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Full Length Research Paper

Assessing the role of RecA protein in the radioresistant bacterium *Deinococcus geothermalis*

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The moderately thermophilic bacterium *Deinococcus geothermalis* exhibits extraordinary resistance to ionizing radiation. RecA protein is considered to be one of the most important participants in radioresistance. To assess the role of the RecA protein in *D. geothermalis*, the recA gene was isolated from *D. geothermalis* and over expressed in *Escherichia coli*. After the *D. geothermalis* RecA protein (GeoRecA) was purified, the recombination activity was investigated *in vitro*. GeoRecA efficiently promoted the strand exchange reaction between homologous linear double-stranded DNA and circular single-stranded DNA substrates at 50°C. Like *Deinococcus radiodurans* RecA protein (DraRecA), GeoRecA could promote DNA strand exchange reaction through normal and inverse pathways. Furthermore, GeoRecA complemented the RecA deficiency of *D. radiodurans*. These results indicate that GeoRecA is a functional homologue of DraRecA and plays an important role in radioresistance. However, unlike DraRecA, GeoRecA could not complement the RecA deficiency of *E. coli*, suggesting that GeoRecA require more strict intracellular conditions than DraRecA does to fulfill its function. This study provides new insights into the role of deinococcal RecA protein in radioresistance.

Key words: *Deinococcus geothermalis*, DNA repair, DNA strand exchange, radio resistance, RecA.

INTRODUCTION

For most organisms, DNA double-strand breaks (DSB) generated by ionizing radiation are lethal or lead to mutagenic effects on cells. However, bacteria of the genus *Deinococcus* that comprises more than 40 distinct species are known for exhibiting extraordinary resistance to ionizing radiation. Among them, only *Deinococcus geothermalis* and *Deinococcus murrayi* are thermophilic bacteria that were isolated from the hot spring at Italy and Portugal, respectively (Ferreira et al., 1997). Although the whole-genome sequence of *D. geothermalis* has been determined (Makarova et al., 2007), the radio resistance mechanisms of *D. geothermalis* remain unclear.

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In *Deinococcus radiodurans* which is the best-studied species among the members of *Deinococcus*, *RecA*-deficient strains exhibited extreme sensitivity to ionizing radiation (Gutman et al., 1994; Narumi et al., 1999; 2001; Satoh et al., 2006).

RecA proteins are highly conserved in almost all microorganisms except for *Buchnera* spp., *Vesicomyosocius okutanii* and *Ruthia magnifica* (Shigenobu et al., 2000; Kuwahara et al., 2007; Newton et al., 2007). The *Escherichia coli RecA* protein (EcoRecA) is one of the best-studied enzymes (Stohl et al., 2002). EcoRecA plays a central role in the repair of stalled replication forks, DSB repair, general recombination, induction of the SOS response and SOS mutagenesis (Cox, 2003). *RecA* protein is one of the most important participants in the radio resistance of *D. radiodurans*. The *D. radiodurans RecA* protein (DraRecA)

could promote the DNA strand exchange reaction and complement *E. coli* RecA deficiency (Narumi et al., 1999; Satoh et al., 2002), indicating that DraRecA and EcoRecA are functional homologues. However, EcoRecA and DraRecA promote the DNA strand exchange reaction by quite different pathway (Kim and Cox, 2002). EcoRecA is ordered such that the single-stranded DNA (ssDNA) is bound first, followed by the double-stranded DNA (dsDNA). In contrast, DraRecA binds the dsDNA first and the homologous ssDNA substrate second. It is believed that the inverse pathway of DraRecA might remarkably be related to the efficient DNA repair mechanism. However, whether the peculiar property of DraRecA is common in RecA proteins of other members of the genus *Deinococcus* is still an open question.

The aim of this study is to assess the role of the *D. geothermalis* RecA protein (GeoRecA) in radioresistance and to highlight the functional similarity and dissimilarity between GeoRecA and DraRecA. To accomplish this purpose, we purified GeoRecA and investigated recombination activity *in vitro*. Furthermore, we employed plasmid complementation assay for GeoRecA. We also employed plasmid complementation assay to assess the *in vivo* function of GeoRecA following γ irradiation.

