

RecA Protein-dependent Proteolysis of Bacteriophage λ Repressor

CHARACTERIZATION OF THE REACTION AND STIMULATION BY DNA-BINDING PROTEINS*

(Received for publication, May 22, 1981)

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RecA protein (the wild type form of the protein, *i.e.* the product of the *recA*⁺ gene), purified to homogeneity by a novel ATP elution step (Cox, M. M., McEntee, K., and Lehman, I. R. (1981) *J. Biol. Chem.* 256, 4676-4678), cleaves bacteriophage λ repressor in a reaction requiring a nucleoside triphosphate, single-stranded polynucleotide and divalent cation. (d,r)ATP, (d,r)UTP, rATP[γ S], rUTP[γ S], and rGTP[γ S] serve as cofactors for recA protease activity, whereas (r,d)GTP, (r,d)CTP, and dTTP do not. The reaction is inhibited by ADP, UDP, and dTTP, all of which bind to recA protein. In the presence of ATP or other hydrolyzable nucleoside triphosphates, the rate of repressor cleavage is greatly enhanced by substituting Mn²⁺ ion for Mg²⁺, an effect which is correlated with a reduction in ATP hydrolysis. The polynucleotide requirement is satisfied by ϕ X174 DNA, poly(dT), poly(dU), and poly(dC). Polyribonucleotides and oligodeoxynucleotides are significantly less effective and duplex DNA inhibits the cleavage of λ repressor in the presence of single-stranded DNA. A ratio of 2-3 single-stranded nucleotides/recA protein monomer is optimal for proteolysis and the reaction is inhibited at higher ratios. Single-stranded DNA binding protein of *Escherichia coli* and gene 32 protein of bacteriophage T4 prevent the inhibition of proteolysis by excess single-stranded DNA. However, a defective single-stranded DNA binding protein isolated from a *lexC* mutant of *E. coli* does not reverse this inhibition by excess polynucleotide. These results provide evidence for a role of single strand DNA-binding protein in the mechanism of bacteriophage λ induction *in vivo*.

The SOS response, a complex and coordinate alteration of metabolic processes in the bacterium *Escherichia coli*, is elicited when DNA is damaged or its replication is blocked (1, 2) and involves induction of the synthesis of several gene products (3), including the recA protein.¹ At least some of

these genes are controlled at the transcriptional level by the *lexA* protein, a repressor which is inactivated in response to DNA damage (4). Bacteriophage λ is also induced in response to DNA damage, and the λ repressor is inactivated analogously to the *lexA* repressor. Roberts *et al.* (5) first determined the mechanism of λ repressor inactivation by showing that recA protein was a protease that cleaved the λ repressor protein. Subsequently, recA protein-dependent proteolytic cleavage of the *lexA* repressor (4) and the bacteriophage P22 repressor (6) have been demonstrated.

In addition to its protease activity, the recA protein promotes the hybridization of SS² DNA molecules (7) as well as formation of D-loops by hybridizing SS DNA to complementary regions within duplex DNA molecules (8, 9). This biochemical versatility is consistent with genetic studies of *recA* mutants, which are defective in DNA recombination and repair (presumably requiring the hybridization function) as well as the induction of SOS functions and prophages (10). A special class of *recA* mutation, termed *lexB*, is defective in SOS and prophage induction activities but normal in DNA recombination (11), demonstrating that the protease and hybridization activities of recA protein are genetically and functionally distinct. Another interesting mutation in the *recA* gene, *tif-1*, is able to induce SOS functions and prophages in the absence of DNA damage (12).

The protease activity of the recA protein is unusual in its requirement for ATP (but not its hydrolysis) and SS polynucleotides (5, 13, 14). Remarkable substrate specificity is observed for the recA protease: cleavage of the λ , *lexA*, and P22 repressors occurs at single sites within the polypeptides. The precise endopeptidase site has been determined in the case of λ repressor (15). No cleavage of other proteins by recA endopeptidase has been observed in crude extracts or partially purified fractions (6, 13).

The requirement for a SS polynucleotide for *in vitro* protease activity is consistent with the notion that the inducing signal *in vivo* is SS DNA regions, generated as a result of DNA damage. It has been suggested that this SS DNA is produced as a replication fork encounters the DNA lesion, leaving postreplication gaps (16), or as a result of DNA degradation, thereby creating SS oligonucleotides (17). Several distinct genetic loci are implicated in induction of SOS functions and prophages (other than *recA* and *lexA* genes) including *recB*, *recC*, *ssb* (*lexC*), and *recF*, and the products of the *recB*, *recC*, and *ssb* genes have been identified. The *recB* and *recC* genes encode exonuclease V, a multifunctional nuclease that presumably has a role in generating the inducer of SOS functions through DNA degradation (17). The product of the *ssb* (or *lexC*) gene is a SS DNA-binding protein (SSB) that is

* This work was supported by Grant GM 06196 from the National Institutes of Health and by Grant PCM 74-00856 from the National Science Foundation. During a portion of this work, the authors were fellows of the Jane Coffin Childs Memorial Fund. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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¹ In this paper "recA protein" refers to the wild type form of the protein, *i.e.* the product of the *recA*⁺ gene.

