Group II Chaperonin in a Thermophilic Methanogen, *Methanococcus thermolithotrophicus*

CHAPERONE ACTIVITY AND FILAMENT-FORMING ABILITY*

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A gene encoding 544 amino acids for a subunit of group II chaperonin (thermosome) was cloned from a thermophilic methanogen, *Methanococcus thermolithotrophicus*. The deduced amino acid sequence showed 66.5, 56.1, and 20.1% similarities to those of Methanopyrus kandleri and Thermoplasma acidophilum and group I chaperonin of *Escherichia coli*, respectively. We call this chaperonin MTTS (*M. thermolithotrophicus* thermosome). The MTTS gene was expressed in *E. coli*. The purified recombinant MTTS seemed to be monomeric on gel filtration in the absence of Mg$^{2+}$ and ATP. The monomer assembled to an oligomer (complex) in the presence of 50 mM MgCl$_2$, 0.25 mM ATP, and 0.3 M (NH$_4$)$_2$SO$_4$. It was eluted immediately before the elution volume of *E. coli* GroEL tetradecamer on gel filtration with a TSK-gel G3000SWXL column. This reconstructed MTTS complex showed the cylindrical structure with two stacked rings in electron microscopy. The MTTS complex formed filamentous structures in the presence of Mg$^{2+}$ and ATP at the protein concentration above 3.0 mg/ml. This filament formation was reversible. The MTTS filament was dissociated to the complex by dilution to the protein concentration of 0.2 mg/ml, even in the presence of Mg$^{2+}$ and ATP. The MTTS complex exhibited weak ATPase activity with the hydrolysis rate of 74 mol of ATP hydrolyzed/mol of MTTS complex/min at 70 °C. The MTTS complex promoted the refolding of chemically denatured thermophilic archaeal citrate synthase and glucose dehydrogenase at 50 °C in an ATP-dependent fashion. The analysis of nucleotide specificity of chaperone activity of MTTS suggested that it was coupled with hydrolysis of ATP, CTP, or UTP.

Several protein factors have influence on folding of other proteins both in vivo and in vitro (1). These factors are peptidyl prolyl cis-trans isomerase, protein disulfide isomerase, and the other proteins, called molecular chaperones. Molecular chaperones (2) are able to bind a wide range of denatured or otherwise unstable intermediate state of folding polypeptides and introduce them to a native and functional state in energy-dependent or independent fashion. Chaperonins form a major class of molecular chaperones with an apparent molecular mass of 60 kDa and form a multisubunit-cylindrical particle.

Chaperonins are divided into two groups, group I and group II (3). Group I chaperonins (GroEL family) are found in bacteria, mitochondria, and chloroplasts. These are composed of one or two different subunits and form tetradecameric cylindrical particle composed of two stacked 7-fold symmetric rings. On the other hand, group II chaperonins are found in eukarya and in archaea and also have two stacked symmetric ring structures. However, numbers of subunit compositions and rotational symmetries of the ring structures are different among group II chaperonins.

T complex polypeptide 1 (TCP-1)$^1$ is a subunit of a heterooligomeric ring complex, chaperonin-containing TCP-1 (CCT) (4), localized in the eukaryotic cytosol. This complex has seven to nine different polypeptides ($M_r$ 50,000–68,000) (5, 6) with pseudo 8-fold symmetric structure (7). The CCT is involved in the folding of actin, tubulin, and firefly luciferase in vitro (8–10) and binding to newly synthesized actin and tubulin monomers in vivo (11).

