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Enhanced DNA binding affinity of RecA protein from *Deinococcus radiodurans*

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ABSTRACT

Deinococcus radiodurans (Dr) has a significantly more robust DNA repair response than *Escherichia coli* (Ec), which helps it survive extremely high doses of ionizing radiation and prolonged periods of desiccation. DrRecA protein plays an essential part in this DNA repair capability. In this study we directly compare the binding of DrRecA and EcRecA to the same set of short, defined single (ss) and double stranded (ds) DNA oligomers. In the absence of cofactors (ATPγS or ADP), DrRecA binds to dsDNA oligomers more than 20 fold tighter than EcRecA, and binds ssDNA up to 9 fold tighter. Binding to dsDNA oligomers in the absence of cofactor presumably predominantly monitors DNA end binding, and thus suggests a significantly higher affinity of DrRecA for ds breaks. Upon addition of ATPγS, this species-specific affinity difference is nearly abolished, as ATPγS significantly decreases the affinity of DrRecA for DNA. Other findings include that: (1) both proteins exhibit a dependence of binding affinity on the length of the ssDNA oligomer, but not the dsDNA oligomer; (2) the salt dependence of binding is modest for both species of RecA, and (3) in the absence of DNA, DrRecA produces significantly shorter and/or fewer free-filaments in solution than does EcRecA. The results suggest intrinsic biothermodynamic properties of DrRecA contribute directly to the more robust DNA repair capabilities of *D. radiodurans*.

1. Introduction

Escherichia coli RecA (EcRecA) has been extensively characterized as an enzyme involved in the preservation of genomic integrity via its role in homologous recombination, and comparisons of EcRecA to other bacterial homologues have revealed significant structural and functional conservation (reviewed in [1,2]). RecA has been determined to be essential for DNA repair after radiation damage in the radiation resistant bacterium *Deinococcus radiodurans*, participating in both homologous recombination and a unique repair pathway known as extended synthesis dependent strand annealing (ESDSA) [3,4].

A handful of studies of *D. radiodurans* RecA (DrRecA) have revealed many similarities, and a few notable variations from the EcRecA biochemical mechanism. Catalysis of strand exchange by DrRecA is initiated from dsDNA rather than ssDNA, in contrast to the typical initiation from ssDNA found for EcRecA and other RecA species [5]. DrRecA filaments formed on dsDNA are shorter than those formed by EcRecA [6]. The extension of DrRecA filaments is slow compared to EcRecA, while the initial nucleation of DrRecA is faster [6]. DrRecA can form an "inactive" RecA-ATP-dsDNA complex that does not immediately hydrolyze ATP upon binding, unlike EcRecA [7]. Plus, DrRecA shows variations in its interactions with its cognate single-stranded binding protein (SSB) relative to the *E. coli* system [7]. In addition to these enzymatic studies of DrRecA, directed evolution studies have emphasized the primacy of RecA in the overall radiation resistance process by identifying mutations of RecA as one of the most prominent acquired adaptations in *E. coli* that have evolved to be more radiation resistant [8]. One question not yet fully answered, however, is: what specific molecular properties of DrRecA contribute to the enhanced DNA repair abilities of *D. radiodurans*?

By directly examining the thermodynamics of the binding of DrRecA and EcRecA to the same DNA constructs under identical solution conditions, this study reveals further differences and similarities between the two proteins. For example, DrRecA binds DNA significantly tighter than does EcRecA in the absence of ATP. In contrast to EcRecA where ATP (or ATPγS) causes an increase in DNA affinity, addition of ATPγS significantly weakens the association between DrRecA and DNA. Further, the findings described herein indicate that both proteins bind preferentially to ssDNA at longer

Abbreviations: EcRecA, *Escherichia coli* RecA; DrRecA, *Deinococcus radiodurans* RecA; Mg-acetate, magnesium acetate; K-acetate, potassium acetate; ssDNA, single-stranded DNA; dsDNA, double-stranded DNA; SSB, single stranded DNA binding protein; ESDSA, extended synthesis dependent strand annealing.

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Table 1
DNA substrates used for these studies.

