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Biochemical Basis of the Constitutive Repressor Cleavage Activity of recA730 Protein

A COMPARISON TO recA441 AND recA803 PROTEINS*

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The *recA730* mutation results in constitutive SOS and prophage induction. We examined biochemical properties of recA730 protein in an effort to explain the constitutive activity observed in *recA730* strains. We find that recA730 protein is more proficient than the wild-type recA protein in the competition with single-stranded DNA binding protein (SSB protein) for single-stranded DNA (ssDNA) binding sites. Because an increased aptitude in the competition with SSB protein has been previously reported for recA441 protein and recA803 protein, we directly compared their *in vitro* activities with those of recA730 protein. At low magnesium ion concentration, both ATP hydrolysis and lexA protein cleavage experiments demonstrate that these recA proteins displace SSB protein from ssDNA in a manner consistent with their *in vivo* repressor cleavage activity, *i.e.* recA730 protein > recA441 protein > recA803 protein > recAwt protein. Additionally, a correlation exists between the proficiency of the recA proteins in SSB protein displacement and their rate of association with ssDNA. We propose that an increased rate of association with ssDNA allows recA730 protein to displace SSB protein from the ssDNA that occurs naturally in *Escherichia coli* and thereby to become activated for the repressor cleavage that leads to SOS induction. RecA441 protein is similarly activated for repressor cleavage; however, in this case, significant SSB protein displacement occurs only at elevated temperature. At physiological magnesium ion concentration, we argue that recA803 protein and wild-type recA protein do not displace sufficient SSB protein from ssDNA to constitutively induce the SOS response.

The recA protein of *Escherichia coli* is required for both genetic recombination (for reviews see Cox and Lehman, 1987; Radding, 1988; Kowalczykowski, 1991a) and induction of the unlinked genes of the SOS system (see Little and Mount, 1982; Walker, 1984; Sassanfar and Roberts, 1990). Induction of the SOS system occurs when recA protein stimulates cleavage of the lexA repressor protein in response to treatments that damage DNA or inhibit DNA replication (Witkin, 1976). Lambdoid prophage are similarly induced when recA protein stimulates cleavage of their lytic repressor proteins (Roberts *et al.*, 1978). *In vitro*, recA protein is active in the stimulation of repressor cleavage when bound to ssDNA[1] and ATP or dATP, suggesting that activation *in vivo* results from the binding of recA protein to single-stranded regions produced in damaged DNA (Craig and Roberts, 1980; Phizicky and Roberts, 1981; Sassanfar and Roberts, 1990).

There are mutations in the *recA* gene that affect the regulation of the SOS response (Tessman and Peterson, 1985). Some mutant recA proteins are constitutively activated for repressor cleavage, *i.e.* DNA-damaging treatment is not required for SOS or prophage induction. The recA441 protein exhibits constitutive repressor cleavage activity most notably when the temperature is elevated to 42 °C (Goldthwait and Jacob, 1964; Kirby *et al.*, 1967). Craig and Roberts (1980) demonstrated that, like the wild-type protein, recA441 protein requires ATP and polynucleotide to stimulate repressor cleavage *in vitro*. Phizicky and Roberts (1981) suggested that recA441 protein forms an active complex with ssDNA and NTP more efficiently than recAwt protein and, as a result, can utilize polynucleotide present in undamaged cells for the stimulation of repressor cleavage. In a previous report (Lavery and Kowalczykowski, 1988), we compared biochemical properties of recA441 protein with those of recAwt protein in an effort to explain the constitutive SOS induction observed in *recA441* strains. We found that recA441 protein is more proficient than recAwt protein in the competition with SSB protein for sites on ssDNA, particularly at low magnesium ion concentration and elevated temperature. This enhanced ability could not be attributed to an increased ssDNA binding affinity, leading us to conclude that kinetic properties of recA protein association with ssDNA were responsible for the recA441 protein advantage. We proposed that the constitutive repressor cleavage activity of recA441 protein results from an enhanced ability to displace SSB protein from the limited ssDNA that naturally occurs in *E. coli* and thereby become activated for repressor cleavage.

The *recA730* allele was derived from *recA441* through conjugation experiments designed to transfer the *recA441* gene from *E. coli* K12 into a B/r derivative (Witkin *et al.*, 1982). Unlike recA441 protein, the recA730 protein displays constitutive repressor cleavage activity at all temperatures examined. In this study, we compare the *in vitro* properties of

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[1] The abbreviations used are: ssDNA, single-stranded DNA; dsDNA, double-stranded DNA; etheno M13 DNA, M13 DNA containing 1,N^6-ethenoadenosine and 3,N^4-ethenocytidine residues; poly(dT), polydeoxythymidilic acid; SSB protein, *E. coli* single-stranded DNA-binding protein.

recA730 protein with those of recAwt protein. We find that, similar to recA441 protein, recA730 protein is more proficient than recAwt protein in the competition with SSB protein for ssDNA binding sites and argue that this is the basis for the constitutive SOS induction observed in *recA730* strains.

An increased ability to compete with SSB protein seems to be a common theme for mutant recA proteins that exhibit enhanced activity *in vivo*. The recA803 protein, a partial suppressor of the recombination deficiency in *recF* mutant strains (Madiraju *et al.*, 1988), also demonstrates increased proficiency in this competition.[2] However, in contrast to *recA441* and *recA730* strains, constitutive SOS induction is not observed in *recA803* strains (Madiraju *et al.*, 1988). This appears to contradict our hypothesis that constitutive repressor cleavage activity by mutant recA proteins results from an increased ability to displace SSB protein from the ssDNA that occurs naturally in *E. coli* (see Lavery and Kowalczykowski, 1988). In order to address this issue, we directly compared the *in vitro* properties of these three mutant recA proteins. At physiological magnesium ion concentration, we find the recA proteins to displace SSB protein from ssDNA in a manner consistent with their *in vivo* repressor cleavage activity, *i.e.* recA730 protein > recA441 protein > recA803 protein > recAwt protein. We therefore elaborate on our previous hypothesis and propose that a threshold degree of SSB protein displacement is required by recA protein in order for constitutive SOS induction to be observed.

MATERIALS AND METHODS

Chemicals and Buffers—All chemicals were reagent grade, and solutions were made using glass-distilled water. ATP and ADP were purchased from Boehringer Mannheim and dissolved as concentrated stocks at pH 7.5. Unless otherwise indicated, reactions were performed in TD buffer (25 mM Tris acetate and 0.1 mM dithiothreitol) and contained magnesium acetate as indicated.

Proteins—Wild-type recA protein was purified from strain JC122772 (Uhlin and Clark, 1981), recA730 protein was purified from strain SC30-SP51 (Garvey *et al.*, 1985), and recA441 protein was purified from strain BEU397 (see Lavery and Kowalczykowski, 1988). Wild-type recA protein and recA730 protein were purified using a preparative procedure[3] based on spermidine precipitation (Griffith and Shores, 1985). RecA441 protein was purified using the procedure described by Cox *et al.* (1981). RecA803 protein was provided by Dr. Madiraju and Dr. Clark of The University of California, Berkeley and further purified by DNA cellulose column chromatography. SSB protein was purified from strain RLM727 as described (LeBowitz, 1985). Protein concentrations were determined using molar extinction coefficients of 2.7×10^4 M^{-1} cm^{-1} for recA protein and 3×10^4 M^{-1} cm^{-1} for SSB protein (Ruyechan and Wetmur, 1976), both at 280 nm. LexA protein was purified from strain JL652 (Little, 1984) using the preparative procedure described by Schnarr *et al.* (1985). Protein concentration was determined using a molar extinction coefficient of 7300 M^{-1} cm^{-1} at 280 nm.

Lactate dehydrogenase and pyruvate kinase were purchased from Sigma as ammonium sulfate suspensions. Working solutions of these enzymes were made by centrifuging a homogeneous sample of the suspension and dissolving the protein pellet in reaction buffer.

DNA—Single- and double-stranded DNA were purified from bacteriophage M13 mp7 using the procedures described by Messing (1983); the duplex DNA was linearized by digestion with *Eco*RI restriction endonuclease. Molar nucleotide concentrations were determined using extinction coefficients of 8780 M^{-1} cm^{-1} for ssDNA and 6500 M^{-1} cm^{-1} for dsDNA, both at 260 nm. Etheno M13 DNA was prepared from the phage DNA as described (Menetski and Kowalczykowski, 1985); its concentration was determined using an extinction coefficient of 7000 M^{-1} cm^{-1} at 260 nm. Poly(dT) was purchased from P-L Biochemicals and dissolved as a concentrated stock in TE buffer (10 mM Tris-HCl and 1 mM EDTA, pH 7.5); molar nucleotide concentration was determined using an extinction coefficient of 8540 M^{-1} cm^{-1} at 259 nm.

