Influence of the C-Terminal Tail of RecA Proteins from Alkaline pH-Resistant Bacterium Deinococcus Ficus

Hsiu-Fang Fan,* Shu Su, Ying-An Kuo, and Cyuan-Ji Chen

ABSTRACT: Deinococcus fuscus CC-FR2-10T, resistant to ultraviolet, ionizing radiation, and chemicals which may cause DNA damage, was identified in Taiwan. The expression level of D. fuscus RecA, which has 92% sequence identity with Deinococcus radiodurans (Dr.) RecA, will be upregulated upon UV radiation. Multiple sequence alignment of RecA proteins from bacteria belonging to Escherichia coli and the Deinococcus genus reveals that the C-terminal tail of D. fuscus RecA is shorter and contains less acidic residues than E. coli RecA. D. fuscus RecA exhibits a higher ATPase activity toward single-stranded (ss) DNA and efficiently promotes DNA strand exchange that a filament is first formed on ssDNA, followed by uptake of the double-stranded (ds) substrate. Moreover, D. fuscus RecA exhibits a pH reaction profile for DNA strand exchange similar to E. coli ΔC17 RecA. Later, a chimera D. fuscus C17E.coli RecA with more acidic residues in the C-terminal tail was constructed and purified. Increased negativity in the C-terminal tail makes the pH reaction profile for Chimera D. fuscus C17E.coli RecA DNA strand exchange exhibit a reaction optimum similar to E. coli RecA. To sum up, D. fuscus RecA exhibits reaction properties in substrate-dependent ATPase activity and DNA strand exchange similar to E. coli RecA. Our data indicate that the negativity in the C-terminal tail plays an important role in the regulation of pH-dependent DNA strand exchange activity.

INTRODUCTION

Deinococcus fuscus, a pale-pink strain (CC-FP2-10T) from the rhizosphere of the tree Ficus religiosa L., was identified in 2006.1 D. fuscus has been shown to survive at highly alkaline pH of up to 10 and to be resistant to UV irradiation (254 nm, 8–10 cm for 10 min).1 The RecA expression level was upregulated, and RecA protein is important for the survival of D. fuscus under UV irradiation.1 These phenomena suggest that D. fuscus RecA plays an important role in repairing DNA damage in the harsh environment. The D. fuscus RecA protein, with 357 amino acid residues, contains a central core domain, a smaller N-terminal domain and a C-terminal domain.1 A homology search performed using the National Center for Biotechnology Information (NCBI) BLAST program revealed that the amino acid sequence of D. fuscus RecA shares 92% identical sequence to Deinococcus radiodurans (Dr.) RecA and 62.0% identity and 77.0% similarity to Escherichia coli RecA. Moreover, the length of the RecA C-terminal tail of different Deinococcus species is variable, and a shorter C-terminal tail with fewer negatively charged residues is found for some Deinococcus species (Figure 1).

The RecA protein is essential for repairing the damaged chromosomal DNA by mediating homologous recombination.3−7 In the canonical model, E. coli RecA can bind on single-stranded (ss) DNA to form a nucleoprotein filament. Later, a RecA-nucleoprotein filament can search for homologous sequence from another double-stranded (ds) DNA. The last step is RecA dissociation after the completion of strand exchange.5,8,9 On the contrary, Dr. RecA more efficiently promotes DNA strand exchange via inverse established pathway.10 Higher ATP hydrolysis rates are determined for the binding of E. coli RecA filaments to ssDNA, while Dr. RecA can hydrolyze ATP more rapidly on dsDNA than on ssDNA at pH 7.5.11,12 Another notable characteristic is that the pH reaction profile for DNA strand exchange shifts to a higher pH condition for E. coli ΔC17 RecA and Dr. RecA.12−15