13mer	5′-*TCGCAGCCGTCC A-3′
13/13mer	5′-*TCGCAGCCGTCC A-3′ 3′- AGCGTCGGCAGG T-5′
20mer	5′-*TCGCAGCCGTCCAAGGGTTT-3′
20/20mer	5′-*TCGCAGCCGTCCAAGGGTTT-3′ 3′- AGCGTCGGCAGGTTCCCAAA-5
63mer	5′-*TACGCAGCGTACATGCTCGTGACTGGGATAACCGTGCCGTTTGCCGACTTTCGCAGCCGTCCA-3′
63/63mer	5′-*TACGCAGCGTACATGCTCGTGACTGGGATAACCGTGCCGTTTGCCGACTTTCGCAGCCGTCCA-3′ 3′-ATGCGTCGCATGTACGAGCACTGACCCTATTGGCACGGCAAACGGCTGAAAGCGTCGGCAGGT-5′

*Denotes the position of the ROX label.

DNA lengths, suggesting that DrRecA's kinetic preference for initiating strand exchange from dsDNA [5] is more complicated than a pure binding preference. The results of these direct DNA binding studies extend the understanding of the species specific properties of RecA that contribute to its central role in enhanced DNA repair.

2. Materials and methods

E. coli and D. radiodurans RecA purification – clones of EcRecA and DrRecA were gifts from the Michael Cox laboratory at the University of Wisconsin. Protein purification was carried out using procedures from the Cox laboratory as previously described [9,10]. Protein concentrations were determined using the Bradford method [11], which agrees within error with determinations made using published extinction coefficients [9,10].

DNA oligomers – DNA was purchased from Integrated DNA Technologies (IDT) and ssDNA templates were 5′ end labeled with Rhodamine X. The oligomers used are indicated in Table 1. For dsDNA constructs, unlabeled complementary strands were annealed to the ROX labeled strands, and confirmed by gel electrophoresis. Calculated ΔG values for the most prominent predicted secondary structures for each of the ssDNA substrates as determined by the mfold program [12] are: −0.2 Kcal/mol for 13mer; −0.2 Kcal/mol for 20mer; and −3.0 Kcal/mol for 63mer, indicating that only the ss63mer might contain some secondary structure.

Fluorescence anisotropy titrations – all titrations were performed using a Horiba Fluoromax-4 spectrofluorometer. Unless otherwise indicated, titration buffer contained 25 mM potassium–acetate and 10 mM Tris–acetate, pH 8.0, at 25 °C. See Ref. [13] for a review of the titration method. For titrations performed in the presence of ATPγS or ADP, 1 mM magnesium acetate and 100 μM cofactor were added to all solutions.

Filament formation kinetics – to examine RecA free filament formation, turbidity measurements were carried out as described by Wilson and Benight [14]. Absorbance was measured every minute for 30 min at 320 nm on a Shimadzu UV-1650 pc spectrophotometer at 25 °C with a protein concentration of 1 μM, and a fixed slit width of 2 nm.

Data analysis – the program KaleidaGraph (Synergy Software) was used to fit binding data to the Hill equation:

$$\theta = \theta_{max} \frac{[P]^{\alpha}}{(K_{50})^{\alpha} + [P]^{\alpha}} \quad (1)$$

