THE DEAD-Box PROTEIN Csha IN STAPHYLOCOCCUS AUREUS CONTAINS ATP-INDEPENDENT DNA STRAND ANNEALING AND EXCHANGE ACTIVITIES

HANH THI DIEU NGUYEN, TAN-VIET PHAM, NGOC-AN NGUYEN, GIA-BUU TRAN
Institute of Biotechnology and Food technology, Industrial University of Ho Chi Minh City.

Abstract. DEAD-box proteins (DBPs) that are usually RNA helicases have important roles in eukaryotic and bacterial RNA metabolism. Recent studies have reported that certain prokaryotic DBPs exhibit ATP-independent nucleic acid displacement and annealing activities. We investigated one putative RNA helicase, CshA DEAD-box protein, from vancomycin-resistant Staphylococcus aureus strain Mu 50 for ATP-independent activities on nucleic acids. We herein report that CshA has two novel ATP-independent activities - annealing of complementary single-stranded DNA (ssDNA) and strand exchange on short double-stranded DNA (dsDNA). These DNA strand annealing and exchange activities are independent of Mg2+ ion or ATP binding and hydrolysis. ssDNA annealing activity as well as versatile DNA strand exchange activity of CshA suggests a possible role in dsDNA break repair processes.

Key words. DEAD-box protein, *Staphylococcus aureus*, RNA helicase, CshA, DNA strand exchange activity, DNA strand annealing activity

1. INTRODUCTION

DEAD-box proteins are an important class of proteins that are widely distributed in both prokaryotes and eukaryotes. These proteins are characterized as putative RNA helicases involved in nearly all RNA metabolic processes, including transcription, splicing, RNA transport, ribosome biogenesis, translation, RNA decay and even viral infections [1-5]. DEAD-box proteins contain nine conserved amino acid motifs that are essential for RNA binding, RNA-dependent ATP hydrolysis, and ATP-dependent RNA unwinding. Because of their important roles in RNA metabolisms, the functions of some DEAD-box proteins in cellular processes have been investigated during the last two decades.

In addition to playing important roles in RNA processing, some DEAD-box proteins also act on DNA substrates. A member of DEAD-box protein family in yeast, Dbp9p, which is required for ribosomal RNA biogenesis, shows DNA unwinding activity [6]. DHH1, another DEAD-box protein from yeast, plays a role in recovery from G1/S cell cycle arrest after DNA damage [7, 8]. A member of DEAH-box protein akin to DEAD-box protein MPH1 from yeast is involved in an error-free DNA damage bypass pathway that requires components from a homologous recombination system [9, 10]. Additionally, DEAD-box protein DDX1, found primarily in the nucleus, is recruited to sites of double-stranded DNA (dsDNA) breaks and interact with RIF1 in early DNA damage response [11, 12].

To date, detailed characterization of DEAD-box proteins has been limited to RNA helicase function in eukaryotes and *E. coli*. To provide further understanding of putative roles of the DEAD-box proteins with nucleic acids, we studied a DEAD-box protein from *Staphylococcus aureus* strain Mu50. *Staphylococcus aureus* is a prominent infectious bacterium that causes hospital-acquired and post-surgical wound infections. Isolated in 1997, Mu50 was one of the first methicillin-resistant *S. aureus* strains reported to have reduced susceptibility to vancomycin [13, 14]. Basic Local Alignment Search Tool (BLAST) protein searches of the *S. aureus* Mu50 genome database have identified two open reading frames (one with 506 and the other with 448 amino acids) that encode putative DEAD-box proteins predicted to be ATP-dependent RNA helicases and its crystal complex structures with AMP has been reported [15-17]. One of the DEAD-box proteins from *S. aureus* with 506 amino acids, identified as CshA, has been known to be involved in biofilm formation [18] and cold adaptation[19]. Recently, CshA has been identified as a potential RNA helicase component of RNA degradosome in bacteria and more recently, CshA has been reported to have a contrary role which protects a small number of mRNAs and 22 small RNAs from degradation by MazFsa endoribonuclease [20-23]. However, molecular functions in addition to those as an RNA helicase remain unknown.

In this study, we characterized the activities of a DEAD-box protein CshA from *S. aureus* Mu 50 on deoxyribonucleic acid substrates. We observed that the protein stimulated DNA annealing and strand exchange activities, which are independent of ATP hydrolysis. These biochemical properties of CshA on various DNA substrates indicate that the protein is likely involved in the process of homologous recombination of duplex DNA.

