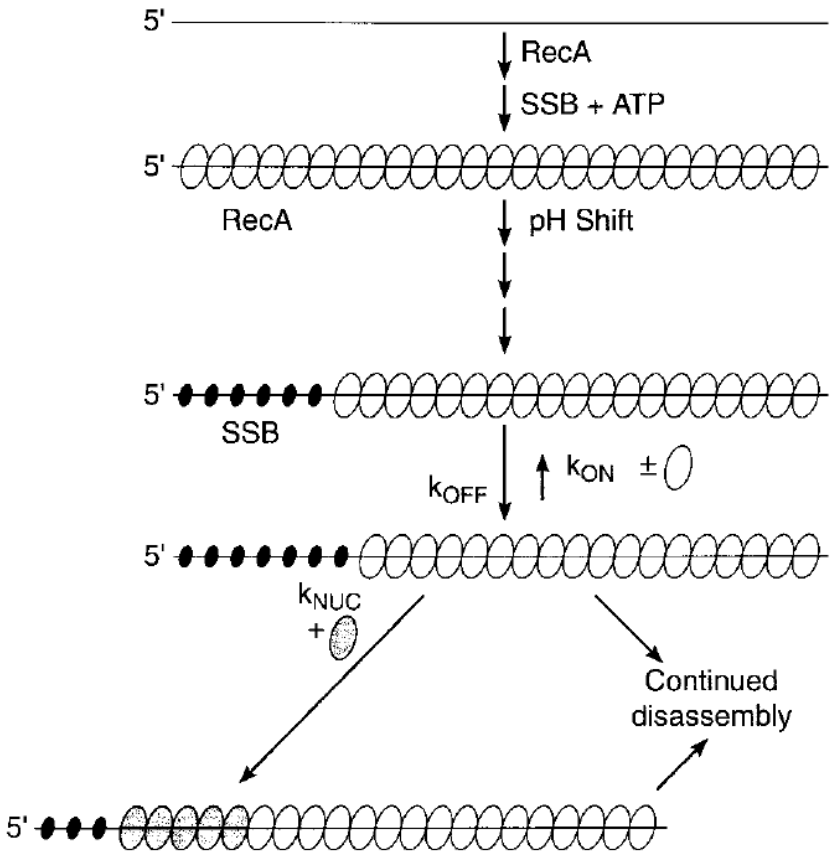

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Quantitative Analysis of the Kinetics of End-dependent Disassembly of RecA Filaments from ssDNA

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On linear single-stranded DNA, RecA filaments assemble and disassemble in the 5′ to 3′ direction. Monomers (or other units) associate at one end and dissociate from the other. ATP hydrolysis occurs throughout the filament. Dissociation can result when ATP is hydrolyzed by the monomer at the disassembly end. We have developed a comprehensive model for the end-dependent filament disassembly process. The model accounts not only for disassembly, but also for the limited reassembly that occurs as DNA is vacated by disassembling filaments. The overall process can be monitored quantitatively by following the resulting decline in DNA-dependent ATP hydrolysis. The rate of disassembly is highly pH dependent, being negligible at pH 6 and reaching a maximum at pH values above 7.5. The rate of disassembly is not significantly affected by the concentration of free RecA protein within the experimental uncertainty. For filaments on single-stranded DNA, the monomer k_{cat} for ATP hydrolysis is 30 min^{-1}, and disassembly proceeds at a maximum rate of 60-70 monomers per minute per filament end. The latter rate is that predicted if the ATP hydrolytic cycles of adjacent monomers are not coupled in any way.

Keywords: RecA protein; filament; assembly; disassembly; kinetic analysis

Introduction

The bacterial RecA protein is critical to the processes of recombinational DNA repair, homologous genetic recombination, and the induction of the SOS response to DNA damage (Cox, 1998; Kowalczykowski & Eggleston, 1994; Roca & Cox, 1997). The *Escherichia coli* RecA protein is a polypeptide chain with 352 amino acid residues with a molecular mass of 37,842 Da. RecA is an ancient protein present in virtually all bacteria, with structural and functional homologues in all classes of organisms (Brendel *et al.*, 1997; Roca & Cox, 1997).