The group II chaperonins have also been reported from thermophilic archaea, e.g. *Sulfolobus shibatae* TF55 (12, 13), *Pyrodictium occultum* (14, 15), *Sulfobus solfataricus* (16, 17), *Thermoplasma acidophilum* (7, 18), *Methanopyrus kandleri* (19), *Methanococcus jannaschii* (20), *Pyrococcus* sp. KOD1 (21), and *Thermococcus* strain KS-1 (22). Chaperonins from archaea except those of methanogens are composed of two distinct subunits. *T. acidophilum* chaperonin, which forms double-layered 8-fold symmetric rings, is composed of two kinds of subunits ($M_r$ 58,000 and 60,000) (23). This chaperonin exhibits weak ATPase activity and is able to bind denatured proteins (7). *S. solfataricus* chaperonin with 9-fold symmetric rings composed of two kinds of subunits ($M_r$ 56,000–68,000) (16, 24) promotes protein foldings (17) and protects thermal aggregation of proteins in ATP-dependent fashion. (17, 24). *Thermococcus* strain KS-1 chaperonin was reported to be composed of two homologous subunits. Each of them forms a homo-oligomeric complex with weak ATPase activity, binds to the protein folding intermediates, and releases them in an ATP-dependent manner (22). The β-subunit of *Pyrococcus* sp. KOD1 chaperonin protects thermal inactivation of yeast alcohol dehydrogenase in ATP-dependent or -independent fashion (21). It has also been reported that *S. shibatae* chaperonin forms a cytoskeleton-like filamentous structure in the presence of Mg-ATP at the protein concentration of 1.0 mg/ml (25). The chaperonin filament-like structure was observed also in the cells of *S. shibatae* (25).

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The nucleotide sequence(s) reported in this paper has been submitted to the GenBank™/EBI Data Bank with accession number(s) AB154345.

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$^1$ The abbreviations used are: TCP-1, T complex polypeptide 1; CS, citrate synthase; GDH, glucose dehydrogenase; PCR, polymerase chain reaction; PAGE, polyacrylamide gel electrophoresis; AMP-PNP, adenosine 5’-(β,y-imino)triphosphate.
Cloning of Chaperonin Gene from M. thermolithotrophicus—M. thermolithotrophicus DSM2095 was grown at 65 °C as described previously (28). Genomic DNA was prepared as described previously (29). A partial chaperonin gene was amplified by PCR from the genome of M. thermolithotrophicus with various primers designed from the consensus amino acid sequences of other archaeal chaperonins. After determination of the partial nucleotide sequence (about 740 base pairs) of the chaperonin, the partial chaperonin gene was used as the probe for the screening of the whole chaperonin gene from the genomic library. To construct the genomic library of M. thermolithotrophicus, the genomic DNA was digested with BamHI and ligated into the corresponding site of pUC18. The library was screened by colony hybridization. Prehybridization and hybridization were carried out at 65 °C, and the filters (Hybond N+, Amersham, UK) were washed with 0.2 × SSC, 0.1% SDS at 65 °C. Positive clones were detected by using the probe labeled with a DIG DNA labeling and detection kit (Boehringer Mannheim). The inserted DNA fragments from positive clones were sequenced using a Dye termination cycle sequencing kit (Perkin-Elmer) and a DNA sequencer (type ABI 373, Perkin-Elmer).

Expression of Chaperonin Gene in E. coli—The chaperonin gene of M. thermolithotrophicus was expressed in E. coli using a pET11a expression vector (Novagen). The chaperonin gene having Ncol and BamHI sites at its 5′- and 3′-terminals, respectively, was prepared by PCR using the forward primer 5′-GGCCATGCGACGCTAACAGCA3′ and the reverse primer 5′-CCGGATCTTACATCATTCCGCCCATAC3′. After amplification, the DNA fragment recovered was digested with Ncol and BamHI and then ligated into Ncol-BamHI sites of pET-11d vector (Novagen). The resultant recombinant M. thermolithotrophicus chaperonin (MTTS) expression vector, pETTS, was introduced into E. coli BL21(DE3) (Novagen). The E. coli BL21(DE3) harboring pETTS was grown in 2 L (yeast extract, 10 g; bactotryptone, 20 g; NaCl, 5 g; and 5.3 mM MgCl2) at 37 °C. When the A600 of the culture reached 1.2–1.5, 1 mM isopropyl-β-D-thiogalactoside was added. The culture was further incubated for an additional 4 h at 37 °C.