² The abbreviations used are: SS, single strand; DS, double strand; ATP[γ S], adenosine-5'-O-(3-thiotriphosphate); SSB, single strand DNA-binding protein.

required for DNA replication and repair of damage (18–20). Baluch *et al.* (21) have recently demonstrated that SSB is required *in vivo* for recA protein and phage λ induction by ultraviolet irradiation.

Previous studies of the recA protein protease activity have concentrated on the *tif* mutant form of recA protein. Here we present an analysis of the protease activity of the wild type recA protein which has been extensively characterized for DNA binding, nucleoside triphosphate hydrolysis, and hybridization activities. We have also investigated the effects of SSB on recA protein-dependent proteolysis of λ repressor and find that SSB from wild type, but not from *lexC113* mutant, cells stimulates λ repressor cleavage by recA protein in the presence of excess single-stranded DNA.

EXPERIMENTAL PROCEDURES

Enzymes—The recA protein was purified by ATP elution from DNA cellulose as described (22) and was greater than 98% pure. λ repressor (23) was generously provided by Dr. A. D. Kaiser. Homogeneous SSB was a gift of Robert Fuller. The *lexC* protein was as described (24). *dnaC* protein, *dnaB* protein, protein *n*, protein *n'*, and *rep* protein (25) were from the laboratory of Dr. A. Kornberg. DNA polymerase I was provided by Stuart Scherer, ρ factor was provided by Ron Conaway (all of this department) and polynucleotide phosphorylase was provided by Dr. Michael Smith (University of British Columbia). DNA ligase was as described (26). Gene 32 protein of bacteriophage T4 was from Dr. Bruce Alberts (University of California, San Francisco). Pyruvate kinase was purchased from Sigma.

Nucleic Acids—Bacteriophage ϕ X174 single-stranded DNA and double-stranded DNA of the plasmid pZ6b were prepared as described (27). Homopolymers and oligonucleotides were purchased from P-L Biochemicals and Grand Island Biological Co. Concentrations of nucleic acids were determined from their extinction coefficients (28, 29).

Protease Assay—Reaction mixtures (30 μ l) containing 20 mM Tris (pH 7.5), 1 mM dithiothreitol, 30 mM NaCl, either 1 mM ATP and 3 mM $MnCl_2$ or 160 μ M ATP[γ S] and 3 mM $MgCl_2$, polynucleotide, λ repressor, and recA protein as indicated were incubated at 37 °C in plastic Eppendorf tubes. Reactions were stopped by addition of 6 μ l of a solution containing 5% sodium dodecyl sulfate, 4% dithiothreitol, 0.05% bromophenol blue, and 25% glycerol. The samples were applied to a 15% polyacrylamide gel (30) and electrophoresed at 25 to 50 mA for approximately 3 h. Gels were stained for 10 min in a solution containing 45.5% methanol, 0.91% acetic acid, and 2.5 mg/ml of Coomassie brilliant blue R250. Following destaining in 10% methanol, 7% acetic acid, the gels were scanned spectrophotometrically using a Quick Scan Jr. densitometer equipped with integrator and the extent of cleavage was calculated.

RESULTS

Characterization of the Protease Activity of the Wild Type recA Protein—Proteolytic cleavage of λ repressor by recA protein required ATP or ATP[γ S], a divalent cation, and a single-stranded polynucleotide, analogous to the *tif-1* mutant enzyme requirement (14). With ATP as a cofactor, efficient repressor cleavage occurred only with Mn^{2+} ion and not with Mg^{2+} , Ca^{2+} , or Zn^{2+} ions (Fig. 1a). This contrasts with the SS DNA-dependent ATPase activity of recA protein which is optimal with Mg^{2+} ion and shows considerably reduced activity with Mn^{2+} ion (Fig. 1b). The failure of Mg^{2+} ion to stimulate the protease activity is related to increased ATP hydrolysis since repressor cleavage was enhanced in the presence of Mg^{2+} ion by either raising the initial ATP concentration or including an ATP-regenerating system (pyruvate kinase/phosphoenolpyruvate; data not shown).

