Protein Expression and Purification

First characterization of extremely halophilic 2-deoxy-D-ribose-5-phosphate aldolase*

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Highlights

- Extreme halophilic DERA expressed in *Escherichia coli* requires NaCl for activation.
- Preparation of crude extract in NaCl produced a large amount of inactive enzyme.
- After purification without salt, dialysis against NaCl successfully prevents the formation of the inactive enzyme.
- The procedure makes it possible to reveal for the first time, the characteristics of a halophilic DERA.

ABSTRACT

2-Deoxy-D-ribose-5-phosphate aldolase (DERA) catalyzes the aldol reaction between two aldehydes and is thought to be a potential biocatalyst for the production of a variety of stereo-specific materials. A gene encoding DERA from the extreme halophilic archaeon, *Haloarcula japonica*, was overexpressed in *Escherichia coli*. The gene product was successfully purified, using procedures based on the protein's halophilicity, and characterized. The expressed enzyme was stable in a buffer containing 2 M NaCl and exhibited high thermostability, retaining more than 90% of its activity after heating at 70°C for 10 min. The enzyme was also tolerant to high concentrations of organic solvents, such as acetonitrile and dimethylsulfoxide. Moreover, *H. japonica* DERA was highly resistant to a high concentration of acetaldehyde and retained about 35% of its initial activity after 5-hours' exposure to 300 mM acetaldehyde at 25°C, the conditions under which *E. coli* DERA is completely inactivated. The enzyme exhibited much higher activity at 25°C than the previously characterized hyperthermophilic DERAs (Sakuraba *et al*., 2007). Our results suggest that the extremely halophilic DERA has high potential to serve as a biocatalyst in organic syntheses. This is the first description of the biochemical characterization of a halophilic DERA.

Keywords: halophile, *Haloarcula japonica*, 2-deoxy-D-ribose-5-phosphate aldolase, archaea, organic solvent, aldehyde

INTRODUCTION

2-Deoxy-D-ribose-5-phosphate aldolase (DERA; EC 4.1.2.4) catalyzes a reversible aldol reaction that generates 2-deoxy-D-ribose-5-phosphate (DRP) using acetaldehyde and D-glyceraldehyde-3-phosphate as substrates [1]. The enzyme has been proposed to function in the metabolic link between the nucleosides and central carbon metabolism in bacterial, archaeal, and mammalian cells [2]. In *Bacillus cereus*, the enzyme reportedly plays a key role in the utilization of the pentose moiety of exogenous nucleosides [3, 4]. In *Salmonella typhimurium* and *Escherichia coli*, the gene encoding DERA belongs to the *deo* regulon that contains four genes encoding enzymes involved in nucleoside catabolism [5, 6]. In mammalian cells, on the other hand, the inhibition of DERA leads to a specific inhibition for the incorporation of labeled purine and pyrimidine into DNA [7]. With archaea, the presence of DERA has so far been described in *Aeropyrum pernix* [8], *Thermococcus kodakaraensis* [9], *Pyrobaculum aerophilum* [10], and *Hyperthermus butylicus* [11], which are all hyperthermophiles. In *T. kodakaraensis*, DERA and a newly identified phosphopentomutase were found to be involved in a metabolic link between central carbon metabolism and pentose biosynthesis and catabolism [9].

DERA is unique in catalyzing the aldol condensation reaction between two aldehyde molecules as both the aldol donor and acceptor components. Its broad substrate specificity is an attractive characteristic for producing a variety of stereo-specific materials [12]. The reactions catalyzed by DERA are becoming commercially important in developing therapeutic agents, including antiviral nucleotides, cholesterol-lowering drugs, and anticancer drugs [13-15]. However, practical application of the enzyme from a mesophilic organism such as *E. coli* is still

limited by its poor resistance to high aldehyde concentrations useful for biocatalysis [16]. We have previously characterized hyperthermophilic DERAs from both the archaeon *P. aerophilum* and the bacterium *Thermotoga maritima* (paeDERA and tmaDERA) [10]. The structures and activities of the two enzymes were then compared with those of the enzyme from *E. coli* (ecoDERA). The two hyperthermophilic DERAs exhibit extremely high thermostability, retaining full activity after incubation for 10 min at up to 90°C, while ecoDERA is markedly inactivated by incubation at temperatures >60°C. Moreover, both the hyperthermophilic DERAs are highly resistant to high concentrations of acetaldehyde [10]. Structural comparison reveals that the strong hydrophobic intersubunit interaction is likely responsible for the extremely high stability of the hyperthermophilic DERAs [8, 10]. However, the hyperthermophilic DERAs showed much less activity than ecoDERA at 25°C. Therefore, if the aldehyde-resistant enzyme is present in mesophilic organisms, the enzyme might have much higher activity at low temperature, which would make it a potential biocatalyst for synthetic organic chemistry.

Extreme halophilic archaea require NaCl above 2.5 M for growth and accumulate salt within cells at concentrations equivalent to or greater than that of the environment [17, 18]. Their enzymes are therefore specialized to function at high salt concentrations, at which ordinary proteins may aggregate and lose activity. The structural and biochemical characteristics of several halophilic enzymes have shown that enhancing solvation is essential for maintaining their solubility and activity in low water activity [19, 20]. The low water activity conditions mimic an aqueous-organic solvent mixture, and consequently halophilic enzymes generally retain considerable activity in organic solvents [19, 21, 22]. Moreover, some of the halophilic enzymes are also tolerant to

extreme pH and heat [23-25]. These observations led us to investigate DERA from mesophilic and extreme halophilic archaea. For this study, the gene encoding a DERA homolog in the genome of an extreme halophilic archaeon, *Haloarcula japonica* TR-1 (JCM7785^T), has been identified. We succeeded in the gene expression, purification, and characterization of the *H. japonica* DERA (hjaDERA). This paper is the first report on the biochemical characteristics of a halophilic DERA.