In vitro, RecA protein promotes a set of DNA strand exchange reactions that mimic its presumed role in recombination and recombinational DNA repair. The active species in this reaction is a helical filament of RecA protein bound to DNA, formed as the first step in the process. There is one RecA monomer bound per three nucleotides or base-pairs of DNA, and six monomers per helical turn. Bound double-stranded DNA (dsDNA) is extended by 50% and underwound to 18 bp per turn.

An understanding of filament assembly and disassembly is a prerequisite for a broader description of how RecA filaments form where they are needed for recombinational activities *in vivo*. Most models for recombination focus on the invasion of 3′ ends (Anderson & Kowalczykowski, 1997*a*; Dixon & Kowalczykowski, 1991; Meselson & Radding, 1975; Resnick, 1976; Smith, 1991; Szostak *et al.*, 1983), in part because of their potential utility in priming DNA synthesis. A 3′ end bias in DNA pairing reactions has been demonstrated with bacterial enzymes *in vitro* (Dixon & Kowalczykowski, 1991; Dutreix *et al.*, 1991; Konforti & Davis, 1991), attributed to the 5′ to 3′ polarity of RecA filament assembly (Register & Griffith, 1985; Shan *et al.*, 1997) which would be expected to leave 3′ ends more uniformly coated with RecA protein than 5′ ends. In addition to its implications for recombination models, information about the assembly and disassembly is needed to provide a baseline for the

Abbreviations used: ssDNA, single-stranded DNA; dsDNA, double-stranded DNA; EDTA, ethylenediamine tetraacetic acid; OAc, acetate ion; SSB, the single-stranded DNA-binding protein of *E. coli*.

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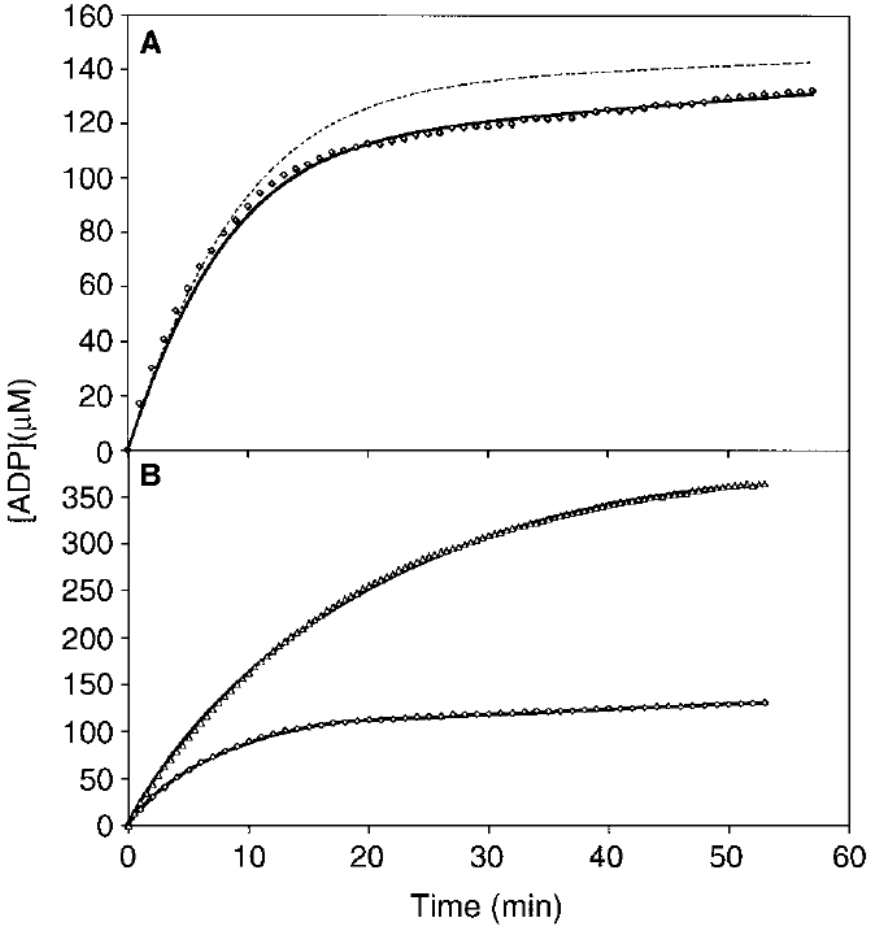