MATERIALS AND METHODS

Strains, plasmids and growth conditions

The bacterial strains and plasmids used in this study are listed in Table 1. *D. radiodurans* and *D. geothermalis* cells were grown at 30 and 45°C, respectively, in TGY broth containing 0.5% tryptone-peptone, 0.1% glucose and 0.3% yeast extract with agitation, or on TGY agar solidified with 1.5% agar. Three µg/ml of chloramphenicol was supplemented to the medium in order to maintain plasmids in *D. radiodurans* cells. *E. coli* strains were grown in Luria-Bertani (LB) broth-Miller or on LB agar-Lennox at 37°C. For the selection of *E. coli* cells transformed with plasmids, 100 µg/ml of ampicillin was added to the medium.

Cloning and construction of expression plasmid of *D. geothermalis recA*

To amplify the *recA* gene (*GeorecA*) from the *D. geothermalis* DSM11300 genomic DNA by PCR reaction, priming was carried out using *PfuTurbo* HotStart DNA polymerase (Stratagene) with primers GeorecA-5Nde (5'-GAGACCATATGAGCAAGGACAACCC-3') and GeorecA-3Bam (5'-GCCCGGATCCCTCACCGCTTACTCT-3') possessing *Nde*I and *Bam*HI restriction sites (underlined in the sequences), respectively. PCR product (1,077 bp) was then digested with *Nde*I and *Bam*HI to adapt the termini for in-frame insertion of *GeorecA* into the *Nde*I-*Bam*HI sites in the pET3a vector (Novagen). The resulting expression plasmid was designated pET3a-GeorecA.

The *GeorecA* expression plasmids in *D. radiodurans* were constructed as follows. The *Nde*I and *Bam*HI-digested *GeorecA* PCR product was inserted into the *Nde*I-*Bam*HI sites in the pRAD1 vector (Meima et al., 2000). The resulting plasmid was designated pRGE1. The *pprA* promoter region (208 bp) was amplified by PCR from pZA8 (Narumi et al., 2004) with primers PDpprAF2 and PDpprAR1 (Ohba et al., 2005), digested with *Nde*I, and inserted into the *Nde*I-site in the pRGE1 to yield plasmid pGEO5.

To amplify the promoter region and *recA* gene (*EcorecA*) from the *E. coli* JM110 genomic DNA by PCR reaction, priming was carried out using *PfuTurbo* HotStart DNA polymerase (Stratagene) with primers ErecAF1 (5'-TGATGGGGAAAACTCGCA-3') and ErecAR1 (5'-CACGATCTGTGACGTCCTT-3'). PCR product (5,539 bp) was inserted into the *Sma*I site in the pUC19 vector (Takara Bio). The resulting plasmid was designated pECrecA11. The *EcorecA* expression plasmids in *E. coli* were constructed as follows. The DNA region containing *EcorecA* promoter and structural gene (2,154 bp) was amplified by PCR from pECrecA11 and primers ECrecAp-F1 (5'-GCGGCGACGGGCATATCAAC-3') plus ECrecAR2 (5'-AACAGGATCCTTAAAAATCTTCG-3'), digested with *Bam*HI, and inserted into the *Bam*HI-site in the pUC19 to yield plasmid pH-EcorecA.

The *GeorecA* expression plasmids in *E. coli* were constructed as follows. The upstream of *EcorecA* region (1,102 bp) including promoter was amplified by PCR from pECrecA11 and primers ECrecAp-F1 and ECrecAp-R1 (5'-CGATAGCCATATGTACTCCTGTCATGCC-3') and digested with *Nde*I and *Bam*HI. The *Nde*I and *Bam*HI-digested *GeorecA* and *EcorecA* promoter PCR products were inserted into the *Bam*HI-site in the pUC19 to yield plasmid pH-GeorecA.

The DNA sequence of the expression plasmids was checked to confirm the absence of errors.

Bioinformatics

Homology search was done using bioinformatics tools on the web site:http://www-archbac.u-psud.fr/genomics/GenomicsToolBox.html. FeatureMap3D helped the analysis of homologous structures in the PDB (Wernersson et al., 2006).