ATPase Assay—The hydrolysis of ATP was monitored using a spectrophotometric assay that couples the production of ADP to the oxidation of NADH (Kreuzer and Jongeneel, 1983; see Kowalczykowski and Krupp, 1987). Reactions were performed in TD buffer containing magnesium acetate, recA protein, and ssDNA as indicated, 0.2 mg NADH/ml, 1 mM ATP, 1.5 mM phosphoenolpyruvate, and 30 units/ml each of pyruvate kinase and lactate dehydrogenase.

LexA Protein Cleavage Assay—The cleavage of lexA protein was determined using the SDS-polyacrylamide gel assay described previously (Little *et al.*, 1980; Lavery and Kowalczykowski, 1988). Extent of lexA protein cleavage was determined spectrophotometrically using a Zeineh Soft Laser scanning densitometer with a tungsten lamp and 595-nm filter. The percentage of intact lexA protein present at each time point was computed using a Hewlett-Packard 3390A integrator. The ratio of the area of the intact lexA protein peak to the sum of the areas of the three peaks corresponding to intact lexA protein and the two lexA protein fragments was determined.

Reactions were performed in TD buffer containing magnesium acetate, NaCl, recA protein and DNA as indicated, 15 μM lexA protein, 1 mM ATP, 1.5 mM phosphoenolpyruvate, and 12.5 units of pyruvate kinase/ml.

DNA Binding Assays—The binding of recA protein to etheno M13 DNA was monitored as described (Menetski and Kowalczykowski, 1985; Menetski *et al.*, 1988). RecA protein titrations of etheno M13 DNA were performed at 25 °C by adding aliquots of recA protein stock to 3 μM etheno M13 DNA in buffer (25 mM Tris-HCl, 10 mM $MgCl_2$, and 0.1 mM dithiothreitol). Salt titrations were carried out at 25 °C by adding aliquots of concentrated NaCl to complexes of recA protein and etheno M13 DNA in buffer (25 mM Tris-HCl, 10 mM $MgCl_2$, 0.1 mM dithiothreitol, and ATP or ADP as indicated). An ATP regenerating system consisting of 1.5 mM phosphoenolpyruvate and 8 units of pyruvate kinase/ml was present in reactions containing ATP.

Measurement of SSB Protein Displacement—The kinetics of SSB protein dissociation from ssDNA were monitored by measuring SSB protein fluorescence at 340 nm (see Kowalczykowski *et al.*, 1987). SSB protein was added to TD buffer containing 10 mM magnesium acetate, 1 mM ATP, 1.5 mM phosphoenolpyruvate, and 15 units of pyruvate kinase/ml; the fluorescence of SSB protein (excitation = 290 nm; emission = 340 nm) was recorded. Then ssDNA was added, and the quenching of SSB protein fluorescence due to binding to ssDNA was measured. RecA protein was added and the increase in SSB protein fluorescence due to displacement from ssDNA was recorded over time. The fraction of SSB protein displaced was determined by comparing the observed fluorescence change due to displacement with the previously measured quenching of SSB protein fluorescence by ssDNA addition.

Association Experiments—The rate of recA protein association with ssDNA was determined using etheno M13 DNA to monitor binding (Menetski and Kowalczykowski, 1990). Reactions were performed in 25 mM Tris-HCl, 0.1 mM dithiothreitol, 150 mM NaCl, 1 mM ATP, and $MgCl_2$ as indicated. RecA protein was added to etheno M13 DNA equilibrated in buffer; the increase in etheno M13 DNA fluorescence due to recA protein binding was monitored.

The experimental data were analyzed by the nonlinear least squares routine in the Data Evaluation System 80 program (DAES80), from R and L Software. This program was used to fit the data to a single exponential equation plus a variable endpoint of the form

$$F = Ae^{-kt} + B$$

where F is the fluorescence signal at time t, A is the amplitude of the fluorescence change due to recA protein binding, k is the observed first order rate constant, and B is the endpoint.

RESULTS

RecA Protein Concentration Dependence of ssDNA-dependent ATPase Activity—The binding of recA protein to ssDNA and ATP yields a ternary complex that is capable of stimulating repressor cleavage; since a consequence of this binding is the hydrolysis of ATP, the formation of this complex is easily monitored by measuring ATPase activity. A comparison of ATP hydrolysis by recA730 protein and recAwt protein in the presence of etheno M13 DNA, which cannot form second-

[2] Madiraju, M. V. V. S., Lavery, P. E., Kowalczykowski, S. C., and Clark, A. J. (1993) *Biochemistry*, in press.

[3] S. C. Kowalczykowski, manuscript in preparation.

ary structures due to modification of cytosine and adenine residues, is shown in Fig. 1. The observed rate of ssDNA-dependent ATP hydrolysis increases with increasing recA protein concentration until the available DNA is saturated and then becomes independent of recA protein concentration. The recA protein concentration dependence curves demonstrate the same DNA binding stoichiometry for the wild-type and recA730 proteins (an apparent site size of about 3.1 nucleotides/recA protein monomer). At both low and high recA protein concentrations, the observed rates of ATP hydrolysis by recA730 protein and recAwt protein are the same, suggesting that the k_{cat} values for ATP hydrolysis are the same for these two recA proteins (37 μM ATP/min). At recA protein concentrations around 1.0 μM, ATP hydrolysis by recA730 protein is greater than by recAwt protein. This suggests that, whereas the k_{cat} values for ATP hydrolysis are the same for these two recA proteins, recAwt protein has difficulty saturating the DNA.

A comparison of the recA protein concentration dependence of M13 ssDNA-dependent ATPase activity is also consistent with this conclusion (Fig. 2). At all recA protein concentrations examined in the absence of SSB protein, the observed rate of ATP hydrolysis by recA730 protein is greater than by recAwt protein (Fig. 2, *open symbols*). This indicates that more of the M13 ssDNA is available to recA730 protein than to recAwt protein and suggests that recA730 protein is more proficient at binding to ssDNA containing secondary structures.

In the presence of SSB protein, again, ATP hydrolysis by recA730 protein is greater than by recAwt protein (Fig. 2, *filled symbols*). This suggests that recA730 protein is more proficient than recAwt protein in the competition with SSB protein for ssDNA binding sites. A close examination of the recA protein concentration dependence curves indicates that the rate of ATP hydrolysis by recAwt protein is gradually increasing at recA protein concentrations above 1.0 μM and is approaching that observed for recA730 protein. Thus, higher recA protein concentrations appear to be required in order for recAwt protein to compete as effectively with SSB protein as recA730 protein does.

Effect of Magnesium Ion Concentration on the Competition between recA Protein and SSB Protein for ssDNA—The effect of SSB protein on recAwt protein-ssDNA complexes is strongly dependent on magnesium ion concentration; at 1 mM magnesium acetate, SSB protein displaces recA protein from M13 ssDNA (Kowalczykowski *et al.*, 1987), resulting in an inhibition of ATPase activity, rather than the stimulation observed at 10 mM magnesium acetate (Kowalczykowski and Krupp, 1987). In contrast, the M13 ssDNA-dependent ATPase activity of recA441 protein (Lavery and Kowalczykowski, 1988) and recA803 protein[2] is not inhibited by SSB protein at 1 mM magnesium ion, indicating that these recA proteins resist being displaced from ssDNA. To determine if recA730 protein is similarly resistant to displacement by SSB protein, we examined M13 ssDNA-dependent ATPase activity under various conditions. The results in Table I show that, like recA441 protein and recA803 protein, M13 ssDNA-dependent ATP hydrolysis by recA730 protein is not inhibited by SSB protein at 1 mM magnesium acetate. Since the k_{cat} values for ATP hydrolysis appear to be the same for all of these recA proteins (Lavery and Kowalczykowski, 1988; this study),[2] the differences in rates of ATP hydrolysis shown in Table I must indicate differences in the amount of recA protein bound to ssDNA under each of the conditions examined.