Purification of Recombinant MTTS from E. coli—The harvested E. coli cells harboring pETTS were sonicated in 50 mM potassium phosphate buffer (pH 7.0) containing 10 mM EDTA-Na2 and the supernatant was incubated at 65 °C for 90 min to precipitate E. coli proteins. The supernatant was collected by centrifugation (10,000 × g for 20 min at 4 °C), and then (NH4)2SO4 was added at the final concentration of 1.3 M. After the centrifugation, the supernatant was applied to a TSKgel Ether-5PW column (7.5 mm, inner diameter × 7.5 cm, Tosoh, Tokyo). The proteins were separated using a linear gradient of 1.5 to 0.0 M (NH4)2SO4 in 25 mM Hepes-KOH buffer (pH 7.0) overnight at 4 °C and then applied to a TSKgel SuperQ-5PW column (7.5 mm, inner diameter × 7.5 cm, Tosoh, Tokyo). The proteins were separated with a linear gradient of 0 to 0.5 M NaCl in 25 mM Hepes-KOH (pH 7.3). Na2-EDTA was added into the collected MTTS fraction at the final concentration of 10 mM, and then the solution was incubated at room temperature for 1 h. The solution was dialyzed against 25 mM Hepes-KOH (pH 6.8) overnight at 4 °C. The dialysate (>1 mg protein/ml) was treated with 50 mM MgCl2, 0.25 mM ATP, and 0.3 M (NH4)2SO4 and then incubated at 50 °C for an additional 45 min for Ultrosoft PLTK filter (Millipore). The concentrated solution was applied to a TSKgel G3000SWXL column (7.8 mm, inner diameter × 30 cm, Tosoh, Tokyo) in 25 mM Hepes-KOH (pH 6.8) containing 50 mM MgCl2, 0.2 mM ATP, and 0.3 M (NH4)2SO4. The purity of MTTS was analyzed by SDS- and native-PAGE. The MTTS fractions were collected and concentrated to the protein concentration above 3.0 mg/ml with an Amicon Ultrafilter PLTUR. The purified chaperonin was stored at 4 °C until used.

Electron Microscopy—An aliquot of chaperonin solution was applied to carbon-coated formvar membrane on copper grids for 5 min, washed with 25 mM Hepes-KOH (pH 6.8) containing 50 mM MgCl2 and 5% glycerol for 10 s, and then negatively stained with 2% uranyl acetate for 10 s. Air-dried samples were observed in a Hitachi H-7000 electron microscope operated at 75 kV.

ATPase Assay—The assay mixture (100 μl) of ATPase activity of chaperonin containing 50 mM MgCl2, 2 mM ATP, 300 mM KCl, and 0–50 μg of chaperonin in 50 mM Hepes-KOH (pH 6.8). The mixture was incubated at 40–90 °C for 30 min. The reaction was terminated by the addition of 10% (v/v) peracetic acid. After centrifugation, released Pi in supernatant was measured using 96-well microplates by malachite green-modified Pi light method (30) with a modification by subtracting spontaneous Pi release. The assay mixture contained 30 μl of the terminated reaction mixtures, 200 μl of color reagent (0.034% (v/v) malachite green hydrochloride, 1.05% (v/v) ammonium molybdate, and 1% (v/v) Triton X-100) and 25 μl of 34% (v/v) sodium citrate·2H2O. The assay mixtures were incubated at room temperature for 30 min, and then the absorbances at 540 nm were measured. Non-enzymatic release of P was measured for the reactions at high temperature was not negligible, and the ATPase activity was calculated by subtracting spontaneous P release.

Time Course Analysis of Protein Folding in the Presence of MTTS—CS (EC 4.1.3.7) and GDH (EC 1.1.1.119) from T. acidophilum (Sigma) were denatured in 6 M guanidine hydrochloride, 5 mM dithiothreitol, and 50 mM Hepes-KOH (pH 7.5) at 50 °C for 30 min and then at room temperature for 3 min. The refolding was started by 60-fold dilution with a dilution buffer, 50 mM Hepes-KOH buffer (pH 7.5) containing 300 mM KCl, 50 mM MgCl2, and 2 mM ATP in the presence or absence of MTTS at 50 °C. The dilution buffers were preincubated for 15 min at 50 °C before the refolding reaction. It was postulated that a possible contamination of a small amount of ATP from stocked solution of MTTS was completely hydrolyzed during the preincubation at 50 °C for 15 min. 1 min before the refolding reaction, 1/50 volume of 100 mM ATP was added to the diluted reaction mixture.