Here, comprehensive experiments were carried out to investigate the reaction properties of E. coli RecA, D. fuscus RecA, E. coli ΔC17 RecA (in which the last 17 residues in the C-terminus are deleted), and the chimera D. fuscus C17E.coli RecA in which the C-terminal tail is replaced with the corresponding residues of the E. coli RecA C-terminus [(Figure S1)] simultaneously. Similar to E. coli RecA, D. fuscus RecA exhibited higher ATPase activity toward ssDNA and preferentially promoted DNA strand exchange via a canonical pathway. A dramatic shift in the pH-dependent strand exchange reaction profile was observed for D. fuscus RecA and...
Figure 1. Multiple sequence alignment of RecA proteins from bacteria belonging to E. coli (E-coli): WP_000963143.11, Deinococcus fuscus (D-fuscus): WP_022801718.1, Deinococcus maricopensis (D-maricopensis): WP_013557980.1, Deinococcus deserti (D-deserti): WP_012694062.1, Deinococcus radiodurans (D-radiodurans): WP_010888966.1, Deinococcus wulumuqiensis (D-wulumuqiensis): WP_017870593.1, Deinococcus grandis (D-grandis): WP_058978423.1, and Deinococcus gobiensis (D-gobiensis): WP_014686240.1. The alignment was performed using ESPript 3.0 program. Solid and dash boxes represent positions of polymeric domains and DNA binding domains respectively. Over lines and symbols indicates the secondary structure of E-coli RecA(2REB). The red squares indicate the negatively charged amino acids in this region. The blue squares indicate the positively charged amino acids in this region.
nM 427-nt dsDNA (at pH = 6.5 (Deinococcus gobiensis 90% identity with Deinococcus reticulitermitis Deinococcus deserti 92% identity with D. radiodurans E. coli RecA)).

Bacteria Belonging to E. coli

RESULTS

Table 1. Primer Sequences Used to Obtain the Experimental DNA Sequences

| DNA substrate | template | primer sequence |
|---------------|----------|----------------|
| 335bp dsDNA   | 3XF3XH   | 5′-TGAGTGATAACACTGCAGGCA |
| 427bp ssDNA   | 3XF3XH   | 5′-ACTACGATACGGGAGGCC |
| 433bp ssDNA (Non-homology) | 3XF3XH | 5′-ACTACGATACGGGAGGCC |
| 500bp ssDNA   | pGEM3Z601 | 5′-Cy3-CTTCTCGAGGTCGCTGTTCAATACA |
| Cy3           | pGEM3Z601 | 5′-Phosp-CGCCATCATGAATCCAGTAAG |
| 500bp ssDNA   | pGEM3Z601 | 5′-Phosp-CGCCATCATGAATCCAGTAAG |

Table 2. Rates of ATP (or dATP) Hydrolysis

| substrate | pH 6.5 | pH 7 | pH 7.5 | pH 8 | pH 8.5 | pH 6.5 (dATP) | pH 6.5 (E. coli RecA) |
|-----------|--------|------|--------|------|--------|---------------|---------------------|
| ssDNA     | 621.2 ± 177.2 | 532.6 ± 63.9 | 500.6 ± 63.9 | 434.0 ± 129.7 | 489.7 ± 56.8 | 450.7 ± 36.8 | 32.7 ± 4.2 |
| dsDNA     | 5.93 ± 0.53 | 4.57 ± 0.12 | 3.52 ± 0.42 | 3.16 ± 0.63 | 1.78 ± 0.60 | 8.42 ± 0.40 | 8.88 ± 0.3 |
|           | 52.8 ± 99.4 | 512.3 ± 75.0 | 510.3 ± 75.0 | 514.7 ± 231.3 | 582.7 ± 147.8 | 502.7 ± 120.8 | 49.5 ± 6.5 |

“RecA and E. coli RecA shares 93% identity with Deinococcus marinopiscis RecA, 92% identity with Deinococcus radiodurans RecA and Deinococcus reticulitermitis RecA, 91% identity with Deinococcus wulamaiensis RecA and Deinococcus grandis RecA, 90% identity with Deinococcus gobiensis RecA, and 62.0% identity and 77.0% similarity with E. coli RecA. The multiple sequence alignment of RecA proteins of the Deinococcus genus and E. coli is shown in Figure 1 and suggests that the reaction behaviors of D. ficus RecA may share many similarities with those of Dr. RecA. Moreover, it is obvious that the C-terminal tail of Deinococcus RecA proteins is shorter and lacks negatively charged residues compared to E. coli RecA (Figure 1). Therefore, we utilized comprehensive biochemical experiments to investigate the reaction properties of E. coli RecA and D. ficus RecA simultaneously.

