Nucleic Acids Research, 1997, Vol. 25, No. 4 719–726

Characterization of a RecA/RAD51 homologue from the hyperthermophilic archaeon *Pyrococcus* sp. KOD1

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Received November 26, 1996; Revised and Accepted December 20, 1996

ABSTRACT

The *Pk-rec* gene, encoding a RecA/RAD51 homologue from the hyperthermophilic archaeon *Pyrococcus* sp. KOD1, was expressed in *Escherichia coli*. The recombinant *Pk*-REC was purified to homogeneity and was shown to be in a dimeric form. A striking property of the purified recombinant *Pk*-REC was the unusual DNase activity on both single- and double-stranded DNAs along with the ATPase activity. The reaction product of this DNase activity was mononucleotides. The optimum temperature and pH for the DNase activity were 60°C and 8–8.5, respectively. In addition, the metal ion requirement for DNase activity was different from that for the ATPase activity. The protein exhibited no DNase activity in the presence of Zn^{2+} ion, which was one of the most preferable divalent cations for ATPase activity. Another unique characteristic of the recombinant protein was that the reaction product of ATPase activity was AMP instead of ADP. *Pk*-REC may represent a common prototype of the RecA family proteins with high RecA-like activity.

INTRODUCTION

Genetic recombination is one of the processes which allows for natural selection in the evolution of organisms. Most of the genetic recombination studies have been carried out using prokaryotic systems, particularly the bacterium *Escherichia coli,* as well as some lower eukaryotes. The bacterial RecA protein is known to play an essential role in homologous recombination, DNA strand exchange, DNA repair and coprotease activity in response to DNA damage resulting in the SOS response, prophage induction and LexA cleavage (1–4). Purified RecA catalyzes the strand exchange reactions between a single-stranded DNA (ssDNA) and a double-stranded DNA (dsDNA) or between two dsDNAs, which is believed to be the central mechanism of homologous genetic recombination. RecA also demonstrates nucleoside triphosphatase activity. All of these activities have been extensively characterized *in vitro* and all require the presence of DNA and nucleoside triphosphate. In lower eukaryotes (*Saccharomyces cerevisiae*) two classes of genes are involved in genetic recombination. One class includes the RAD52 epistasis group of genes (5–7) involved in mitotic DNA repair and meiotic recombination. Among these gene products, RAD51 protein is structurally and functionally similar to RecA protein of *E.coli* (8,9). The genes belonging to the other class are essential only for meiotic recombination. Among these gene products, Dmc1 is structurally and evolutionary related to RAD51 and RecA.

Genes for RecA/RAD51 protein homologues have recently been reported from the third primary kingdom, archaea (10,11). It is of extreme interest to determine the enzymatic properties of hyperthermophilic archaeal RecA/RAD51 homologues, because hyperthermophilic archaea can grow in extreme environments which are probably similar to that of the primitive earth and, therefore, it is expected that enzymes from these cells may represent prototypes of enzymes which belong to the same protein family. Since some eukaryotic genes, such as the TATA-binding protein gene, have also been found in archaea (12–15), a similar transcriptional system seems to be shared by eukarya and archaea. Moreover phylogenetic trees based upon aminoacyl-tRNA synthetase (16), H^+-ATPase (17) and elongation factors genes (18) show that archaea and eukarya share a more common ancestor than either does with bacteria. We have previously cloned the *recA*/*rad51* homologue gene (*Pk-rec*) from the hyperthermophilic archaeon *Pyrococcus* sp. KOD1 and analyzed its sequence (11). It was found that *Pk*-REC lacks the N- and C-terminal domains as compared to other members of this protein family. It has been suggested that for the *E.coli* RecA protein the N-terminal domain contributes to its self assembly (19) and the C-terminal domain contributes to regulate the binding affinities to both single- and double-stranded DNAs (20). Nevertheless, recombinant *Pk*-REC complemented the UV light sensitivity of *E.coli recA* mutant strain (11), suggesting that *Pk*-REC is functional *in vivo* and its structure may be an ancestral prototype of bacterial and eukaryotic enzymes. In this report, we have purified recombinant *Pk*-REC and analyzed its enzymatic properties. The unique biochemical activities of this protein are discussed.