2. MATERIALS AND METHODS

2.1. Cloning, expression, and purification of C-His6-CshA

The gene encoding the full-length (506 amino acids) DEAD-box helicase (NCBI accession number NP_372605.1) was amplified from genomic DNA of *S. aureus* Mu50 strain with polymerase chain reaction using specific primers. The forward primer contained an NdeI restriction site and had the sequence 5′-CCCCATATGCAAAATTTAAAGAACTAGGG-3′, whereas the reverse primer contained an XhoI site and had the sequence 5′-CCCCTCGAGTTTTTGATGGTCAGCAAATG-3′. The PCR product was then sub-cloned between the NdeI and XhoI sites of a pET-22b vector (Novagen, Darmstadt, Germany). This construct contains an additional hexa-histidine tag (LEHHHHHH) at the C-terminus for purification purposes. The recombinant plasmid was transformed into *E. coli* Rosetta (DE3) strain (Novagen), and the cells were grown in a shaking incubator at 37°C in Luria broth medium supplemented with 50 μg/mL ampicillin. Protein expression was induced by adding 0.5 mM isopropyl-D-1-thiogalactopyranoside when the cells reached an optical density at 600 nm of approximately 0.6. The culture was further incubated at 16°C for 4 h. The cultured cells were harvested, and the recombinant CshA was purified to homogeneity with Ni^{2+}-chelating affinity chromatography and subsequent size-exclusion chromatography, as described previously for a purification of N-terminal domain of DEAD-box RNA helicase from *S. aureus* strain Mu50 [24].

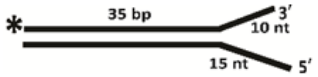
2.2. Nucleic acid substrates

The DNA oligonucleotides listed in Table 1 were chemically synthesized and purchased (Cosmo Genetech, Seoul, Korea). The oligonucleotides with an asterisk (see Table 1) were 5′-^{32}P-labeled with T4 polynucleotide kinase (10 U, Takara, Tokyo, Japan) and 1 μL of [γ-^{32}P] ATP (3,000 Ci/mmol, GE Healthcare, Piscataway, NJ, USA) in 20 μL of reaction buffer containing 50 mM Tris-HCl, pH 8.0, 10 mM $MgCl_2$, and 5 mM dithiothreitol (DTT) at 37°C for 1 h. The labeled oligonucleotides were subsequently purified via phenol/chloroform extraction and subsequent ethanol precipitation. DNA duplexes (Table 2) were prepared by annealing two DNA oligonucleotides in which the mixture of complementary oligonucleotides was heated at 95°C for 5 min and cooled slowly at room temperature for 30 min.

Table 1. Oligonucleotides used in this study

Oligonucleotide number (length, nts)	**Sequence (5′→3′)**
1* (35)	TTGACTTCATGACCTATAGTGAGTCGTATTAGTCC
2 (35)	GGACTAATACGACTCACTATAGGTCATGAAGTCAA
3* (45)	TTGACTTCATGACCTATAGTGAGTCGTATTAGTCCTTTTTTTTTT
4 (50)	TTTTTTTTTTTTTTGGACTAATACGACTCACTATAGGTCATGAAGTCAA

Table 2: Structure of DNA substrates

Name of duplex DNA	Oligonucleotides used	Structure *(*) 5'-[^{32}P] end labeling*
5'-tail 35D*/50D	**1* + 4**	
3'-tail 45D*/35D	**3* + 2**	
Forked 45D*/50D	**3* + 4**	