DNA- and pH-Dependent ATPase Activities. The most prominent characteristics of Dr. RecA proteins are the dramatically different DNA binding preference, the different substrate-dependent ATPase activity, and the different reaction pathway of DNA strand exchange from E. coli RecA. Because D. ficus also belongs to the Deinococcus genus, we measured the DNA-dependent ATP hydrolysis rates of D. ficus RecA. The hydrolysis activities of E. coli RecA were also investigated under the same condition and served as a reference (Figure 2A and Table 2). A smaller ATP hydrolysis

Figure 2. Substrate-dependent ATP hydrolysis rate; (A) substrate-dependent ATP hydrolysis rates were measured under different concentrations of ATP with the EnzCheck phosphate assay kit. The experimental data were fitted with Michaelis–Menten kinetics. Experiments were carried out with 4 μM E. coli RecA or D. ficus RecA, 30 nM 427-nt dsDNA or 427-nt ssDNA in complete reaction buffer with different concentrations of ATP at pH = 6.5 (~0.31 RecA/nt). (B) pH- and substrate-dependent ATP hydrolysis rates. Experiments were carried out with 4 μM D. ficus RecA, 30 nM 427-nt dsDNA ( ), or 427-nt ssDNA ( ) in complete reaction buffer with 2 mM ATP at different pH conditions. The experiments were performed at 22 °C. The same experiments were performed with 2 mM dATP at pH 6.5 for comparison. (C): 427-nt ssDNA) and (D): 427-nt dsDNA).

E. coli ΔC17 RecA, which is consistent with previous reports. In contrast, the optimal pH-dependent strand exchange profile shifted to acidic conditions for E. coli RecA and the chimera D. ficus C17 E. coli RecA. Our experimental results indicate that the negativity in the C-terminal tail of RecA proteins directly regulates the pH-dependent DNA strand exchange activity.

Multiple Sequence Alignment of RecA Proteins from Bacteria Belonging to E. coli and the Deinococcus Genus. A homology search performed using the National Center for Biotechnology Information (NCBI) BLAST program revealed that the amino acid sequence of D. ficus RecA shares 93% identity with Deinococcus marinopiscis RecA, 92% identity with Deinococcus deserti RecA, D. radiodurans RecA and Deinococcus reticulitermitis RecA, 91% identity with Deinococcus wulamaiensis RecA and Deinococcus grandis RecA, 90% identity with Deinococcus gobiensis RecA, and 62.0% identity and 77.0% similarity with E. coli RecA. The multiple sequence alignment of RecA proteins of the Deinococcus genus and E. coli is shown in Figure 1 and suggests that the reaction behaviors of D. ficus RecA may share many similarities with those of Dr. RecA. Moreover, it is obvious that the C-terminal tail of Deinococcus RecA proteins is shorter and lacks negatively charged residues compared to E. coli RecA (Figure 1). Therefore, we utilized comprehensive biochemical experiments to investigate the reaction properties of E. coli RecA and D. ficus RecA simultaneously.
rate of \(35 \mu M/min\) and \(K_{\text{cat}}\) of \(8.88 \text{ min}^{-1}\) was observed for \(E. coli\) RecA, compared to previous studies.\(^{18}\) There are several differences including experimental temperature (22 vs 37 °C), DNA substrate used (linear 427-nt vs circular M13mp18), SSB (no SSB vs one SSB monomer per 10 nucleotides of ssDNA), and different buffer systems (without vs with the ATP regeneration system). Those factors might result in a smaller ATP hydrolysis rate observed in our system. Higher ATPase activity was found when \(E. coli\) RecA was bound to ssDNA (Figure 2A and Table 2), and this phenomenon is consistent with previous reports validating the reliability of this ATPase measurement.\(^{11,16,17}\) In this study, \(D. fuscus\) RecA exhibited a similar substrate preference in terms of ATPase activity to that of \(E. coli\) RecA at pH 6.5 (Figure 2A). Moreover, higher dATP hydrolysis rates were observed for \(D. fuscus\) RecA with either ssDNA or dsDNA as a cofactor, and similar behaviors were observed for \(D. recA\) (Figure 2B).\(^{12}\) With either ssDNA or dsDNA as substrates, a higher ATP hydrolysis rate was found at pH 6.5 under our experimental conditions (Figure 2B). To rule out the influence of pH-dependent conformational change, the secondary structures of \(D. fuscus\) RecA under different pH conditions were examined by CD spectroscopy (Figure 3). The absorbance exhibits a significant change at pH 6.0, indicating some changes in the \(\alpha\)-helix content. The theoretical pl of \(D. fuscus\) RecA is 5.756 (<pH 6.0) calculated based on sequence (Protein Tool-Prot pi). Therefore, there could be some changes in its structure at pH near pl. However, the overall shapes of the CD spectra are quite similar from pH 7.0 to pH 9.0, indicating no significant difference in secondary structure. The following experiments were carried out at pH between 7.0 and 9.0.

