

Full Length Research Paper

RecA-mediated cleavage reaction of Lambda repressor and DNA strand exchange require an active extended filament conformation but not ATP hydrolysis

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Accepted 4 January, 2010

DNA pairing and strand exchange activities are essential for genetic recombination. When DNA is damaged, RecA proteins bind to DNA in the presence of ATP and catalyze the specific proteolytic cleavage of Lambda repressor. The cleavage reaction induces and regulates the expression of DNA repair genes. In this work, it has been examined by introducing sites directed mutagenesis (in the ATP catalytic domain or in the DNA binding loop of RecA), the ability of RecA protein to hydrolyze ATP or to cleave Lambda repressor either in the presence of DNA or in the presence of high salt concentration, and the ability of RecA to promote DNA strand exchange. It was observed that mutant E96D does not hydrolyze ATP at all, but fulfills RecA functions such as cleavage of Lambda repressor and strand exchange in the presence of DNA. However mutant E158K hydrolyzes ATP as well in the presence of high salt concentration as in the presence of DNA, but does not fulfill RecA functions. These observations suggest that ATP hydrolysis is not required for the cleavage of Lambda repressor and the genetic recombination, but is necessary for the release of RecA from DNA before DNA repair.

Key words: ATP-hydrolysis, genetic recombination, cleavage, nucleoprotein, DNA repair genes.

INTRODUCTION

The integrity of a genome is maintained by bacterial cells. This is allowed by the SOS response (induction of the expression of DNA repair genes like RecBCD) which is induced by conditions that cause massive DNA damage or inhibit accurate DNA duplication (Radman, 1975; Sassanfar and Roberts, 1990). Two major proteins regulate the SOS response, the repressor LexA and the coprotease RecA. RecA protein is essential for homologous genetic recombination in *Escherichia coli* (Roca and Cox, 1997). In the presence of ATP (Adenosine Triphosphate), RecA protein binds to ssDNA (single-strand DNA) depending on the type of DNA damage and forms a nucleofilament. The nucleofilament promotes DNA pairing and strand exchange activities which are essential for genetic recombination (Friedberg et al., 2006; Kowalczykowski, 1991; Roca and Cox, 1997; Walker, 1984). *In vivo*, in response to DNA damage, RecA nucleofilament becomes activated binds and catalyzes the self-cleavage of LexA protein at a specific site. The cleavage induces the SOS response and regulates the expression of DNA repair genes (Friedberg et al., 2006; Little,

1984; Luo et al., 2001). The activated RecA-ssDNA-ATP complex acts as a co-protease (Little, 1984; Luo et al., 2001). The binding of the active RecA filament to LexA functional homologues UmuD mutagenesis protein (Little and Mount, 1982; Peat et al., 1996a, b) and several phage repressors (Bell et al., 2000; De Anda et al., 1983; Eguchi et al., 1988) undergo also self-cleavage as well as under SOS activation. Lambda repressor binds to RecA-DNA complex at a notch between subunits. The active RecA-DNA nucleoprotein complex requires ATP or an ATP analog, and hydrolyzes ATP but the self-cleavage reaction does not require ATP hydrolysis (Craig and Roberts, 1981). ATP hydrolysis is important for some RecA processes such as disassembly of the nucleoprotein filament and strand exchange (Cox et al., 2006; Lusetti and Cox, 2002; Shan et al., 1996). In the absence of DNA or in the presence of high RecA also requires salt concentration requires also ATP or an ATP analog and hydrolyzes ATP.

The ability to hydrolyze ATP in a DNA-dependent manner is crucial to RecA function in the cell. However, the

way in which ATP hydrolysis contributes to RecA function(s) is not fully understood (Gruenig et al., 2008). In this study, the role of ATP hydrolysis was determined by analyzing the RecA-mediated ATP hydrolysis, RecA-mediated cleavage reaction of Lambda repressor and RecA-mediated strand exchange as well in the presence of DNA as in the presence of high salt concentration. Biochemical characterization has revealed that RecA hydrolyzes ATP as well in the presence of DNA as in the presence of high salt concentration. But at high salt concentration, in the absence of DNA, RecA hydrolyzes ATP but does not cleave Lambda repressor. In addition, the data show that mutant E96D that contacts the bound nucleotide does not hydrolyze ATP but exhibits RecA functions such as cleavage of Lambda repressor and DNA recombination, while mutant E158K hydrolyzes ATP but does not fulfill RecA functions. The results of these studies indicate that ATP hydrolysis is not required for the cleavage of Lambda repressor and DNA recombination.