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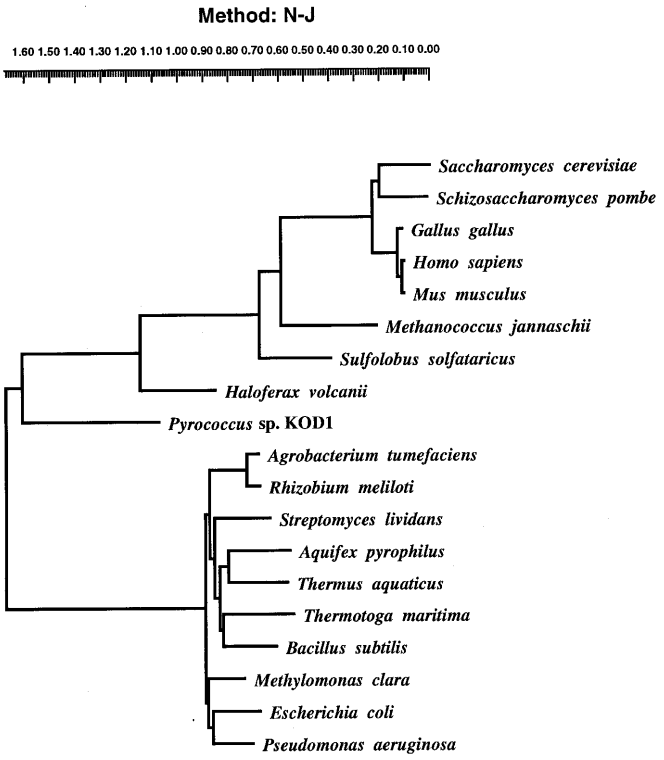


Figure 1. Phylogenetic tree for RecA/RAD51 protein like gene family estimated by neighbour joining method. The tree was constructed by the program of ODEN and Biosearch/Sinca. Segments corresponding to an evolutionary distance of 0.1 are shown. Each name at the terminus represents the species from which the protein originated.

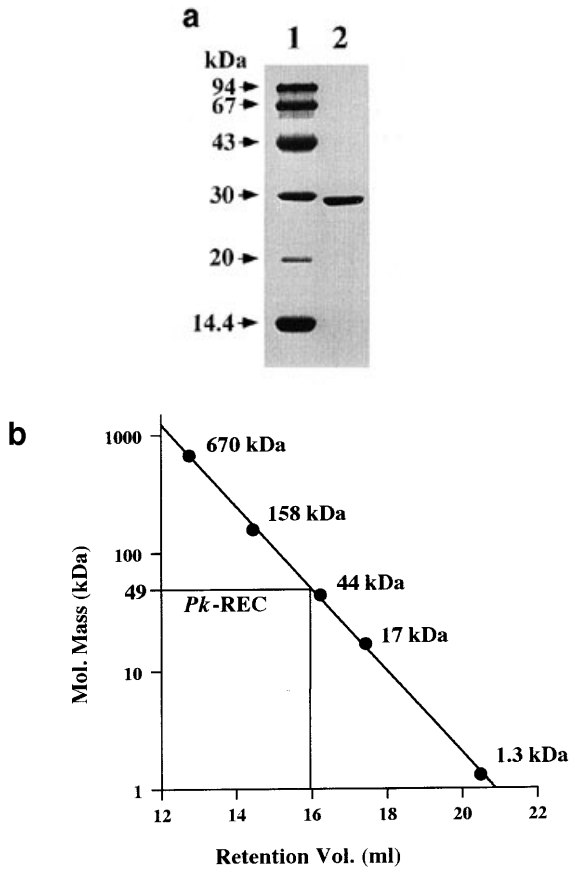


Figure 2. Homogeneity and molecular mass determination of recombinant *Pk*-REC. (**a**) Coomassie brilliant blue stained SDS–PAGE of purified recombinant *Pk*-REC. Lane 1, molecular mass standards labelled on the left side. Lane 2, purified recombinant *Pk*-REC. (**b**) Molecular mass determination by gel filtration chromatography.

MATERIAL AND METHODS

Bacterial strains and plasmids

Pyrococcus sp. KOD1 was isolated from a solfataric hot spring in Kagoshima, Japan (21). *Escherichia coli* strain JM109 was used as a host for subcloning the gene fragments and DNA manipulations. *Escherichia coli* strain HMS174(DE3)pLysS. [F⁻*recA hsdR* (r_{k12}-m_{k12}^{+})Rif^{R} (DE3)pLysS(Cm^{R})] was used as a host to express the *Pk-rec* gene by using pET-8c expression vector (Novagen, Madison, WI).

Media

L broth [10 g tryptone (Difco Lab., Detroit, MI), 5 g yeast extract (Difco Lab.) and 5 g NaCl in 1 liter of deionized water, pH 7.2) was used for *E.coli* culture. NZCYM medium (10 g NZ amine, 5 g NaCl, 5 g yeast extract, 1 g casamino acids and 2 g $MgSO_4 7H_2O$ in 1 liter deionized water pH 7.0) was used for the expression of the gene by induction with 1 mM isopropyl-β-D-thioglactopyranoside (IPTG) (Sigma, St Louis, MO). Ampicillin (Sigma) was routinely used at a final concentration of 50 μg/ml.

General DNA manipulations

Restriction endonucleases were purchased from Takara Shuzo Co. (Kyoto, Japan), DNA polymerase and T4 DNA ligase were purchased from Pharmacia Biochemicals Inc. (Uppsala, Sweden). Digestion of DNA with restriction enzymes and analysis of DNA fragments by agarose gel electrophoresis were performed under standard conditions (22). Transformation was performed by the calcium chloride procedure as described by Sambrook *et al.* (22). Mini scale preparation of *E.coli* plasmid DNA was done by the alkaline lysis method (22) and large scale plasmid DNA preparation was performed by using Qiagen plasmid Maxi kit (Qiagen Inc., Chatsworth, CA).