In contrast to native M13 ssDNA, when etheno M13 DNA

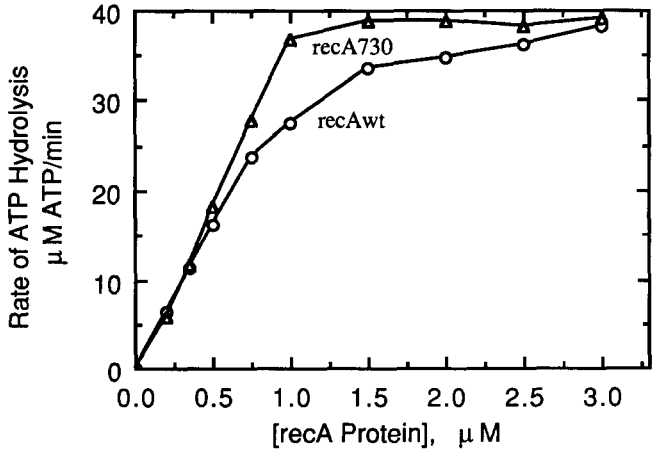

FIG. 1. **RecA protein concentration dependence of etheno M13 DNA-dependent ATPase activity.** The rate of ATP hydrolysis (micromolar ATP/min) was measured as described under "Materials and Methods" at 10 mM magnesium acetate and 37 °C. Reactions contained 3.3 μM etheno M13 DNA and recA protein as indicated. *Circles* indicate reactions containing recAwt protein; *triangles* indicate reactions containing recA730 protein.

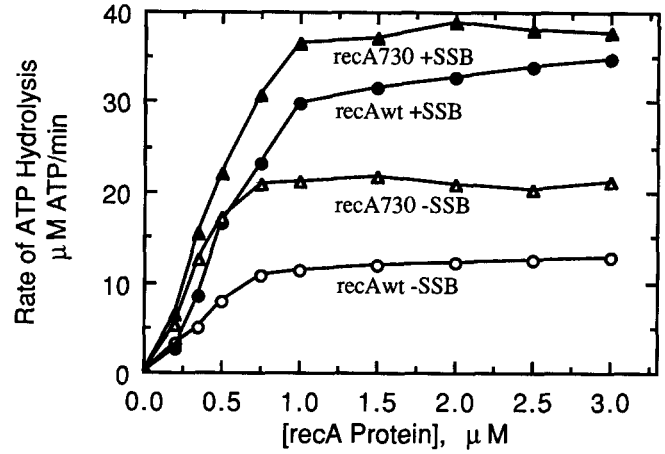

FIG. 2. **RecA protein concentration dependence of M13 ssDNA-dependent ATPase activity.** The rate of ATP hydrolysis (micromolar ATP/min) was measured as described under "Materials and Methods" at 10 mM magnesium acetate and 37 °C. Reactions contained 3.3 μM M13 ssDNA, 0.6 μM SSB protein where indicated, and recA protein as indicated. *Circles* indicate reactions containing recAwt protein; *triangles* indicate reactions containing recA730 protein. *Open symbols* indicate reactions performed in the absence of SSB protein; *filled symbols* indicate reactions performed in the presence of SSB protein. SSB protein, when present, was added after recA protein had attained a steady-state rate of ATP hydrolysis.

TABLE I

ssDNA-dependent ATPase activity in the presence of SSB protein

The rate of ATP hydrolysis (micromolar ATP/min) was determined as described under "Materials and Methods"; reactions contained magnesium acetate as indicated, 3 μM M13 ssDNA, 2 μM recA protein, and 0.6 μM SSB protein. SSB protein was added after recA protein had attained a steady-state rate of ATP hydrolysis.

Protein	Rate of ATP hydrolysis					
	1 mM magnesium ion			10 mM magnesium ion		
	32 °C	37 °C	42 °C	32 °C	37 °C	42 °C
	μM ATP/min			*μM ATP/min*		
recAwt	<0.5	<0.5	<0.5	18.9	28.7	38.9
recA730	25.0	36.3	43.0	25.8	33.3	44.4
recA441	23.0	34.5	43.2	10.2	27.2	37.5
recA803	12.4	20.9	34.1	21.6	27.4	38.8

or poly(dT) is used as polynucleotide cofactor, the ATPase activity of recAwt protein is inhibited by the addition of SSB protein at all magnesium ion concentrations examined (Kowalczykowski and Krupp, 1987). However, there is no effect of SSB protein addition on etheno M13 DNA- or poly(dT)-dependent ATP hydrolysis by recA730 protein (data not shown). Thus, SSB protein does not displace recA730 protein from any polynucleotide examined. This sets recA730 protein apart from recA441 protein and recA803 protein; the latter two mutant proteins are resistant to displacement from M13 ssDNA by SSB protein at 1 mM magnesium ion (see Table I) but are displaced from etheno M13 DNA and poly(dT), albeit at slower rates than recAwt protein (Lavery and Kowalczykowski, 1988).[2]

ATP Hydrolysis in Reactions where ssDNA Is Precoated with SSB Protein—The outcome of the competition between recA protein and SSB protein for ssDNA binding sites is dependent on the order in which the two proteins are added to DNA (Kowalczykowski and Krupp, 1987). When ssDNA is precoated with SSB protein, it acts as an obstacle to recA protein binding, and a lag is observed prior to ATP hydrolysis; the length of the lag varies with experimental conditions and is assumed to parallel the kinetics of SSB protein displacement by recA protein (Lavery and Kowalczykowski, 1988). Figs. 3*A* and 4*A* compare the rates of SSB protein displacement by recAwt protein, recA730 protein, recA441 protein, and recA803 protein deduced from examination of ATPase activity (the inverse of the lag time prior to steady-state ATP hydrolysis is reported). Figs. 3B and 4B compare the steady-state rate of ATP hydrolysis following the lag for these recA proteins; this rate is indicative of how much recA protein is bound to the ssDNA (*i.e.* how much SSB protein has been displaced).

At both 1 and 10 mM magnesium acetate, recA730 protein displaces SSB protein from ssDNA more quickly and more completely than the other recA proteins, regardless of temperature (Figs. 3 and 4). At 42 °C, the rate of ATP hydrolysis following the lag is equal to that observed when SSB protein is added to the DNA after recA protein (see Table I), suggesting that recA730 protein can displace all of the SSB protein from ssDNA under these conditions.

At 1 mM magnesium acetate, recAwt protein is unable to displace SSB protein from ssDNA, regardless of temperature (Fig. 3). Under these conditions, both recA803 protein and recA441 protein are able to displace some SSB protein from the DNA. However, the data indicate that recA441 protein displaces SSB protein from ssDNA more quickly and more completely than recA803 protein, especially at elevated temperature. At 1 mM magnesium acetate, the recA proteins can be ranked by their abilities to displace SSB protein as follows: recA730 protein > recA441 protein > recA803 protein > recAwt protein (Fig. 3).

At 10 mM magnesium acetate, recAwt protein can displace some SSB protein from ssDNA (Fig. 4). Under these conditions, the data indicate that recA803 protein displaces SSB protein from ssDNA more quickly and more completely than recA441 protein, especially at lower temperature. At 10 mM magnesium acetate, the recA proteins can be ranked by their abilities to displace SSB protein as follows: recA730 protein > recA803 protein > recA441 protein > recAwt protein (Fig. 4).

Direct Measurements of recA Protein Displacement of SSB Protein from ssDNA—The preceding assumes that ATPase activity parallels the displacement of SSB protein from ssDNA by recA protein. In order to test the validity of this assumption, we directly examined this displacement by monitoring SSB protein fluorescence after recA protein addition; the dissociation of SSB protein from ssDNA is accompanied by an increase in its fluorescence. The results in Fig. 5 show that, for both recAwt protein and recA730 protein, the increase in fluorescence that signifies dissociation of SSB protein from M13 ssDNA precedes the observation of ATP hydrolysis, *i.e.* a significant amount of SSB protein is displaced from the DNA before the hydrolysis of ATP is observed. This may reflect a requirement for relatively large clusters of recA protein molecules to be formed on the ssDNA before ATP hydrolysis can occur. Although ATP hydrolysis lags behind SSB protein dissociation, the time-dependent increase in the observed rate of ATP hydrolysis parallels the increase in fluorescence, indicating SSB protein dissociation. Additionally, the extent of SSB protein displacement deduced from an examination of ATP hydrolysis agrees with that determined from fluorescence measurements (Fig. 5).