MATERIALS AND METHODS

Cloning, protein expression, and purification. Within the *H. japonica* TR-1 genomic sequence determined by Prof. Kaoru Nakasone (Faculty of Engineering, Department of Biotechnology and Chemistry, Kinki University, Hiroshima, Japan; unpublished work), a gene (Hja_deoCnt) whose predicted amino acid sequence exhibits 28% identity with that of paeDERA was identified. The nucleotide sequence information of that gene was kindly supplied by Prof. Nakasone and deposited in the DDBJ/EMBL/GenBank data bank under accession no. LC121519. The gene encoding hjaDERA was amplified using PCR. The oligonucleotide primers used to amplify the gene fragment were 5'- CCCGC**CATATG**GACGATATACCAGACCGC-3', which contains a unique *Nde*I restriction site (bold) overlapping the 5' initiation codon, and 5'-TT**GGATCC**TCAGTAGCCGTCGGTCGTGT-3', which contains a unique *Bam*HI restriction site (bold) proximal to the 3' end of the termination codon. Chromosomal *H. japonica* DNA was isolated as described previously [26] and used as the template. The amplified 0.7-kb fragment was digested with *Nde*I and *Bam*HI and ligated with the expression vector pColdI (Takara Bio, Japan) previously linearized with NdeI and BamHI to generate pC-hjaDERA, which was then used to transform the *E. coli* strain

BL21 (DE3) codon plus-RIPL (Agilent Technologies, Santa Clara, CA, USA). The transformants were cultivated at 37°C in 1 L of SB medium (1.2% tryptone peptone, 2.4% yeast extract, 1.25% K_2HPO_4, 0.38% KH_2PO_4 and 0.5% glycerol) containing 100 mg ampicillin/L until the optical density at 600 nm reached 0.6, after which expression was induced by adding 1 mM isopropyl-β-D-thiogalactopyranoside to the medium, and the cultivation was continued for an additional 24 h at 15°C. The cells were harvested by centrifugation, suspended in 10 mM Tris-HCl buffer (pH 7.5) and disrupted by sonication, after which the cell debris was removed by centrifugation (15,000 × g for 30 min). The resulting supernatant, which served as the crude extract, was loaded onto a Protino Ni-IDA column (Macherey-Nagel, Germany) that had been equilibrated with 10 mM Tris-HCl buffer (pH 7.5). The column was then washed with the same buffer, and the enzyme was eluted with a linear gradient of 0–0.5 M imidazole in the buffer. The enzyme-containing fractions were checked using SDS-PAGE and activity measurement. The active fractions were collected and dialyzed against 10 mM Tris-HCl buffer (pH 7.5) containing 2 M NaCl. The resultant solution was concentrated by ultrafiltration (Amicon Ultra 30K; Merck Millipore, Germany) and loaded onto a Superdex 200 26/60 column (GE Healthcare Bio-Sciences AB, Uppsala, Sweden) previously equilibrated with 10 mM Tris-HCl buffer (pH 7.5) containing 2 M NaCl and eluted with the same buffer.

Determination of enzyme activity, kinetic parameters, and protein concentration. DERA activity was determined by measuring the oxidation of NADH in a coupled assay using triose-phosphate isomerase and glycerol-3-phosphate dehydrogenase. The standard reaction mixture consisted of 100 mM citrate-Na citrate buffer (pH 6.4)

containing 1 M NaCl, 0.15 mM NADH, 2 mM DRP, 8 units of triose-phosphate isomerase (rabbit muscle), 2.5 units of glycerol-3-phosphate dehydrogenase (rabbit muscle), and the hjaDERA (1–2 μL) in a final volume of 1.00 mL. After incubating the reaction mixture for 3 min at 37°C without the DERA preparation, the reaction was started by adding the enzyme. The disappearance of NADH was monitored from the decrease in absorbance at 340 nm using a Shimadzu UV-mini 1240 spectrophotometer equipped with a thermostat (extinction coefficient ε= 6.22 mM^{-1} cm^{-1}). The Michaelis constants were determined from Lineweaver-Burk plots [27] of data obtained from the initial rate of D-glyceraldehyde-3-phosphate formation at 37°C. The protein concentration was determined using the Bradford method, with bovine serum albumin serving as the standard [28].

Polyacrylamide gel electrophoresis and molecular mass determination. SDS-PAGE (12.5% acrylamide slab gel, 1 mm thick) was carried out using the procedure of Laemmli [29], after which the protein band was stained with Sil-Best Stain Kit (Nacalai Tesque, Kyoto, Japan) for silver staining. The molecular mass of the purified enzyme was determined using a Superdex 200 26/60 column (GE Healthcare) with 10 mM Tris-HCl buffer (pH 7.5) containing 2 M NaCl as the elution buffer. Gel filtration standards (Bio-Rad Lab., Hercules, CA, USA) were used as the molecular mass standards. The subunit molecular mass was determined by SDS-PAGE using nine marker proteins (6.5–200 kDa) (Takara Bio).

