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Analysis of Double Stranded DNA-dependent Activities of *Deinococcus radiodurans* RecA Protein

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In this study, the double-stranded DNA-dependent activities of *Deinococcus radiodurans* RecA protein (Dr RecA) were characterized. The interactions of the Dr RecA protein with double-stranded DNA were determined, especially dsDNA-dependent ATP hydrolysis by the Dr RecA protein and the DNA strand exchange reaction, in which multiple branch points exist on a single RecA protein-DNA complex. A nucleotide cofactor (ATP or dATP) was required for the Dr RecA protein binding to duplex DNA. In the presence of dATP, the nucleation step in the binding process occurred more rapidly than in the presence of ATP. Salts inhibited the binding of the Dr RecA protein to double-stranded DNA. Double-stranded DNA-dependent ATPase activities showed a different sensitivity to anion species. Glutamate had only a minimal effect on the double-stranded DNA-dependent ATPase activities, up to a concentration of 0.7 M. In the competition experiment for Dr RecA protein binding, the Dr RecA protein manifested a higher affinity to double-stranded DNA than was observed for single-stranded DNA.

Keywords: *Deinococcus radiodurans*, RecA protein, DNA-dependent ATP hydrolysis, DNA strand exchange, Tau plot

Deinococcus radiodurans is a polyextremophile (Richmond *et al.*, 1999), showing remarkable resistance to a range of severe damage induced by ionizing radiation, desiccation, ultraviolet radiation (UV), oxidizing agents, or electrophilic mutagens (Minton, 1994). This bacterium is well-known for its resistance to ionizing radiation. It is capable of surviving acute exposure to gamma radiation exceeding 1,700,000 rads without lethality or induced mutation (Daly *et al.*, 1994), and is also capable of vigorous growth even in the presence of chronic irradiation (6,000 rads per hour) (Lange *et al.*, 1998). The *RecA* gene of *D. radiodurans* appears very similar to those found in *Thermus* spp. and other gram-positive bacteria. RecA or RecA-like proteins play a critical role in biological processes that require homologous DNA pairing and recombination (Cox, 1999). *D. radiodurans* RecA is normally constitutively expressed at low levels in *D. radiodurans*, and is only expressed transiently at high levels after the infliction of extreme DNA damage (Carroll *et al.*, 1996). A recent analysis of predicted expression levels (based on codon usage) of the main repair proteins of *D. radiodurans* and *E. coli* revealed that neither set of repair genes is likely to be expressed at high levels. There is one exception to this trend, however. The RecA protein has been predicted to be expressed abundantly in both organisms; the level of *E. coli* RecA expression was predicted to be high, and RecA expression in *D. radiodurans* was predicted to be remarkably high (Makarova *et al.*, 2001). A functional RecA is crucial to the expression of the resistance phenotypes of *D. radiodurans* (Minton, 1996). For example, the disruption of *D. radiodurans RecA* greatly diminishes its ability to recover from acute DNA damage (Carroll *et al.*, 1996; Minton, 1996), and prevents its growth in the presence of chronic radiation (Venkateswaran *et al.*, 2000). The biological functions of the RecA protein of *D. radiodurans* have been closely associated with the efficient repair of DSBs. In many respects, the *D. radiodurans* (Dr) RecA protein is similar to other bacterial RecA proteins. It forms helical filaments on DNA, hydrolyzes both ATP and dATP, and promotes DNA strand exchange (Kim *et al.*, 2002). It binds to double-stranded DNA

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(dsDNA) more readily than does the *E. coli* RecA protein. Whereas the biological properties of the Dr RecA protein have been evaluated in detail, the molecular characterics of the Dr RecA nucleoprotein filament, an active species in the RecA reaction, have yet to be elucidated. The characterizations of molecular functions, in particular binding to duplex DNA, as well as the response of DNA strand exchange to ATP hydrolysis, constitute a source of insight into the principal role of RecA protein, which is involved in specialized participitation for the radiation resistance phenotype. In this study, the interactions of the Dr RecA protein with double-stranded DNA were determined.

Materials and Methods

Enzymes and reagents

The native Dr RecA protein was purified as described (Kim *et al.*, 2002), and its concentration was determined with the extinction coefficient of ε_{280} = 0.372 A_{280} ml/mg. *E. coli* SSB was obtained from Sigma and stored frozen at -70°C in a buffer containing 20 mM Tris-HCl (pH 8.4), 0.15 M NaCl, 1 mM EDTA, 1 mM β-mercaptoethanol, and 50% glycerol. The SSB concentration was determined with the extinction coefficient of ε_{280} = 1.5 A_{280} ml/mg. The restriction endonucleases were obtained from New England Biolabs. Tris buffer, phage DNAs, phosphoenolpyruvate, pyruvate kinase, lactic dehydrogenase, NADH, ATP, and dATP were all purchased from Sigma.