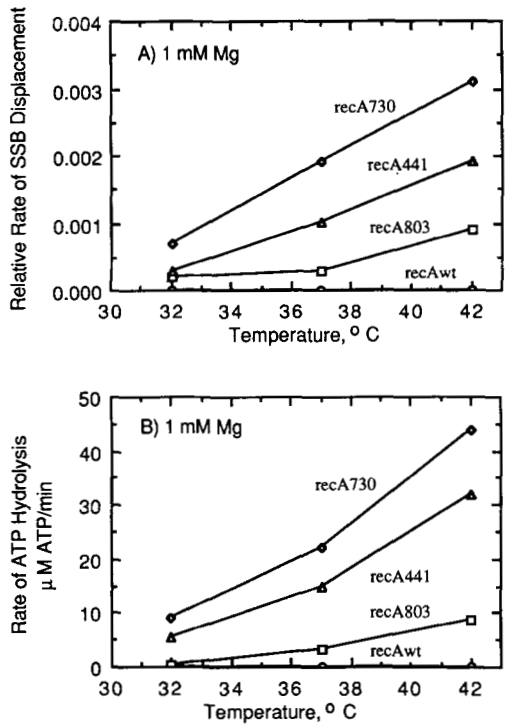

FIG. 3. **Temperature dependence of SSB protein displacement at 1 mM magnesium acetate.** ATP hydrolysis was measured as described under "Materials and Methods" at 1 mM magnesium acetate. Reactions contained 3 μM M13 ssDNA, 0.6 μM SSB protein, and 2 μM recA protein; the SSB protein was added to ssDNA prior to the addition of recA protein. *A* indicates relative rates of SSB protein displacement; the inverse of the lag times (in seconds) prior to steady-state ATP hydrolysis are reported. *B* reports the rate of ATP hydrolysis (micromolar ATP/min) following the lag phase. *Circles* indicate recAwt protein; *diamonds* indicate recA730 protein; *triangles* indicate recA441 protein; *squares* indicate recA803 protein.

Experiments comparing the activity of recA730 protein in the displacement of SSB protein from M13 ssDNA and etheno M13 DNA give similar results (Fig. 6). The increase in fluorescence that signifies dissociation of SSB protein from both M13 ssDNA and etheno M13 DNA precedes the observation of ATP hydrolysis by recA730 protein. Once again, the time-dependent increase in the observed rate of ATP hydrolysis parallels the increase in fluorescence, indicating SSB protein dissociation, and the extent of SSB protein displacement deduced from ATPase activity agrees with that determined from fluorescence measurements.

Stimulation of lexA Repressor Protein Cleavage by recA

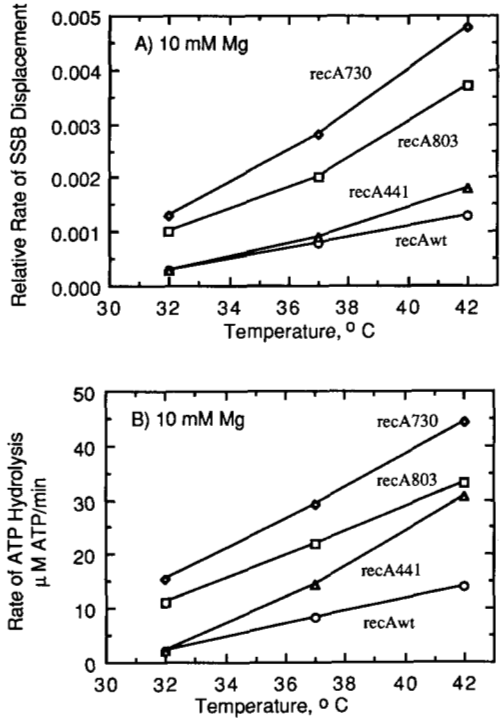

FIG. 4. **Temperature dependence of SSB protein displacement at 10 mM magnesium acetate.** ATP hydrolysis was measured as described under "Materials and Methods" at 10 mM magnesium acetate. Reactions contained 3 μM M13 ssDNA, 0.6 μM SSB protein, and 2 μM recA protein; the SSB protein was added to ssDNA prior to the addition of recA protein. *A* indicates relative rates of SSB protein displacement; the inverse of the lag times (in seconds) prior to steady-state ATP hydrolysis is reported. *B* reports the rate of ATP hydrolysis (micromolar ATP/min) following the lag phase. *Circles* indicate recAwt protein; *diamonds* indicate recA730 protein; *triangles* indicate recA441 protein; *squares* indicate recA803 protein.

Protein—Since the ternary complex of recA protein, ssDNA, and ATP required for ATP hydrolysis is the same complex that is necessary for the stimulation of repressor cleavage (Craig and Roberts, 1980), we expected the behavior of the wild-type and mutant recA proteins in lexA protein cleavage to parallel that observed in ATPase activity. This reasoning requires the k_{cat} values for repressor cleavage by each of these recA proteins to be the same. Consistent with reports that excess ssDNA is inhibitory to the cleavage of repressors (Craig and Roberts, 1980; Takahashi and Schnarr, 1989), an examination of ssDNA-dependent lexA protein cleavage using limiting recA protein concentrations was not useful to compare the k_{cat} values for lexA protein cleavage by the wild-type and mutant recA proteins (data not shown). Therefore, we examined lexA protein cleavage using a molar excess of recA protein relative to ssDNA (3 μM recA protein and 3 μM etheno M13 DNA). Under these conditions, the observed rates of lexA protein cleavage by recAwt protein, recA730 protein, recA441 protein, and recA803 protein were within 6% of one another (data not shown), demonstrating comparable k_{cat} values for lexA protein cleavage by these recA proteins.

When M13 ssDNA is employed as the polynucleotide cofactor in the absence of SSB protein, the observed rate of lexA protein cleavage by all of the recA proteins is greater at 1 mM magnesium acetate than at 10 mM magnesium acetate (Fig. 7*A*). This is consistent with the stability of secondary structures in ssDNA being less at 1 mM magnesium acetate, allowing more recA protein to bind the DNA and be active in lexA protein cleavage. At both magnesium ion concentrations, the rate of lexA protein cleavage is greatest by recA730 protein, least by recAwt protein, and intermediate by recA441 protein and recA803 protein.

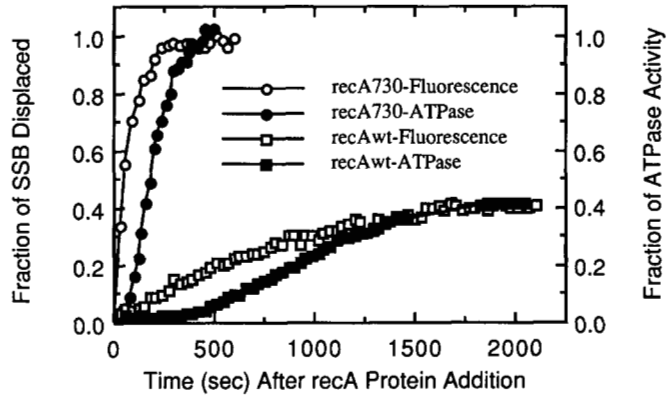

FIG. 5. **Direct measurement of SSB protein displacement from M13 ssDNA compared with ATPase activity.** Displacement of SSB protein by recA protein was measured fluorimetrically as described under "Materials and Methods" at 10 mM magnesium acetate and 42 °C. The fraction of SSB protein displaced from ssDNA was determined by dividing the fluorescence reading at each time point by the value for SSB protein fluorescence quenching observed upon ssDNA addition. The rate of ATP hydrolysis (micromolar ATP/min) was measured as described under "Materials and Methods" at 10 mM magnesium acetate. The fraction of recovered ATPase activity was determined by dividing the rate of ATP hydrolysis at each time point by the rate of ATP hydrolysis observed in reactions where SSB protein is added to ssDNA after recA protein. Reactions contained 3 μM M13 ssDNA, 0.3 μM SSB protein, and 1.5 μM recA protein; the SSB protein was added to ssDNA prior to the addition of recA protein. *Squares* indicate reactions performed with recAwt protein; *circles* indicate reactions performed with recA730 protein. *Open symbols* indicate the fraction of SSB protein displaced determined from fluorescence measurements; *filled symbols* indicate the fraction of recovered ATPase activity.