Effects of temperature and pH on enzyme stability and activity. The optimal temperature for the reaction was determined by performing the standard assay at

temperatures ranging from 20 to 70°C. To determine the effect of temperature on its stability, the enzyme was incubated for 10 min at different temperatures in 10 mM Tris-HCl buffer (pH 7.5) containing 2 M NaCl. After centrifugation (15,000 × g for 5 min), the residual activity in the supernatant was determined using the standard assay method. To determine the effect of pH on its stability, the enzyme was incubated in different pH buffers for 30 min at 50°C, and the remaining activity was again determined under the standard assay conditions. The buffers (200 mM) used for these assays were citrate-Na citrate (pH 5.3–6.8), KH_2PO_4-K_2HPO_4 (pH 6.3–7.9), Tris-HCl (pH 7.7–8.9), glycine-NaOH (pH 8.9–11.1), K_2HPO_4-K_3PO_4 (pH 11.4–12.2), and KCl-NaOH (pH 12.3–12.6). To determine the optimal pH for enzyme activity, citrate-Na citrate (pH 4.9–6.4) and citrate-Na_2HPO_4 (pH 5.9–6.8) buffers (100 mM) were used at 37°C.

Stability of the enzyme in water-miscible organic solvents and acetaldehyde. To determine the effects of organic solvents on enzyme stability, the enzyme (0.5 mg/mL) in 10 mM Tris-HCl buffer (pH 8.0) containing 2 M NaCl and various concentrations of methanol, ethanol, acetonitrile, or dimethylsulfoxide (DMSO) was incubated at 25°C, after which the residual activity was determined using the standard assay method. To examine the effects of acetaldehyde on enzyme stability, the enzyme (0.5 mg/mL) in 10 mM Tris-HCl buffer (pH 8.0) containing 2 M NaCl and 300 mM acetaldehyde was incubated at 25°C, after which the residual activity was determined at appropriate intervals using the standard assay method. Prior to analyzing the enzyme activity, the organic solvent or acetaldehyde was removed from the enzyme solution

using a combination of dilution in the 10 mM Tris-HCl buffer containing 2 M NaCl and concentration by ultrafiltration (Amicon Ultra 30K), which was repeated several times.

RESULTS

Purification of recombinant hjaDERA. An expression system for the hjaDERA gene (pC-hjaDERA) was constructed using the pColdI vector (Fig. 1), within which the recombinant protein was encoded as a fusion protein with a His-tag at its N terminus. In preliminary studies, the recombinant *E. coli* cells were disrupted in 2 M NaCl. The crude extract exhibited a high level of DERA activity. In the following Ni-chelating chromatography step, however, a large amount of the absorbed enzyme was eluted as an inactive protein by a linear gradient of imidazole, even though the purification buffers contained 2 M NaCl (data not shown). The elution profile of the next gel filtration chromatography step indicated that a peak of the inactive protein was present besides the normal peak of the active enzyme. Although the inactive protein was supposed to have a larger molecular mass than the active enzyme, the two proteins were indistinguishable in size as determined with SDS-PAGE analysis (Fig. 2A). Therefore, the cell disruption and Ni-chelating chromatography steps were carried out without NaCl and the resulting eluate was dialyzed against a high concentration of NaCl. Dialysis against the buffers containing different concentrations of NaCl (ranging from 0.5 to 3 M) showed that the most suitable concentration was 2 M (Fig. 3). When the resulting dialysate was subjected to gel filtration chromatography, the enzyme was eluted as a single peak with DERA activity (Fig. 2B). Table 1 shows a typical result of the purification. The purified enzyme showed a single band on SDS-PAGE (Fig. 4).

About 19 mg of the purified enzyme was obtained from 1 L *E. coli* cells cultured in the 1 L medium.

Molecular mass and subunit structure. SDS-PAGE showed the subunit molecular mass of hjaDERA to be about 29 kDa (Fig. 4), which is consistent with the molecular mass (26,593 Da) calculated from the amino acid sequence including a His-tag. The native molecular mass of about 55.5 kDa determined by gel filtration suggests the native enzyme is a homodimer.

Catalytic properties. The enzyme activity in different concentrations of NaCl was examined. The enzyme activity increased with the increase in concentration from 0.5 to 1 M, but gradually decreased in concentration from 1 to 3 M (data not shown). Thus, 1 M NaCl was included in the standard reaction mixture. HjaDERA showed typical Michaelis-Menten kinetics at 37°C; the Km value for DRP was 1.12 ± 0.18 mM and the Vmax value was 20.7 ± 2.6 μmol/min/mg. At 25°C, these values were calculated to be 1.02 ± 0.22 mM and 8.92 ± 1.4 μmol/min/mg, respectively. Evaluation of the catalytic activity at different pH values revealed the enzyme to be maximally active at around pH 6.4 (Fig. 5A). The optimal temperature of the enzyme reaction was about 60°C (Fig. 5B). When we examined the thermostability of the enzyme, we found that hjaDERA retained more than 90% of its activity after heating at 70°C for 10 min (Fig. 5C). At 80°C, however, the activity was almost lost. When hjaDERA was heated for 30 min at 50°C, the enzyme showed no loss of activity at pH ranging from 6.3 to 12.3 (Fig. 5D).

