Biochemical and Biophysical Research Communications 389 (2009) 622–627

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Property of cold inducible DEAD-box RNA helicase in hyperthermophilic archaea

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ARTICLE INFO

Article history:
Received 5 September 2009
Available online 13 September 2009

Keywords:
Archaea
Hyperthermophile
DEAD-box protein
Cold adaptation
Thermococcus kodakaraensis

ABSTRACT

TK0306 (*Tk*-DeaD) of hyperthermophilic archaeon *Thermococcus kodakaraensis* is annotated as the DEAD-box helicase gene; nevertheless, its ortholog has not been identified in closely related genera, *Pyrococcus* spp., which generally grow at higher temperature than *T. kodakaraensis*, suggesting that the cold-inducible RNA helicase of *Tk*-DeaD functions under cold stress conditions. Quantitative RT-PCR revealed that *Tk-deaD* was more dominantly transcribed at 60 °C than at 85 °C and 93 °C in both logarithmic and stationary phases. Immunoblot analyses revealed that *Tk*-DeaD was detected only in logarithmic-phase cells cultivated at 60 °C but hardly detected at 85 °C and 93 °C in both phases. *Tk*-DeaD expression is, hence, post-transcriptionally regulated and appears under vigorous growth conditions at 60 °C. Recombinant *Tk*-DeaD purified to homogeneity started to unfold at 20 °C, fully unfolded at 70 °C, and exhibited maximal ATPase activity and unwinding activity specific for single-strand paired RNA at 50 °C, which is lower than the growth limit of *T. kodakaraensis*.

Introduction

Protection from stress is important for the survival of living organisms. One major stress factor is temperature change. Under heat stress conditions, many heat shock proteins, including molecular chaperons, are produced to rescue and/or to degrade denatured unfolded proteins [1,2]. Under cold stress conditions, cold shock-inducing proteins, including RNA chaperonins, DNA gyrase, and ribonuclease, are produced in bacterial cells, such as *Escherichia coli* [3,4]. In addition to these cold shock proteins, one major cold shock protein is the DEAD-box protein family, which contains the distinctive Asp-Glu-Ala-Asp (DEAD) sequence; however, not all DEAD-box proteins are cold shock proteins. DEAD-box proteins are known to belong to the group of RNA helicase, the enzyme that participates in the unwinding of folded RNA coupling with NTP hydrolysis. There are several reports on cold shock-induced DEAD-box proteins from bacteria, such as *E. coli* [5], *Anabaena* sp. (cyanobacteria) [6], and *Bacillus subtilis* [7]. The biological characteristics of cold shock-induced DEAD-box RNA helicase from *E. coli*, called CsdA, were thoroughly studied, and it has been hypothesized that CsdA plays a role in some processes, such as the biogenesis of the 50S ribosomal subunit, translation initiation, or the formation of degradosome with RNase E under cold shock conditions [8–10].

RNA or DNA helicases are classified into superfamilies (SFs) based on the homology of their amino acid sequences [11]. RNA helicases in the DEAD-box family generally belong to SF2. SF2 helicases have some highly conserved NTP -, substrate- or metal-binding motifs, such as Walker A and Walker B motifs [12,13]. In addition to these motifs, the Q motif and the aromatic amino acid residue generally found in 17 amino acids upstream from the Q motif are unique to the DEAD-box family helicase and are important for ATPase activity [14]. In archaea, many DEAD-box proteins have been annotated during genome analysis. It has been reported that the DEAD-box protein was induced by cold shock and low-temperature cell-growth conditions in the hyperthermophilic archaeon *Methanocaldococcus jannaschii* [15] and psychrophilic archaeon *Methanococcoides burtonii* [16]. These reports suggest that DEAD-box RNA helicase in archaea is involved in cell adaptation to cold stress environments. As growth temperatures fall in hyperthermophilic archaea, transcribed mRNA is assumed to form

Abbreviations: SF, superfamily; RT-PCR, reverse-transcriptional PCR; CD, circular dichroism; dsRNA, double-stranded RNA; ssRNA, single stranded RNA; dsDNA, double-stranded DNA; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis.