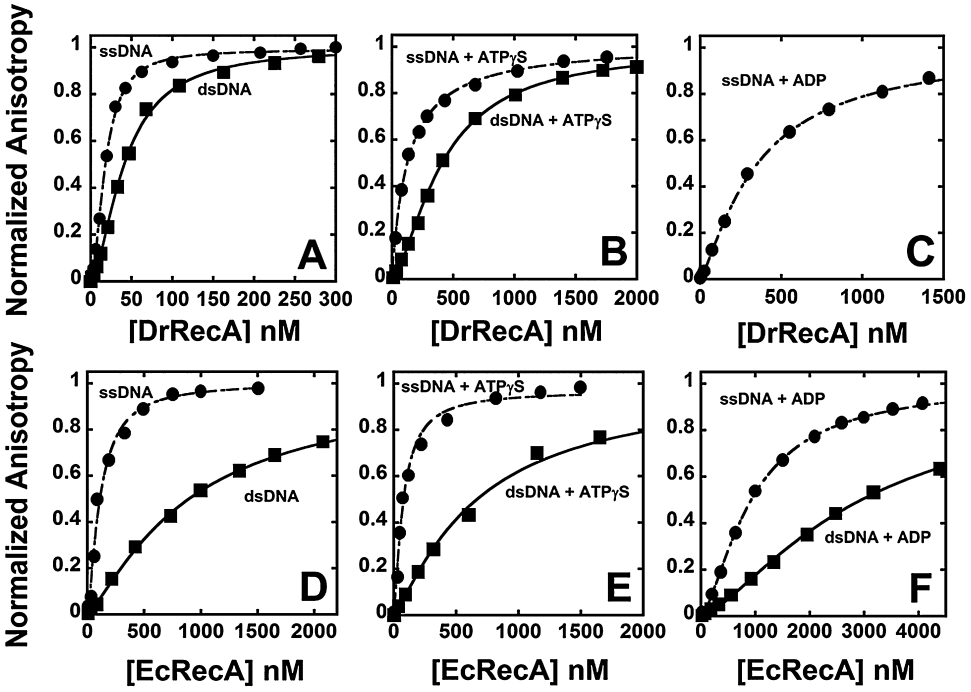

Fig. 1. Representative equilibrium binding titrations fit to the Hill equation are shown for DrRecA (A–C) and EcRecA (D–F) binding to 63mer ssDNA (circles) and 63/63mer dsDNA (squares) in 25 mM K-acetate, 10 mM Tris–acetate, pH 8.0 in the absence of cofactor (A and D), in the presence of 100 μM ATPγS + 1 mM Mg-acetate (B & E), and in the presence of 100 μM ADP + 1 mM Mg-acetate (C and F).

where θ is the fractional saturation; P is the total protein concentration; K_{50} is the apparent dissociation constant and midpoint of the binding curve; and α is the Hill coefficient. Fluorescence anisotropy changes for a DNA fragment that binds multiple proteins may or may not be directly linearly proportional to the fractional saturation (or binding density) on the DNA [15]. Simulations of the propagated effect of the largest potential deviations modeled by Lohman and Bujalowski [15] predict up to a possible 1.4 fold deviation in the determined K_d values for binding, and a potential change of up to ±0.1 in the Hill coefficient, if such nonlinearity exists in the data herein. Due to these potential errors, the binding affinities are reported herein as K_{50} values rather than absolute K_d values. Even if the largest modeled nonlinear deviations between anisotropy and fractional saturation were embedded within this data, the 1.4 fold potential deviation in binding affinities would not alter any conclusions of this study, since (1) all differences between RecA species, and even the smaller differences between ssDNA binding affinities, are much larger than this potential deviation, and (2) all changes from such potential nonlinearity will most likely change the K_{50} values in the same direction.

3. Results

Comparative binding affinities– Fig. 1 shows equilibrium binding curves for DrRecA and EcRecA to ssDNA and dsDNA 63mer oligomers in the absence of cofactor, and in the presence of ATPγS or ADP. Any titration for one RecA species is carried out under identical solution conditions with the other RecA species, and the relative concentration ranges in the different panels of Fig. 1 reflect the differences in binding affinity. The fitted K_{50} and Hill coefficients are shown in Table 2, and report that in the absence of cofactor, DrRecA can bind dsDNA over 20 fold tighter than EcRecA, and can bind ssDNA almost 9 fold tighter than EcRecA. These relationships are illustrated in graphic form in Fig. 2.