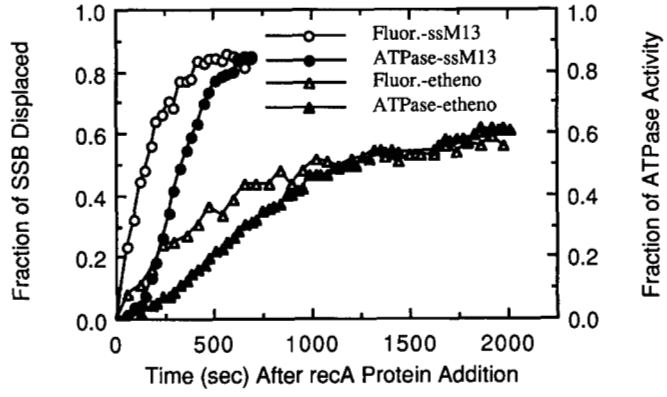

FIG. 6. **Direct measurements of SSB protein displacement from etheno M13 DNA and M13 ssDNA compared with ATPase activity.** Reactions were performed at 37 °C as described in Fig. 5 and contained 0.3 μM SSB protein, 1.5 μM recA730 protein, and 3 μM etheno M13 DNA or M13 ssDNA, as indicated. *Triangles* indicate reactions performed with etheno M13 DNA; *circles* indicate reactions performed with M13 ssDNA. *Open symbols* indicate the fraction of SSB protein displaced determined from fluorescence measurements; *filled symbols* indicate the fraction of recovered ATPase activity.

In order to compare the amount of ternary complex present during these lexA protein cleavage reactions, we examined ssDNA-dependent ATPase activity in the presence of lexA protein (Fig. 7*B*). Under all conditions examined, the observed rate of ATP hydrolysis parallels the observed rate of lexA protein cleavage (Fig. 7). This demonstrates that differences in the observed rates of M13 ssDNA-dependent lexA protein cleavage are the result of differences in the amount

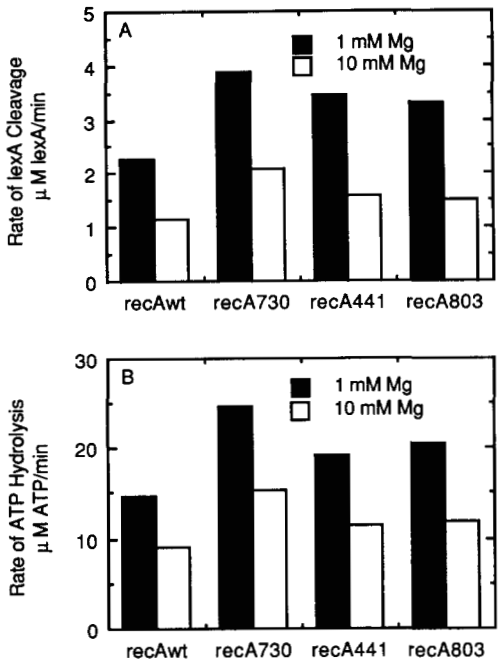

FIG. 7. **LexA protein cleavage *versus* ATPase activity.** The maximum rate of lexA protein cleavage was determined from a reaction time course of the gel assay described under "Materials and Methods." The rate of ATP hydrolysis was measured as described under "Materials and Methods." Reactions were performed at 37 °C and contained 50 mM NaCl, magnesium acetate as indicated, 3 μM M13 ssDNA, 2 μM recA protein, and 15 μM lexA protein added to start the cleavage reaction. *A* indicates rates of lexA protein cleavage (micromolar lexA protein/min); *B* indicates rates of ATP hydrolysis (micromolar ATP/min). *Filled bars* indicate reactions performed at 1 mM magnesium acetate; *open bars* indicate reactions performed at 10 mM magnesium acetate.

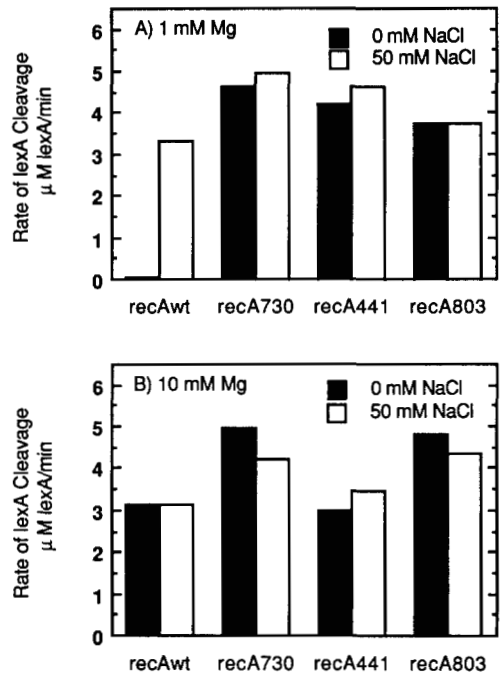

FIG. 8. **LexA protein cleavage in the presence of SSB protein.** The maximum rate of lexA protein cleavage was determined at 37 °C from a reaction time course of the gel assay described under "Materials and Methods." Reactions contained magnesium acetate and NaCl, as indicated, 3 μM M13 ssDNA, 0.6 μM SSB protein, 2 μM recA protein, and 15 μM lexA protein. RecA protein and ssDNA were incubated in reaction buffer at 37 °C for 2 min; SSB protein was then added, and the mixture was incubated at 37 °C for 5 min; lexA protein was then added to start the cleavage reaction. *A* indicates reactions performed at 1 mM magnesium acetate; *B* indicates reactions performed at 10 mM magnesium acetate. *Filled bars* indicate reactions performed at 0 mM NaCl; *open bars* indicate reactions performed at 50 mM NaCl.

of ternary complex present, rather than differences in the intrinsic abilities of the recA proteins to stimulate cleavage of lexA protein. RecA730 protein forms the greatest amount of ternary complex and accordingly stimulates cleavage of lexA protein at the greatest rate. RecAwt protein forms the least amount of ternary complex, whereas recA441 protein and recA803 protein form intermediate amounts.

LexA Protein Cleavage in the Presence of SSB Protein—In the presence of SSB protein, the results obtained from lexA protein cleavage reactions (Fig. 8) parallel those obtained from ATPase activity (see Table I). At 1 mM magnesium acetate, recA730 protein and recA441 protein are more proficient than recA803 protein and recAwt protein in lexA protein cleavage, whereas at 10 mM magnesium acetate, recA730 protein and recA803 protein are more proficient than recA441 and recAwt protein. Thus, the data confirm our conclusion that differences in the observed rates of repressor cleavage are the result of differences in the abilities of the mutant recA proteins to bind ssDNA and form the ternary complex active in repressor cleavage.

Our examination of lexA protein cleavage revealed an unanticipated effect of NaCl on the ability of recAwt protein to cleave lexA protein in the presence of SSB protein. Somewhat surprisingly, when cleavage reactions are performed in the presence of SSB protein at 1 mM magnesium acetate, recAwt protein is able to stimulate cleavage of lexA protein at 50 mM NaCl, but not at 0 mM NaCl (Fig. 8*A*); this difference is not observed at 10 mM magnesium acetate (Fig. 8*B*). Similarly, the addition of NaCl has virtually no effect on the observed rate of lexA protein cleavage by the recA730, recA441, and recA803 proteins, regardless of magnesium ion concentration (Fig. 8). An examination of M13 ssDNA-dependent ATPase activity gave comparable results (data not shown). Thus, the inability of recAwt protein to compete with SSB protein for ssDNA binding sites at 1 mM magnesium acetate is overcome by the addition of 50 mM NaCl. When NaCl was omitted from reactions performed in the absence of SSB protein, the observed rates of lexA protein cleavage were essentially the same as when NaCl was included (data not shown).

LexA Protein Cleavage in Reactions Where ssDNA Is Precoated with SSB Protein—We have previously argued that the constitutive repressor cleavage activity of recA441 protein can be attributed to its ability to be activated for cleavage by displacing SSB protein from ssDNA (Lavery and Kowalczykowski, 1988). To determine if the repressor cleavage properties of recA730 protein and recA803 protein fit this hypothesis, we compared the abilities of these recA proteins in lexA protein cleavage reactions where M13 ssDNA is precoated with SSB protein. At 32 °C and 1 mM magnesium acetate, there is no observed cleavage of lexA protein by recAwt protein, recA441 protein, or recA803 protein within 60 min; there is some cleavage by recA730 protein under these conditions (Fig. 9, *A1*). At 10 mM magnesium acetate, both recA730 protein and recA803 protein are able to cleave lexA protein, with cleavage being more rapid by recA730 protein; no cleavage of lexA protein by either recAwt protein or recA441 protein is observed under these conditions (Fig. 9, *A2*).

