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# Biochemical Properties of the *Escherichia coli* recA430 Protein

## Analysis of a Mutation that Affects the Interaction of the ATP–recA Protein Complex with Single-stranded DNA

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The biochemical properties of the recA430 protein have been examined and compared to those of wild-type recA protein. We find that, while the recA430 protein possesses ssDNA-dependent rATP activity, this activity is inhibited by the *Escherichia coli* single-stranded DNA binding protein (SSB protein) under many conditions that enhance wild-type recA protein rATPase hydrolysis. Stimulation of rATPase activity by SSB protein is observed only at high concentrations of both rATP (>1 mM) and recA430 protein (>5 μM). In contrast, stimulation of ssDNA-dependent dATPase activity by SSB protein is less sensitive to protein and nucleotide concentration. Consistent with the nucleotide hydrolysis data, recA430 protein can carry out DNA strand exchange in the presence of either rATP or dATP. However, in the presence of rATP, both the rate and the extent of DNA strand exchange by recA430 protein are greatly reduced compared to wild-type recA protein and are sensitive to recA430 protein concentration. This reduction is presumably due to the inability of recA430 protein to compete with SSB protein for ssDNA binding sites under these conditions. The cleavage of lexA repressor protein by recA430 protein is also sensitive to the nucleotide cofactor present and is completely inhibited by SSB protein when rATP is the cofactor but not when dATP is used. Finally, the steady-state affinity and the rate of association of the recA430 protein–ssDNA complex are reduced, suggesting that the mutation affects the interaction of the ATP-bound form of recA protein with ssDNA. This alteration is the likely molecular defect responsible for inhibition of recA430 protein rATP-dependent function by SSB protein. The biochemical properties observed in the presence of dATP and SSB protein, i.e. the reduced levels of both DNA strand exchange activity and cleavage of lexA repressor protein, are consistent with the phenotypic behavior of *recA430* mutations.

### 1. Introduction

The *recA* gene of *Escherichia coli* is required for both SOS induction and genetic recombination (Clark, 1973). Many mutations in this gene have been isolated and characterized (Clark, 1982; Wang & Tessman, 1986). These mutations were studied *in vivo* to determine the mechanistic pathways of recombination (Clark *et al.*, 1984) and in an attempt to map functional domains of this interesting protein (Devoret *et al.*, 1983; Wang & Tessman, 1986; Kawashima *et al.*, 1984). Recent studies demonstrate that the biochemical analysis of purified mutant proteins can offer insight into the physical and mechanistic requirements of recA protein during SOS induction and *in vitro* recombination reactions (Moreau & Roberts, 1984; Lavery & Kowalczykowski, 1988; Kowalczykowski *et al.*, 1989; Kowalczykowski & Krupp, 1989).

recA protein catalyzes the biochemically unique reaction of DNA strand exchange. This reaction typically involves the exchange of a ssDNA§ molecule (circular M13 ssDNA) with a homologous

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§ Abbreviations used: ssDNA, single-stranded DNA; dsDNA, double-stranded DNA; SSB protein, single-stranded DNA binding protein; PEP, phosphoenol pyruvate; RFI, relative fluorescence increase.



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partner in a dsDNA molecule (linear M13 dsDNA) to form a heteroduplex DNA product. The dsDNA product contains one DNA strand from the original dsDNA molecule and one from the ssDNA molecule to yield a nicked or gapped circular duplex DNA molecule. This reaction has been shown to require either rATP or dATP (Cox & Lehman, 1981*b*; Weinstock, 1982) and is generally thought to be representative of events occurring in the *in vivo* recombination process.

The *recA430* allele of *E. coli* displays altered induction of both the SOS response and the lytic growth of lambdoid bacteriophages (Devoret *et al.*, 1983). Bacteria containing this *recA* allele show a slightly reduced recombination frequency (Morand *et al.*, 1977). Thus, the *in vivo* data suggest that the *recA430* mutation is in a region of the protein involved in the interaction of recA protein with substrate repressor proteins and which affects recombination functions only slightly. The *recA430* mutation results in the change of amino acid residue 204 from a glycine to a serine and, consequently, this region of the protein was functionally assigned as a repressor interaction domain (Kawashima *et al.*, 1984). No other recA protein functions are associated with this region.

In this paper, the biochemical properties of the protein product of the *recA430* gene are characterized. We find that the recA430 protein can catalyze the *in vitro* DNA strand exchange and lexA repressor cleavage reactions, consistent with *in vivo* data described above. However, the rate and the extent of DNA strand exchange catalyzed by recA430 protein are sensitive to the type and concentration of nucleoside triphosphate present, the recA430 protein concentration, and the presence of SSB protein. The ssDNA-dependent NTPase and the lexA repressor cleavage activities of recA430 protein displays similar sensitivities. Together, these data suggest that the reduced ability of recA430 protein to carry out the DNA strand exchange reaction correlates with an inability to compete with SSB protein. The physical basis for this defect appears to result from reductions in the apparent steady-state affinity for ssDNA and in the rate of association with ssDNA in the presence of rATP.

## 2. Materials and Methods

### (a) *Chemicals*

All chemicals used were reagent grade and solutions were made in glass distilled water. rATP was purchased from Boehringer-Mannheim and dATP was purchased from Sigma Chemical Company; both were dissolved as concentrated stock solution at pH 7·5. Concentrations of adenine nucleotides were determined spectrophotometrically using an extinction coefficient of $1{\cdot}54 \times 10^4$ M$^{-1}$ cm$^{-1}$ at 260 nm.

### (b) *Proteins*

recA430 protein was purified from *E. coli* strain JC12768 containing a plasmid, pBEU46 (which is pBEU2 (Uhlin *et al.*, 1983), carrying the *recA430* allele). Wild-type recA protein was purified from *E. coli* strain JC12772 (Uhlin & Clark, 1981). These *E. coli* strains were obtained from A. J. Clark at the University of California, Berkeley. Both proteins were purified using a preparative protocol (Kowalczykowski, unpublished results) based on spermidine precipitation (Griffith & Shores, 1985). Protein concentrations were determined using an extinction coefficient of $2{\cdot}7 \times 10^4$ M$^{-1}$ cm$^{-1}$ at 280 nm.

SSB protein was purified from *E. coli* strain RLM 727 using a preparative protocol provided by Dr Roger McMacken of the Johns Hopkins University. The concentration of SSB protein was determined using an extinction coefficient of $3 \times 10^4$ M$^{-1}$ cm$^{-1}$ at 280 nm.

lexA protein was purified from *E. coli* strain JL652 (Little, 1984) using the method of Schnarr *et al.* (1985). Protein concentration was determined using an extinction coefficient of 7300 M$^{-1}$ cm$^{-1}$ at 280 nm.

### (c) *DNA*

Phage M13 ssDNA and replicative form dsDNA were isolated as described by Messing (1983). The replicative form was linearized using *Eco*RI restriction endonuclease. The concentrations of ssDNA and dsDNA were determined using extinction coefficients at 260 nm of 8784 and 6500 M$^{-1}$ cm$^{-1}$, respectively.

Etheno M13 DNA was made as described by Menetski & Kowalczykowski (1985). The concentration of etheno M13 DNA was determined using an extinction coefficient of $7{\cdot}0 \times 10^3$ M$^{-1}$ cm$^{-1}$ at 260 nm (Menetski & Kowalczykowski, 1987).

