa, probably intact polypeptides, remained irrespective of the presence of potassium ion. In contrast, 60-kDa polypeptides were degraded nearly completely in the absence of nucleotide or the presence of ADP. Intriguingly, the αWT complex digested with thermolysin retained its quaternary structure and was able to capture denatured GFP but did not exhibit ATP-dependent folding activity (data not shown). These results are fully consistent with the previous findings for CCT (10). Similar results were obtained in the experiments using protease K (Fig. 1B). In the presence of potassium ion, ATP was not effective in protecting the chaperonin from protease K compared with the digestion by thermolysin (Fig. 1, A and B). We speculate that the difference is caused by the excess amount of protease K (ratio of chaperonin/protease K, 6/1 (w/w)). The digestion might
have proceeded in the protease-sensitive state during the ATP hydrolysis cycle.

Then, the same protease digestion experiments were performed using \( \alpha \text{G65S} \) (Fig. 1, C and D). The mutant is fixed in the protease-sensitive state irrespective of nucleotide species and potassium ion. In this mutant, the loss of protein-folding activity is likely to be due to the absence of ATP-dependent conformational change (20).

Proteinase K-digested fragments of \( \alpha \text{WT} \) were subjected to amino acid sequencing to determine the sites of cleavage (Table I). Fragment B, Fragment E, and a part of Fragment D were produced by digestion in the helical protrusion. It is thought that Fragment C was produced after proteinase K invaded into the central cavity based on the crystal structure of \( T \). KS-1 chaperonin. The region adjacent to Fragment F is so exposed to the bulk solution that it is labile. Thus, the helical protrusion in the apical domain was the area most susceptible to proteolysis in the entire structure. Therefore, the changes in protease sensitivity caused by ATP and AMP-PNP would be due to a conformational change of the helical protrusion.

Previously, we have determined the crystal structure of the mutant \( \alpha \text{G65C/I125T} \), which exhibited almost the same characteristics as \( \alpha \text{G65S} \) (18, 20). Whereas this mutant lacks an ability to refold the captured non-native proteins in the presence of ATP, the crystal structure obtained in ammonium sulfate solution indicated that it takes the closed conformation, with the helical protrusions in both rings pointing toward the center of the cavity. Then, the protease digestion experiment was performed in the presence of 1.2 M ammonium sulfate. Both thermolysin and proteinase K were active even in the presence of such a high concentration of ammonium sulfate (data not shown). Both the wild type and \( \alpha \text{G65C} \) were highly resistant to cleavage under these conditions irrespective of the

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and mutant proteins to the protease-resistant form, probably the closed conformation.

**Tryptophan Fluorescence Enhancement Associated with ATP Binding**—To examine the movement of the helical protrusion during the functional cycle further, we constructed mutants, αL265W and αG65S/L265W. The target residue for replacement with tryptophan, leucine 265, is located at the tip of the helical protrusion. The fluorescence spectrum of α-chaperonin was almost flat due to the lack of tryptophan residues (data not shown). Thus, by monitoring the fluorescence of the mutants, a change in the local environment around the tip of the helical protrusion would be elucidated. Both mutants could capture acid-denatured GFP and heat-denatured citrate synthase. αL265W was able to facilitate the refolding of GFP in an ATP-dependent manner despite the fact that the yield was less than half that of αWT. Protease experiments showed that αL265W also took a protease-resistant conformation on binding ATP (data not shown). These results indicated that αL265W has the ability to undergo the same ATP-induced conformational changes as αWT. αG65S/L265W exhibited similar functional and structural characteristics to αG65S (data not shown).

Fig. 3 (A and B) shows the fluorescence spectra of αL265W under various conditions. The addition of ATP caused a large enhancement (about 68%) of tryptophan fluorescence intensity in both buffers. Although the changes to the maximal emission wavelength were only marginal (Table II), there seemed to be a significant change in the environment around Trp-265 associated with ATP. The fluorescence change in αL265W induced by AMP-PNP in TKM buffer was smaller than that in TNM buffer. It correlates well with the difference of protease sensitivity in the presence of AMP-PNP with and without potassium ion (Fig. IA). On the other hand, no detectable change was induced by the addition of ADP under either set of conditions. Also, the presence of ammonium sulfate resulted in a significant enhancement of fluorescence (Table II). Fig. 3 (C and D) shows the fluorescence spectra of ααG65S/L265W. When ATPase activity was suppressed in the TNM buffer, the fluorescence intensity of αG65S/L265W decreased in the presence of ATP and AMP-PNP. The effect of AMP-PNP was unchanged in the presence of potassium ion. Although the fluorescence intensity of αG65S/L265W was increased by the hydrolysis of ATP, the change was smaller than that for αL265W. As was the case for αL265W, the presence of ammonium sulfate substantially enhanced the fluorescence intensity (Table II).