ATPase assay

ATP hydrolysis was monitored as described previously (Kim *et al.*, 2002). All reactions were done at 37°C. ATP (or dATP) hydrolysis was measured by a coupled spectrophotometric assay. Absorbance measurements were obtained on a spectrophotometer equipped with thermojacketed cuvette holders attached to a constant-temperature water circulator. The reaction mixtures contained 25 mM Tris-acetate (pH 7.5), 1 mM DTT, 5% glycerol, 3 mM potassium glutamate, and 10 mM magnesium acetate. Concentrations of RecA protein, DNA, and ATP (or dATP) are shown in the figure legends. In cases in which the pH was varied, the buffer used was a 25 mM concentration of either Tris-acetate or MES-NaOH. An ATP regenerating system (3 mM phosphoenolpyruvate, 10 U/ml of pyruvate kinase, and 3 mM potassium glutamate) and a coupling system (2 mM NADH and 10 U/ml of LDH) were also included. When dATP was employed, the ATP regenerating and coupling enzymes were increased to 25 U/ml of pyruvate kinase and 60 U/ml of lactate dehydrogenase. The reactions were initiated by the addition of ATP after all other components had been incubated for 10 min at 37°C. The regeneration of ATP from ADP and phosphoenolpyruvate is coupled with the conversion of NADH to NAD^+ (in the reaction catalyzed by lactate dehydrogenase), which can be monitored by a decrease in absorbance at 380 nm. Although the absorbance maximum for NADH was observed at 340 nm, absorbances were measured at 380 nm, in order to maintain then within the linear absorbance range of the spectrophotometer for an extended length of time, as required by these experiments. Rates of ATP hydrolysis (μM/min) were calculated from the ΔA_{380}/min obtained at steady state, using an extinction coefficient of 1,210 M^{-1} cm^{-1} at 380 nm for NADH. In most cases, absorbances were measured continuously over a period of 1 h.

DNA strand exchange reactions

The RecA-dependent DNA strand exchange reaction was carried out as described (Kim *et al.*, 2002) between circular ssDNA and the linear dsDNA (derived from either ϕX174 or M13mp18). Reactions were conducted at 37°C in solutions containing 25 mM Tris-acetate (pH 8.1 for Dr RecA protein reactions), 1 mM DTT, 5% glycerol, 3mM potassium glutamate, 10 mM magnesium acetate, and an ATP-regenerating system (10 units/ml of pyruvate kinase/3.3 mM phosphoenolpyruvate). DNA, SSB, ATP, and Dr RecA protein concentrations are provided for each experiment. The standard protocol (modified as described in the figure legends) began with a 5 min preincubation of ssDNA with the Dr RecA protein at 37°C. This preincubation was followed by the addition of ATP and SSB. After an additional 5 min of incubation, linear duplex DNA was added to initiate the DNA strand exchange reactions. Aliquots (20 μl unless otherwise indicated) of the strand exchange reactions were removed at each time point, and the reactions were stopped by addition of 5 μl of gel loading buffer (0.125% bromophenol blue, 25 mM EDTA, 25% glycerol, and 5% SDS). These aliquots were stored on ice until after the last time point was taken. The samples were electrophoresed in a 0.8% agarose gel with TAE buffer, then stained with ethidium bromide.

Results and Discussion

Characterization of the interaction of Dr RecA protein with dsDNA

Binding kinetics were indirectly monitored via DNA-dependent ATPase assays, as previously described (Pugh and Cox, 1987). For double-stranded DNA, this technique can be used to measure DNA binding accurately. The measurement of DNA binding by monitoring ATP hydrolysis is premised on the assumption that RecA monomers bound to duplex DNA can hydrolyze ATP. Estimates of binding obtained in