As temperature increases, considerably more cleavage of lexA protein is observed (Fig. 9). At 37 °C, recA730 protein is able to cleave lexA protein more effectively than the other

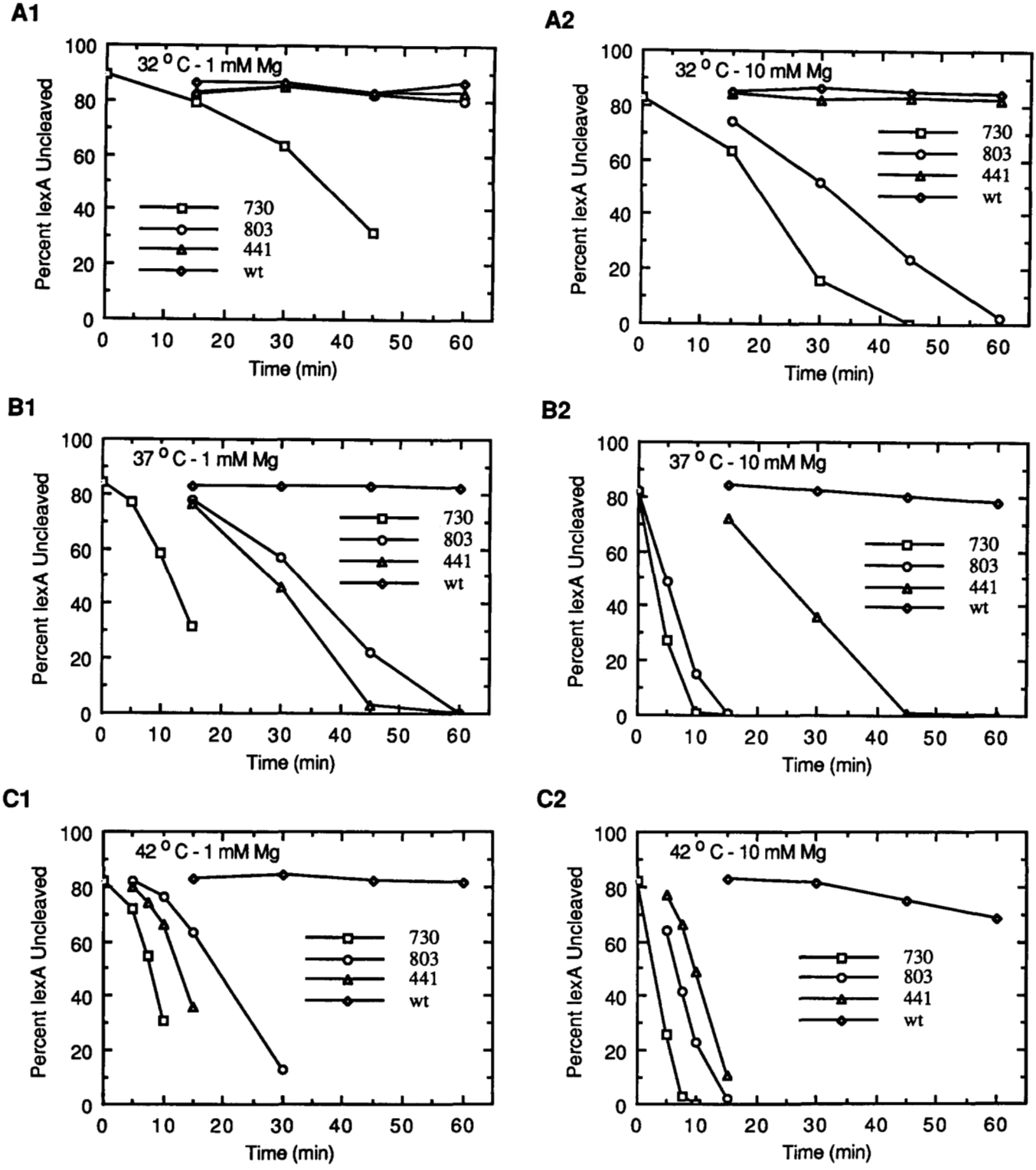

FIG. 9. **LexA protein cleavage in reactions where ssDNA is precoated with SSB protein.** Reactions were performed in TD buffer containing magnesium acetate as indicated, 3 μM M13 ssDNA, 15 μM lexA protein, 0.6 μM SSB protein, and 2 μM recA protein. LexA protein and SSB protein were added to ssDNA in reaction buffer; recA protein was added to start the cleavage reaction. *A*, *B*, and *C* indicate reactions performed at 32, 37, and 42 °C, respectively. *A1*, *B1*, and *C1* indicate reactions performed at 1 mM magnesium acetate, whereas *A2*, *B2*, and *C2* indicate reactions performed at 10 mM magnesium acetate. *Squares* indicate reactions containing recA730 protein; *circles* indicate reactions containing recA803 protein; *triangles* indicate reactions containing recA441 protein; and *diamonds* indicate reactions containing recAwt protein.

recA proteins (Fig. 9*B*). At 1 mM magnesium acetate, cleavage by recA441 protein is only slightly more rapid than by recA803 protein (Fig. 9, *B1*), whereas at 10 mM magnesium acetate, cleavage by recA803 protein is significantly more rapid than by recA441 protein (Fig. 9, *B2*).

At 42 °C, recA730 protein is again more proficient at lexA protein cleavage than the other recA proteins (Fig. 9*C*). Compared with recA803 protein, the ability of recA441 protein in lexA protein cleavage is improved more dramatically by increasing the temperature to 42 °C; at 1 mM magnesium acetate, cleavage by recA441 protein is significantly faster than by recA803 protein (Fig. 9, *C1*), whereas at 10 mM magnesium acetate, cleavage by recA441 protein is only slightly slower than by recA803 protein (Fig. 9, *C2*).

The rankings of the recA proteins deduced from this examination of lexA protein cleavage are consistent those deduced from our examination of ATPase activity (see Figs. 3 and 4), *i.e.* at 1 mM magnesium acetate, recA730 protein > recA441 protein > recA803 protein > recAwt protein and at 10 mM magnesium acetate, recA730 protein > recA803 protein > recA441 protein > recAwt protein. Thus, as expected, the behavior of the wild-type and mutant recA proteins in lexA protein cleavage parallels that observed in ssDNA-dependent ATPase activity.

LexA Protein Cleavage in the Presence of dsDNA—Lu and Echols (1987) reported lexA protein cleavage by recA441 protein in the presence of dsDNA and argued that this is significant to the constitutive SOS induction observed in *recA441* strains. To address this hypothesis, we compared the ability of dsDNA to act as a cofactor in the stimulation of lexA protein cleavage by recAwt protein, recA730 protein, recA441 protein, and recA803 protein. At 37 °C, at both 1 and 10 mM magnesium acetate, no cleavage of lexA protein was observed within 60 min by recAwt protein in the presence of dsDNA (data not shown). Very slow cleavage of lexA protein was observed by recA730 protein, recA441 protein, and recA803 protein under these conditions (<5% of the rate observed in the presence of ssDNA); the rate of lexA protein cleavage was virtually the same for each of these recA proteins and was independent of magnesium ion concentration (data not shown).

The Binding of recA730 Protein to Etheno M13 DNA—To determine if the enhanced activities of recA730 protein could be attributed to differences in ssDNA binding characteristics, we compared the binding of recA730 protein and recAwt protein with etheno M13 DNA, a modified ssDNA whose fluorescence increases upon recA protein binding. RecA protein titrations of etheno M13 DNA indicated that recA730 protein and recAwt protein bind ssDNA with virtually the same stoichiometry (data not shown). Since the salt titration midpoint (defined as the concentration of NaCl required to dissociate one-half of the recA protein-DNA complex present) is related to the affinity of a protein for ssDNA (Menetski and Kowalczykowski, 1985), we performed salt titrations of recA protein-etheno M13 DNA complexes. Table II compares salt titration midpoint values for recA730 protein and recAwt protein. In the absence of nucleotide cofactor and in the presence of ADP, the salt titration midpoint values are essentially the same for the two recA proteins; in the presence of ATP, the salt titration midpoint value for recA730 protein is actually a bit lower than for recAwt protein. Thus, the enhanced properties of recA730 protein cannot be attributed to an increased affinity for ssDNA.

The Kinetics of Wild-type and Mutant recA Protein Association with Etheno M13 DNA—Previous examination of mutant recA proteins suggested that the ability to compete with SSB protein for binding sites is related to the association rate of recA protein with ssDNA; a decreased rate of association was correlated with a decreased ability to compete, whereas an increased rate of association was correlated with an increased ability to compete (Menetski and Kowalczykowski, 1990; see Kowalczykowski, 1991b). To further address this issue, we compared the association properties of recAwt protein, recA730 protein, recA441 protein, and recA803 protein (Fig. 10). At 1 mM magnesium chloride, recA730 protein associates with etheno M13 DNA considerably faster than the other recA proteins (Fig. 10*A*). Under these conditions,

TABLE II

The binding of recA protein to etheno M13 DNA

Reactions were performed as described under "Materials and Methods" and contained 3 μM etheno M13 DNA, 1 μM recA protein, 0.5 mM ADP, as indicated, and 1 mM ATP as indicated.