Panels B and E of Fig. 1 show that the presence of ATPγS, a non-hydrolyzable ATP analog, significantly decreases the binding affinity of DrRecA. In contrast, the presence of ATPγS slightly increases the affinity of EcRecA for DNA. The presence of ATPγS thus causes convergence of the relative affinities of the two RecA species. The finding that the highest affinity binding state for DrRecA is in the absence of ATP is a reversal of what has long been established for EcRecA, where several studies have reported that the binding of EcRecA to contiguous (unbroken) dsDNA requires the presence of ATP or a non-hydrolyzable ATP analog such as ATPγS [16–18]. For EcRecA binding to these shorter DNA oligomers, ATP is clearly not required, but does enhance the binding of the *E. coli* protein. Because binding of EcRecA to contiguous dsDNA has long been established to require ATP, the binding of both EcRecA and DrRecA to dsDNA observed here is likely primarily due to binding at the ends of the short DNA oligomers. Viewed in this light, the results suggest that in the absence of cofactor, dsDNA breaks might be a preferred binding target for DrRecA protein. In a recent review, Slade and Radman note that almost all (97%) of the radiation induced ds breaks in *D. radiodurans* are "single-event" breaks [19], meaning they will be blunt-end or very nearly blunt-end breaks.

Binding of the two species of RecA to 63mer DNA in the presence of ADP is also shown in Fig. 1, and reported in Table 2. The presence of ADP strongly decreases the affinity of EcRecA for DNA, and has a similar effect on DrRecA. Only data for DrRecA binding to ssDNA are shown because the presence of ADP inhibited reliable binding measurements of DrRecA for dsDNA with our assay.

Length dependence of ssDNA binding – for the 63 and 63/63mer DNAs in Fig. 1, affinity is tightest for ssDNA for both species of RecA, but the relative difference between the affinity for ssDNA over dsDNA is larger for EcRecA. This relative ssDNA:dsDNA affinity changes with DNA length, however, and Fig. 3 shows the

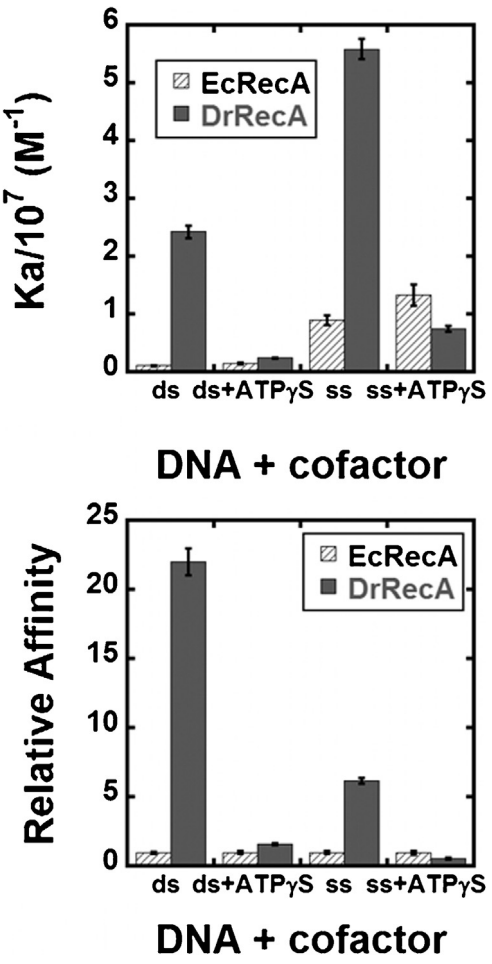

Fig. 2. Top panel graphically illustrates the differences in binding affinities, plotted here as Ka valutes, for EcRecA (striped bars) and DrRecA (solid bars) to ds and ss 63mer DNA in the presence and absence of ATPγS cofactor. The bottom panel shows the data re-scaled as normalized relative binding affinities, where for each pair, the affinity of EcRecA (open bars) is normalized to 1.0 and the relative affinity of DrRecA is shown as the corresponding solid bar.

effect of oligomer length on the DNA binding affinity of DrRecA and EcRecA in the absence of cofactor. Both proteins show a significant length dependence in their affinity for ssDNA (panels A and C), and little or no length dependence for dsDNA binding (panels B and D). The lack of dsDNA length dependence again emphasizes that for these short oligomers, dsDNA binding is likely end-binding. Also again, the differing concentration axes of the plots re-emphasizes the significantly tighter binding of DrRecA versus EcRecA to all DNA constructs in the absence of cofactor. Fig. 3E shows that increasing ssDNA binding affinity with increasing length predicts a switchover point in the range of 13–30 nucleotides for both RecA species. At DNA lengths shorter than this range, dsDNA binding will be tighter, while at longer lengths, ssDNA binding will be favoured by both proteins.

