

Hydrolysis of Nucleoside Triphosphates Catalyzed by the *recA* Protein of *Escherichia coli*

CHARACTERIZATION OF ATP HYDROLYSIS*

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Both single- and double-stranded DNA stimulate the hydrolysis of ATP catalyzed by the *recA* protein of *Escherichia coli*. However, the reactions differ in their pH optima, response to *recA* protein concentration, salt sensitivity, and degree of inhibition by ADP, all of which reflect different requirements for the prehydrolytic binding of single- and double-stranded DNA by the *recA* protein. Single- and double-stranded DNA stimulate hydrolysis of the same nucleoside triphosphates, principally (r,d)ATP and (r,d)UTP, suggesting that a single hydrolytic site is utilized in both single- and double-stranded DNA-dependent reactions. *recA* protein also catalyzes detectable ATP hydrolysis in the absence of exogenous DNA, although the rate is reduced 2 to 3 orders of magnitude. This DNA-independent hydrolysis shows the same nucleotide specificity at pH 6.2 and 7.5, although the rate of hydrolysis depends upon the pH.

Recent *in vitro* studies of the *recA* protein of *Escherichia coli* have illuminated its role in homologous recombination and in the repair of DNA. Two remarkably different activities are associated with this 38,000 molecular weight polypeptide. First, the *recA* protein promotes hybridization of complementary DNA strands. Either two single strands can be annealed to produce DS¹ DNA (1) or a single strand can be annealed to a homologous sequence within DS DNA (strand assimilation) to produce a triple-stranded D-loop structure (2, 3). Although both reactions require ATP, they differ significantly in other respects which presumably reflect the added complexity of binding and unwinding DS DNA in the assimilation reaction (4, 5). Second, certain repressor proteins are inactivated by proteolytic cleavage in a reaction dependent upon *recA* protein, ATP, and single-stranded polynucleotides (6, 7). Although it is surprising that a single protein can participate in two such dissimilar reactions, these activities nevertheless

correlate with the function *in vivo* of the *recA* protein as deduced from genetic studies. The hybridization reactions account for the direct participation of *recA* protein in strand exchange during DNA recombination and postreplication repair, while repressor inactivation is in accord with the regulatory role of *recA* protein in prophage induction and in the induction of other (SOS) functions in response to DNA damage (8).

As noted above, both hybridization and repressor inactivation are dependent upon ATP. Previous studies had, in fact, shown the *recA* protein to possess DNA-dependent ATPase activity (1, 9-11). However, the role of ATP appears to be complex. Although both annealing of single strands and assimilation can be stimulated under certain conditions by the analog ATP(γ S) (12),² which is not appreciably hydrolyzed by *recA* protein (11, 13), ATP is a much more effective cofactor, and ATP(γ S) will, under most circumstances, inhibit the ATP-stimulated hybridization reactions (1, 11). In contrast, ATP(γ S) is a very effective cofactor for repressor inactivation (9). Furthermore, ATP (and ATP(γ S)) causes an oligomerization of *recA* protein in the absence of DNA (10, 14) and affects its binding to and dissociation from DNA (3-5, 14). These observations indicate that both the binding and hydrolysis of ATP have important consequences for the enzymatic activities associated with the *recA* protein.

To provide further insight into the interaction of ATP with *recA* protein, we present here a detailed characterization of the ATPase activity of the *recA* protein. We have found that although ATP hydrolysis appears to occur at the same site on the enzyme in the presence of either SS or DS DNA, these two reactions are distinguishable by a number of criteria that are related to the different requirements for interaction of SS and DS DNA with the *recA* protein. We have also found that *recA* protein hydrolyzes ATP in the absence of a DNA cofactor, but at a much reduced rate. Analysis of this DNA-independent reaction should provide some insight into the role of DNA in ATP hydrolysis.

EXPERIMENTAL PROCEDURES

Materials—DEAE-cellulose (DE52) was obtained from Whatman; hydroxylapatite (Bio-Gel HTP) from Bio-Rad; plastic-backed polyethyleneimine cellulose sheets (Polygram MN 300) from Brinkmann; nucleoside triphosphates from P-L Biochemicals and Sigma; [³H]ATP from Amersham; [α -³²P]ATP from ICN; maleic acid from Sigma; calf thymus DNA from Calbiochem; and Polymin P from BASF Wyandotte Corp., Parsippany, NJ.

Bacteriophage P22 DNA and supercoiled plasmid DNA were prepared as described previously (1). The plasmids employed were pBR322 (15) and pZ6b, a derivative of pBR322 (constructed by Richard Mulligan of this department) in which the 346-nucleotide

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¹ The abbreviations used are: DS, double-stranded; ATP(γ S), adenosine-5'-O-(3-thiotriphosphate); BSA, bovine serum albumin; PEI, polyethyleneimine; SDS, sodium dodecyl sulfate; SS, single-stranded; NTP, nucleoside triphosphate.