	Salt titration midpoint[a]		Relative fluorescence increase[b]	
	recAwt	recA730	recAwt	recA730
	mM			
No cofactor	260	270	1.82	1.74
ADP	135	145	1.72	1.70
ATP	975	825	2.20	2.10

[a] The salt titration midpoint is the concentration of NaCl (millimolar) required to dissociate one-half of the recA protein-etheno M13 DNA complex present.

[b] The relative fluorescence increase is the ratio between the fluorescence of the recA protein-etheno M13 DNA complex at the beginning of a salt titration and the fluorescence of the dissociated components at the end of a salt titration.

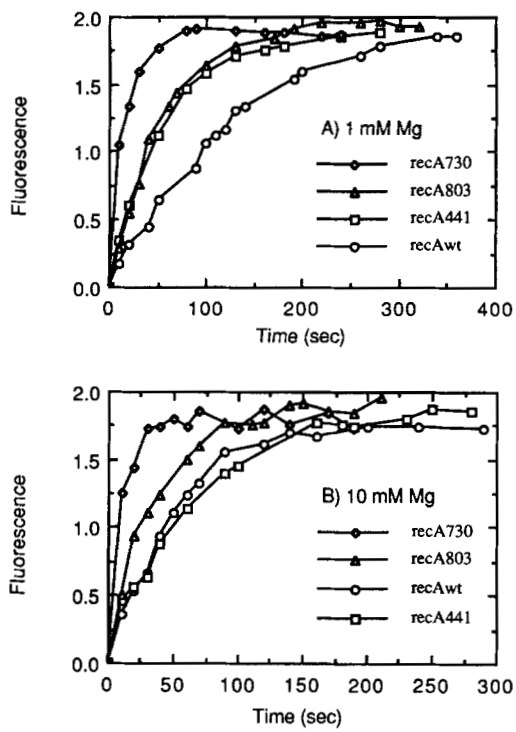

FIG. 10. **The association of recA protein with etheno M13 DNA.** Reactions were performed at 25 °C as described under "Materials and Methods;" 0.1 μM recA protein was added to 6 μM etheno M13 DNA, and the increase in etheno M13 DNA fluorescence due to recA protein binding was monitored over time. *Circles* indicate reactions containing recAwt protein; diamonds indicate reactions containing recA730 protein; *squares* indicate reactions containing recA441 protein; *triangles* indicate reactions containing recA803 protein. *A* indicates reactions performed at 1 mM magnesium chloride; *B* indicates reactions performed at 10 mM magnesium chloride.

recAwt protein associates with the DNA more slowly than the other recA proteins; the rates of recA441 protein and recA803 protein association are somewhat greater than that of recAwt protein.

At 10 mM magnesium chloride, recA730 protein again associates with etheno M13 DNA faster than the other recA proteins (Fig. 10*B*). While the rates of association of the other recA proteins increase with increasing magnesium ion concentration (see Chabbert *et al.*, 1987), the rate of recA441 protein association is virtually unaffected by increasing magnesium ion concentration to 10 mM (Fig. 10, compare *A* and *B*). As a result, recA441 protein associates with the DNA more slowly than the other recA proteins under these conditions. These conditions of high magnesium ion concentration and low temperature (25 °C) are those where recA441 protein has the most difficulty competing with SSB protein for ssDNA binding sites (see Table I).

Since the abilities of recA441 protein and recA730 protein to displace SSB protein from ssDNA increase dramatically with increasing temperature, we compared the effect of increasing temperature on the association properties of the wild-type and mutant recA proteins (Fig. 11). While the rate of association with etheno M13 DNA increases with increasing temperature for all of the recA proteins, the rates of recA730 protein and recA441 protein association increase more dramatically. At low temperatures, the rates of recA441 protein and recA803 protein association are comparable. However, as temperature increases to 40 °C, the rate of recA441 protein is considerably greater than that of recA803 protein. The effect of increasing temperature on the association kinetics of these recA proteins is much the same as the effect of increasing temperature on their abilities to displace SSB protein from ssDNA (see Fig. 3). Thus, there does indeed seem to be a correlation between the rate of recA protein association with ssDNA and the ability to compete with SSB protein for binding sites.

DISCUSSION

Constitutive induction of the SOS response is observed in both *recA441* and *recA730* strains; the constitutive repressor cleavage activity of recA441 protein is observed most notably at elevated temperature, whereas that of recA730 protein is observed at all temperatures that have been examined. In a previous study, we examined biochemical properties of recA441 protein in an effort to explain the constitutive SOS induction seen in *recA441* strains (Lavery and Kowalczykowski, 1988). We argued that the repressor cleavage properties of recA441 protein could be attributed to an increased ability to displace SSB protein from the ssDNA that occurs naturally in *E. coli*; the SSB protein displacement ability of recA441 protein is significantly enhanced at elevated temperature. In this study we examine biochemical properties of recA730 protein in a similar effort to explain the constitutive SOS induction seen in *recA730* strains. We find that recA730 protein is more proficient than both recAwt protein and recA441 protein at displacing SSB protein from ssDNA and is quite competent at low temperature. Thus, it is attractive to hypothesize that the basis for the enhanced repressor cleavage properties of these two mutant recA proteins is similar. However, the recA803 protein also displays an increased ability to displace SSB protein from ssDNA *in vitro*,[2] yet constitutive SOS induction is not observed in *recA803* strains (Madiraju *et al.*, 1988). To reconcile these observations, we directly compare the abilities of these three mutant recA proteins to form the ternary complex active in repressor cleavage under a variety of conditions. Our examination of both lexA protein cleavage activity and ATPase activity at low magnesium ion concentration indicates that the hierarchy of these recA proteins in their abilities to displace SSB protein from ssDNA is consistent with their *in vivo* repressor cleavage properties, *i.e.* recA730 protein > recA441 protein > recA803 protein > recAwt protein.

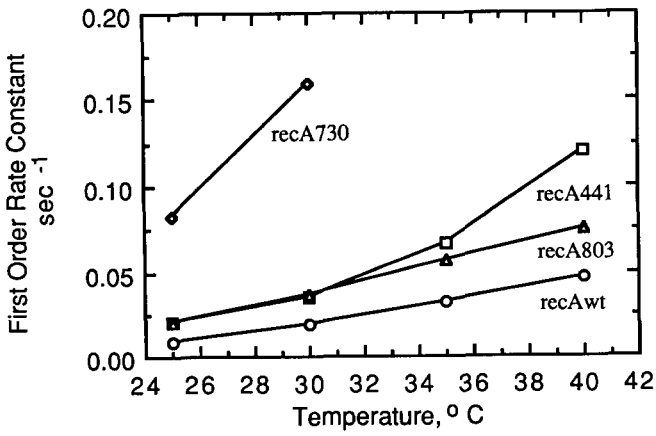

FIG. 11. **Temperature dependence of recA protein association with etheno M13 DNA.** The apparent first order rate constant (s^{-1}) for recA protein association with etheno M13 DNA was determined as described under "Materials and Methods." Reactions were performed at 1 mM magnesium chloride and contained 0.2 μM recA protein and 6 μM etheno M13 DNA. *Circles* indicate reactions containing recAwt protein; *diamonds* indicate reactions containing recA730 protein; *squares* indicate reactions containing recA441 protein; *triangles* indicate reactions containing recA803 protein.

LexA protein cleavage experiments indicate that the k_{cat} values for cleavage are the same for the wild-type and mutant recA proteins. However, the recA proteins differ in their stimulation of lexA protein cleavage under a variety of conditions. RecA730 protein is the most active in lexA protein cleavage, whereas recAwt protein is the least active. RecA441 protein and recA803 protein are intermediate in their lexA protein cleavage, with recA441 protein generally showing enhanced activity relative to recA803 protein under conditions of low magnesium ion concentration and elevated temperature. Since k_{cat} values for lexA protein cleavage are the same, these differences in the observed rate of cleavage must be attributable to differences in the abilities of the recA proteins to form the complex with ssDNA and ATP that is required for the stimulation of repressor cleavage. Consistent with this, lexA protein cleavage activity parallels ATPase activity when examined under equivalent conditions; the ternary complex required for repressor cleavage is active in the hydrolysis of ATP. Since the hydrolysis of ATP can be easily monitored in a continuous assay (Kowalczykowski and Krupp, 1987), a thorough comparison of the abilities of the various recA proteins to form this ternary complex can be accomplished by examining ATPase activity.