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doi:10.1016/j.bbrc.2009.09.038

unfavorable stem-like folded structures, resulting in a change of translation efficiency. When we compared the range of growth temperatures among (hyper) thermophilic archaea, an obvious distinction was observed in the lower-limit growth temperatures between thermophilic archaea with and without harboring DEAD-box RNA helicase, as shown in Fig. 1. In Thermococcales order, TK0306 in *Thermococcus kodakaraensis* is annotated as a DEAD-box protein (*Tk*-DeaD); nevertheless, no ortholog of TK0306 has been identified in closely related genera, *Pyrococcus* sp. strains, which generally grow at higher temperatures than *T. kodakaraensis* [17]. These differences strongly suggest that DEAD-box RNA helicase makes adaptation possible for hyperthermophilic archaea under cold stress conditions. However, it is unclear what kinds of substrates *Tk*-DeaD recognizes, such as double-stranded RNA (dsRNA) or single stranded RNA (ssRNA). Whether *Tk*-DeaD possesses thermostability, as other constitutively expressed thermostable proteins do, is also unclear. In the present study, we focused on *Tk*-DeaD of *T. kodakaraensis*, and its expression profiles were analyzed. Recombinant *Tk*-DeaD was then purified to homogeneity, and protein properties, including thermostability, were investigated.

Materials and methods

RNA isolation and reverse transcription PCR. T. kodakaraensis strain KOD1 were grown anaerobically in MA–YT medium with an addition of pyruvate (0.5%) [18]. Total RNA from *T. kodakaraensis* was isolated from cells harvested at the early log growth phase (cells grown at 85 °C or 93 °C, OD_{660} = 0.2–0.3; cells grown at 60 °C, OD_{660} = 0.1) or stationary phase (cells grown at 85 °C or 93 °C, OD_{660} = 0.8; cells grown at 60 °C, OD_{660} = 0.3) using RNeasy Midi Kit (Qiagen). Each total RNA (0.4 μg) in 10 μl of a reaction mixture was used for reverse transcription at 55 °C for 30 min with a reverse primer TK0306RT-R (5′-CGAGTATCTTAGTTTTCCTGGCC-3′) designed for TK0306 gene. Reverse transcriptase was purchased from Roch Diagnostics. cDNA samples synthesized by the reverse transcription were amplified by PCR with TK0306 gene-specific primers, TK0306RT-F (5′-CAGGTCGCTCAGGGGCAAAAGGA-3′) and TK0306RT-R. The PCR conditions were 23 cycles of 15 s at 96 °C, 30 s at 57 °C and 30 s at 68 °C. Three independent reverse-transcriptional PCR (RT-PCR) reactions were conducted to analyze the PCR products (494 bp) by 1% (w/v) agarose gel electrophoresis. As a control to ensure that the signal intensities reflected the initial levels of each transcript directly, RT-PCR reactions were performed with the same total RNAs and the 16S rRNA gene-specific primers (16SrDNA-F, 5′-ACACCCGTAGGGCGTCGGGATGT-3′; 16SrDNA-R, 5′-CGTATTCGCCGCGCGATGATGACACGCGGG-3′) under the PCR conditions described above, except for the reaction cycle (16 cycles) and annealing temperature (60 °C). The resulting 174 bp fragments were analyzed in the same way.

