DOI 10.5012/bkcs.2011.32.6.1925

Molecular Cloning and Chaperone Activity of DnaK from Cold-adapted Bacteria, KOPRI22215

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Received April 6, 2011, Accepted April 21, 2011

Psychrophilic bacteria have acquired cold-resistance in order to protect themselves against freezing temperatures, which would otherwise be lethal. DnaK/DnaJ/GrpE systems are molecular chaperones which facilitate proper folding of newly synthesized proteins. Efficient folding processes are of great importance especially in a cold environment, such as the Arctic. In order to understand the protection mechanisms of psychrophilic bacteria against cold temperatures, we have explored a genome of KOPRI22215, tentatively identified as *Psychromonas arctica*, whose genome sequence has not yet been discovered. With an aim of searching for a coding gene of DnaK from KOPRI22215, we have applied a series of polymerase chain reactions (PCR) with homologous primers designed from other Psychromonas species and LA PCR *in vitro* cloning. 1917 bp complete coding sequence of dnaK from KOPRI22215 was identified including upstream promoter sites. Recombinant plasmids to overexpress PaDnaK along with EcDnaK (DnaK of *E. coli*) were then constructed in pAED4 vector and the pET-based system to induce PaDnaK expression by IPTG. Characterization assays of expressed PaDnaK were carried out by measuring survival rates upon 4 day incubation at 4 °C: a refolding assay as molecular chaperone, and ATPase assay for functional activity. Taking account of all the data together, we conclude that PaDnaK was identified, successfully expressed, and found to be more efficient in providing cold-resistance for bacterial cells.

Key Words : DnaK, Psychophilic bacterium, Cold-resistance, Inverse PCR, IPTG induction

Introduction

Psychrophilic bacteria have acquired cold-resistance in order to protect themselves against otherwise lethal freezing temperatures. Psychrophilic bacteria have been compared with mesophilic bacteria to provide clues for cold-adaptation.[1] The potential importance of molecular chaperones in efficient folding processes has been addressed for subzero temperatures.[2] For example, cold shock proteins (Csps) known as RNA chaperones were induced by temperatures dropping by 10 °C.[3] When expressed in *Escherichia coli* (*E. coli*), it dramatically increased the host's cold resistance more than 10 times. The *tig* gene encoding trigger factor with a peptidyl-propyl cis-trans isomerase (PPIase) activity was also identified.[4] Biochemical analysis showed that the expressed PaTF was functional in PPIase assay as well as *in vivo* cold-resistance assay.[5]

Amongst heat shock proteins (Hsps) acting as molecular chaperones, Hsp 70 systems (DnaK/DnaJ/GrpE) are known to play a crucial role in rescuing bacterial cells from cold environments by facilitating the proper folding of proteins when synthesized.[6] DnaK consists of an N-terminal ATPase domain and a C-terminal substrate binding domain.[7] DnaK interacts with DnaJ and GrpE to form a ternary complex as a functional unit. The ATP-bound form of DnaK shows low affinity for unfolded protein substrates whereas the ADP form of DnaK displays high affinity for them.[8] Co-chaperone DnaJ stimulates the ATPase activity of DnaK while GrpE dissociates ADP out of the complex and then releases folded protein substrate.[9]

It is interesting to note that even in mesophilic *E. coli*, DnaK/DnaJ/GrpE is involved in the recovery rate of the induced culture after freezing treatment in a similar fashion to that of the heat-shocked culture.[10] It can be envisioned that DnaK/DnaJ/GroEL can prevent irreversible denaturation by chaperoning the unfolded polypeptides during freezing. On the other hand, the level of DnaK expression in psychrophilic bacterial is rather diverse. The *dnaK* and *dnaJ* genes from *Colwellia maris* were down regulated by a temperature shift from 26 °C to 10 °C.[11] The proteomic study showed that in *Pseudoalteromonas haloplanktis* the psychrophilic trigger factor (TF) rescues the chaperone function as both DnaK and GroEL are down regulated to 4 °C.[12] In contrast to these findings, other groups reported opposite results in which DnaK was up-regulated upon cold treatment. The proteomic analysis of *Chaetoceros neogracile*, an Antarctic alga, upon cold treatment exhibited increased levels of DnaK and ClpB.[13] It was worth noting that the dnaK-deleted mesophilic *E. coli* strains were not viable at cold temperatures, but when complemented with cold-adapted chaperones GroEL/GroES from the psychrophilic *Oleispira Antarctica* they were able to survive at low temperatures.[14]

