Molecular Cloning of groESL Locus, and Purification and Characterization of Chaperonins, GroEL and GroES, from Bacillus brevis

Masao Tokunaga,†‡ Yoichi Shiraishi‡ Masatake Odachi,§ Makoto Mizukami,§ Hiroko Tokunaga,‡ John S. Philo, † Tsutomu Arakawa, † Matsujiro Ishibashi,§ Ryoichi Tanaka,§ and Hiroaki Taka †

†Laboratory of Applied and Molecular Microbiology, Faculty of Agriculture, Kagoshima University, 1-21-24 Korimoto, Kagoshima 890-0065, Japan
‡Department of Research & Development, Higeta Shoyu Co., Choshi, Chiba 288-0041, Japan
†Alliance Protein Laboratories, 3957 Corte Cancion, Thousand Oaks, California 91360 USA

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The groESL locus of a protein-hypersecreting bacterium, Bacillus brevis, was cloned by PCR using primers designed based on the DNA sequence of a B. subtilis homolog. GroEL protein was purified to apparent homogeneity and its ATPase activity was characterized: it hydrolyzed ATP, CTP, and TTP in this order of reaction rate, and its specific activity for ATP was 0.1 μmole/min/mg protein. Purified GroEL forms a tetradecamer. GroEL was estimated to contain 22% α-helix, 24% β-sheet, and 19% turn structures, by CD measurement. GroES protein was also highly purified to examine its chaperonin activity. GroEL protected from thermal inactivation of and showed refolding-promoting activity for malate dehydrogenase, strictly depending on the presence of ATP and GroES.

Key words: groESL; GroEL; GroES; hrcA; Bacillus brevis

Considering that plenty of new genes will be made available by progress in various genome projects, development of highly efficient expression and production systems for heterologous proteins is under vigorous investigation. B. brevis is one of such hopeful host-vector systems to produce intact, biologically functional foreign proteins intra- and extracellularly. Uda and his co-workers first isolated this protein-hyperproducing bacteria, secreting up to 20 g/liter of endogenous proteins into the culture medium.1,2) This Gram-positive bacterium is very useful for the extracellular production of heterologous proteins with establishment of a novel host-vector system: several mammalian proteins (~2 g per liter) as well as several bacterial proteins (1.0~3.5 g per liter) were successfully produced extracellularly.3,4) The highly efficient secretion of proteins requires many factors including molecular chaperones to allow secretory precursor proteins to maintain secretion-competent conformations for export.5,6,7) Since B. brevis secretes a large amount of heterologous as well as homologous proteins into the culture medium, we reason that the molecular chaperones in B. brevis have very important roles in these processes. Co-expression of chaperonin genes might accelerate the secretion efficiency of heterologous proteins from B. brevis, and purified GroESL proteins must assist in the refolding of unfolded recombinant proteins in vitro. Indeed, in Bacillus subtilis, over-production of major chaperones increased the secretory production yield of a single-chain antibody by 60%.8) Immobilized chaperones were used as a continuous refolding machinery.9,10) We have reported previously for B. brevis, the purification and characterization of the major molecular chaperone DnaK and its co-chaperones, and cloning of their genes.11) However, the molecular chaperons GroEL and GroES have not been characterized in B. brevis.

In this paper, we describe the purification and characterization of the molecular chaperons, GroEL and GroES proteins, and cloning of groESL locus from B. brevis.