In the refolding mixture of denatured CS, the final concentrations of single polypeptide of CS and MTTS in the refolding mixtures were 0.33 and 5.3 μM, respectively. In the refolding of GDH, the final concentrations of single polypeptide GDH and MTTS were 0.33 and 9.75 μM, respectively. The molar ratios of CS and GDH single polypeptide to the MTTS complex, which was assumed to be hexadecameric, were 1:1 and 1:1.8, respectively. To see the effect of ATP on the arrested refolding reactions by MTTS, 1/100 volume of 400 mM ATP was added to the refolding mixture at 10 min after start of the reaction in the CS refolding and 15 min in the GDH refolding. Activities of CS or GDH in the refolding mixture were measured at the indicated periods.

Enzyme Assays—CS activity was measured according to Sere et al. (31). The assay mixture contained 0.1 mM 5,5′-dithiobis(2-nitrobenzoic acid), 0.047 mM acetyl CoA, and 0.23 mM oxaloacetic acid in 0.1 M Tris-HCl buffer (pH 8.1). The change of absorbance at 412 nm was monitored for 30 s at 50 °C. The assay mixture for GDH contained 1% (v/v) glucose, 0.5 mM NADP+ in 0.1 M Tris-HCl buffer (pH 8.1). The change of absorbance at 340 nm was monitored for 30 s at 50 °C.

Nucleotide Specificity for Chaperone Activity of MTTS—To investigate the nucleotide specificity of chaperone activity of MTTS, CS was employed as the substrate. The final concentration of CS single polypeptide in the refolding mixtures was 0.33 μM. In the refolding reaction mixtures, ADP, AMP, AMP-PNP, GTP, CTP, UTP, and UDP were used as the nucleotides. The final concentrations of the nucleotides were 2.0 mM. After 45 min of reactions, the CS activities in the reaction mixtures were assayed. When the CS refoldings were arrested, 1/100 volume of 400 mM ATP was added, and then reaction mixtures were incubated at 50 °C for an additional 45 min. After these second reactions in the presence of ATP, CS activities in the reaction mixtures were measured.
RESULTS

Cloning of Chaperonin Gene from *M. thermolithotrophicus*—The chaperonin gene of *M. thermolithotrophicus* encoding 544 amino acids was cloned (Fig. 1). The putative promoter sequence, \((T/A)(T/A)TATATA\) box (32), was found at approximately 70 base pairs upstream from the initiation codon ATG. The putative archaeal T-rich transcription termination sites (33) were found approximately 20 base pairs downstream from the stop codon (TAA). The amino acid sequence of MTTS shows 78.7, 66.5, and 20.0% identities with chaperonins of *M. jannaschii*, *M. kandleri*, and *E. coli*, respectively. The ATP binding site \((GDGTTT)\) (34) was found in MTTS amino acid sequence. The unique GG\(X\) (\(X\) is M, D, or S) repeat motif containing three times repeat of GGM was found in its C terminus. No other

**Fig. 1.** DNA sequence and the deduced amino acid sequence of MTTS. The putative archaeal promoter is boxed. The ATP binding site (GDGTTT) is shown in boldface. The putative archaeal T-rich transcription termination site is underlined with a solid line. The GG\(X\) motif is double-underlined.
chaperonin gene was detected by either PCR using various primers designed from the consensus archaeal chaperonin sequences or hybridization at 65 °C of the M. thermolithotrophicus genomic libraries with a 0.74-kilobase pair partial sequence of the MTTS as a probe under the washing condition of 0.1% SDS, 0.2 × SSC.