### (d) *lexA cleavage assay*

The cleavage of lexA protein was determined as described by Little *et al.* (1980) and modified by Lavery & Kowalczykowski (1988). Reaction buffer contained 20 mM-Tris·HCl (pH 7·5), 10 mM-$MgCl_2$, 50 mM-NaCl, and 1 mM-dithiothreitol. The experiments were done by adding 3 $\mu$M-M13 ssDNA, 1 mM-rATP or dATP, and 1·5 $\mu$M-recA protein to reaction buffer. An rATP and dATP regenerating system, containing 6 mM-phosphoenolpyruvate (PEP) and 17 units of pyruvate kinase/ml, was added to each reaction. SSB protein (0·6 $\mu$M) was added after approximately 1 min and the reaction was started by the addition of 10 $\mu$M-lexA protein. Time points were removed and analyzed by electrophoresis on 15% (w/v) polyacrylamide gels. The protein bands were quantified, after Coomassie staining, by a Zeineh scanning densitometer using a 595 nm filter. Percentage cleavage was determined as the percentage of the protein fragments relative to the total (protein fragments and intact lexA protein) for each time point.

### (e) *NTP hydrolysis assay*

The rate of rATP or dATP hydrolysis was determined using an enzyme-coupled spectrophotometric assay described by Kreuzer & Jongeneel (1983). Typical buffer conditions consisted of 20 mM-Tris acetate (pH 7·5), 0·1 mM-dithiothreitol, and 10 mM-magnesium acetate at 37 °C. The assay also contained 50 units of pyruvate kinase/ml, 25 units of lactate dehydrogenase/ml and 1·5 mM-PEP. Changes in these conditions are cited in the Figure legends.

The apparent stoichiometry of recA protein binding to ssDNA was determined from NTP hydrolysis data by measuring the concentration of recA protein required to saturate the rate of DNA-dependent NTP hydrolysis. This was done by drawing a line through the initial linear region of the recA protein concentration dependence data and determining the point at which this line intersects a line drawn through end-points of the data (parallel to the $x$-axis). If the endpoints were still increasing slightly, this horizontal line was estimated as a line to which the data were asymptotically approaching. The maximum rate of hydrolysis for a single recA protein monomer ($k_{cat}$ or turnover number) is determined by dividing the maximum rate of hydrolysis observed by the total amount of protein bound (as determined from the apparent binding stoichiometry) to obtain the pseudo-1st-order rate constant $k_{cat}$.

### (f) *DNA strand exchange assay*

The DNA strand exchange reaction was monitored using an agarose gel assay (Cox & Lehman, 1981*a*; Roman & Kowalczykowski, 1986). However, in this paper, electrophoresis was conducted using 0·8% (w/v) agarose gels in the absence of ethidium bromide and later stained to visualize the DNA bands. This procedure allows the product of the DNA strand exchange reaction, gapped circular duplex DNA, to be separated from various intermediate species. Intermediates in this reaction are plectonemic joint molecules containing both strands of the dsDNA molecule and the invading ssDNA molecule (Cox & Lehman, 1981*a*; Menetski, Bear & Kowalczykowski, unpublished results).

Reaction buffer contained 25 mM-Tris acetate (pH 7·5), 1 mM-dithiothreitol, 6 mM-magnesium acetate, and 1 mM-NTP at 37°C. The concentrations of M13 ssDNA and linear dsDNA were 5 μM and 10 μM, respectively. The concentrations of recA protein and SSB protein were 3 μM and 0·45 μM, respectively. An NTP regenerating system, containing 25 units of pyruvate kinase/ml and 1·5 mM-PEP, was included in all reactions.

In the presence of high concentrations of magnesium acetate (10 mM) and low concentrations of PEP (<1·5 mM), wild-type protein can form a DNA species that does not migrate into a 0·8% agarose gel (not shown). This species is probably a consequence of several rounds of strand invasion using the end of the displaced ssDNA molecule and represents a network-like structure held together by base-pairing (Chow *et al.*, 1988; Lavery & Kowalczykowski, 1990). This species makes the quantification of the rate of intermediate and product formation difficult. Thus, the rates are reported at lower magnesium ion concentration (6 mM), where the formation of this high molecular weight structure does not occur.

DNA strand exchange reaction products were quantified from photographic negatives using a Zeineh soft laser scanning densitometer. The percentage of product and intermediate formation was determined as the amount of either species divided by the total dsDNA for each time point. The maximum rates of intermediate and product formation are reported.

### (g) *ssDNA binding experiments*

The binding of recA430 protein to ssDNA was determined using the fluorescently modified M13 ssDNA, etheno M13 DNA (Menetski & Kowalczykowski, 1985; Menetski *et al.*, 1988). Typical binding experiments were conducted using 10 μM-etheno M13 DNA and 1 μm-recA protein (wild-type or recA430) in binding buffer (25 mM-Tris acetate (pH 7·5), 6 mM-magnesium acetate, 1 mM-dithiothreitol) at 25°C. When rATP or dATP was present, a regenerating system was included containing 1·5 mM-PEP and 5 units of pyruvate kinase/ml. The relative fluorescence increase was determined as the final fluorescence of the saturated protein–DNA complex divided by the fluorescence of the dissociated etheno M13 DNA and recA protein at the end of the salt titration. The salt titration mid-point for each protein–DNA complex was determined as described by Menetski *et al.* (1988).

### (h) *Association experiments*

The association rate of recA protein with ssDNA can be determined using etheno M13 DNA. At high salt concentrations (>100 mM-NaCl), the rate of association of recA protein to ssDNA is slow enough to monitor using manual mixing methods. Standard reaction conditions were 20 mM-Tris·HCl (pH 7·5), 4 mM-$MgCl_2$, 0·1 mM-dithiothreitol, 150 mM-NaCl at 25°C. The etheno M13 DNA concentration was 6 μM and the nucleotide concentration was 500 μM. recA protein (0·1 μM) was added to the DNA to initiate the reaction. The concentration of protein is <10% saturation of the ssDNA to avoid any complications that might arise from excluded binding site phenomenon.

## 3. Results

### (a) *DNA strand exchange catalyzed by recA430 protein*

Morand *et al.* (1977) reported that strains carrying the *recA430* allele display an almost (30%) wild-type level of recombination activity. Since the DNA strand exchange activity of recA protein is thought to reflect the *in vivo* recombination function of this protein, this activity of recA430 protein was examined. Figure 1(a) shows the time-course for DNA strand exchange catalyzed by wild-type recA protein in the presence of rATP. There is an initial rapid increase in the formation of strand exchange intermediates (open circles), which peaks at approximately ten minutes. These intermediates represent various types of plectonemic joint molecules that are stable during gel electrophoresis (Menetski, 1988). After approximately ten minutes, these intermediates are converted into product molecules (gapped circular duplex DNA; filled circles). In contrast to wild-type recA protein, recA430 protein carries out the DNA strand exchange reaction much less efficiently (Fig. 1(a)). In the presence of rATP, less than 10% of the input M13 dsDNA is converted to intermediates (open triangles) and these intermediates are formed at a nine-fold slower rate (Table 1; line A and F); product formation is not detectable.