this way correlate quite well with other measures of binding, such as DNase protection, light scattering, and DNA unwinding assays (Pugh *et al.*, 1988). The time course of ATP hydrolysis by the Dr RecA protein in the presence of dsDNA is shown in Fig. 1 as a measure of the course of DNA binding. Reaction progress curves showed that the relatively low initial velocities increase gradually to the steady-state velocities in the DNA-dependent ATP hydrolysis reaction. At a pH of 7.5, the Dr RecA protein bound gradually to dsDNA through a slow initiation step, followed by a rapid propagation of binding throughout the entire DNA molecule. Both reactions achieved the same steady state of ATP hydrolysis, after a lag. This lag (8 min) for a reaction with 10 μM RecA protein was significantly longer than the lag (3 min) for the reaction with 20 μM RecA protein. The lag for dsDNA-dependent ATP hydrolysis is consistent with the results previously obtained in reactions with the *E. coli* RecA protein, and principally reflects the slow nucleation step in binding. The shorter lag observed in a reaction with more RecA protein revealed that faster binding occurred in a reaction with a greater quantity of Dr RecA protein. In the dsDNA-dependent reaction, a lag was detected prior to the establishment of a steady state under most conditions. For ATP, steady-state hydrolysis was observed with a lag of less than 3 min at pH 6.5 under standard reaction conditions. The lag increased to just over 13 min at a pH of 7.5. At pH 8.5, the lag disappeared, but the rate slowed markedly. For dATP, the reaction at pH 6.5 was relatively slow, but exhibited no evident lag. A slight lag and a higher rate were observed at pH 7.5, and a longer lag and a higher ultimate rate was observed at a pH of 8.5 (data not shown).

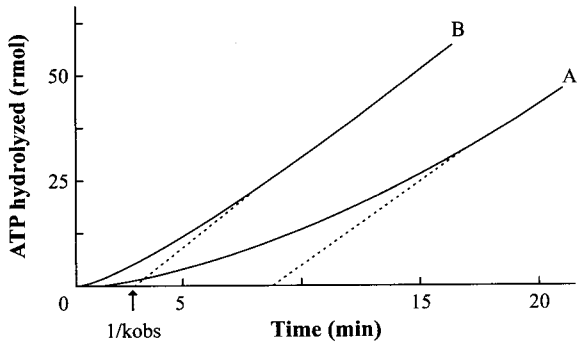

Fig. 1. Time-course of dsDNA-dependent ATP hydrolysis by Dr RecA protein at pH 7.5. Reaction mixture contained 25 mM Tris-acetate, pH 7.5, 10 μM linear dsDNA, 2 mM ATP, 10 μM Dr RecA protein (A) or 20 μM Dr RecA protein (B). The time lag (Tau;1/kobs)) in the approach to steady state ATP hydrolysis (A) or dATP hydrolysis (B) was plotted via the extrapolation of the linear steady state rate back to the *x* axis where zero ATP is hydrolyzed.

In order to characterize in detail the binding process of the Dr RecA protein to dsDNA, the Tau analysis was conducted. An equation derived for the binding mechanism of Fig. 2 has been previously described (Pugh and Cox, 1987). The time lag (τ) in this system (as in Fig. 1) is determined via the extrapolation of the linear steady state rate back to the *x* axis, at which zero ATP is hydrolyzed. The time value at the intersection with the *x* axis is designated as Tau (τ), and it represents the average time required for the formation of a complete RecA-dsDNA

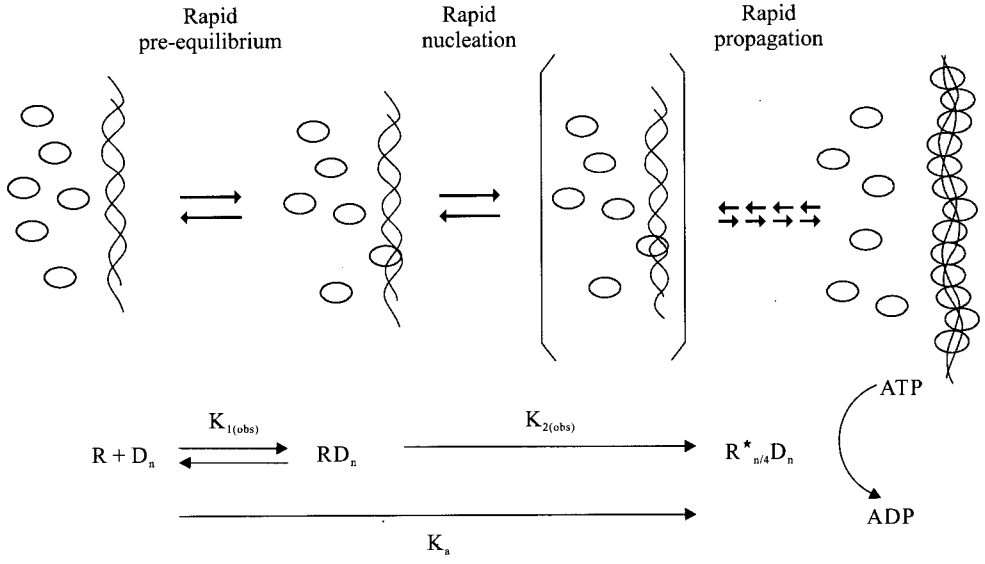

Fig. 2. Binding pathway of RecA protein to duplex DNA (Pugh and Cox, 1988). The general mechanism includes rapid pre-equilibrium and a slow nucleation step. R and D represent the RecA protein and DNA, respectively.