**Reaction Pathway of RecA-Promoted DNA Strand Exchange.** It has been reported that \(D. recA\) proteins can promote DNA strand exchange in the exact inverse of an established normal pathway in which preformed ssRecA nucleoprotein filament interacts with dsDNA molecules.\(^{10}\) Next, we investigated the reaction pathway of RecA-promoted DNA strand exchange with electrophoresis mobility shift assay for \(E. coli\) RecA and \(D. fuscus\) RecA side by side (Figure 4A,B). Under our experimental conditions, shorter DNA molecules were used (427-nt vs 7229-nt M13mp8).\(^{12}\) SSB did not affect the DNA strand exchange efficiency for both RecA proteins (Figure 4B,C). Moreover, \(E. coli\) RecA and \(D. fuscus\) RecA proteins preferentially promoted DNA strand exchange via a canonical pathway (Figure 4B,C).

**pH-Dependent RecA-Mediated Strand Exchange Reaction Profile.** A dramatic change in the pH profile of the RecA-mediated DNA strand exchange reaction was observed for \(E. coli\) ΔC17 RecA and \(D. recA\).\(^{12–15}\) Moreover, a faster nucleation rate on dsDNA is observed for \(E. coli\) ΔC17 RecA,\(^{13,16,19}\) attributed to the suppression of electrostatic repulsion upon the deletion of acidic residues in its C-terminus.\(^{14,15}\) These observations suggest that residues in RecA’s C-terminus might play an important role in shifting the pH reaction profile of the DNA strand exchange reaction toward higher pH. To verify the influence of the C-terminal tail, pH-dependent RecA-mediated strand exchange reactions were performed for \(E. coli\) RecA, \(D. fuscus\) RecA, \(E. coli\) ΔC17 RecA (in which the last 17 residues in the C-terminus are deleted), and the chimera \(D. fuscus\) C17-\(E. coli\) RecA [in which the C-terminal tail is replaced with the corresponding residues of the \(E. coli\) RecA C-terminus (Figure S1)] side by side (Figure 5). To avoid the base-induced destabilization of DNA strands, control experiments were performed in complete reaction buffer without RecA, and no detectable strand exchange product was observed at pH values between 7.0 and 9.0 (Figure S2A,B). Besides, no detectable strand exchange product was observed in the absence of ATP or in the presence of nonhomologous ssDNA, confirming the ATP dependent and sequence dependent activities of RecA proteins (Figure S2C,D). The DNA strand exchange activity promoted by \(E. coli\) RecA and chimera \(D. fuscus\) C17-\(E. coli\) RecA reaches an optimum near pH 7.0, trails off gradually, and is nearly abolished at pH > 8.5. In contrast, the entire pH-dependent reaction profile shifts toward higher pH for \(D. fuscus\) RecA and \(E. coli\) ΔC17 RecA, approaching an optimum ≥pH 8.5 (Figures 5B and 6C). These observations supported our hypothesis that the negativity in the C-terminal tail affects the optimum pH reaction profile of the RecA-mediated DNA strand exchange reaction.

**DISCUSSION**

The characterization and reaction behaviors of \(D. fuscus\) RecA are described and compared to \(E. coli\) RecA here. A higher ATPase activity was observed for \(D. fuscus\) RecA with ssDNA as the cofactor (Figure 2 and Table 2). According to previous studies, *Deinococcus geothermalis* RecA (sharing 87.6% sequence identity with Dr. RecA and 89.0% sequence identity with *D. fuscus* RecA) and *Deinococcus murrayi* RecA (sharing 86.9% sequence identity with Dr. RecA and 89.0% sequence identity with *D. fuscus* RecA) exhibited higher ATP hydrolysis rates on ssDNA than on dsDNA at pH = 7.5.\(^{20}\) Therefore, a higher ATP hydrolysis rate of RecA protein with dsDNA as the substrate is not necessarily a general feature for RecA proteins from bacteria belonging to the *Deinococcus* genus.