While the 13 and 20mer ssDNA oligomers will not form any secondary structure, there is the question of whether secondary structure formation within the ssDNA 63mer might affect the binding results. However, since the measured affinity of ssDNA 63mer is tighter than dsDNA 63mer for both proteins, both in the absence and presence of ATPγS, complete absence of any potential secondary structure could only increase this ssDNA > dsDNA affinity difference.

K-acetate dependence of binding – the 20mer oligonucleotide substrates were used to examine if changing salt concentration

Table 2
DNA binding parameters from fits to the Hill equation.

	DrRecA			EcRecA		
DNA substrate	K_{50} (nM)	Hill coefficient	ΔG_{app} (Kcal/mol)*	K_{50} (nM)	Hill coefficient	ΔG_{app} (Kcal/mol)*
13mer	166 ± 14	1.6 ± 0.1	−9.3	1008 ± 29	2.1 ± 0.1	−8.2
13/13mer	46.9 ± 1.5	2.7 ± 0.2	−10.0	986 ± 49	1.6 ± 0.1	−8.2
20mer	49.7 ± 2.5	1.9 ± 0.2	−10.0	440 ± 12	2.2 ± 0.1	−8.7
20/20mer	35.1 ± 0.9	2.5 ± 0.2	−10.2	731 ± 24	1.1 ± 0.1	−8.4
63mer	17.9 ± 0.4	1.9 ± 0.1	−10.6	111 ± 7	1.4 ± 0.1	−9.5
63/63mer	41.1 ± 1.3	1.7 ± 0.1	−10.1	895 ± 38	1.3 ± 0.1	−8.3
63mer ATPγS	133 ± 7	1.0 ± 0.1	−9.4	75 ± 7	1.4 ± 0.2	−9.7
63/63mer ATPγS	407 ± 11	1.6 ± 0.1	−8.7	670 ± 59	1.2 ± 0.1	−8.4
63mer ADP	355 ± 15	1.3 ± 0.1	−8.8	929 ± 18	1.5 ± 0.1	−8.2
63/63mer ADP	ND	ND	ND	2943 ± 114	1.4 ± 0.1	−7.6

* All ΔG values are ± < 0.1 Kcal/mole.