**Model of the Functional Cycle of T. KS-1 a Subunit Chaperonin**—The protease digestion experiments and tryptophan fluorescence spectroscopic studies presented above clearly demonstrate the following structural characteristics of archaeal group II chaperonins. First, ATP induces a striking structural change to αWT and αL265W irrespective of potassium ion. In the nucleotide-free or ADP-bound state, the chaperonin was highly

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**TABLE I**

Assignment of α WT-derived fragments based on amino acid sequencings

| Fragment | Assignment | Location |
|----------|------------|----------|
| B        | Leu<sup>209</sup>, Glu<sup>273</sup> | Helical protrusion |
| C        | Arg<sup>18</sup>, Leu<sup>27</sup> | Equatorial domain |
|          | Tyr<sup>19</sup>, Asn<sup>28</sup> |           |
|          | Val<sup>20</sup>, Ile<sup>29</sup> |           |
|          | Asp<sup>23</sup>, Ala<sup>12</sup> |           |
| D        | Lys<sup>27</sup>, Gly<sup>206</sup> | Equatorial domain |
|          | Asp<sup>95</sup>, Thr<sup>59</sup> |           |
|          | Lys<sup>274</sup>, Ile<sup>283</sup> | Helical protrusion |
| E        | Leu<sup>209</sup>, Glu<sup>273</sup> | Helical protrusion |
| F        | Val<sup>143</sup>, Asp<sup>530</sup> | Intermediate domain |
| A        | Ala<sup>-1</sup>, Asn<sup>-3</sup> | Proteinase K |

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**Fig. 2.** Protease resistance of αWT and αG65C induced by high concentration of sulfate ion. A, αWT and αG65C were preincubated with or without 1 mM of the different nucleotides (ATP, AMP-PNP, and ADP) at 65 °C for 5 min, and then thermolysin was added to the chaperonin solution. Incubation proceeded in TNM buffer containing 1.2 M ammonium sulfate. The detailed procedures are described under “Materials and Methods.” Lane M, molecular weight marker; lane W, αWT; lane F, nucleotide- and ammonium sulfate-free form of αWT; lanes 1 and 5, without addition of nucleotide; lanes 2 and 6, incubated with ATP; lanes 3 and 7, incubated with AMP-PNP; lanes 4 and 8, incubated with ADP. B, αG65C was preincubated with or without 1 mM ATP at 65 °C for 5 min, and then thermolysin was added to the chaperonin solution. Incubation proceeded in TNM buffer without or with 1.2 M salt (ammonium sulfate, sodium sulfate, and ammonium chloride). The detailed procedures are described under “Materials and Methods.” Lane M, molecular weight marker; lane C, αG65C; lane 1, without addition of nucleotide; lane 2, incubated with ATP; lane 3, incubated with 1.2 M ammonium sulfate; lane 4, incubated with 1.2 M ammonium sulfate and ATP; lane 5, incubated with 1.2 M sodium sulfate; lane 6, incubated with 1.2 M sodium sulfate and ATP, lane 7, incubated with 1.2 M ammonium chloride; and lane 8, incubated with 1.2 M ammonium chloride and ATP.

Nucleotides (Fig. 2A). It was shown that sodium sulfate has almost the same effect as ammonium sulfate, but ammonium chloride does not (Fig. 2B). A small angle neutron scattering study also suggested that *Thermoplasma* chaperonin takes the closed conformation in the presence of a high concentration of sulfate (29). Therefore, we concluded that a high concentration of sulfate ion induced the conformational change of wild type
sensitive to proteases. Addition of ATP induced a change to the relatively protease-resistant form (Figs. 1 and 2). The fluorescence intensity of \(\alpha\)H9251L265W was enhanced by the addition of ATP (Fig. 3). So, it is concluded that \(\alpha\)H9251-chaperonin undergoes a large conformational change in the presence of magnesium ion and ATP and that the hydrolysis of ATP is dispensable for this change. The amino acid sequences of the digested fragments suggested that the proteases first attacked the helical protrusion region, which composes the built-in lid (Table I). It seems that this region is very flexible in the nucleotide-free and ADP-bound state, and ATP or AMP-PNP binding induces a transition to a rigid state. Based on the GFP refolding assay, the change of tryptophan fluorescence (Fig. 3), and the crystal structure,\(^2\) we concluded that the conformational change induced by binding ATP brings about the closure of built-in lid, which would be critical for protein folding. It was shown that the conformation of CCT bound to \(\alpha\)-actin and \(\beta\)-tubulin in the presence of AMP-PNP is similar to that of the thermosome x-ray structure (11). In contrast, \(\alpha\)G65S does not exhibit significant conformational change. The lack of protein folding activity by the mutant would be caused by the loss of conformational change in the ATP hydrolysis cycle.