(*) Asterisks indicate the positions of radiolabels

2.3. DNA filter-binding assay

5′-^{32}P-labeled DNA substrates (ssDNA-45mer, 3′-tail 45D/35D) were prepared and assayed for protein binding using an alkali-treated nitrocellulose and diethylaminoethanol double-filter method [25]. Protein (recombinant CshA or bovine serum albumin (BSA) at a final concentration of 0.15 mg/mL) was added to radioactive DNA substrates (10 nM) in reaction buffer (20 μL) containing 50 mM Tris-HCl, pH 7.5, 25 mM NaCl, 2 mM DTT, and 4 mM $MgCl_2$ with or without 4 mM ATP or adenosine 5′-(β,γ-imido) triphosphate (Sigma-Aldrich, St. Louis, MO, USA). Ethylenediaminetetraacetic acid (EDTA; 10 mM) was supplemented to reactions that required no Mg^{2+} condition to scavenge contaminating Mg^{2+}. The reactions were incubated at 4°C for 30 min and then filtered under vacuum onto double filters composed of positively charged Hybond N^+ membrane (GE Healthcare, Buckinghamshire, UK) beneath nitrocellulose membranes (GE Healthcare) in a 96-well dot-blot apparatus (Bio-Rad, Hercules, CA, USA). The filter membranes were washed twice with the reaction buffer at room temperature. Filters were dried and assayed for radioactivity remaining in protein-bound DNAs and free DNAs retained on the nitrocellulose membrane and the hybond N^+ membrane, respectively. Radioactivity was quantified on a Cyclone PhosphorImager (Packard Instrument Co., Meriden, CT, USA), and the fraction of protein-bound DNAs was determined using the amount of DNA remaining on the nitrocellulose membrane relative to the total amount of DNA present in both membranes.

2.4. Duplex DNA unwinding assay

Duplex DNA unwinding reaction mixtures contained 50 mM Tris-HCl, pH 7.5, 25 mM NaCl, 2 mM DTT, 0.15 mg/mL BSA, 4 mM $MgCl_2$, 10 nM ^{32}P-labeled forked dsDNA (45D/50D) substrate, various concentrations of CshA (0, 0.01, 0.05, 0.1, 0.5, 1.0 μM), and 200 nM trap DNA that was an unlabeled complementary single stranded DNA (ssDNA; No. 3 in Table 1) in the presence or absence of 4 mM ATP. The duplex DNA unwinding reaction was incubated at room temperature for 30 min and stopped with a quenching buffer (100 mM EDTA, pH 8.0, 0.4% sodium dodecyl sulfate, 20% glycerol, 0.1% bromophenol blue, 0.1% xylene cyanol). The quenched reaction mixtures were applied to 15% nondenaturing polyacrylamide gel, and the reaction products were analyzed using a PhosphorImager (Packard Instrument). A control reaction was carried out similarly without the protein, and the reaction mixture was heated at 90°C for 10 min ("Boiled" in Fig. 2A).

2.5. DNA strand exchange assay

DNA strand exchange assay was performed under various conditions with or without cofactors (Mg^{2+}, ATP, or both). Labeled fork 45D*/50D dsDNA (10 nM) was incubated in a buffer containing 50 mM Tris-HCl, pH 7.5, 25 mM NaCl, 2 mM DTT, and 0.15 mg/mL BSA with or without CshA (1 μM). We used 35D ssDNA (50 nM) as an unlabeled complementary oligonucleotide to the labeled 45D strand of the fork substrate in the strand exchange reaction. Cofactors (4 mM Mg^{2+} or 4 mM ATP) were supplemented to the

reaction mixture depending on the conditions, as indicated in the figure legend. The reaction mixtures were incubated at room temperature for 5, 15, 30, and 60 min and stopped with the quenching buffer used in the DNA unwinding reaction. The reaction products were separated via loading onto 15% non-denaturing polyacrylamide gel and visualized using the PhosphorImager (Packard Instrument Co., Meriden, CT, USA).

2.6. DNA strand annealing assay

Standard DNA strand annealing was performed at 25°C for the times indicated in the figure legend. The reaction mixture contained 50 mM Tris-HCl, pH 7.5, 25 mM NaCl, 2 mM DTT, 0.15 mg/mL BSA, 0.1 nM of ^{32}P-labeled ssDNA (indicated in the figure), and 0.25 nM of unlabeled complementary ssDNA with or without 0.1 μM CshA. The reactions were stopped with the quenching buffer. Reaction mixtures were immediately loaded onto 15% nondenaturing polyacrylamide gel running at 80 V, and the annealed products were detected using the PhosphorImager (Packard Instrument Co.).