² G. M. Weinstock, K. McEntee, and I. R. Lehman, unpublished results.

region between the *Hind*III and *Bam* HI restriction endonuclease sites was replaced with a 291-nucleotide segment containing the Z6b *Hae* III fragment from phage ϕ X174. ϕ X174 SS viral DNA was prepared by extraction of the virus with phenol-SDS at 65 °C. DNA nucleotide concentrations were determined by taking an A_{260} of 1 to be 50 μ g/ml of DS DNA and 36 μ g/ml of SS DNA (1 μ g = 3.1 nmol of nucleotide).

Buffers—R buffer is 20 mM Tris-HCl (pH 7.5), 20 mM KCl, 10% (v/v) glycerol, 1 mM dithiothreitol, and 0.1 mM EDTA. Maleate buffer, pH 6.2, contained 20 mM maleic acid and 33 mM NaOH. In the presence of 10 mM Mg^{2+} , the pH fell to 6.0.

Purification of *recA* Protein—*recA* protein was purified by selective extraction from Polymin P followed by phosphocellulose chromatography as described previously (1). This material (Fraction II) was greater than 90% pure and analysis on SDS-polyacrylamide gels indicated that no contaminant comprised more than 1% of the total protein.

Fraction II was purified further by DEAE-cellulose chromatography. Five mg of protein were applied to a 5-ml column of DE52 in R buffer containing 50 mM KCl and eluted at a rate of 2 bed volumes/h with a 50-ml gradient (50 to 300 mM) of KCl in R buffer. *recA* protein (>50%) eluted at 180 mM KCl. All DNA-dependent ATPase activity eluted at the position of *recA* protein, indicating that Fraction II contained no contaminating DNA-dependent ATPase. However, DNA-independent ATPase activity associated with *recA* protein was resolved from a second, low level DNA-independent ATPase. A small amount of polynucleotide phosphorylase activity was also separated from *recA* protein by this procedure.

The peak of *recA* protein was pooled (Fraction III) and further purified on a column of hydroxylapatite. Fraction III (3 mg) was applied to a 3-ml hydroxylapatite column equilibrated with 20 mM sodium phosphate (pH 6.8), 10% (v/v) glycerol, and 1 mM dithiothreitol. Protein was eluted at a rate of 1 bed volume/h with a 30-ml gradient (20 to 300 mM) of sodium phosphate (pH 6.8). *recA* protein eluted at 160 mM sodium phosphate, and all three ATPase activities co-purified with the *recA* protein (Fig. 1). The hydroxylapatite fraction was concentrated by precipitation with ammonium sulfate (70% saturation) and dialyzed against R buffer (Fraction IV).

Fraction IV contained only a single contaminant (*M*, approximately 100,000) amounting to less than 1% of the total protein. In addition to being free of contaminating ATPases and polynucleotide phosphorylase, Fraction IV lacked the low level DNA endonuclease and protein kinase activities found in some Fraction II preparations. In addition to ATPase, Fraction IV contained UTPase and SS DNA annealing and assimilation activities and could inactivate bacteriophage λ repressor protein.

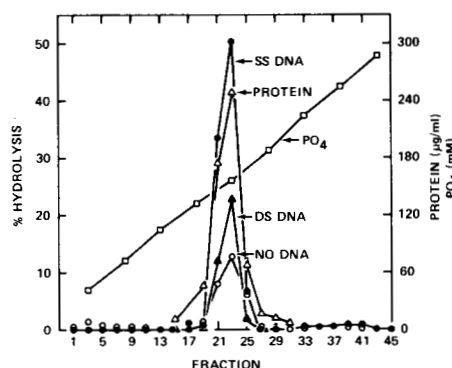


FIG. 1. Hydroxylapatite chromatography of *recA* protein. Fractions were assayed as described under "Experimental Procedures." SS DNA-dependent ATPase activity was measured in Tris-HCl (pH 7.5) and reaction mixtures contained 317 μ M ATP, 217 μ M heat-denatured calf thymus DNA, and 10 μ l of each fraction; incubation was for 1 h. DNA-independent ATPase activity was measured in Tris-HCl (pH 7.5) and contained 5 μ M ATP and 50 μ l of each fraction; incubation was for 7 h. DS DNA-dependent ATPase activity was measured in sodium maleate (pH 6.2) and contained 317 μ M ATP, 31 μ M P22 DS DNA, and 10 μ l of each fraction (dialyzed into R buffer); incubation was for 1 h. Protein concentration was determined by the method of Lowry *et al.* (17) using BSA as a standard. ●—●, SS DNA-dependent ATPase; ○—○, DNA-independent ATPase; ▲—▲, DS DNA-dependent ATPase; △—△, protein; □—□, phosphate.