Protein purification

E. coli BLR(DE3) carrying pET3a-GeorecA was cultivated in LB broth-Miller containing ampicillin. At an optical density of approximately 0.4 at 600 nm, isopropyl--D-1-thiogalactopyranoside was added to a final concentration of 0.4 mM and growth was continued for an additional 3 h. The cells were then harvested by centrifugation. The cell pellet was resuspended in cold buffer containing 50 mM Tris-HCl (pH 8.0), 5 mM ethylenediaminetetraacetic acid (EDTA), 1 mM phenylmethylsulfonyl fluoride and 0.1% protease inhibitor cocktail (Calbiochem), and stored at –80˚C. All subsequent steps were carried out at 4˚C unless otherwise indicated. The suspension was lysed by sonication for 10 min on ice. The debris was removed by centrifugation at 7,000 g, 4˚C for 30 min and ammonium sulfate was added slowly to the supernatant to give 20% saturation. The supernatant was stirred for 1 h and then centrifuged for 30 min. Protein was further purified to apparent homogeneity by column chromatography on Toyopearl Phenyl-650S (Tosoh), HiTrap Heparin (GE Healthcare) and Mono Q HR 5/5 (GE Healthcare). The pooled fractions were concentrated and desalted using an Amicon Ultra-15 filter 30K Centrifugal Filter Device (Millipore) with 50 mM Tris-HCl (pH 7.4).

DNA strand exchange assay

The strand exchange reaction was assayed by assembling the following reaction mixture. First, 20 µM φX174 viral DNA (ssDNA) (New England Biolabs) was preincubated in a buffer containing 20 mM Tris-HCl (pH 7.4), 1 mM dithiothreitol, 10 mM $MgCl_2$, 2 mM ATP, and 0.9 µM *E. coli* single-stranded DNA binding protein (SSB) (GE Healthcare) at 37°C for 5 min, after which, 34 µM GeoRecA protein

Table 1. Strains and plasmids used in this study.

Designation	Relevant description	Source or reference
D. radiodurans		
R_1	Wild-type (ATCC13939)	ATCC
MR_1	Wild-type	Moseley (1967)
rec30	Same as MR_1 but *RecA670*	Narumi et al. (1999)
D. geothermalis	Wild-type (DSM11300)	DSMZ
E. coli		
JM110	Host for plasmid subclones, *recA*$^+$	Takara Bio
JM107	Host for plasmid subclones, *recA*$^+$	Takara Bio
JM109	Host for plasmid subclones, *recA1*	Takara Bio
BLR(DE3)	Host for expression plasmid	Novagen
Plasmid		
pET3a	*E. coli* vector	Novagen
pET3a-GeoRecA	pET3a with *Nde*I-*Bam*HI::1,077-bp PCR product containing *GeorecA*	This study
pRAD1	*E. coli-D. radiodurans* shuttle vector	Meima et al. (2000)
pRGE1	pRAD1 with *Nde*I-*Bam*HI::1,077-bp PCR product containing *GeorecA*	This study
pZA8	pUC19 with 6,005-bp *D. radiodurans* DNA fragment containing *pprA*	Narumi et al. (2004)
pGEO5	pRGE1 containing *D. radiodurans pprA* promoter region and *GeorecA*	This study
pUC19	*E. coli* vector	Takara Bio
pECRecA11	pUC19 with 5,539-bp *E. coli* DNA fragment containing *recA*	Takara Bio
pH-ecoRecA	pUC19 containing *E. coli recA* promoter region and *EcorecA*	This study
pH-GeoRecA	pUC19 containing *E. coli recA* promoter region and *GeorecA*	This study

was added. The incubation continued for an additional 5 to 60 min at various temperatures from 37 to 70°C and the reaction was initiated by adding 30 µM *Hinc*II-digested fragment of *φ* X174 RF I (dsDNA) (New England Biolabs). The reaction was quenched by the addition of a buffer consisting of 20 mM Tris-HCl (pH 7.4), 0.5% SDS, 40 mM EDTA and 2 mg/ml of proteinase K (Qiagen). After being incubated at 37°C for 30 min, the sample was subjected to 1% agarose gel electrophoresis. The substrate DNA and strand exchange products were visualized by staining with ethidium bromide (0.5 µg/ml).