Purification of Recombinant MTTS from E. coli—The MTTS gene was expressed in E. coli BL21(DE3). The recombinant MTTS was purified to homogeneity by heat treatment, hydrophobic interaction chromatography and anion exchange chromatography (Fig. 2). The purified recombinant MTTS eluted at an elution volume of 8.5 ml (67–158 kDa) on gel filtration with a TSKgel G3000SWXL column in 0.15 M NaCl, 50 mM Heps-KOH (pH 6.8) and was thought to be monomer or dimer (data not shown). Oligomeric complex formation of MTTS from its monomer was investigated. After incubation of the purified monomer with 10 mM EDTA-Na2, the solution was dialyzed against 50 mM Heps-KOH (pH 6.8). The dialysate was incubated in 50 mM Heps-KOH (pH 6.8) with 50 mM MgCl2, 0.25 mM ATP, and 0.3 M (NH4)2SO4 at the protein concentration above 3.0 mg/ml at 35 °C for 5 h. A new peak appeared at the elution volume of 6.06 ml immediately after void volume (Fig. 3A). The elution volume of E. coli GroEL was 6.09 ml under the same condition. On native-PAGE, the reconstructed MTTS complex was migrated at the same position with that of GroEL tetradecamer. The ATP hydrolysis rate of the MTTS complex was observed (Fig. 4C). In the chaperone reaction with denatured citrate synthase, neither filament nor chained oligomeric complex was observed. The ring structure of MTTS oligomeric complex was observed with the MTTS concentration of 0.31 mg/ml (Fig. 4D).

ATPase Activity of MTTS—The ATP hydrolysis depending on the amount of MTTS complex was observed at 65 °C (Fig. 5A). However, the monomeric MTTS showed undetectable ATPase activity under the same condition (data not shown). The optimum temperature of the ATPase activity was 60–70 °C (Fig. 5B). The ATP hydrolysis rate of the MTTS complex was 74 mol of ATP hydrolysis/mol of MTTS complex/min at 70 °C. Significant heat inactivation was observed at 90 °C.

Time Course Analysis of Protein Foldings in the Presence of MTTS—The time course of the CS refoldings in the presence and absence of MTTS complex was analyzed (Fig. 6A). After 20 min of incubation, in the presence of ATP and MTTS the recovery of CS activity reached a plateau of approximately 60% of the original activity. In the spontaneous refolding, the final CS recovery reached a plateau after 10 min and was about 17%. The MTTS arrested the refolding of CS in the absence of ATP. After the addition of ATP, it promoted the refolding of CS. The time course analyses of GDH refoldings in the presence and absence of MTTS were also performed (Fig. 6B). Although the spontaneous recovery of GDH was approximately 35%, MTTS-ATP enhanced the recovery up to approximately 60%. In the absence of ATP, MTTS arrested the refolding of GDH. After the addition of ATP, GDH was refolded to the final recovery of approximately 65%.

Nucleotide Specificity for Chaperone Activity of MTTS in
Protein Folding—Although MTTS arrested the refolding of CS in the absence of nucleotides, it promoted the refolding of CS in the presence of ATP, CTP, and UTP (Fig. 7). On the other hand, in the presence of ADP AMP, AMP-PNP, GTP, CDP, and UDP, MTTS arrested the refolding of CS. After the addition of ATP, refoldings of CS in the arrested reactions were promoted (Fig. 7).