ATP[γ S], which also serves as a cofactor for the protease activity, is not appreciably hydrolyzed by recA protein (14, 31, 32), although in the presence of DNA it binds extremely tightly to the enzyme (32) and stabilizes recA protein-DNA complexes (33). With ATP[γ S], both Mg^{2+} and Ca^{2+} ions stimulated the protease; other divalent cations were not

tested. With 1 mM ATP[γ S], optimal cleavage activity occurred at 3 mM Mg^{2+} ion and inhibition was observed above 5 mM Mg^{2+} ion (data not shown), similar to Mn^{2+} ion effects upon the reaction with ATP. The inhibition by Mg^{2+} ion was reversed by Ca^{2+} ion (data not shown).

Repressor cleavage was absolutely dependent on a single-stranded polynucleotide with either ATP (Mn) or ATP[γ S] (Mg) as cofactor. In both cases, optimum activity was observed at a ratio of about 3 single-stranded nucleotides/recA protein monomer (Fig. 2), similar to that reported previously for the *tif* mutant protein in the presence of ATP[γ S] (14). At this stoichiometry the SS DNA is saturated with recA protein (34). DS DNA failed to stimulate proteolysis, even below pH

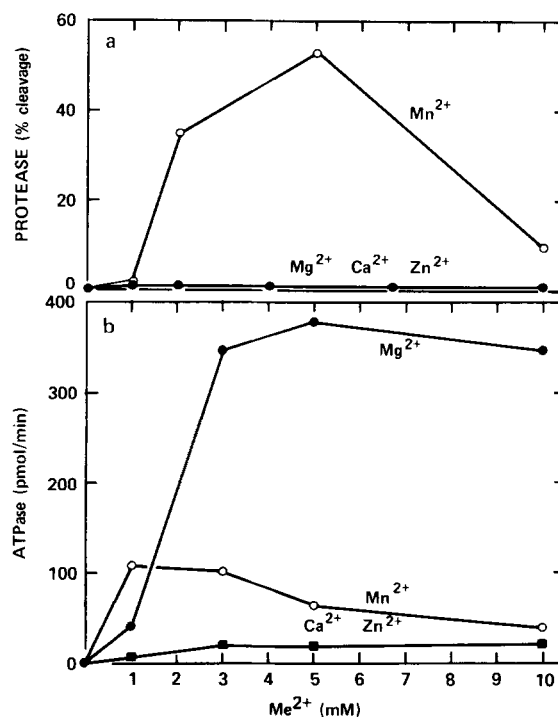


FIG. 1. Divalent cation requirement for protease and ATPase activities of recA protein. *a*, reactions were as described under "Experimental Procedures" with 930 μ M ATP, 13 μ M ϕ X174 SS DNA, 4.4 μ M recA protein, and 5.8 μ M λ repressor. Incubation was for 110 min. *b*, ATPase assays were performed as described previously (27) with 20 mM Tris-HCl (pH 8.1), 20 mM NaCl, 500 μ M ATP, 250 μ M heat-denatured SS calf thymus DNA, and 0.72 μ M recA protein. The rates are determined from time courses.

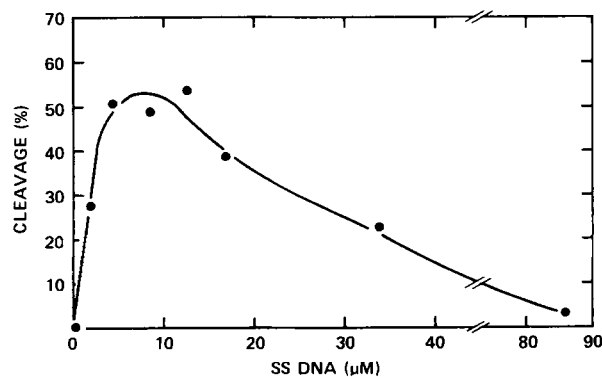


FIG. 2. Requirement for SS DNA in the ATP-dependent protease reaction. Reactions were performed as described under "Experimental Procedures" and contained 930 μ M ATP, 3 mM $MnCl_2$, 4.4 μ M recA protein, 5.8 μ M λ repressor, and the indicated amount of circular ϕ X174 SS DNA. Incubation was for 85 min.