Measurement of cell survival rate

D. radiodurans cells were grown in TGY broth at 30°C with agitation to early stationary phase. Cells were harvested by centrifugation at 7,000 g, 4°C for 5 min, washed twice with 10 mM sodium phosphate buffer (PB; pH 7.0) and resuspended in the same buffer. Aliquots (0.1 ml) of the cell suspension were dispensed into test tubes and irradiated at room temperature for 1 to 2 h with ^{60}Co γ rays at dose rates from 0.1 to 4 kGy per h that were regulated by changing the distance of the samples from the γ ray source. Irradiated samples were appropriately diluted with 10 mM PB, spread onto TGY agars and incubated at 30°C for 3 days prior to the enumeration of colonies.

E. coli cells were grown at 37°C in LB broth-Miller, harvested, washed and resuspended as described above. Aliquots (0.1 ml) of the cell suspension were irradiated at room temperature for 1 h with ^{60}Co γ rays at dose rates from 0.1 to 0.2 kGy per h. Irradiated sample was diluted appropriately with the same buffer, spread on LB Agar-Lennox and incubated for 18 h at 37°C prior to the enumeration of colonies.

RESULTS AND DISCUSSION

Sequence and structure of *D. geothermalis* RecA

To construct the expression plasmid, we cloned the *recA* gene from the thermophilic radio resistant bacterium *D. geothermalis* (GeoRecA). The predicted GeoRecA is consistent with the amino acid sequence of *D. geothermalis* DSM11300 RecA protein (GenBank monomer protein contains 358 amino acid residues and

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EcoRecA   1     MAIDENK-----------QKALAAALGQIEKQFGKGSIMRLGEDRSMDVETISTGSLSLD   49
DraRecA   1     MSKDATKEISAPTDAKERSKAIETAMSQIEKAFGKGSIMKLGAESKLDVQVVSTGSLSLD   60
GeoRecA   1     MSKDNPKDFGTPSDSKERLKAIETAMTQIEKAFGKGSIMRLGAESKLDVQAVSTGSLSLD   60
                *  *  *             **    *  **** ******* **      **   ********

EcoRecA   50    IALGAGGLPMGRIVEIYGPESSGKTTLTLQVIAAAQREGKTCAFIDAEHALDPIYARKLG   109
DraRecA   61    LALGVGGIPRGRITEIYGPESGGKTTLALAIVAQAQKAGGTCAFIDAEHALDPVYARALG   120
GeoRecA   61    LALGVGGIPRGRITEIYGPESGGKTTLALSVIAQAQRAGGTCAFIDAEHALDPVYARSLG   120
                 *** ** * *** ******* ***** *   * **  * ************** *** **

EcoRecA   110   VDIDNLLCSQPDTGEQALEICDALARSGAVDVIVVDSVAALTPKAEIEGEIGDSHMGLAA   169
DraRecA   121   VNTDELLVSQPDNGEQALEIMELLVRSGAIDVVVVDSVAALTPRAEIEGDMGDSLPGLQA   180
GeoRecA   121   VNTDELLVSQPDNGEQALEIMELLVRSGAIDVVVVDSVAALTPRAEIEGEMGDSLPGLQA   180
                *  * ** **** *******   * **** ** ********** *****  ***  ** *

EcoRecA   170   RMMSQAMRKLAGNLKQSNTLLIFINQIRMKIGVMFGNPETTTGGNALKFYASVRLDIRRI   229
DraRecA   181   RLMSQALRKLTAILSKTGTAAIFINQVREKIGVMYGNPETTTGGRALKFYASVRLDVRKI   240
GeoRecA   181   RLMSQALRKLTAILSKTGTAAIFINQVREKIGVMYGNPETTTGGRALKFYASVRLDVRKI   240
                * **** ***   *    *  ***** * ***** ********* ********** * *

EcoRecA   230   G-AVKEGENVVGSETRVKVVKNKIAAPFKQAEFQILYGEGINFYGELVDLGVKEKLIEKA   288
DraRecA   241   GQPTKVGNDAVANTVKIKTVKNKVAAPFKEVELALVYGKGFDQLSDLVGLAADMDIIKKA   300
GeoRecA   241   GQPVKLGNDAVGNTVKVKTVKNKVAPPFKEVELTLLYGKGFDQLSDLVTLAADMDIIKKA   300
                *   * *   *       * **** * ***  *    ** *      ** *      * **