Materials and Methods

Strains and culture medium. Bacillus brevis HPD31 was grown in T2M medium (10 g of glucose and polypepton, 5 g of meat extract, 2 g of yeast extract, 10 mg of FeSO₄ and MnSO₄, and 1 mg of ZnSO₄ per liter). HPD31 cells cultured at 45 C for 72 h and a hrcA-disrupted mutant of B. brevis

† To whom correspondence should be addressed. Masao Tokunaga Fax: 81-99-285-8634; E-mail: tokunaga@chem.agri.kagoshima-u.ac.jp

Abbreviations: GST, glutathione S-transferase; 14-mer, tetradecamer; DTT, dithiothreitol
DNA isolation and cloning of groESL locus. The method of genomic DNA isolation from *B. brevis* was described elsewhere. The cloning of the groESL locus was done by 3 successive PCRs using the genomic DNA as a template and 3 sets of primers, P1-P6 (Fig. 1). The first set of primers, P1 and P2, was designed on the basis of the published nucleotide sequence of groEL gene of *B. subtilis*. The forward primer, P1 (5'-GGTTAAGTAACTT-CAGCCAAAA-3'), covered the part near the 5' end region of the gene starting 75 nucleotides downstream of the initiation codon. The backward primer, P2 (5'-TTACATCATTTACCCATTGCCGGCC-AT-3') covered the 3' end region containing the translation termination codon. About a 1.4-kb fragment was amplified after the PCR. The remaining parts of the locus, 5' upstream region containing groES gene and the 3' downstream region of groEL, were further cloned by "inverse PCR" as follows. The analysis of the nucleotide sequence of the PCR product showed that there were 2 PvulII sites in the sequence. Therefore, in the next step, using the upstream region of the first PvulII site (373 bp from the initiation codon) as a probe, genomic Southern blot analysis of the chromosomal DNA, which had been digested with PvulII, was done. As a result, a third PvulII site was found about 1 kb upstream from the first site, indicating that the complete 5' end of groEL and whole groES gene resided on the 1-kb fragment between the two PvulII sites. The PvulII-PvulII 1-kb fragment was isolated from the agarose gel and self-ligated to make it circular. A second PCR was done using the circularized 1-kb fragment as the template and the following primers: 5'-GAAAAAGCTTCTGATCACGTG-3' (P3) and 5'-CTCAAGTACCCATCGGTCG-3' (P4). P3 primer covered the PvulII site plus an upstream region toward the 3' end of the groEL gene and P4 covered from near the 5' end of the cloned fragment in the opposite direction. To clone the 3' end of groEL gene, a third PCR was done with the same strategy as that described above. A unique Psrl site was found toward the 3' end of the cloned groEL gene. Genomic Southern blot analysis of chromosomal DNA digested with Psrl, using the downstream sequence of Psrl site as the probe, showed that a second Psrl site existed at about 0.9 kb downstream of the first one. PCR was done, using the circularized Psrl-Psrl 0.9-kb fragment by self-ligation as the template, and the following primer set: 5'-TATGGGATTTGAGTCTGATGGGCGG-3' (P5) and 5'-GGTAGATACCTTTTCTACTGCAG-3' (P6). P5 primer covered a 1583-1608 bp region away from the initiation codon toward the termination, while P6 1231-1207 bp covered the opposite direction. By the PCR, about a 0.6-kb fragment was amplified. It was confirmed that the fragment contained the 3' end of the groEL gene by analyzing the nucleotide sequence. Thus, the whole groESL operon of *B. brevis* was cloned. The nucleotide sequence of groESL from *B. brevis* HPD31 found in this study has been deposited in the EMBL, GenBank, DDBJ databases under the accession number AB038650.

Construction of plasmid pGST-GroES. pGEX-4T-1 (Amersham-Pharmacia) was digested with BamHI and EcoRI. The groES gene was amplified by PCR with the forward primer, 5'-CCCGGATCCCTTGCGTTC-3' and a BamHI site followed by the coding sequence starting at Leu23, and a backward primer (5'-CCGGAATTCTTAAACGTAGTACCGAGG-3'), which contains the coding sequence up to the COOH-terminal Gly and an EcoRI site. The PCR product was cut with BamHI and EcoRI, and ligated into the vector to generate pGST-GroES.