DNA manipulation and purification of recombinant Tk-DeaD. DNA manipulations were carried out by the previously described method [18]. The gene was amplified with *T. kodakaraensis* genomic DNA as a template and two oligonucleotide primers TK0306EX-F (5′-TGACATATGAGTTTTATATAACTTGG-3′, underlined sequences correspond to NdeI site) and TK0306EX-R (5′-AGGATCCTCAA TATCTCCTCCTTC-3′, underlined sequences correspond to BamHI site). DNA fragment obtained by NdeI and BamHI digestion was inserted into pET21a(+) (Novagen) at the corresponding sites, and the resulting plasmid was used to transform *E. coli* BL21-CodonPlus(DE3)-RIL (Stratagene). The recombinant *E. coli* cells were grown in Luria–Bertani (LB) medium [10 g/l of tryptone,10 g/l of yeast extract and 5 g/l of NaCl (pH 7.0)] containing ampicillin (50 μg/ml) and chloramphenicol (25 μg/ml) at 37 °C, and the expression of *Tk*-DeaD was induced by the addition of 0.1 mM isopropyl-β-D-thiogalactopyranoside. After a further incubation for 4 h at 37 °C, the cells were harvested by centrifugation and resuspended in 50 mM Tris–HCl buffer (pH 7.6) containing 1 mM EDTA, 10% glycerol and 200 mM ammonium sulfate. The cell suspension was then sonicated and centrifuged (10,000 rpm, 15 min). The soluble cell extract was applied onto a HiTrap SP column (5 ml) (GE Healthcare). *Tk*-DeaD was eluted with a linear gradient of NaCl (500 to 800 mM) in Tris–HCl buffer (pH 7.6) and the eluted sample was further fractionated with phosphocellulose column (Whatman). *Tk*-DeaD was finally dialyzed against 50 mM Tris–HCl buffer (pH 7.6) containing 200 mM NaCl. Protein concentration was determined with Protein Assay Kit (Bio-Rad) according to the instructions from the manufacturer using bovine serum albumin as a standard.

Immunoblot experiment of Tk-DeaD. The polyclonal antisera against *Tk*-DeaD were obtained from Female New Zealand White rabbit (Japan SLC) and designed as anti-*Tk*-DeaD. Total cytoplasmic protein extracts of *T. kodakaraensis* cells were prepared by sonication of *T. kodakaraensis* cells cultivated at 60 °C, 85 °C and 93 °C,

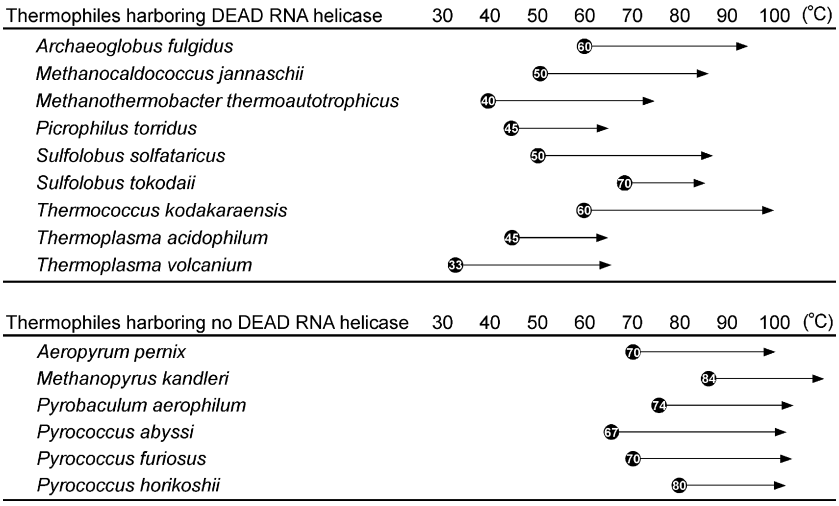

Fig. 1. Comparison of growth ranges of various thermophilic archaea. Archaea presented in this figure are as follows: *Aeropyrum pernix* [25], *Archaeoglobus fulgidus* [19], *Methanocaldococcus jannaschii* [19], *Methanopyrus kandleri* [19], *Methanothermobacter thermautotrophicus* [26], *Picrophilus torridus* [27], *Pyrobaculum aerophilum* [19], *Pyrococcus abyssi* [19], *Pyrococcus furiosus* [20], *Pyrococcus horikoshii* [19], *Sulfolobus solfataricus* [19], *Sulfolobus tokodaii* [28], *Thermococcus kodakaraensis* [29], *Thermoplasma acidophilum* [19], and *Thermoplasma volcanium* [19]. Circled numbers indicate the lowest growth temperatures and arrows indicate the growth ranges.

followed by centrifugation (10,000 rpm, 10 min). The cytoplasmic extracts (20 μg each) were, respectively, subjected to electrophoresis in 0.1% SDS-12% polyacrylamide gels (SDS–PAGE) and electroblotted onto a polyvinylidene difluoride membrane (Bio-Rad). Membranes were blocked using 5% skimmed milk and incubated with the rabbit anti-*Tk*-DeaD serum at 1:10,000 dilutions, respectively. The analysis of expression level was performed using alkaline phosphatase-conjugated goat anti-rabbit IgG (Cappel) and a BCIP/NBT stock solution (Roche Diagnostics) according to the manufacturer's instructions.