3. RESULTS

3.1. S. aureus CshA binds to duplex DNA with overhangs

DEAD-box protein CshA from *S. aureus* is predicted to be an ATP-dependent RNA helicase. However, we detected no RNA helicase activity of any means with several types of duplex RNA substrates (data not shown). Thus, we attempted to test whether the protein binds to DNA instead of RNA. DNA-binding activities of CshA were examined using a double-filter binding assay, in which an upper nitrocellulose membrane retains protein-bound nucleic acids. Based on the quantification of the density of ^{32}P-labeled DNA present on the nitrocellulose membrane (see Fig. 1A), CshA has DNA-binding affinity; in the presence of Mg^{2+}, CshA binds to ssDNA as well as dsDNA substrates. However, DNA-binding affinity of CshA is higher in the case of dsDNA substrate than in the case of ssDNA substrate. The effect of ATP hydrolysis energy on DNA binding was examined under several conditions. The protein showed significant DNA binding activity in the presence of Mg^{2+}, which is not affected by the presence or absence of ATP (see Fig. 1A). The presence of nonhydrolyzable ATP analog adenosine 5′-(β,γ-imido) triphosphate (AMP-PNP) did not significantly affect DNA binding affinity. The result of quantitative analysis shows the percentage of protein-bound DNA in the condition with Mg^{2+} is about two-fold higher than the percentage of protein-bound DNA in the condition without Mg^{2+} (Fig. 1B). Thus, DNA binding by CshA is mainly dependent on Mg^{2+}, which requires neither nucleotide binding nor hydrolysis energy.

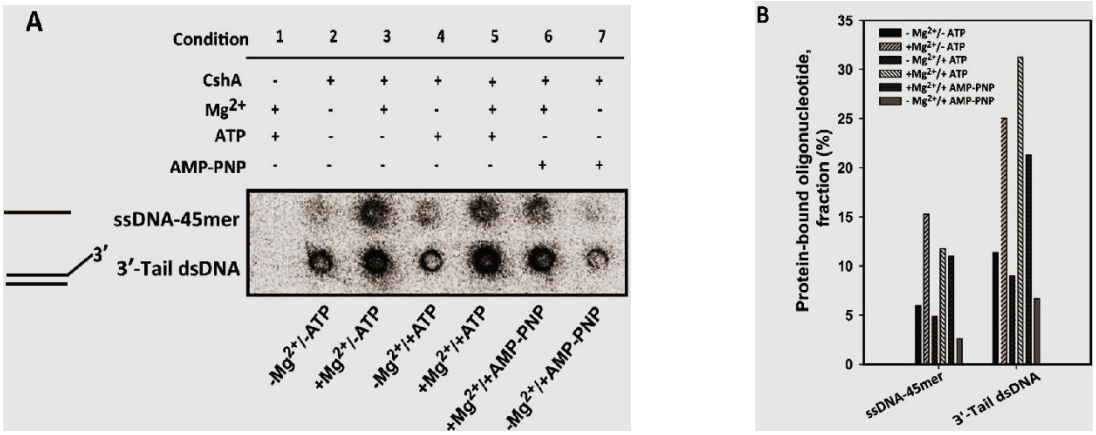


Figure 1. DNA binding by the recombinant CshA from S. aureus. (A) DNA binding activity of CshA with various DNA substrates under various conditions with or without 4 mM Mg2+, or 4 mM ATP/adenosine 5′-(β,γ-imido) triphosphate (AMP-PNP), or both. 32P-labeled DNA substrate (10 nM) was incubated with CshA (2 μM) at 4°C for 30 min, and the reaction mixtures were applied to the filter apparatus as described in Materials and Methods. (A) Shown is the nitrocellulose membrane (left panel) retaining 32P-labeled DNA substrates bound to protein in the filter binding assay, and (B) quantitative analysis shows the percentage of protein-bound DNA (right panel).

3.2. DNA strand exchange is promoted by S. aureus CshA

Because *S. aureus* CshA has a strong DNA-binding affinity to forked dsDNA, we examined the duplex DNA unwinding activity of CshA on forked dsDNA in the presence of an excess amount of trap DNA (i.e., unlabeled strand). Interestingly, the expected ssDNA product was released in both the presence and the absence of ATP (Fig. 2A). However, in the absence of the trap DNA, CshA showed no release of ssDNA under reaction conditions with ATP (see Fig. 2B). Given these results, we hypothesized that CshA has DNA strand exchange activity rather than duplex DNA unwinding activity. The ssDNA strand exchange activity replaces one strand in a duplex DNA with another complementary ssDNA to release the ssDNA strand from a duplex DNA. Although the results for both the ssDNA strand exchange and the duplex DNA unwinding reactions are the same release of an ssDNA strand, strand exchange activity is by nature different from helicase activity; DNA strand exchange activity requires no energy from nucleoside triphosphate (NTP) hydrolysis in the reaction.