MATERIALS AND METHODS

Aluminum nitrate, sodium fluoride (NaF), adenosine diphosphate (ADP), ATP, ATP γ S, nicotinamide adenosine dehydrogenase (NADH), phosphoenolpyruvate (PEP), pyruvate kinase, lactic dehydrogenase, and calf Thymus double strand DNA were from Sigma-Aldrich. Restriction enzymes, Φ X174 dsDNA and ssDNA were from New England Biolabs. Ethylenediaminetetra acetic acid (EDTA) and Dithiothreitol (DTT) were from Research Products International. The GTG-repeating 15-mer oligonucleotide was from Integrated DNA Technologies. All other reagents were Fisher Scientific ACS grade.

Expression and purification of the untagged RecA

DNA for the RecA was PCR-amplified from genomic DNA and ligated into the NdeI and BamHI sites of pET-9a. The resulting plasmid expresses RecA protein without tag. Site-directed mutagenesis to incorporate E96D, A179V and E158K mutations was performed as described by Ndjonka et al. (2003). The mutations and the integrity of the insert were verified by DNA sequencing. Expression and purification of RecA protein and mutants were performed as described by Ndjonka and Bell (2009). The protein concentration was determined spectrophotometrically using an extinction coefficient of 15220 M⁻¹ cm⁻¹ at 280 nm. The homogeneity of the enzyme preparation was analyzed by a 12.5% gel Glycine-SDS-PAGE (Sodium dodecyl sulfate polyacrylamide gel electrophoresis). The proteins were visualized by Coomassie Brilliant Blue staining.

ATP hydrolysis

The ATPase assay was performed using an Ultrospec 2100 *pro* UV/VIS spectrophotometer (Amersham Biosciences) and consisted to measure the OD₃₄₀ of the dehydrogenation of NADH in the presence of Φ X174 ssDNA or high salt concentration (Sodium Acetate: NaAc) (Rajan et al., 2006). 500 μ l reaction mixture contained 25 mM Tris-HCl, pH 7.4, 10 mM MgCl₂, 1 mM DTT, 1 mM ATP, 2 mM PEP, 0.5 mM NADH, 30 units/ml each of pyruvate kinase and lactic dehydrogenase, 3 μ M (nucleotides) ssDNA Φ X174 (or 1.8 M NaAc). The reaction was initiated by adding 0.8 μ M RecA, and 0.3 μ M single strand binding protein (SSB) was added after RecA. The

reactions were carried out at 37°C with an electrically heated cell holder. The OD₃₄₀ was monitored at 1 min interval using the spectrophotometer. The cell path length was 0.5 cm. The NADH extinction coefficient at 340 nm of 6.3 mM⁻¹cm⁻¹ was used to calculate the rates of ATP hydrolysis. Each reaction was performed in triplicate.

Cleavage assay with ATP regeneration system and Lambda cl₁₀₁₋₂₂₉ DM

The RecA-mediated cleavage reaction of Lambda (λ) repressor was performed using a modified previously described procedure (Ndjonka and Bell, 2009). 50 μ l reaction was pre-incubated in 20 mM Tris-HCl pH 7.4, 30 μ M (nucleotides) 15-Mer GTG ssDNA oligonucleotide or calf Thymus double strand DNA, (1 mM ADP, 2 mM Al(NO₃)₃, 10 mM NaF) or (1 mM ATP, 30 units/ml Pyruvate kinase, 10 mM Phosphoenolpyruvate in the ATP regeneration system), 2 mM MgCl₂, 50 mM NaCl and 5 μ M *E. coli* RecA at 25°C (ADPAIF₄) or 37°C (ATP) for 30 min. 10 μ M Lambda repressor (which contains two mutations, P158T and A152T. In this construct cl₁₀₁₋₂₂₉ DM, the DM stands for double mutant (Ndjonka and Bell, 2006)) was added to the above mixture and incubated at 25°C (ADPAIF₄), 37°C (ATP) for another 20 min. The reaction was terminated at indicated time points by addition of 0.25x volume of 5x SDS-PAGE loading buffer and immediately heated at 95°C for 5 min. The proteins were loaded onto a 15% SDS polyacrylamide gels and visualized by Coomassie brilliant staining. Stained gels were scanned and the intensity of each band was quantified as indicated by Ndjonka and Bell (2006). Each reaction was carried out in triplicate.