In this work, with the aim of investigating the functional role of DnaK in providing cold-resistance in psychrophilic bacteria, we have identified the pa*dnak* gene encoding PaDnaK from *Psychromonas arctica* (KOPRI22215) whose

genome sequence was not determined.[15] Fortunately, genome sequences of *Psychromonas ingrahamii* have recently been discovered.[16] With no genome information available, a series of polymerase chain reactions (PCR), including a gradient PCR and LA PCR *in vitro* cloning, were applied to pinpoint the p*adnaK* encoding gene from the whole genomic DNA. We have further developed the expression systems of PaDnaK protein in *E.coli* and were able to confirm the presence of PaDnaK. Characterizations of expressed PaDnaK were carried out by measuring survival rates upon extensive incubation at 4 °C, refolding assay as molecular chaperone, and ATPase assay for functional activity. Considering that DnaK is not fully functional in the absence of DnaJ, we present here putative functional roles of PaDnaK in cold-adaption even better than EcDnaK.

Experimental Section

Strains and Equipment. Psychrophilic bacterium (KOPRI22215) was donated by KOPRI, Korea Polar Research Institute. Monitoring of cell growth and measurement of enzyme activity were carried out with UV-VIS spectrophotometer (Hewlett Packard 8452A). Gel electrophoresis was performed by BioRad Mini-gel and Power Pac 300. Gradient polymerase chain reaction (PCR) was performed with homologous primers in the 96 Gradient Thermal Block (A-2042, Bioneer).

PCR Amplification of DnaKs. KOPRI22215 was grown at 15 °C in Difco™ Marine broth medium. BL21(DE3) was cultured in LB medium (Trypron 10 g, Yeast extract 5 g, NaCl 10 g per liter). In order to isolate genomic DNA, cells were treated with G-spin™ for bacteria genomic DNA exraction kit (iNtRON). Since we had no genomic information for KOPRI22215 available, we designed the homologous primers [5'-GCACAACGTCAAGCAACTAAAGATGCAGG-3' (forwarding primer; F1), 5'-GCAACCGCTTCATCAGG-GTTAACATCTTT-3' (reversing primer; R1)] to target homologous regions of DnaKs based on sequence comparison of DnaK-encoding genes known from other Psychromonas species. The PCR mixture is composed of 1 μL of genomic DNA, 2 μL of each dNTP (2.5 mM), 0.4 μL of each primer (10 pmol each), 2 μL of 10x pfu buffer and 0.4 μL of pfu polymerase (Takara) in a final volume of 20 μL. Cycles of gradient PCR consisted of 1 min denaturation at 94 °C, 1 min annealing at a gradient temperature between 48 °C and 58 °C, and 5 min extension at 72 °C. The PCR product was analyzed in 1.0 % agarose gel stained with ethidium bromide (EtBr). The DNA band corresponding to a desired molecular weight was excised to purify amplified DNA with a Geneclean turbo kit (MP Bio) and sequenced by Cosmo GenTech (Seoul, Korea). Using the partially identified internal region of the putative dnaK gene from KOPRI22215, we applied the LA (long and accurate) PCR in vitro cloning technology (Takara) to further identify the upstream (5'-terminus)and downstream region (3'-terminus) of the encoding gene. To amplify the 5'-terminus, Hind III Cassette was ligated into 5' terminal of HindIII cut genomic DNA. Primers used for the 1st PCR were a cassette primer (5'-GTACATATTGTCGT-TAGAACGCGTAATACGACTCA-3', C1) for Hind III and a specific primer (S1) [5'-GCAACCGCTTCATCAGGGTT-AACATCTTT-3'(R1)]. Annealing was done at 55 °C for 30 sec and extension was performed at 72 °C for 1 min using Takara LA Taq polymerase. The 2nd PCR was carried out with 1 μL of the 1st PCR product and a new set of primers (5'-CGTTAGAACGCGTAATACGACTCACTATAGGGAGA-3', C2) and SR1 (5'-CCTAAGTCGTATACAGCAACAA-CGCTGTCGCCT-3') probed for the inner region of 1st primer binding sites. To amplify the 3'-temunus, (5'-GTACAT-ATTGTCGTTAGAACGCGTAATACGACTCA-3', C1) and (5'-GCACAACGTCAAGCAACTAAAGATGCAGG-3', F1) were used for the 1st PCR and (5'-CGTTAGAACGCGTAA-TACGACTCACTATAGGGAGA-3', C2 and 5'-ACCTGCC-TTACATCACTGCA GATGCATCAG GTC-3', SF1) were used for the 2nd PCR. Both the 5'-terminus and 3'-terminus region of the encoding gene were identified by sequencing each DNA fragment obtained by the 2nd PCR experiments.