In the presence of dATP, recA430 protein can produce more intermediate and product species than are observed in the presence of rATP (Fig. 1(b)). Approximately 30% of the input M13 dsDNA is converted into intermediate (open triangles) in 40 minutes, and greater than 40% is converted into product after 80 minutes (filled triangles). However,

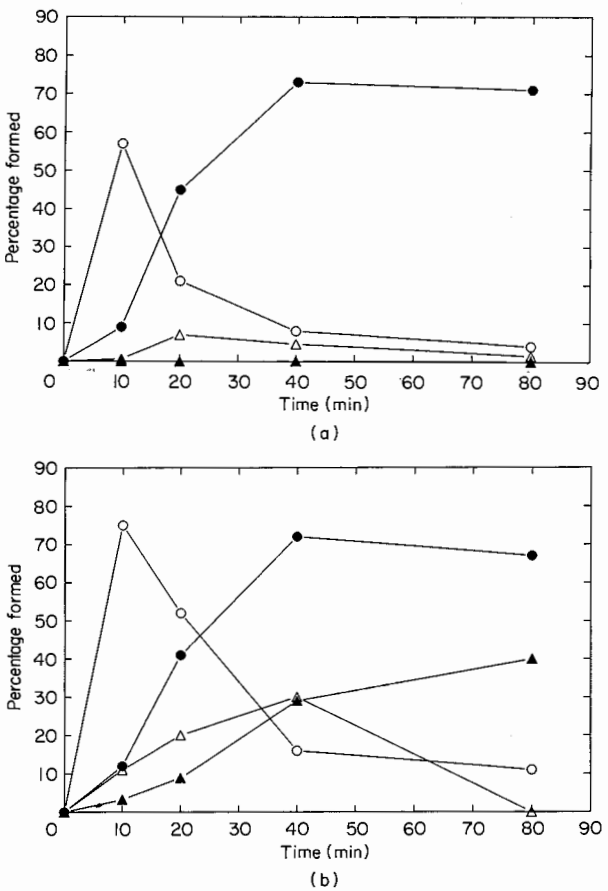


**Figure 1.** The DNA strand exchange activity of recA430 protein in the presence of rATP and dATP. Strand exchange experiments were done as described in Materials and Methods. The percentage of both products (gapped duplex molecules) and intermediates (joint molecules) are shown as a function of time: intermediate formation by wild-type recA protein (open circles), product formation by wild-type recA protein (filled circles), intermediate formation by recA430 protein (open triangles), and product formation by recA430 protein (filled triangles). (a) rATP; (b) dATP.

**Table 1**
*Effect of rATP and dATP on the recA430 protein-catalyzed DNA strand exchange reaction*

| Reaction conditions† | Nucleotide | Intermediate formation (pM/min) | Product formation (pM/min) |
|---|---|---|---|
| RecA430 protein | | | |
| A: 1 | rATP | 4·4 | <0·4 |
| B: 1 | dATP | 7·6 | 7·0 |
| C: 2, 1·5 mM-PEP | rATP | 21 | 6·6‡ |
| D: 3, 1·5 mM-PEP | rATP | 1·5 | 3·4 |
| E: 2, 6 mM-PEP | rATP | <0·1 | <0·1 |
| Wild-type recA protein | | | |
| F: 1 | rATP | 38 | 24 |
| G: 1 | dATP | 49 | 19 |

The DNA strand exchange reactions were carried out as described in Materials and Methods. Rates of intermediate and product formation are given in units of pM-dsDNA molecules/min and are reproducible to ±10%.

† Reactions were conducted under the following conditions (1) Standard DNA strand exchange assay conditions (i.e. 3 μM-recA protein, 0·45 μM-SSB protein, 5 μM-ssDNA, 6 mM-magnesium acetate, 1·5 mM-PEP). (2) Same as condition (1) (above) except using 6 μM-recA protein, 0·9 μM-SSB protein, 10 μM-ssDNA, 10 mM-magnesium acetate, and PEP concentration as indicated. (3) Same as condition (1) (i.e. 3 μM-recA protein, 0·45 μM-SSB protein, 5 μM-ssDNA) except using 10 mM-magnesium acetate and 1·5 mM-PEP.

‡ This value is an underestimate due to formation of the network species, which fails to enter the gel.

the rates of both intermediate and product formation are still slower than those of wild-type recA protein even in the presence of dATP (Table 1, lines B and G).

During the course of these experiments, we observed that recA430 protein-catalyzed DNA strand exchange, in the presence of rATP, is sensitive to solution conditions such as PEP and recA430 protein concentrations. At a higher PEP concentration (6 mM-PEP), no intermediate or product formation is observed (Table 1, line E). Reducing the PEP concentration to 1·5 mM substantially increases both the rate of intermediate and product formation (line C). Reducing the recA430 protein concentration from 6 μM (line C) to 3 μM (line D) reduces the rate of intermediate formation by approximately tenfold and product formation by twofold. In contrast, reducing wild-type recA protein concentrations by twofold has no significant effect on the rates of intermediate and product formation (data not shown). Also, wild-type recA protein can catalyze DNA strand exchange at 6 mM-PEP under conditions that completely inhibit recA430 protein (not shown). Thus, the recA430 protein-catalyzed DNA strand exchange reaction is sensitive to the type of NTP present and to both the PEP and recA protein concentrations.

### (b) *The NTPase activity of recA430 protein is more sensitive to inhibition by SSB protein*

The hydrolysis of rATP by recA protein is required during the DNA strand exchange reaction under typical conditions (Cox & Lehman, 1981*b*). Consequently, the impaired ability of recA430 protein to catalyze DNA strand exchange in the presence of rATP could be due to a decreased ability to hydrolyze rATP. In the absence of SSB protein, increasing the recA430 protein concentration up to approximately 0·5 μM increases the rate of rATP hydrolysis (Fig. 2; open triangles); above this concentration, the M13 ssDNA becomes saturated and the rate is independent of protein concentration. The data yield an apparent stoichiometry of 5·8 (±0·3) nucleotides/recA430 protein monomer. This value is in excellent agreement with that determined for wild-type recA protein (5·6 for M13 ssDNA; Menetski & Kowalczykowski, 1989; Lavery & Kowalczykowski, 1988). The $k_{cat}$, or turnover

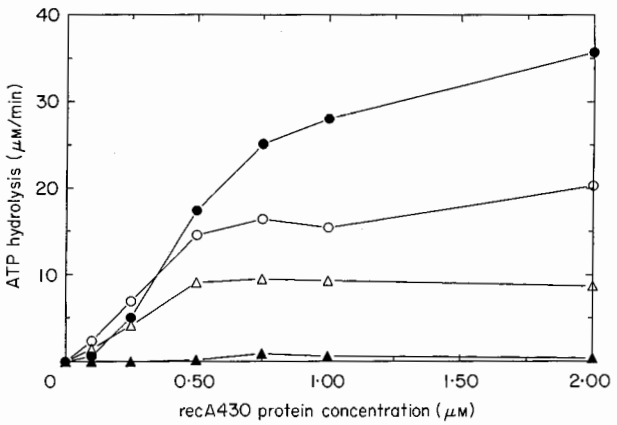


**Figure 2.** Effect of rec430 protein concentration on nucleotide hydrolysis. Nucleotide hydrolysis experiments were done as described in Material and Methods. The concentration of M13 ssDNA was 3 μM, NTP was 500 μM and SSB protein, when added, was 0·6 μM: rATP without SSB protein (open triangles); rATP with SSB protein (filled triangles); dATP without SSB protein (open circles); and dATP with SSB protein (filled circles).

number (normalized for the amount of DNA-bound recA, see Materials and Methods), for rATP hydrolysis is 18 μM-rATP per minute per μM-recA430 protein, which is nearly the same as that determined for wild-type recA protein (20 μM-rATP/min/μM recA protein; Menetski & Kowalczykowski, 1989). Thus, in the absence of SSB protein, the characteristics of rATP hydrolysis appear to be very similar to those of wild-type recA protein.