nucleoprotein complex, and corresponds to a reciprocal of the overall binding rate constant (k_{obs}). The lag time for binding or Tau (τ) is derived via the following equation;

$$\text{Tau } (= 1/k_{obs}) = 1/k_2 + 1/ka[R]$$

in which K_1 represents the pre-equilibrium in step 1, k_2 is the nucleation step, $ka = K_1 \times k_2$, and [R] is the total concentration of RecA protein. The individual constants can be determined by plotting Tau versus 1/[R] (Pugh and Cox, 1987). The reciprocal of the Tau intercept is k_{2obs} and the reciprocal of the slope is ka. Kl can be derived from the relationship $K_1 = ka/k_{2obs}$. When the binding of the Dr RecA protein to dsDNA was assessed via the analysis of dsDNA-dependent ATP hydrolysis, the Dr RecA protein was shown to bind to DNA in a range of pH between 6.5 and 8.5 (Kim *et al.*, 2002). The time lag (Tau) in the approach to steady state ATP hydrolysis is plotted as a function of the reciprocal of the total concentration of RecA protein (Fig. 3A). Reactions were carried out at pH 7.5 under standard reaction conditions with 10 μM DNA and the indicated RecA protein concentration. In the presence of ATP at pH 7.5, the association constant ($K1_{obs}$) for the Dr RecA protein with dsDNA was calculated to 4.7×10^4/M, the rate constant ($k2_{obs}$) for the slow nucleation step was 0.34/min. These kinetic parameters, $K1_{obs}$, and $k2_{obs}$ for DNA binding in the presence of dATP, were 7.6×10^3/M and 7.6/min, respectively (Fig. 3B). These data indicate that the Dr RecA proteins bind to DNA with a 6-fold higher affinity in the presence of ATP than in the presence of dATP upon initial weak interaction. However, the subsequent nucleation step, which is the rate-limiting step for the overall process, occurred at a 20-fold faster rate in the presence of dATP than in the presence of ATP. The overall process for the formation of active nucleoprotein progressed with a 4 times higher rate in the presence of dATP than in the presence of ATP. These kinetic parameters of the *E. coli* RecA protein were measured to be 2.3×10^5/M for K1 and 0.16/min for k2 (Kim *et al.*, 1989). When the reactions for dsDNA-dependent ATP (or dATP) hydrolysis by Dr RecA protein were initiated by adding ATP (or dATP) after the incubation of Dr RecA protein with dsDNA for the corresponding lag time, the reactions were required for the lag time to approach the steady state of ATP hydrolysis in the timecourse of ATP hydrolysis. This shows that the order of addition of reaction components had no effect on the time course of the reaction with dsDNA, thereby indicating that ATP is required for binding. When the time courses of ATP hydrolysis in the presence of ssDNA as a measure of the course of DNA binding were monitored, all reactions achieved a steady state of ATP hydrolysis without any significant lag. This is principally reflective of the rapid formation of nucleoprotein occurring in the binding of the Dr RecA protein binding to ssDNA with no measurable kinetic barrier (data not shown).