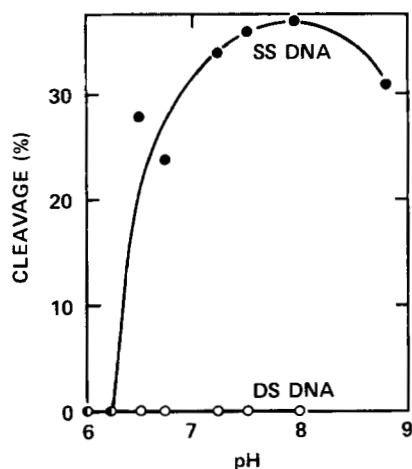


FIG. 3. pH-dependence of λ repressor proteolysis with SS and DS DNA cofactors. Reactions were performed as described under "Experimental Procedures" and contained 3 mM $MgCl_2$, 160 μM ATP[γ S], 5.4 μM λ repressor, 4.4 μM recA protein, and either 13 μM ϕ X174 SS DNA or pZ6b DS DNA. Buffers, at 20 mM, were sodium maleate (pH 6.0–6.7) or Tris-HCl (pH 7.2–8.8). Incubation was for 60 min. pH values were measured at 20 mM and 25 $^{\circ}C$ and were not corrected for the effect of Mg^{2+} ion.

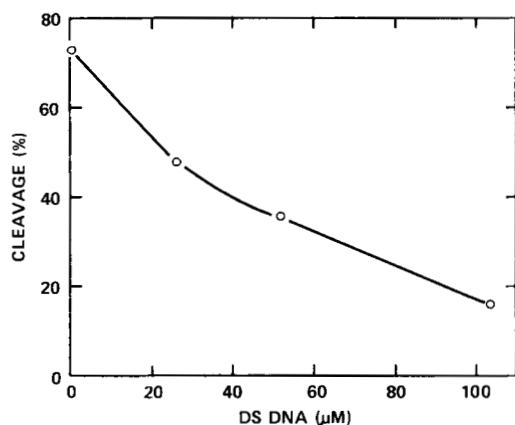


FIG. 4. Inhibition of recA protein-dependent proteolysis by DS DNA. Reactions were performed as described under "Experimental Procedures" and contained 2 mM ATP[γ S], 3 mM $MgCl_2$, 13 μM ϕ X174 SS DNA, 4.4 μM recA protein, 3.9 μM λ repressor, and the indicated amount of pZ6b DS DNA. Incubation was for 120 min.

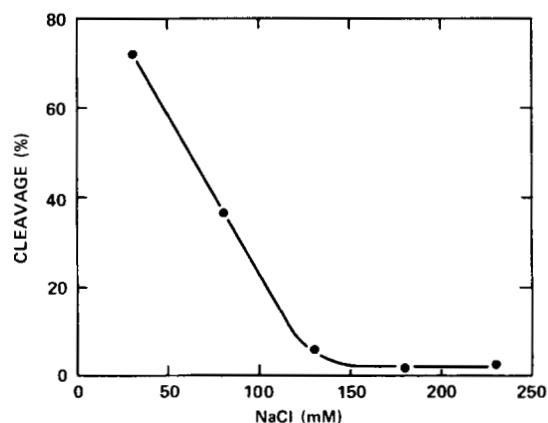


FIG. 5. Salt sensitivity of recA protein-dependent proteolysis. Reactions were performed as described under "Experimental Procedures" and contained 160 μM ATP[γ S], 3 mM $MgCl_2$, 4.4 μM recA protein, 13 μM ϕ X174 SS DNA, 5.8 μM λ repressor, and the indicated amount of NaCl. Incubation was for 120 min.