Effects of water-miscible organic solvents and acetaldehyde on enzyme stability. The effect of organic solvents on the enzyme stability was examined by incubating the enzyme with methanol, ethanol, acetonitrile, or DMSO at 25°C. After incubation for 10 min, the enzyme retained more than 80% of its activity in DMSO or acetonitrile even at a concentration up to 50% (Fig. 6A). Under similar conditions, the enzyme retained its activity in methanol (30%) or ethanol (20%). Moreover, the enzyme retained more than 80% of its activity after incubation for 50 min with DMSO or acetonitrile (at a concentration of 50%) and with methanol or ethanol (at a concentration of 20%) (Fig. 6B). The stability of hjaDERA against acetaldehyde was tested. The enzyme retained 35% of its activity after exposure for 5 h to 300 mM acetaldehyde at 25°C in 2 M NaCl, while more than 80% of the activity was lost after incubation for 20 h (Fig. 7). Prior to analyzing the enzyme activity, a combination of dilution in the buffer containing 2 M NaCl and concentration by ultrafiltration was used to remove the organic solvent or acetaldehyde from the enzyme solution. We confirmed that this treatment had no effect on the specific activity of the standard enzyme.

DISCUSSION

Since enzymes from halophiles generally require a high salt concentration for activity and stability, a major problem is the difficulty in expressing them in *E. coli*. Also, the expressed product generally requires reactivation or refolding under the high salt conditions [30, 31]. Furthermore, conventional purification techniques are incompatible with high salt conditions. In the present study, we succeeded in the gene expression and purification of hjaDERA, which made it possible to report the first characteristics of an extremely halophilic DERA. Upon disruption of the recombinant *E. coli* cells in 2 M

NaCl, hjaDERA was not fully activated. The following gel filtration chromatography and SDS-PAGE analysis indicated that an inactive aggregate form of the enzyme was produced under the conditions used (Fig. 2A). After purifying the enzyme without NaCl, dialysis of the enzyme against the buffer containing 2 M NaCl successfully prevented formation of the inactive form (Fig. 2B). With nucleoside diphosphate kinase from *Halobacterium salinarum*, it has been reported that the recombinant enzyme expressed in *E. coli* is fully activated upon disruption of the recombinant *E. coli* cells in 4 M NaCl [30]. Therefore, a unique feature of the reactivation exhibited by hjaDERA may provide useful information for expressing and purifying extreme halophilic enzymes.

The catalytic and molecular properties of hjaDERA, ecoDERA, tmaDERA, and paeDERA are summarized in Table 2. It has been reported that ecoDERA is largely inactivated by incubation at temperatures >60°C, whereas tmaDERA and paeDERA from two hyperthermophiles (*P. aerophilum* and *T. maritima*, respectively) retain full activity even when heated for 10 min at 90°C [10]. When tmaDERA and paeDERA were heated for 30 min at 50°C, they show no loss of activity at pH levels ranging from 5.0 to 11.0, while ecoDERA shows significant loss of activity at pH levels higher than 10.0 and lower than 5.5 [10]. These results indicate that tmaDERA and paeDERA show thermal and pH stability levels that are much higher than that of ecoDERA. In contrast, the Vmax values of tmaDERA and paeDERA for DRP cleavage are only 1.7 and 0.4%, respectively, that of ecoDERA at 25°C (Table 2). In this study, we found hjaDERA retains more than 90% of its activity after heating at 70°C for 10 min. This result suggests hjaDERA has significantly higher thermostability than ecoDERA. The enzyme showed no loss of activity at pH ranging from 6.3 to 12.3 when heated for 30 min at 50°C, indicating that hjaDERA has higher pH stability than ecoDERA, while ecoDERA

exhibits higher pH stability at pH ranging from 5.5 to 6.3. As expected, hjaDERA exhibited high catalytic activity at 25°C; the Vmax value of hjaDERA is about 9 and 36 times higher than those of tmaDERA and paeDERA, respectively. Moreover, the enzyme is highly resistant to organic solvents, such as acetonitrile and DMSO. These observations indicate that extremely halophilic DERAs may have a high potential to serve as biocatalysts in organic syntheses.

Both tmaDERA and paeDERA have been reported to be highly resistant to acetaldehyde, retaining 46 and 53% of their initial activity, respectively, after exposure for 20 h to 300 mM acetaldehyde at 25°C [10]. In contrast, ecoDERA was almost completely inactivated after exposure for 2 h under the same conditions (Fig. 7) [10]. The stability of hjaDERA against acetaldehyde was less than that of tmaDERA and paeDERA, but higher than that of ecoDERA: hjaDERA retained 35% of its initial activity after exposure for 5 h to 300 mM acetaldehyde at 25°C (Fig. 7) and after incubation for 20 h, more than 80% of the activity was lost. However, the initial activity of hjaDERA at 25°C was much higher than that of tmaDERA and paeDERA. Practical application of ecoDERA has so far been limited due to its instability against the substrate aldehyde [16]. In addition, a low catalytic activity of hyperthermophilic DERAs at low temperatures like 25°C is a major disadvantage for use in bioreactors. Clearly, the higher capability of hjaDERA as a catalyst at low temperatures as well as its high stability against aldehyde and organic solvents may be potentially useful for further development of the application of extremely halophilic DERAs.

Footnote

Acknowledgements

*This work was supported in part by research funding from the Japan Society for the Promotion of Science (KAKENHI grant No. 15K07395 to H. S.).