Second, sulfate ion fixes both \(\alpha\)WT and \(\alpha\)G65S in a state highly homologous to that of \(\alpha\)WT bound to ATP. Gutsche et al. (29) pointed out that high ammonium sulfate conditions induce the closed conformation, based on a small angle neutron scattering study. Although \(\alpha\)G65 mutants are fixed in the lid-open state, the crystal structure of the mutant \(\alpha\)-chaperonin was determined to be a closed form. The contradiction would be explained by the fact that the crystallization buffer included a

**Fig. 3. Fluorescence spectra of tryptophan mutants.** Spectra were obtained with excitation at 295 nm at a protein concentration of 0.25 \(\mu\)M at 65 °C. For each spectrum, an average of five scans was obtained. The details of experiments are described under "Materials and Methods." A, \(\alpha\)L265W in TNM buffer; B, \(\alpha\)L265W in TKM buffer; C, \(\alpha\)G65S/L265W in TNM buffer; D, \(\alpha\)G65S/L265W in TKM buffer. Black dotted line, without addition of nucleotides; black line, incubated with ATP; gray dotted line, incubated with AMP-PNP; and gray line, incubated with ADP.
Conformational Change of Archaeal Group II Chaperonin

TABLE II

Fluorescence parameters of α265W and αG65S/L265W

|           | Maximum of Try fluorescence | Fluorescence at emission maximum |
|-----------|-----------------------------|---------------------------------|
| nm        | arbitrary units              |                                  |
| αL265W, without potassium ion |                             |                                  |
| Nucleotide-free | 344               | 5.89                             |
| + ATP      | 343               | 9.94                             |
| + AMP-PNP  | 343               | 9.72                             |
| + ADP      | 344               | 5.86                             |
| + 1.2 M ammonium sulfate | 343               | 9.26                             |
| + 1.2 M ammonium sulfate and ATP | 342 | 10.3                             |
| αL265W, with potassium ion |                             |                                  |
| Nucleotide-free | 344               | 5.59                             |
| + ATP      | 343               | 9.43                             |
| + AMP-PNP  | 344               | 6.60                             |
| + ADP      | 344               | 5.41                             |
| + 1.2 M ammonium sulfate | 342               | 9.19                             |
| + 1.2 M ammonium sulfate and ATP | 342 | 10.0                             |
| αG65S/L265W, without potassium ion |                             |                                  |
| Nucleotide-free | 351               | 6.32                             |
| + ATP      | 344               | 5.77                             |
| + AMP-PNP  | 343               | 5.24                             |
| + ADP      | 344               | 6.41                             |
| + 1.2 M ammonium sulfate | 342               | 10.8                             |
| + 1.2 M ammonium sulfate and ATP | 342 | 13.3                             |
| αG65S/L265W, with potassium ion |                             |                                  |
| Nucleotide-free | 344               | 6.38                             |
| + ATP      | 343               | 7.51                             |
| + AMP-PNP  | 344               | 5.37                             |
| + ADP      | 344               | 6.64                             |
| + 1.2 M ammonium sulfate | 342               | 13.2                             |
| + 1.2 M ammonium sulfate and ATP | 342 | 11.1                             |

high concentration of ammonium sulfate.2

Third, the conformations in the nucleotide-free state and the ADP-bound state are the same or very similar to each other. This is consistent with previous results of electron microscopy and small angle neutron scattering measurement showing that the binding of ADP does not induce a major change in structure among group II chaperonins (12–14, 29). Melki et al. (30) pointed out that there are large differences between ADP–AlF4–CCT, corresponding to the state of transition of ATP hydrolysis, and ADP-CCT in electron microscopic images and hydrodynamic properties.

Fig. 4 is a schematic model for the conformational change to αWT induced by ATP binding and hydrolysis based on the above. It is thought that αWT is in a lid-closed state, which results in the promotion of protein folding, following binding with the non-native protein and ATP. Judging from the above results, it could be expected that the binding of ATP to αG65S doesn’t lead to the conformation with the built-in lid closed. Gutsche et al. (14) suggested that the interaction of the thermostime with ATP is at least a two-step process and that the thermostime would be an open toroidal complex similar to ATP-bound GroEL in the first step. The fluorescence intensity of αG65S/L265W decreased when ATP and AMP-PNP were present in TNM buffer. Although the reason for the decrease is not clear, the intermediate form, mentioned above, may exist before the lid-closed form. If this is the case, the possibility exists that the conformational changes stop during this intermediate state, at least when there is no substrate. Because the actions of the ADP-bound form are very similar to that of the nucleotide-free form, both are considered to be nearly the same structure. It is thought that the inorganic phosphate released by ATP hydrolysis causes α-chaperonin to return to the open conformation.

Recently, Meyer et al. (31) have shown that the lid closure of

CCT is induced not by the binding but by the hydrolysis of ATP. Their results clearly show that no conformational change occurs in the presence of AMP-PNP. They explained that the discrepancy between their results and previous observations using archaenal chaperonins results from ATP contamination of the AMP-PNP.

We have also found that the AMP-PNP and ADP used in this study were contaminated with less than 1% and 2% of ATP, respectively. But, the conformational change would not be caused by the ATP hydrolysis, because it occurred even under the conditions where the hydrolysis of ATP was restricted. The discrepancy might be explained by the structural difference between CCT and archaenal chaperonins.

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