Comparison of Homologous DnaK Proteins. The DNA sequence obtained by PCR amplification was translated to the corresponding polypeptides using Translate tool (ExPASy Proteomic Server). Alignments with other homologous DnaK proteins among Psychromonas species were examined using ClustalW2 (EBI server).

Cloning of PaDnaK in Protein Expression Vector Systems. Two primers flanking the entire dnaK gene (padnaK) were designed to possess Nde1 and BamH1 sites [5'-CATATGCCAGCGTGCAGATAGTCAACTAAACAA-GTA-3' (F2 with Ndel), 5'-ACTGGATCCGGGGTTACCT-AATTATTTTTTCTCGTC-3' (R2 with HindIII)]. Gradient PCR was performed with pfu polymerase (Promega). The amplified PCR product was cloned into pBluscript SK II which was previously digested with EcoR V by T4 DNA ligase. The white colony resulting from disruption of lacZ gene in the plasmid was selected to provide the *padnaK* insert for cloning into a pAED4 expression vector. The *padnaK* insert digested with NdeI and BamH1 was ligated into compatible ends of pAED4 vector by T4 DNA ligase and transformed into DH5α competent cells. For over-expression and simple purification, we also developed the pET-28a vector based expression system for producing the His-tagged PaDnaK. In a similar fashion, gradient PCR was performed by using a new set of primers containing Nde1 and HindIII sites [5'-GAGAGTTCATATGGGTAAAATTAT-CGG-3' (F3), 5'-GGGGAAGCTTAATTATTTTTTCTCGTC-3' (R3)] to provide a full length insert of padnaK/pET28a. After it was transformed into DH5a, the right colony was confirmed to drop the insert out by incubating with the same restriction enzymes. DnaK from *E. coli* (EcDnaK) was amplified similarly except using primers [5'-AGTGGAGACG-TTCATATGGGTAAAATA-3' (F4 with Nde1), 5'-CGAAG-CTTG GGC GAT TAT TTT TTG TCT-3' (R4 with HindIII)].

Overexpression of PaDnaK Protein by IPTG Induction. BL21(DE3) was transformed with the resulting plasmid (pdnaK/pAED4) and incubated with 0.03 mM IPTG (isopropyl β-D-1-thiogalactopyranoside) at 30 °C for 3 hrs. Cells