Phylogenetic analysis

Alignment of amino acid sequences and estimation of the number of amino acid substitutions per site or evolutionary distances between sequences of extant species was measured by calculating the proportion of amino acid difference between the sequences compared (23); positions where gaps are present in any of the aligned sequence were excluded from the analysis. Based on the evolutionary distance matrix, a phylogenetic tree was inferred by neighbour joining method. To obtain the reliability of the inferred phylogenetic tree, the bootstrap method (24) was also applied. The bootstrap resamplings were repeated 1000 times, and for each resampling a tree was inferred. The bootstrap probability that a particular tree topology occurs during the resampling was evaluated. Amino acid sequences in the conserved main central

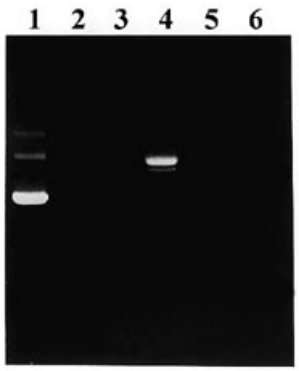


Figure 3. DNase activity of recombinant *Pk*-REC for both single- and double-stranded DNAs. In lanes 1–3 double-stranded DNA (ϕX174 vector plasmid DNA, New England Biolabs., Beverly, MA) and in lanes 4–6 single-stranded DNA (M13 mp19 phage DNA , Pharmacia) was used as a substrate. Lanes 1 and 4, a negative control (a reaction mixture from which *Pk*-REC was omitted. Lanes 3 and 6, ATP was omitted from the reaction mixture.

domain was used for the calculation with *Pk*-REC using the ODEN program (National Institute of Genetics, Mishima, Japan) and Bioresearch/Sinca (Fujitsu, Tokyo, Japan).

Production and purification of *Pk*-REC

Escherichia coli strain HMS174(DE3)pLysS carrying the expression plasmid containing *Pk-rec* gene was grown overnight at 37°C in NZCYM medium containing ampicillin. The preculture was inoculated (1%) into fresh NZCYM medium and the cultivation was continued till the optical density at 660 nm reached 0.35. The culture was then induced with 1 mM (final concentration) IPTG and incubated for another 4 h at 37°C. Cells were harvested by centrifugation at 6000 *g* for 10 min and washed with 50 mM sodium phosphate buffer (pH 7.0). The cell pellet was resuspended in the same buffer and cells were disrupted by sonication. Soluble and insoluble fractions were separated by centrifugation (15 000 *g* for 30 min). The recombinant *Pk*-REC was recovered mainly in soluble fraction and purified by HiTrap Q, Mono Q and HiTrap Heparin affinity columns (Pharmacia) with NaCl/50 mM sodium phosphate buffer of increasing NaCl molarity for elution. All purification steps were performed at 4°C. Purity of the protein was examined by SDS–PAGE using the Phast system and Phast gel (Pharmacia). A mixture of [rabbit muscle phosphorylase b (94 000), bovine serum albumin (67 000), egg white ovalbumin (43 000), bovine erythrocyte carbonic anhydrate (30 000), soya bean trypsin inhibitor (20 100) and bovine milk α-lactalbumin (14 400)] was used for molecular weight standards. Protein concentration was determined with bicinchoninic acid (BCA) protein assay kit (Pierce, Rockford, IL) according to the manufacturer's instructions using bovine serum albumin as a standard.

The molecular mass of the native enzyme was determined by analytical gel filtration chromatography using Superose 6 [HR 10/30] column (Pharmacia). Molecular weight standards [bovine thyroglobulin (670 000), bovine γ-globin (158 000), chicken ovalbumin (44 000), horse myoglobin (17 000) and vitamin B12 (1500)] were purchased from BioRad (BioRad Lab., Hercules, CA).