Primer extension analysis. The non-RI primer extension analysis was carried out according to the reported procedure [18]. IR Dye-labeled primer (5′-CTGAAGCACTGGATAGTCC-3′) was mixed with RNA (60 μg) and reverse transcriptase reaction was carried out with the Transcriptor Reverse Transcriptase (Roche) for 30 min at 50 °C. The reverse transcripts were resuspended in loading buffer containing 95% formamide, 20 mM EDTA and 0.05% bromophenol blue. After heat treatment at 95 °C for 5 min, samples were loaded on 6% polyacrylamide gel containing 7 M urea.

ATPase assay of Tk-DeaD. ATPase activity of *Tk*-DeaD was determined by measuring the volume of free phosphate released by ATP hydrolysis. Nucleic acid dependency of ATPase activity was determined by using dsRNA, ssRNA, or single stranded DNA (ssDNA). 5′- and 3′-overhanged dsRNA was prepared by annealing 63mer RNA (ssRNA63, 5′-UGGGCUGCAGGUCGACUCUAGAGGAUCCCCGGGCG AGCUCGAAGUCGGGUCUCCCUAUAGUGA-3′) and 33mer complementary RNA (ssRNA33, 5′-CCGACUUCGAGCUCGC CCGGGGAUCCU CUAGAG-3′). ssRNA63 and ssDNA63 (5′-TGGGCTGCAGGTCGACTCT AGAGGATCCCCGGGCGAGCTCGAAGTCGGGTCTCCCTATAGTGA-3′) were used as ssRNA and ssDNA substrates, respectively. The reaction mixture (10 μl) composed of 5 nM oligonucleotide, purified *Tk*-DeaD (0.2 μM), 1 mM ATP, 2 mM $MgCl_2$, 2 mM DTT, and 4 unit of Ribonuclease inhibitor from human placenta (Takara bio) in 50 mM HEPES buffer (pH 7.6) was incubated at 30–70 °C for 30 min. After incubation, the reaction mixture was kept on ice to stop the enzymatic reaction. The concentration of free phosphate in the reaction mixture was measured by BIOMOL GREEN™ (BIOMOL).

Helicase assay of Tk-DeaD. To prepare ^{32}P-labeled oligonucleotide, 50 pmol of ssRNA63 was phosphorylated and radiolabelled by incubation with [γ^{-32}P] ATP (6000 Ci/mmol) (ParkinElmer) by T4 polynucleotide kinase (Takara bio) at 37 °C for 30 min. The reaction product was purified by centrifugation through MicroSpin G-25 columns (GE-healthcare). To prepare single-strand paired (stem-looped) RNA, ssRNA63 was heated and cooled as well. Unwinding assays were performed at 50 °C for 30 min in a total volume of 10 μL of 50 mM HEPES buffer (pH7.6) containing 2 mM $MgCl_2$, 2 mM DTT, 1 mM ATP, labeled RNA, 4 unit of Ribonuclease inhibitor and 0.2 μM enzyme. After incubation, reaction mixture was immediately kept on ice. The reaction products were resolved on 7% acrylamide-gels (native polyacryl amide gel) at 250 V for 50 min and the radioactivity of folded or unwound was analyzed using BAS-2500 and BAS-IP SR 2040 (FUJIFILM) or exposure to X-ray films.

Circular dichroism (CD) spectroscopic analysis. CD spectroscopic experiments were performed on a J-820 CD spectropolarimeter (JASCO) at 20 °C. Far-UV CD spectra in the 200–260 nm region were measured using a 1.0 cm pathlength-quartz cuvette at 0.05 nm steps over the wavelength range designated with a scan speed of 50 nm/min. The experiments were performed with 2 μM protein in distilled water (pH 7.6) containing 300 mM NaCl. Thermal denaturation curve of the purified enzyme was determined by monitoring the change in the CD value at 222 nm using solution containing protein at 2 μM in 300 mM NaCl (pH 7.6) in the cuvette as the temperature increased at a rate of 0.5 °C/min from 20 °C to 96 °C. All spectra were corrected for buffer contributions to the signals.