The M13 ssDNA-dependent rATPase activity of wild-type recA protein is stimulated by SSB protein (Kowalczykowski & Krupp, 1987). At this rATP concentration, no rATP hydrolysis by recA430 protein is observed in the presence of SSB protein at the recA430 protein concentrations tested (Fig. 2, filled triangles). Similar inhibitory behavior has been observed for the mutant recA142 protein (Kowalczykowski *et al.*, 1989). This result implies that SSB protein completely displaces recA430 protein from ssDNA in the presence of rATP.

The recA430 protein concentration dependence of dATPase activity is also shown in Figure 2. In the absence of SSB protein, the apparent stoichiometry is 4·1 nucleotides per recA430 protein monomer (open circles). This value is comparable to that determined for wild-type recA protein (3·4; Menetski & Kowalczykowski, 1989). The differences in binding stoichiometries observed in the presence of rATP *versus* dATP reflect the extent to which the recA proteins can utilize regions of ssDNA secondary structure as an effector for NTPase activity. The $k_{cat}$ for dATP hydrolysis is 30 μM-dATP per minute per μM-recA430 protein, which also compares favorably with the wild-type value (29 μM-dATP/min/μM-recA protein; Menetski & Kowalczykowski, 1989). Thus, in the absence of SSB protein, the dATPase activity of recA430 protein is similar to that of wild-type recA protein.

In the presence of SSB protein, the protein concentration dependence of dATP hydrolysis appears to be sigmoidal (Fig. 2, filled circles). At low recA430 protein concentrations (<0·25 μM), addition of SSB protein inhibits dATP hydrolysis. Above approximately 0·5 μM-recA430 protein, SSB protein stimulates dATP hydrolysis. Wild-type recA protein dATPase activity is not inhibited by SSB protein at low protein concentrations (Menetski & Kowalczykowski, 1989). These data suggest that, compared to wild-type recA protein, the competition of recA430 protein with SSB protein for limited ssDNA binding sites is more sensitive to both the type of NTP and recA430 protein concentration.

### (c) *Nucleotide concentration dependence of recA430 protein NTPase activity*

Figure 3(a) shows that the rate of rATP hydrolysis by recA430 protein increases with increasing nucleotide concentration. The half-maximal rATP concentration (apparent $K_m$) is approximately 85 μM-rATP and is slightly larger than that of wild-type recA protein (60 μM-rATP; Menetski *et al.*, 1988).

In the presence of SSB protein, the recA430 protein concentration has an effect on rATP hydrolysis (Fig. 3(a)). At 1μM-recA430 protein, addition of SSB protein completely inhibits rATP hydrolysis at rATP concentrations as high as 2 mM (data not shown). However, at 5 μM-recA430 protein, SSB protein stimulates rATPase activity at rATP concentrations greater than 800 μM. In comparison, the rATPase activity of wild-type recA protein (at 1 μM) is stimulated by SSB protein above 200 μM-rATP (Kowalczykowski & Krupp, 1987).

Examination of the dATPase activity of recA430 protein shows that the apparent $K_m$ value is approximately 100 μM-dATP in the absence of SSB protein (Fig. 3(b)); this value is twofold higher than that observed for wild-type recA protein (50 μM-dATP; Menetski *et al.*, 1988). In contrast to the rATPase activity, dATP hydrolysis is stimulated by SSB protein at either 1 or 5 μM-recA430 protein. The concentration of dATP required (200 μM) to induce SSB protein stimulation of dATP hydrolysis is greater than for wild-type recA protein (70 μM; Menetski & Kowalczykowski, 1989). These data suggest that recA430 protein may not interact with nucleotide cofactors as effectively as wild-type recA protein and that this defect is particularly pronounced in the presence of SSB protein.

### (d) *Effect of magnesium acetate and sodium chloride concentrations on nucleotide hydrolysis by recA430 protein*

In the presence of SSB protein, hydrolysis of rATP by wild-type recA protein is influenced by salt

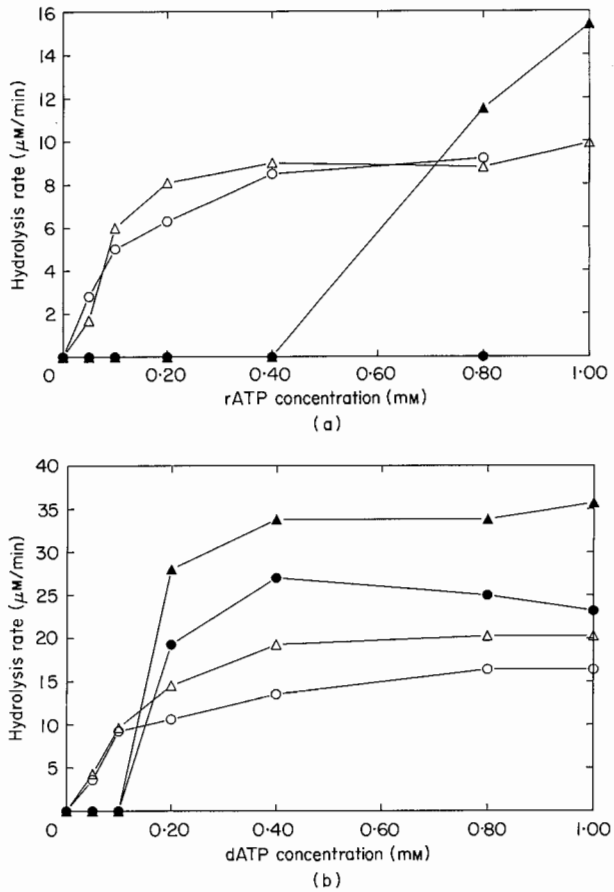


**Figure 3.** Effect of nucleotide concentration on nucleotide hydrolysis. Nucleotide hydrolysis experiments were done as described in Materials and Methods. The concentration of M13 ssDNA was 3 μM, recA430 protein was either 1 or 5 μM, and SSB protein, when added, was 0·6 μM. (a) rATP, in the absence of SSB protein both at 1 μM-recA430 protein (open circles) and at 5 μM-recA430 protein (open triangles); rATP, in the presence of SSB protein both at 1 μM-recA430 protein (filled circles) and at 5 μM-recA430 protein (filled triangles). (b) dATP, in the absence of SSB protein at both 1 μM-recA430 protein (open circles) and at 5 μM-recA430 protein (open triangles); dATP, in the presence of SSB protein both at 1 μM-recA430 protein (filled circles) and 5 μM-recA430 protein (filled triangles).

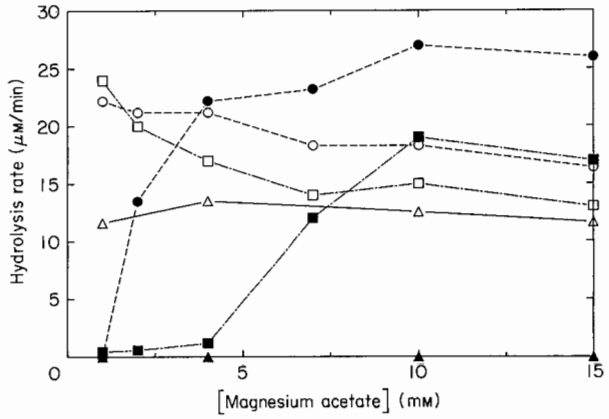


**Figure 4.** Effect of magnesium acetate concentration on nucleotide hydrolysis by recA430 protein. Nucleotide hydrolysis experiments were done as described in Materials and Methods (3 μM-M13 ssDNA and 0·6 μM-SSB protein, when added) except that magnesium acetate concentration was varied. Results are presented for: rATP hydrolysis by 1 μM-recA430 protein at 500 μM-rATP in the absence (open triangles) and presence (filled triangles) of SSB protein; rATP hydrolysis by 5 μM-recA430 protein at 1 mM-rATP in the absence (open squares) and presence (filled squares) of SSB protein; dATP hydrolysis by 1 μM-recA430 protein at 500 μM-dATP in the absence (open circles) and presence (filled circles) of SSB protein.

concentration (Kowalczykowski & Krupp, 1987). Below 2 mM-magnesium acetate, SSB protein completely inhibits rATP hydrolysis by wild-type recA protein, while above 2 mM-magnesium acetate SSB protein stimulates hydrolysis.