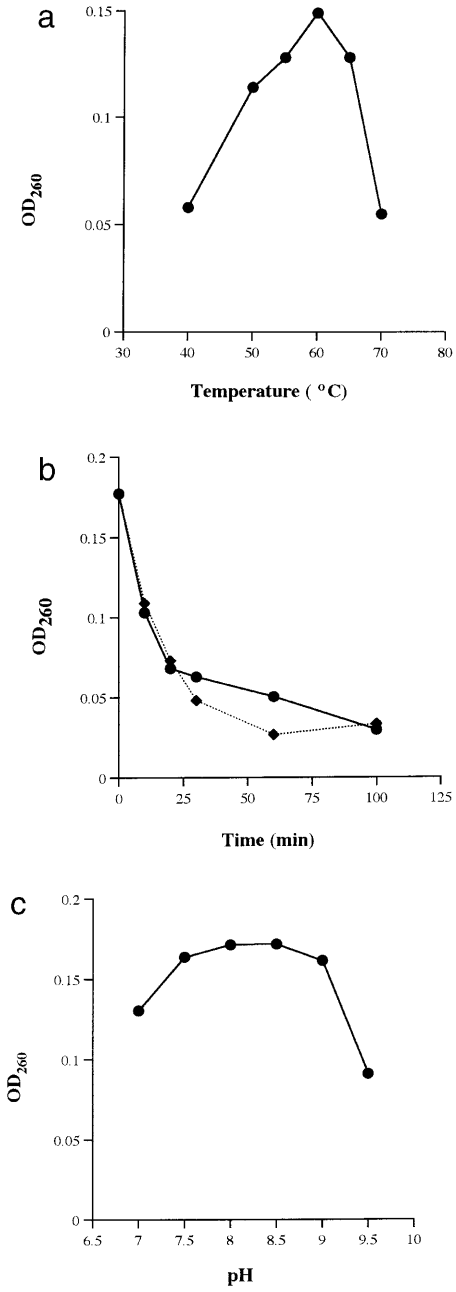
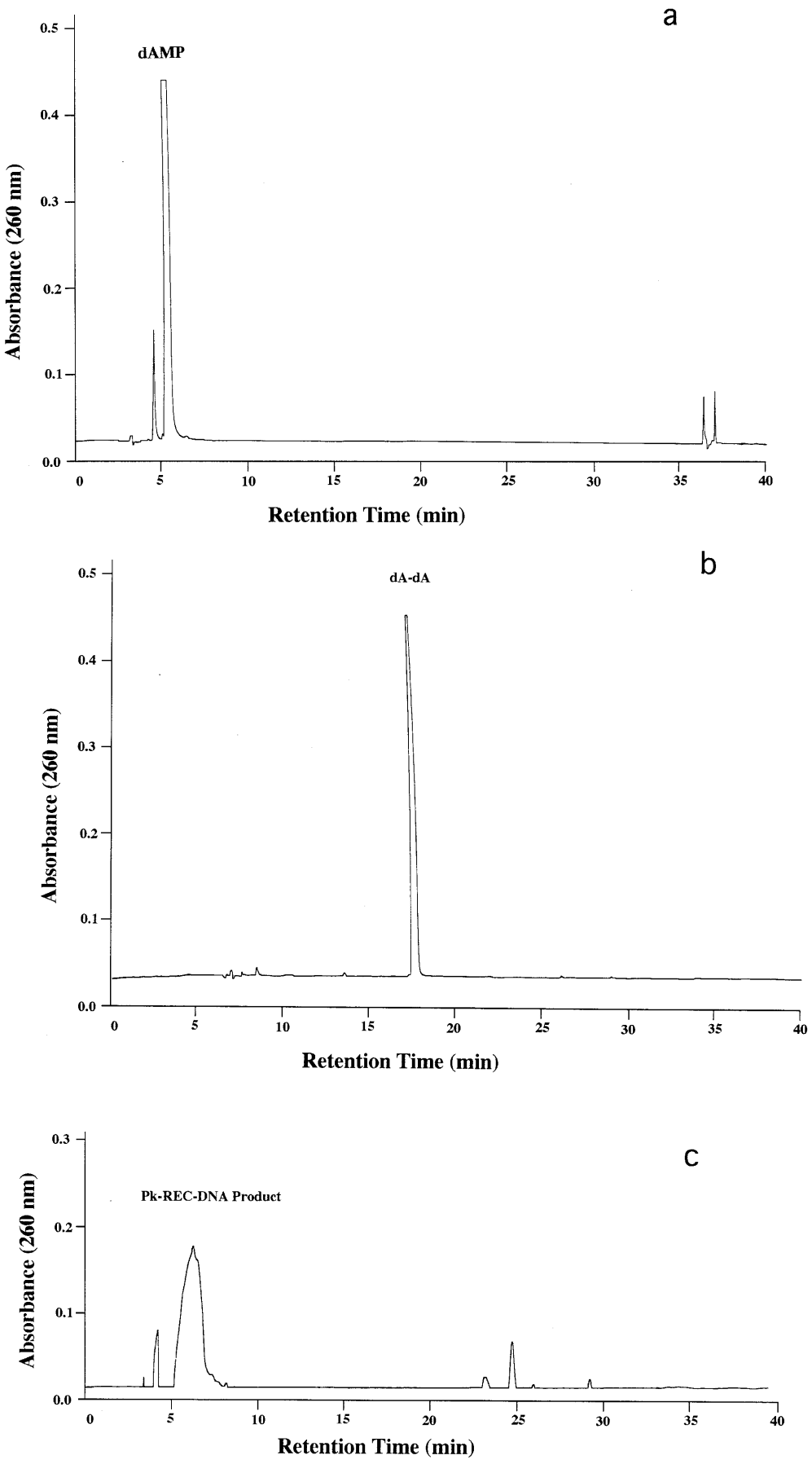


Figure 4. Characteristics of recombinant *Pk*-REC. (**a**) Effect of temperature on the DNase activity, (**b**) thermostability of the enzyme at 60°C (●) and 80°C (◆), (**c**) effect of pH on the enzyme activity.