Abbreviations: DERA, 2-deoxy-D-ribose-5-phosphate aldolase; DRP, 2-deoxy-D-ribose-5-phosphate; paeDERA, *Pyrobaculum aerophilum* DERA; ecoDERA, *Escherichia coli* DERA; tmaDERA, *Thermotoga maritima* DERA; hjaDERA, *Haloarcula japonica* DERA; DMSO, dimethylsulfoxide

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Figure legends

Fig. 1 An expression vector for hjaDERA gene (pC-hjaDERA).

Fig. 2. Elution profiles of gel filtration chromatography and SDS-PAGE of effluent. (A) The cell disruption, Ni-chelating chromatography, and Superdex 200 26/60 column chromatography steps were carried out in 2 M NaCl. (B) The cell disruption and Ni-chelating chromatography steps were carried out without NaCl and the resulting eluate was dialyzed against 2 M NaCl. After that, the Superdex 200 26/60 column chromatography step was carried out in 2 M NaCl. The effluent from the column was monitored with a UV detector at a wavelength of 280 nm.

Fig. 3. Activation of hjaDERA by dialysis against NaCl. The eluate from Ni-chelating chromatography was dialyzed against the buffers containing different concentrations of NaCl.

Fig. 4. SDS-PAGE of recombinant hjaDERA. Lane 1, marker proteins; lane 2, purified hjaDERA.

Fig. 5. Optimal pH level and temperature for DRP cleavage and thermal/pH stability. (A) The DRP cleavage assay was performed at various pH levels at 37°C. Citrate-Na citrate (•) (pH 4.9–6.4) and citrate-Na_2HPO_4 (○) (pH 5.9–6.8) buffers (100 mM) were used. (B) The optimal temperature for the reaction was determined by performing the standard assay at temperatures ranging from 20 to 70°C. (C) After incubation for 10 min

at the indicated temperatures, the remaining activity was assayed. (D) The enzyme was incubated for 30 min at 50°C in buffers of various pH levels, after which the remaining activity was assayed. The buffers (200 mM) used for these assays were citrate-Na citrate (□) (pH 5.3–6.8), KH_2PO_4-K_2HPO_4 (■) (pH 6.3–7.9), Tris-HCl (▲) (pH 7.7–8.9), glycine-NaOH (♦) (pH 8.9–11.1), K_2HPO_4-K_3PO_4 (○) (pH 11.4–12.2), and KCl-NaOH (●) (pH 12.3–12.6).

Fig. 6. Effects of water-miscible organic solvents on hjaDERA stability. (A) The enzyme was incubated with various concentrations of organic solvents at 25°C for 10 min in 2 M NaCl, after which residual activity was determined using the standard assay method. The organic solvents used were methanol (□), ethanol (■), acetonitrile (▲), and DMSO (♦). (B) The enzyme was incubated with organic solvents at 25°C in 2 M NaCl, and the DRP cleavage activity was assayed at appropriate intervals. The organic solvents used were 20% methanol (□), 20% ethanol (■), 50% acetonitrile (▲), and 50% DMSO (♦).

Fig. 7. Effect of acetaldehyde on enzyme stability. The hjaDERA preparation containing 2 M NaCl were incubated at 25°C with (●) or without (○) 300 mM acetaldehyde, and the DRP cleavage activity was assayed at appropriate intervals. The stability of ecoDERA against 300 mM acetaldehyde was plotted (♦) [10].

Table 1 Purification of recombinant hjaDERA

Step	Total protein (mg)	Total activity (μmol/min)	Specific activity (μmol/min/mg)
Crude extract	529	11.8	0.0223
Ni-chelating chromatography	29.2	0.456	0.0156
Dialysis against NaCl	28.2	353	12.5
Gel filtration	18.8	302	16.1

Table 2 Catalytic properties of hjaDERA, ecoDERA, tmaDERA, and paeDERA

	hjaDERA	ecoDERA [10]	tmaDERA [10]	paeDERA [10]
Optimum pH	6.4	7.5	6.5	6.0
Optimum temperature (°C)	60	-	-	-
pH stability	6.3–12.3	5.5–10.0	5.0–11.0	5.0–11.0
Thermostability (°C)	70	60	90	100
Vmax (μmol/min/mg)	8.92 ± 1.4 (25°C) 20.7 ± 2.6 (37°C)	58 ± 2.0 (25°C) -	1.00 ± 0.07 (25°C)	0.25 ± 0.03 (25°C) -
Km for DRP (mM)	1.02 ± 0.22 (25°C) 1.12 ± 0.18 (37°C)	0.23 ± 0.01 (25°C)	0.02 ± 0.003 (25°C)	0.066 ± 0.004 (25°C)