Purification of GroEL and GroES proteins from *B. brevis*. Cells (20 g wet weight), suspended in 100 ml of 50 mM Tris-HCl buffer, pH 8.0, containing 25 mM KCl, 2 mM MgCl2 (TKM buffer) and a protease inhibitor mix (1 tablet of Complete in 50 ml, Boehringer), were disrupted by sonic oscillation, and then centrifuged at 12,000×g for 15 min. The supernatant was ultracentrifuged at 200,000×g for 1 h, and the supernatant was fractionated by the addition of ammonium sulfate. GroEL purification was followed by SDS-polyacrylamide gel electrophoresis (PAGE). Proteins, recovered at 30–60% of saturated ammonium sulfate, were dissolved in 10 ml of TKM buffer, and put on a Sephacyr S-300 column (2.6 × 90 cm) equilibrated with 50 mM Tris-HCl buffer, pH 8.0, containing 0.15 M KCl, 2 mM MgCl2, and 1 mM...
phenylmethylsulfonyl fluoride (5 ml of sample was put on for one run). The neighboring fractions containing GroEL were concentrated with an Amicon membrane filter Y-30, and put on an ATP-agarose column (12 ml resin) equilibrated with TKM buffer. After this was washed with 2 and 1 column volumes of TKM buffer with and without 0.3 M KCl, respectively, the column was eluted with TKM buffer containing 3 mM ATP. The eluates were put on the HPLC DEAE-5PW column (TOSOH) after concentration with an Amicon filter, and the column was eluted with a linear gradient of NaCl from 0 to 0.5 M in 50 mM Tris-HCl buffer, pH 8.0. The fractions eluted at 0.38 M NaCl containing GroEL were collected, and put on an HPLC gel filtration column (G3000SWXL, TOSOH) equilibrated with 20 mM Tris-HCl buffer, pH 7.2, containing 0.2 M KCl (300 µg each for one run). The GroEL protein eluted at the void volume was apparently homogeneous.

GroES protein was purified from an hrcA-disruptor mutant. Protein fractions containing GroES after Sephacryl S-300 gel filtration of the purification procedure described above were concentrated by an Amicon Y-10 filter, and put on DEAE-5PW (6 mg protein per run) with gradient elution of 0 to 0.5 M NaCl in 50 mM Tris-HCl buffer, pH 8.0. The GroES fractions eluted at 0.25 M NaCl from DEAE column were concentrated and put on a hydroxyapatite HPLC column (Mitsuioatsu) with gradient elution of 0.01 to 0.25 M Na-phosphate buffer, pH 7.0. The eluted GroES fraction at 0.13 M Na-phosphate buffer was concentrated and put on a second DEAE-5PW column. The GroES fraction eluted at 0.21 M NaCl was used as a final preparation. This final GroES sample contains one faint unidentified protein with molecular mass of 44 kDa: the purity of GroES is greater than 97% by SDS-PAGE.

**Assay for ATPase activity of GroEL.** A reaction mixture (total 25 µl) containing 1 mM ATP, 50 mM KCl, 5 mM Mg(CH_3COO)_2, 30 mM Tris-HCl buffer, pH 7.8, and GroEL was incubated at 37 C for 40 min, and then 17 µl of 2 M perchloric acid was added to stop the enzyme reaction. After incubation on ice for 30 min, samples were centrifuged at 12,000 x g for 1 min to remove insoluble materials, and 25 µl of this supernatant was used for inorganic phosphate (Pi) measurement by the method of Lancett et al. One unit was defined as one µmole of Pi liberation per minute.

**Sedimentation velocity analysis of GroEL.** Sedimentation velocity experiments were done at 20 C in a Beckman Optima XL-A analytical centrifuge using absorbance scans at 230 nm and 2-channel charcoal epon centerpieces. The data were analyzed using the programs DCDT + version 1.10 or SVEDBERG version 6.35. The protein partial specific volume and the buffer density and viscosity were calculated using the program SEDINTERP version 1.04.