DISCUSSION

Archaeal chaperonins other than those of methanogens are composed of α and β subunits that are highly homologous to each other (Table I). In the present study, only one chaperonin (MTTS, thermosome from *M. thermolithotrophicus*) gene was cloned even after many trials to detect other subunit genes of chaperonin by PCR or hybridization. These suggest that the *M.*
**thermolithotrophicus** genome encodes only one chaperonin gene. *M. jannaschii* has only one chaperonin gene in its genome (20). A chaperonin that has two-stacked 8-fold symmetric ring structure was reported from a hyperthermophilic methanogen, *M. kandleri* (19). This chaperonin is composed of one kind of subunit. MTTS has a GGX (X is S or D) repeat motif containing three times repeat of GGM in its C terminus (Fig. 1), which is found in most group I chaperonins. In group II chaperonins, a similar motif has been found in the α subunit of a hyperthermophilic archaeon, *Thermococcus* strain KS-1 chaperonin (22), and the two different subunits of a halophilic archaeon, *Haloferax volcanii* chaperonin (35). The truncation of C-terminal 16 amino acid residues (i.e. GAAGGMGGMGGGMGM) from GroEL of *E. coli* did not affect its oligomeric structure but reduced the rate of ATP hydrolysis (36). On the other hand, whereas introduction of wild-type GroEL gene to temperature-sensitive *E. coli* mutants reduced their temperature sensitivity, the truncated gene lacks this ability (36).

Some archaeal oligomeric chaperonin complexes have been purified from thermophilic archaea in the absence of Mg-ATP. Some archaeal chaperonin genes have been expressed in *E. coli*. The recombinant homo- or hetero-oligomeric chaperonin complexes from *T. acidophilum* and *Thermococcus* strain KS-1 have also been purified in the absence of Mg-ATP (18, 22). We considered that the MTTS complex produced in *E. coli* was dissociated to monomer during the purification without Mg-ATP. In vitro dissociation and reassembly of *T. acidophilum* recombinant chaperonin was reported (18). Homo-oligomers of α and β chaperonin subunits of *T. acidophilum* dissociate to monomer at pH 3.0 but reassemble to the homo-oligomeric complex in the presence of Mg<sup>2+</sup> and ATP at room temperature overnight at pH 6.8. In the assembly of the MTTS subunit to oligomeric complex at 35 °C and pH 6.8 for 5 h, Mg<sup>2+</sup>, ATP, and (NH₄)₂SO₄ were indispensable. In the presence of (NH₄)₂SO₄, hydrophobic interaction of MTTS subunit molecules may increase. However, the reconstruction condition in this study was not physiological. Other factors may be required for the formation of the MTTS complex at 65 °C.

Recently, the purified chaperonin (TF55) from *S. shibatae* formed cytoskeleton-like filaments at the protein concentration...
of 1 mg/ml in the presence of Mg\(^{2+}\) and nucleotides in vitro under physiological condition (25). This chaperonin filament was also found in the cell of S. shibatae. We found that the reconstructed MTTS complex formed filamentous structure similar to that of S. shibatae at the protein concentration above 3.0 mg/ml in the presence of 50 mM MgCl\(_2\), 0.2 mM ATP, and 0.3 mM (NH\(_4\))\(_2\)SO\(_4\) (Fig. 4B). Although TF55 has \(\alpha\) and \(\beta\) subunits (13, 25), our study showed that a recombinant MTTS, which is composed of a single subunit, is also able to form the filament in the presence of Mg-ATP. This MTTS filament formation was reversible. The MTTS filament was easily dissociated to the oligomeric complex even in the presence of Mg-ATP by dilution to the protein concentration of 0.2 mg/ml (Fig. 4C). We observed the double ring structure of the MTTS during the refolding reaction of the denatured CS both in the presence (Fig. 4D) and absence (data not shown) of ATP. No filament was observed. The MTTS concentration in the reaction mixture of the refolding experiment (Figs. 4D and 6A) was 0.31 mg/ml, which was far below the concentration for filament formation. Under this condition, MTTS filament might spontaneously dissociate to the complex. It is not clear whether MTTS filament has the chaperonin activity or not. Although MTTS may form filament in the M. thermolithotrophicus cells like that in S. shibatae, it is remained to be studied. It is reported that E. coli GroEL interacts with substrate proteins in the central cavity of ring structures (2, 37). If the MTTS functions in the same way as the group I chaperonin, it would not have chaperone activity in the filamentous form. Because MTTS filament formation and dissociation to complex were reversible, it is interesting to study interaction of MTTS filament with denatured proteins. The MTTS filament may be dissociated to the oligomeric complex in the interaction with the denatured proteins.