Method…

Prepare RecA filament at low pH

pH shift to 8 at time 0

monitoring **d[ADP] /dt**

k_{cat} is set to be 28 min^{-1}

$[D-ends]$: the concentration of disassembling DNA ends

= the concentration of DNA molecules

$[\mathrm{Re}cA]$: the total concentration of RecA monomers

$[\mathrm{Re}cA_B]$: the concentration of RecA that is bound to the DNA

$[DNA]$: the concentration of available (unbound) DNA binding sites

n_{TOT} : the total number of RecA binding sites per DNA molecule = 2693/3 =898

n_{BOUND} : the average number of binding sites occupied by RecA per DNA molecule

n_{GAP} : the average number of unoccupied binding sites per molecule

k_{cat} : ATP hydrolysis by the RecA protein

k_{NUC} : nucleation rate

k_{OFF} : monomer dissociation rate

$$\frac{\partial[\mathrm{Re}cA_B]}{\partial t} = k_{NUC}[D-ends](n_{GAP})\left(\frac{n_{GAP}}{2}\right)[\mathrm{Re}cA] - k_{OFF}[D-ends]$$

$$n_{TOT} = n_{GAP} + n_{BOUND} = n_{GAP} + \left(\frac{[\mathrm{Re}cA_b]}{[D-ends]}\right)$$

$$\frac{\partial[\mathrm{Re}cA_B]}{\partial t} = \frac{1}{2}k_{NUC}[D-ends]\left(n_{TOT-}\frac{[\mathrm{Re}cA_B]}{[D-ends]}\right)[\mathrm{Re}cA] - k_{OFF}[D-ends]$$

Assumptions

- **3’ extension is very fast**
- **The concentration of REcA protein is kept in sufficient excess re lative to binding sites on the DNA**

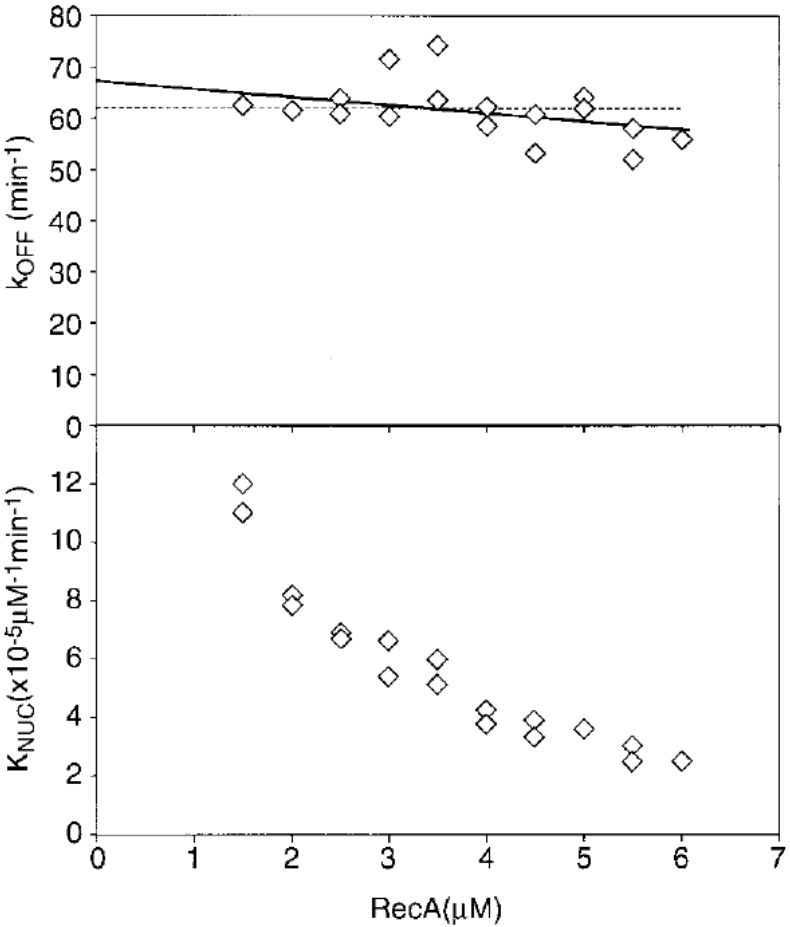
$$[\mathrm{Re}cA_B] = [D-ends]\left(n_{TOT} - \sqrt{\frac{2k_{OFF}}{k_{NUC}[\mathrm{Re}cA]}}\tanh\left(\sqrt{\frac{k_{NUC}[\mathrm{Re}cA]k_{OFF}}{2}}t\right)\right)$$