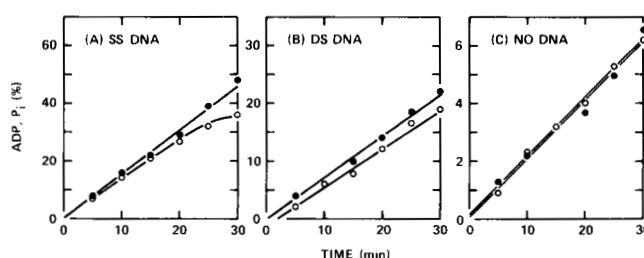


FIG. 2. Kinetics of ATP hydrolysis. Reactions were performed as described under "Experimental Procedures" and also contained 60 μ Ci/ml of $[\gamma\text{-}^{32}\text{P}]\text{ATP}$. PEI-cellulose strips were developed in 1 M acetic acid and 0.4 M LiCl to increase separation between ADP and P_i . A, SS DNA-dependent ATPase reaction in Tris-HCl (pH 7.5) containing 950 μ M ATP, 217 μ M heat-denatured calf thymus DNA, and 2.2 μ M Fraction II *recA* protein. B, DS DNA-dependent ATPase reaction in sodium maleate (pH 6.2) containing 950 μ M ATP, 31 μ M P22 DNA, and 2.1 μ M Fraction III *recA* protein. C, DNA-independent ATPase reaction in Tris-HCl (pH 7.5) containing 5 μ M ATP and 11 μ M Fraction III *recA* protein. ●—●, ADP; ○—○, P_i .

The specific activities of the SS DNA-dependent ATPase in Fractions II, III, and IV were essentially identical. However, several different preparations of Fraction II showed 2- to 3-fold differences in the specific activity of the DS DNA-dependent ATPase, although all were greater than 90% pure.

recA protein concentration was determined using $E_{280}^{1\%} = 5.16$ ($A_{280} = 1 = 51 \mu$ M *recA* protein), as calculated from the amino acid composition (16). Measurements of *recA* protein concentration by the method of Lowry *et al.* (17) using BSA as standard gave about two-thirds the concentration estimated from the absorbance. A_{280}/A_{260} ratios varied from 1.44 to 1.65 among different Fraction II preparations.

recA protein was stable for at least 6 months when stored at 4 °C in R buffer or at -70 °C in R buffer containing 50% (v/v) glycerol. The protein was unstable in sodium maleate (pH 6.2) at 37 °C ($t_{1/2}$ approximately 15 min). However, in the presence of ATP, or at 30 °C without ATP, *recA* protein was stable at this pH ($t_{1/2} > 3$ h). DNA had no effect on this inactivation.

ATPase Assay—Reaction mixtures (60 μ l) contained 20 mM buffer (either Tris-HCl or sodium maleate unless otherwise specified), 10 mM $MgCl_2$, 1 mM dithiothreitol, DNA as indicated, $[\text{H}^3]\text{ATP}$ (concentration as specified, 67 μ Ci/ml), and *recA* protein. Reactions in Tris buffer also contained 30 mM NaCl. Reaction mixtures were incubated in plastic Eppendorf tubes at 30 °C (significant adsorption of *recA* protein to borosilicate tubes was observed even in the presence of 100 μ g/ml of BSA). Aliquots (1 μ l) were spotted onto PEI-cellulose strips (0.5 cm \times 5 cm) containing ATP and ADP markers and developed in 1 M formic acid and 0.5 M LiCl. ATP and ADP were identified with an ultraviolet lamp, cut out, and counted in scintillation fluid (0.1 g of 1,4-bis[2-(5-phenyloxazolyl)]benzene (POPOP); 4 g of 2,5-diphenyloxazole (PPO)/1 toluene). In this solvent system, ATP remains near the origin, ADP migrates about halfway to the solvent front, and P_i runs ahead of ADP.

ATP concentrations were determined using $\epsilon_{259} = 15,400$ at pH 7.

RESULTS

Hydrolysis of ATP to ADP and P_i —The *recA* protein catalyzes several modes of ATP hydrolysis, operationally distinguished by the DNA cofactor and pH. In all cases, ATP was hydrolyzed solely to ADP and P_i (<2% AMP after 30% of ATP hydrolyzed to ADP) which were produced at similar rates (Fig. 2).

In the presence of SS DNA at pH 7.5, hydrolysis of ATP proceeded at a constant velocity until the rate decreased as a consequence of ADP inhibition (>50% hydrolysis with 1 mM initial ATP concentration). Hydrolysis required Mg^{2+} ; Mn^{2+} showed lower activity (30% of maximum rate at 2 mM Mn^{2+}), while Ca^{2+} and Zn^{2+} were essentially inactive (<10% activity, data not shown). At pH 6.2, the kinetics of hydrolysis was similar except that a slightly greater extent was observed, possibly because ADP inhibition was less effective. In either

case, the reaction continued until more than 90% of the ATP was hydrolyzed. In contrast, with DS DNA at pH 6.2, the maximum extent of hydrolysis was about 60% and the extent decreased at lower *recA* protein or ATP concentrations (Fig. 3*a* and *b*). The relatively low extent of hydrolysis is probably a consequence of product inhibition since addition of ADP also limited the extent (Fig. 3*c*). For both SS and DS DNA-dependent ATP hydrolysis, a pulse of ATP into an ongoing