Results and discussion

Comparison of the growth temperatures of thermophilic archaea with and without DEAD-box RNA helicases

Many archaea are known to have DEAD-box RNA helicase homologs in the genome. We listed archaeal strains whose growth temperature ranges had been precisely examined and classified into two groups based on the presence or absence of a DEAD-box RNA helicase ortholog, as shown in Fig. 1. It has become clear that archaea harboring DEAD-box RNA helicase generally possess lower growth limit temperatures than those that do not harbor DEAD-box RNA helicase, with the exception of *Sulfolobus tokodaii*. *S. tokodaii* carries the DEAD-box RNA helicase ortholog called ST2459. However, we speculate that ST2459 is not functionally expressed in *S. tokodaii* for the following reasons. All conserved motifs are observed in the primary structure in ST2459; however, a critical aromatic residue (phenylalanine), which is present in about 17 amino acid residues upstream from the motif Q [14], is not present in the ST2459. In addition, the initial methionine was not identified in a suitable position. An in-frame initiation codon appears in an unfavorable position that corresponds to 12 amino acids upstream from motif Q, resulting in a more imperfect amino terminal-deleted version than that of other DEAD-box RNA helicases. Hence, we speculated that mutation occurred and the previously presented initiation codon for ST2459 could be lost, resulting in the elimination of helicase activity. In fact, *S. tokodaii*, unlike other *Sulfolobus* sp. strains, does not grow at such low temperatures. In the euryarchaeal order Thermococcales, the minimum growth temperature of *T. kodakaraensis* (60 °C) is lower than those of *Pyrococcus abyssi* (67 °C) [19], *Pyrococcus furiosus* (70 °C) [20] and *Pyrococcus horikoshii* (80 °C) [19]. The gene TK0306, which presumably encodes the DEAD-box RNA helicase, is found in the *T. kodakaraensis* genome and not in *Pyrococcus* spp. TK0306 encodes a protein of 406 amino acids (46 kDa) and shares high amino acid sequence similarity with a previously characterized bacterial RNA helicase from *E. coli* (CsdA, 43% identical), [5]. Therefore, TK0306 was designated as *Tk-deaD*.

Expression profiles of Tk-deaD

As reported above, *Tk*-DeaD is expected to contribute to the lower temperature adaptation of hyperthermophilic archaea. To obtain information concerning the temperature dependency of *Tk-deaD* transcription, RT-PCR analysis was carried out using the total RNA from *T. kodakaraensis* cells at 93 °C, 85 °C, or 60 °C, as described in Materials and methods. The mRNAs were extracted from both the logarithmic and stationary phases. As shown in Fig. 2A, signals corresponding to *Tk-deaD* transcripts were more dominantly detected in cells grown at 60 °C than at 85 °C and 93 °C (Fig. 2A). These results indicate that *Tk-deaD* is transcribed in larger amounts at lower temperatures. As shown in Fig. 2C, the transcriptional start site was identified at 158 nucleotides upstream of the initiation codon of *Tk-deaD*, indicating that a length of 5′-untranslated region (5′-UTR) is 158 nucleotides. In addition, to conduct research at the protein level, polyclonal antisera against *Tk*-DeaD were prepared, and immunoblot experiments were carried out. In the logarithmic phase, *Tk*-DeaD was detected only in the cells cultivated at 60 °C and hardly detected in those at 85 °C and 93 °C (Fig. 2B). In the stationary phases, *Tk*-DeaD expression was not detected at any temperature. These results showed that *Tk*-DeaD appears in *T. kodakaraensis* cell in the logarithmic phase but disappeared in stationary phase at 60 °C. *Tk*-DeaD is considered to function under conditions that promote vigorous growth at lower temperature. Stage-specific expression at 60 °C occurs at the post-transcriptional level. It is unclear how *Tk*-DeaD expression