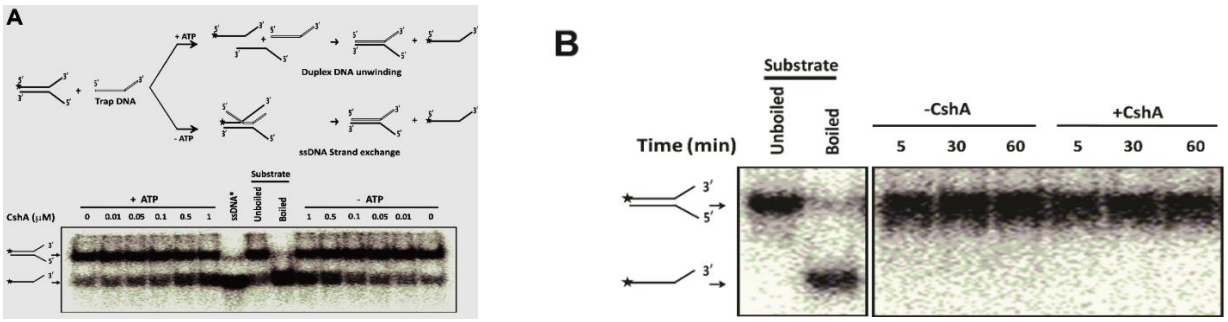


Figure 2. CshA shows DNA strand exchange activity rather than dsDNA unwinding. (A) For the dsDNA unwinding assay, various concentrations of CshA were incubated with 10 nM of 32P-labeled forked dsDNA (Nos. 3 and 4) in buffer containing 50 mM Tris-Cl, pH 7.5, 25 mM NaCl, 2 mM dithiothreitol (DTT), 0.15 mg/mL bovine serum albumin (BSA), 4 mM Mg2+, and excess unlabeled oligonucleotide (No. 3, 250 nM) as a trap. The reaction was performed in the presence and absence of 4 mM ATP for 30 min at room temperature. The reaction products were analyzed with 15% nondenaturing polyacrylamide gel electrophoresis (PAGE). (B) The same dsDNA unwinding assay was performed in a reaction without the trap DNA. 32P-labeled forked dsDNA (Nos. 3 and 4; 10 nM) was incubated with or without 1 μM of CshA in buffer containing 50 mM Tris-HCl, pH 7.5, 25 mM NaCl, 2mM DTT, 0.15 mg/mL BSA, 4 mM Mg2+, and 4 mM ATP for various amounts of time. The control substrates were the 32P-labeled forked dsDNAs that were boiled at 95°C for 10 min. Asterisks indicate the positions of radiolabels.

To confirm the DNA exchange activity of CshA, we investigated DNA strand exchange activity on the forked DNA under various reaction conditions without Mg^{2+} and ATP (Fig. 3). The forked dsDNA (45D/50D) formed by annealing complementary oligonucleotides Nos. 3 and 4 (see Table 1) was used in the reaction containing another complementary oligonucleotide (No. 2; 35D). If CshA had ssDNA strand-exchange activity, the strand exchange product 3′-tail dsDNA (45D/35D) would be formed by replacing one of the duplex strands with a third complementary strand (35D). A schematic diagram of DNA strand exchange assay between a labeled forked dsDNA (oligonucleotide Nos. 3 and 4) and a complementary ssDNA-35mer (oligonucleotide No. 2) is shown in Fg.3A. An increase in the strand-exchange product in the presence of CshA occurred, whereas the strand exchange product did not accumulate in the absence of the enzyme (Fig. 3B). Moreover, CshA catalyzed the DNA strand exchange in the absence of Mg^{2+} ion and ATP. This result clearly shows that the strand exchange products (45D/35D) resulted from the strand exchange activity of CshA, not from duplex DNA unwinding activity followed by strand annealing, which is a nucleoside triphosphate–dependent helicase function.