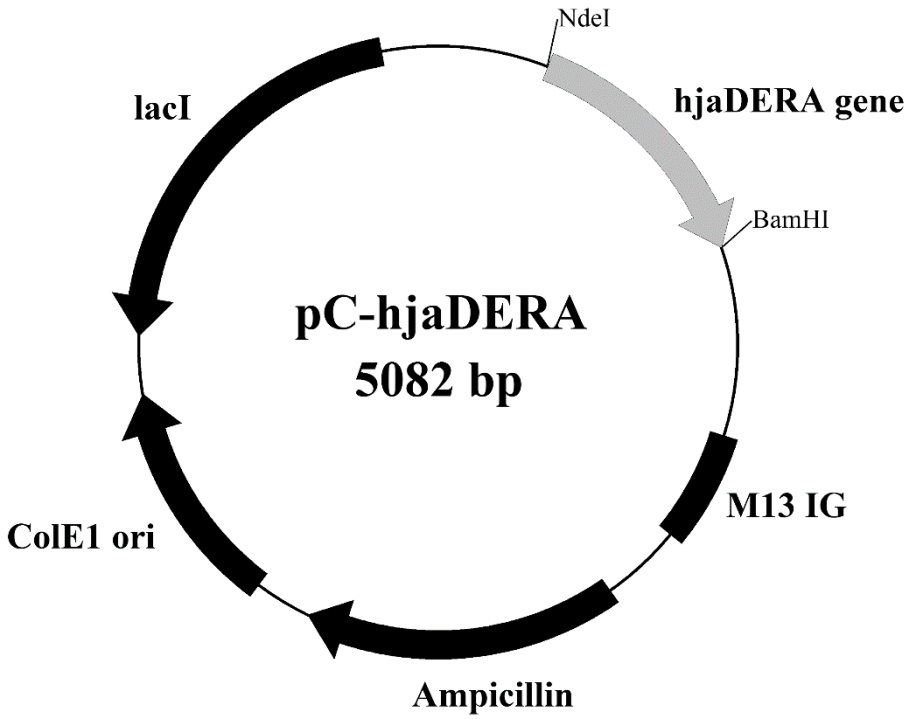
NdeI
hjaDERA gene
BamHI
lacI
pC-hjaDERA
5082 bp
M13 IG
ColE1 ori
Ampicillin

Fig. 1

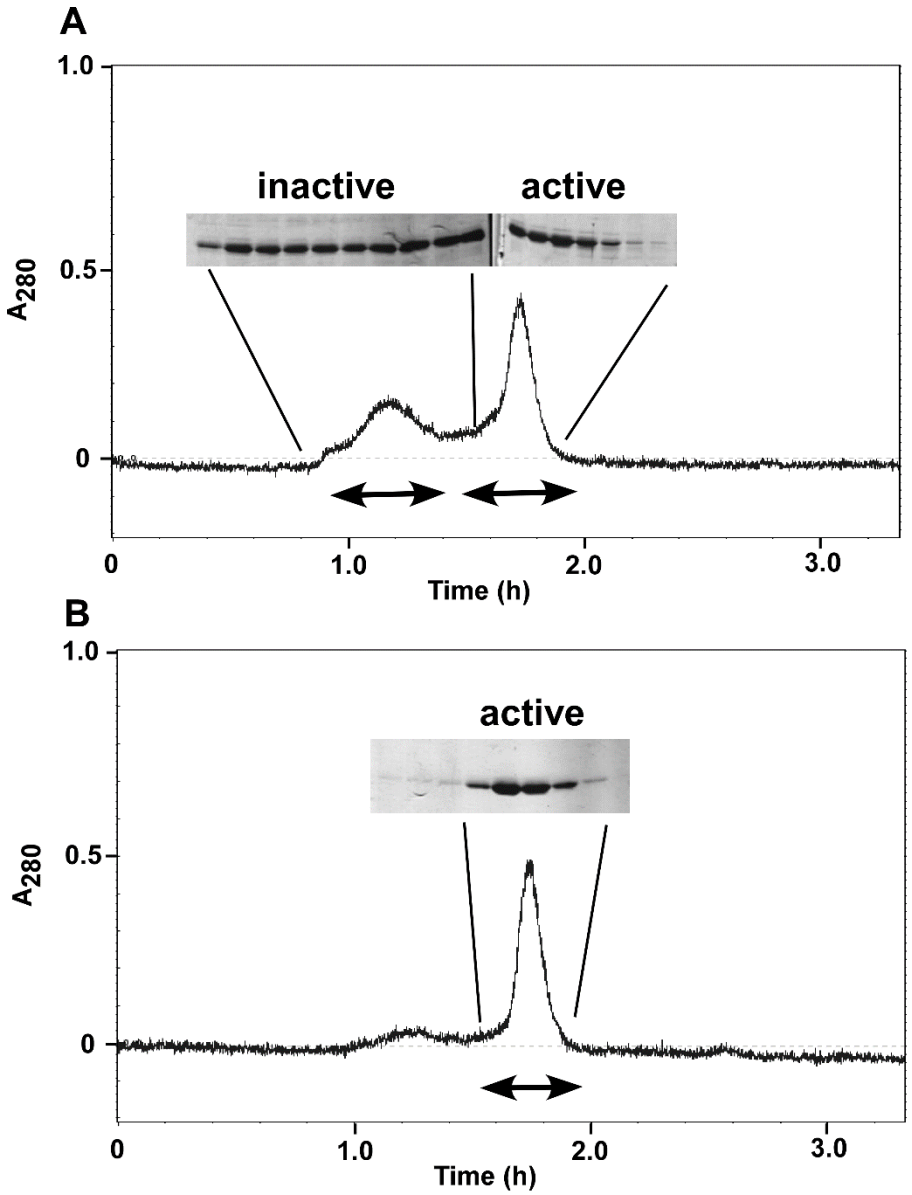
A
1.0
0.5
0
A280
inactive
active
0
1.0
2.0
3.0
Time (h)
B
1.0
0.5
0
A280
active
0
1.0
2.0
3.0
Time (h)