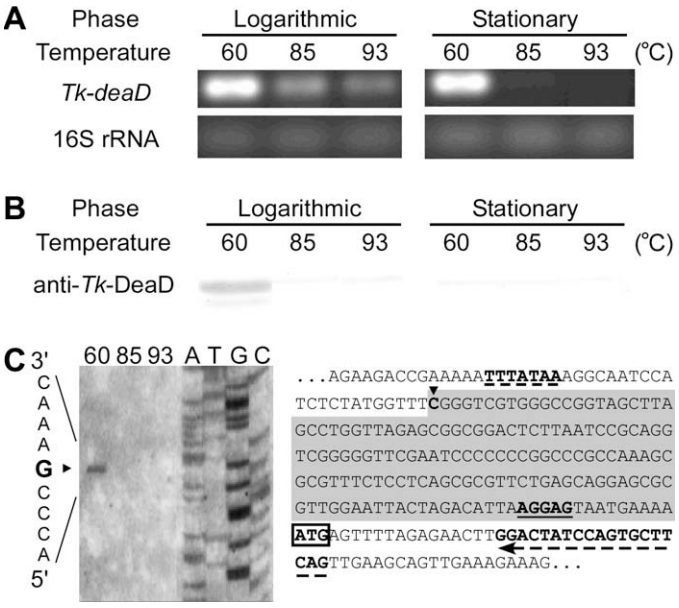

Fig. 2. Expression profiles of *Tk-deaD* at various growth temperatures. (A) Comparison of mRNA levels. RT-PCR reactions were performed with total RNAs from cells in logarithmic and stationary phases cultivated at 60 °C, 85 °C, and 93 °C. The levels of the *Tk-deaD* transcripts were evaluated as the signal intensities of fragments amplified with the respective gene-specific primers. As a control, the levels of 16S rRNA were examined. (B) Comparison of protein levels. Immunoblot analysis with anti-*Tk*-DeaD was performed using cytoplasmic extracts obtained from cells by the methods described in Materials and methods. (C) Mapping of the transcription start site for *Tk-deaD.* Primer extension reactions were performed with total RNAs isolated from *T. kodakaraensis* cultivated at 60 °C, 85 °C, and 93 °C. The extended reverse transcripts were analyzed with sequencing ladders generated using the same primer and template (lanes A, T, G and C). The transcription start site is represented by a triangle. The 5′-UTR is shaded. The TATA element is broken underlined. The initiation codon is boxed. 16S rRNA binding region is underlined. The primer binding region is indicated by a broken arrow.

is controlled. The 5′-UTR might be involved in the cold inducibility of *Tk*-DeaD. Transcripts of some cold shock genes, such as *cspA, cspB, cspG, cspI*, and *csdA* from *E. coli* and *dead* from *M. burtonii,* possess a long 5′-UTR [3,16]. CspA, which is a transcriptional and translational enhancer, is one of a well-studied cold shock protein. The expression of CspA is mainly regulated at the post-transcriptional level, but its promoter is not involved in cold induction. The 5′-UTR of *cspA* is known to form a single-strand paired form called a stem-loop structure. Under cold shock, the transcript with the long 5′-UTR forms another stem-loop structure and is stabilized, resulting in enhancing translation [21]. The 5′-UTR also functions as a target for RNase, and the deletion of 5′-UTR causes instability to transcripts [4]. The translations of these cold shock genes in a cooler environment are also supported by other Csps (CspA to CspI) [10]. However, no obvious Csp homolog is found on the *T. kodakaraensis* genome. The mechanism for cold adaptation in *T. kodakaraensis* seems different from that for bacteria. A typical stem-loop structure was not found in 5′-UTR of *Tk-DeaD.* CpkA was also reported as a unique cold-inducible chaperonin in *T. kodakaraensis*, and its expression is regulated at the transcriptional level [18]. When we compared promoter regions, including 5′-UTR of *Tk-deaD* with *cpkA*, no significant conserved sequence was observed, except for the TATA box and BRE regions. These two genes are, hence, regulated in different manners.