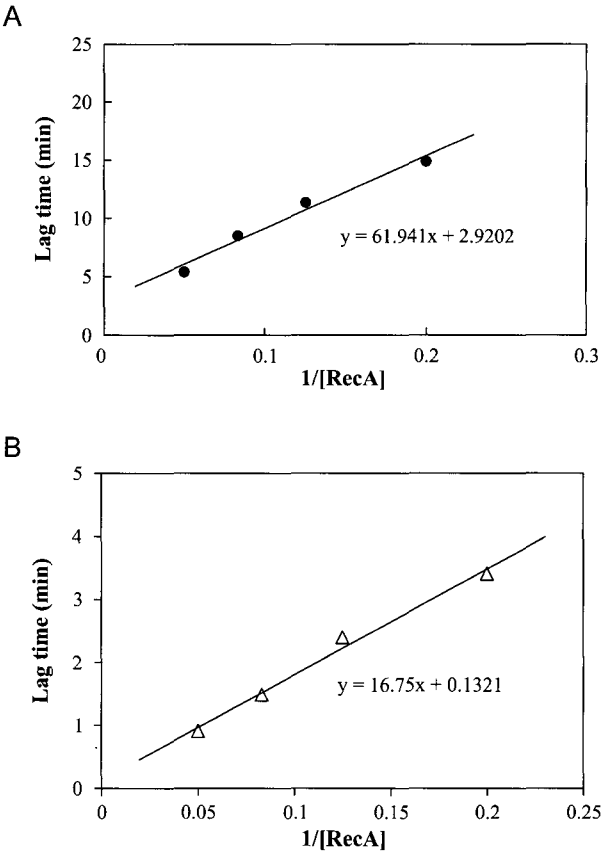

Fig. 3. Tau plot for Dr RecA protein binding to dsDNA in the presence of ATP. The time lag (Tau) in the approach to steady state ATP hydrolysis (A) or dATP hydrolysis (B) is plotted as a function of the reciprocal of the total RecA protein concentration. Reactions were carried out at pH 7.5 under standard reaction conditions with 10 μM DNA, 2 mM ATP (or dATP) and the indicated concentration of RecA protein.

Effect of salt on the DNA-dependent ATPase

D. radiodurans exhibits the resistance to the severe damage associated with desiccation as well as by ionizing radiation, ultraviolet radiation (UV), and oxidizing agents. Thus, the bacteria could survive in dried environments. The relative salt concentration inside the cell would increase under dehydration conditions. The activities of the Dr RecA protein were determined under a variety of salt concentration conditions. The Dr RecA protein is a DNA-dependent ATPase. Dr RecA monomers bound to duplex DNA were determined to hydrolyze ATP at a kcat of 12 per min (Kim *et al.*, 2002). In the absence of DNA, ATP hydrolysis by the

Dr RecA protein occurs at a negligible rate (k_{cat} of 0.0001 per min). Once bound to DNA, the Dr RecA protein exhibits ATP (or dATP)-hydrolyzing activity. ATP hydrolysis by the RecA protein would play a crucial role in processes in which the RecA protein is involved. Although heterogeneity in the base sequence might affect RecA binding to some extent, the Dr RecA protein binds to dsDNA with sequence-non-specificity upon binding. This indicates that the RecA proteins interact with the sugar-phosphate backbone of DNA through ionic binding. Salt environments may have the most significant effects on the activity of Dr RecA protein bound to DNA. As is the case with the majority of DNA-binding proteins, DNA-dependent ATP hydrolysis by RecA protein was found to be affected by the ionic environment (Fig. 4). The DNA-dependent reaction was salt-sensitive, and evidenced 50% inhibition at 50 mM NaCl. A greater extent of inhibition (90% reduction in the rate of ATP hydrolysis) was noted in the presence of 100 mM NaCl (data not shown). DNA-dependent ATPase activities manifested different degrees of sensitivity to cation concentrations. KCl (potassium in cationic form) at 100 mM inhibited dsDNA-dependent ATP hydrolysis by 35%. With increasing KCl concentrations, greater reductions in the rate of ATP hydrolysis were observed. ATP hydrolysis was completely inhibited in the presence of 400 mM KCl. The dsDNA-dependent ATP hydrolysis was inhibited in the presence of salt, but exhibited different sensitivity to ionic species at the same salt concentrations. K-acetate also exerted an inhibitory effect on dsDNA-dependent ATP hydrolysis, up to a concentration of 400 mM. Hydrolytic activity

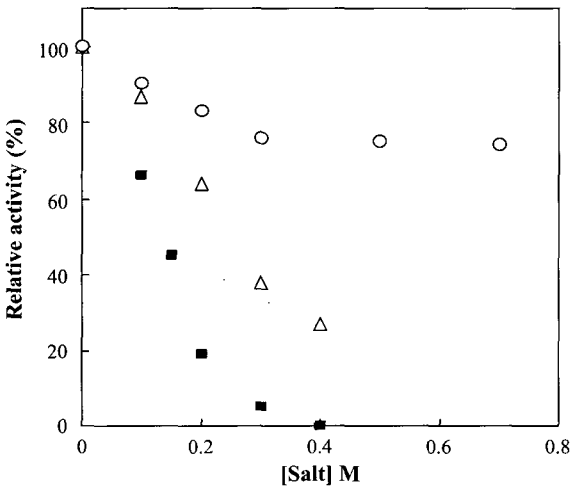

Fig. 4. Effect of salt on dsDNA-dependent ATP hydrolysis at pH 7.5. ATP hydrolysis reactions were performed at pH 7.5 in the presence of 2 μM RecA protein, 10 μM dsDNA, 2 mM ATP, and the indicated concentrations of various salts; K-glutamate (○), K-acetate (△) and KCl (■).