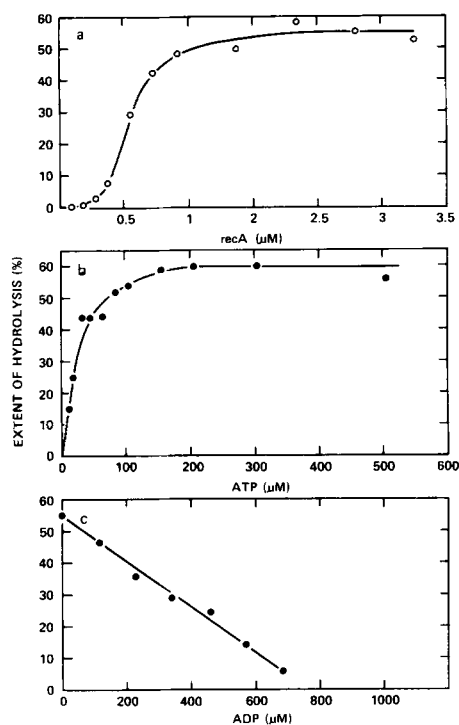


FIG. 3. Extent of DS DNA-dependent ATP hydrolysis. All reactions were performed as described under "Experimental Procedures" in sodium maleate (pH 6.2) using Fraction II *recA* protein. Extent of hydrolysis was determined from a time course of the reaction. *a*, 450 μ M ATP and 22 μ M P22 DS DNA. *b*, 450 μ M ATP, 26 μ M pBR322 DS DNA and 2.2 μ M *recA* protein. *c*, 450 μ M ATP, 22 μ M P22 DS DNA, and 2.2 μ M *recA* protein. Incubation was at 30 °C in *a* and *b* and 37 °C in *c*.

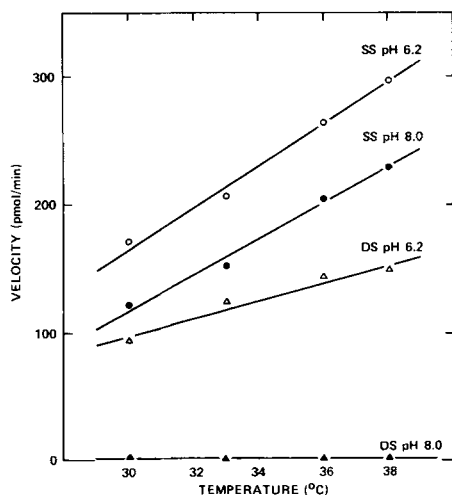


FIG. 4. Temperature dependence of ATP hydrolysis. Reaction (30 μ l) were performed as described under "Experimental Procedures" in either Tris-HCl (pH 8.0) or sodium maleate (pH 6.2) containing 512 μ M ATP, 1.1 μ M Fraction IV *recA* protein, and 42 μ M DNA (ϕ X174 SS or pZ6b DS).

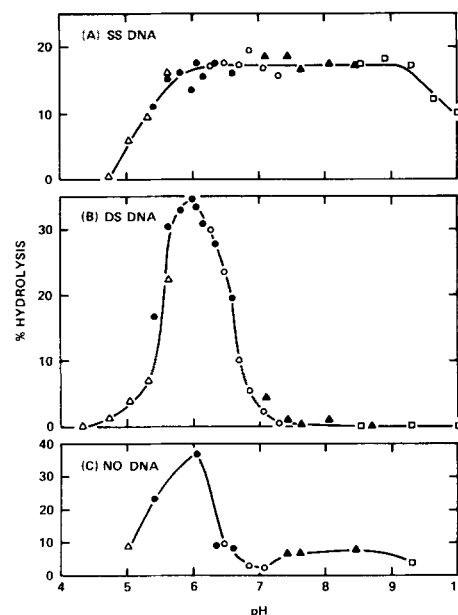


FIG. 5. pH dependence of ATP hydrolysis. Reaction mixtures (30 μ l) for measurement of SS DNA-dependent ATPase activity contained 20 mM buffer, 10 mM $MgCl_2$, 1 mM dithiothreitol, 30 μ g/ml of BSA, 1 mM ATP, 74 μ M ϕ X174 SS DNA, and 2.9 μ M Fraction II *recA* protein; incubation was for 15 min. Assays of DS DNA-dependent ATPase activity were the same except that 37 μ M P22 DNA was used, *recA* protein concentration was 5.8 μ M, and incubation was for 30 min. In reaction mixtures (20 μ l) for assay of DNA-independent ATPase activity, BSA was omitted, ATP was 50 μ M, Fraction IV *recA* protein was added to 6 μ M, and incubation was for 60 min. pH was measured at 20 mM buffer in the presence of 10 mM $MgCl_2$ at 25 °C. Δ — Δ , Sodium acetate; \bullet — \bullet , sodium maleate; \circ — \circ , potassium phosphate; \blacktriangle — \blacktriangle , Tris-HCl; \square — \square , glycine-NaOH.

reaction resulted in hydrolysis of the pulsed ATP with no significant lag (<2 min, data not shown). However, in the DS DNA-dependent reaction, addition of ATP following the onset of ADP inhibition failed to stimulate additional hydrolysis.