DNA strands exchange

The recombination reaction contained 25 mM Tris Acetate pH 7.5, 10 mM magnesium acetate, 3 mM potassium glutamate, 21 μ M ssDNA Φ X174, 5% glycerol, 1 mM DTT, 30 units/ml phosphor-creatine, 11 mM creatine phosphokinase, 7 μ M RecA, 3 mM ATP and 0.3 μ M SSB. This mixture was incubated at 37°C for 10 min, at which point the reaction was initiated with 21 μ M dsDNA Φ X174 previously linearized with PstI and incubated at 37°C. At the indicated time points 10 μ l aliquots were removed and quenched by adding 1/3 volume of stop buffer containing 60 mM EDTA, 5% SDS, 6.25% glycerol and 0.05% bromophenol blue. Before loading on a 0.8% agarose gel, the samples were incubated at 42°C for 30 min and run overnight at 2 V/cm in 0.5x TBE (Tris boric acid EDTA) buffer. The DNA bands were visualized by soaking the gel in 0.5x TBE buffer and 1% ethidium bromide for 30 min after washing for 2 h in water. Gels were scanned and the intensity of each band was quantified with Kodak digital science™ 1D image analysis software. The percentage of nicked circular DNA formed was calculated from the ratio of the net intensities of the bands corresponding to the nicked circular DNA formed to the intermediate joint molecule, the ssDNA and the linear double stranded DNA (dsDNA). Each reaction was carried out in triplicate.

RESULTS AND DISCUSSION

The study was carried out to determine the role of ATP hydrolysis in RecA functions. First, the RecA-mediated ATP hydrolysis was studied either in the presence of DNA or in the presence of high salt concentration to understand the effect of high salt concentration relative to DNA in ATP hydrolysis. Secondly, the RecA-mediated cleavage reaction was studied either in the presence of

Table 1. ATPase rate of RecA wild type and mutants A179V, E158K and E96D in the presence of ssDNA Φ X174 or of 1.8 M sodium acetate.

RecA	K _{cat} or ATPase rate (min ⁻¹)	
	DNA (ssDNA Φ X174)	Salt (1.8 M sodium acetate)
WT	25.6 ± 1.2	20.4 ± 1.2
A179V	33.5 ± 2.3	41.1 ± 0.6
E158K	22.8 ± 0.2	26.8 ± 0.1
E96D	0.5 ± 0.02	0.8 ± 0.01

The ATPase assay was performed as described in materials and methods. The high salt activation may mimic the ionic interaction of the protein with DNA. WT is wild type.

DNA (calf Thymus long double strand DNA, 15-Mer GTG ssDNA oligonucleotide) or in the presence of high salt concentration as well with ATP regeneration system and $cl_{101-229}$ DM as with ATPyS, AMP-PNP or ADPAIF₄ to document the role of ATP hydrolysis in cleavage reaction. Finally, the RecA-mediated DNA strand exchange was studied to determine the impact of ATP hydrolysis relative to DNA strand exchange.

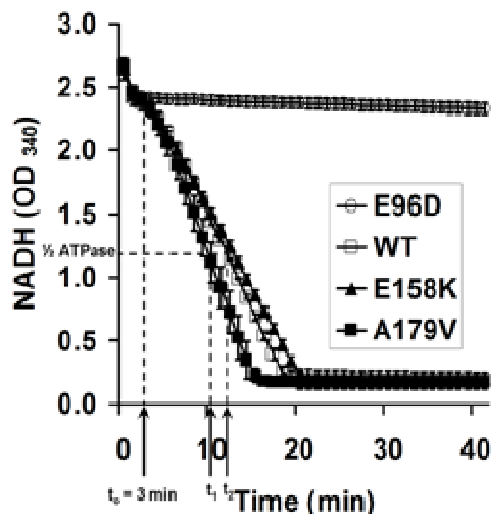
RecA-mediated ATP hydrolysis and cleavage reaction

The ATP hydrolysis of RecA and three mutants were examined and the results are depicted in Figures 1 (A and B) and Table 1. RecA does not hydrolyze ATP in the absence of DNA or high salt (result not shown); this finding is in accordance with the previous observed results of Pugh and Cox (1988). RecA wild type and mutants A179V and E158K exhibit ATPase activity as well in the presence of DNA as in the presence of 1.8 M sodium acetate. In the presence of DNA, RecA wild type, mutants A179V and E158K exhibit an apparent K_{cat} of 25.6 ± 1.2, 33.5 ± 2.3 and 22.8 ± 0.2 min⁻¹ respectively (Table 1). In the presence of 1.8 M sodium acetate, RecA wild type and mutant E158K exhibit a comparable K_{cat} as observed in the presence of DNA. However, in the presence of high salt concentration, mutant A179V displays a 1.2-fold more ATP hydrolytic activity than A179V in the presence of DNA (Table 1). This finding is comparable to previous reported ATPase activity in the presence of DNA with a K_{cat} varying between 25 to 32 min⁻¹ (Gruenig et al., 2008; Pugh and Cox, 1988) and a K_{cat} of 25 min⁻¹ in the presence of sodium acetate (Pugh and Cox, 1988). The similarity between ATPase properties of RecA wild type and mutant E158K either in the presence of DNA or in the presence of high salt concentration indicates that the mutation does not interfere with ATP hydrolysis and that the salt may play a role comparable to that of the DNA (Pugh and Cox, 1988). The increase of the K_{cat} observed with the mutant A179V can be explained by the fact that this mutation may allosterically change the conformation of RecA. This change