were collected and resuspended in 20 μL 5 x sample buffer [60 mM Tris-Cl (pH 6.8), 25% Glycerol, 2% SDS, 14.4 mM 2-Mercaptoethanol, 0.1% Bromophenol blue] and 80 μL of lysis buffer [50 mM Tris-HCl (pH 7.6), 100 mM NaCl, 1 mM EDTA, 1 mg/mL lysozyme, 15% (W/V) sucrose]. Protein lysates were separated on 10% SDS-PAGE followed by Coomassie Brilliant Blue (CBB) staining. In the case of BL21(DE3) transformed with padnaK/pET28a, cells were incubated with 1 mM IPTG at 30 °C for 4 hrs. Overexpressed proteins were further purified by Ni^{2+}-NTA (nitrolotriacetate) affinity chromatography.[17] Briefly, collected cell pellets were resuspended in 10 μL Binding buffer [50 mM Tris-Cl (pH 8), 250 mM NaCl, 8 mM imidazole] containing 1 mg/mL lysozyme. After 30 min incubation in ice and 10 min rocking at 4 °C, 5 μg/mL DNase and 1% Triton X-100 were added. After 10 min incubation at 4 °C, the supernatant was collected and filtered through a 0.45 μm syringe filter. The filtered samples were loaded to a Ni^{2+}-NTA column which was in advance equilibrated with 4 volumes of binding buffer. The column was then washed with10 volumes of binding buffer, 6 volumes of washing buffer [50 mM Tris-Cl (pH 8), 250 mM NaCl, 20 mM imidazole], and finally 6 volumes of elution buffer [50 mM Tris-Cl (pH 8), 250 mM NaCl, 400 mM imidazole]. The eluted fraction was buffer-changed to Buffer A [25 mM Tris-Cl (pH 7.5), 10 mM KCl, 5 mM $MgCl_2$] using Amicon Ultracel −10 K (Milipore).

Cold-Resistance by Spotting Assay. BL21 (DE3) transformed with pAED4, padnaK/pAED4 and ecdnaK/pAED4 at $OD_{600} \approx 0.25$ was incubated with 0.03 mM IPTG for 3 hrs at 30 °C. 10^8 cells in 5 mL volume were spotted onto LB^{amp} plates with serial 10-fold dilutions. Each plate was incubated at 4 °C for 0-4 days, transferred to 30 °C for 16 hrs before the sizes and densities of the surviving spots were examined. In parallel, counting assays were carried out by spreading 150 μL cells into LB^{amp} plates and examining the survival rates of cells which had experienced the same cold treatment.

Assay of Chaperone Activity. The activity of purified PaDnaK as a molecular chaperone was assayed by measuring the refolding rate of citrate synthase (CS, Sigma).[18] CS was used to combine oxaloacetate and acetyl CoA to make citrate, which in turn reacted with DTNB [5,5'-dithiobis (2-nitrobenzoic acid)] to absorb light of wavelength 412 nm. The ability of refolding of denatured CS in the presence of chaperone PaDnaK was examined as a measure of chaperone activity. 0.3 mM CS was denatured by a denaturation buffer [8.0 M Urea, 0.1 M Tris-Cl (pH 8.0), 20 mM DTT] at 25 °C for 1 hr. Denatured CS was diluted by a renaturation buffer [0.1 M Tris-Cl (pH 8.0), 10 mM $MgCl_2$, 10 mM KCl, 2 mM ATP] and after 2 hr incubation at 25 °C, the refolded CS activity was determined by measuring the absorbance at 412 nm for reaction mixtures containing 700 mL reaction buffer [0.1 M Tris-Cl (pH 8.0), 2 mM EDTA, 0.1 mM DTNB, 0.23 mM oxalacetate, 0.047 mM acetyl CoA] and 300 mL of refolding mixture.