EcoRecA   289   GAWYSYKGEKIGQGKANATAWLKDNPETAKEIEKKVRELLLSNPN--STPDFSVDDSEGV   346
DraRecA   301   GSFYSYGDERIGQGKEKTIAYIAERPEMEQEIRDRVMAAIRAGNAGEAPALAPAPAAPEA   360
GeoRecA   301   GSFYSYGEERIGQGKEKAIAYIAERPELEQEIRDRVLAAIKEGRD----PIAAVPETPAL   356
                *  ***  * *****    *     **   **   *

EcoRecA   347   AETNEDF                                                       353
DraRecA   361   AEA----                                                       363
GeoRecA   357   AE-----                                                       358
                **
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Figure 1. Multiple amino acid sequence alignment of EcoRecA, DraRecA and GeoRecA. Multiple alignment was determined using the CLUSTAL W program (Thompson et al., 1994), EcoRecA, *E. coli* RecA (Horii et al., 1980), DraRecA, *D. radiodurans* RecA (Narumi et al*.,* 1999) and GeoRecA, *D. geothermalis* RecA (this study). Dashes indicate gaps in the alignment. Numbers on both sides represent the coordinates of each protein. Asterisks indicate identical residues. Boxes and over lines represent positions of polymeric domains and DNA binding domains respectively, which are proposed for EcoRecA, (Karlin and Brocchieri, 1996).

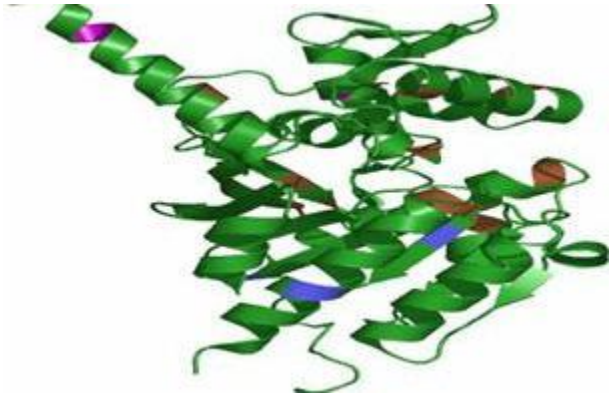

Figure 2. The PDB structure 1XP8 of the homologous protein *D. radiodurans* RecA. Green, Perfect match with GeoRecA (*D. geothermalis* RecA); Brown, Mismatch (low significance: ex, valine (hydrophobic aliphatic) → isoleucine (hydrophobic aliphatic); Violet, Mismatch (high significance: ex, alanine (hydrophobic aliphatic) → threonine (polar neutral)); Blue, Sequence gap in query sequence.

accession No. ABF46432). Figure 1 shows the alignment of amino acid sequence of GeoRecA, DraRecA and EcoRecA. GeoRecA was more similar to DraRecA (87.4% identity) than to EcoRecA (57.8% identity). However, many residues are invariants strongly attesting to the functional and structural importance of these segments containing the DNA binding and polymeric domains (Figure 1) (Karlin and Brocchieri, 1996).

The overall fold of GeoRecA, DraRecA and EcoRecA is similar but different in specific regions. We exemplify this case by the structural mismatch regions between GeoRecA and DraRecA (Figure 2). Also, for example,