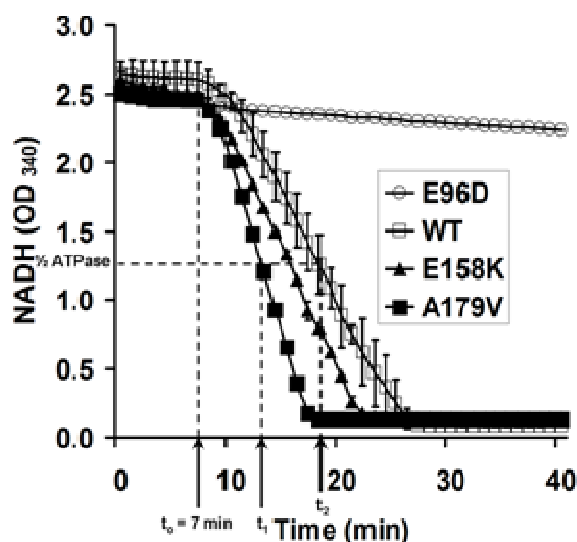
of conformation could in turn affect RecA filament assembly, ATP hydrolysis and both primary and secondary DNA binding properties of RecA and may result in an increasing of the ATP hydrolysis (VanLoock et al., 2003a). A179V has been reported as a tight DNA binding mutant that also accelerates the cleavage reaction but nothing up to now has been reported about the increasing of ATP hydrolysis rate (Kowalczykowski, 1991).

Binding of RecA protein to DNA involves interactions with the phosphate backbone of the DNA as reported by (Bell, 2005; Leahy and Radding, 1986). Since the activation of RecA protein promotes ATP hydrolysis with DNA, the high salt activation may mimic the ionic interaction of the protein with DNA (Pugh and Cox, 1988). It is possible that stimulation is produced by binding of ions at the DNA-binding site which is supposed to interact with DNA phosphate backbone. Like DNA, this ionic interaction may activate the ATP hydrolysis.

Mutant E96D exhibits an ATPase activity as low to almost undetectable either in the presence of DNA or in the presence of high salt concentration (Figures 1A and B). This mutant does not improve the capacity to hydrolyze ATP. In opposite to the result reported by Rehrauer and Kowalczykowski (1993), E96D does not attenuate ATP hydrolysis but fails to hydrolyze ATP. This mutation eliminates the binding of ATP to E96D (Campbell and Davis, 1999), and destabilizes ATP hydrolysis since this mutation occurs in one of the six regions of RecA that contacts the bound nucleotide (Bell, 2005). This region is a loop containing the catalytic E96 which activates the attacking water molecule (Bell, 2005). Figure 2 shows that wild type, mutants A179V and E96D proteins facilitate cleavage of Lambda repressor protein in the presence of ATP (Table 1), mutant A179V increases the rate of ATPase activity and displays coprotease activity of Lambda repressor at almost 49% as indicated in Figure 2A. In contrast mutant E96D has no ATP hydrolytic activity (Figure 1), but cleaves almost totally Lambda repressor in the presence of DNA (GTG₁₅) in an ATP regeneration system (Figure 2A). This result shows that E96D does not prevent the capacity to form long active extended filament in the presence of ATP and that E96D facilitates Lambda repressor cleavage as well as wild-



(A) ATP-Hydrolysis in the presence of ssDNA Φ X174



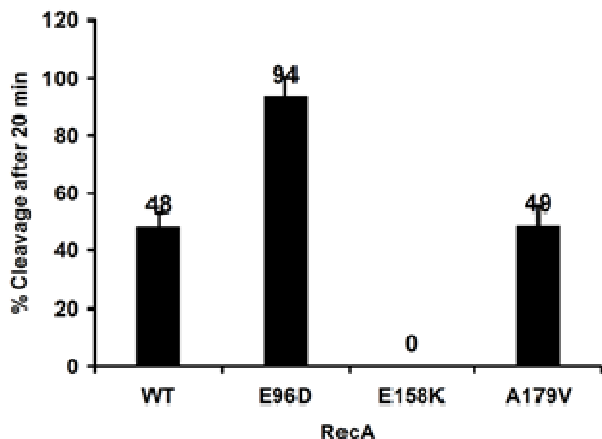
(B) ATP-Hydrolysis in the presence of 1.8 M Sodium Acetate