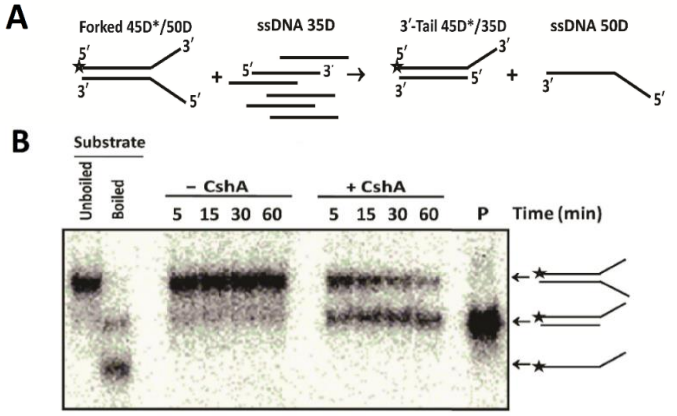


Figure 3. DNA strand exchange activity of CshA. (A) DNA strand exchange activity in the absence of 4 mM Mg2+ and 4 mM ATP. (A) A schematic diagram of DNA strand exchange assay between a labeled forked dsDNA (oligonucleotide Nos. 3 and 4) and a complementary ssDNA-35mer (oligonucleotide No. 2). (B) Reactions were carried out in a buffer containing 10 nM of 32P-labeled forked dsDNA, 50 nM unlabeled complementary ssDNA with 1 μM of CshA at room temperature for various amounts of time (5, 15, 30, and 60 min). The DNA strand exchange products at each reaction time were resolved in 15% non-denaturing polyacrylamide gel. P: positive controls showing expected DNA strand exchange product.

3.3. S. aureus CshA catalyzes strand annealing of complementary ssDNA into dsDNA

CshA displayed DNA strand-exchange activity, which is independent of ATP. Several previous reports have found that strand exchange activity is often accompanied by ssDNA strand annealing activity [26, 27]. Thus, we investigated whether *S. aureus* CshA has ssDNA strand annealing activity by incubating CshA with two partially complementary ssDNAs (oligonucleotides Nos. 1 and 4). A schematic diagram of DNA strand annealing assay between a labeled oligonucleotides No. 1 and a complementary unlabeled ssDNA-oligonucleotide No. 4 is shown in Fig. 4A. The presence of the enzymatic DNA strand annealing activity would form the 5′-tail dsDNA product (D35/D50) as a function of time. The reaction was carried out for various time periods at room temperature, and the reaction products were analyzed by non-denaturing 15% polyacrylamide gel electrophoresis. The analysis of reaction products showed that a 5′-tail dsDNA product (D35/D50) forms efficiently in the presence, but not in the absence of CshA, and the amount of reaction product increases as incubation time increases (see Fig. 4B). The DNA strand annealing activity by CshA was detected in both the absence of Mg^{2+} and ATP, suggesting that DNA strand annealing activity is independent of Mg^{2+} or ATP hydrolysis.

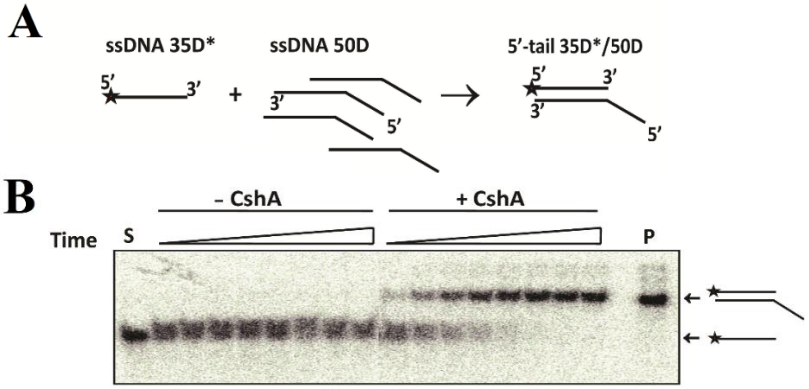


Figure 4. ssDNA strand annealing by CshA. (A) A schematic diagram of DNA strand annealing assay between a labeled oligonucleotides No. 1 and a complementary unlabeled ssDNA-oligonucleotide No. 4. (B) Nondenaturing polyacrylamide gel electrophoresis (PAGE) analysis of DNA strand annealing reaction. Reactions were carried out in 50 mM Tris-HCl, pH 7.5, 25 mM NaCl, 2 mM DTT, 0.15 mg/mL BSA, 0.1 nM 32P-labeled ssDNA, 0.25 nM of unlabeled complementary ssDNA, and 0.1 μM of CshA in the absence of 4 mM Mg2+ and ATP at room temperature for increasing times (0.1, 0.25, 0.5, 1, 2, 5, 10, and 15 min). S and P denote the ssDNA substrates and the annealed DNA products, respectively.