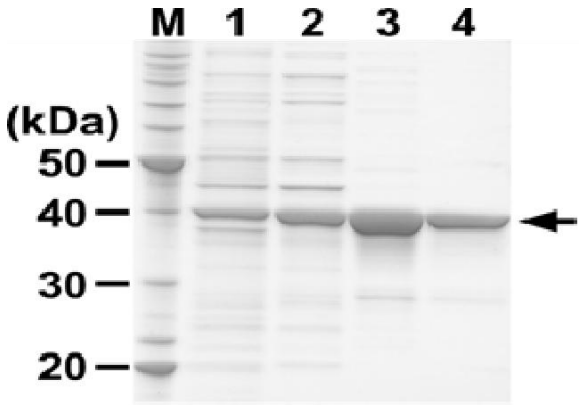

Figure 3. Purification of *D. geothermalis* RecA protein. Samples (10 µg) were subjected to 12.5% SDS-PAGE and stained with a coomassie brilliant blue. Lane M, 1 protein marker (precision plus protein standards from Bio-Rad); lane 1, total cellular proteins from *E. coli* BLR(DE3)/pET3a-GeorecA induced by IPTG; lane 2, pooled GeoRecA fractions from phenyl 650S column; lane 3, pooled GeoRecA fractions from HiTrap Heparin HP column; lane 4, pooled GeoRecA fractions from Mono S column. On the left, relative molecular masses (kDa) of the standard proteins are shown. Arrow on the right indicates the position of the 38-kDa band of GeoRecA.

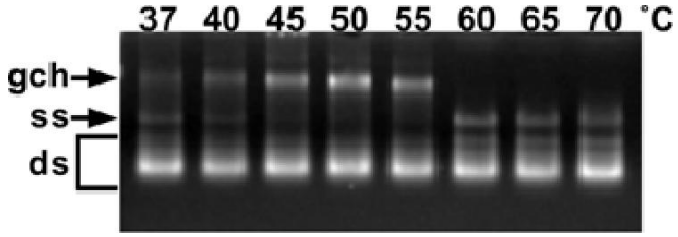

Figure 4. Optimum temperature of GeoRecA for the promotion of DNA strand exchange reaction. Reactions were carried between circular ssDNA and the linear dsDNA derived from *Hin*cII-digested φX174 RF I to yield an expected product φX174 RFII (nicked circular dsDNA) at various temperatures from 37 to 70°C in the buffer as described in Materials and Methods. The positions of dsDNA (ds), ssDNA (ss) and the complete strand exchange product (gapped circular heteroduplex DNA, gch) are indicated.

compared to EcoRecA, the inner surface along the central axis of DraRecA protein filament has an increased positive electrostatic potential (Rajan and Bell, 2004). The theoretical pI/Mw (http://www.expasy.org/tools/pi_tool.html) for GeoRecA is 5.48/38,156.78 (358 amino acid residues including the initiating methionine), 5.45/38,144.76 (363 amino acid residues including the initiating Met) for DraRecA, and 5.09/37,973.37 (353 amino acid residues including the initiating Met) for EcoRecA.

DNA strand exchange

To investigate the recombination activity of GeoRecA *in vitro*, we purified the protein as described in Materials and Methods. The purified protein migrated on a SDS-polyacrylamide gel with an apparent molecular mass of 38 kDa (Figure 3). This is close to the molecular mass (38,156 Da) calculated from DNA sequence data.

To check whether the purified GeoRecA possesses recombination activity *in vitro*, a DNA strand exchange assay was performed. The assays were carried out at various temperatures from 37 to 70°C. GeoRecA promoted an efficient strand exchange reaction between homologous circular ssDNA and linear dsDNA substrates to yield complete strand exchange products (Figure 4). GeoRecA most efficiently promoted the strand exchange reaction at 50°C. The optimum temperature of 50°C for

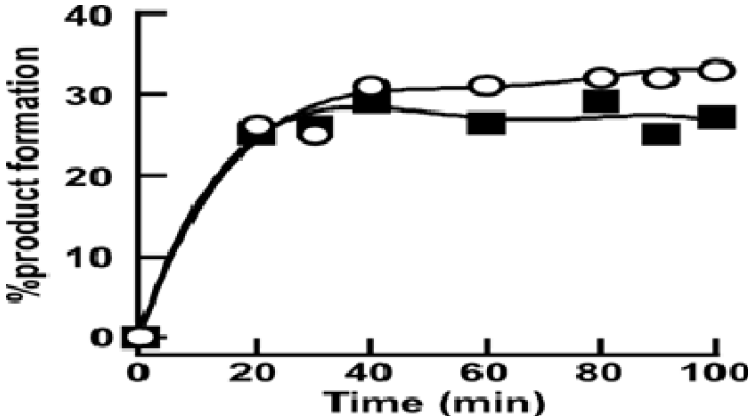

Figure 5. GeoRecA promotes DNA strand exchange reaction *via* inverse pathway. Reactions were carried out at 50°C, pH 7.4 between circular ssDNA (ss) and the linear dsDNA (ds). In the ds to ss reactions (closed squares), the GeoRecA protein was preincubated with the linear dsDNA and ATP for 5 min. The ssDNA was then added to start the reaction and the *E. coli* SSB protein was added 5 min later. In the ss to ds reactions (open circles), GeoRecA was preincubated with the ssDNA, ATP and *E. coli* SSB for 5 min. Then dsDNA was added to start the reaction.