Effect of temperature on the Tk-DeaD structure

Tk-DeaD was expressed in *E. coli* cells, and the recombinant form was purified to homogeneity, as shown in Fig. 3A. The thermostability of *Tk*-DeaD was evaluated by CD spectrum analysis. The far-UV CD spectra of the purified *Tk*-DeaD are shown in Fig. 3B. The spectrum at 20 °C showed minimal values at 222 nm, which is the typical pattern of an α-helical protein. However, the minimal value at 208 nm, as another typical pattern of the α-helical structure, was not detected due to unexpected noise. The thermal denaturation curve was obtained by monitoring the change in the CD value in a 1 cm cuvette (4 mL size) at 222 nm in the range of 20 °C to 96 °C. As shown in Fig. 3C, the CD value at 222 nm was gradually increased, responding to the temperature increase. The *Tk*-DeaD did not show typical thermotolerance, unlike the thermostable proteins so far reported. An insoluble aggregation form was observed at above 80 °C.

ATPase activity

Helicase unwinds folded DNA and/or RNA, generally coupling with NTP hydrolysis. To examine the enzymatic property of *Tk*-DeaD, ATPase activity was examined by monitoring the released phosphate from ATP. First, nucleic acid dependency was investigated on the ATPase activity. The activity was obtained only by the addition of RNA to the reaction mixture, and the highest activity was observed in the presence of ssRNA (Fig. 4A). On the other hand, a low level of activity was obtained in the presence of DNA. These results indicated that the ATPase activity of *Tk*-DeaD depends on the presence of RNA. The highest activity was shown when the reaction was carried out at 50 °C (Fig. 4B). The optional temperature of *Tk*-DeaD seemed lower than those of other enzymes from *T. kodakaraensis* [22–24].

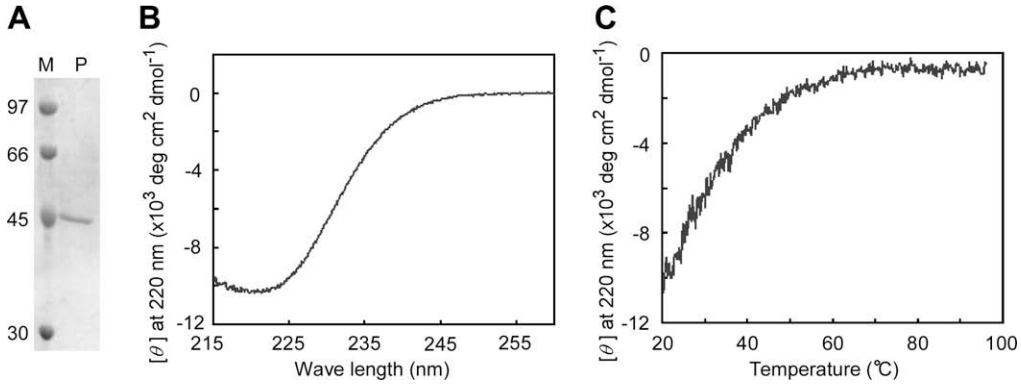

Fig. 3. Thermostability evaluation of *Tk*-DeaD. (A) SDS–PAGE of purified recombinant protein. Lane M, molecular mass marker; lane P, *Tk*-DeaD. (B) Far-UV CD spectra at 20 °C. (C) Thermal denaturation curve of the protein. The change of the CD value was monitored by the methods described in Materials and methods.