**Circular dichroism analysis of GroEL.** Circular dichroism (CD) measurements were done on a Jasco J-715 spectropolarimeter with a Peltier cell holder and a PTC-348WI temperature controller. A cuvette with 0.1 cm pathlength was used throughout. A scan rate at 10 nm/min and an average of 10 scans were used with a time constant of 4 sec. Far UV CD was measured using a GroEL at 1.1 mg/ml in 50 mM Tris-HCl, 25 mM KCl, and 2 mM MgCl_2, at pH 8.0 in the absence and presence of 0.11 mM ATP. The solvent spectrum was subtracted from the sample spectrum and the CD signal after subtraction was converted to mean residue ellipticity using the mean residue weight of 105.6. Thermal melting was done at a scan rate of 20 C/h at 222 nm. Protein concentrations were measured spectrophotometrically using the extinction coefficient of 0.089.

**Chaperonin assay for protection and reprofiling of thermal denaturation of pig heart mitochondrial malate dehydrogenase.** Pig heart mitochondrial malate dehydrogenase (MDH, 89 nm, 1.25 µg) was incubated at 37 C for 4 h in 200 µl of 38 mM Tris-HCl buffer, pH 7.6, containing 1.8% (vol/vol) glycerol, 36 mM NaCl, 7.7 mM KCl, 5.4 mM MgCl_2, and 1.5 mM diithiothreitol (DTT) with and without GroEL (175 nm, 28 µg), GroES (279 nm, 3.9 µg) and 1.5 mM ATP. Every 1 h incubation, residual malate dehydrogenase activity in 20-µl portions was measured. The reaction mixture (0.5 ml) contains 95 mM potassium phosphate buffer, pH 6.8, 0.2% bovine serum albumin (BSA), 645 µM oxaloacetic acid, 1 mM DTT, and 300 µM NADH, and the decrease of absorbance at 340 nm was monitored at 37 C. For the refolding assay, GroES was added to the MDH which was first incubated with GroEL and ATP for 4 h at 37 C, and MDH activity was measured every 30 min.

**Others.** SDS-PAGE was done as described by Laemmli, and protein bands were stained with Coomassie brilliant blue. Western blotting and immuno-staining with 4-chloronaphthol and ECL kit (Amersham-Pharmacia) have been described. Glutathione S-transferase(GST)-GroES fusion protein was purified from E. coli JM109 cells harboring pGST-GroES according to the manufacturer’s instruction, and was injected into a white rabbit with complete Freund’s adjuvant to raise antibodies. The amount of protein was measured as described by Lowry et al. The amount of protein in the band stained with Coomassie blue after SDS-PAGE was also measured by densitometry or by using NIH Image software with bovine serum albumin as the standard. For comparison, purified GroEL and GroES
proteins derived from E. coli were purchased from TAKARA.

Results and Discussion

Cloning and nucleotide sequence of groESL locus of B. brevis

We cloned a 2.8-kb DNA fragment containing the groESL locus of B. brevis by PCR and inverse PCR technology (Fig. 1). Cloned nucleotide sequence of the groESL operon of B. brevis (deposited as AB038650) had high similarity to that of B. subtilis (Fig. 2). The homology scores of groES and groEL were 70 and 75%, respectively. The alignment of the two nucleotide sequences showed that in the upstream region of groES gene of B. brevis, homologous sequence of −35 and −10 region of B. subtilis groESL operon existed (Fig. 2A). The sequence of a 9-bp inverted repeat, or CIRCE element,20 to which the HrcA protein is thought to bind, was exactly the same between the two species. Thus, the transcription of groESL operon of B. brevis may be under the same regulation system as that of B. subtilis. Namely, a sigmaA like factor may recognize the promoter sequence of hrcA gene which resides in front of grpE gene of dnaK operon10 and the gene product of hrcA

Fig. 2. Nucleotide Sequence of B. brevis groESL Operon.