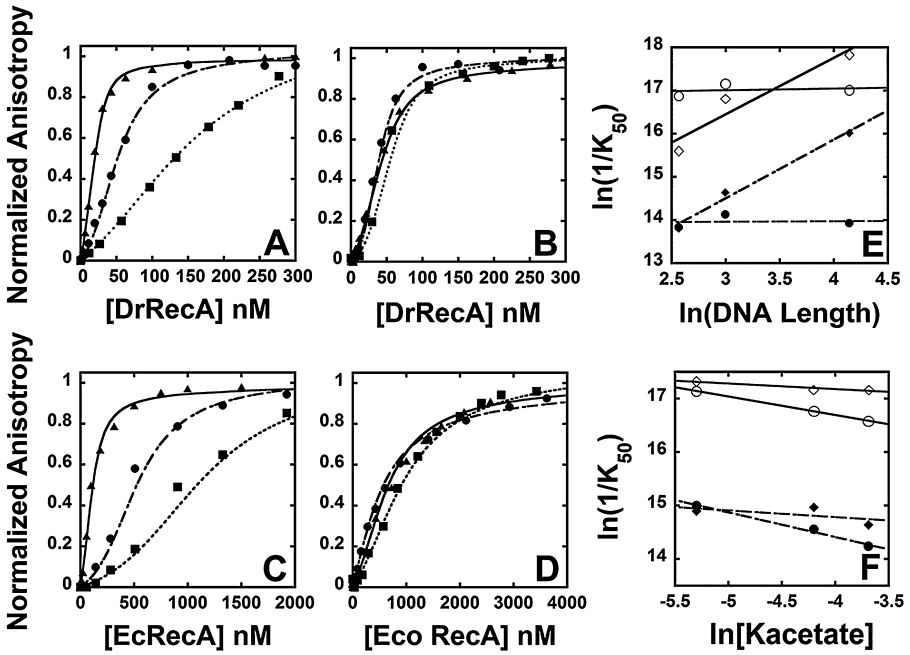

Fig. 3. Length dependence of ssDNA and dsDNA binding by RecA. Representative equilibrium binding titrations fit to the Hill equation are shown for DrRecA (A and B) and EcRecA (C and D) binding to ssDNA(A and C) and dsDNA(B and D) in 25 mM K-acetate, 10 mM Tris–acetate, pH 8.0 in the absence of cofactor. Triangles show 63 nucleotide substrates, circles show 20 nucleotide substrates, and squares show 13 nucleotide substrates. Panel E shows the length dependence data in Panels A–D plotted as ln (DNA length) versus ln K_{50}. Panel F shows the K-acetate dependence of ssDNA and dsDNA binding by RecA in the absence of cofactor. Open symbols with solid lines show DrRecA. Closed symbols with dashed lines show EcRecA. Diamonds show ssDNA while circles show dsDNA. The slopes of the dsDNA plots are −0.24 ± 0.02 and −0.46 ± 0.05 for DrRecA and EcoRecA, respectively. The slopes of the ssDNA plots are −0.10 ± 0.03 and −0.12 ± 0.17 for DrRecA and EcoRecA, respectively. Slopes of the salt linkage plots (ln $1/K_{50}$ versus ln [K-acetate]) report the net ion release upon protein-DNA interaction.

significantly affected the binding of either DrRecA or EcRecA. As can be seen in Fig. 3F, the predicted linkages at low salt (25 mM to 5 mM) indicate very little change in binding affinity for any of the DNA substrate-RecA homologue combinations used here. This indicates that the relationships reported in this study should remain consistent across the low salt ranges typically used for studies of RecA.

Cooperativity of binding – the data of Table 2 also reveal subtle but consistent differences between the cooperativity of binding of the two RecA species to these short DNA oligomers. When binding dsDNA, at all lengths, the binding cooperativity for DrRecA is always stronger than the binding cooperativity of EcRecA as indicated by higher values of the Hill coefficient. This is seen both in the presence and absence of ATPγS. Interestingly, higher cooperativity is seen for DrRecA binding to the shorter 20/20mer and 13/13mer substrates than to the 63/63mer substrate, while higher cooperativity is seen for the shorter ssDNA constructs relative to the 63mer for EcRecA.

Filament formation kinetics – under appropriate conditions, RecA can spontaneously form filaments without DNA, a process that has been shown to be competitive with DNA binding [20]. Such competition, if present, would lead to an increase in the observed K_{50} values. The filament formation curves shown in Fig. 4 indicate that under the solution conditions used for the DNA binding experiments little or no free filament formation occurs. In contrast, we also examined solution conditions that are known to support free filament formation (i.e., 10 mM $MgCl_2$ with no monovalent salt or nucleotide cofactor) and show that there is a significant change in scattering intensity that reaches a maximum at approximately 30 min for EcRecA and 20 min for DrRecA. It is notable that the maximal scattering intensity for DrRecA free filaments is significantly lower than the maximal scattering intensity for EcRecA free filaments, suggesting that DrRecA either forms fewer, or shorter free filaments in solution. This result is interesting in light of the prediction, based on nucleation and filament extension kinetics, that DrRecA will also form shorter filaments on DNA than will EcRecA [6,7], as it suggests that the shorter filament length is an intrinsic oligomerization property of the DrRecA protein even in the absence of DNA.