There is little available information on ATPase activity of chaperonins from methanogens (Table I). Although chaperonin from M. kandleri has an ATP binding site (GDGT1T), ATPase activity has not been detected (19). The MTTS complex that has the GDGT1T motif exhibited weak ATPase activity with the hydrolysis rate of 74 mol of ATP hydrolysis/mol of MTTS complex/min at 70 °C. This activity is similar to those of E. coli GroEL and \(\alpha\) and \(\beta\) homo-chaperonins from Thermococcus strain KS-1 (22), but 20-fold higher than that of T. acidophilum chaperonin (3.7 mol of hydrolyzed ATP/mol of thermostable/min at 60 °C) (7). The ATPase activity of the monomeric MTTS was not detected. These data indicated that the ring structure is required for MTTS to exhibit ATPase activity.

Although some group II chaperonins have been isolated from thermophilic archaea, their protein folding activities are different (Table I). S. solfataricus chaperonin promotes the folding of some thermophilic archaeal proteins in an ATP-dependent fashion (17). The recombinant homo-oligomers composed of \(\alpha\) or \(\beta\) subunits from Thermococcus strain KS-1 chaperonin bind to folding intermediates of thermophilic isopropylmalate dehydrogenase and release them in an ATP-dependent fashion (22). Chaperonin purified from T. acidophilum binds to the denatured luciferase and dihydrofolate reductase but does not release them even in the presence of ATP (7). The recombinant \(\alpha\) and \(\beta\) homo-oligomeric chaperonin from Sulfolobus sp. strain 7 also binds to folding intermediates of isopropylmalate dehydrogenase but does not release them in an ATP-dependent fashion (38). Although two chaperonins from methanogens, M. jannaschii and M. kandleri are known (19, 20), their chaperone functions have not been shown (Table I). The MTTS complex binds to folding intermediates of thermophilic archaeal CS and GDH and releases them in an ATP-dependent fashion (Fig. 6). There are few reports on GroE-like chaperone activity of native or recombinant archaeal chaperonins in protein folding in vitro. No gene-encoding co-chaperonin, such as GroES homolog, was found in archaeal genomes (20, 39, 40). Our data support that group II chaperonins do not require a cofactor like GroES in their protein folding activity.

On the other hand, some archaeal chaperonins protect thermal inactivation of proteins. S. solfataricus chaperonin protects

![Image](77x335 to 270x449)

**TABLE I**

Structural and functional features of archaeal chaperonins

The characters of both homo-oligomers in protein folding were similar; ATP hydrolysis rates of chaperonins from Sulfolobus sp. strain 7 and P. occultum were calculated with the estimation that molecular mass of all subunits as 59 kDa. + and –, positive and negative, respectively.

| Organism                      | Native or recombinant chaperonin | Subunit composition | Symmetry | ATPase activity (mol Pi release/mol complex/min) | Protein folding | Filament formation | Reference |
|-------------------------------|----------------------------------|---------------------|----------|-------------------------------------------------|----------------|--------------------|----------|
|                               |                                  |                     |          | Arrest | Folding |                                      |          |
| Crenarchaeota                 |                                  |                     |          | +     | +       | +                         | NR       | +                  | 12, 25   |
| S. shibatae                   | N\(^a\)                           | 2                   | 9        | 30     | +       | +       | NR       | +                  | 16, 17   |
| S. solfataricus               | N\(^a\)                           | 2                   | 9        | α, β < 2.1 | +       | +       | NR       | -                  | 38       |
| Sulfolobus sp. strain 7       | R\(^a\)                           | 2                   | 9        | 77     | +       | -       | NR       | NR                 | 14       |
| P. occultum                   |                                  | 2                   | 9        | +     | +       | +       | NR       | -                  |          |
| Euryarchaeota                 |                                  |                     |          | +     | +       | +       | NR       | -                  |          |
| T. acidophilium               |                                  | 2                   | 8        | 3.7    | -       | -       | NR       | +                  | 22       |
| Thermococcus strain KS-1     | R\(^a\)                           | 2                   | 8        | α, 127; β, 56 | +       | +       | NR       | NR                 | 19       |
| M. kandleri                   |                                  | 1                   | 8        | Undetectable | NR       | NR       | NR       | +                  |          |
| M. jannaschii                 |                                  | 1                   | NR       | NR     | NR       | NR       | +        | -                  |          |
| M. thermolithotrophicus       |                                  | 1                   | 8        | 74     | +       | +       | NR       | -                  | This study |