Figure 1. ATPase activity of RecA in the presence of DNA compared with the ATPase activity of RecA in the presence of high salt concentration. The RecA-catalyzed ATP hydrolysis either in the presence of ssDNA or in the presence of high salt was monitored over time. The reactions were performed as described under materials and methods. Time zero (t_0) corresponds to the time of RecA/SSB addition (arrow) (A): ATP hydrolysis in the presence of DNA (ssDNA Φ X174). Reactions were monitored spectrophotometrically for about 3 min without RecA protein. Mutant A179V and RecA wild type hydrolyze the half of ATP after 8 min (t_1) and 10 min (t_2) respectively after the addition of RecA. (B) ATP hydrolysis in the presence of 1.8 M salt (NaAc). RecA was added after 7 min (t_0). Mutant A179V and RecA wild type respectively hydrolyze the half of ATP after 6 min (t_1) and 12 min (t_2) after the addition of RecA.

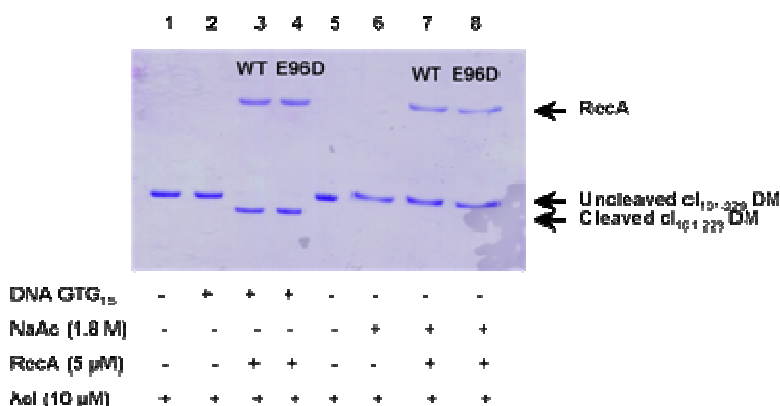
ATP. Thus, SOS induction does not require ATP hydrolysis by the RecA protein (Gruenig et al., 2008).

Mutant E158K displays an ATP hydrolytic activity either in the presence of DNA or in the presence of high salt (Figure 1), but this mutant does not display co-protease activity of Lambda repressor (Figure 2A), probably because DNA may not bind at this binding site of the mutant, but may bind to the second binding site, since it was possible by gel shift assay to show binding of DNA to this mutant (result not shown). This mutant may form a filament that may not be extended and as well not active. This finding can be explained by the fact that the mutation occurs in one of the two DNA binding domain of RecA (Chen et al., 2008). These results also show that the ATP hydrolysis is not important for the self-cleavage reaction of Lambda repressor. Figure 2B reinforces these observations. RecA wild type hydrolyzes ATP but can not cleave Lambda repressor when the DNA is replaced by high salt concentration as indicated in Figure 2B. This result also shows that the presence of DNA and its binding to RecA is important for the formation of the nucleofilament. And cleavage can only occur, when the nucleofilament formed is an extended active filament (Galkin et al., 2009; VanLoock et al., 2003b). By using two different ATP analogs, (a slowly hydrolyzed ATP γ S or a non hydrolysable AMP-PNP and ADPAIF $_4$) (Figure 3), RecA mediates the cleavage of Lambda repressor as well in the presence of ssDNA (GTG $_{15}$) as in the presence of calf long dsDNA (Figure 3A). Cleavage of Lambda repressor is faster on GTG 15 mers (Rajan et al., 2006). With ATP γ S (slowly hydrolyzed) and ADPAIF $_4$ (non hydrolysable) as a cofactor, the cleavage rate is significantly higher for the ssDNA than it is for the dsDNA (Figure 3A). Proficient cleavage occurs 2 min after addition of Lambda repressor and complete cleavage is achieved before 20 min time point in the presence of ssDNA (Figure 3A, lane 2 and 4), while cleavage occurs 60 min after addition of Lambda repressor in the presence of dsDNA (Figure 3A, lane 7 and 9). In contrast, mutant E96D does not cleave Lambda repressor in the presence of ADPAIF $_4$ (Figure 3B, lane 2 and 3), but in the presence ATP γ S. Proficient cleavage in the presence of ssDNA occurs 5 min after addition of repressor and 60 min in the presence of dsDNA (Figure 3B, lane 4 and 9). With AMP-PNP there is no cleavage of Lambda repressor (Figure 3, lane 6 and 10). Figure 3 shows also that ATP hydrolysis is not requested for cleavage reaction, but the presence of ATP, its binding to RecA and the binding of DNA to RecA are very important for the formation of the extended active filament (activated RecA-DNA-ATP complex) as reported by (Craig and Roberts, 1981; Egelman and Stasiak, 1993; Little, 1984; Luo et al., 2001; Rehrauer and Kowalczykowski, 1993; Yu and Egelman, 1992). It has been shown that RecA forms an extended filament with AMP-PNP (DiCapua et al., 1992; Sauer et al., 1982; VanLoock et al., 2003b), but the inability of RecA-AMP-PNP to cleave repressor is due to the fact that this extended filament is inactive (Galkin et al., 2009; Krishna