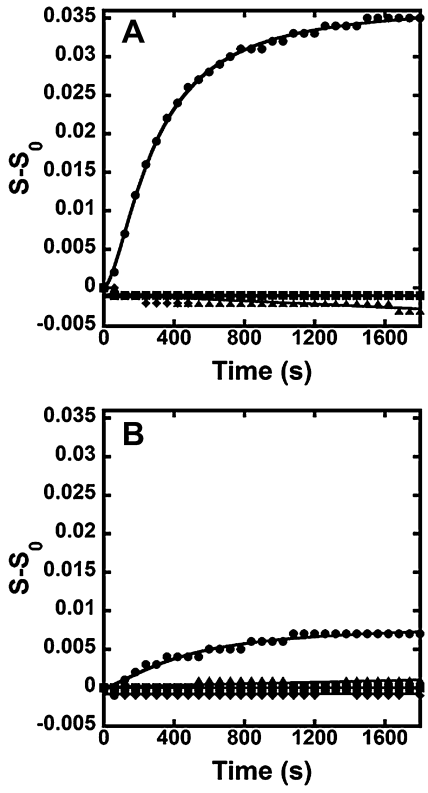

Fig. 4. Free-filament formation kinetics of RecA in the absence of DNA. The change in absorbance over time is plotted for (A) EcRecA, and (B) DrRecA under various solution conditions. All solutions contained 10 mM Tris, pH 8.0 and 1 μM RecA protein. Circles are in the presence of 10 mM $MgCl_2$; squares are in the presence of 25 mM potassium-acetate; diamonds are in the presence of 25 mM potassium-acetate, 1 mM $MgCl_2$, and 100 μM ATPγS; and triangles are in the presence of 25 mM potassium-acetate, 1 mM $MgCl_2$, and 100 μM ADP.

4. Discussion

Comparatively examining the DNA binding behavior of homologous proteins reveals information about how each homologue might be specifically adapted for its own intracellular and physiological environment. The question has been posed as to whether some or all of *D. radioduran's* DNA repair proteins are actually intrinsically more efficient at DNA repair than those from non-radiation resistant bacteria, or whether they are effectively equivalent but much better protected from radiation damage [19]. This study demonstrates that at least as regards initial binding of DNA, DrRecA is significantly more effectual than EcRecA under many conditions.

Previous studies of EcRecA DNA binding have been conducted using a variety of techniques, including fluorescence anisotropy [20,21,22], and our results for ssDNA binding of EcRecA fall in the same affinity range as those previously reported for ss39mers by Bar-Ziv and Libchaber [23]. Most previous studies of EcRecA binding have been performed in the presence of ATPγS or ATP; and ATP hydrolysis has also often been used as an indirect method for monitoring binding, however, both our data and recent data from Ngo et al., demonstrate that RecA binding probably should not be monitored this way [7].

The present study is one of the first to thermodynamically examine direct binding of DrRecA, and to examine binding of either RecA species to short defined dsDNA oligomers. Binding of EcRecA to contiguous dsDNA has long been reported to require the presence of ATP or a non-hydrolysable ATP analogue such as ATPγS [16–18]. We find that both RecA proteins strongly bind dsDNA oligomers in the absence of any type of ATP cofactor, most likely because they are both end-binding to the DNA, but that somewhat surprisingly, DrRecA binds such dsDNA oligomers over an order of magnitude more tightly than does EcRecA. Also, while the binding results herein for EcRecA to ssDNA follow the previously established hierarchy of substrate selectivity relative to cofactor presence (ATPγS > no cofactor > ADP) [22], DrRecA binding demonstrates an altered binding hierarchy (no cofactor > ATPγS > ADP), with the largest change between the no cofactor and ATPγS states in DrRecA (7.4 fold), but between the no cofactor and ADP states for EcRecA (8.4 fold).

The binding results thus indicate that if intracellular adenosine availability is low, DrRecA will be significantly (9–20 fold) more targeted toward binding of damaged DNA than would EcRecA. Several studies have highlighted the damage to free nucleotides by ROS [24,25], the breakdown and export of nucleotides, especially ATP, upon irradiation of *D. radiodurans* [reviewed in 19], and expansion within *D. radiodurans* in the number of Nudix hydrolayses, which may be involved in the processing and excretion of damaged nucleotides [19,26,27]. All of these studies suggest depletion of the free nucleotide pools, creating low adenosine nucleotide availability, and suggesting the potential physiological benefits of enhanced RecA binding activity under such conditions.