was retained at a level of approximately 35% at 300 mM K-acetate. Generally, a lesser extent of inhibition was noted in reactions with K-acetate than reactions with KCl at same concentration. Potassium glutamate exerted a stimulatory effect on ssDNA-dependent ATP hydrolysis, to a concentration of 300 mM (data not shown). Double-stranded DNA-dependent ATPase activity by the Dr RecA protein, however, evidenced only minimal inhibition with 300 mM K-glutamate. The extent of inhibition effected by K-glutamate did not increase at higher concentrations of up to 800 mM. In the presence of KCl or K-acetate, dsDNA-dependent ATP hydrolysis by the Dr RecA protein was inhibited with a higher sensitivity to salt concentrations, but this was not the case with K-glutamate. This indicates that the ionic strength of the reaction system did not exert any direct effects on dsDNA-dependent ATP hydrolysis. The dsDNA dependent-ATP hydrolytic reaction was less efficient in acetate solution than in glutamate solution. The combined effects of ionic species and ionic strength may affect the Dr RecA protein-mediated reactions. In the presence of glutamate, the observed reduction of ATP hydrolysis might be interpreted as a direct effect on the intrinsic activity of ATPase, but the observed lower degree of inhibition would indicate that K-glutamate stabilizes the binding of the RecA protein to dsDNA via an enhancement of affinity to DNA. In a previous study (Kim *et al.*, 1989), it was demonstrated that Na-glutamate causes an increase in the binding equilibrium constant for the association of *E. coli* RecA protein with duplex DNA in binding to dsDNA. According to the results of this study, the significant effect of salt on Dr RecA protein activities may not constitute a general ionic strength effect, but rather an ion-specific effect. It has been shown that the binding of the RecA protein to DNA occurs primarily via an electrostatic interaction with the phosphate backbone of DNA. Salts can function either as competitors or effectors of DNA binding. The inhibition of ATP hydrolytic activities has not been associated with increasing salt concentrations, but rather with interference with DNA binding. The observation of tighter DNA binding in glutamate solution may allow for the Dr RecA protein to remain unaffected by increasing ionic strength. The nature of glutamate (Arakawa and Timasheff, 1984) may serve to enhance the interaction of the Dr RecA protein with dsDNA, which is dependent on the bulk salt concentration, by stabilizing the RecA complex with DNA.

Analysis of binding competition

The evident difference in the k_{cat} for ATP hydrolysis on dsDNA as opposed to ssDNA constitutes a convenient characteristic by which the two complexes

might be distinguished in mixed solutions. This assay was employed in order to characterize binding in competition experiments at pH 7.5. The time course of ATP hydrolysis with an ssDNA cofactor was generally linear with time under these conditions. Using dsDNA as a cofactor, a lag was noted prior to the establishment of a steady state, under most conditions. At pH 6.5, steady-state ATP hydrolysis was observed with a lag of less than 3 min. This lag increased to just over 13 min at pH 7.5. At pH 8.5, the lag disappeared, but the rate slowed remarkably. The analysis of complexes of Dr RecA protein with DNA in the reactions, under which a condition competition occurred between ssDNA and dsDNA for Dr RecA protein

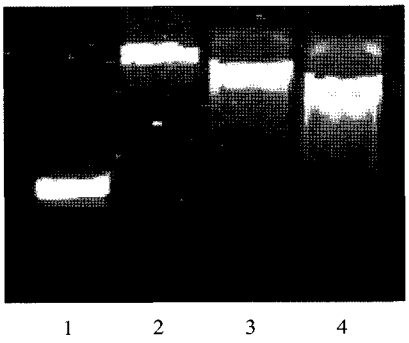

Fig. 5. Gel mobility shift assay. The reaction mixture contained 25 mM Tris-acetate (pH 7.5), 2 mM ATP, and (1) 20 μM linear dsDNA of ϕX174 (2) 20 μM linear dsDNA of ϕX174 and 5 μM Dr RecA protein (3) 20 μM linear dsDNA of ϕX174, 10 μM ssDNA of M13mp8 and 5 μM Dr RecA protein (4) 20 μM linear dsDNA of ϕX174, 20 μM ssDNA of M13mp8 and 5 μM Dr RecA protein.