type and mutant A179V, and do so in the presence of



(A) % Cleavage of λ cl₁₀₁₋₂₂₉ DM with ATP regeneration system and GTG₁₅



(B) Cleavage of λ cl₁₀₁₋₂₂₉ DM with ATP, GTG₁₅ or High salt concentration

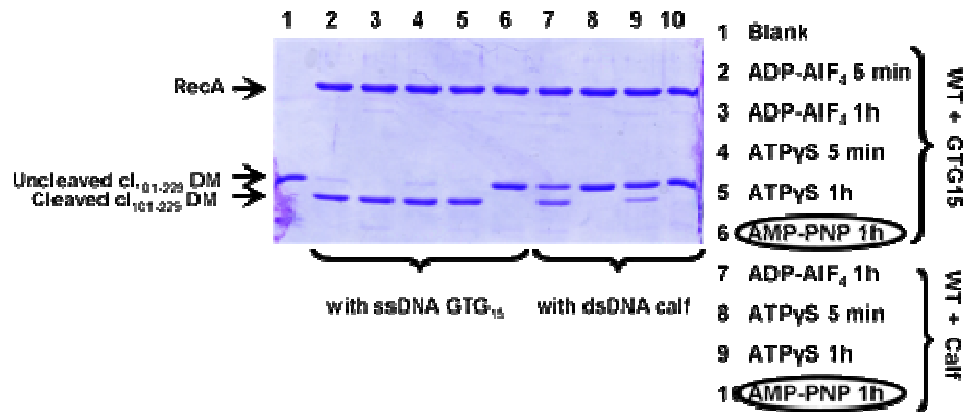
Figure 2. RecA wild type and mutants mediated cleavage reaction. ATP and GTG 15-mer oligonucleotide were used in this reaction. (A) Cleavage of λ cl₁₀₁₋₂₂₉ DM (cl) with ATP in regeneration system and GTG₁₅ repeating oligonucleotide. Notice that the cleavage rate of cl by E96D is significantly increased compared to the cleavage rate by RecA wild type and A179V. E158K does not cleave cl. (B) Cleavage of λ cl₁₀₁₋₂₂₉ DM with ATP in regeneration system. The cleavage reaction of λ cl₁₀₁₋₂₂₉ DM was compared either in the presence of DNA (GTG₁₅) or in the presence of high salt concentration. In Lane 3 and 4, RecA and E96D cleave λ cl₁₀₁₋₂₂₉ DM in the presence of DNA, however in Lane 7 and 8 there is no cleavage in the presence of high salt. This result shows that ATP hydrolysis is not required for the cleavage reaction of repressor. Lane 1 and 5 are negative control, while lane 2 and 6 are positive control.

et al., 2007; VanLoock et al., 2003b).

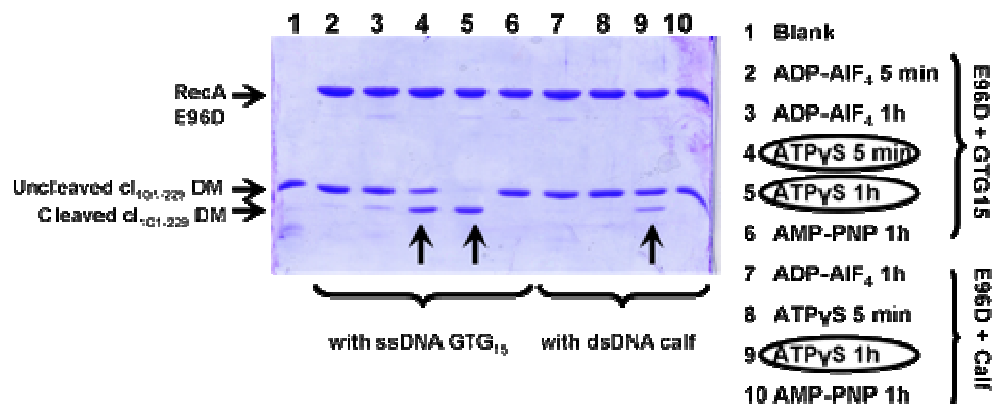
RecA-mediated strand exchange, formation of joint molecule intermediates and nicked circular product