A: A comparison of the upstream sequence of groESL operon between B. brevis (B.b.) and B. subtilis (B.s.). Homologous regions including −35, −10 and ribosome-binding site (SD), are boxed. An inverted repeat which is composed of 9 nucleotides, or CIRCE element, is shown by arrows. The initiation codons of groES of each bacteria are also boxed. B: Nucleotide sequence and deduced amino acid sequence of B. brevis groESL operon, starting from the initiation codon of groES. Putative SD sequence of groEL is underlined.
may work as a negative regulator by binding the CIRCE element. Actually, as in the case of *B. subtilis*, we have observed a sharp increase of GroEL protein production caused by the disruption of the *hrcA* gene (data not shown). The homology score in the deduced amino acid sequence between *B. brevis* and *B. subtilis* was 76% for GroES and 80% for GroEL, demonstrating that the major molecular chaperones were highly conserved between the two *Bacillus* species.

**Purification of *B. brevis* chaperonins, GroEL and GroES**

Purifications of GroEL and GroES proteins were monitored by western blotting using anti-*E. coli* GroEL antiserum and anti-GST-*B. brevis* GroES fusion protein-antiserum prepared here. *B. brevis* GroES was not cross-reactive with commercial anti-*E. coli* GroES-antiserum (Sigma or StressGen). As shown in Fig. 3A, GroEL protein was purified by the procedures described in Materials and Methods to apparent homogeneity with a yield of about 11 mg from 20 g (wet weight) of cells. The amino-terminal amino acid sequence of GroEL protein was AKQVKFSEDARRSML, which was identical to the expected sequence deduced from the DNA sequence of the *groEL* gene. The molecular mass of *B. brevis* GroEL was calculated to be 57,310 from the deduced amino acid sequence. The final GroEL preparation was dialyzed against 50 mM Tris-Cl buffer, pH 8.0, stored in portions frozen at −20°C.

To overproduce GroES protein, we have constructed *hrcA*-disrupted mutant of *B. brevis* (Mizukami and Takagi, paper in preparation). This mutant in *B. subtilis* has been shown to overproduce GroES protein as well as other major molecular chaperons by several-fold. As shown in Fig. 3B, GroES protein was highly purified. Although the GroES protein in Fig. 3B (14% gel) was stained as a smear band, it was demonstrated that no proteins other than GroES were detected in the GroES band of final preparation by using an other gel system and by NH2-terminal amino acid sequence analysis. The NH2-terminal amino acid sequence, MNLPLGDRVV, was exactly same as that deduced from DNA sequence of the *groES* gene. The molecular mass of *B. brevis* GroES was calculated to be 10,127.

**Characterization of the ATPase activity of GroEL**

We studied the enzymatic properties of GroEL-ATPase activity. The ATPase activity increased linearly both with increasing GroEL concentration up to 5 μg/assay mixture, and with increasing incubation time up to 40 min at 2 μg GroEL/assay mixture. The optimum reaction temperature was 60°C, while the optimum reaction pH was around 7.0 to 8.0. The GroEL-ATPase activity was stable in the buffer solutions at pH from 5.0 to 8.0, and lost its activity below 4.0. The GroEL-ATPase required K+ ion, and was severely inhibited with Na+ ion (data not shown). The specific activity of GroEL-ATPase was found to be around 0.1 Unit/mg protein at 37°C. The substrate specificity of GroEL-ATPase is shown in Table 1. It hydrolyzed CTP and TTP with 59 and 10% efficiency relative to the efficiency for ATP, but did not react with nucleoside di- and monophosphates. We have demonstrated that the GroEL from halophilic bacteria hydrolyzed ATP but not CTP, UTP and GTP[21] and *E. coli* GroEL was reported to hydrolyze ATP, CTP and UTP,[22] suggesting that the substrate specificity of GroEL ATPase activity was slightly different among those isolated from different origins.