Unlike Dr. RecA, *D. fuscus* RecA proteins can more efficiently promote DNA strand exchange via a canonical pathway (Figure 4B,C). *D. geothermalis* RecA has been reported to promote DNA strand exchange reactions through both pathways without detectable differences.\(^{21}\) In contrast, Wanarska et al. reported that *D. geothermalis* RecA and *D. murrayi* RecA preferentially promote DNA strand exchange via a canonical pathway.\(^{22}\) Under our experimental conditions, equal amounts and equal lengths of ssDNA and dsDNA exchanged with RecA.
molecules were used in the presence of saturated amounts of RecA (∼0.31 RecA/nt). It has been reported that ATP hydrolysis activity tightly regulates RecA nucleoprotein filament and affects homologous recombination efficiency. Dr. RecA proteins can hydrolyze ATP rapidly on dsDNA than on ssDNA at 6.5 < pH < 8.5. In the previous single-molecule experiment, a less continuous nucleoprotein filament has been reported for Dr. RecA, and a similar behavior has been observed for D. fiscus RecA and chimera D. fiscus C17_E. coli RecA (unpublished data). Besides, a higher binding affinity toward ssDNA than dsDNA has been found for Dr. RecA and E. coli RecA. These studies suggest that the dramatically different strand exchange behavior reported for Dr. RecA has presumably linked to its higher dsDNA-dependence ATPase activity instead of filament stability or DNA binding affinity. Therefore, a higher ATP hydrolysis rate on ssDNA has been found for D. fiscus RecA (Figure 3 and Table 2), supporting preferential DNA strand exchange via a canonical pathway. Based on phylogenetic analysis using the 16S rRNA gene sequence, D. fiscus exhibits a higher sequence similarity with D. radiodurans (94.3%). However, the lexA-imuB-dnaE2 gene cassette contributing to the TLS (translesion synthesis) polymerase activity was found in D. fiscus but not in D. radiodurans. Therefore, the responses under UV stress are different between D. fiscus and D. radiodurans. Despite a higher amino acid sequence similarity being found between Dr. RecA and D. fiscus RecA, there are still some differences within the C-terminal domain and N-terminal domain. These observations suggest that the reaction behaviors could be different among these two RecA proteins.

The negativity in the C-terminal tail of E. coli RecA proteins has been reported to play an important role in the rate of nucleation onto dsDNA at physiological pH. According to sequence alignment, Dr. RecA has less negativity in the C-terminal tail than E. coli RecA (Figure 1), and a faster nucleation rate on dsDNA has also been reported for Dr. RecA on a single-molecule level. More interestingly, a shift in the optimal profile of pH-dependent DNA strand exchange has