DNase activity

Deoxyribonuclease (DNase) reaction mixture (30 µl), containing double (ϕX174 vector plasmid) or single (M13 mp19) stranded, linear or circular DNA (5 µg) and purified recombinant *Pk*-REC (0.1 µg), were incubated at 60°C for 10 min in 30 mM Tris–HCl (pH 8.0) and 10 mM $MgCl_2$. For each reaction sample, 5 µl sample loading buffer [40 mM Tris-acetate (pH 8.0), 1 mM EDTA, 50% glycerol and a trace of bromophenol blue] was added, and stored on ice to stop the reaction, and then analyzed by 1% agarose gel electrophoresis in Tris-acetate/EDTA buffer or


a
dAMP
Absorbance (260 nm)
Retention Time (min)
b
dA-dA
Absorbance (260 nm)
Retention Time (min)
c
Pk-REC-DNA Product
Absorbance (260 nm)
Retention Time (min)

15% native polyacrylamide gel in Tris-borate/EDTA buffer (22). DNA was visualized under ultraviolet light by ethidium bromide staining. DNase activity was also determined by measuring the liberated acid soluble oligo- or mononucleotides (25). For quantitative assay, the reaction mixture contained 50 µg DNA, 1 µg *Pk*-REC, 30 mM Tris–HCl (pH 8.0), 10 mM $MgCl_2$, and sterilized water to make a total volume of mixture 60 µl. The reaction was carried out at 60°C and stopped after 30 min by the addition of 60 µl acid lanthanum reagent composed of 0.02 M $La(NO_3)_3$ in 0.2 N HCl. The precipitates were removed by centrifugation, and the absorbance of the supernatant solution was measured by a model DU 260 spectrophotometer (Beckman, Fullerton, CA) at 260 nm against a blank sample incubated without enzyme. The reaction product of DNase activity was also analyzed by HPLC using Wakopak WS-DNA (4.6 × 150mm, Wako, Osaka, Japan) column with increasing concentration of acetonitrile in 0.1 M triethylamine-acetate (TEAA) buffer at pH 7.0 (50/50 v/v). Deoxyadenosine monophosphate (dAMP), deoxy-adnyryl deoxyadenosine (dA-dA) and a randomly synthesized 27 nucleotide oligomer were used as size standards. Absorbance was monitored at 260 nm.

ATPase assay

ATPase activity of *Pk*-REC was assayed in a reaction mixture containing 20 mM Tris–HCl (pH 8.0), 2 mM DTT, 0.1 mM ATP, 2 mM $MgCl_2$, 100 µg/ml BSA, 0.1 µg *Pk*-REC and water to make a final volume of 25 µl. Each reaction contained 100 nCi [α-^{32}P]ATP. Reaction mixtures were prepared on ice and incubations were at 55°C for 30 min followed by storage on ice: 1 µl was then spotted directly onto Polygram® CEL 300 PEI thin layer chromatography plates (Macherey-Nagel, GmbH & Co., Duren, Germany). The substrate and products of the reaction were separated by one dimensional chromatography using 1 M LiCl. The reaction product of ATPase activity was also examined by HPLC using TSKgel DEAE-2SW (4.6 × 250 mm) column (Tosoh, Tokyo, Japan) using an increasing molarity of sodium phosphate buffer (pH 3.0) in acetonitrile (80/20 v/v). Adenosine triphosphate (ATP), adenosine diphosphate (ADP) and adenosine monophosphate (AMP) were used as standards. Absorbance was measured at 260 nm.

Effect of metal ions

The metal ions in the purified recombinant *Pk*-REC solution were removed by chelating with 20 mM EDTA and subsequent dialysis for 36 h at 4°C against MilliQ water. DNase and ATPase activities of the dialyzed protein were examined as described above with the slight modification that Mg^{2+} was replaced by Ca^{2+}, Cd^{2+}, Co^{2+}, Cu^{2+}, Fe^{2+}, Mn^{2+}, Ni^{2+}, Pb^{2+}, Sr^{2+} or Zn^{2+} for DNase activity, and by Co^{2+} or $Zn^{2+}$for ATPase activity. The salts used to analyze the effect of metal ions on the enzymatic activities were $CaCl_2$, $CdCl_2$, $CoCl_2$, $CuSO_4$, $FeSO_4$, $MgCl_2$, $MnCl_2$, $NiCl_2$, $PbCl_2$, $SrCl_2$ and $ZnCl_2$.

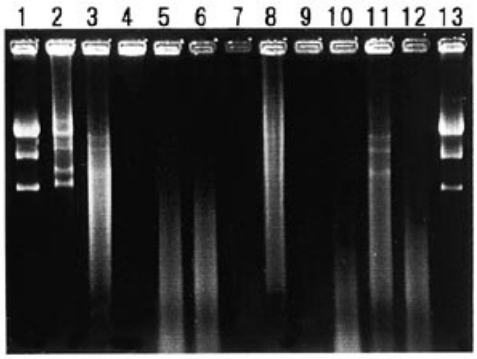


Figure 6. Effect of metal ion on the DNase activity of recombinant *Pk*-REC. Recombinant *Pk*-REC was treated with 20 mM EDTA and dialyzed overnight before use. Reactions were carried out without *Pk*-REC (lane 1), without any metal ion (lane 2), and in the presence of 10 mM (final concentration) of Ca^{2+} (lane 3), Co^{2+} (lane 4), Cd^{2+} (lane 5), Cu^{2+} (lane 6), Fe^{2+} (lane 7), Mn^{2+} (lane 8), Mg^{2+} (lane 9), Ni^{2+} (lane 10), Pb^{2+} (lane 11), Sr^{2+} (lane 12) and Zn^{2+} (lane 13).