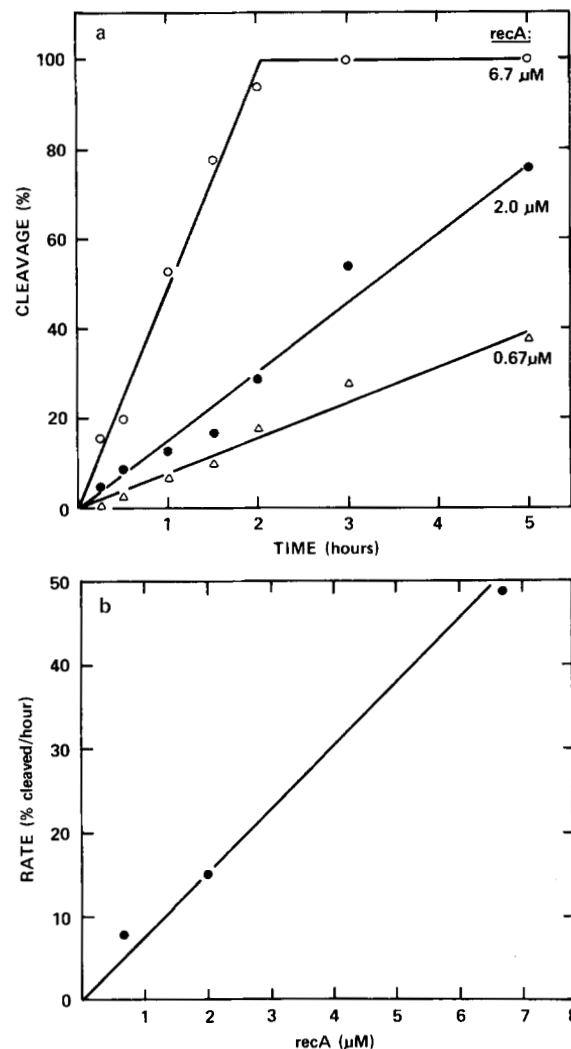


FIG. 6. Kinetics and recA protein dependence of λ repressor proteolysis. Reactions (200 μl) were performed as described under "Experimental Procedures" with 3 mM $MgCl_2$, 1 mM ATP[γ S], circular ϕ X174 SS DNA at a ratio of 3 nucleotides/recA monomer, 5.8 μM λ repressor, and the indicated amount of recA protein. *a*, kinetics of the reaction; *b*, recA protein dependence of the cleavage reaction.

TABLE I
Specificity of the nucleoside triphosphate requirement for protease activity of recA protein

Reactions were performed as described under "Experimental Procedures" with 3 mM $MnCl_2$, 4.4 μM recA protein, 13 μM ϕ X174 SS DNA, and 5.8 μM λ repressor. Incubation was for 135 min.

NTP	Concentration μM	λ repressor cleaved %
rATP	930	48
dATP	1000	53
rGTP	1085	1
dGTP	1130	<1
rCTP	1000	1
dCTP	940	2
rUTP	990	17
dUTP	1000	13
dTTP	1220	<1
rATP[γ S]	167	61
rADP	950	<1
rUDP	990	<1
dTMP	960	<1
dAMP	1010	<1

7 (Fig. 3) where DS DNA binding to recA protein and stimulation of ATP hydrolysis are optimal (27, 33). In fact, DS DNA inhibited the SS DNA-dependent cleavage of λ repressor (Fig. 4). Under the conditions of this experiment, SS DNA stimulates binding of DS DNA to recA protein (31) and thus it appears likely that this inhibition is due to the interaction of DS DNA with recA protein rather than with λ repressor.

Proteolysis was sensitive to monovalent ions (Fig. 5). The reaction was inhibited by concentrations of spermidine greater than 1 mM; lower concentrations had no effect (data not shown). RecA protein-dependent proteolysis with either ATP or ATP[γ S] as cofactor was completely abolished by 10 mM *N*-ethylmaleimide. This result is noteworthy in that the formation of stable recA protein-ATP[γ S] complexes is not sensitive to *N*-ethylmaleimide (32).

The rate of proteolysis in the presence of ATP[γ S] as cofactor was proportional to recA protein concentration and proceeded at a constant rate until all of the repressor was cleaved (Fig. 6). In addition, this experiment demonstrates turnover of the recA protein. Complete cleavage did not occur with ATP as a cofactor, presumably as a consequence of ATP hydrolysis. Proteolysis with either ATP (Mn) or ATP[γ S] as cofactor, however, showed the same initial rate, which corresponded to one cleavage event/recA monomer/130 min at 37 °C. It should be noted that at the high concentrations of λ repressor used in these experiments the rate of repressor cleavage is probably limited in part by the dimerization of the repressor monomer (6).

Specificity of the Nucleoside Triphosphate Requirement

TABLE II

Inhibition by nucleoside triphosphates of ATP-dependent proteolysis of λ repressor by recA protein

Reactions were as in Table I and contained 930 μ M rATP. Incubation was for 90 min.

Inhibitor	Concentration μ M	λ repressor cleaved %
None		40
rUTP	990	32
rGTP	1085	25
rCTP	1000	13
dGTP	1130	16
dCTP	940	20
dUTP	1000	16
dTTP	1220	2
rADP	950	9
rUDP	990	5
dAMP	1010	38
dTMP	960	39

TABLE III

Polynucleotide specificity of the protease activity of recA protein

Reactions were performed as described under "Experimental Procedures" and contained 3 mM MgCl₂, 160 μ M ATP[γ S], 1 mM potassium phosphate, 5.8 μ M λ repressor, and 4.4 μ M recA protein. Polynucleotides were present at 10 μ M (nucleotides); oligonucleotides were present at 24 μ M. Incubation was for 80 min.