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![Fig. 3](image-url)

**Fig. 3.** SDS-polyacrylamide Gel Electrophoretogram of *B. brevis* GroEL and GroES Samples from Each Purification Step. A. Purification steps for GroEL. MW, molecular weight standard; lane 1, crude homogenate; lane 2, supernatant after ultracentrifugation; lane 3, ammonium sulfate precipitate (30–60%); lane 4, after the Sephacryl S-300 gel filtration; lane 5, after the ATP agarose column; lane 6, after the DEAE-5PW column; lane 7, final preparation after the G3000SWgel filtration column. To lanes 1–4, 15 μg of protein was applied; to lanes 5–7, 3 μg of protein was applied. B. Purification steps for GroES. MW, molecular weight standard; lane 1, crude homogenate; lane 2, supernatant after ultracentrifugation; lane 3, ammonium sulfate precipitate (30–60%); lane 4, after the Sephacryl S-300 gel filtration; lane 5, after the DEAE-5PW column; lane 6, after the hydroxyapatite column; lane 7, final preparation after the second DEAE-5PW column. To lanes 1–3, 20 μg of protein was applied; to lanes 4 and 5, 10 μg of protein was applied; to lanes 6 and 7, 5 μg of protein was applied.
Table 1. Substrate Specificity of GroEL-ATPase

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Subunit and secondary structures of B. brevis GroEL protein

The solution mass and conformation of B. brevis GroEL were investigated by sedimentation velocity. Figure 4 shows the sedimentation coefficient distribution, g(s*), for a sample loaded at ~60 μg/ml. This material appears to contain only one single species with a sedimentation coefficient, s_{20,w}, of 22.6 S, very similar to values of 22-23 S reported for the tetradecamer (14-mer) double ring structure of GroEL from E. coli. The minimum width of the peaks in the g(s*) distribution can be used to estimate the diffusion coefficient and therefore the molar mass assuming there is no peak broadening due to heterogeneity nor any significant association-dissociation reactions during the experiment. A fit to these data, shown as the solid line in Fig. 4, implies a mass of 840 kDa, which is consistent with the predicted mass of 802 kDa for the 14-mer within the ~5% error expected for this approach. A more accurate analysis can be obtained by directly fitting the raw velocity scans using the program SEDBERG (results not shown), which gives a diffusion coefficient, D_{20,w}, of 2.72 × 10^{-7} cm^2 s^{-1} and a mass of 793 ± 7 kDa, in excellent agreement with the mass of a 14-mer. This consistency of the mass estimates from velocity analysis with the predicted mass also implies good homogeneity of the sample.

The far UV CD spectrum of GroEL 14-mer was taken at 25 C. As shown in Fig. 5A, α, it has a CD spectrum characteristic of the presence of α-helix. Curve fitting shows that the protein has 22% α-helix, 24% β-sheet, and 19% turn.

The sample was thermally melted, scanning at 20 C/h from 25 C to 75 C. The result indicates a sharp cooperative transition (Fig. 5B). The melting temperatures were calculated by taking the first derivative to the transition. The onset of transition is 63 C, the mid-temperature 67 C and the end temperature 70 C. The mid melting point of E. coli GroEL was reported to be 60 C. There is no visible precipitate after heating to 75 C, consistent with no apparent change in High Tension (HT) voltage (not shown). Figure 5A, c also shows that the heat-denatured protein has a substantial secondary structure as indicated by the ellipticity at 222 nm. This sample was cooled to 25 C and incubated at this temperature for more than 30 min. The cooled sample shows a significant secondary structure, although the helical content is reduced (18% α-helix, 28% β-sheet, and 19% turn, Fig. 5A, c). The rescanning of the cooled sample showed no thermal transition (data not shown), indicating that thermal unfolding of GroEL is irreversible under the conditions used. The temperature dependency of secondary structure were parallel with the thermal stability and optimum reaction temperature of GroEL ATPase (data not shown).

The addition of 0.11 mM ATP did not alter the CD pattern (Fig. 5A, b) and thermal melting of GroEL (data not shown), indicating the gross secondary structure of B. brevis GroEL was not changed by the addition of ATP. This result was consistent with that obtained for E. coli GroEL. It was reported that the transition from binding-active to folding-active conformation of E. coli GroEL involves its dramatic conformational change by the binding of both nucleotide and GroES. However, nucleotide binding alone to the E. coli GroEL, in the absence of GroES, has not been observed to produce this extent of conformation change.