In the presence of SSB protein, the rATPase activity of 1 μM-recA430 protein is inhibited completely at all magnesium acetate concentrations tested (Fig. 4, filled triangles). At 5 μM-recA430 protein, rATP hydrolysis is inhibited by SSB protein below 4 mM-magnesium acetate, and full stimulation of the hydrolysis rate is not observed until 10 mM-magnesium acetate (Fig. 4, filled squares). Therefore, even at the higher recA430 protein concentration, higher concentrations of magnesium acetate are required to observe SSB protein-dependent stimulation of rATP hydrolysis when compared to wild-type recA protein.

The dATPase activity of recA430 protein is inhibited by SSB protein below 2 mM-magnesium ion, whereas stimulation occurs above 2 mM-magnesium acetate (Fig. 4, filled circles). In comparison, dATP hydrolysis by wild-type recA protein is stimulated by SSB protein at all magnesium acetate concentrations from 1 to 15 mM (Menetski & Kowalczykowski, 1989).

The effect of NaCl was also examined (data not shown). In the absence of SSB protein, the rate of rATP hydrolysis decreases as NaCl concentration increases at both 1 μM and 5 μM-recA430 protein, and is inhibited by 50% at approximately 140 mM and 275 mM-NaCl, respectively. In the presence of SSB protein, rATP hydrolysis by 1 μM-recA430 protein is inhibited at all salt concentrations tested. At 5 μM-recA430 protein, SSB protein stimulates rATP hydrolysis to NaCl concentrations greater than 500 mM (50% inhibition is observed at 310 mM-NaCl).

In the absence of SSB protein, the rate of dATP hydrolysis is inhibited by 50% at approximately 300 mM-NaCl. In the presence of SSB protein, dATPase activity increases about 20% at 200 mM-NaCl and is inhibited by 50% (of maximum) at approximately 410 mM-NaCl. Therefore, increasing NaCl concentration does not alter the observed pattern of SSB protein effects on NTPase activity.

(e) *dsDNA-dependent nucleotide hydrolysis by recA430 protein*

Wild-type recA protein is able to hydrolyze either rATP (Roman & Kowalczykowski, 1986; Kowalczykowski *et al.*, 1987; Pugh & Cox, 1987, 1988) or dATP (Menetski & Kowalczykowski, 1989) in a dsDNA-dependent reaction. The dsDNA-dependent NTPase activity has two characteristic features (Kowalczykowski *et al.*, 1987; Pugh & Cox, 1988): a rate-limiting lag in nucleotide hydrolysis, which represents a DNA "opening" or unwinding event, and a steady-state rate of nucleotide hydrolysis following the lag, presumably using the "open" dsDNA to stimulate nucleotide hydrolysis. The data in Table 2 show that the lag times for nucleotide hydrolysis are approximately 1·5-fold longer for recA430 protein than for wild-type recA protein with either rATP or dATP. The lag in nucleotide hydrolysis is reduced (approximately 80%) in the presence of dATP for both proteins.

The steady-state rates of nucleotide hydrolysis for both recA430 protein and wild-type recA protein are also shown on Table 2. The final rate of dsDNA-dependent rATP hydrolysis by recA430 protein is half that of the wild-type recA protein (which, under these conditions, is below the rate observed at saturation of the dsDNA). The final steady-state rate of dsDNA-dependent dATP hydrolysis by recA430 protein is the same as wild-type recA protein. These data suggest that either less recA430 protein–dsDNA complex is formed or the $k_{cat}$ (turnover number) for rATP hydrolysis in the dsDNA-dependent reaction is reduced compared to wild-type. Since the rate of ssDNA-dependent rATP hydrolysis is the same for recA430 and wild-type recA protein, the dsDNA-dependent hydrolysis data suggest that less recA430 protein–dsDNA complex is formed.

(f) *In the presence of dATP, recA430 protein can utilize ssDNA that is complexed with SSB protein as substrate for NTP hydrolysis*

The binding of SSB protein to M13 ssDNA prior to the addition of recA protein greatly inhibits the hydrolysis of rATP (Kowalczykowski & Krupp, 1987; Lavery & Kowalczykowski, 1988). This inhibition is manifest as a lag in nucleotide hydrolysis and a reduced rate of hydrolysis after the lag. The lag in nucleotide hydrolysis probably corresponds to the actual displacement of SSB protein from the ssDNA. The final steady-state rate of nucleotide hydrolysis represents the intrinsic rate of nucleotide hydrolysis as well as the steady-state amount of recA protein bound to the ssDNA after displacement. The addition of dATP to reaction mixtures containing rATP decreases the lag in hydrolysis and increases the steady-state rate of nucleotide hydrolysis for the wild-type protein (Menetski & Kowalczykowski, 1989).

Figure 5 shows the effect of dATP on SSB protein displacement by recA430 protein, in both the absence and the presence of 500 μM-rATP. As dATP concentration is increased, the length of the lag in nucleotide hydrolysis decreases (Fig. 5(a)). In the presence of 500 μM-rATP, the length of the lag at low dATP concentrations is greater than that observed in the absence of rATP; high dATP concentrations decrease the length of the lag to a value similar to that obtained in the absence of rATP. This limiting value is about 1·7-fold greater than that observed for wild-type recA protein under similar conditions (300 s; Menetski & Kowalczykowski, 1989). Figure 5(b) shows that the steady-state rate of NTP hydrolysis is sigmoidal with respect to dATP concentration in both the presence and the absence of additional rATP. The dATP concentration at half-maximal stimulation is approximately 175 μM and 200 mM-dATP in the absence or presence of rATP, respectively. In contrast, the wild-type recA protein displays hyperbolic behavior (Menetski & Kowalczykowski, 1989). Also, unlike wild-type recA protein, the final steady-state rate of NTP hydrolysis by recA430 protein at saturation is slower in the presence of 500 μM-rATP than in its absence.

Thus, as inferred from these NTP hydrolysis data, rATP reduces the ability of recA430 protein to displace SSB protein (both the rate and extent of displacement) when dATP is present. In contrast, rATP enhances the displacement of SSB protein by wild-type recA protein when dATP is present (Menetski & Kowalczykowski, 1989). These data also imply that recA430 protein displaces SSB protein from ssDNA more slowly than does wild-type recA protein under all conditions.

**Table 2**

*Characteristics of dsDNA-dependent NTP hydrolysis by recA430 protein*

| | rATP | | dATP | |
|---|---|---|---|---|
| | Lag (s) | Rate (μM/min) | Lag (s) | Rate (μM/min) |
| recA(wt) | 980 | 8·0 | 200 | 22 |
| recA430 | 1500 | 4·9 | 300 | 21 |

NTP hydrolysis was determined as described in Materials and Methods. The reactions contained 6 μM-linear M13 dsDNA, 2·1 μM-recA protein and 1 mM-nucleoside triphosphate. Standard strand exchange assay buffer was used, except that the magnesium acetate concentration was 10 mM and PEP concentration was 1·5 mM. Lags were determined by the intersection of a line drawn through the linear region of the curve and a horizontal line drawn through the initial absorbance. The rate reported is the final linear steady-state rate after the lag. This rate was determined to be constant for at least 300 s. wt, wild-type.