Although the biological roles of the strand exchange activity has not been defined precisely, the activity may be involved in replication fork regression during the repair of damaged DNA replication forks [14] or in homologous recombination repair through double holiday junction (dHJ) [15, 22, 23, 24, 25, 26, 28]. In support of our conjecture, several DNA helicases with DNA strand exchange activity that facilitates homologous recombination have been implicated in DNA double-strand breaks repair [16] and replication blockage release [19]. The combination of strand exchange and annealing is likely to be involved in the formation of dHJ. A BLM ortholog in budding yeast harboring ssDNA strand annealing and strand exchange activities that are both ATP-independent has been demonstrated to play a role in the SDSA pathway as well as in dHJ branch migration for DNA repair after dsDNA breaks [20]. This study suggested that CshA from *S. aureus* must be validated to understand the roles of CshA in maintaining genome stability in bacteria, which would benefit the development of a new antibiotic reagent against the drug-resistant bacteria.

4. CONCLUSIONS

In summary, DEAD-box protein CshA from *S. aureus* exhibited the DNA-binding affinity on both ssDNA substrate and dsDNA substrate in which 3'-tailed dsDNA substrate showed stronger binding affinity. The results showed that DNA binding by CshA is mainly dependent on Mg^{2+}, which requires neither nucleotide binding nor hydrolysis energy. Moreover, the CshA exhibits DNA strand exchange and DNA strand annealing activities. These DNA strand annealing and exchange activities are independent of Mg^{2+} ion or ATP binding and hydrolysis. This showed the diversity of biochemical characteristic of DEAD-box proteins and contributed in insights of roles of DEAD-box proteins in all nucleic acid metabolic processes in organisms, as well as the putative role of CshAin maintaining genome stability in bacteria, which would benefit the development of a new antibiotic reagent against the drug-resistant bacteria. To determine the role of CshA in the DNA repair or RNA mechanism, the in vivo models will be applied with specific mutant on the gene coding for CshA DEAD-box protein.

REFERENCES

[1] Linder, P., Dead-box proteins: a family affair--active and passive players in RNP-remodeling. Nucleic Acids Res, 2006. 34(15): p. 4168-80.

[2] Rocak, S. and P. Linder, DEAD-box proteins: the driving forces behind RNA metabolism. Nat Rev Mol Cell Biol, 2004. 5(3): p. 232-41.

[3] de la Cruz, J., et al., Unwinding RNA in Saccharomyces cerevisiae: DEAD-box proteins and related families. Trends Biochem Sci, 1999. 24(5): p. 192-8.

[4] Khemici, V. and P. Linder, RNA helicases in RNA decay. Biochemical Society transactions, 2018. 46(1): p. 163-172.

[5] Meier-Stephenson, V., et al., DEAD-box helicases: the Yin and Yang roles in viral infections. Biotechnology and Genetic Engineering Reviews, 2018. 34(1): p. 3-32.

[6] Kikuma, T., et al., Dbp9p, a member of the DEAD box protein family, exhibits DNA helicase activity. J Biol Chem, 2004. 279(20): p. 20692-8.

[7] Bergkessel, M. and J.C. Reese, An essential role for the Saccharomyces cerevisiae DEAD-box helicase DHH1 in G1/S DNA-damage checkpoint recovery. Genetics, 2004. 167(1): p. 21-33.

[8] Erguden, B., Dhh1 is a member of the SESA network. Yeast, 2018.

[9] Schurer, K.A., et al., Yeast MPH1 gene functions in an error-free DNA damage bypass pathway that requires genes from Homologous recombination, but not from postreplicative repair. Genetics, 2004. 166(4): p. 1673-86.

[10] Ang, J.S., et al., Dosage Mutator Genes in Saccharomyces cerevisiae: A Novel Mutator Mode-of-Action of the Mph1 DNA Helicase. Genetics, 2016. 204(3): p. 975-986.

[11] Li, L., et al., A role for DEAD box 1 at DNA double-strand breaks. Mol Cell Biol, 2008. 28(20): p. 6413-25.

[12] Li, L., et al., Role for RIF1-interacting partner DDX1 in BLM recruitment to DNA double-strand breaks. DNA Repair (Amst), 2017. 55: p. 47-63.

[13] Hiramatsu, K., et al., Methicillin-resistant Staphylococcus aureus clinical strain with reduced vancomycin susceptibility. J Antimicrob Chemother, 1997. 40(1): p. 135-6.

[14] Hiramatsu, K., et al., Dissemination in Japanese hospitals of strains of Staphylococcus aureus heterogeneously resistant to vancomycin. Lancet, 1997. 350(9092): p. 1670-3.

[15] Kuroda, M., et al., Whole genome sequencing of meticillin-resistant Staphylococcus aureus. Lancet, 2001. 357(9264): p. 1225-40.