The RecA wild type, mutants A179V, E96D and E158K recombination activity was assessed. The DNA strand-exchange of wild type RecA and mutants was analyzed and compared (Figure 4 and Table 2). RecA wild type

and mutants A179V, E96D converted the circular single-stranded DNA (cssDNA) and the linear double-stranded DNA (ldsDNA) to joint molecule intermediates (JM) and nicked circular (NC) duplex (Figure 4A). The nicked circular product of DNA strand exchange was evident at 5 min with wild-type (WT) RecA, mutants E96D and A179V with 30, 20 and 5% nicked products formed respectively (Figure 4B). 55 min later 1.33, 2.8 and 8.4-fold nicked products are formed with wild type RecA, E96D and A179V respectively (Figure 4B). The mutant E158K is not



(A) RecA WT and λ cl₁₀₁₋₂₂₉ DM Coprotease Assay with GTG₁₅, Calf Thymus and ADP-AIF₄, ATPyS, AMP-PNP



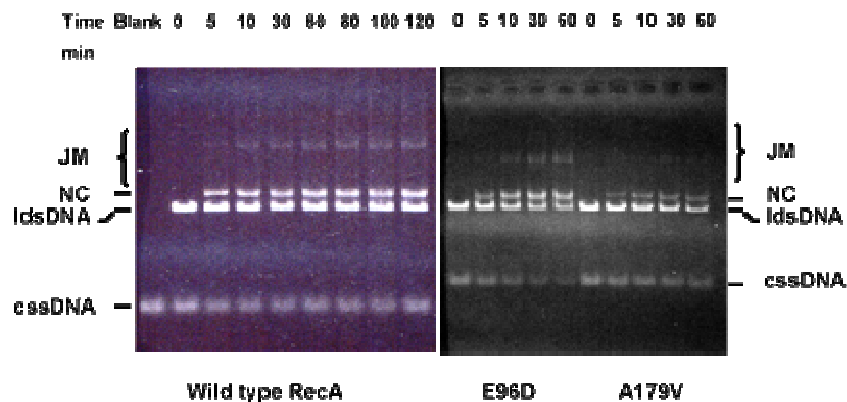
(B) RecA E96D and λ cl₁₀₁₋₂₂₉ DM Coprotease Assay with GTG₁₅, Calf Thymus and ADP-AIF₄, ATPyS, AMP-PNP

Figure 3. RecA wild type and mutant E96D mediated cleavage reaction. The reaction in the presence of ATP analogs (a slowly hydrolyzed ATPyS or a non hydrolysable AMP-PNP and ADPAIF₄), 15-mer GTG oligonucleotide or long double strand DNA (Calf DNA). (A) Wild type RecA-mediated cleavage reaction of λ cl₁₀₁₋₂₂₉ DM with ATP analogs, GTG₁₅ and calf DNA. Excepted AMP-PNP (lane 6 and 10), RecA cleaves Lambda repressor with all the other ATP analogs as well with ssDNA as with long dsDNA. However the cleavage with dsDNA takes more time to occur. (B) Mutant E96D-mediated cleavage reaction of λ cl₁₀₁₋₂₂₉ DM with ATP analogs, GTG₁₅ and calf DNA. E96D cleaves wild type Lambda repressor only with ATPyS as well with ssDNA as with dsDNA (lanes 4, 5 and 9). Using ADPAIF₄ and GTG₁₅, cleavage occurs after more than 1h reaction (lane 3).

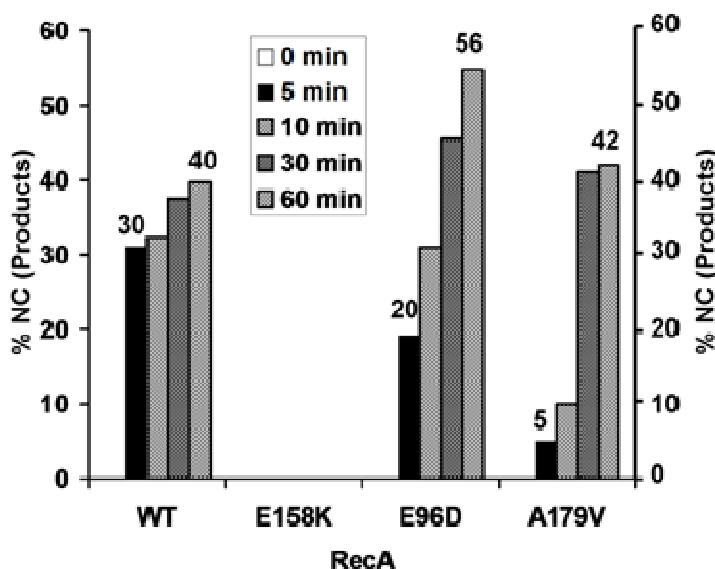
able to convert cssDNA and ldsDNA to joint molecules and nicked products (Figure 4B and Table 2). Since this mutant occurs in one of the two DNA binding loop (Kowalczykowski, 1991), it fails to bind DNA properly and this failure may prevent homologous pairing to take place. The mutant E96D functions poorly in the presence of ATP, but the fact that its forms long extended filament promotes DNA pairing and strand exchange at almost 60% after 60 min (Table 2). These results suggest that upon ATP binding, mutant E96D enables a conformation