(g) *The affinity of recA430 protein for ssDNA is modulated by nucleotide cofactors*

The diminished ability of recA430 protein to compete with SSB protein for ssDNA binding sites could result from a lower apparent ssDNA binding affinity. A measure of the apparent affinity of recA

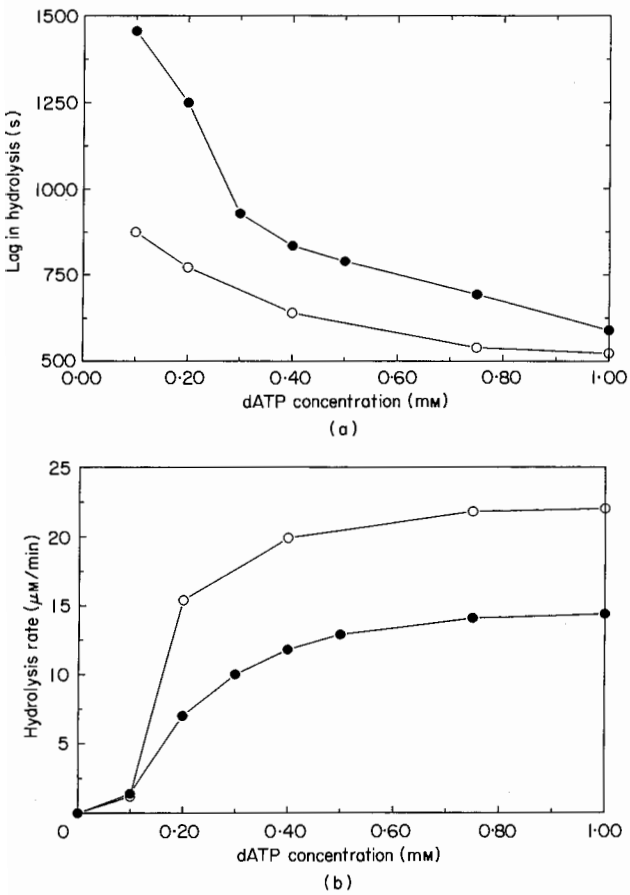


**Figure 5.** Displacement of SSB protein from ssDNA by recA430 protein. Nucleotide hydrolysis experiments were done as described in Materials and Methods (3 μM-M13 ssDNA, 1 μM-recA430 protein and 0·6 μM-SSB protein) except SSB protein was added to the ssDNA prior to the addition of recA430 protein. (a) The lag in nucleotide hydrolysis observed in the absence (open circles) and presence (filled circles) of 500 μM-rATP. Lags were determined as described (Menetski & Kowalczykowski, 1989). (b) The rate of steady-state nucleotide hydrolysis after the lag. The final rate of hydrolysis observed in the absence (open circles) and presence (filled circles) of 500 μM-rATP.

protein for ssDNA can be determined from the salt concentration required to disrupt 50% of the protein–DNA complex (Menetski & Kowalczykowski, 1985). In the absence of a nucleotide cofactor, 300 mM-NaCl is required to dissociate one-half of the recA430–etheno M13 DNA complex (Table 3). This concentration is identical with that determined for wild-type recA protein.

Both rATP and dATP increase the apparent affinity of wild-type recA protein for ssDNA (Table 3; Menetski & Kowalczykowski, 1985; Menetski *et al.*, 1988). The data in Table 3 show that the steady-state affinity of recA430 protein for ssDNA also increases in the presence of either rATP or dATP, although the increases are less than those of the equivalent wild-type recA protein complexes. In the presence of ADP, the salt titration midpoint

**Table 3**
*Effect of nucleotide cofactors on the binding of recA430 to ssDNA*

| Nucleotide cofactor | STMP† (mM-NaCl) | | RFI‡ | |
|---|---|---|---|---|
| | 430 | wt | 430 | wt |
| No cofactor | 300 | 300 | 2·1 | 2·1 |
| rATP | 430 | 570 | 2·3 | 2·3 |
| dATP | 400 | 600 | 2·5 | 2·6 |
| rADP | 160 | 160 | 2·0 | 2·1 |

Binding experiments were conducted as described in Materials and Methods and contained 500 μM-nucleotide. Values for recA430 protein (430) and wild-type (wt) recA protein are given.

† Salt titration mid-point (STMP) values are reproducible to ±20 mM-NaCl.

‡ Relative fluorescence increase (RFI) of the recA protein–etheno M13 DNA complex was determined as described in Materials and Methods.

of the recA430 protein–etheno M13 DNA complex is reduced (160 mM) relative to the value observed in the absence of cofactor; this value is identical with that determined for wild-type recA protein.

Another characteristic of the recA protein–etheno M13 DNA complex is the relative fluorescence increase (RFI) upon complex formation. The RFI of the wild-type recA protein–DNA complex is higher in the presence of either rATP or dATP than in the presence or absence of nucleoside diphosphates (Table 3). This difference in fluorescence of the etheno M13 DNA is thought to correspond to induction of an alternative conformation of the DNA-bound recA protein (Menetski & Kowalczykowski, 1985; Menetski *et al.*, 1988). Both rATP and dATP increase the fluorescence of the recA430 protein–etheno M13 DNA complex (Table 3), suggesting that the rATP- and dATP-induced conformations of recA430 and wild-type recA proteins are similar. Thus, the effect of nucleotide cofactors on the ssDNA binding properties of recA430 protein are similar to those of wild-type recA protein. The significant exception is that the salt titration midpoint values in the presence of rATP or dATP are lower, implying a lower steady-state affinity of the mutant protein for ssDNA relative to wild-type protein.

### (h) *The rate of asssociation of recA430 protein with ssDNA is much slower than that of wild-type recA protein*

The rate of recA protein association with poly(etheno-dA) has been shown to be influenced by factors such as pH, magnesium ion concentration and polynucleotide length (Chabbert *et al.*, 1987). The data in Figure 6 show that, in the presence of rATP, the rate of recA430 protein association with etheno M13 DNA is slower than for wild-type recA protein. The half-time for wild-type recA protein binding in the presence of rATP is 28 seconds, while the half-time for recA430 protein is almost three times

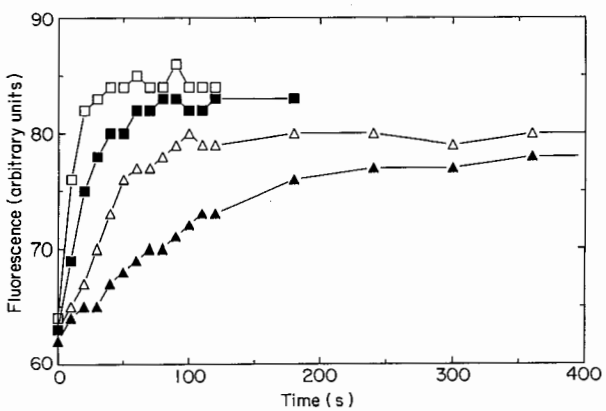


**Figure 6.** Time-course for the association of recA protein with etheno M13 DNA in the presence of rATP and dATP. Association was observed as described in Materials and Methods. Etheno M13 DNA concentration was 6 $\mu$M and recA protein concentration was 0·1 $\mu$M. The observed increase in fluorescence is plotted as a function of time for: wild-type recA protein in the presence of either rATP (open triangles) or dATP (open squares); recA430 protein in the presence of either rATP (filled triangles) or dATP (filled squares).

greater, 81 seconds. When dATP is substituted for rATP, the association half-times decrease to 7 and 15 seconds for wild-type and recA430 protein, respectively. Thus, the NTP–wild-type recA protein complex can associate with ssDNA faster than the equivalent NTP–recA430 protein complex. In addition, the association rate of dATP–recA430 protein exceeds that of the rATP–wild-type recA protein. These data demonstrate that the mutation in the recA430 protein reduces the rate at which this protein associates with ssDNA.