Polynucleotide	λ repressor cleaved %
None	<1
Poly(rA)	<1
Poly(dA)	1
Poly(rU)	<1
Poly(dU)	31
Poly(dT)	31
Poly(rC)	<1
Poly(dC)	9
dT ₁₂	<1
dA ₁₂	<1

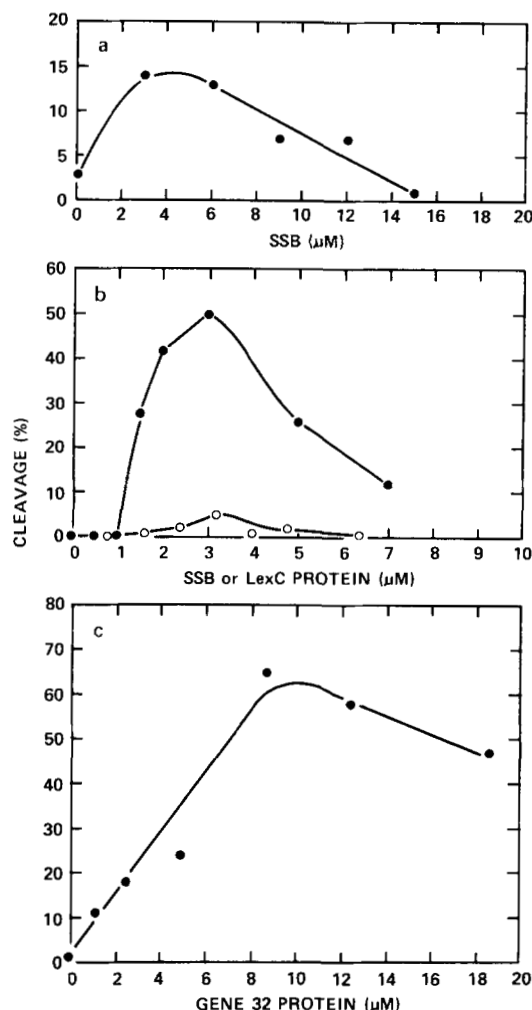


FIG. 7. Effect of SS DNA binding proteins on recA protease activity. *a*, reactions contained 930 μ M ATP, 3 mM MnCl₂, 4.4 μ M recA protein, 5.8 μ M λ repressor, 84 μ M ϕ X174 SS DNA, and the indicated amount of SSB. Incubation was for 85 min. *b*, reactions contained 160 μ M ATP[γ S], 3 mM MgCl₂, 4.4 μ M recA protein, 5.8 μ M λ repressor, 43 μ M poly(dT), and the indicated amount of SSB (●—●) or lexC (○—○) protein. Incubation was for 120 min. *c*, reactions contained 200 μ M ATP[γ S], 3 mM MgCl₂, 5.3 μ M recA protein, 7.0 μ M λ repressor, 101 μ M ϕ X174 SS DNA, and the indicated amount of gene 32 protein. Incubation was for 70 min.

for Proteolysis—In the presence of Mn²⁺ ion, rATP and dATP were the preferred cofactors for the proteolytic reaction but significant activity was also observed with rUTP and dUTP; other nucleoside triphosphates, diphosphates, and monophosphates were not active as cofactors (Table I). This specificity parallels that of the NTPase activity of recA protein (27) and indicates that the same nucleoside triphosphate binding site is involved in both reactions. (r,d)GTP, (r,d)CTP, and (r,d)UTP were modest inhibitors of ATP-dependent proteolysis; however, dTTP, ADP, and UDP were more potent inhibitors (Table II), similar to their effect on the ATPase activity of recA protein (35). dAMP and dTMP had no effect.

As noted above, proteolysis with ATP[γ S] was as efficient as with ATP (Mn). The maximum rate of proteolysis with ATP[γ S] occurred when ATP[γ S] was present at equimolar concentrations with recA protein (data not shown) as expected if proteolysis requires formation of stable, stoichiometric recA protein-ATP[γ S] complexes (32). Proteolysis was also observed with UTP[γ S] and GTP[γ S] cofactors (data not shown).

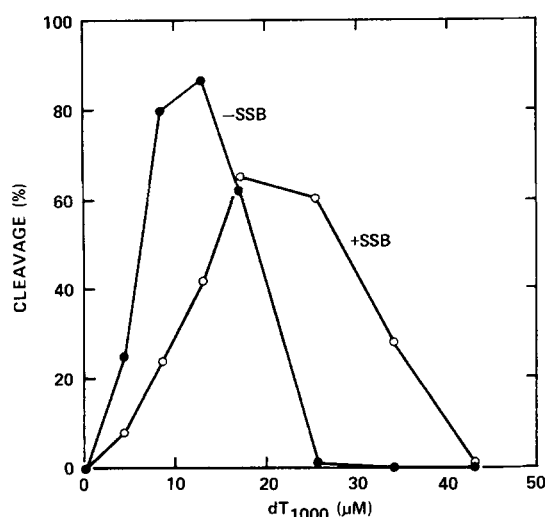


FIG. 8. Effect of SSB on the SS DNA optimum for recA protease cleavage of phage λ repressor. Reactions were performed as described under "Experimental Procedures" and contained 4.4 μ M recA protein, 5.8 μ M λ repressor, 160 μ M ATP[γ S], 3 mM MgCl₂, either 1 μ M or no SSB, and the indicated amount of dT₁₀₀₀. Incubation was for 120 min.