The ATP ase Activity of DnaK. In order to examine if purified recombinant PaDnaK is functionally intact, we have measured the ATPse activity of N-terminal PaDnaK using Malachite green method.[19] In this method, Absorbance at 660 nm was an indicator for a complex of malachite green, molybdate, and free orthophosphate.[20] 20 mg DnaK was dissolved in reaction buffer [10 × reaction buffer: 500 mM HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid), 300 mM KCl, 300 mM NH_4Cl, 10 mM dithiothreitol, 50 mM $Mg(acetate)_2$] containing 0.5 mg/mL Bovine serum albumin and 10 mM ATP. The reaction mixtures were incubated for 30 min at 4 °C, 20 °C, and 37 °C. 100 mL of aliquot is added to 800 mL of malachite green-ammonium molybdate reagent (3 volumes of 0.045% malachite green hydrochloride, 1 volume of 4.2% ammonium molybdate in 4 N HCl, and 1/50 volume of 1% Triton X-100). After 1 min incubation, 100 mL of 34% citric acid was added and incubated for 1 hr at room temperature. Samples were spectroscopically measured at 660 nm.

Results and Discussions

Identification of the *dnak* Gene from KOPRI22215. Since there was no genomic information available, we designed a set of homologous primers coding for DnaK proteins known from other *Psychromonas* species. Using a gradient PCR, a fragment of between 458 and 1104 bp position was successfully amplified (Figure 1(a)). Subsequent LA PCR in vitro cloning technology gave us the *dnak* gene from KOPRI22215 (padnaK). The full length of encoding *padnaK* from genomic DNA was amplified and separated in 1.0% agarose gel boxed in lane 1 in Figure 1(b). Upon DNA sequencing, we found that the *padnaK* gene is 1917 nucleotides in length and codes for PaDnaK of 638 amino acid residues, with a predicted molecular mass of 68,846 Da (Genebank accession number: JF813789). Sequence com-

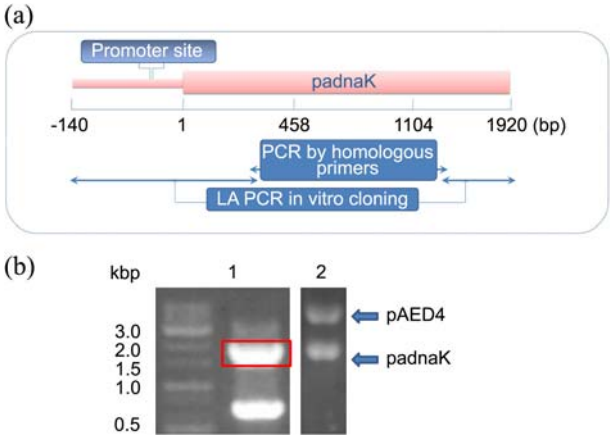

Figure 1. Identification of PaDnaK encoding gene from KOPRI22215. (a) The full length of *padnaK* gene (1917 bp) was amplified by a series of modified PCR technologies, including a gradient PCR and LA PCR in vitro cloning. (b) PCR ampilification of genomic DNA yielded 2.0 kbp fragment of full length padnaK gene boxed in lane 1. The padnaK gene was ligated in pAED4 expression vector to give padnaK/pAED4 plasmid which was double digested at Nde1 and BamH1sites. The sizes of inserts and vector were compared in 1.0% agarose gel with DNA size marker.

parison of PaDnaK using ClustalW2 (EBI) gave us 77% sequence homology with that of *E. coli* (EcDnaK) and 89% sequence homology with other Psychromonas species (PsDnaK). In order to find putative promoter sites of *padnaK* gene, we have searched the upstream region up to −140 bp by Promoter Prediction (UC-Berkeley), but fail to find any promoter in this region except the ribosome binding site (GGAGA) upstream of ATG where translation starts (data not shown). Since dnaK/dnaJ/GrpE encoding genes are often closely organized in bacterial chromosomes as demonstrated in other bacteria, more systematic primer extension analysis further downstream to elucidate locations of promoters may be needed..