state which allows homologous pairing to take place.

In conclusion, this work shows that Lambda cleavage requires only the formation of the RecA–ssDNA–NTP ternary complex, but not hydrolysis of the bound nucleotide. ATP hydrolysis is used to enhance or to promote a wide range of cellular processes. Hence three requirements are needed for the full functions of RecA to occur. The first requirement is the RecA binding ATP, the second is the RecA binding DNA and the third is the ATP hydrolysis in SOS induction: in response to DNA dam-



(A) DNA Strand exchange in the presence of ATP



(B) Percentage of nicked products (NC) formed

Figure 4. RecA wild type and mutants mediates homologous pairing. The recombination activities were evaluated as described in materials and methods. (A) DNA strand exchange in the presence of ATP. Time points were taken at 0, 5, 10, 30, 60, 80, 100 and 120 min. Gels were scanned and the intensity of each band was quantified as described in materials and methods. RecA wild type and mutants A179V, E96D converted the circular single-stranded DNA (cssDNA) and the linear double-stranded DNA (IdsDNA) to joint molecule intermediates (JM) and nicked circular (NC) duplex. (B) Percentage of nicked products formed. The percentage of nicked circular DNA formed was calculated as described under materials and methods. The nicked circular product formed was determinate at 60 min for wild-type RecA, mutants E96D and A179V with 40%, 56% and 42% respectively. Mutant E158K is not able to convert cssDNA and IdsDNA to joint molecules and nicked products.

age, ATP binds to RecA. The binding of ATP causes an allosteric change in RecA, allowing RecA to adopt a high affinity state for DNA binding and forming the active extended nucleofilament. Then repressor binds to RecA nucleofilament. The binding of repressor to RecA catalyzes their specific proteolytic cleavage which regulates

the expression of DNA repair genes (Friedberg et al., 2006; Little, 1984; Luo et al., 2001). The cleavage of repressor inactivates the Lambda repressor from binding to the SOS operator sequences and results in the transcription of the SOS genes, helping the cell to manage the DNA damage. Hence the ATPase activity of RecA is

Table 2. Relationship between ATP hydrolysis and cleavage reaction of Lambda repressor and strand exchange of RecA wild type and mutants A179V, E158K and E96D in the presence of ssDNA Φ X174 or of 1.8 M sodium acetate.

RecA	DNA (ssDNA Φ X174)		Salt (1.8 M Sodium Acetate)		Strand exchange
	ATP hydrolysis	Cleavage after 20 min in the presence of ATP	ATP hydrolysis	Cleavage after 20 min in the presence of ATP	Nicked products formed after 60 min
WT	+++	++	+++	-	++
A179V	++++	++	++++	-	++
E158K	+++	-	+++	-	-
E96D	-	++++	-	-	+++

The ATP hydrolysis has no effect on the cleavage reaction of Lambda repressor and the DNA strand exchange. (-): 0%, (++): 40%, (+++): 60%, (++++): > 60%.

stimulated allowing RecA to be released from the damaged DNA. The DNA repair genes can then repair the damages *via* recombination, excision repair or mutagenesis. The RecA stops to be activated, and Lambda repressor can control the transcription of the SOS genes again (Cirz et al., 2006; Little and Mount, 1982; Matic et al., 2004; McKenzie et al., 2000). The ATPase activity of RecA is then necessary to release RecA monomers from the damaged DNA before DNA repair. The released monomers can then re-bind at sites of gaps in the filament, allowing recombination to proceed (Menetski et al., 1990).

ACKNOWLEDGMENTS

This work was done at Ohio State University College of Medicine (OSU: USA). The author acknowledges Dr Charles Bell in OSU. The author is also grateful to M. Amadou Vamoulke, General Manager of CRTV for the opportunity he gave to N.D. to integrate the University of Ngaoundere in Cameroon.

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