Specificity of the Polynucleotide Requirement for Proteolysis—Proteolysis by the recA protein was stimulated by deoxyribopolymers while ribopolymers had little effect (Table III). Polydeoxypyrimidines were most active as cofactors. Furthermore, short oligonucleotides (dT₁₂) were not active as cofactors. Thus, proteolysis by the wild type recA protein shows a similar polynucleotide specificity as does its ATPase activity (27). However, this specificity differs from that of the *tif* mutant protein³ (14).

Substrate Specificity of the Protease—Several purified *E. coli* proteins were tested as substrates for the recA protein protease in the presence of ATP[γ S]. No recA protein-dependent cleavage, as detected by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, was observed for any of the following purified proteins: SSB, dnaC protein, protein n, dnaB protein, rep protein, protein n', DNA polymerase I, DNA ligase, ρ factor, or polynucleotide phosphorylase. Furthermore, none of these proteins inhibited λ repressor cleavage by recA protein. Functional assays of these proteins were not performed after incubation with recA protein; consequently, proteolytic cleavage events which alter the activity without appreciably changing the electrophoretic mobility of the protein would not have been detected.

Effect of the *E. coli* SSB on Proteolysis—As described previously, excess SS DNA inhibits recA protein-dependent proteolysis of λ repressor (Fig. 2; Ref. 14). Likewise, strand assimilation is inhibited by excess SS DNA and the inhibition is overcome by SSB (24, 36) which binds to the excess SS DNA. As shown in Fig. 7, SSB also overcame inhibition of the recA protease activity by excess SS DNA. The stimulatory effect of SSB was seen in both ATP- and ATP[γ S]-dependent reactions. In both cases an optimum SSB concentration was observed, above which SSB inhibited the reaction. The gene 32 protein of bacteriophage T4 also overcame the SS DNA inhibition of recA protein-dependent proteolysis (Fig. 7c), similar to its effect on the assimilation reaction³ (36). However, SSB protein purified from the *lex*C113 mutant of *E. coli* only weakly stimulated proteolysis (Fig. 7b). The mutant *lex*C binding protein also failed to stimulate the strand-assimilation reaction (24).

As shown in Fig. 8, the effect of SSB is to shift the DNA optimum to higher concentrations while having little effect on the maximum rate, which is determined by the recA protein concentration. Higher concentrations of DNA were required to inhibit proteolysis in the presence of SSB but inhibition was observed, indicating that SSB does not alter the recA protein but exerts its effect by covering the DNA. The shift in the optimum in Fig. 8 indicates that about 10 nucleotides are bound/SSB monomer; thus, the DNA is nearly saturated with SSB (37).

DISCUSSION

Further Evidence for the Protease Activity of the recA⁺ Protein—Our results further support the conclusion that proteolytic cleavage of λ repressor is catalyzed by recA protein. Thus, homogeneous recA protein purified by a procedure that differs from those used previously (22) contains an endopeptidase activity that cleaves λ repressor. The nucleoside triphosphate specificity that is observed for proteolysis is very similar to the SS DNA-dependent hydrolytic activity of recA protein. Preliminary characterization of mutant forms of the recA protein purified by this procedure is consistent with this view. The *tif*-1 mutant protein is altered in its polynucleotide requirements for both ATPase and repressor cleavage activities.⁴ The *lex*B30 mutant protein fails to cleave λ repressor *in vivo* (11) and *in vitro* but is active for recombination *in vivo* and strand pairing *in vitro*³ (7, 11).

Under the conditions described in this paper, recA protein cleaves an equimolar amount of λ repressor in about 2 h; *in vivo*, the cleavage of λ repressor is complete in about 30 min (38). However, in our reactions the concentration of repressor is at least 50-fold higher than *in vivo* (39), whereas the recA protein concentration is comparable to that found in uninduced cells.⁵ In view of the fact that at high concentrations, repressor dimerization may inhibit its proteolysis by recA protein (6, 40), the rate of cleavage that we observe *in vitro* is not inconsistent with the *in vivo* rate of λ induction.