A comparison of ssDNA-dependent ATP hydrolysis by recAwt protein and recA730 protein suggests that recA730 protein is more proficient at competing with both secondary structures and SSB protein for ssDNA binding sites. Although increased aptitude in the competition for ssDNA binding sites has been previously reported for recA441 protein and recA803 protein, it has not been observed to the extent displayed by recA730 protein. The enhanced ability of recA730 protein in this competition includes an increased proficiency in the displacement of SSB protein from ssDNA. Examination of ATP hydrolysis, in reactions where ssDNA is precoated with SSB protein, indicates that recA730 protein displaces SSB

protein from ssDNA more quickly and more completely than all other recA proteins examined. At high magnesium ion concentration (10 mM magnesium acetate), recA803 protein is somewhat more proficient than recA441 protein in SSB protein displacement. However at low magnesium ion concentration (1 mM magnesium acetate), the ability of recA441 protein in SSB protein displacement is significantly greater than that of recA803 protein, especially at elevated temperature. Although the ability of all of the recA proteins to displace SSB protein increases with increasing temperature, that of recA730 protein and recA441 protein increases more dramatically. This has been previously reported for recA441 protein and is consistent with the temperature-inducible phenotype observed in *recA441* strains (Lavery and Kowalczykowski, 1988). Although recA730 protein displaces SSB protein more effectively with increasing temperature, it is very proficient in this displacement even at low temperature.

Since the *in vivo* concentration of free magnesium ion is generally thought to be in the range of a few millimolar, and since recA441 protein shows enhanced activity relative to recA803 protein at 1 mM magnesium acetate, we argue that the observations made at low magnesium ion concentration are significant. We propose that recA protein must displace a threshold amount of SSB protein from ssDNA in order for cleavage of lexA protein to be sufficient to induce the SOS response (see Fig. 3). RecAwt protein and recA803 protein are unable to attain this threshold and therefore do not promote constitutive induction of the SOS response. RecA441 protein is only able to attain this degree of SSB protein displacement at elevated temperature, which accounts for the temperature inducibility of SOS induction in *recA441* strains. Consistent with the constitutive SOS induction observed in *recA730* strains, recA730 protein is extremely proficient at SSB protein displacement and is able to attain this threshold even at low temperature.

The preceding assumes that an investigation of ATPase activity is a legitimate way to measure the ability of recA protein to displace SSB protein from ssDNA. We have addressed the validity of this assumption. We directly measured the displacement of SSB protein from ssDNA by monitoring the increase in fluorescence that accompanies its dissociation from the DNA and compared this with the time-dependent increase in ATP hydrolysis by recA protein. Somewhat unexpectedly, we find that ATP hydrolysis lags behind the fluorescence increase that signifies SSB protein dissociation. However, the time-dependent increase in ATP hydrolysis parallels the dissociation of SSB protein from ssDNA, and the extent of SSB protein displacement deduced from measurements of ATP hydrolysis agrees with that determined from fluorescence measurements. This suggests that a sufficient quantity of SSB protein must be displaced before recA protein can be activated for ATP hydrolysis. The binding of recA protein may occur via a mechanism involving a nucleation step in which recA protein monomers (or small aggregates) bind to ssDNA sites vacated by SSB protein. This is followed by a growth phase in which further recA protein binds to the ssDNA; this growth results in the formation of clusters large enough to be active in ATP hydrolysis (Kowalczykowski, 1986). The rate of growth is proportional to the rate at which SSB protein dissociates from ssDNA. Both the rate of nucleation and the rate at which recA protein clusters grow appear to be affected by mutations in the recA protein; ATP is hydrolyzed more rapidly in reactions containing recA730 protein than in reactions containing recAwt protein, and the rate at which the observed rate of ATP hydrolysis increases is also greater in reactions containing recA730 protein. Despite the temporal difference in appearance, both the rate and extent of SSB protein displacement determined from ATPase assays correlate with those determined from fluorescence measurements. Therefore, examination of ATPase activity provides a useful measure of the ability of recA proteins to displace SSB protein from ssDNA.

The increased competitiveness of recA730 protein for DNA binding sites cannot be attributed to an increased affinity for ssDNA. Similarly, the enhanced properties of recA441 protein and recA803 protein could not be attributed to increased affinities for ssDNA (Lavery and Kowalczykowski, 1988.[2] However, the displacement of SSB protein from ssDNA by these recA proteins correlates with their rates of association with ssDNA; an increased proficiency at SSB protein displacement coincides with an increased rate of association with ssDNA. The abilities of recA441 protein and recA730 protein to displace SSB protein increase dramatically with increasing temperature; this is paralleled by dramatic increases in their rates of association with ssDNA with increasing temperature. RecA730 protein is much more proficient than the other recA proteins at SSB protein displacement, and recA730 protein associates with ssDNA much more rapidly than the other recA proteins. At elevated temperature and low magnesium ion concentration, recA441 protein displaces SSB protein from ssDNA more proficiently than recA803 protein, and recA441 protein associates with ssDNA more quickly than recA803 protein under these conditions. Similarly, recAwt protein has more difficulty displacing SSB protein from ssDNA than the other recA proteins, and recAwt protein associates with ssDNA more slowly than the other recA proteins. Thus, this physical property of recA protein (ssDNA association rate) appears to determine its ability to displace SSB protein from ssDNA. It is likely to reflect both the ability of recA protein to nucleate on ssDNA sites vacated by SSB protein and the binding of additional recA protein during the growth phase.

Lu and Echols (1987) have reported slow dsDNA-dependent cleavage of lexA protein by recA441 protein and proposed that this contributes to the constitutive SOS induction seen in *recA441* strains. Our examination of lexA protein cleavage activity in the presence of dsDNA suggests that constitutive SOS induction is not the result of dsDNA-dependent repressor cleavage. At 37 °C, the abilities of recA730 protein, recA441 protein, and recA803 protein to stimulate cleavage of lexA protein are essentially equal in the presence of dsDNA. Since recA730 protein is the only protein to display constitutive repressor cleavage activity under these conditions, this argues that constitutive SOS induction is not caused by this slow dsDNA-dependent cleavage of repressors.

Our observations at physiologically significant low magnesium ion concentration are consistent with the following hypothesis. The very rapid association of recA730 protein with ssDNA enables it to displace SSB protein from the ssDNA that occurs naturally in *E. coli* and to thereby become activated for repressor cleavage under all conditions. However, only at elevated temperature is the association of recA441 protein with ssDNA rapid enough for SSB protein displacement to be sufficient to result in a degree of repressor cleavage that is adequate to induce the SOS response. Although recA803 protein associates with ssDNA somewhat more rapidly than recAwt protein, this difference is not great enough to produce the level of SSB protein displacement required for constitutive SOS induction.

The increased rate of recA803 protein association with ssDNA is also postulated to be the basis for the partial suppression by *recA803* of recombination defects in *recF*

strains.[2] In this hypothesis, recF protein is proposed to assist recA protein in the utilization of ssDNA exposed by the nuclease activity of the recJ protein (Lovett and Kolodner, 1989). The mutant recA803 protein, due to its increased rate of association with ssDNA, is able to utilize this ssDNA in the absence of recF protein. Consistent with this hypothesis, the *recA441* allele (Thomas and Lloyd, 1983; Volkert *et al.*, 1984) and the *recA730* allele[4] also partially suppress defects in *recF* strains; the increased rates at which recA441 protein and recA730 protein associate with ssDNA would similarly enable each to utilize ssDNA in the absence of recF protein. Direct comparison shows that *recA730* is more effective than *recA441* in the suppression of defects in *recF* strains[4]; this is consistent with the greater rate ofecA730 protein association with ssDNA observed in this study.

Since recA803 protein is able to suppress defects in *recF* without promoting constitutive SOS induction, and recA441 protein is similarly able to suppress defects in *recF* at low temperature (Volkert *et al.*, 1984), a condition where it does not promote constitutive SOS induction, we argue that less of an increased rate of association with ssDNA is required to suppress defects in *recF* than is required to constitutively induce the SOS response. The rates at which recA803 protein and recA441 protein (at low temperature) associate with ssDNA allow increased utilization of the ssDNA produced by the nuclease activity of recJ protein in the absence of recF protein but are not sufficient for displacement of SSB protein adequate to induce the SOS response. The more rapid rates at which recA730 protein and recA441 protein (at high temperature) associate with ssDNA would be sufficient for both of these functions.

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