DNA Repair 31 (2015) 91–96

Contents lists available at ScienceDirect

DNA Repair

journal homepage: www.elsevier.com/locate/dnarepair

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

Jaycob D. Warfel[1], Vince J. LiCata*

Department of Biological Sciences, Louisiana State University, Baton Rouge, LA 70803, USA

ARTICLE INFO

Article history:
Received 23 March 2015
Received in revised form 3 May 2015
Accepted 4 May 2015
Available online 5 May 2015

Keywords:
Homologous recombination
DNA repair
DNA binding protein

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.

* Corresponding author. Tel.: +1 225 578 5233.
E-mail address: licata@lsu.edu (V.J. LiCata).
[1] Present address. Pennington Biomedical Research Center, Louisiana State University, Baton Rouge, LA 70808 U.S.A.

http://dx.doi.org/10.1016/j.dnarep.2015.05.002
1568-7864/

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:

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

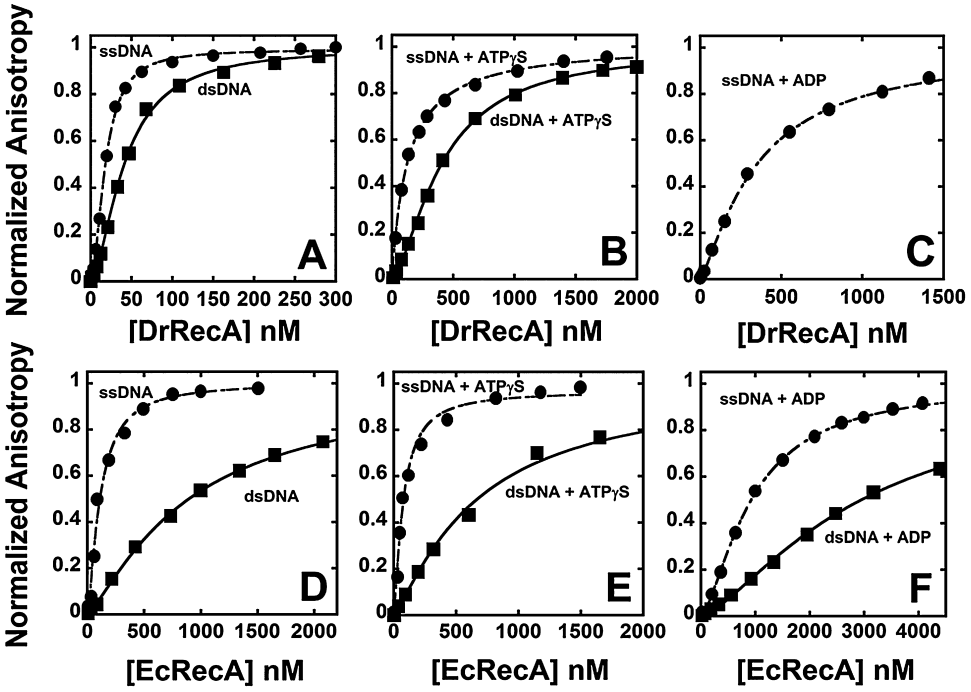

Fig. 1. Representative equilibrium binding titrations fit to the Hill equation are shown for DrRecA (A–C) and EcRecA (D–F) binding to 63mer ssDNA (circles) and 63/63mer dsDNA (squares) in 25 mM K-acetate, 10 mM Tris–acetate, pH 8.0 in the absence of cofactor (A and D), in the presence of 100 μM ATPγS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