RESULTS

Phylogenetic analysis

The phylogenetic tree was constructed by comparing the amino acid sequence of *Pk*-REC with those of known RecA/RAD51 homologues from bacteria, eukaryotes and other archaea. This phylogenetic tree, shown in Figure 1, reveals that KOD1 obviously diverged from other organisms before eukaryotes, bacteria and other archaea with an inclination for archaea. This result suggests that *Pk-rec* gene is a common prototype of *recA*-like gene family.

Pk-rec gene expression in *E.coli*

In order to characterize the enzymatic properties of *Pk*-REC, the encoding gene was overexpressed by utilizing the T7 promoter expression system of *E.coli* strain HMS174(DE3)pLysS. Gene expression was induced with 1 mM IPTG at 37°C and *Pk*-REC was successfully produced in soluble form. Most of the host proteins were precipitated by heat treatment at 80°C for 15 min. The *Pk*-REC protein, in the soluble fraction after heat treatment, was purified by ion exchange and affinity chromatography. The protein was eluted at 0.35 M NaCl (pH 7.0) through MonoQ column and at 0.45 M NaCl (pH 7.0) through HiTrap Heparin affinity column. The purified recombinant *Pk*-REC protein gave a single band on SDS–PAGE (Fig. 2a). The molecular weight of the protein estimated from SDS–PAGE is ~28 kDa while the weight calculated from amino acid sequence is 24 kDa. In order to examine whether the protein exists in monomeric form, the molecular weight of the protein in the native state was analyzed

Figure 5. Reaction product of DNase activity of recombinant *Pk*-REC. Randomly synthesized 27 nucleotide double-stranded DNA fragment was used as a substrate and the reaction product was analyzed by HPLC using Wakopak WS-DNA column. dAMP and dA-dA shown in (**a**) and (**b**), respectively, were used as standard markers for HPLC elution comparison, (**c**) shows the reaction product of *Pk*-REC DNase activity.