[16] Ohta, T., et al., Nucleotide substitutions in Staphylococcus aureus strains, Mu50, Mu3, and N315. DNA Res, 2004. 11(1): p. 51-6.

[17] Chen, X., et al., Crystal structures of the N-terminal domain of the Staphylococcus aureus DEAD-box RNA helicase CshA and its complex with AMP. Acta Crystallographica Section F, 2018. 74(11): p. 704-709.

[18] Oun, S., et al., The CshA DEAD-box RNA helicase is important for quorum sensing control in Staphylococcus aureus. RNA Biol, 2013. 10(1): p. 157-65.

[19] Hunger, K., et al., Cold-induced putative DEAD box RNA helicases CshA and CshB are essential for cold adaptation and interact with cold shock protein B in Bacillus subtilis. J Bacteriol, 2006. 188(1): p. 240-8.

[20] Roux, C.M., et al., Characterization of components of the Staphylococcus aureus mRNA degradosome holoenzyme-like complex. J Bacteriol, 2011. 193(19): p. 5520-6.

[21] Jankowsky, E., Methods in Enzymology- Volumn 511: RNA Helicase. Academic Press, USA, 2012: p. 369-381.

[22] Kim, S., et al., Characterization of RNA Helicase CshA and Its Role in Protecting mRNAs and Small RNAs of Staphylococcus aureus Strain Newman. Infection and Immunity, 2016. 84(3): p. 833.

[23] Giraud, C., et al., The C-terminal region of the RNA helicase CshA is required for the interaction with the degradosome and turnover of bulk RNA in the opportunistic pathogen Staphylococcus aureus. RNA biology, 2015. 12(6): p. 658-674.

[24] Lee, S.Y., et al., Cloning, purification, crystallization and preliminary X-ray crystallographic analysis of the N-terminal domain of DEAD-box RNA helicase from Staphylococcus aureus strain Mu50. Acta Crystallogr Sect F Struct Biol Cryst Commun, 2010. 66(Pt 12): p. 1674-6.

[25] Wong, I. and T.M. Lohman, A double-filter method for nitrocellulose-filter binding: application to protein-nucleic acid interactions. Proc Natl Acad Sci U S A, 1993. 90(12): p. 5428-32.

[26] Zhao, X. and C. Jain, DEAD-box proteins from Escherichia coli exhibit multiple ATP-independent activities. J Bacteriol, 2011. 193(9): p. 2236-41.

[27] Muftuoglu, M., et al., Cockayne syndrome group B protein has novel strand annealing and exchange activities. Nucleic Acids Res, 2006. 34(1): p. 295-304.

[28] Wu, Y., Unwinding and rewinding: double faces of helicase? J Nucleic Acids, 2012. 2012: p. 140601.

PROTEIN DEAD-BOX CSHA CỦA *STAPHYLOCOCCUS AUREUS* CÓ CÁC HOẠT TÍNH TRAO ĐỔI MẠCH VÀ LIÊN KẾT MẠCH DNA MÀ KHÔNG PHỤ THUỘC ATP

Tóm tắt. Các protein DEAD-box (DBPs) thư VÀ LIÊN KẾT MẠCH DNAnealing anannealing activity DNA strand exchange activity of CshA suggests a possible role in dsDNA break repair processes. nt of Mgit ATP-independent nucleic acid displacement and annealing activities. ay thế và liên kết các mạch acid nucleic không phụ thuộc ATP. Chúng tôi đã nghiên cứu các hoạt tính không phụ thuộc ATP trên mạch acid nucleic của một protein DEAD-box tương tự RNA helicase, CshA, từ chủng Staphylococcus aureus Mu 50 kháng kháng sinh vancomycin. Chúng tôi nhH DNANA strand a2 holing activity DNA strandATP – liên kết các mạch đơn DNA có trình tự bổ sung và trao đổi mạch trên các mạch đôi DNA ngắn. Các hoạt tính trao đổi mạch hay liên kết mạch DNA này thì không phụ thuộc vào ion Mg2+ và s0 kháng kháng sinh vancomycinHoChúng tôi nhH DNANA strand a2 holing nh không thụ thuộc ATP – liên kết các mạch đơn DNA có trình tự bổ sung và trao đổi mạch trên các mạch đô

Từ khoá. DEAD-box protein, *Staphylococcus aureus*, RNA helicase, CshA, DNA strand exchange activity, DNA strand annealing activity

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