Fig. 2

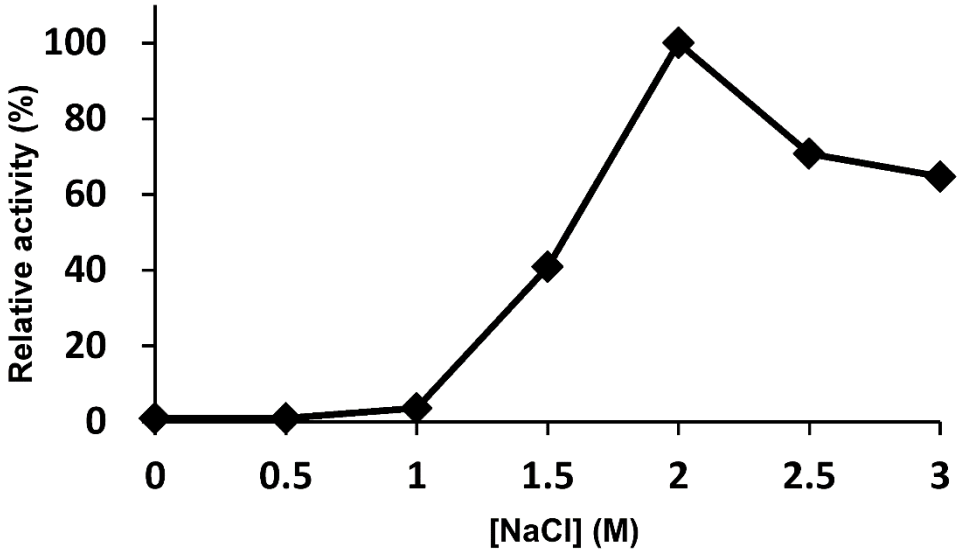

Fig. 3

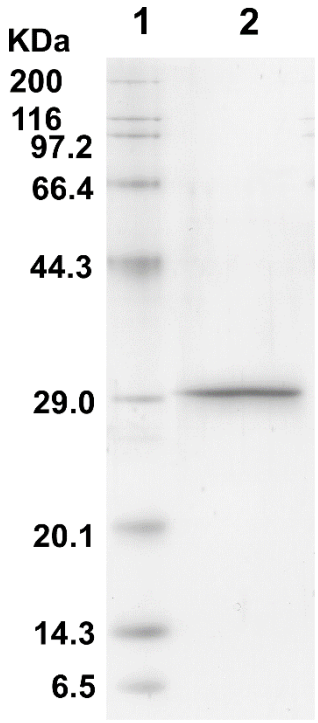

Fig. 4

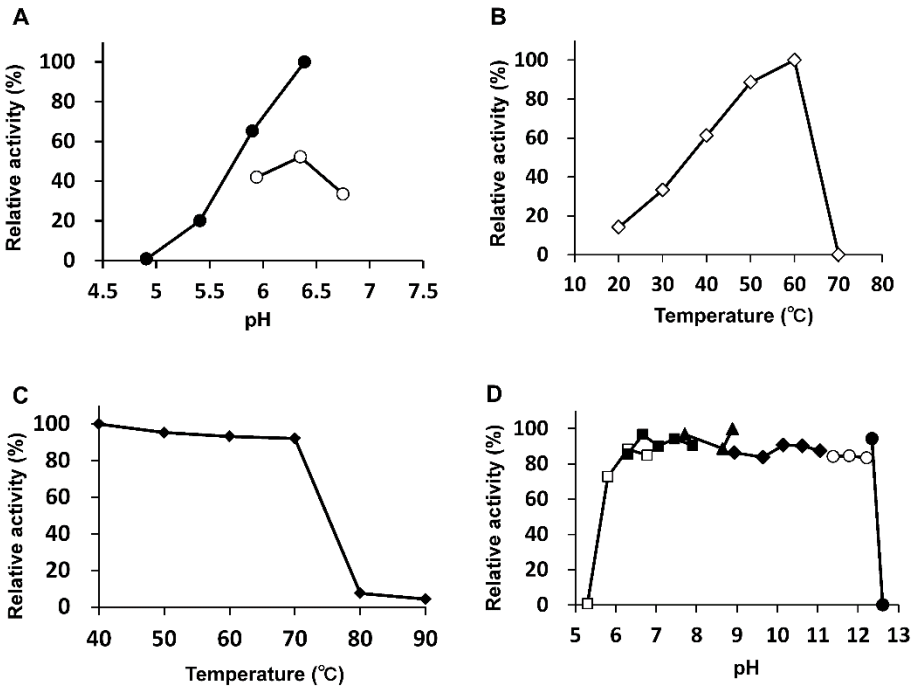
A
Relative activity (%)
100
80
60
40
20
0
4.5
5
5.5
6
6.5
7
7.5
pH
B
Relative activity (%)
100
80
60
40
20
0
10
20
30
40
50
60
70
80
Temperature (℃)
C
Relative activity (%)
100
80
60
40
20
0
40
50
60
70
80
90
Temperature (℃)
D
Relative activity (%)
100
80
60
40
20
0
5
6
7
8
9
10
11
12
13
pH