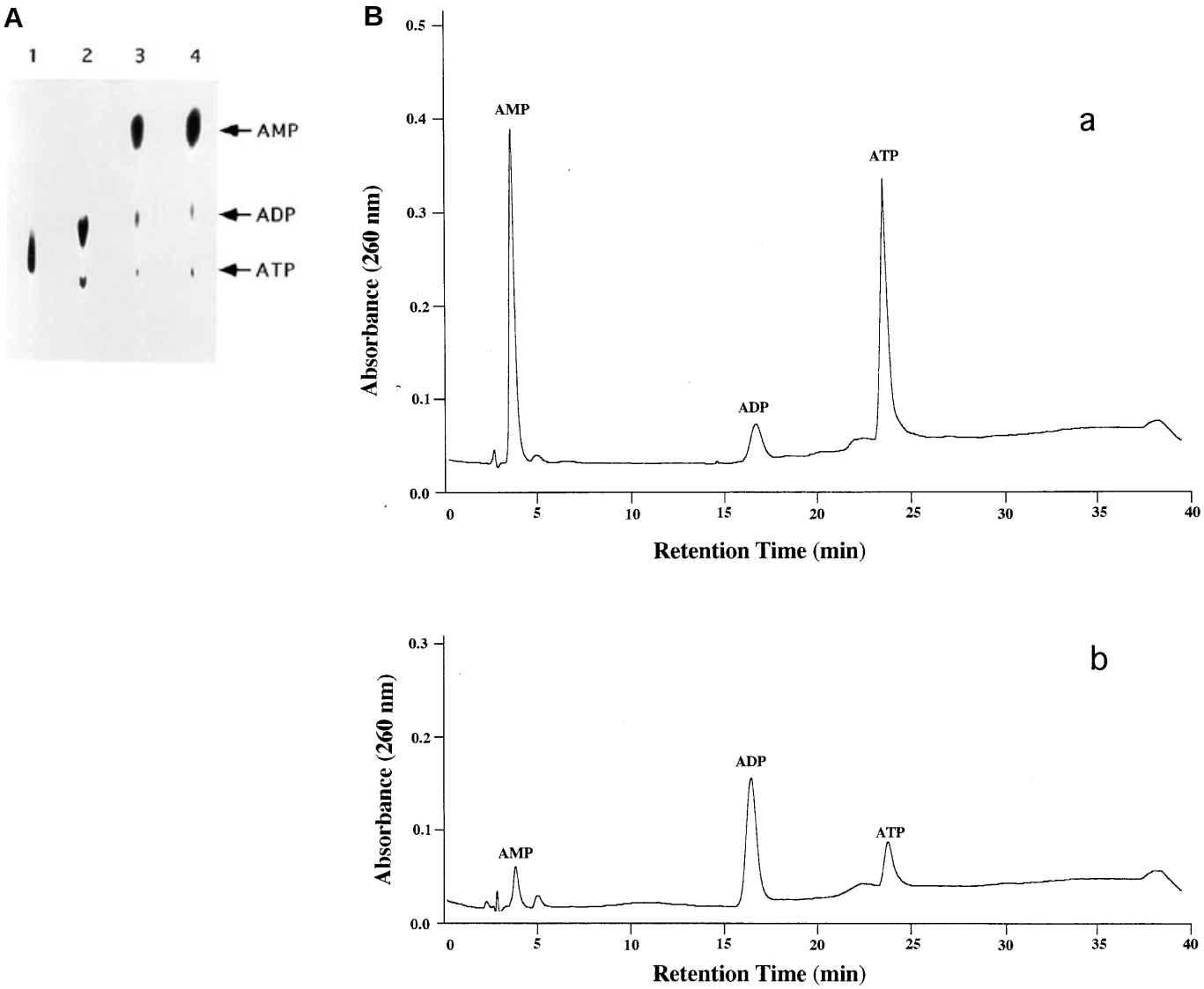


Figure 7. Reaction product of ATPase activity of *Pk*-REC. (**A**) Separation of the reaction product by thin layer chromatography. Lane 1, negative control with no *Pk*-REC protein. Lane 2, positive control containing *E.coli* RecA protein instead of *Pk*-REC. Lane 3, *Pk*-REC was added in the reaction mixture with ϕX174 DNA (120 µM). Lane 4, Reaction mixture containing *Pk*-REC without DNA. (**B**) Analysis of the reaction product by TSKgel DEAE-2WS column chromatography in comparison with *E.coli* RecA, (a) *Pk*-REC (b) *E.coli* RecA. Small peak at AMP in (b) was resulted from non-enzymatically degraded fraction of ATP.

by gel filtration chromatography, as well. It was determined to be 49 kDa (Fig. 2b), suggesting that *Pk*-REC exists as a dimer.

DNase activity

When the purified recombinant *Pk*-REC was tested for its DNA binding activity, a common and basic characteristic of RecA protein family, we found that it has DNA degrading (DNase) activity. DNase activity was detected both for single- and double-stranded DNAs (Fig. 3). It was also observed that DNase activity of *Pk*-REC is independent of ATP. *Pk*-REC has no detectable bias for either double-stranded or single-stranded DNA. When RNA, instead of DNA, was used as a substrate, no nuclease activity was observed. Enzyme activity was further assayed at various temperatures and 60°C proved to be the optimum (Fig. 4a). Heat stability of the enzyme was examined at 60 and 80°C and it was found that the enzyme is rather heat labile having an enzymatic half life of 20 min for both 60 and 80 °C (Fig. 4b). The pH profile of *Pk*-REC activity showed a broad plateau extending from pH 7.5 to 9 with maximal activity at pH 8–8.5 (Fig. 4c). The final reaction product of *Pk*-REC DNase activity appeared to be mononucleotides. This was observed in the reaction using a 27 bp double-stranded DNA fragment as a substrate and analyzing the reaction product on 15% native polyacrylamide gel (data not shown) as well as on HPLC (Fig. 5). When a dinucleotide (dA-dA) was used as a substrate, no activity was observed. Concerning the ionic strength, DNase activity of *Pk*-REC gradually decreased as the concentration of Tris–HCl in the reaction mixture increased beyond 60 mM. It was fully inhibited in the presence of 200 mM Tris–HCl. When the enzyme activity was examined in the presence of Ca^{2+}, Co^{2+}, Cd^{2+}, Cu^{2+},

Fe^{2+}, Mn^{2+}, Mg^{2+}, Ni^{2+}, Pb^{2+}, Sr^{2+} and Zn^{2+}, *Pk*-REC exhibited DNase activity to a variant degree in the presence of all these tested metal ions except for Zn^{2+}. No DNase activity, even no binding, was observed in the presence of Zn^{2+} (Fig. 6). In order to confirm that the observed DNase activity is not derived from any undetectable contaminant from the host cells, the comparable amounts of host cells carrying the vector only were treated in the same way as the cells containing the *Pk-rec* gene. The crude lysate was treated for 15 min at 80°C and the resulting supernatant was assayed for DNase activity. No DNase activity was observed.

ATPase activity

ATP hydrolyzing activity by *Pk*-REC was compared in the presence and absence of DNA and no detectable difference was observed. These results indicate that, unlike other RecA proteins, the ATPase activity of *Pk*-REC is independent of both single-stranded and double-stranded DNAs. The reaction product of *Pk*-REC activity was migrated on TLC plates with a R_f value equivalent to AMP (Fig. 7A). The reaction product was further analyzed by HPLC and there it also eluted with a retention time completely matching AMP (Fig. 7B). Some of the divalent metal cations (Co^{2+}, Mg^{2+} and Zn^{2+}) were compared as a metal cofactor for *Pk*-REC ATPase activity. Of them Zn^{2+} proved to be the most effective (data not shown).