ATP hydrolysis in the presence of SS DNA had a similar temperature profile at either pH 6.2 or 8.0 and differed from that observed with DS DNA at pH 6.2 (Fig. 4). At pH 8.0, no DS DNA-dependent ATP hydrolysis was observed with Fraction IV, although with Fraction II hydrolysis was observed after a 10-min lag. This hydrolysis reached only 10 to 20% the rate observed at pH 6.2.

ATP hydrolysis also occurred at pH 8.0 in the absence of DNA, although at a much reduced rate. As shown in Fig. 2*C*, ADP and P_i were produced at similar rates despite the fact that less than one hydrolytic event occurred per enzyme molecule. This finding suggests that neither phospho- nor adenyl enzyme intermediates accumulated under these conditions.³ The velocity of this reaction was constant for 4 to 5 h, and at least 4 mol of ADP were formed/mol of enzyme. DNA-independent hydrolysis at pH 6.2 showed kinetics similar to those at pH 8.0 (data not shown), but the velocity was somewhat greater at the lower pH (see Fig. 5).

The SS DNA-dependent ATPase activity co-purifies with *recA* protein and is altered by mutations in the *recA* gene (1, 10). The DS DNA-dependent and DNA-independent ATPase activities also co-purified with *recA* protein. Thus, when Fraction II was chromatographed on DEAE-cellulose and the peak

³ Low level phosphorylation of *recA* protein (Fraction II) ($\sim 10^{-3}$ phosphate/*recA* monomer) was observed in both the absence and presence of SS or DS DNA. However, neither Fraction III nor IV was phosphorylated, suggesting that the phosphorylation observed with Fraction II was due to a contaminating protein kinase activity.

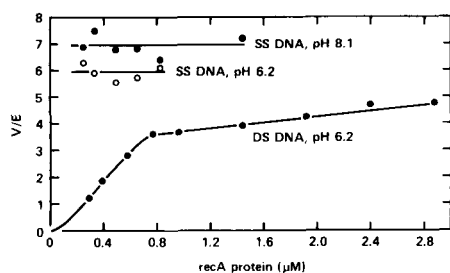


FIG. 6. Effect of recA protein concentration on turnover number. Reactions were as described under "Experimental Procedures" and contained 500 μ M ATP and the indicated amounts of Fraction II recA protein. SS DNA-dependent ATPase reactions were in Tris-HCl (pH 8.1) or sodium maleate (pH 6.2). Measurements of DS DNA-dependent ATPase were in sodium maleate (pH 6.2). A time course was performed at each recA protein concentration and the initial velocity determined.

TABLE I

Dependence of DS DNA-dependent ATP hydrolysis on recA protein concentration

Reactions (30 μ l) were performed as described under "Experimental Procedures" in sodium maleate (pH 6.2) containing 465 μ M ATP. DS DNA was pZ6b; recA protein was Fraction II. Velocities were determined by measuring the time course of the reaction.

recA protein	DNA	Nucleotides/ recA protein monomer	V/E
μ M			
2.2	308	140	5.3
2.2	62	28	4.5
0.44	308	700	1.3
0.44	62	140	1.8
0.44	12	28	1.0

fractions rechromatographed on hydroxylapatite, all three ATPase activities co-eluted with recA protein (Fig. 1). Furthermore, the ratio of the ATPase activities was essentially the same in Fractions III and IV. Thus, we conclude that all three ATPase activities are intrinsic to the recA protein.

Effect of pH on ATP Hydrolysis—SS DNA-dependent ATPase activity showed essentially no change in activity in the pH range from 6 to 9 (Fig. 5A). In contrast, the DS DNA-dependent ATPase showed a sharp optimum near pH 6 (Fig. 5B). This pH dependence was the same with both linear and supercoiled DS DNA and accounts for the low levels of DS DNA-dependent ATPase activity previously reported (1, 2, 9, 10).⁴ A shift from pH 6.2 to pH 8.0 caused immediate cessation of DS DNA-dependent ATP hydrolysis (data not shown), indicating a continuous requirement for the lower pH.

DNA-independent ATP hydrolysis also showed maximum activity near pH 6 (Fig. 5C); however, some hydrolysis occurred at pH 7.5. DNA-independent hydrolysis at either pH 6.2 or pH 7.5 was unaffected by 5 mM potassium phosphate, making it unlikely that a polynucleotide cofactor was synthesized by a contaminating polynucleotide phosphorylase.

All three ATP hydrolytic activities were impaired below pH 6. This was due, at least in part, to instability of recA protein under these conditions since the extents as well as the rates of hydrolysis were diminished.

Effect of recA Protein Concentration on ATP Hydrolysis—The turnover number (moles of ADP formed/mol of recA protein/min) of the SS DNA-dependent ATPase reaction was independent of recA protein concentration for concentrations above 0.2 μ M recA protein (Fig. 6), indicating that the velocity

of hydrolysis is directly proportional to recA protein concentration. Below 0.2 μ M, the turnover number decreased, possibly due to dissociation of recA protein oligomers (10) or enzyme inactivation. In contrast, the turnover number for ATP hydrolysis in the presence of DS DNA at pH 6.2 increased with recA protein concentration, indicating that the velocity was a nonlinear function of enzyme concentration. A similar effect was observed with both supercoiled and linear DS DNA. As shown in Table I, this effect was due to the recA protein concentration and not the ratio of recA protein to DNA. Inasmuch as the SS DNA-dependent reaction is directly proportional to recA protein concentration at both pH 6.2 and pH 8.1, the variation in turnover number is not simply due to the lower pH but must instead reflect a difference in the interaction between recA protein and the SS or DNA cofactor.