Protection and refolding of thermal denaturation of malate dehydrogenase by GroESL and ATP

Since the purified GroEL forms a 14-mer and has
Chaperonin GroESL from B. brevis

Fig. 5. The Far UV Circular Dichroism Spectra and Thermal Melting of B. brevis GroEL.
A. The mean residue ellipticity is plotted against wavelength; a, in the absence of ATP before thermal scan; b, in the presence of 0.11 mM ATP before thermal scan; c, in the absence of ATP after thermal scan. B. Thermal scanning of B. brevis GroEL described in Materials and Methods.

Fig. 6. Chaperonin Assay for B. brevis GroESL Proteins for Malate Dehydrogenase.
A. Protection of thermal inactivation of malate dehydrogenase. a, control experiment with refolding buffer; b, in the presence of bovine serum albumin; c, GroEL (175 nM)/ATP; d, GroES (279 nM)/ATP; e, GroEL/GroES; f, GroEL/GroES/ATP; g, GroEL/GroES/CTP. B. Refold-promoting assay for thermal denatured malate dehydrogenase. a, same as f in Fig. 6A; b, same as c in Fig. 6A; c, same as b, but GroES was added after 4 h incubation (shown by an arrow).

ATPase activity, the chaperone activity of GroESL was studied. We examined the effects of GroEL and GroES on the thermal inactivation of pig heart mitochondrial MDH. Hartman et al. reported that this enzyme was inactivated rapidly with incubation at 37°C. We used this assay system for B. brevis GroEL and GroES.

MDH lost 90% of its activity after incubation at 37°C for 4 h (Fig. 6A, a). However, this thermal inactivation was largely prevented in the presence of GroEL/GroES/ATP (Fig. 6A, f). GroEL/ATP, GroES/ATP, and GroEL/GroES without ATP (Fig. 6A, c, d, and e) showed no protection, nor did bovine serum albumin (BSA, Fig. 6A, b) or BSA/ATP (not shown) show any protective effects. These data clearly demonstrate that B. brevis GroEL interacts with
GroES, and protects thermal inactivation of MDH in an ATP-dependent manner. We found that inactivated MDH, incubated with GroEL/ATP at 37 °C for 4 h, was reactivated by the addition of GroES (Fig. 6B, c). This data suggested that GroEL alone could bind and trap the unfolded intermediates of MDH at 37 °C, and could release them by the cooperation of GroES and ATP. It is most likely that GroEL and GroES recognize the early unfolding intermediates of MDH and promote their refolding in the presence of ATP. Although GroEL hydrolyzed CTP with 59% efficiency of ATP, the presence of ATP was essential for the refolding of MDH (Fig. 6A, g). B. brevis GroEL also showed a refolding-promoting activity for urea-denatured α-glucosidase (data not shown). All these data clearly suggested that B. brevis GroESL can be used for in vitro refolding machinery.

In this report, we studied the purification, the fundamental enzymatic and physicochemical properties of B. brevis chaperonins, GroEL and GroES, and further demonstrated their refold-promoting activity for denatured proteins. This is a first report in Bacillus species that both GroEL and GroES proteins are purified and resolved their interactions in vitro. We also cloned and sequenced the B. brevis groESL locus, and suggested the gene regulation by CIRCE regulon. Here, we reported that the thermal-melting point of B. brevis GroEL was 7 °C higher than that of E. coli GroEL, indicating that it will be a good reagent for the protein-refolding in vitro. We have previously reported that another major chaperone system consisting of DnaK, DnaJ, and GrpE of B. brevis interacts with unfolded proteins.10 We are now investigating the effects of co-expression of these major chaperones, DnaK and GroEL, and their co-chaperones on the secretory efficiency of various recombinant proteins from B. brevis.

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