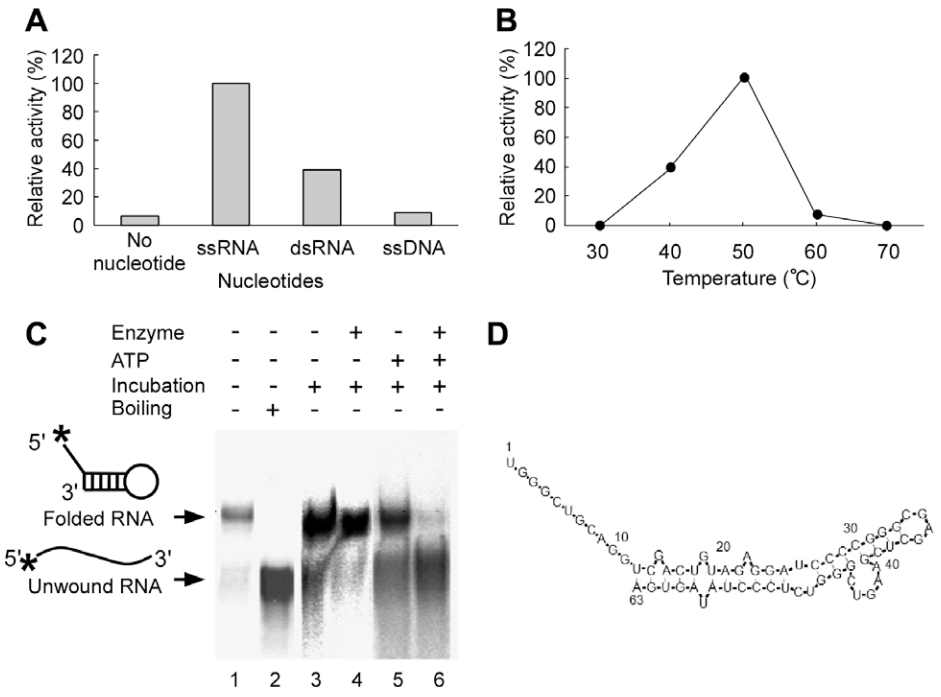

Fig. 4. Enzyme characteristics of *Tk*-DeaD. (A) Nucleic acid dependency for ATPase activities. ATP was incubated at 50 °C with *Tk*-DeaD in the presence of ssRNA, dsRNA, or ssDNA. Released free phosphates were measured, and relative values were indicated. (B) Effect of temperature on the ATP hydrolysis. ATP was incubated at indicated temperatures with *Tk*-DeaD in the presence of ssRNA. (C) Unwinding activity of recombinant *Tk*-DeaD. Unwinding reactions were performed as described in Materials and methods. "Incubation" indicates treatment at 50 °C for 30 min. "Boiling" indicates treatment at 100 °C for 15 min that causes oligonucleotides to unwind. (D) The predicted stem-loop structure of ssRNA63. The free-energy change ($-\Delta G$) of the structure was calculated to be 23.7 kcal/mol.

Helicase activity of Tk-DeaD

To confirm the helicase activity of *Tk*-DeaD, an unwinding assay was performed. When the ssRNA (ssRNA63) was loaded onto an acrylamide gel, two signals were detected (Fig. 4C, lane 1). The boiled RNA appeared at the position of the lower band (Fig. 4C, lane 2), indicating that the upper and lower bands are structured folded form (stem-looped RNA) and unwound form, respectively. An unwinding assay was performed at 50 °C with the folded substrate. As a result, the band shift was observed when ssRNA was used as the substrate (Fig. 4C, lane 6), showing that *Tk*-DeaD is an RNA helicase and unwinds the folded ssRNA structure. The 5′-overhanged dsRNA, 3′-overhanged dsRNA, 5′- and 3′-overhanged RNA were also prepared and used as nucleic acid substrates. However, *Tk*-DeaD did not unwind the dsRNAs (data not shown). *Tk*-DeaD appears to contribute to the unfolding of an unfavorable stem-loop like structure of RNA probably formed at the cold stressed conditions.

Conclusion

Tk-DeaD is identified as a DEAD-box RNA helicase in *T. kodakaraensis*, and its ortholog is not identified in closely related genera, *Pyrococcus* spp., which grow at higher temperature than *T. kodakaraensis*. *Tk*-DeaD is expressed under cold stress temperature of 60 °C but not at 85 °C and 93 °C in *T. kodakaraensis*. *In vitro* enzymatic analysis revealed that *Tk*-DeaD possesses thermolabile properties, unlike other thermostable enzymes from *T. kodakaraensis*. It exhibited unwinding activity for single-strand stem-looped RNA in an ATP-dependent manner. *Tk*-DeaD is considered to be a cold-inducible RNA helicase that functions under cold stress conditions.

Acknowledgments

This study was supported by Grants of Japan Society for the Promotion of Science (JSPS) KAKENHI (20580090) and a Grant-in-Aid for Creative Scientific Research (Project No. 18GS0421).

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