The turnover numbers for DNA-independent ATP hydrolysis at both pH 6.2 and 7.5 were independent of recA protein concentration from 1.6 μ M to 6.6 μ M recA protein (data not shown). Lower protein concentrations have not been investigated.

Effect of Monovalent Ions on ATP Hydrolysis—The SS and DS DNA-dependent activities showed different sensitivities to monovalent ion concentration (Fig. 7). The SS DNA-dependent reaction was relatively resistant to salt, being 50% inhibited at 300 mM KCl or NH_4Cl . In contrast, the DS DNA-dependent reaction showed an abrupt inhibition at about 100 mM salt.

Inhibition of ATP Hydrolysis by ADP—The SS and DS DNA-dependent ATPase activities were inhibited to different degrees by ADP (Fig. 8). At equal ADP and ATP concentrations, SS DNA-dependent ATPase activity at pH 6.2 was reduced less than 2-fold. In contrast, DS DNA-dependent activity was reduced 5-fold. This difference is related to the different Hill coefficients for ATP in the two reactions (18).

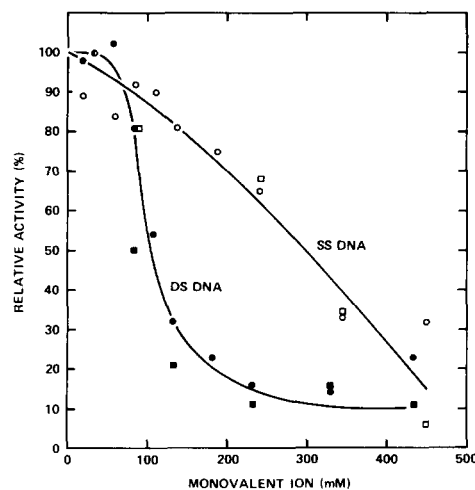


FIG. 7. Effect of monovalent ions on ATPase activity. Assays of ATPase activity were performed in sodium maleate (pH 6.2) as described under "Experimental Procedures." Reaction mixtures (30 μ l) also contained 30 μ g/ml of BSA, 500 μ M ATP, and the indicated concentrations of KCl or NH_4Cl . SS DNA-dependent ATPase reactions contained 296 μ M ϕ X174 SS DNA, and 2.9 μ M Fraction II recA protein. Incubation was for 10 min. DS DNA-dependent ATPase reactions contained 7.4 μ M P22 DNA and 5.9 μ M Fraction II recA protein. Incubation was for 30 min. One-hundred% activity represents 30 and 34% hydrolysis for the SS DNA- and DS DNA-dependent reactions, respectively. The pH of the buffer was adjusted for each salt concentration to be between pH 6.0 and 6.2. \circ — \circ , KCl; \square — \square , NH_4Cl , SS DNA-dependent ATPase; \bullet — \bullet , KCl; \blacksquare — \blacksquare , NH_4Cl , DS DNA-dependent ATPase.

⁴ In a previous publication (3), the pH of DS DNA binding and ATPase assays was incorrectly reported as pH 7.5 instead of approximately pH 6.

Hydrolysis of Other Nucleoside Triphosphates—The *recA* protein hydrolyzes a variety of nucleoside triphosphates in addition to ATP (Table II) (1). In all reactions, (r,d)ATP was most efficiently hydrolyzed but (r,d)UTP and, to a lesser extent, (r,d)CTP were also hydrolyzed. dTTP and (r,d)GTP were relatively inert to hydrolysis. Although the relative rates at which the different NTPs were hydrolyzed varied slightly with reaction conditions, the same general specificity was observed, providing further evidence that all of the hydrolytic activities are due to the *recA* protein. In addition, both the rate and extent of hydrolysis of dNTPs were somewhat greater than for rNTPs, and in the absence of DNA, dATP was hydrolyzed much more rapidly than was ATP.

Polynucleotide Cofactor Requirement for DNA-dependent ATP Hydrolysis—A variety of polydeoxynucleotides can stimulate the ATPase activity of *recA* protein (Table III). Polydeoxypyrimidines and poly(dA) were most effective while poly(dG) was inactive. Polyribonucleotides were poor cofactors, although poly(rU) and poly(rC) showed some activity. Thus, the SS polynucleotide cofactor shows sugar, but not