Fig. 5

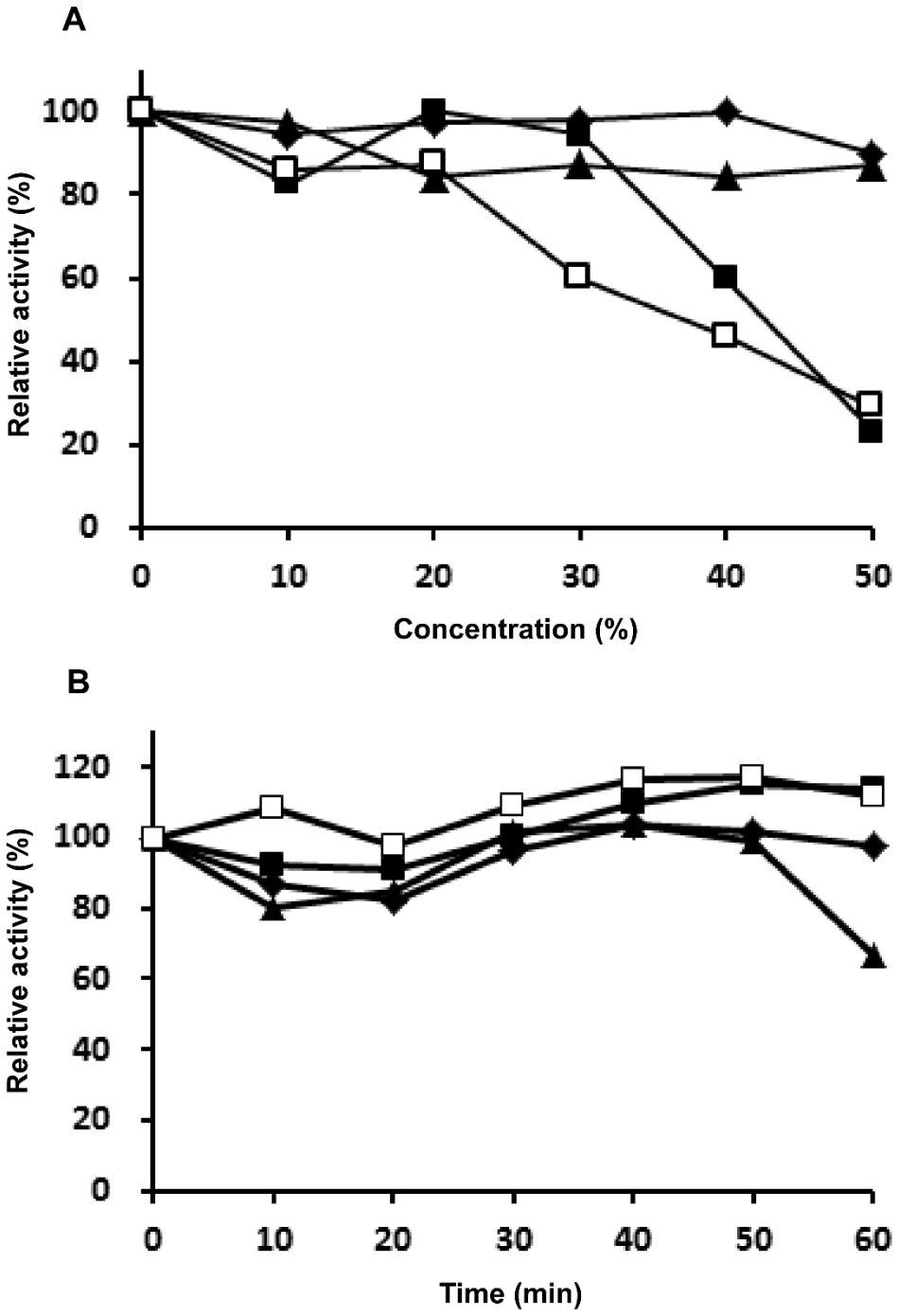
A
Relative activity (%)
100
80
60
40
20
0
0
10
20
30
40
50
Concentration (%)
B
Relative activity (%)
120
100
80
60
40
20
0
0
10
20
30
40
50
60
Time (min)

Fig. 6

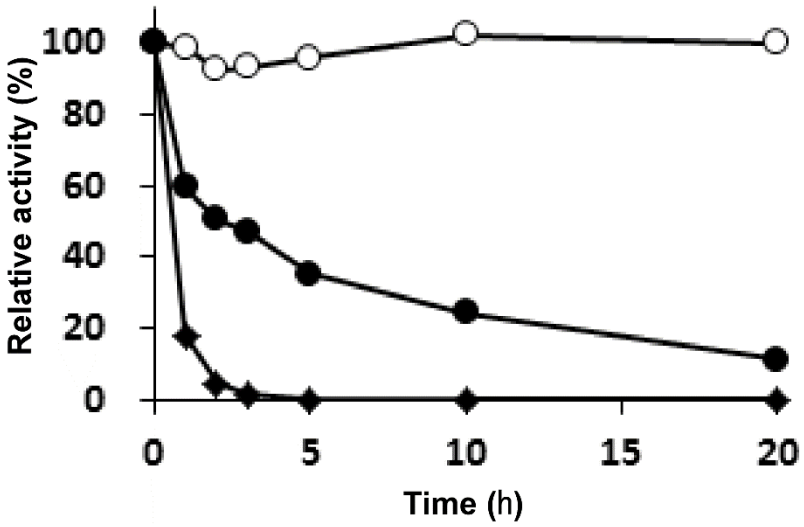

Fig. 7