Figure 4. DNA strand exchange reactions promoted by D. fiscus RecA and E. coli RecA. (A) Fluorescence-based RecA-mediated DNA strand exchange process. Fine solid line and coarse solid line indicate the DNA strands in the dsDNA molecule. Dash line indicates the ssDNA with the homologous sequence to the fine solid line in the dsDNA molecule. Cy3 was labeled on the DNA strand with the same sequence to the incoming ssDNA and indicated by the black circle. (B) RecA-mediated DNA strand exchange reactions were checked in 2% agarose gel and imaged with typhoon 9400. M indicates DNA marker. (i) In the absence of SSB. (ii) In the presence of SSB. (C) Quantitative analysis of RecA-mediated DNA strand exchange products in the absence of SSB. The strand exchange efficiency was calculated with the intensity ratio of ssDNA vs sum of ssDNA and dsDNA. Each experimental condition was repeated for three times in complete reaction buffer at pH = 7.5 with 1.6 μM RecA (∼0.31 RecA/nt), 10 nM 500-nt ssDNA, 10 nM 500-nt Cy3-dsDNA, and 2 mM ATP. Reaction was run for 45 min and quenched with 40 mM EDTA, 0.5% SDS, and 2 mg/mL proteinase K. n = 3 (repetition). *** = p < 0.005.
been observed for both \( E. \text{coli} \Delta C17 \) RecA and \( D. \text{ficus} \) RecA.\(^{12,13} \) Here, we compared the pH-dependent DNA strand exchange efficiency promoted by \( E. \text{coli} \) RecA, \( D. \text{ficus} \) \( C17 \) \( E. \text{coli} \) RecA, \( D. \text{ficus} \) RecA, and \( E. \text{coli} \) \( \Delta C17 \) \( E. \text{coli} \) RecA simultaneously. For \( D. \text{ficus} \) RecA and \( E. \text{coli} \) \( \Delta C17 \) \( E. \text{coli} \) RecA with less negativity in the C-terminal tail, the optimal pH of DNA strand exchange shifted to the base condition, consistent to previous studies.\(^{12,13} \) For \( E. \text{coli} \) RecA and \( D. \text{ficus} \) \( C17 \) \( E. \text{coli} \) RecA with more negativity in the C-terminal tail, the profile of pH-dependent DNA strand exchange reaches an optimum near acid condition (Figure 5B,C). Even though \( D. \text{ficus} \) RecA exhibits only 62.0% identity and 77.0% similarity with \( E. \text{coli} \) RecA, replacement of the C-terminal tail with the last 17-residues in \( E. \text{coli} \) RecA totally shifts the pH-dependent reaction profile of \( D. \text{ficus} \) RecA a lot. These phenomena corroborate our statement that the increased negative charge density in the C-terminal tail significantly affects the pH-dependent reaction profile of DNA strand exchange promoted by RecA proteins.

Our statement can be further confirmed by structural analysis. Here, a simulated structure of \( D. \text{ficus} \) RecA was built based on the sequence (Figure 6A, Swiss model). The superposition between \( D. \text{ficus} \) RecA and \( E. \text{coli} \) RecA overlaps well with an rms of 0.071 Å, while the superposition of \( E. \text{coli} \) RecA and \( D. \text{ficus} \) RecA exhibits significant movements in the C-terminal domain, which plays an important role in the coordination of ATP-mediated conformational change and serves as a gateway for the entry of dsDNA molecules, with an rms of 1.012 Å (Figure 6A). From previous molecular modeling, the interactions of the acidic tail of \( E. \text{coli} \) RecA (334−352) with the C-terminal residues that regulate the second DNA entrance were observed, also supporting the regulatory gateway role of acidic tail of \( E. \text{coli} \) RecA.\(^{27−29} \) Under higher pH, there are less protons within reaction environment. The electrostatic repulsions between negative residues of RecA proteins and phosphate backbone of DNA molecules become bigger, suppressing the binding of RecA proteins to DNA molecules and leading to a weaker strand exchange efficiency. An increased positive charge on the

Figure 5. pH-dependent RecA-mediated DNA strand exchange reactions. (A) DNA strand exchange promoted by RecA at different pH conditions; 4 \( \mu \text{M} \) RecA proteins were premixed with 30 nM 427-nt ssDNA at different pH conditions for 15 min. Later, the reaction was initiated after addition of 30 nM 335-nt dsDNA. Reaction was quenched with 0.05% SDS, 40 mM EDTA, and 2 mg/mL proteinase K at different time points. (B) These experiments were performed in the complete reaction buffer at specific pH conditions with 4 \( \mu \text{M} \) \( E. \text{coli} \) RecA, \( D. \text{ficus} \) RecA, \( E. \text{coli} \) \( \Delta C17 \) RecA, and chimera \( D. \text{ficus} \) \( C17 E. \text{coli} \) RecA, respectively, at a reaction time of 60 min. The reaction products are 427/335-nt hybrid DNA molecule. (C) Quantified DNA strand exchange yield obtained from the intensity of 427/335-nt hybrid DNA to the sum intensity of 427-nt ssDNA and 335-nt dsDNA without the addition of RecA proteins shown in (F). \( n=3 \) (repetition), ** \( p<0.005 \) and *** \( p<0.05 \).