Conditions

- **2693nt ssDNA linear**
- **1.5 uM nucleotides of ssDNA**
- **1.5 uM RecA**
- **0.15 uM SSB**
- **pH shift from 6.346 to 7.98**

$$\frac{\partial[ADP]}{\partial t} = k_{cat}[\mathrm{Re}cA_B]$$

$$[ADP] = k_{cat}[D-ends]\left(n_{TOT}\cdot t - \frac{2}{k_{NUC}[\mathrm{Re}cA]}\ln\left(\cosh\left(\sqrt{\frac{k_{NUC}[\mathrm{Re}cA]k_{OFF}}{2}}t\right)\right)\right)$$



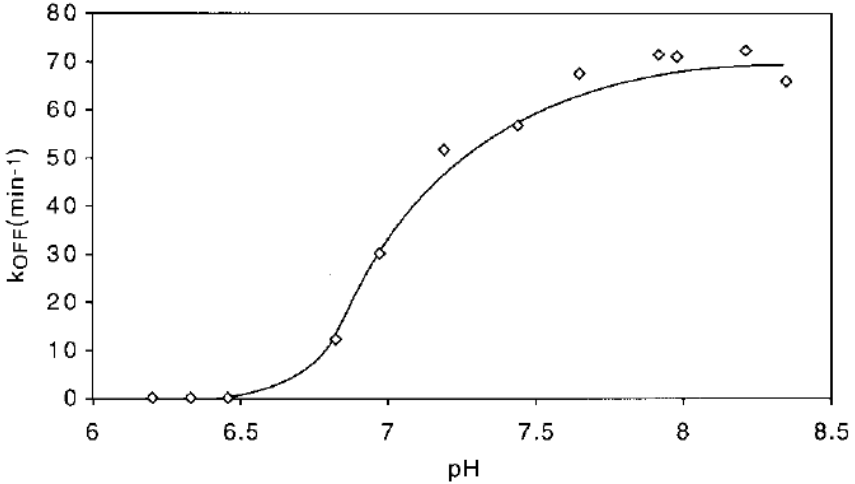
K_{ON} is minimal

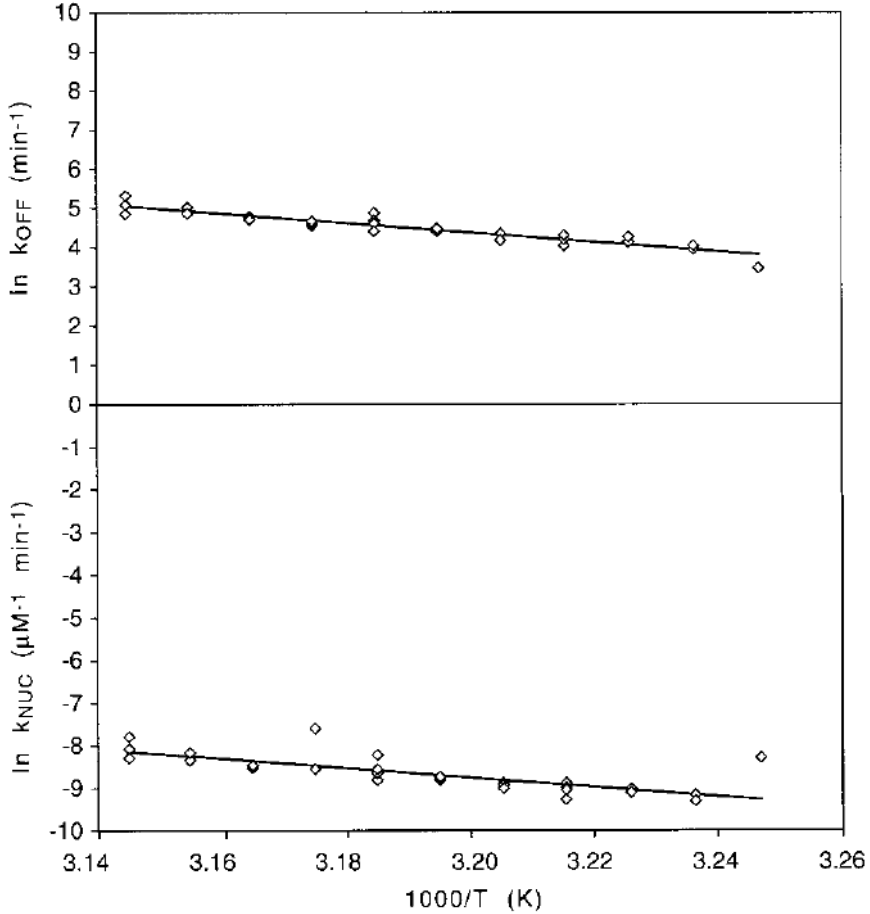