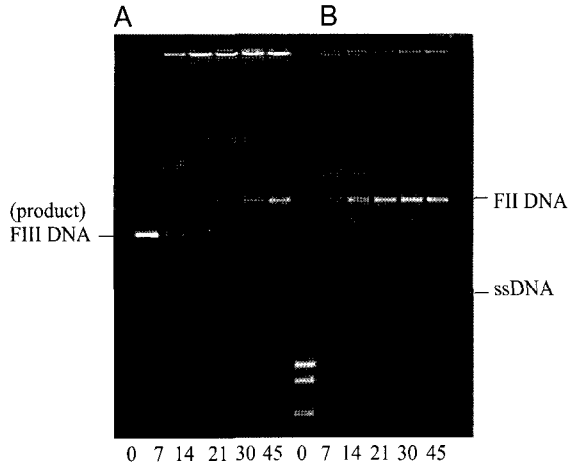

Fig. 6. Dr RecA protein-mediated DNA strand exchange at pH 7.5. Reaction mixtures contained 25 mM Tris-acetate (pH 7.5), 5 μM ssDNA, 2.5 μM Dr RecA protein, 2 mM ATP, 0.5 μM SSB and 10 μM full length dsDNA (7.2 kb) (A) and 3- fragmented DNA (1.9, 2.5, and 2.8 kb) (B).

binding, was carried out by the electrophoretic mobility shift assay in the presence of both DNAs, which were present at concentrations of 10 μM or 20 μM (30 μM total when mixed) for ssDNA and dsDNA, respectively. The concentration of RecA protein (2.5 μM) is sufficient to bind to approximately 7.5 μM of ssDNA or 15 μM of dsDNA. In cases in which both DNAs are present, the RecA protein is sufficient to bind no more than half of the DNA present. The results of an experiment with both DNAs are provided in Fig. 5. Dr RecA protein bound to ϕX174 linear dsDNA at pH 7.5 in the presence of ATP, and the complex was moved with more retarded mobility than free dsDNA upon electrophoresis (lane 2). When both linear dsDNA (ϕX174) and M13mp ssDNA (with the same nucleotide concentration) was present in the reaction, the Dr RecA protein was determined to bind to dsDNA, the mobility of which was retarded. The mobility of the complexes of dsDNA with Dr RecA protein formed in the presence of ssDNA (lane 3) was increased slightly when compared to that seen with the complexes formed in the presence of dsDNA. This may indicate that the Dr RecA protein bound to a lesser extent in the presence of ssDNA. Double-stranded DNA was relatively fully coated by the Dr RecA protein only in the presence of dsDNA. Double-stranded DNA was also retarded on electrophoresis when a 2-fold excess of ssDNA was present in the reaction (lane 4). This shows that dsDNA was favored in cases of Dr RecA protein binding in competition with ssDNA. Combined with the results of DNA-dependent ATP hydrolysis, it was shown that, in cases in which both DNAs were present, the binding of the Dr RecA to ssDNA is kinetically rapid with no lag time, but the complexes were not thermodynamically favorable, and binding to dsDNA occurred slowly with a lag time, although the complexes of RecA protein with dsDNA were favorable. Competition with dsDNA and ssDNA did not follow patterns reminiscent of the results reported for the RecA protein of *E. coli*. In a previous study, it was shown that the *E. coli* RecA protein has much higher affinity to dsDNA than to ssDNA (Pugh and Cox, 1987).

Strand exchange reaction with multiple branch points

The repair of DNA damage is of central importance to cells. DNA damage by radiation is primarily characterized by double-strand breaks, which induce DNA fragmentation. The fragmented DNAs are then joined together in the original order during the repair process. Damage as the result of radiation can be efficiently and properly repaired in cases in which the repair by recombination with the intact homologous DNA is involved in the repair process. In order to