The tighter dsDNA binding of DrRecA relative to EcRecA shown here could be related to its role in the unique process of extended synthesis dependent strand annealing (ESDSA) in *D. radiodurans*. Radman and co-workers have shown that DNA repair within *D. radiodurans* begins with ESDSA, and have identified DrRecA as being integral in several steps in this synthetic process, including enhancing the initial DNA degradation necessary to produce ssDNA overhangs [4,19,28]. Enhanced binding to the ds break sites where DNA degradation is initiated may be a key requirement in the initiation of ESDSA, and may help explain why EcRecA, which would bind more weakly, does not fully compliment the oxidative stress resistance phenotype of DrRecA knockout mutants [29].

This study also demonstrates that in the absence of cofactor, DrRecA binds to ssDNA nearly an order of magnitude more tightly than does EcRecA. EcRecA has long been known to form filaments on ssDNA in the absence of cofactor, and a number of previous binding studies of EcRecA to short ssDNA oligomers have been published [23,30,31]. RecA will thus also be significantly more likely to be found in ssDNA filaments in *D. radiodurans* than in *E. coli*.

Previous work has also indicated a clear length dependence for the binding of EcRecA to ssDNA [31]. Our data confirm this behavior and demonstrate that DrRecA shares the same characteristic. It should be noted that the finding that DrRecA binds longer lengths of ssDNA more tightly than dsDNA does not refute the finding from Kim and Cox that DrRecA prefers to use an "inverse pathway" to initiate strand exchange from dsDNA [5], although it does refine the understanding of those results. These results are compatible for several reasons: (1) The preference of DrRecA for initiating strand exchange from dsDNA [5] is a kinetic result, not a binding affinity measurement i.e., the fact that equilibrium binding to target "A" is tighter than binding to target "B" is not incompatible with the fact that the reaction may proceed more efficiently (or exclusively) from "B". (2) The kinetic data actually track the end result of a multi-step reaction, of which only the very first step is DNA binding, and Kim and Cox report that the initial binding to ssDNA in these reactions is faster than initial binding to dsDNA [5]. (3) Our binding data also show that DrRecA discriminates less between dsDNA and ssDNA at longer DNA lengths than does EcRecA (the relative ssDNA:dsDNA binding affinities are closer together for DrRecA at the longer DNA lengths tested here). This result indicates that for longer DNAs, in a mixture of ssDNA and dsDNA, DrRecA will bind significantly to both, whereas the binding population in a similar mixture with EcRecA would be more heavily dominated by ssDNA bound complexes.

It is also notable that the recent characterization of mutant RecA proteins that arise during forced evolution of radiation-resistance

in *E. coli* revealed some characteristics that suggest compatibility with the properties of DrRecA identified in the present study. Although no direct binding experiments were performed, the radiation-resistance induced EcRecA D276 mutants found by Hsu et al., like DrRecA itself [6], exhibited both (1) faster filament nucleation on DNA and (2) shorter filament formation [32]. Although the filament nucleation process characterized in the studies by Piechura et al., is a much more composite process than a simple on-rate for DNA binding, it is still intriguing to note that a faster on-rate in a binding equilibrium process, in the absence of any off-rate changes, would produce tighter binding (Kd = off-rate/on-rate). Likewise, the shorter filaments formed by the radiation-resistant EcRecA mutants [32], like the shorter filaments formed by DrRecA itself [6], mirror the intrinsically shorter free-filament formation by DrRecA suggested in the present study.

More so than many DNA binding proteins, the behavior of RecA has been found to be profoundly affected by all aspects of its environment, including the buffer used [33], the temperature and pH of the solution [8,21], and the sequence of the DNA (even though it is ostensibly non-specific) [23]. Thus, any study of RecA should be only cautiously extrapolated beyond the specific conditions of that study without direct evidence that particular changes would have minimal effect (such as demonstrating the weak dependence on K-acetate concentration shown herein). In this study, we chose frequently used solution conditions in previous studies of RecA to directly compare the binding of EcRecA and DrRecA to short ssDNA and dsDNA oligomers, and found a number of interesting molecular differences and similarities between the two species of RecA. It is likely that a broader survey of solution conditions and substrates will continue to reveal other species-specific characteristics of the two proteins.

Conflict of interest

None.

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