### (i) *lexA protein cleavage by recA430 protein is decreased relative to wild-type recA protein*

The *recA430* allele has been described as showing different patterns of SOS and lamboid bacteriophage induction (Devoret *et al.*, 1983). Those studies showed that the recA430 protein will cleave the lexA repressor *in vivo*, but at a slower rate than does the wild-type recA protein. Eguchi *et al.* (1988) have shown that the *in vitro* pattern of repressor protein cleavage by recA430 protein follows that deduced from *in vivo* experiments (i.e. $\phi$80 cI>lexA->lambda cI repressors).

Studies of lexA repressor cleavage have demonstrated that SSB protein affects the observed cleavage rates (Lavery & Kowalczykowski, 1988; Palmer & Kowalczykowski, unpublished results). Therefore, we examined the effect of SSB protein on the catalysis of lexA protein cleavage by recA430 protein. In the presence of rATP, the rate of lexA protein cleavage is approximately 20% of that observed with wild-type recA protein (Table 4); when SSB protein is added, lexA cleavage by recA430 protein is inhibited. When dATP is substituted for rATP, the rate of lexA repressor cleavage by both recA proteins is increased (Table 4); unlike the rATP-dependent reaction, catalysis of lexA cleavage by recA430 protein is reduced, but not completely inhibited, by the addition of SSB protein. Thus, dATP is a better cofactor for recA protein enzymatic function and is required for lexA protein cleavage by recA430 protein when SSB protein is present.

**Table 4**

*Cleavage of lexA repressor in the presence of either rATP or dATP*

| | rATP ($\mu$M/min) | | dATP ($\mu$M/min) | |
|---|---|---|---|---|
| | Without SSB | With SSB† | Without SSB | With SSB† |
| recA(wt) | 0·95 | 1·3 | 1·3 | 2·0 |
| recA430 | 0·18 | <0·1 | 0·58 | 0·47 |

The lexA cleavage reaction was performed as described in Materials and Methods. Error on these values is approximately 10%. wt, wild-type.

† SSB protein (0·6 $\mu$M) was added to the reactions after the addition of M13 ssDNA and recA protein.

## 4. Discussion

In this report we have analyzed the biochemical properties of the recA430 protein. This mutant recA protein shows a strong preference *in vitro* for the nucleotide cofactor dATP in DNA strand exchange, NTP hydrolysis, and lexA repressor cleavage reactions. This result is surprising since wild-type recA protein does not show such a strong preference; however, under sub-optimal conditions, dATP is a more effective cofactor for wild-type protein function as well (Menetski & Kowalczykowski, 1989). Analysis of the recA430 protein NTPase activity suggests that this mutant protein does not compete effectively with SSB protein for ssDNA binding sites in the presence of rATP, but substitution of dATP for rATP enables recA430 protein to compete with SSB protein. This difference in competition with SSB protein is sufficient to explain the enhancement of recA430 protein-catalyzed DNA strand exchange, NTP hydrolysis, and lexA protein cleavage activities upon substitution of dATP for rATP.

DNA strand exchange requires the binding of recA protein to ssDNA (Muniyappa *et al.*, 1984; Cox & Lehman, 1981*a*,*b*). This binding has been termed presynapsis and represents the first step of the DNA strand exchange reaction (Gonda & Radding, 1983). Since the binding of recA protein to ssDNA activates its ATPase activity, ATP hydrolysis can be used to indicate formation of a recA protein–ssDNA complex. Our rATP hydrolysis data show that this activity of recA430 protein is inhibited by SSB protein under many of the conditions typically used

for DNA strand exchange and rATP hydrolysis experiments. This indicates that formation of the recA protein–ssDNA complex essential for these activities is blocked by SSB protein. As expected, recA430 protein cannot catalyze the DNA strand exchange reaction very well under these conditions (e.g. at 3 $\mu$M-recA430 protein in the presence of rATP). However, if the recA430 protein concentration is increased, then both ATP hydrolysis and DNA strand exchange activities are observed in the presence of SSB protein. This parallel between the rATPase activity and DNA strand exchange activity suggests that, in the presence of rATP and SSB protein, the ability of recA430 protein to form an active presynaptic complex is the molecular event that is sensitive to inhibition by SSB protein.

dATP enhanced the ability of wild-type recA protein to compete with SSB protein for ssDNA (Menetski & Kowalczykowski, 1989). Similarly, substitution of dATP for rATP allows recA430 protein to function in the presence of SSB protein under conditions that are non-permissive using rATP. When dATP is used, DNA strand exchange occurs more rapidly, SSB protein stimulates dATP hydrolysis, and recA430 protein displaces SSB protein from a preformed SSB protein–ssDNA complex. Thus, dATP restores the ability of recA430 protein to compete effectively with SSB protein and permits formation of a functional recA protein–ssDNA complex.

Our data suggest that recA430 protein may rely heavily on dATP as a cofactor for recombination *in vivo*. Under non-induced conditions, the total concentration of recA protein *in vivo* is estimated to be approximately 3 $\mu$M; the free concentration will be lower (Menetski & Kowalczykowski, 1985). At this protein concentration *in vitro*, the rATP–recA430 protein complex is a poor competitor of SSB protein for ssDNA binding sites under all conditions. Since the cellular concentration of SSB protein is presumed to be in excess of the ssDNA concentration, dATP may be required to enhance the properties of recA430 protein and allow *in vivo* recombination at nearly normal frequencies. In the presence of rATP *in vitro*, addition of dATP enhances the ability of recA430 protein to compete with SSB protein; stimulation of SSB protein displacement by dATP is half-maximal at approximately 200 $\mu$M-dATP (Fig. 5). This dATP concentration is close to that estimated as the *in vivo* concentration (175 $\mu$M-dATP; Bochner & Ames, 1982) and implies that recA430 protein function would be very responsive to changes in the dATP concentration. Thus, the intracellular concentration of dATP is high enough to allow recA430 protein to compete effectively with SSB protein and, consequently, to participate in homologous recombination at basal concentrations of protein. This suggestion that dATP is required for recA protein function is not without precedent. The recA protein analog from *Bacillus subtilis* requires dATP for catalysis of DNA strand exchange *in vitro*; rATP will not substitute for dATP (Lovett & Roberts, 1985). Thus, dATP may be a more physiologically significant nucleotide cofactor for the *E. coli* recA protein function than has been previously appreciated (for a discussion, see Roberts *et al.*, 1982).

Consistent with the above suggestion, the biochemical activities of recA430 protein observed in the presence of both dATP and SSB protein (both of which are intracellular constituents) parallel the *in vivo* behavior of this mutation (Morand *et al.*, 1977; Devoret *et al.*, 1983). Under these conditions, the DNA strand exchange activity of recA430 protein is about 40% of wild-type protein activity and the *in vivo* recombination function, as defined by the recombination index determined from conjugal crosses, is about 30% of the wild-type level. lexA repressor cleavage by recA430 protein is 24% of wild-type protein activity, which also compares favorably with the observation that induction of recA430 protein synthesis *in vivo* is 30% of the wild-type amount.