Inhibition by Single-stranded DNA and the Effect of SSB—Single-stranded DNA is required stoichiometrically for both repressor cleavage (14) and strand assimilation (8) reactions and, when present in excess, inhibits both reactions (14, 24). Previously it was suggested that the inhibition of assimilation is due to competition between the excess SS DNA and DS DNA for binding to recA protein (24). However, it seems unlikely that a similar competition exists between the site for binding λ repressor and SS DNA. The stoichiometric requirement for SS DNA in strand assimilation has also been attributed to a requirement for melting secondary structure in the SS DNA (36); however, here we report inhibition of λ repressor cleavage by poly(dT), which lacks such secondary structure. High concentrations of SS DNA are not inhibitory to the recA protein *per se* since neither the ATPase activity nor the strand-reassociation activity is inhibited by excess SS DNA (7, 35). In addition, it appears that high concentrations of oligonucleotides, unlike polynucleotides, are significantly less inhibitory (in Ref. 14, compare Table I and Fig. 6).

It is not clear whether inhibition requires binding of the excess SS DNA to recA protein. RecA protein has the capacity to bind more than 3 nucleotides/monomer. Thus, when SS DNA is present at optimum stoichiometry, the protein is capable of binding DS DNA (24, 31). If this DS DNA binding is related to binding of excess SS DNA, it can explain the inhibitory effect of DS DNA on proteolysis. Furthermore, in two-stage binding experiments, about 10 SS DNA nucleo-

³ Unpublished results.

⁴ K. McEntee, manuscript in preparation.

⁵ C. Paoletti, personal communication.

tides/recA monomer are required in a preincubation to inhibit subsequent SS DNA binding.³ Thus, it is possible that binding of additional SS DNA converts recA protein to a form which is unable to interact with other macromolecules. This could be due, for instance, to conformational changes in the protein or sequestering of protein in complexes with DNA. Alternatively, if the additional DNA is not bound, inhibition could still result from sequestering or from an altered oligomeric form of recA protein bound along the DNA. The lack of inhibition by oligonucleotides is consistent with any of these views.

The effect of SSB on the protease reaction appears to be to bind to the excess SS regions and abolish their inhibitory effect. The observation that high concentrations of SSB inhibit proteolysis suggests that recA protein binds poorly to SS DNA that is covered with SSB. The data of Figs. 7B and 8 are consistent with this notion. Optimal activity occurs when the SS DNA just saturates the recA protein and SSB. At lower SSB concentrations there is inhibition due to free SS regions while at higher SSB concentrations the inhibition would be due to the competition between SSB and recA protein for SS DNA. Thus, this model suggests that no interaction between SSB and recA protein is necessary for repressor cleavage *in vitro*. Consistent with this, we find that the gene 32 protein of bacteriophage T4 substitutes for SSB. A similar effect of this phage-coded protein has been reported for strand assimilation promoted by recA protein (36).

The observation that SS DNA covered with SSB is not an effector for the protease reaction *in vitro* has implications for the *in vivo* reaction. SS DNA at the replication fork is likely to be complexed with SSB and thus will not be a good effector for the induction of SOS functions by recA protein. However, DNA damage will generate additional SS DNA regions lowering the SSB:SS DNA ratio *in vivo*.

The increased amount of SS DNA regions could be bound with recA protein in order to stimulate the recA protease and derepress SOS functions including the *recA* gene. Thus the level of SSB determines a threshold value of SS DNA needed to activate recA protein. A prediction of this model is that strains containing plasmids that overproduce SSB will show an altered dose response for recA protein induction. Implicitly assumed in this model is that the synthesis of SSB is not itself regulated by DNA damage, a possibility that has not been tested directly.

Recently Baluch *et al.* (21) demonstrated the *lexC113* and *ssb1* mutant strains are defective in their ability to synthesize recA protein and induce prophage λ following ultraviolet treatment. In the case of *ssb1* mutant strains, ultraviolet induction of recA protein synthesis is blocked at 42 °C, a temperature at which the binding protein is nonfunctional *in vivo* (19) and *in vitro* (18). The *lexC113* mutation prevents ultraviolet induction of recA protein synthesis and prophage λ at all temperatures (21). Our observations are consistent with these results. Our results that excess SSB inhibits recA protein dependent proteolysis of phage λ repressor is compatible with the idea that when SS DNA regions (gaps) are limiting, recA protein and SSB compete for these binding sites. A more detailed analysis of the interaction between recA protein and both wild type and mutant forms of SSB (*lexC113* and *ssb1*) should elucidate the mechanism of these effects.

Acknowledgments—We thank Dr. I. R. Lehman for his support and encouragement.

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