Overexpression of PaDnaK in *E. coli*. In order to overexpress PaDnaK in *E. coli,* we prepared the *padnaK* insert by PCR amplification with two primers containing Nde1 and BamH1 sites at 5'-end and 3- end, respectively. PCR products were separated in agarose gel, showing the right size of DNA band at the expected 2.0 kbp (boxed in lane 1 of Figure 1(b)). The PCR amplified DNA insert was extracted from the excised gel piece and digested by Nde1 and BamH1 for cloning into pAED4 vector containing complementary cohesive ends. Upon ligation by T4 DNA ligase followed by transformation into DH5α, positive clones were selected by rapid screening or colony PCR with F2 and R2. The padanK/pAED4 plasmid was purified and double-digested with Nde1 and BamH1 to display vector and insert bands (lane 2 in Figure 1B). The padnaK/pAED4 plasmid was then transformed into BL21(DE3) for overexpression of PaDnaK under the control of T7 promoter. Induction of padnaK/pAED4 by 0.03 mM IPTG was demonstrated in 10% SDS-PAGE, showing that PaDnaK was successfully overproduced at ~70 kDa (lane 4 in Figure 2(a)). Auto-induction of paDnaK/pET28a plasmid was negligible, if any (lane 3 in Figure 2(a)).

With an aim of purifying PaDnaK in affinity chromatography, we constructed another recombinant His-tagged PaDnaK expression system in pET28a. Cloning of padnaK/pET28a was similarly carried out and overexpression of PaDnaK in the pET system by 1 mM IPTG was performed in BL21 (DE3). Expressed PaDnaK underwent Ni^{2+}-column chromatography followed by Amicon-10 concentration, giving us the pure form of PaDnaK in a good yield (lane 2 in Figure 2(b)). EcDnaK used as controls was also amplified,

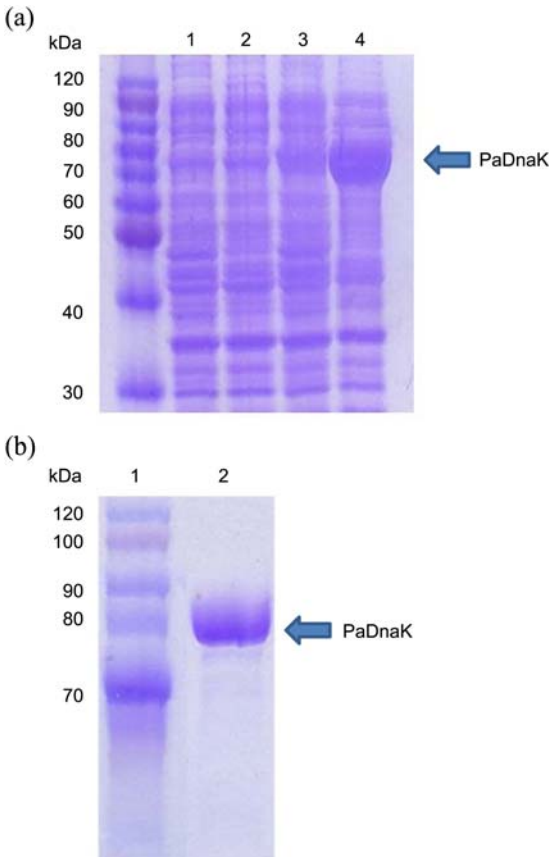

Figure 2. Overexpression of PaDnaK by IPTG induction. (a) BL21 (DE3) transformed with pAED4 vector control (lane 1 and 2) and padnaK/pEAD4 (lane 3 and 4) were cultured overnight and induced without IPTG (lane 1 and 3) or with 0.03 mM IPTG (lane 2 and 4). PaDnaK was overexpressed by 0.03 mM IPTG at 30 °C for 3 hrs. (b) Induction of BL21(DE3) transformed with padanK/pET28a was similarly carried out with 1 mM IPTG at 30 °C for 4 hrs. Affinity chromatograpy by Ni^{2+}-NTA agarose gel subsequently yielded the pure form of PaDnaK.