determine the role of the Dr RecA protein in that process, it was attempted to determine the capacity of the Dr RecA protein to promote the DNA strand exchange reaction between circular single-stranded DNA, and the linear pieces of double-stranded DNAs produced with the whole length linear dsDNA by restriction digestion. The sum of length of the restriction fragments of the linear duplex DNA in each set corresponds to that of the full-length linear duplex DNA. The final product (FII DNA; nicked circular DNA) was observed in reactions both with full-length linear dsDNA (FIII DNA) and with 3 restriction DNA fragments (Fig. 6). The time course of DNA strand exchange reaction reveals that the final product (F II DNA) was formed in both reactions. When the 3 restriction DNA fragments (Fig. 6B) were combined, a significant amount of final product (nicked circular duplex DNA; FII DNA)) appeared after 7 min of incubation, which is 3-4 fold earlier than the time point in the reaction containing single, full-length linear duplex DNA in the presence of ATP (Fig. 6A), in which the final product appeared only after 20 min. This indicates that the fragmented DNAs could homologously align with ssDNA, and pair with ssDNA through the entire length of ssDNA in the Dr RecA protein-mediated DNA strand exchange reaction. Subsequently, efficient strand exchange could occur. Thus one strand, derived from ssDNA, in the heteroduplex product DNA remained intact as it was in its original state, and the other strand, which had been derived from dsDNA, harbored 2 nicks. These nicks could be sealed in the ongoing process of repair. These characteristics demonstrate that the Dr RecA protein may play a central role in the initial steps of the DNA repair process, in which double-strand breaks generated in the DNA by radiation, were efficiently repaired.

Acknowledgment

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References

Arakawa, T. and S.N. Timasheff. 1984. The mechanism of Na-glutamate, lysine-HCl, and piperazine-N,N'-bis (2-ethanesulfonic acid) in the stabilization of tubulin and microtubule formation. *J. Biol. Chem.* 259, 4979-4986.

Carroll, J.D., M.J. Daly, and K.W. Minton. 1996. Expression of *RecA* in *Deinococcus radiodurans*. *J. Bacteriol.* 178, 130-135.

Cox, M.M. 1999. Recombinational DNA repair in bacteria and the RecA protein. *Prog. Nucleic Acids Res. Mol. Biol.* 63, 310-366.

Daly, M.J., L. Ouyang, P. Fuchs, and K.W. Minton. 1994. *In vivo* damage and *RecA*-dependent repair of plasmid and chromosomal DNA in the radiation-resistant bacterium *Deinococcus radiodurans*. *J. Bacteriol.* 176, 3508-3517.

Kim, J.I., J. Heuser, and M.M. Cox. 1989. Enhanced RecA protein binding to Z DNA represents a kinetic perturbation of a general duplex DNA binding pathway. *J. Biol. Chem.* 264, 21848-21856.

Kim, J.I., A.K. Sharma, S.N. Abbott, E.A. Wood, D.W. Dwyer, A. Jambura, K.W. Minton, R.B. Inman, M.J. Daly, and M.M. Cox. 2002. RecA Protein from the extremely radioresistant bacterium *Deinococcus radiodurans*: Expression, Purification, and Characterization. *J. Bacteriol.* 184, 1649-1660.

Lange, C.C., L.P. Wackett, K.W. Minton, and M.J. Daly. 1998. Engineering a recombinant *Deinococcus radiodurans* for organopollutant degradation in radioactive mixed waste environments. *Nat. Biotechnol.* 16, 929-933.

Makarova, K.S., L. Aravind, Y.I. Wolf, R.L. Tatusov, K.W. Minton, E.V. Koonin, and M.J. Daly. 2001. Genome of the extremely radiation-resistant bacterium *Deinococcus radiodurans* viewed from the perspective of comparative genomics. *Microbiol. Mol. Biol. Rev.* 65, 44-79.

Minton, K.W. 1994. DNA repair in the extremely radioresistant bacterium *Deinococcus radiodurans*. *Mol. Microbiol.* 13, 9-15.

Minton, K.W. 1996. Repair of ionizing-radiation damage in the radiation resistant bacterium *Deinococcus radiodurans*. *Mutat. Res.* 363, 1-7.

Pugh, B.F. and M.M. Cox. 1987. Stable binding of RecA protein to duplex DNA. Unraveling a paradox. *J. Biol. Chem.* 262, 1337-1343.

Pugh, B.F., B.C. Schutte, and M.M. Cox. 1988. Extent of duplex DNA underwinding induced by RecA protein binding in the presence of ATP. *J. Mol. Biol.* 205, 487-492.

Richmond, R.C., R. Sridhar, and M.J. Daly. 1999. Physicochemical survival pattern for the radiophile *Deinococcus radiodurans*: a polyextremophile model for life on Mars. *SPIE* 3755, 210-222.

Venkateswaran, A., S.C. McFarlan, D. Ghosal, K.W. Minton, A. Vasilenko, K. Makarova, L.P. Wackett, and M.J. Daly. 2000. Physiologic determinants of radiation resistance in *Deinococcus radiodurans*. *Appl. Environ. Microbiol.* 66, 2620-2626.