$$\left(k_{OFF}\right)_{obs} = k_{OFF} - k_{ON}\left[\mathrm{Re}\,cA\right]$$

$$k_{ON} = \frac{k_{OFF} - \left(k_{OFF}\right)_{obs}}{\left[\mathrm{Re}\,cA\right]}$$

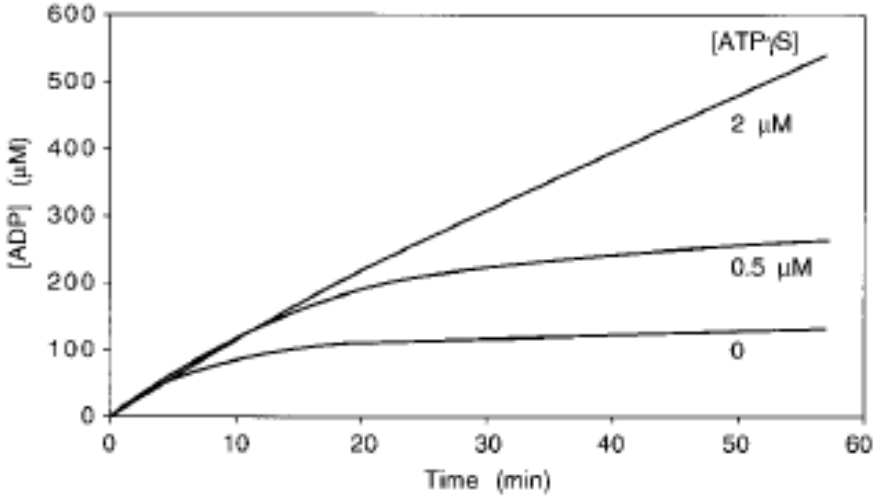
k_{ON} is determined as 1.875 uM^{-1}

pH and Temperature dependence





Adding small amount of ATPgS



$$\frac{\partial[\mathrm{Re}cA_B]}{\partial t} = k_{NUC}[DNA]\left(\frac{n_{GAP}}{2}\right)[\mathrm{Re}cA] - k_{OFF}[D-ends]$$

$$[DNA] = [D-ends](n_{GAP})$$