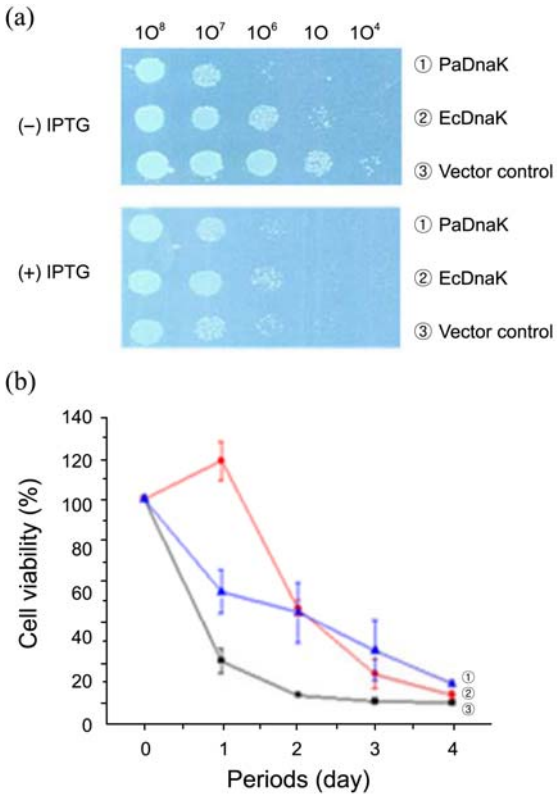

Figure 3. Counting assay for *in vivo* cold-resistance provided by PaDnaK. (a) BL21(DE3) transformed with padnaK/pAED4, ecdnak/pAED4 and pAED4 vector only were induced with or without IPTG. 10^8 cells from each treatment were serially diluted and spotted onto LB^{amp} plates. (b) Counting assays were performed by spreading aliquots of cells with dnaK genes expressed in LB^{amp} plates. The incubation time at 4 °C ranged from 0 day to 4 days. After a period of cold treatment, LB^{amp} plates were incubated at 30 °C for 16 hrs before counting the number of cells.

cloned both in pAED4 and pET28a, expressed and successfully purified in a similar way to PaDnaK (data not shown).

Counting Assay for *in vivo* Cold-Resistance Provided by PaDnaK. For the purpose of investigating if PaDnaK provides cold resistance to mesophilic *E. coli*, we carried out a counting assay for viable cells upon *in vivo* cold incubation for transgenic *E. coli* cells transformed with *padnaK* along with *ecdnaK* or empty pAED4 vector. Each transformant was cultured in the presence of 0.03 mM IPTG for 3 hrs to make sure that PaDnaK or EcDnaK was induced. The expression of each DnaK was confirmed by SDS-PAGE each time (data not shown). After 10^8 cells were spotted onto LB^{amp} plates with serial 10-fold dilutions, incubated at 4 °C for 4 days, and transferred to 30 °C for 16 hrs incubation, the densities of surviving cell spots were examined (Fig. 3(a)). Even in the absence of IPTG induction, *E. coli* cells with *dnak* genes became lethal because of auto-induction of DnaK proteins. In the presence of IPTG induction, only the negative control with cells transformed by pAED4 vector underwent severe damage upon cold treatment. It was interesting to observe that cells with *PaDnaK* exhibited no dramatic change in cell viability whereas the viability of cells with EcDnaK significantly decreased. In parallel, we counted the number of viable cells by spreading 150 mL cells in LB^{amp} plates (Fig. 3(b)). The incubation time at 4 °C ranged from 0 day to 4 days. Both PaDnaK and EcDnaK provided better viability against cold treatment than that of the vector control no matter how long cold incubation persisted. After one day of treatment the number of viable cells expressed with EcDnaK increased the cell viability. However, cells with PaDnaK expressed were severely damaged as much as the vector control. The viabilities of most cells decreased dramatically especially in the absence of any DnaK expressed. Even if short incubation with PaDnaK at cold temperature made cells unhealthy, longer incubation rescues cells from otherwise lethal damages. It has been reported that cells with overexpressed DnaK became lethal as we observed in the case of PaDnaK.[21] This is attributed to a defect in cell septation and the formation of cell filaments at a moderate level of DnaK. Co-expression of DnaJ was reported to overcome this bacteriocidal effect.[21] Our results suggest that even without copartner DnaJ protein expressed, PaDnaK played a significant role in conferring cold-resistance to mesophilic bacteria, *E. coli.*