\(^a\) N, native; R, recombinant; NR, not reported; NP, not prepared; R\(^a\), native chaperonin is composed of two distinct subunits, but recombinant \(\alpha\) and \(\beta\) homo-oligomers were characterized.
thermal aggregation of denatured proteins by binding to them and then releasing them in an ATP-dependent fashion (17). The recombinant chaperonin composed of β subunit from *Pyrococcus KOD1* stabilizes mesophilic enzymes in both an ATP-dependent and -independent fashion (21). Now, we are investigating whether MTTS complex protects thermal inactivation of some proteins.

The nucleotide specificity of chaperone activity of group II chaperonins has not been reported. In the presence of ATP, CTP, and UTP, MTTS elevated the final recovery of CS but arrested the refolding of CS with ADP, AMP, AMP-PNP, GTP, CDP, and UDP. After the arrest, CS refolding was stimulated by the addition of ATP (Fig. 7). This indicates that MTTS cannot release the refolding intermediates of CS with these nucleotides. AMP-PNP is a non-ATP hydrolysis analogue of ATP. These results suggest that the chaperone activity of MTTS is coupled with hydrolysis of ATP, CTP, or UTP. The arrest and folding activities of some archaean chaperonins are variable (Table I). The α and β homo-oligomeric chaperonins from *Sulfolobus* sp. strain 7 (38) and *T. acidophilum* chaperonin (7) can bind to the folding intermediates but cannot release them even in the presence of ATP. It would be caused by the fact that those chaperonins have little or no detectable ATPase activity.

On the other hand, *E. coli* GroEL can release the proteins such as dihydrofolate reductase and β-lactamase in the presence of ATP, or even nonhydrolyzable ATP analogs, in the absence of GroES (41–43). *E. coli* chaperonin GroE (GroEL-GroES complex) and GroEL exhibit ADP-dependent chaperone activity in the folding of yeast enolase and other proteins (44, 45). It is considered that ADP-dependent chaperone activity of GroE is not coupled with hydrolysis. However, ADP-dependent chaperone activity was not observed in MTTS (Fig. 7). The GroEL-GroES complex also exhibits effective chaperone activity with not only ATP but also CTP and UTP. However, GroEL utilizes ATP more efficiently than CTP and UTP (44). MTTS is able to effectively utilize not only ATP but also CTP and UTP with approximately the same activity without a co-factor like GroES (Fig. 7).

It is considered that several molecular chaperones other than GroEL-GroES, which are DnaK, DnaJ, and GrpE, cooperate with GroEL-GroES from the post-translational stage to the matured stage of polypeptides in the bacterial cytosol (46, 47). HSP70 (DnaK homolog) and HSP60 (GroEL homolog) cooperate in the protein folding in mitochondria (48). However, the archaean genome information showed that chaperonin (thermosome) and FKBP (FK506 binding protein)-type peptidyl prolyl cis-trans isomerases are the major components of their molecular chaperones and peptidyl prolyl cis-trans isomerases, which are important factors involved in protein folding (20, 39). No homologs of DnaK, DnaJ, GrpE, and GroES are found in their genomes except in that of *M. thermoautotrophicum*. Our present study showed that a single subunit chaperonin, MTTS, exhibits the weak ATPase activity and chaperone activity coupled with ATP-hydrolysis in protein folding in *vitro*. On the other hand, we had already purified FKBP-type peptidyl prolyl cis-trans isomerase from *M. thermolithotrophicus*, named MTFK (FKBP from *M. thermolithotrophicus*), and cloned the corresponding gene (28). It is interesting to study the synergistic interaction of MTFK and MTTS in protein folding.

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