GeoRecA protein activity as shown in Figure 4 is consistent with the fact that *D. geothermalis* can grow between 45 and 50°C (Ferreira et al., 1997).

EcoRecA initiates DNA strand exchange with a filament bound to the ssDNA, followed by uptake of the duplex substrate (normal pathway). In contrast, the pathway of DNA strand exchange promoted by DraRecA is the exact inverse (Kim and Cox, 2002). We examined which pathway of the DNA strand exchange reaction GeoRecA employed. Like DraRecA, GeoRecA promoted DNA strand exchange reaction through both pathways (Figure 5). Whereas, the inverse pathway is clearly major in DraRecA mediated DNA strand exchange pathway (Kim and Cox, 2002), GeoRecA mediated reactions showed no significant difference through both pathways under our conditions (Figure 5). However, these results suggested that the inverse DNA strand exchange pathway which is different from other bacterial RecA-mediated pathway might be related to the remarkable efficient DNA repair mechanism of the genus *Deinococcus*.

Complementation of *RecA* deficiencies of *D. radiodurans* and *E. coli*

To investigate the function of GeoRecA protein *in vivo*, we constructed the *GeorecA* expression plasmid under the control of the radiation responsive *pprA* promoter from *D. radiodurans* as described in Materials and Methods. Transcriptome analysis revealed that the *pprA* gene exhibited a *recA*-like activation pattern following γ irradiation (Liu et al., 2003). Because the *recA* promoter is not defined yet, the *pprA* promoter was used for expression plasmid instead. The *GeorecA* expression plasmid was introduced into DNA repair deficient mutant *D. radiodurans* strain rec30 carrying *recA670* mutation (Narumi et al., 1999). Then, the sensitivity of strain rec30 carrying the *GeorecA* expression plasmid following γ irradiation was compared between strains R_1 (carrying the wild-type *recA*) and rec30. Strain rec30 exhibited much more sensitive to γ irradiation than strain R_1, consistent with previous studies (Narumi et al., 1999). In contrast, strain rec30 carrying the *GeorecA* expression plasmid was resistance to γ irradiation but slightly sensitive than that of strain R_1 carrying pRAD1 (no insert) (Figure 6), indicating that the GeoRecA protein partially complements *D. radiodurans recA670* mutation. This result suggested that GeoRecA exhibited low recombination activity under the growth condition of *D. radiodurans*, and is consistent with our observation of *in vitro* RecA-mediated DNA strand exchange reaction (Figure 4). It has also been shown that EcoRecA provides partial complementation to a *D. radiodurans recA* null mutant (Schlesinger, 2007).

The *D. radiodurans recA* gene has been shown to complement *E. coli recA1* (Narumi et al., 1999). Whether the *GeorecA* complements the deficiency was tested. For this purpose, we constructed the *GeorecA* and *EcorecA* expression plasmids under the control of the *E. coli recA* promoter as described in Materials and Methods. Then, *E. coli* strain JM109 carrying *recA1* was transformed with these expression plasmids. The *EcorecA* expression plasmid was used as control. *E. coli* strains JM107 and JM109 are isogenic except for the *recA* gene. As shown in Figure 7, the JM109 transformant carrying the *EcorecA* expression plasmid was as resistant to γ rays as strain JM107 (as for JM109 but $recA^+$) carrying pUC19 (no insert). On the other hand, the