Biochemical Characteristics of Functional PaDnaK. We performed biochemical assays to evaluate if the purified PaDnaK was functional at a cold temperature. First, the chaperone activity of PaDnaK in renaturation of previously denatured CS was examined *in vitro* (Fig. 4(a)). Renaturation by PaDnaK (145% of control) was more efficient than that of EcDnaK (130% of control) as demonstrated in its 0 day condition. In order to investigate if PaDnaK is kept stable in cold conditions, PaDnaK and EcDnaK were incubated at 4 °C for 4 days. It was demonstrated that PaDnaK still contains 120% of vector control whereas EcDnaK lost most of its chaperone activities. Secondly, we checked the temperature dependence of ATPase activity which is a characteristic for the N-terminal domain of DnaK in other systems. It was earlier reported that the ATPase activity of cold-induced SheDnaK from Psychrotropic bacterium *Shewanella* sp *AC10* had a bell shaped temperature dependence, optimal at 37 °C , whereas EcDnaK dramatically increased from 20 °C to 70 °C.[22] It was interesting to note that at 15 °C, SheDnaK displayed higher ATPase activity than EcDnaK. When we measured the ATPase activities of PaDanK and EcDnaK by Malachite green assay, we observed that PaDnaK exhibited 10% higher ATPase activity than that of EcDnaK (Fig. 4(b)) throughout the conditions from 4 °C to 37 °C. Considering that co-chaperone DnaJ may be indispensible for DnaK function in some species, *padnaJ* gene encoding PaDnaJ in KOPRI22215 should be identified and further investigated as a partner component of the *in vitro* analysis, including chaperone activity as well as the ATPase activity of PaDnaK.

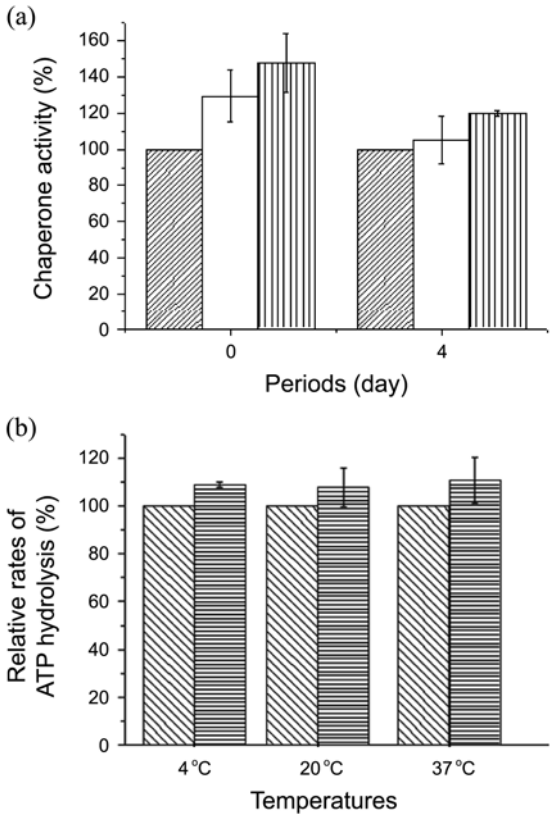

Figure 4. Biochemical characteristics of functional PaDnaK. (a) Chaperone activity of PaDnaK and EcDnaK was carried out by reactivation of previously denatured CS immediately upon purification (day 0) or after 4 days' incubation at 4 °C (day 4). The left column (▨) represents the buffer control, the middle one (□) EcDnaK, and the right one (|||) PaDnaK. (b) The ATPase activities of PaDnaK (▨) and EcDnaK (▤) were measured by Malachite green assay.

Acknowledgments. This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2010-0007956).

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