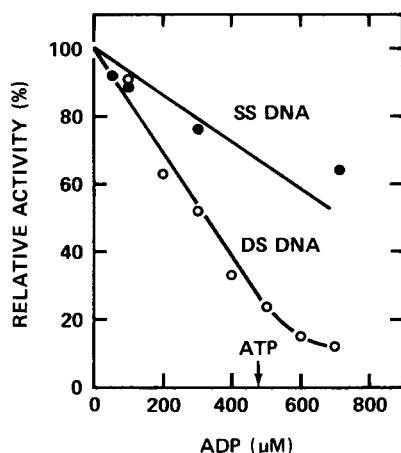


FIG. 8. Inhibition of ATPase activity by ADP. Reactions were performed as described under "Experimental Procedures" and contained the indicated concentration of ADP. Assays were in sodium maleate (pH 6.2) and contained 475 μ M ATP (arrow), 2.2 μ M Fraction II *recA* protein, and either 217 μ M heat-denatured calf thymus DNA or 31 μ M P22 DS DNA. Velocities were measured by a time course of the reaction. One-hundred% activity corresponded to 1066 and 707 pmol of ADP formed/min for ATPase reactions with SS DNA and DS DNA, respectively.

TABLE II

Hydrolysis of nucleoside triphosphates by recA protein

SS and DS DNA-dependent ATPase reactions in Tris-HCl (pH 7.5) and sodium maleate (pH 6.2), respectively, contained 500 μ M NTP and 0.79 μ M Fraction IV *recA* protein; DNA-independent ATPase reactions contained 50 μ M NTP (pH 7.5) or 100 μ M NTP (pH 6.2) and 4.8 μ M Fraction IV *recA* protein. A time course was performed for each NTP. The rate of ATP hydrolysis, arbitrarily defined as 100% activity, was 7.8, 2.0, 9×10^{-3} , and 2×10^{-2} mol of ADP formed/mol of *recA* protein/min for reactions with SS DNA, DS DNA, no DNA (pH 7.5), or no DNA (pH 6.2).

NTP	SS DNA (pH 7.5)	DS DNA (pH 6.2)	No DNA	
			(pH 7.5)	(pH 6.2)
rATP	=100%	=100%	=100%	=100%
dATP	121	157	435	449
rGTP	2	7	8	3
dGTP	4	25	8	3
rUTP	48	62	18	24
dUTP	79	86	30	41
dTTP	4	7	14	13
rCTP	27	40	12	6
dCTP	40	62	14	12

TABLE III

Polynucleotide cofactors for SS DNA-dependent ATP hydrolysis by recA protein

Reactions in Tris-HCl (pH 8.1) were as described under "Experimental Procedures" and contained 1.4 μ M Fraction II *recA* protein and either 500 μ M or 1 mM ATP. Velocities were determined from a time course of the reaction.

Polynucleotide	Concentration	V/E
	μ M	ADP/ <i>recA</i> protein monomer/min
Calf thymus DNA (heat denatured)	514	7.4
ϕ X174 SS DNA	18	6.4
Poly(dA)	4.2	4.8
	19	6.9
	142	9.0
Poly(dG)	12	<0.1
Poly(dI)	19	7.4
Poly(dT)	4.2	8.9
	19	11.8
	92	11.1
dT ₁₂	92	<0.1
Poly(dC)	19	13.0
Poly d(A-T)	31	0.46
	129	1.3
Poly d(G-C)	26	<0.1
Poly(dA)·poly(dT)	26	1.1
Poly(dG)·poly(dC)	26	0.69
Poly(rA)	92	<0.1
	513	<0.1
Poly(rU)	4.2	0.17
	462	4.9
Poly(rC)	4.2	1.6

sequence, specificity (10).

Although (dT)₁₂ was inactive as a cofactor, a 74-nucleotide *Hae* III restriction endonuclease fragment from ϕ X174 viral strand DNA stimulated the ATPase activity (data not shown). Thus, there is also a size requirement for the SS DNA cofactor (19).

DISCUSSION

DNA-dependent Hydrolysis of ATP—SS and DS DNA both stimulate the *recA* protein-catalyzed hydrolysis of ATP, but the reactions differ in their responses to pH, ionic strength, ADP, and *recA* protein concentration. Similar distinctions have been observed for the binding of *recA* protein to SS and DS DNA in the presence of the nonhydrolyzable analog ATP(γ S) (14). Thus, the differences between the SS and DS DNA-dependent ATPase reactions reflect, at least in part, different requirements for prehydrolytic binding of SS and DS DNA to *recA* protein. The two types of reactions exhibit the same nucleotide specificity. Moreover, since UTP, GTP, and dTTP are competitive inhibitors of ATPase activity (18), NTP hydrolysis very likely occurs at a common site on the enzyme. This conclusion is strengthened by the observation that ATP(γ S) binds tightly to a single site per enzyme monomer (13). We, therefore, conclude that although the mechanism of interaction of *recA* protein with SS and DS DNA differs, once bound, both DNAs cause the conformational change(s) in *recA* protein necessary to stimulate NTP hydrolysis at a single active site.

Effect of pH—pH affects several aspects of *recA* protein function. Thus, binding to DS DNA (14), DS DNA-dependent ATPase activity, and DNA-independent ATPase activity all have pH optima near pH 6. Furthermore, SS DNA-dependent

ATP hydrolysis shows a different dependence on ATP and DNA concentrations at pH 6.2 compared to pH 8.0 (18). We do not know if the pH affects more than one step or possibly a step common to all hydrolytic reactions. We have, however, observed differences in the oligomeric structure of recA protein at pH 6.2 and 7.5 (14).