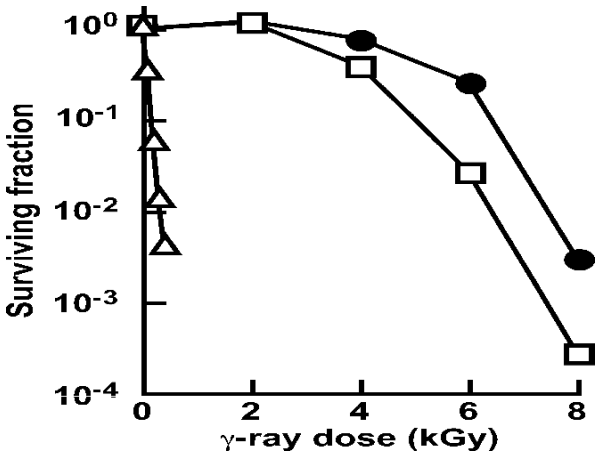

Figure 6. Sensitivity of *D. radiodurans* strains to γ-rays. Closed circles, strain R_1 (wild-type) carrying pRAD1; open triangles, strain rec30 (*recA670*); open squares, strain rec30 (*recA670*) carrying pGEO5.

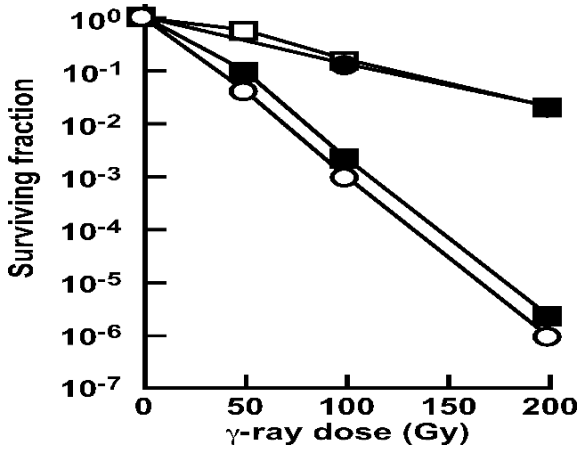

Figure 7. Sensitivity of *E. coli* strains to γ-rays. Closed circles, *E. coli* JM107 carrying pUC19; closed squares, *E. coli* JM109 (*recA1*) carrying pUC19; open squares, *E. coli* JM109 (*recA1*) carrying pH-EcorecA; open circles, *E. coli* JM109 (*recA1*) carrying pH-GeoRecA.

sensitivity of JM109 transformant carrying the *GeorecA* expression plasmid was equal to that of strain JM109 carrying pUC19, though the transformant produced abundant amounts of the plasmid-encoded GeoRecA protein (data not shown). This result indicated that GeoRecA could not complement *E. coli* RecA deficiency, whereas DraRecA could. However, it should be considered that the regulation of growth stage-specific expression level of RecA protein is critical in the DNA damage response mechanism. Therefore, the inability of complementation might be related to the expression level of GeoRecA that possibly could not be controlled enough in *E. coli*. GeoRecA may require more strict intracellular conditions than DraRecA does to fulfill its function.

Conclusion

In this study, the role of GeoRecA in radioresistance and the functional similarity and dissimilarity between GeoRecA and DraRecA were assessed through DNA strand exchange and plasmid complementation assays. The optimal temperature at which GeoRecA most efficiently promoted the strand exchange reaction between homologous linear double-stranded DNA and circular single-stranded DNA substrates was 50°C. GeoRecA could promote DNA strand exchange reaction through normal and inverse pathways, and it could complement the RecA deficiency of *D. radiodurans*, indicating that GeoRecA is a functional homologue of

DraRecA and plays an important role in radio resistance. However, unlike DraRecA, GeoRecA could not complement the RecA deficiency of *E. coli*. This result suggests that GeoRecA require more strict intracellular conditions than DraRecA does to fulfill its function. Our findings from this study provide new insights into the role of deinococcal RecA protein in radioresistance and the potential use of thermostable RecA protein as a reagent in DNA engineering such as the targeted DNA cleavage, the asymmetric linker attachment and the multiplex PCR reaction (Koob et al., 1992; Shigemori, 2005; Shigemoti et al., 2005). Further research is required to puzzle out the nature of GeoRecA proteins. Our effort is currently being directed towards generating a *GeorecA* disruptant strain to investigate gene disruption effect on radioresistance and functional complementation by EcoRecA and DraRecA.

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