The ssDNA binding studies provide some insight into the physical basis for the enzymatic defects. In the presence or absence of ADP, the ssDNA binding affinity of recA430 protein is identical with wild-type protein. However, in the presence of rATP or dATP, the apparent steady-state ssDNA binding affinity of recA430 protein is lower than that of wild-type recA protein. This suggests that either the rate of association with or dissociation from ssDNA (or both) is altered in the presence of nucleoside triphosphates. Consistent with this finding is the observation that the rate at which recA430 protein associates with ssDNA is slower than that of wild-type recA protein in the presence of rATP. Substitution of dATP for rATP increases the rate of both wild-type and mutant recA protein association with ssDNA. The association of recA protein with ssDNA has been proposed to occur by a slow nucleation–elongation mechanism (Chabbert *et al.*, 1987). Since the molecular nature of the rate-limiting step for the association of recA protein with ssDNA is not totally clear, the reason for the reduced rate observed with recA430 protein is unknown. However, the finding that this mutant protein shows an altered rate of association with ssDNA that is dependent on the type of nucleoside triphosphate present suggests that this is an important property of the protein; further studies should help to define the molecular events that underlie the association process.

The ssDNA binding properties of recA430 protein are somewhat similar to those of recA142 protein (Kowalczykowski *et al.*, 1989); namely, the apparent affinity in the presence or absence of ADP is the same as wild-type recA protein, but it is reduced (relative to wild-type protein) in the presence of ATP. As discussed previously (Kowalczykowski *et al.*, 1989), this suggests that the low affinity ssDNA binding state of the mutant proteins is unaltered. However, the apparent DNA binding affinity of the high-affinity ssDNA binding state is reduced by the mutation. In agreement, since the transition to the high-affinity state is thermodynamically coupled to

ATP binding, a reduction in apparent ATP affinity (i.e. an increase in the $K_m$) is also observed. The amino acid substitutions are in a similar location (the recA430 protein substitution is at residue 204, Gly→Ser (Kawashima *et al.*, 1984), and recA142 is at residue 225, Ile→Val (Dutreix & Devoret, personal communication)), suggesting that these residues define a region of the protein important to the high-affinity ssDNA binding state. An alteration in the high-affinity ssDNA binding state could result from perturbation of the ATP binding site, the ssDNA binding site of the high-affinity conformer, or the region involved in the conformation change. Further studies are required to distinguish between these possibilities.

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## References

Bochner, B. R. & Ames, B. N. (1982). *J. Biol. Chem.* **257**, 9759–9769.

Chabbert, M., Cazenave, C. & Helene, C. (1987). *Biochemistry*, **26**, 2218–2225.

Chow, S. A., Rao, B. J. & Radding, C. M. (1988). *J. Biol. Chem.* **263**, 200–209.

Clark, A. J. (1973). *Annu. Rev. Genet.* **7**, 67–86.

Clark, A. J. (1982). *Biochimie*, **64**, 669–675.

Clark, A. J., Sandler, S. J., Willis, D. K., Chu, C. C., Blanar, M. A. & Lovett, S. T. (1984). *Cold Spring Harbor Symp. Quant. Biol.* **49**, 453–462.

Cox, M. M. & Lehman, I. R. (1981*a*). *Proc. Nat. Acad. Sci., U.S.A.* **78**, 3433–3437.

Cox, M. M. & Lehman, I. R. (1981*b*). *Proc. Nat. Acad. Sci., U.S.A.* **78**, 6018–6022.

Devoret, R., Pierre, M. & Moreau, P. L. (1983). *Mol. Gen. Genet.* **89**, 199–206.

Eguchi, Y., Ogawa, T. & Ogawa, H. (1988). *J. Mol. Biol.* **202**, 565–573.

Gonda, D. K. & Radding, C. M. (1983). *Cell*, **34**, 647–654.

Griffith, J. & Shores, C. G. (1985). *Biochemistry*, **24**, 158–162.

Kawashima, H., Horii, T., Ogawa, T. & Ogawa, H. (1984). *Mol. Gen. Genet.* **193**, 288–292.

Kowalczykowski, S. C. & Krupp, R. A. (1987). *J. Mol. Biol.* **193**, 97–113.

Kowalczykowski, S. C. & Krupp, R. A. (1989). *J. Mol. Biol.* **207**, 735–747.

Kowalczykowski, S. C., Clow, J. & Krupp, R. A. (1987). *Proc. Nat. Acad. Sci., U.S.A.* **84**, 3127–3131.

Kowalczykowski, S. C., Burk, D. L. & Krupp, R. A. (1989). *J. Mol. Biol.* **207**, 719–733.

Kreuzer, K. N. & Jongeneel, C. V. (1983). In *Methods in Enzymology* (Wu, R., Grossman, L. & Moldave, K., eds), vol. 100, pp. 144–160, Academic Press, New York.

Lavery, P. E. & Kowalczykowski, S. C. (1988). *J. Mol. Biol.* **203**, 861–874.

Lavery, P. E. & Kowalczykowski, S. C. (1990). *J. Biol. Chem.* in the press.

Little, J. W. (1984). *Proc. Nat. Acad. Sci., U.S.A.* **81**, 1375–1379.

Little, J. W., Edmiston, S. H., Pacelli, L. Z. & Mount, D. W. (1980). *Proc. Nat. Acad. Sci., U.S.A.* **77**, 3225–3229.

Lovett, C. M. & Roberts, J. W. (1985). *J. Biol. Chem.* **260**, 3305–3313.

Menetski, J. P. (1988). Ph.D. thesis, Northwestern University.

Menetski, J. P. & Kowalczykowski, S. C. (1985). *J. Mol. Biol.* **181**, 281–295.

Menetski, J. P. & Kowalczykowski, S. C. (1987). *J. Biol. Chem.* **262**, 2085–2092.

Menetski, J. P. & Kowalczykowski, S. C. (1989). *Biochemistry*, **28**, 5871–5881.

Menetski, J. P., Varghese, A. & Kowalczykowski, S. C. (1988). *Biochemistry*, **27**, 1205–1212.

Messing, J. (1983). *Methods Enzymol.* **101**, 20–78.

Morand, P., Blanco, M. & Devoret, R. (1977). *J. Bacteriol.* **131**, 572–582.

Moreau, P. L. & Roberts, J. W. (1984). *Mol. Gen. Genet.* **198**, 25–34.

Muniyappa, K., Shaner, S. L., Tsang, S. S. & Radding, C. M. (1984). *Proc. Nat. Acad. Sci., U.S.A.* **81**, 2757–2761.

Pugh, B. F. & Cox, M. M. (1987). *J. Biol. Chem.* **262**, 1337–1343.

Pugh, B. F. & Cox, M. M. (1988). *J. Mol. Biol.* **203**, 479–493.

Roman, L. J. & Kowalczykowski, S. C. (1986). *Biochemistry*, **25**. 7375–7385.

Roberts, J. W., Phizicky, E. M., Burbee, D. G., Roberts, C. W. & Moreau, P. L. (1982). *Biochimie*, **64**, 805–807.

Schnarr, M., Pouyet, J., Granger-Schnarr, M. & Daune, M. (1985). *Biochemistry*, **24**, 2812–2818.

Uhlin, B. E. & Clark, A. J. (1981). *J. Bacteriol.* **148**, 389–390.

Uhlin, B. E., Schweikart, V. & Clark, A. J. (1983). *Gene*, **22**, 255–265.

Wang, W. B. & Tessman, E. S. (1986). *J. Bacteriol.* **168**, 901–910.

Weinstock, G. M. (1982). *Biochimie*, **64**, 611–617.

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