Since binding of recA protein to DS DNA at pH 8.0 is stimulated by SS DNA (11), a simple explanation for the effect of the lower pH is that it elicits a change in the protein similar to that promoted by SS DNA. However, the requirement for SS DNA is not completely eliminated at pH 6.2 since ATP hydrolysis at pH 6.2 still requires a DNA cofactor. Thus, there may be two effects of DNA on the enzyme; one accomplished by acidic pH or SS (but not DS) DNA and another requiring either SS or DS DNA but unaffected by pH.

These effects are consistent with the model for strand assimilation first proposed by Shibata *et al.* (11) and which we have adapted for this discussion (Fig. 9). The model illustrates a possible relationship between the forms of the enzyme involved in strand annealing and assimilation. In particular, at pH 7.5, SS DNA and ATP are required to activate the enzyme (stage I) and allow it to bind DS DNA (stage II). Strand assimilation, which requires ATP hydrolysis, then ensues (stage III). We imagine that SS DNA annealing is an analogous process in which SS DNA replaces DS DNA in stage II.

This model suggests a simple relationship between the various ATPase reactions described in this paper. At pH 6, we imagine the stage I transition to be DNA-independent, allowing DS DNA to interact with recA protein (in stage II) in the absence of SS DNA. At pH 8, DS DNA fails to stimulate ATP hydrolysis because the stage I transition is not stimulated by DS DNA and is rate-limiting. This also provides an explanation for the pH dependence of the rate of DNA-independent ATP hydrolysis. We imagine that in the absence of DNA at pH 8, stage I is rate-limiting but, at pH 6, stage I becomes independent of DNA, and the DNA-dependent stage II transition is then rate-limiting. Thus, ATP hydrolysis in the absence of DNA, although never as rapid as in the presence of DNA, has a greater velocity at the lower pH.

This model demonstrates one possible relationship between the hydrolytic reactions catalyzed by recA protein at different pH values and with different DNA cofactors. However, further experiments are necessary to test the validity of this scheme.

DNA-independent Hydrolysis of ATP—The DNA-independent hydrolysis of ATP by recA protein occurs at approximately 0.1 to 1% of the rate of the DNA-requiring reaction, depending upon the pH. It is clear that the DNA-independent

ATPase activity is due to the recA protein since it co-purifies with the DNA-dependent ATPase activities. Moreover, the DNA-independent ATPase activity has a nucleotide specificity and pH profile that are analogous to the DNA-dependent reactions. Contamination of the recA protein with small amounts of DNA, despite purification through Polymyxin P, phosphocellulose, DEAE-cellulose, hydroxylapatite, and two ammonium sulfate precipitations, could account for this reaction. This possibility cannot be ruled out directly because the amount of DNA required to give the low rate of hydrolysis observed would be below the level of detection. However, the characteristics of ATP hydrolysis in the absence of exogenous DNA argue against contamination of the recA protein with DNA. In particular, because of the extremely tight binding of ATP(γ S) to recA protein in the presence of DNA, it is possible to inhibit irreversibly the DNA-dependent ATPase activity by preincubation of recA protein with ATP(γ S) and DNA prior to the addition of ATP (13). However, preincubation with ATP(γ S) in the absence of DNA does not inhibit DNA-independent ATPase activity at either pH 6.2 or 7.5, thus making it unlikely that there is significant contamination of the recA protein with DNA.

While more elaborate explanations are possible, we believe that this activity represents a genuine DNA-independent ATPase activity intrinsic to the recA protein. Allowing that noncovalent binding of DNA either induces or stabilizes a new conformational state of the enzyme, it is likely that there will be an equilibrium between the two states in the absence of DNA. Thus, if this equilibrium determines the rate-limiting step for DNA-independent hydrolysis, and assuming that all recA protein molecules participate, the observed velocities of hydrolysis imply that DNA shifts this equilibrium by 100- to 1000-fold, depending on the pH.

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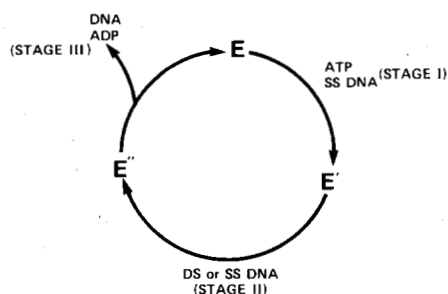


FIG. 9. Possible relationship between DNA binding and ATP hydrolysis at pH 7.5. In stage I at pH 7.5, recA protein (*E*) binds SS DNA and ATP, converting it into a form (*E'*) which can bind a 2nd DNA molecule in stage II. At pH 6.2, the conversion of *E* to *E'* is independent of SS DNA although ATP is still required. *E'* binds to DS DNA (in strand assimilation) or to SS DNA (in strand annealing). In stage III, hydrolysis of ATP and release of product occur at both pH values.