Figure 6. Structural analysis (A) stereo view of superimposed \( E. \text{coli} \) RecA (cyan, 2REB), \( D. \text{RecA} \) (green, 1xp8), and \( D. \text{ficus} \) RecA (magentas, simulated by Swiss model) (B) Electrostatic surface potential of \( E. \text{coli} \) RecA, \( D. \text{RecA} \), and \( D. \text{ficus} \) RecA calculated by APBS. The surface is colored according to electrostatic potential at 3 \( kT \) with positively charged regions blue and −8 \( kT \) with negatively charged regions red.
surface is observed for both *D. fusc* RecA and Dr. RecA based on electrostatic potential calculation (Figure 6B, APBS). The significantly higher positively charged regions may dictate the formation of more stable nuclei and higher binding affinity toward DNA molecules. The amino acid sequence of *D. fusc* RecA exhibits a predicted net charge +1.2 and +6 higher than those of Dr. RecA and *E. coli* RecA, respectively, also suggesting that the proton sensitivity of *D. fusc* RecA is lower than that of Dr. RecA and *E. coli* RecA and supporting a higher DNA strand exchange efficiency observed in the basic condition (Figure 5). In the future, more experiments are required to further investigate the regulation mechanism of negativity in the C-terminal tail.

**MATERIALS AND METHODS**

**Proteins.** *E. coli* RecA was purchased from New England Biolabs (NEB, M0249S, native *E. coli* RecA without histidine tag accession ID: WP_000963143.1) and used without further purification. *D. fusc* RecA and chimer *D. fusc* CI7G_080RecA are also designed and constructed based on pET-27b (+) with ampicillin/mL and 12.5 μg of tetracycline/mL at 37 °C. BLR was inoculated into LB medium containing 100 μg of ampicillin/mL and 0.45 μg/mL solid ammonium sulfate to extract RecA. The predicted pellet was dissolved with bufer A containing 300 mM potassium glutamate, 5% glycerol, 2 mM DTT, 2 mM ATP, an ATP regeneration system (2 unit/mL pyruvate kinase and 1.5 mM 2-phosphoenolpyruvate), and 2 mg/mL BSA at a specified pH. For the DNA substrate preference experiment, 10 nM 500-nt ssDNA or 500-nt Cy3-dsDNA was preincubated with 1.6 μM RecA (~0.31 RecA/nt) in complete reaction buffer for 10 min with or without an additional 5 min of incubation in the presence of 50 nM *E. coli* SSB (Promega, M3011). The reaction was initiated after the addition of homologous 500-nt or 335-nt dsDNA and quenched with 40 mM EDTA, 0.5% SDS and 2 mg/mL protease K at a reaction time of 30 min. The reaction products were checked on 2% agarose gel and quantified with a Typhoon 9400 & Trio (GE Healthcare). For the pH-dependent experiment, 30 μM 427-nt ssDNA molecules were preincubated with 4 μM RecA (~0.31 RecA/nt) in complete reaction buffer. The experiment was initiated after the addition of homologous 335-nt dsDNA and quenched with 40 mM EDTA, 0.5% SDS and 2 μg/mL protease K at a reaction time of 30 min. The experiments were performed at 37 °C. The reaction products were checked on 2% agarose gel stained with GelRed (Biotium) and quantified with ImageJ.

**Circular dichroism Studies of Secondary Structure.** RecA proteins were prepared in complete reaction buffers containing 25 mM Tris-HCl (pH 9.0, 8.0 and 7.0) or 25 mM phosphate (pH 8.0, 7.0 and 6.0), 10 μM magnesium acetate, 3 mM potassium glutamate, 5% glycerol, 2 mM DTT, and 2 mM ATP at specific pH. All CD experiments were performed with a final RecA concentration of 0.2–0.3 μg/μL at 22 °C.

**ASSOCIATED CONTENT**

**Supporting Information**

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.0c02865.

SDS-PAGE analysis of the fractions obtained from expression and purification of RecA proteins and control experiments of EMSA assay for the RecA-mediated strand exchange reaction (PDF)

**Accession Codes**

*E. coli*: WP_000963143.1 and *D. fusc* RecA: WP_022801718.1.
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S.S., Y.-A.K., and C.-J.C. have contributed equally to this work. Y.-A.K., S.S., and C.-J.C. contributed to performed purification, EMSA, and data analysis. H.-F.F. contributed to the design of the experiments, independent analysis, manuscript writing, and presentation.

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