DISCUSSION

The gene locus encoding *Pk*-REC was found adjacent to the 16S rRNA and ribosephosphate pyrophosphokinase genes (unpublished data) on the KOD1 genome. *Pk*-REC consists of only a central domain of proteins in this family from bacteria, eukarya, and even from other archaea (11) and is almost equally apart from bacteria and eukarya with slight inclination towards the latter. Putative proteins named RadA from acidophilic, halophilic and methanogenic hyperthermophilic archaea are inclined to a higher degree towards eukarya (Fig. 1) (10) and are larger in size as compared to *Pk*-REC (11). The amino acid sequence comparison suggested *Pk*-REC to be a common prototype of RecA/RAD51 group proteins. RecA family members usually exist in aggregates and N-terminal domain in RecA is reported to contribute for its self assembly (19,26). Since *Pk*-REC lacks the N-terminal domain of RecA, it is understandable that it does not aggregate in a multimeric form but instead forms a dimer. Since the previous work on the C-terminal truncated RecA protein showed enhanced DNA binding activities (20), the comparison of *Pk*-REC with RecA suggested it to have high RecA like activity. In fact, it complemented *recA* defect of *E.coli in vivo* (11). When the purified recombinant *Pk*-REC protein was examined for its DNA binding activity, we found that it had DNase activity and this activity was specific for deoxyribose sugar since no activity was observed when RNA was used as a substrate. The optimum temperature for enzyme activity was shown to be 60°C, although the optimum growth temperature of the strain KOD1 is 95°C. This suggests the existence of a protein stabilizing system in KOD1 cells. The reaction product of DNase activity was shown to be a mononucleotide, indicating that *Pk*-REC cleaves the phosphodiester bond non-specifically. When a dinucleotide (dA-dA) was used as a substrate, no cleavage was observed, probably because the reaction rate was dramatically reduced. This phenomenon is a common characteristic of other non specific DNases.

It was found that Mg^{2+} can be replaced by other divalent cations, such as Ca^{2+}, Cd^{2+}, Cu^{2+}, Mn^{2+}, Ni^{2+}, Pb^{2+} and Sr^{2+} but DNase activity was reduced to some extent, however when Mg^{2+} was substituted with Co^{2+} or Fe^{2+} no detectable change was observed. It should be noted that the protein exhibits little DNase activity in the presence of Zn^{2+} ion. Because the protein exhibits a very weak DNase activity even in the absence of any metal ion, it seems likely that Zn^{2+} ion binds to the Mg^{2+} binding site of the protein and inhibits the DNase activity.

ATP hydrolysis is a common characteristic of the RecA protein family with ADP as the reaction product and DNA is usually required as a cofactor (27). Moreover, it was shown that the ATP hydrolyzing reaction by *Pk*-REC is independent of DNA. Likewise, the DNase activity does not depend on ATP. The analyses of metal ion requirement showed that Zn^{2+} was not utilized as a metal cofactor for DNase activity but was the most effective metal cofactor for the ATPase activity. This may suggest that the protein possesses two different catalytic sites for DNase and ATPase activities. Alternatively, these catalytic sites may be overlapped and the protein possesses only one metal binding site. Preference of the enzyme for metal ions may vary for different substrates. The DNase activity is not due to contamination of nucleases from the *E.coli* cells, since the cell lysate prepared from *E.coli* HMS174(DE3)pLysS in the similar way as *Pk*-REC has no DNase activity. Furthermore, when double amino acid replacements were introduced in the ATPase active site (positions correspond to Lys 72 and Thr 73 in *E.coli* RecA), the mutated protein lost all ATPase activity and showed a dramatic decrease in DNase activity (20-fold decrease in V_{max}) (personal communication). This result strongly demonstrates that *Pk*-REC has both ATPase and DNase activities and the active sites of these activities seems closely related. Another unique characteristic of *Pk*-REC was its reaction product of ATPase activity. The reaction product of RecA like ATPase activity from both bacteria and eukarya is ADP while the major reaction product of *Pk*-REC ATPase activity is AMP. Since ADP is also detected as a minor product, *Pk*-REC may be able to cleave both sites of the β-phosphate group in ATP. Previously, *Pk*-REC was shown to complement the UV sensitivity of an *E.coli recA* null mutant strain. All of these results demonstrate that *Pk*-REC is highly functional both *in vivo* and *in vitro*. Archaea in general and hyperthermophilic archaea in particular, are thought to evolve from, and still live in environments very similar to that of primitive earth. Cells at that time might have been exposed to harsh conditions including fairly strong UV light. Under this stress it would be essential to maintain effective DNA repair systems. From the perspective of evolution, the primitive highly thermal environments might have given rise to this unique RecA like characteristic followed by the transfer of DNase activity to other enzymes with the reduction of growth temperatures and the development of metabolic systems.

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